Culture and growth of two benthic diatoms species isolated from the Salar del Huasco (North of Chile, 20° S) at different conditions of temperature, light and nutrient

Cultivo y crecimiento de dos especies de diatomeas bentónicas aisladas del Salar del Huasco (Norte de Chile, 20° S) a diferentes condiciones de temperatura, luz y nutrientes

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ABSTRACT

Benthic diatoms are cultured usually under laboratory conditions to be used as a food source for other organisms, of aquaculture interest or for biotechnological applications. Laboratory experiments demonstrate the incidence of the physical and chemical variables on abundance and growth rates of diatoms. While macronutrients are usually selected and dosed into common culture mediums to meet the general requirements of a wide range of diatoms, the availability and optimization of micronutrients are more susceptible to each organism's particular physiological conditions. The aim of this study was to characterize the growth of two species of benthic diatoms isolated from the Salar de Huasco in batch cultures at different conditions of temperature (10, 15 and 20 °C), light intensity (40, 80 and 120 µmol m^{-2} s^{-1}) and concentrations of silica (1.06 x 10^{-4} M Na_2SiO_3 x $9H_2O$ and 2.12 x 10^{-4} M Na_2SiO_3 x $9H_2O$) and selenium (10^{-8} M H_2SeO_3) in f/2 medium, on a light-dark cycle of 18:6 h. Both *Nitzschia epithemioides* Grunow in Cleve & Grunow (1880) as *Nitzschia* sp. showed higher maximum cell densities (692800 ± 107704 and 649600 ± 68942 cells ml^{-1}, respectively) and exponential growth rates (1.80±0.56 and 0.97±0.32 div. d^{-1}, respectively) at the highest temperature (20°C). The light intensity to which the cultures were exposed had no effect on cell density and exponential growth rate in both taxa. Regarding to nutrients, an increase in silicates concentrations on the culture medium could promote the growth of *N. epithemioides* and *Nitzschia* sp. since a tendency was observed to higher cell densities (1.08 x 10^6 ± 84,639 and 1.32 x 10^6 ± 109,038 cells ml^{-1}, respectively) and exponential growth rates (1.98 ± 0.44 and 0.95 ± 0.18 div. d^{-1}, respectively) with respect at the normal f/2 medium and f/2 medium plus selenium. Simultaneous addition of silicate and selenium to the culture medium increased the maximum cell density of the two strains under study, but this increase was significant (p = 0.05) only for *N. epithemioides* and among the normal f/2 medium (719200 ± 116895 cells ml^{-1}) and the f/2 medium with double the amount of silicate and selenium (1498800 ± 209599 cells ml^{-1}). However, the exponential growth rates were not significantly different when compared to those of the control (without the addition of silicate and selenium). In conclusion both *N. epithemioides* and *Nitzschia* sp shown an increased cell density and exponential growth rate at 20 °C. The different light intensities not significantly influenced the growth of both taxa. An increase in the concentration of silicates or simultaneous increase in silicates and selenium in the culture medium can be considered as possible strategy to increase cell density of benthic diatom *N. epithemioides* in batch cultures.

Keywords: Growth characteristics, culture, *Nitzschia*, selenium, silicate.

RESUMEN

Las diatomeas bentónicas usualmente son cultivadas bajo condiciones de laboratorio para ser utilizadas como fuente de alimento de otros organismos de interés acuícola o para aplicaciones biotecnológicas. Experimentos de laboratorio demuestran la incidencia de las variables físicas y químicas sobre la abundancia y tasas de crecimiento de las diatomeas. Mientras los macronutrientes están generalmente seleccionados y dosificados en los medios de cultivo más comunes para satisfacer los requerimientos generales de un amplio rango de diatomeas, la disponibilidad y optimización de micronutrientes son más susceptibles a las condiciones fisiológicas particulares de cada organismo. El objetivo de este estudio fue caracterizar el crecimiento de dos especies de diatomeas bentónicas aisladas del Salar de Huasco en cultivos tipo batch, a diferentes condiciones de temperatura (10, 15 y 20 °C), intensidad de la luz (40, 80 y 120 µmol m^{-2} s^{-1}), y

