Request Date: 09-NOV-2007 MOBAC
Expires at ONE AM on: 14-NOV-2007
ILL Number:

TGQ or OCLC #:

ILL Number: 1998234
Rota Call Numbers: USC2:S&E Stacks QK568.T67 H38 ; S&E Stacks QK568.T67 H38 v.1:1 2002;
Material Type: Serial
Title: Harmful algae.
Article Author: Navarro, J. M.;Munoz, M. G.;Contreras, A. M.BACD, J.: Temperature as a factor regulating growth and toxin content in the dinofiagellate Alexandrium catenella
Vol./Issue: 5 6, 6, 5
Part Pub. Date: 2006
Pages: 762-
Pub. Place: Amsterdam ; New York : Elsevier, c2002-
Requester: CALIFORNIA STATE UNIV, MONTEREY BAY - Interlibrary Loan
Patron ID:
Patron Barcode:
Patron e-mail:
Service Level:
Delivery Method: ARIEL/TRICOR
Request Note: Please ARIEL when possible (198.189.249.141) - thank you!
FAX/ARIEL:(831) 582-3875
EMAIL:library_document_delivery@csumb.edu
OCLC Req. Ex. Source: ILLiad
Need by Date: 09-DEC-2007
Verification Source: <TN:17902><ODYSSEY:198.189.237.236/ILL
OCLC
Supplier Reference: ILLNUM:37008383

TGQ or OCLC #: 37008383
ID: MB@

ISBN/ISSN: 15689883

Address: CSU Monterey Bay/Library/100 Campus Center/Seaside, CA 93955-8001
Patron Name: Taylor, Bethany
Patron Address:
Patron Phone:
Service Type: Copy non returnable
Max Cost: USD25
Payment Type: IFM

Copyright Compliance: CCG

Requester Symbol: OCLC:MB@

Available online at www.sciencedirect.com
ScienceDirect
Harmful Algae 5 (2006) 762–769
HARMFUL ALGAE
www.elsevier.com/locate/hal

Temperature as a factor regulating growth and toxin content in the dinoflagellate *Alexandrium catenella*

J.M. Navarro *, M.G. Muñoz, A.M. Contreras

Instituto de Biología Marina "Dr. Jürgen Winter", Universidad Austral de Chile, Casilla 567, Valdivia, Chile

Received 24 October 2005; received in revised form 23 January 2006; accepted 7 April 2006

Abstract

Controlled laboratory culture of *Alexandrium catenella* was used to determine the effects of a range of temperatures between 10 and 16 °C on the growth and saxitoxin content of this dinoflagellate, using strain ACC02 isolated from seawater at Aysen, XI Region, Southern Chile. Cell cultures were made using L1 culture medium at 30‰ salinity, and a photon flux density of 59.53 μmol m^2 s^{-1}. The results showed that the duration of the exponential growth phase was determined by the experimental temperature, with maximum cell concentrations obtained at 12 °C; significantly lower cell concentrations and growth rates were obtained at 16 °C. Cell dry weight and chlorophyll *a* values followed cell growth trends. The toxicity of *A. catenella* was variable at all the experimental temperatures, with a tendency towards having an inverse relation to temperature, with the highest values occurring at 10 °C and the lowest at 16 °C. The optimal range of temperature for the growth of the Chilean strain of *A. catenella* differed from rates reported for this species isolated at other latitudes, and was correlated with natural temperature conditions predominant in the environment from which it was isolated. The inverse relation of toxicity with temperature in the laboratory was broadly reflected in observations on the toxicity of this dinoflagellate in the field, and coincided with results from the literature.

Keywords: *Alexandrium catenella*; Dinoflagellate; Temperature; Growth rates; Toxin content; PSP; HABs

1. Introduction

Harmful algal blooms (HABs) are phenomena which occur on a cosmopolitan scale in the sea, often producing serious consequences for public health and the well being of aquatic organisms where they occur. They may have serious productive and economic impacts on artisan and/or industrial fisheries. Initiation, development and decline of HABs are due to interactions of multiple biological, oceanographic and meteorological factors which are as yet poorly understood. Various authors have discussed the importance of temperature in the dynamics of HABs, with general agreement that this parameter plays a fundamental role both in the germination of cysts, and in vegetative growth of the cells. Steidinger (1997) suggested that temperature could affect division rates, photosynthesis and respiration, cell size, and other factors in species participating in these blooms. Anderson et al. (1984), suggested that temperature was one of the main environmental factors which affected the development of dinoflagellate blooms, with optimal values of 5–8 °C observed at high latitudes. In a study of *Alexandrium ostenfeldii* cultivated between 9.1 and 26.5 °C, Jensen and Moestrup (1997) observed growth between 11.3 and 23.7 °C; the optimal temperature for the culture was 20 °C, giving a growth rate of 0.3 divs d^{-1}. Temperature may also be an important factor in the production of paralytic shellfish toxin (PSP); Cembella (1998) showed that low temperatures contributed to high

* Corresponding author. Tel.: +56 63 221556; fax: +56 63 221455.
E-mail address: jnavarro@uach.cl (J.M. Navarro).

