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Salinity and temperature effects on the growth and chlorophyll-a content of some planktonic algae

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- *Abstract:* The effect of salinity (0-40 ‰) and temperature (11-36°C, at 5°C intervals) variations on maximum growth rate (div d^{-1}), maximum yield (log_{10} cell number) and chlorophyll- *a* content (pg $cell^{-1}$) of four planktonic algae was examined under laboratory conditions. *Phaeodactylum tricornutum* grew over the entire range of experimental salinities, at 11-26 °C. The highest maximum growth rates (1.6 div d^{-1}) occurred between 9-30 ‰ and 16-26 °C. Optimum salinity range for maximum yield (7.0) was found at 9-35 ‰, under 16 °C. *Tetraselmis gracilis* reproduced from 4 to 40 ‰ at 11-31 °C, with the highest values of maximum growth rate (1.6 div d^{-1}) and maximum yield (6.1) occurring at salinities between 14-40 ‰ at 11-21 °C and 11-16 °C, respectively. *Minutocellus polymorphus* and *Chaetoceros* sp grew between 9-40 ‰ and 11-31 °C. Their highest maximum growth rates (2.1 and 2.6 div d^{-1}, respectively) were found at 31°C, between 20-35 ‰ and 20-40 ‰, respectively. The highest maximum yields for *M. polymorphus* (7.2) were recorded between 16-21 °C at 20-40 ‰ and for *Chaetoceros* sp (6.8), between 25-40 ‰ at 16-31°C. Chlorophyll-*a* content per cell was not conspicuously associated to temperature and salinity for the four species. At low salinity extremes, when cell division was inhibited, an increase in the amount of chlorophyll-*a* per cell was detected.

- *Resumo*: Estudou-se o efeito de variações de salinidade (0-40 ‰) e temperatura (11-36°C, em intervalos de 5°C) sobre a taxa máxima de crescimento (div d^{-1}), o rendimento máximo (log_{10} nº cel ml^{-1}) e o conteúdo de clorofila-*a* (pg cel^{-1}) de quatro espécies de algas planctônicas, sob condições de laboratório. *Phaeodactylum tricornutum* cresceu em toda a amplitude de salinidade experimental e entre 11-26°C. As mais altas taxas de crescimento (1.6 div d^{-1}) foram obtidas entre 9-30 ‰ e 16-26°C. O ótimo de salinidade para o rendimento máximo (7.0) foi observado entre 9- 35‰, à 16°C. *Tetraselmis gracilis* se reproduziu nas salinidades de 4 a 40 ‰ e nas temperaturas de 11 a 31°C, com os mais altos valores de taxas máximas de crescimento (1.6 div d^{-1}) e rendimento máximo (6.1), ocorrendo entre 14-40 ‰ , nas temperaturas entre 11-21°C e 11-16°C, respectivamente. *Minutocellus polymorphus* e *Chaetoceros* sp cresceram entre 9-40 ‰ e 11-31 °C. Os valores mais altos para as taxas máximas de crescimento (2.1 e 2.6 div d^{-1}, respectivamente) foram obtidos à 31 °C, entre 20-35 ‰ e 20-40‰, respectivamente. Os máximos rendimentos para *M. polymorphus* (7.2) foram observados entre 16-21 °C e 20-40 ‰ e para *Chaetoceros* sp (6.8), entre 25-40 ‰ e 16-31 °C. Nas quatro espécies estudadas, o conteúdo de clorofila-a por célula não se associou claramente às variações de temperatura e salinidade. Nos extremos baixos de salinidade, em que a divisão celular foi inibida, verificou-se um aumento da concentração celular de clorofila-*a*.

- *Descriptors*: Biossays, Phytoplankton, Salinity effects, Temperature effects, Growth, Chlorophyll, *Phaeodactylum tricornutum, Tetraselmis gracilis, Chaetoceros* sp, *Minutocellus polymorphus*.