concentraciones de sílice (1.06 x 10^{-4} M Na_2SiO_3 x $9H_2O$ y 2.12 x 10^{-4} M Na_2SiO_3 x $9H_2O$) y selenio (10^{-8} M H_2SeO_3) en medio f/2, a un ciclo 18:6 h luz-oscuridad. Tanto *Nitzschia epithemioides* Grunow in Cleve & Grunow (1880) como *Nitzschia* sp, exhibieron las mayores densidades celulares máximas (692800 ± 107704 y 649600 ± 68942 células mL^{-1}, respectivamente) y tasas de crecimiento exponencial (1,80 ± 0,56 y 0,97 ± 0,32 div. d^{-1}, respectivamente) a la temperatura más elevada (20 °C). La intensidad de luz a la cual los cultivos fueron expuestos no afectó la densidad celular y tasa de crecimiento exponencial en ambos taxa. En cuanto a los nutrientes, un incremento en la concentración de silicatos en el medio de cultivo, podría favorecer el crecimiento de *N. epithemioides* y *Nitzschia* sp. ya que se observó una tendencia al aumento en la densidad celular (1,08 x 10^6 ± 84.639 y 1,32 x 10^6 ± 109.038 células mL^{-1}, respectivamente) y tasa de crecimiento exponencial (1,98 ± 0,44 y 0,95 ± 0,18 div. d^{-1}, respectivamente) con respecto a los medios f/2 normal y f/2 más selenio. La adición simultanea de silicato y selenio al medio de cultivo aumentó las densidades celulares máximas de las dos cepas bajo estudio, pero este aumento fue significativo ($p = 0,05$) sólo para *N. epithemioides* y entre los medios de cultivo f/2 normal (719200 ± 116895 células mL^{-1}) y medio f/2 con el doble de silicato y selenio (1498800 ± 209599 células mL^{-1}). Sin embargo, las tasas de crecimiento exponencial no tuvieron diferencias significativas con el control (sin adición de silicato y selenio). En conclusión tanto *N. epithemioides* y *Nitzschia* sp. muestran un aumento en la densidad celular y la tasa de crecimiento exponencial a 20 °C. Las diferentes intensidades de luz evaluadas no influyeron significativamente en el crecimiento de ambos taxa. Un aumento en la concentración de silicatos o aumento simultáneo de silicatos y selenio en el medio de cultivo puede ser considerado como posible estrategia para incrementar la densidad celular de la diatomea bentónica *N. epithemioides* en cultivos discontinuos.

Palabras clave: Características de crecimiento, cultivo, *Nitzschia*, selenio, silicato.

INTRODUCTION

Diatoms are the main microalgal component in biomass and in biodiversity of marine and freshwater aquatic ecosystems, making up a part of phytoplankton and phytobenthos. Their contribution to the global primary production in these ecosystems is highly significant (Round *et al*. 1990). The communities of benthic diatoms, predominantly pennate, are much more difficult to sample and quantify, because they are strongly adhered to different types of substrates and in varied types of environments; for this reason, biologists and ecologists have largely disregarded them and there is little data available in the literature (Raniello *et al*. 2007). Nonetheless, benthic diatoms are cultured usually under laboratory conditions to be used as a food source for other organisms, such as abalone and sea urchin (Dunstang *et al*. 1994) or for biotechnological applications (Stoermer & Smol 1991).