1568-9883/$ – see front matter
doi:10.1016/j.hal.2006.04.001

levels of PSP production, which in part explained the occurrence of high levels of toxin content from populations of *Alexandrium* at high latitudes. Here, Reguera (2002) suggested that low temperature contributed to the accumulation of toxins per cell, since cell division was responsible for lowering concentrations of toxin per cell by dilution.

South of Chile hosts large populations of filter feeding bivalve molluscs, highly susceptible to contaminative effects of toxins produced by *Alexandrium catenella* blooms. During the last decade, these HABs have increased in frequency, intensity, and area affected, including the coastal zone from Castro (Chiloé Island) to south of the Beagle Channel. From 1991 to the present, several cases of red tides formed of *A. catenella* have occurred in southern Chile, with the latest in the fall of 2002, having maximum concentrations of 700,000 cells L^{-1} (Clement et al., 2002) and toxicity values of above 20,000 μg of STX/100 g tissue.

The objective of the present study was to examine the role of temperature variation typical of southern Chile on the growth and toxin content of an *A. catenella* strain isolated from the region.

2. Materials and methods

2.1. Experimental design

Monoclonal non-axenic *A. catenella* (strain ACC02, Regional Center for Resource Analysis and Environment; CERAM) was employed in a series of growth experiments using static batch cultures. This strain had been isolated in Chile's Aysen Region in 1994 by CERAM, Universidad Austral de Chile. Cell cultures were carried out at four different temperatures (10, 12, 14, and 16 °C) using enriched, filtered and sterilized L1 seawater medium (Guillard and Hargraves, 1993). The salinity was 30‰, and the photoperiod 14/10 h (L/D), with a mean luminous intensity of 59.53 ± 0.25 μmol m^{-2} s^{-1} as measured using a LI-COR L1-250 Light Meter. The light sources were standard fluorescent tubes suspended over the culture bottles and as light level varied within the incubator, the cultures were rotated in their locations every 3 days. In all tests, *A. catenella* cells (32–36 μm) were inoculated into culture media from acclimated (4 months), exponential-phase stock cultures grown at a single temperature (14 °C). Three replicate 5000 mL Duran Schott Mainz bottles, containing 4800 mL culture medium were inoculated with about 600 cells mL^{-1} for each temperature run. Cultures at each temperature were carried out in a culture chamber, controlling the photoperiod and temperature levels.

Sampling of the cultures was carried out every 3 days at the beginning, and every 5 days in the other phases of the experiment to reflect conditions in the cultures through each of the population growth phases. Measurements on the cultures included cell density (cells mL^{-1}), growth rate (divs d^{-1}), total dry weights of cells in the culture (μg mL^{-1}), chlorophyll *a* (μg L^{-1}) and toxin concentration (fmol $cell^{-1}$). As cell concentration increased with time, sample volume for the different parameters declined during the experiments. As *A. catenella* cells were very inhomogenously distributed within the culture, bottles were gently homogenised before samples were taken for the different analyses.

2.2. Cell counts

Aliquots of 10 mL were recovered from each of the three replicate cultures, fixed with 10% seawater-formalin, and allowed to decant in sedimentation chambers for 40 min using the Utermöhl method (Utermöhl, 1958). To get accurate counts, dilutions were done for samples with high cell concentration. Cell counts were carried out using an inverted microscope, and an average from the three replicates was calculated.

2.3. Cell growth rate

Cell growth rate was determined every 3 days within the exponential phase of the cultures when this rate was at a maximum. Values represent the average of three replicates and the calculation was made following Guillard (1973):

$$K_e = \frac{\ln(N_1/N_0)}{t_1 - t_0}$$

where K_e is the growth constant, N the number of cells in the culture (cells mL^{-1}), T the time (days) and ln is the natural log.

2.4. Dry weight

It was determined using given culture volumes (day 0 = 150 mL) which were filtered onto 450 °C ashed, tared, 2.5 cm diameter GF/C glass fiber filters having a nominal porosity of 1.2 μm. Filtered material was washed with isotonic ammonium formate (3%), dried at 70 °C for 48 h, and weighed to determine the total weight of the sample.