- *Descritores*: Bioensaios, Fitoplâncton, Efeitos da salinidade, Efeitos da temperatura, Crescimento, Clorofila, *Phaeodactylum tricornutum, Tetraselmis gracilis, Chaetoceros* sp, *Minutocellus polymorphus*.

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Introduction

A study of the physiological responses of microalgae in relation to variations in salinity (Shimura *et al.*, 1979; Brown, 1982; Brand, 1984; Fabregas *et al.*, 1984, 1985, 1987; Tsuruta *et al.*, 1985; Rendall & Wilkinson, 1986) and temperature (Verity, 1981; Redalje & Laws, 1983; Baars, 1988*a,b*) can reveal some differences on their adaptability to the environment. Ecophysiological studies are helpful in determining environmental conditions which are optimal, favourable or merely tolerable for the growth of species. Moreover, temperature and salinity interactions (Krawiec, 1982; Saks, 1982; Watras *et al.*, 1982; Miller & Kamykowski, 1986) may elucidate the observed patterns of distribution and abundance of planktonic algae in nature (Gessner, 1970; Gessner & Schramm, 1971). Besides, temperature and salinity responses are useful for establishing the appropriate conditions for optimizing cell growth in bioassay procedure (Shubert, 1984; Bonin *et al.*, 1986), in aquaculture systems (Epifanio *et al.*, 1981; Wikfors *et al.*, 1984; Okauchi & Hirano, 1986; Walsh *et al.*, 1987) or in commercial production of chemicals derived from microalgae (Cohen, 1986; Richmond, 1986).

In the present investigation, we report on the influence of temperature and salinity variations on growth responses and chlorophyll-*a* content of four species of planktonic algae isolated from the southeastern coastal areas of Brazil.

Materials and methods

The experimental organisms were obtained from the Culture Collection of the Instituto Oceanográfico da Universidade de São Paulo, SP, Brazil. *Phaeodactylum tricornutum* Bohlin (Bacillariophyceae), strain Phaeo-Ub3 and *Chaetoceros* sp (Bacillariophyceae), strain Chaets-Ub1 were isolated from coastal areas of Ubatuba (SP) in 1972 and 1986, respectively. The *Chaetoceros* sp is a tiny small unicellular species which resembles either *C. calcitrans, C. gracilis* or *C. simplex,* commonly used in aquaculture. *Tetraselmis gracilis* (Kylin) Butcher (Prasinophyceae), strain Tetrag-C1, was isolated from the estuarine region of Cananéia (SP) in 1972. *Minutocellus polymorphus* (Hargraves & Guillard) Hasle, von Stosch & Syvertsen (Bacillariophyceae), strain Minp-CF1, was isolated from coastal areas of Cabo Frio (RJ) in 1981.

Experiments were done at salinities ranging from 0.32 to 40.07 ‰ and temperatures from 11 to 36 °C, at 5 °C intervals. These were done in batch cultures. Stock cultures at these salinities and temperatures were established through stepwise subculturing, from an initial stock at 30 ‰ salinity and 21 °C temperature. The cultures were not bacteria free and grown in 300-ml borosilicate Erlenmeyer flasks, in controlled temperature and light incubators. The light intensity was 125 μE m^{-2} s^{-1}, which was provided by fluorescent lamps (TLD 15w /54). Light was measured with a Lambda Instruments LI-190S quantum sensor and LI-185A quantum meter. A 12:12 light-dark regime was chosen.

All the glassware were first soaked in commercial sulfuric acid. Thereafter, they were washed sequentially with 10% Na_2C0_3, distilled water, 10% HCl, distilled water, dried at 50 °C, covered and autoclaved.

The organisms were grown in artificial seawater medium (ASP 2), formulated by Provasoli *et al.* (1957). In order to obtain the highest salinity (40.07 ‰), the original salinity was altered by the proportional increase in the amounts of the major seawater elements (Na, K, Mg, Ca, B, Cl, S). All these major elements were omitted for the lowest salinity (0.32 ‰). The rest of the salinity range (3.57, 9.02, 14.42, 19.77, 25.18, 30.14 and 35.14 ‰) were made by adding different proportions of the 0.32 and 40.07 ‰ media. Salinity was measured with a refractometer (Goldberg T/C), conductivity and chlorinity. pH values of media were about 7.8 before sterilization. The culture media were autoclaved for 45 minutes, allowed to cool for at least 24 h, whereafter the vitamin solution (sterilized by membrane filtration, Millipore GS) was added maintaining sterility.