The physiological plasticity of diatoms to environmental factors such as temperature, salinity, light intensity and nutrient concentrations is the keys to their ability to survive and grow in different types of environments (Tomas 1996, Dempster & Sommerfeld 1998, Thessen *et al*. 2005, Affan *et al*. 2007). Numerous laboratory experiments demonstrate the incidence of these factors on abundance and growth rates (Méléder *et al*. 2003, Mercado *et al*. 2004, Van der Grinten *et al*. 2005, Affan *et al*. 2007, Raniello *et al*. 2007, D'Alelio *et al*. 2009). Particularly important are the concentrations of silicate (macronutrient) and selenium (micronutrient) in the culture medium for the diatoms (Harrison *et al*. 1988, Round *et al*. 1990, Nelson *et al*. 1995, Tréguer *et al*. 1995, Raniello *et al*. 2007). Silicates are essential as a components of the cell wall and as metabolic regulators. In his absence the proteins, DNA, chlorophyll and carotenoid synthesis are inhibited; photosynthesis and glycolysis are reduced; lipid synthesis also can be enhanced and altered by the availability of silicates (Werner 1978, Taguchi *et al*. 1987, Roessler 1988, Round *et al*. 1990). The role of selenium in cell function is still unclear. Some evidence suggests that it is important in cell division processes and maintaining internal membrane integrity (Doucette *et al*. 1987), while it is also an essential part of the enzyme glutathione peroxidase, which protects cells against the destructive effects of hydrogen peroxide (Overbaugh & Fall 1982, Price & Harrison 1988). While macronutrients are usually selected and dosed into common culture mediums to meet the general requirements of a wide range of diatoms, the availability and optimization of micronutrients are more susceptible to each organism's particular physiological conditions (Keller & Selvin 1987).

Diatoms of the Salar de Huasco, in the northern Chilean Altiplano, are subject to severe changes in physical and chemical environmental variables. The limnic systems in this region are characterized by closed endorheic basins with more evaporation than precipitation (Vila & Mühlhauser 1987, Risacher *et al*. 2003). It has been hypothesized that due to the expansion and contraction of the lakes of the Altiplano, its aquatic organisms have been subjected to drastic changes in both water levels and composition of salts. This has resulted in aquatic systems with physicochemical characteristics that depend heavily on the availability of water (Chong 1988, Keller & Soto 1998, Risacher *et al*. 2003). It is likely that both the quality and the quantity of the water determine the biological diversity, its distribution and its ability to be present in this type of systems. The information on the systematics and ecology of benthic diatoms in limnic bodies of the Altiplano is scarce and mostly restricted to contributions with a strong physiographic orientation (Márquez-García *et al*. 2009).

The aim of this study was to characterize the growth of two species of benthic diatoms isolated from the Salar del Huasco in batch cultures at different conditions of temperature, light intensity and nutrient concentrations. Considering that the water of Salar de Huasco has high concentration of arsenic (ca. 12 mg L^{-1}), the results of this study, among others, will pursue researches to evaluate the ability of these taxa to bioconcentrate and to biotransform inorganic arsenic in to organic compounds that are less toxic.

MATERIALS AND METHODS

Sample site

Water and microalgae samples were collected in summer (November, 2012) from Lagoon Huasco, Salar de Huasco, located in the Altiplano (*ca*. 4000 m altitude), in the north of Chile (20°17'30"S; 68°52'20"W; Fig. 1). Its maximum depth is 40 cm and has unique physiographic and climatic characteristics (Risacher *et al*. 2003).

Physicochemical analyses

Water samples were collected in 1 liter polyethylene containers and then after being filtered (0.45 µm), the following parameters were measured: total dissolved solids (TDS), chloride (Cl), sulfate, (So_4), hardness ($CaCO_3$), nitrate (NO_3), phosphate (PO_4), sodium (Na) and potassium (K), according to standardized protocols (APHA, AWWA, WPCF 1992). *In situ*, measurements of temperature, electrical conductivity, pH, electrical potential and dissolved oxygen were taken using field equipment, WTW Multiline P3. These data were examined to obtain a reference of environmental conditions for growth of diatoms in terms of salinity, temperature, pH and nutrients.

Collection, isolation and establishment of unialgal culture

Lagoon Huasco, with its high elevation, is subject to strong winds, and because the depth at the collection site was not more than 10 cm, it was possible to obtain, by filtering with 20 µm phytoplankton net, microalgae, mix with floating fine sediment. The retained material was deposited in polyethylene containers and transported, cold, to the laboratory.