2.5. Chlorophyll a

A given amount of culture was filtered (day 0 = 50 mL) from each replica onto pretreated 2.5 cm GF/C filters as above. Filters with retained material were wrapped in aluminum foil and stored at −20 °C until analysis (no more than 2 weeks). Extraction of chlorophyll *a* was done following the method of Yentsch and Menzel (1963), which included placing each filter in 10 mL of 90% acetone, and storing in the cold for 12–24 h. The samples were then centrifuged at 4000 rpm for 10 min and read in a Turner Designs TD-700 spectrofluorometer, previously calibrated with standard chlorophyll solutions.

2.6. Toxin analyses

Toxin content was determined on samples containing a minimum of 500,000 *A. catenella* cells, which were settled by centrifugation at 6000 rpm for 20 min. Each pellet was stored in an eppendorf tube at −20 °C until performance of the second step of the procedure which included extraction of the toxin. For this, each pellet was treated with 700 μL of 0.1 N HCl and sonicated in a bath (Aquasonic Model 75T) for 5 min, followed by heating to boiling for 10 min. The samples were then centrifuged at 14,000 rpm for 20 min. The supernatant (500 μL) was removed and made to 1000 μL with distilled water (400 μL) and 1 N HCl (100 μL). The samples were then frozen in eppendorf tubes until the assay was performed. Toxin content in *A. catenella* was assayed using the electrophysiological test of Vélez et al. (2001), where HEK 293 cells (Human Embryonic kidney cells) stably expressing STX-sensitive rat skeletal muscle Na channels were patch clamped in the whole-cell configuration. The equivalent STX concentration was estimated using calibration curves obtained by external perfusion with known concentrations of purified STX; these curves were generated using a stepped series of increasing concentrations of STX-dihydrochloride (US Food and Drug Administration, Office of Seafood).

2.7. Statistical analyses

Prior to evaluating statistical differences among the results obtained on the cultures at the different temperatures we carried out tests for normality and homogeneity of the data using the Shapiro–Wilks and Levene tests, respectively (Zar, 1999). Where normality did not exist in spite of making transformations, we employed Kruskal–Wallis non-parametric statistical tests. Linear regressions were carried out to determine the relationship among number of cells, dry weight, and chlorophyll *a*.

3. Results

3.1. Population growth

The cell concentration increased without interruption at all four experimental temperatures, in all cases presenting a sigmoidal growth curve (Fig. 1). Chain-forming cells were observed mainly during the first week of culture, reducing their presence to less than 7% when cultures reached ca. 5000 cells mL^{-1}. At 14 and 16 °C an initial lag phase was noted, in which there was a slow increase in cell density, continuing at all temperatures into an exponential growth phase whose extension over time varied with the experimental temperature. A stationary phase was observed prior to the senescence and death phases. The *A. catenella* cell concentration at 10 °C (Fig. 1) increased exponentially between days 0 and 55, reaching a maximum of 29,460 ± 460 cells mL^{-1}, followed by a marked decrease in the terminal phase of the culture. The growth rate calculated for 10 °C during the maximum cellular growth phase was 0.18 ± 0.0 divs d^{-1} (Table 1). The highest cell concentration occurred at 12 °C, with a mean growth rate of 0.11 ± 0.0 divs d^{-1} (Table 1). Among the experimental temperatures, cell growth was most rapid at 14 °C, reaching its maximum concentration on day 45 with the highest growth rate (0.30 ± 0.01 divs d^{-1}) observed in the study (Table 1 and Fig. 2). The lowest cellular increase and growth rate was observed at 16 °C (Table 1 and Fig. 2).

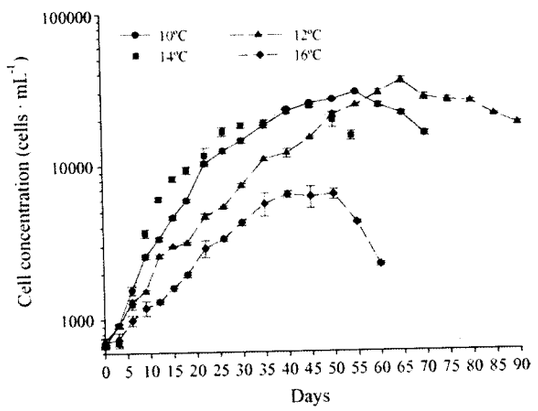

Fig. 1. Growth of *Alexandrium catenella* cultures at four different temperatures. Values are means ± S.E.