Growth kinetics was measured in 200 ml cultures done in triplicate. Cells from the stock cultures preconditioned for five days were used so that initial cell concentration were about 10^4 cells ml^{-1}. At different times (0, 1, 3, 5, 8, 11, 15 and 18 days), culture aliquots were fixed with Lugol's solution and counted by using a hemacytometer. The exponential growth rate, u, was determined for each replicate by the least squares method of linear regression on the logarithmically transformed data (Guillard, 1973). Maximum yield logarithmically transformed was calculated for each replicate. Growth rate and maximum yield averages were obtained for each experimental combination of salinity and temperature.

Culture aliquots from each treatment and at each sampling time were combined and filtered through a GF/F Whatman filter, and chlorophyll-*a* extracted according to Strickland & Parsons (1968), using a Zeiss PMQ II spectrophotometer. The chlorophyll-*a* content obtained in the exponential growth phase was expressed per cell. Daily pH measurements were done for each replicate using a Beckman Electromate pHmeter.

A two-way analysis of variance (ANOVA) was carried out on growth rate and maximum yield averages in

order to test temperature and salinity effects. Differences between averages were tested for statistical significance ($P > 0,05$) with the Tukey method of multiple comparisons (Neter *et al.*, 1990). The results of chlorophyll-*a* content per cell at the exponential growth phase were subjected to the Tukey test for additivity (Neter *et al.*, *op. cit.*) to examine whether or not the two experimental factors interact. In case of a non- interactive model, a two-way analysis of variance (ANOVA) was applied, followed by the Tukey multiple comparison procedure. If the model was interactive, the Tukey method was employed directly, considering the pure error mean square, which represents the random variability of the error, excluding the interaction effects.

Results

The average maximum growth rates (Fig. 1) and maximum yields (Fig. 2) were plotted against salinity for each temperature and test species. The outcome of the statistical calculations are given in Tables 1-2. The coefficients of variation for the replicates were generally low (C.V. < 10%), except at the extremes of salinity and temperature.