To establish unialgal cultures, the isolation method used was single-cell isolation by micropipette and successive washes, according to the protocol described by Andersen (2005, Chapter 6). Basically, the isolation was performed using well slides (with more than one depression) or directly on drops (of culture medium) placed on a slide (in rows) using an inverted microscope (Nikon TS 100). Using sterile Pasteur pipettes, the algal units were isolated and washed one by one, then deposited in test tubes or Petri dishes with f/2 culture medium (Guillard 1975, pH = 8.00, E.C = 52 mS cm^{-1}, TDS = 33300 mg L^{-1}). Finally non-axenic unialgal cultures of the taxa grew in 250 ml Erlenmeyer flasks (ca. 50 ml culture volume), incubated at 120 µmol photons m^{-2} s^{-1} (LI-250 Light Meter, LI-COR. U.S.A.), at 20 ± 2 °C and with a L:D cycle of 18:6 h.

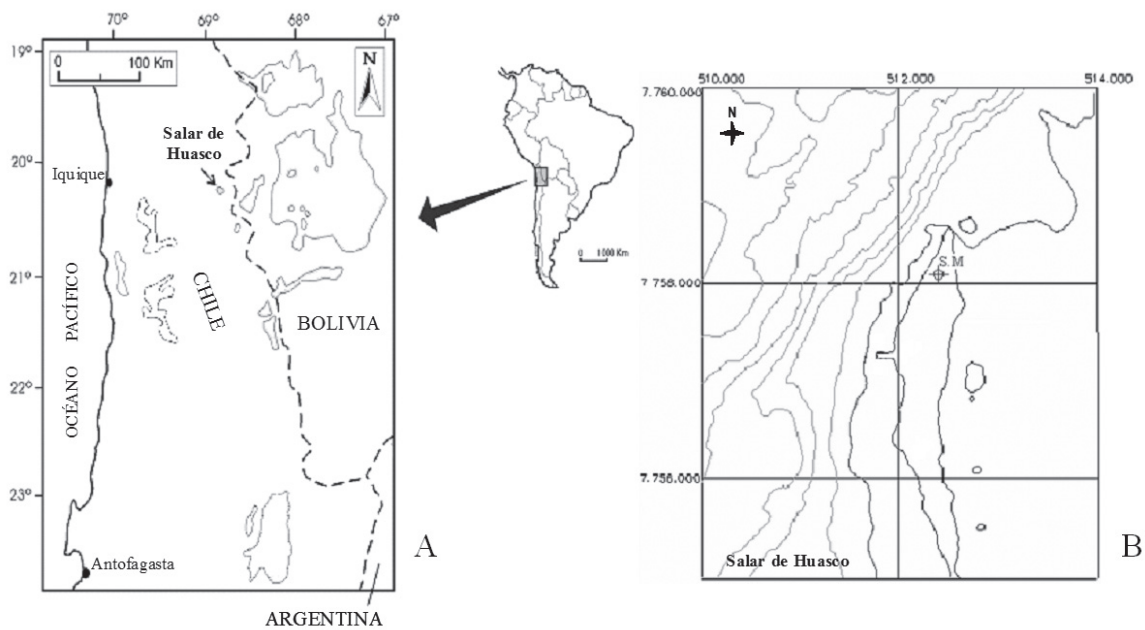

Figure 1. Map of the geographical location of the Salar de Huasco (modified from Chong, 1988).

Figura 1. Mapa de localización geográfica del Salar de Huasco (modificado de Chong, 1988).

Diatom identification

Taxonomic identification was performed based on the distinctive morphological characteristics of each taxon (Cleve-Euler 1953, Hustedt 1930, Krammer & Lange-Bertalot 1988, 1991, 2000, 2004). The morphology and data on cell dimensions of living diatoms were obtained using an Olympus light microscope (model CX 31. Camera, 518CU 5.0M CMOS). To remove the organic matter of the frustules, small volumes of the culture were washed with acid according to the method proposed by Hasle & Fryxell (1970). The frustules were prepared for a scanning electron microscope (SEM) as described by Amato *et al*. (2005) and examined under a scanning electron microscope SEM JEOL JSM6380-LV. The morphology of the frustules were analyzed from photomicrographs taken with a camera (JSM-6830) attached to the SEM microscope.

Strains of benthic diatoms in study were deposited in the Culture Collection of Microalgae, University of Concepción (CCM-UdeC), Chile, under the numbers, CCM-UDEC 296 and CCM-UDEC 297.