Table 1
Maximum cellular concentration (cells mL^{-1}) and growth rate (divs d^{-1}) in *A. catenella* cultivated at different temperatures (values are means ± standard error)

Temperature (°C)	Maximum cellular concentration (cells mL^{-1})	Growth rate (divs d^{-1})
10	29.460 ± 460	0.18 ± 0.00
12	34.850 ± 1.641	0.11 ± 0.00
14	24.000 ± 448	0.30 ± 0.01
16	6.389 ± 300	0.10 ± 0.03

Statistical comparisons of maximum cell concentration at the four temperatures showed no significant differences ($p > 0.05$) among the cultures at 10, 12 and 14 °C, with significantly less values at 16 °C. Tests on the growth rate (Fig. 2) showed significant differences (Kruskal–Wallis test; $H = 9.46$, d.f. 3, $p = 0.039$) only between 14 and 16 °C, with no significant correlations ($p > 0.05$) either with temperature ($R^2 = 0.21$) or maximum cell counts ($R^2 = 0.06$).

3.2. Dry weight

The dry weight of cultured cells increased without interruption at all temperatures (Fig. 3). The maximum levels were inversely proportional to the temperature, with values of: 117.3 ± 0.88, 120.3 ± 3.18, 90.9 ± 0.55 and 23.3 ± 1.38 μg mL^{-1} at 10, 12, 14 and 16 °C, respectively. The statistical analyses showed significantly lower values at 16 °C (Kruskal–Wallis test; $H = 40.91$, d.f. 3, $p = 0.000$) compared with the other temperatures. A direct relationship was observed between cell dry weight and cell concentration, with R^2 values equal to or greater than 0.92 ($p < 0.05$) at 10, 12 and 14 °C, and 0.75 ($p < 0.05$) at 16 °C (Fig. 4). The dry weight per cell was greater in the initial phase (days 0–6), decreasing during the exponential growth phase, and then increasing toward the terminal phase. The highest values for individual cell dry weights were obtained at day 3, at 14 °C (9.64 ± 1.13 ng $cell^{-1}$) and at 10 °C (9.47 ± 0.91 ng $cell^{-1}$). Lower weights were obtained at 12 °C (5.88 ± 0.34 ng $cell^{-1}$ at day 3) and at 16 °C (5.79 ± 1.71 ng $cell^{-1}$ at day 6).

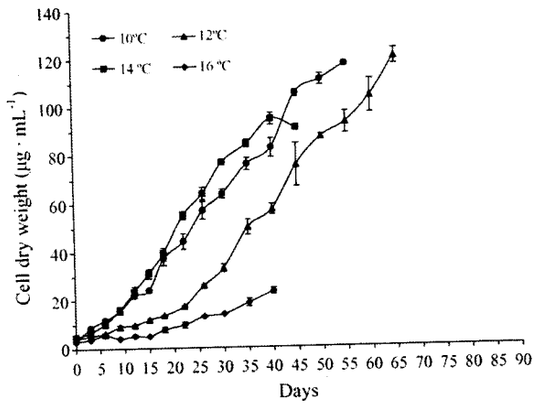

Fig. 3. Temporal fluctuation of the dry weight of *A. catenella* cultured at four different temperatures. Values are means ± S.E.

3.3. Chlorophyll a

Showed uninterrupted increase in cultures at 12, 14 and 16 °C (Fig. 5). At 10 °C a drop in chl *a* was recorded on day 26, again increasing from day 35 to reach a maximum on day 50. The statistical analyses showed significant differences in chl *a* ($p < 0.05$) between 10

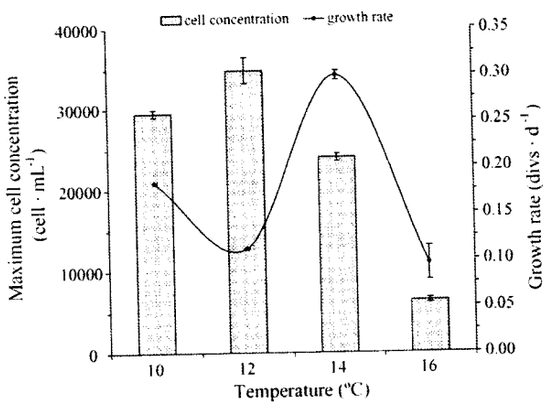

Fig. 2. Maximum cell abundance and growth rates of *A. catenella* cultured at four different temperatures. Values are means ± S.E.

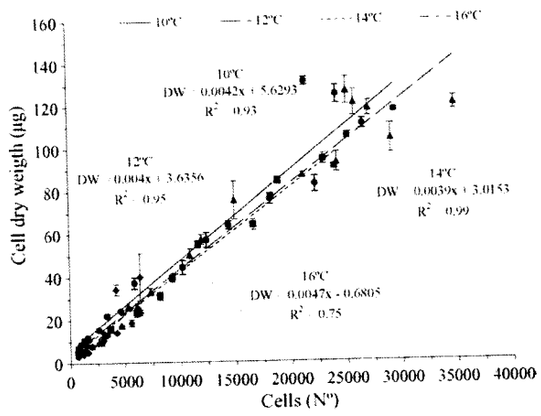

Fig. 4. Dry weight as a function of cell number of *A. catenella* cultured at four different temperatures. Values are means ± S.E.