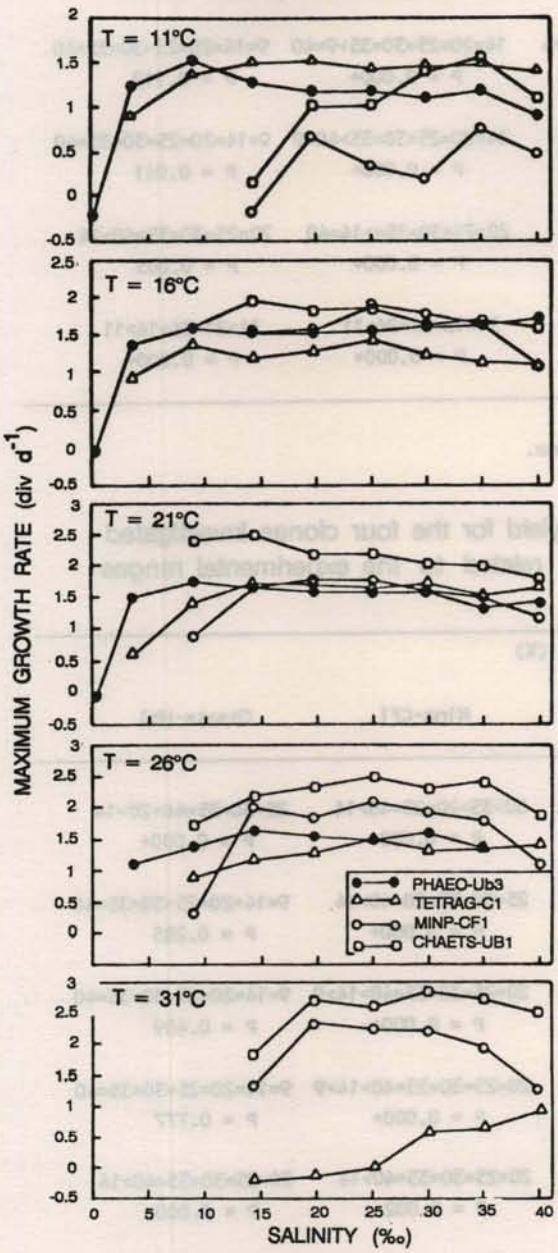

Fig. 1. Maximum growth rate (div d^{-1}) of *Phaeodactylum tricornutum* (Phaeo-Ub3), *Tetraselmis gracilis* (Tetrag-C1), *Minutocellus polymorphus* (Minp-CF1) and *Chaetoceros* sp (Chaets-Ub1) as a function of salinity and temperature.

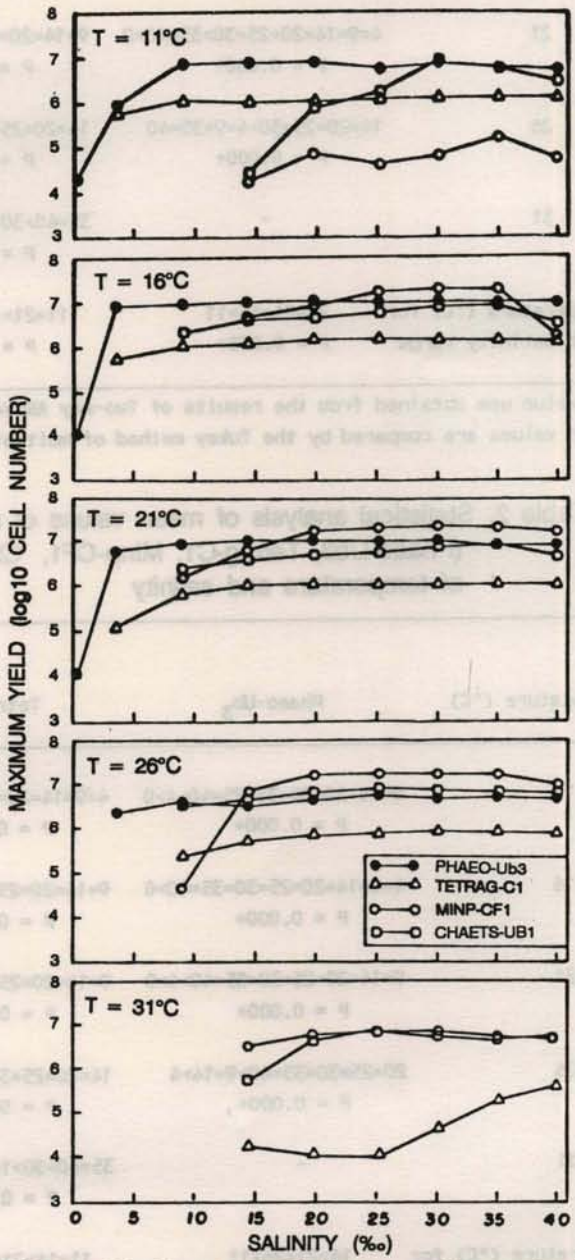

Fig. 2. Maximum yield (log_{10} cell number) of *Phaeodactylum tricornutum* (Phaeo-Ub3), *Tetraselmis gracilis* (Tetrag-C1), *Minutocellus polymorphus* (Minp-CF1) and *Chaetoceros* sp (Chaets- Ub1) as a function of salinity and temperature.