Experimental culture conditions

In order to evaluate growth rate and biomass production of the strains investigated, each taxon was cultured in f/2 + Si medium from Guillard (Guillard 1975). Three culture parameters were tested: temperature (10, 15 and 20 °C), photon flux density (40, 80 and 120 µmol m^{-2} s^{-1}) and different concentrations of selenium and silicate (Table I). All treatments were maintained on a light-dark cycle of 18:6 h and manual shaking twice a day.

For each strain, 1 x 10^4 cells ml^{-1} was inoculated into Erlenmeyer flasks containing 100 ml of culture medium and incubated for 20 days. Four replicates were used. The growth was monitored daily by cell counting (2 ml iodine fixed), under a Zeiss inverted microscope, and using Utermöhl chambers of 1 ml capacity. For this purpose, the samples were sonicated during 20 s. The density of cells per ml was calculated with the following formula:

$$N° \text{ (cells/ml)} = \frac{\text{Total chamber area}}{\text{Counted area}} \times N° \text{ counted cells.}$$

The specific growth rate (k) defined as the increase in cell density per unit time (Guillard 1973) was calculated according to the following equation:

$$k \text{ [div./day]} = 3.322 \log (N_1 - N_0)/t_1 - t_0$$

Where N_1 and N_0 correspond to the cell density at the beginning (t_0) and the end (t_1) of the time interval selected between inoculation and maximum cell density, respectively. The average value of the replicates was used for the growth curves of each studied treatment.

Statistical analyses

To determine significant differences between exponential growth rates and cell densities in the different treatments (temperature, photon flux density, and different concentrations of silicate and selenium in f/2 medium), comparisons were carried out using one-way analysis variance (ANOVA), followed by multiple comparisons with a Tukey's test. A value of $p < 0.05$ was considered significant. All of the statistical analysis was performed using the software SigmaPlot, version 11.0 (Systat Software Inc.).

Table I. Physiographic and climatic characteristics of the basin of the Salar de Huasco (Risacher *et al*. 2003).

Tabla I. Características fisiográficas y climáticas de la cuenca del Salar de Huasco (Risacher *et al*. 2003).

Basin	Salar de Huasco
Elevation (m)	3778
Maximum elevation (m)	5190
Mean temperature (°C)	5
Precipitation (mm/año)	150
Evaporation (mm/año)	1260
TDS min. (mg/L)	108
TDS max. (mg/L)	113,093
Evol. path	SO_4
Area of basin (km^2)	1572
Area of Salar (km^2)	51
Area of water (km^2)	2,5

Table II. Physical and chemical parameters of water from Lagoon Huasco.

Tabla II. Parámetros físicos y químicos del agua de Laguna Huasco.

in situ		Chemical Analysis (mg L^{-1})	
Temperature	12.5 °C	STD	50821
Electrical Conductivity	14.828 mS/cm	Cl	13028
pH	8.65	SO_4	4389
Electric Potential	-93 mV	$CaCO_3$	3109
Dissolved Oxygen	4.57 mg L^{-1}	NO_3	9.32
		PO_4	3.83
		Na	8140
		K	4017
		As	12.5 ± 0.8

*Data analyzed in water samples taken at the collection site of microalgae.
*Datos analizados en las muestras de agua tomadas en el sitio de colección de las microalgas.

RESULTS

Physicochemical analysis

The *in situ* measurements of temperature, electrical conductivity, pH, electrical potential, dissolved oxygen and the chemical analysis of the water (Table II), determined that the water was alkaline, saline and its major ions corresponded to sodium, chlorides and sulfates.

Diatoms identification

Light microscope (LM) observations for both taxa revealed single cells with an isopolar frustule and bilateral symmetry, with oval, elongated valves, rounded at the polar endings. Generally two chloroplasts per cell were observed (Fig. 2 A, B and F).