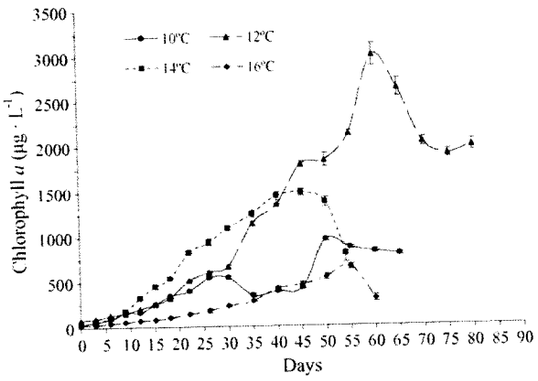

Fig. 5. Temporal fluctuation of chlorophyll *a* in *A. catenella* cultured at four different temperatures. Values are means ± S.E.

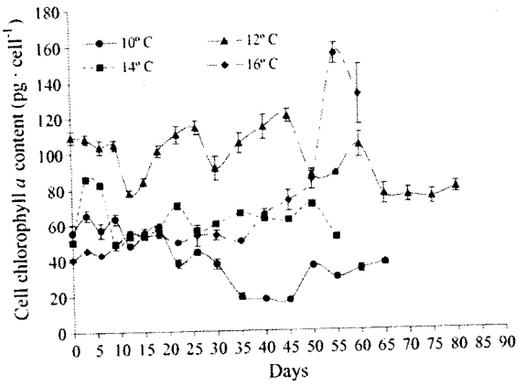

Fig. 7. Chlorophyll *a* per cell of *A. catenella* cultured at four different temperatures. Values are means ± S.E.

and 12 °C (Kruskal–Wallis test; $H = 45.37$, d.f. 3, $p = 0.030$). Chlorophyll *a* values obtained at 16 °C were significantly lower (Kruskal–Wallis test; $H = 45.37$, d.f. 3, $p = 0.000$) than those obtained for the other experimental temperatures. This parameter was highly correlated with cell abundance ($p < 0.05$), with R^2 values equal to or greater than ≥0.93 for cultures at 12 and 14 °C, and lower for 10 °C ($R^2 = 0.76$) and 16 °C ($R^2 = 0.72$) (Fig. 6). Highest values for individual cell chl *a* content (Fig. 7) were observed at 12 °C (73.96–120.19 pg cell^{-1}), while the lower values occurred at 10 °C (16.89–65.35 pg cell^{-1}).

3.4. Cell toxicity

Toxin content of *A. catenella* was highly variable at all the experimental conditions (Fig. 8), showing an inverse relation to the temperature when the maximum values of toxin content were compared (Fig. 9). The highest toxin concentration was registered at 10 °C on day 3 of the experimental period (27.77 ± 4.68 fmolSTX equiv. cell^{-1}). In contrast, the lowest values for toxins were obtained at 16 °C, where the maximum value reached 3.46 ± 0.00 fmolSTX equiv. cell^{-1}, on day 22 of the experimental period. The highest toxin concentration at 12 °C (day 40) and at 14 °C (day 6) were 13.33 ± 4.11 and 7.82 ± 1.65 fmolSTX equiv. cell^{-1}, respectively. The strong increase in toxin content observed between days 0 and 6 at 10, 12 and 14 °C, represented the lag phase preceding the beginning of the exponential growth phase of the cultures (Fig. 8). At 10 and 14 °C the toxin content showed a decline in the late stages of the cultures, while at 12 °C the toxin concentration continued a moderate increase until

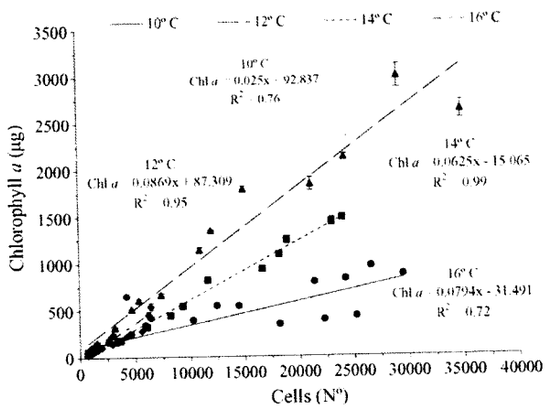

Fig. 6. Chlorophyll *a* as a function of cell number of *A. catenella* cultured at four different temperatures. Values are means ± S.E.