Table 1. Statistical analysis of mean values of maximum growth rate for the four clones investigated (Phaeo-Ub3, Tetrag-C1, Minp-CF1, Chaets-Ub1), related to the experimental ranges of temperature and salinity

	Salinity (%)			
Temperature (°C)	Phaeo-Ub_3	Tetrag-C1	Minp-CF1	Chaets-Ub1
11	9=14>4=20=25=30=35=40>0 P = 0.000+	9=14=20=25=30=35=40>4 P = 0.000+	20=25=30=35=40>14 P = 0.001	20=25=30=35=40>14 P = 0.000+
16	4=9=14=20=25=30=35=40>0 P = 0.000+	9=14=20=25=30=35>4=40 P = 0.000+	14=20=25=30=35=40 P = 0.523	9=14=20=25=30=35=40 P = 0.709
21	4=9=14=20=25=30>35=40>0 P = 0.000+	9=14=20=25=30=35=40>4 P = 0.000+	14=20=25=30=35>9=40 P = 0.000+	9=14=20=25=30=35=40 P = 0.112
26	14=20=25=30>4=9=35=40 P = 0.000+	14=20=25=30=35=40>9 P = 0.000+	14=20=25=30=35>40>9 P = 0.000+	9=14=20=25=30=35=40 P = 0.061
31	-	35=40>30>14=20=25 P = 0.000+	20=25=30=35=>14=40 P = 0.000+	20=25=30=35=40>14 P = 0.005
Temperature (°C) for each salinity range	26=21=16>11 P = 0.000+	11=21>16=26>31 P = 0.000+	31>16=21=26>11 P = 0.000+	31>21=26>16>11 P = 0.000+

P value was obtained from the results of Two-way ANOVA.
Mean values are compared by the Tukey method of multiple comparisons.

Table 2. Statistical analysis of mean values of maximum yield for the four clones investigated (Phaeo-Ub3, Tetrag-C1, Minp-CF1, Chaets-Ub1) related to the experimental ranges of temperature and salinity

	Salinity (%)			
Temperature (°C)	Phaeo-Ub_3	Tetrag-C1	Minp-CF1	Chaets-Ub1
11	9=14=20=30=35>25=40>4>0 P = 0.000+	4=9=14=20=25=30=35=40 P = 0.098	30>35>20=25=40>14 P = 0.000+	25=30=35=40>20>14 P = 0.000+
16	4=9=14=20=25=30=35=40>0 P = 0.000+	9=14=20=25=30=35=40>4 P = 0.027	25=30=35>20=40>14 P = 0.000+	9=14=20=25=30=35=40 P = 0.285
21	9=14=20=25=30=35=40>4>0 P = 0.000+	9=14=20=25=30=35=40>4 P = 0.000+	20=25=30=35=40>14>9 P = 0.000+	9=14=20=25=30=35=40 P = 0.409
26	20=25=30=35=40>9=14>4 P = 0.000+	14=20=25=30=35=40>9 P = 0.000+	20=25=30=35=40>14>9 P = 0.000+	9=14=20=25=30=35=40 P = 0.777
31	-	35=40>30>14=20=25 P = 0.000+	20=25=30=35=40>14 P = 0.002	20=25=30=35=40>14 P = 0.000+
Temperature (°C) for each salinity range	16>21=26>11 P = 0.000+	11=16>21=26>31 P = 0.000+	16=21>26=31>11 P = 0.000+	16=21=26=31>11 P = 0.000+

P value was obtained from the results of Two-way ANOVA.
Mean values are compared by the Tukey method of multiple comparisons.

P. tricornutum, growing over the entire range of experimental salinities and *T. gracilis* reproducing between 4-40‰ were more euryhaline than *M. polymorphus* and *Chaetoceros* sp which grew in a salinity range of 9- 40‰ .

The temperature 36 °C was lethal for the four species. *T. gracilis*, *M. polymorphus* and *Chaetoceros* sp, growing between 11 and 31 °C, were more eurythermic than *P. tricornutum* which was unable to survive at 31°C.