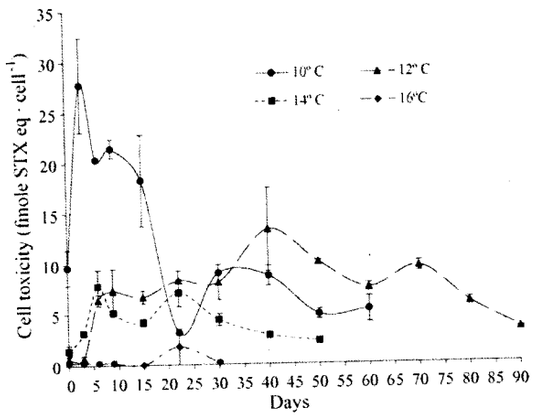

Fig. 8. Temporal fluctuation in the concentration of toxins in *A. catenella* cultivated at four different temperatures. Values are means ± S.E.

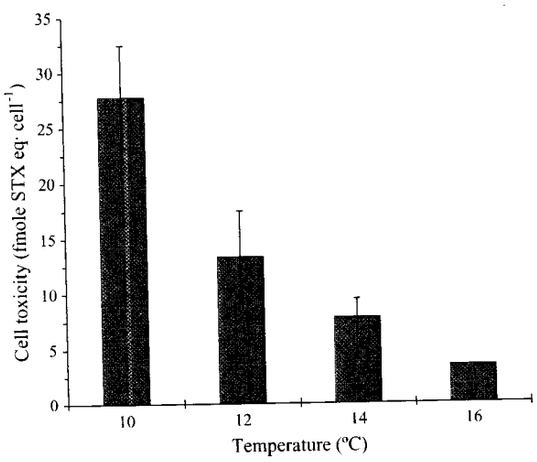

Fig. 9. Maximum concentration of toxin in *A. catenella* cultivated at four different temperatures. Values are means ± S.E.

day 40. The statistical analysis showed that the maximum cellular toxin content measured at 16 °C was significantly less (Kruskal–Wallis test; $H = 40.02$, d.f. 3, $p = 0.000$) than at the other temperatures, and that the cellular toxin content at 14 °C was significantly different than those measured at 10 °C (Kruskal–Wallis test; $H = 40.02$, d.f. 3, $p = 0.020$) and 16 °C (Kruskal–Wallis test; $H = 40.02$, d.f. 3, $p = 0.000$) (Fig. 9).

4. Discussion

Growth of *A. catenella* in culture followed a pattern similar to that described for other species of dinoflagellates (Parker et al., 2002; Juhl, 2005), and was dependent of temperature. Previous studies on the culture of *A. catenella* reported wide variability in total cell concentrations from 18,000 cells mL^{-1} (Siu et al., 1997) to 70,000 cells mL^{-1} (Séchet et al., 2003). The photoperiod employed for our experiments fell within the range cited as adequate for *A. catenella* by Gavin et al. (1997) of 14/10 to 16/8 h L/D. Other studies, however, employed higher intensities of light (Parker et al., 2002; Lim and Ogata, 2005), obtaining similar growth rates to those of the present study, suggesting the capacity of photoacclimation in the growth of *A. catenella* when exposed to low light intensity. Falkowski (1980), Falkowski and Raven (1997) and Ramus (1990) described the capacity for photoacclimation of unicellular algae and macrophytes, where chlorophyll per cell can increase 5 to 10-fold as irradiance decreases. This is in agreement with the results obtained by Matsuda et al. (1996) with *A. catenella*, which suggested that higher growth could be produced at photon flux densities above 50 μmol m^2 s^{-1}, a value lower than the light intensity used in this study (ca. 60 μmol m^{-2} s^{-1}).

According to Ichimi et al. (2001), strains of *Alexandrium tamarense* from Japan can grow within a broad range of temperature, with an optimum of 15 °C. This contrasted with present results, where the growth of *A. catenella* became significantly reduced at 16 °C, suggesting adaptation of this species to the environmental temperatures of southern Chile which rarely reach 16 °C (Navarro and Jaramillo, 1994). The highest mean population growth rate of 0.3 divs d^{-1} obtained at 14 °C, was close to that reported by other authors for this and other species of dinoflagellates. Siu et al. (1997) reported a growth rate of 0.29 divs d^{-1} for *A. catenella* at an optimal temperature of 20–25 °C. Gavin et al. (1997) also found the optimal growth range between 20 and 25 °C, for the same species. In contrast, the ACC02 strain of this species isolated in southern Chile had a lower range of thermal tolerance, with 16 °C representing the upper limit for cell division (0.1 divs d^{-1}), and 14 °C representing the optimal temperature for growth rate. These results suggest that the growth rate and the maximum cell density observed for *A. catenella* at 14 and 12 °C, are related to the optimal temperatures at which natural blooms occur (Molinet et al., 2003), showing the capacity of the species to adapt to a wide range of temperatures.

Increase in cell dry weight during the final phase of the culture was probably due to the accumulation of thecae of dead cells which added to the total weight of the culture and also to the increase in cell size due to lower division rates in the last stages of culture as reported by Jensen and Moestrup (1997) for *A. ostenfeldii*.

The highest values of chlorophyll *a* per cell at 12 °C were probably related to the low growth rate at this temperature, which according to Cembella (1998) avoids partition of cellular components into daughter cells. This condition, however, is not observed at 16 °C, where the lower concentration of chl *a* seems to be related with the negative effect of this temperature, which becomes the upper limiting value in its natural environment.

The toxin content of *A. catenella* was inversely correlated with temperature, in agreement with Cembella (1998), who concluded that the low temperatures contributed to high levels of PSP per cell, explaining the occurrence of higher levels of toxicity in *Alexandrium* populations at higher latitudes. Ichimi et al. (2002) also found an inverse relationship between toxin content and growth rates, introducing uncertainty as to whether the increase in

toxicity is a physiological response to lower temperature, or an indirect response due to the decreases in growth rates at lower temperature. Since the present study did not demonstrate a relation between the concentration of toxin per cell and the growth rate, the increase in toxicity is probably the result of the effects of low temperature on metabolic processes which regulate the production of toxins. However, the high cellular toxin content observed at 10 °C at the early stage of the experiment, could be related with the acclimation process of the cells previously acclimated at 14 °C. Increase in toxin has been described in the exponential growth phase, followed by decreases when the cells entered the stationary phase (Cembella, 1998; Gavin et al., 1997). This is in agreement with the present study for cultures at 10, 12 and 14 °C, where the toxin concentration showed an important increase between day 0 and most of the exponential growth phase. Cell toxin content values may be considered moderate to low, in agreement with Séchet et al. (2003), which determined toxin concentrations in a range of 13.4–40.3 fmol cell^{-1} in cultures of *A. catenella*. Higher toxicities have been reported by MacKenzie et al. (2004), with values of 150 and 329 fmol cell^{-1} in cultures of *A. catenella* and *A. tamarense*, respectively. Although the light intensity utilized in this study was near the lower limit for growth of this species (Matsuda et al., 1996), the present results suggest that temperature occupied a more important regulatory role in the growth of *A. catenella*. The optimal temperature range under the present light conditions was different from that obtained with strains of the same species isolated from other latitudes, suggesting that the ranges of tolerance of *A. catenella* to local environmental conditions such as light, temperature and salinity, could vary with the geographic location of its populations. The optimal range of temperature for the growth of the present dinoflagellate strain correlated well with ambient water temperatures in southern Chile, in which important blooms have been reported in recent years. The temperature was important not only for cellular growth, but was also a determinant factor on the concentration of toxin in the *A. catenella* cultures, where there is substantially less toxin per cell at the highest experimental temperature.

Acknowledgements

We especially thank G. Urrutia, M. Maturana and V. Garrido for their valuable help during the experiments, to M. Seguel for providing the *A. catenella* strain (ACC02) and to J. Toro for providing the chamber with controlled conditions for the cell cultures. We also thank Marco Cordova from the laboratory of Marine Toxins from Universidad de Chile for performing electrophysiological analysis of *Alexandrium* toxic cells and to an anonymous reviewer for the constructive criticisms that helped to improve our work. This study was supported by a research grant to JMN (Fondecyt 1030340).[SES]

References

Anderson, D.M., Kulis, D.M., Binder, B.J., 1984. Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis*: cyst yield in batch cultures. J. Phycol. 20, 418–425.

Cembella, A.D., 1998. Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms, vol. G41. NATO ASI Series, Berlin, pp. 381–403.

Clement, A., Aguilera, A., Fuentes, C., 2002. In: Análisis de marea roja en Archipiélago de Chiloé, contingencia verano 2002, XXII Congreso de Ciencias del Mar, 28–30 de mayo, Valdivia, Chile.

Falkowski, P.G., 1980. Light-shade adaptation in marine phytoplankton. In: Falkowsli, P.G. (Ed.), Primary Productivity in the Sea. Plenum, New York, pp. 99–117.

Falkowski, P.G., Raven, J.A., 1997. Aquatic Photosynthesis. Blackwell Science, USA, p. 375.

Gavin, K., Siu, Y., Young, M.L.C., Chan, D.K.O., 1997. Environmental and nutritional factors which regulate population dynamics and toxin production in the dinoflagellate *Alexandrium catenella*. Hydrobiologia 352, 117–140.

Guillard, R.R.L., 1973. Division rates. In: Stein, J.R. (Ed.), Handbook of Phycological Methods Culture Methods and Growth Measurements. Cambridge University Press, New York, pp. 290–311.