The highest maximum growth rates (1.6 div d^{-1}) for *P. tricornutum* and *T. gracilis* were measured at salinities ranging from 9 to 30 ‰ and 14 to 40 ‰, respectively. For *M. polymorphus* (2.1 div d^{-1}) and *Chaetoceros* sp (2.6 div d^{-1}) these values were found between 20-35‰ and 20-40‰, respectively. The optimum temperatures obtained for maximum growth rate were between 16-26°C for *P. tricornutum*, between 11-21°C for *T. gracilis*, and 31 °C for *M. polymorphus* and *Chaetoceros* sp (Fig. 1).

The highest maximum yields (log_{10} cell number) were found for *M. polymorphus* (7.2) and *P. tricornutum* (7.0) at salinities between 20-40‰ and 9-35‰, respectively. *Chaetoceros* sp and *T. gracilis* showed their maximum values (6.8 and 6.1, respectively) in media with salinities of 25-40‰ and 14-40 ‰, respectively. The optimum temperatures for achieving the highest maximum yields were 16 °C for *P. tricornutum*, 11-16 °C for *T. gracilis*, 16-21°C for *M. polymorphus* and 16- 31°C for *Chaetoceros* sp (Fig. 2).

Chlorophyll-*a* contents per cell against salinity and temperature for each species are shown in Figure 3. The Tukey test for additivity and the two - way analysis of variance showed that temperature and salinity did not have an influence on the chlorophyll-*a* contents per cell for *P. tricornutum* and *T. gracilis*.

For both *M. polymorphus* and *Chaetoceros* sp the Tukey test for additivity pointed out the existence of a significant interaction between temperature and salinity on chlorophyll-*a* results. According to the Tukey method of multiple comparisons, certain temperature-salinity combinations (T = 16°C, S = 14‰ for *M. polymorphus* and T = 11°C, S = 14‰ for *Chaetoceros* sp) produced the highest values (Fig. 3). At low salinity extremes, when cell division was inhibited, an increase in the amount of chlorophyll-*a* per cell was detected for *M. polymorphus*, *Chaetoceros* sp and *P. tricornutum* (Fig. 3).

The culture media showed pH values between 7.3-8.6 for *P. tricornutum*, 7.6-8.4 for *T. gracilis*, 7.4-8.3 for *M. polymorphus* and 7.6-8.3 for *Chaetoceros* sp.

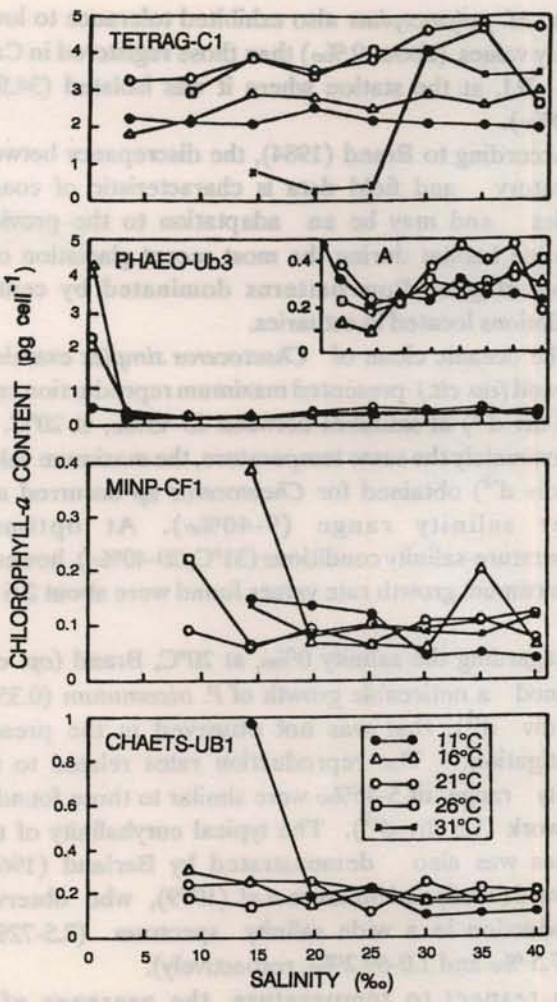

Fig. 3. Chlorophyll-*a* content (pg $cell^{-1}$) at different temperatures for *Tetraselmis gracilis* (Tetrag-C1), *Phaeodactylum tricornutum* (Phaeo-Ub3), *Minutocellus polymorphus* (Minp-CF1) and *Chaetoceros* sp (Chaets-Ub1), as a function of salinity. A. Chlorophyll-*a* data excluding the highest values relative to the salinity of 0.32 ‰ .