Guillard, R.R.L., Hargraves, P.E., 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. Phycologia 32, 234–236.

Ichimi, K., Yamasaki, M., Okumura, Y., Suzuki, T., 2001. The growth and cyst formation of a toxic dinoflagellate, *Alexandrium tamarense*, at low water temperatures in northeastern Japan. J. Exp. Mar. Biol. Ecol. 261, 17–29.

Ichimi, K., Suzuki, T., Ito, A., 2002. Variety of PSP toxin profiles in various culture strains of *Alexandrium tamarense* and change of toxin profile in natural *Alexandrium tamarense* population. J. Exp. Mar. Biol. Ecol. 273, 51–60.

Jensen, M.O., Moestrup, O., 1997. Autoecology of the toxic dinoflagellate *Alexandrium ostenfeldii*: life history and growth at different temperatures and salinities. Eur. J. Phycol. 32, 9–18.

Juhl, A.R., 2005. Growth rates and elemental composition of *Alexandrium monilatum*, red-tide dinoflagellate. Harmful Algae 4, 287–295.

Lim, P.T., Ogata, T., 2005. Salinity effect on growth and toxin production of four tropical *Alexandrium* species (Dinophyseae). Toxicon 45, 699–710.

MacKenzie, L., de Salas, M., Adamson, J., Beuzenberg, V., 2004. The dinoflagellate genus *Alexandrium* (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics. Harmful Algae 3, 71–92.

Matsuda, A., Nishijima, T., Fukami, K., 1996. Effects of nitrogen deficiency on the PSP production by *Alexandrium catenella* under axenic cultures. In: Yasumoto, T., Oshima, Y., Fukuyo, Y. (Eds.), Harmful and Toxic Algal Blooms. Intergubernamental Oceanographic Comisión of UNESCO, pp. 305–308.

Molinet, C., Lafon, A., Lembeye, G., Moreno, C., 2003. Patrones de distribución espacial y temporal de floraciones de *Alexandrium catenella* (Whedon & Kofoid) Balech 1985, en aguas interiores de la patagonia noroccidental de Chile. Rev. Chil. Hist. Nat. 76, 681–698.

Navarro, J.M., Jaramillo, R., 1994. Evaluación de la oferta alimentaria natural disponible a organismos filtradores de la bahía de Yaldad (43°08;73°44′), sur de Chile. Rev. Biol. Mar. 29 (1), 57–75.

Parker, N.S., Negri, A.P., Frampton, D.M.F., Rodolfi, L., Tredici, M.R., Blackburn, S.I., 2002. Growth of the toxic dinoflagellate *Alexandrium minutum* (Dinophyceae) using high biomasa culture systems. J. Appl. Phycol. 14, 313–324.

Ramus, J., 1990. A form-function análisis of photon capture for seaweeds. Hydrobiologica 204/205, 65–71.

Reguera, B., 2002. Establecimiento de un Programa de Seguimiento de Microalgas Toxicas. In: Sar, E.A., Ferrario, M.E., Reguera, B. (Eds.), Floraciones Algales Nocivas en el Cono Sur Americano. Instituto Español de Oceanografía, pp. 21–56.

Séchet, V., Berard, J., Bohec, M., Bougaran, G., Carré, C., Masselin, P., Truquet, P., 2003. Growth and toxicity of *Alexandrium catenella* from the Thau lagoon (France) cultured in stirred tank bioreactors. In: Villalva, A., Reguera, B., Romalde, J.L., Beiras, R. (Eds.), Molluscan Shelfish Safety. Intergovernamental Oceanographic Commission of UNESCO.

Siu, G.K.Y., Young, M.L.C., Chan, D.K.O., 1997. Environmental and nutritional factors which regulate population dynamics and toxin production in the dinoflagellate *Alexandrium catenella*. Hydrobiologia 352, 117–140.

Steidinger, K.A., 1997. Dinoflagellates. In: Tomas, C.R. (Ed.), Identifying Marine Phytoplankton. Florida Department of Environmental Protection. Florida Marine Research Institute, St. Petersburg, Florida, pp. 387–584.

Utermöhl, H., 1958. Zur vervollkommnung der quantitatven Phytoplankton-Methodik. Mitt. Int. Ver. Theor. Angew Limnoll. 9, 1–39.

Vélez, P., Sierralta, J., Alcayata, C., Fonseca, M., Loyola, H., Johns, D.C., Tomaselli, G.F., Marbán, E., Suárez, B.A., 2001. A functional assay for paralytic shellfish toxins that uses recombinant sodium channels. Toxicon 39, 929–935.

Yentsch, C.S., Menzel, D.W., 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Res. 10, 221–231.

Zar, J.H., 1999. Biostatistical Analysis. Prentice-Hall, Upper Saddle River, p. 123.