Discussion

Natural and cultured microalgae can show good agreement in respect to salinity tolerance (Paasche, 1975; Krawiec, 1982; Saks, 1982; Rendall & Wilkinson, 1986). *Tetraselmis gracilis*, a brackish water flagellate, reproducing from 4 to 40‰, revealed a salinity growth range compatible with the salinity regimes occurring in Cananéia (from 1 to 35‰, according to Aidar-Aragão, 1980), its original habitat. However, *P. tricornutum* and *Chaetoceros* sp were able to survive at much lower salinities (about 0 and 9‰, respectively) than those found at Ubatuba coastal waters, where the diatoms strains were collected. In this region, salinity is quite constant (34.03-35.53 ‰), except for some relatively low values occurring bordering the inner parts of the bays (Teixeira,

1973). *M. polymorphus* also exhibited tolerance to lower salinity values (about 9 ‰) than those registered in Cabo Frio - RJ, at the station where it was isolated (34.94 - 35.80‰).

According to Brand (1984), the discrepancy between laboratory and field data is characteristic of coastal species and may be an adaptation to the previous estuarine habitat during the most recent glaciation or a reflect of gene flow patterns dominated by central populations located in estuaries.

The oceanic clone of *Chaetoceros simplex* examined by Brand *(op. cit.)* presented maximum reproduction rates (1.85 div d^{-1}) at salinities between 25-45‰, at 20°C. At approximately the same temperature, the maximum values (2.1 div d^{-1}) obtained for *Chaetoceros* sp occurred at a wider salinity range (9-40‰). At optimum temperature-salinity conditions (31°C; 20-40‰), however, the maximum growth rate values found were about 2.6 div d^{-1}.

Regarding the salinity 0‰, at 20°C, Brand (*op. cit.*) obtained a noticeable growth of *P. tricornutum* (0.35 to 1.24 div d^{-1}), that was not observed in the present investigation. The reproduction rates related to the salinity range of 5-45‰ were similar to those found in this work (1.6 div d^{-1}). The typical euryhalinity of this species was also demonstrated by Berland (1966), Hayward (1968) and Shimura *et al.* (1979), who observed reproduction in a wide salinity spectrum (2.5-72‰, 4.4-87.5 ‰ and 1.0-69.2‰, respectively).

In respect to temperature, the presence of *P. tricornutum* in Ubatuba-SP is associated with 22°C (Vieira, 1975). Indeed, this temperature is near the optimum determined for the same clone in relation to a wide spectrum of physiological processes, under laboratory conditions (Vieira, *op. cit.*; Gaeta, 1985). Based on the results of Vieira (*op. cit.*) and on those found at the present investigation, the lethal temperature for this isolate is between 27 and 30°C. *P. tricornutum* is frequently the dominant species in both enriched laboratory and outdoor mass cultures. Nevertheless, its temperature optimum found in defined laboratory experiments (20°C) is obscured by the interactions of other environmental factors found outdoors, so that *P. tricornutum* can be dominant outdoors over a wide range of temperature, between 0-25°C (Goldman & Ryther, 1976; Goldman, 1977*a*; Goldman & Mann, 1980).

Cell division in *P. tricornutum* is relatively slow, considering its size, i. e., 24 um in length (Bonin *et al.*, 1986). In this study, the maximum value found was 1.84 div d^{-1}, obtained for one of the replicates at 9‰ salinity and 11 °C temperature. The maximum growth rate found by several authors is close to 2 div d^{-1} (Spencer, 1954; Nelson *et al.*, 1979; Sharp *et al.*, 1979; Gaeta, 1985). Ben-Amotz & Gilboa (1980) and Fawley (1984) recorded maximum division rate values of 2.3 (20°C) and 2.16 div d^{-1} (23 °C), respectively. However, Subba Rao (1981) mentioned maxima about 1.0 div d^{-1}. In outdoor tanks, the values are even much lower (Raymont & Adams, 1958).

Clones of *M. polymorphus* (referred as *Bellerochea polymorpha*), isolated from different environments and studied by Hargraves & Guillard (1974), showed salinity and temperature tolerance ranges very similar to those obtained in the present work. For the salinity spectrum of 1.2-32‰, they observed that this diatom was able to reproduce between 8-32‰, 12-32‰ or 16-32‰, with these salinity ranges permitting growth rate estimated as half maximal value or greater. The five clones examined presented a measurable growth from 11°C or 15°C to 30.5°C or 31.5°C. At these highest temperatures, they exhibited rapid and consistent growth responses. In our clone, however, maximum growth rates at 9‰ salinity were equal or lower than half maximal value. It also showed the highest maximum growth rates at 31°C (2.1 div d^{-1}), between 20-35‰, indicating that its high temperature limit might be still higher, as it was found for the tropical clones previously described.

T. gracilis grew almost over the whole experimental salinity range, with maximum growth rates between 14-40‰. As a matter of fact, the genus *Tetraselmis* has been often cited as specially able to tolerate wide salinity fluctuations, since it grows at almost constant rates in a wide salinity spectrum (McLachlan, 1961; Hellebust, 1976; Fabregas *et al.*, 1984). It was also considered to be an eurythermic organism, developing at environmental temperatures between 5-33°C (Okauchi & Fukusho, 1984). However, our results showed a weak growth of *T. gracilis* at 31°C.

Temperature has been considered one of the most important variables affecting algal growth (Eppley, 1972; Raven & Geider, 1988; Kozitskaya, 1989) and the chemical composition of the marine microalgae (Furnas, 1978; Yoder, 1979; Verity, 1981). The highest cellular contents of nitrogen, carbon and chlorophyll have been found at the extremes of the temperature growth range (Goldman & Ryther, 1976; Goldman, 1977*a,b*; Goldman & Mann, 1980), in continuous cultures. However, as it concerns to the cellular chlorophyll-*a* content, this behaviour has not been frequently observed because of the diversity of culture techniques and species and also due to the influence of light-dark cycles (Bonin *et al.*, 1986). Several authors have found maximum cellular chlorophyll contents at the most favourable temperature for growth. This pattern was shown for *P. tricornutum* and *Nitzschia closterium* (Morris & Glover, 1974), *Chaetoceros curvisetum* (Furnas, 1978), *Skeletonema costatum* (Yoder, 1979). For *Leptocylindrus danicus*, the maximum chlorophyll-*a* content was related to the

optimum temperature for the photosynthetic rate (Verity, 1981).

In the present work, salinity and temperature did not have a remarkable effect on the cellular chlorophyll-*a* content of the four species. The highest values were found under stressed temperature-salinity conditions, when cellular division rate was inhibited. A similar behaviour was demonstrated for *Platymonas* sp, which exhibited maximum chlorophyll concentrations at the extremes of the salinity range (McLachlan, 1961). This author pointed out that low salinities seemed to restrict cell division although chlorophyll synthesis was not affected in the same extent.

Conclusions

The four species examined are euryhaline and eurythermic. *P. tricornutum* and *T. gracilis* showed a wider adaptability to salinity changes. In an estuarine environment, where salinity fluctuations are very large, they could have competitive advantages. *M. polymorphus* and *Chaetoceros* sp showed maximum growth rates in higher temperatures. When summer conditions prevail, these species could be favoured. *P. tricornutum* and *T. gracilis* grew better in lower temperatures. The results obtained suggest that temperature and salinity affect the distribution and abundance of phytoplankton in the marine environment.

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