Under scanning electron microscope (SEM), it was possible to identify only one taxon at species level. This was *Nitzschia epithemioides* Grunow in Cleve & Grunow (1880). Its valves were depressed or constrained in the central region (Fig. 2 A-C); cell length 22.97 ± 3.41 µm and width 6.93 ± 1.08 µm; showed fibulae of two types (1) small isodiametrical structures, 9 to 12 in 10 µm (Fig. 2 E), and (2) transversely elongated ribs, some of which pass completely through the valve, 3 to 5 in 10 µm (Fig. 2 D, E). There is a central pair of fibulae farther apart than the others (Fig. 2 E); central raphe endings presented a thickening of the margin (Fig. 2 D).

The other taxon identified at genus level corresponded also to the genus *Nitzschia* (Fig. 2 F-J), cells were smaller than *N. epithemioides*: length 12.61 ± 2.54 µm and width 6.06 ± 0.97 µm; raphe system fibulate, usually along the border of the valve, with fibulae that are small and discrete, square or rectangular (Fig. 2 H-J).

Growth curves at different temperatures

For *Nitzschia epithemioides*, the carrying capacity of the cultures was reached at 18 days of incubation at 10 and 15 °C, and at 16 days at 20 °C (Fig. 3 A). Significant differences ($p \leq 0.05$) were found in cell density for the three temperatures tested. The higher maximum cell density was observed at 20 °C (692800 ± 107704 cells ml^{-1}), followed by 15 °C (337200 ± 64351 cells ml^{-1}) and 10 °C (85008 ± 11115 cells ml^{-1}), respectively. All of the pairwise multiple comparison procedures based on the Tukey Test were significant ($p = 0.05$) for the cultures incubated at 20 and 10 °C and the cultures incubated at 20 and 15 °C. The exponential growth rate (k) was not significantly different among the three temperatures. However, the reported value at 20 °C was approximately double (1.80 ± 0.56 div. d^{-1}), to that reported at 15 and 10 °C (1.09 ± 0.30 and 0.97 ± 0.36 div. d^{-1}, respectively).

For *Nitzschia* sp. the carrying capacity of the cultures was reached at 18 days of incubation of the evaluated temperatures (Fig. 3 B). Significant differences ($p \leq 0.05$) were found in cell density among the three temperatures. The higher maximum cell density was observed at 20 °C (649600 ± 68942 cells ml^{-1}), followed by 15 °C (361900 ± 93734 cells ml^{-1}) and 10 °C (247720 ± 46471 cells ml^{-1}). All of the pairwise multiple comparison procedures based on the Tukey Test were significant ($p = 0.05$) among the incubated cultures at 10 and 20 °C. The exponential growth rate (k) was not significantly different among the temperatures evaluated (0.97 ± 0.32, 0.59 ± 0.29 and 0.47 ± 0.07 div. d^{-1} at 20, 15 and 10 °C).

Growth curves at different light intensities

Growth curves for *Nitzschia epithemioides* at 40, 80 and 120 µmol photons m^{-2} s^{-1} can be seen in Fig. 4 A. The carrying capacity of the cultures was reached at 16 days of incubation at all photon flux densities evaluated. Although, no significant differences ($p \leq 0.05$) were found at 40, 80 y

120 µmol photons m^{-2} s^{-1}, both in maximum cell density (791200 ± 76114, 692800 ± 107704 and 719200 ± 116895 cells ml^{-1}, respectively) and exponential growth rate (1.73 ± 0.38, 1.80 ± 0.56 and 1.38 ± 0.72 div. d^{-1}, respectively), a tendency is observed at higher cell densities and growth rates of microalgae at lower irradiances.

For *Nitzschia* sp. the growth curves can be observed in Fig. 4 B. The carrying capacity of the cultures was reached at 18 days of incubation for all photon flux densities evaluated. No significant differences ($p \leq 0.05$) were found in maximum cell density among 40, 80 and 120 µmol photons m^{-2} s^{-1} (667200 ± 38467, 643800 ± 128640, 709440 ± 128200 cells ml^{-1}, respectively). The exponential growth rate (k) neither showed significant differences (0.77 ± 0.60, 0.87 ± 0.38 y 0.96 ± 0.39 div. d^{-1}, respectively). However, its value tended to increase in the higher irradiances.

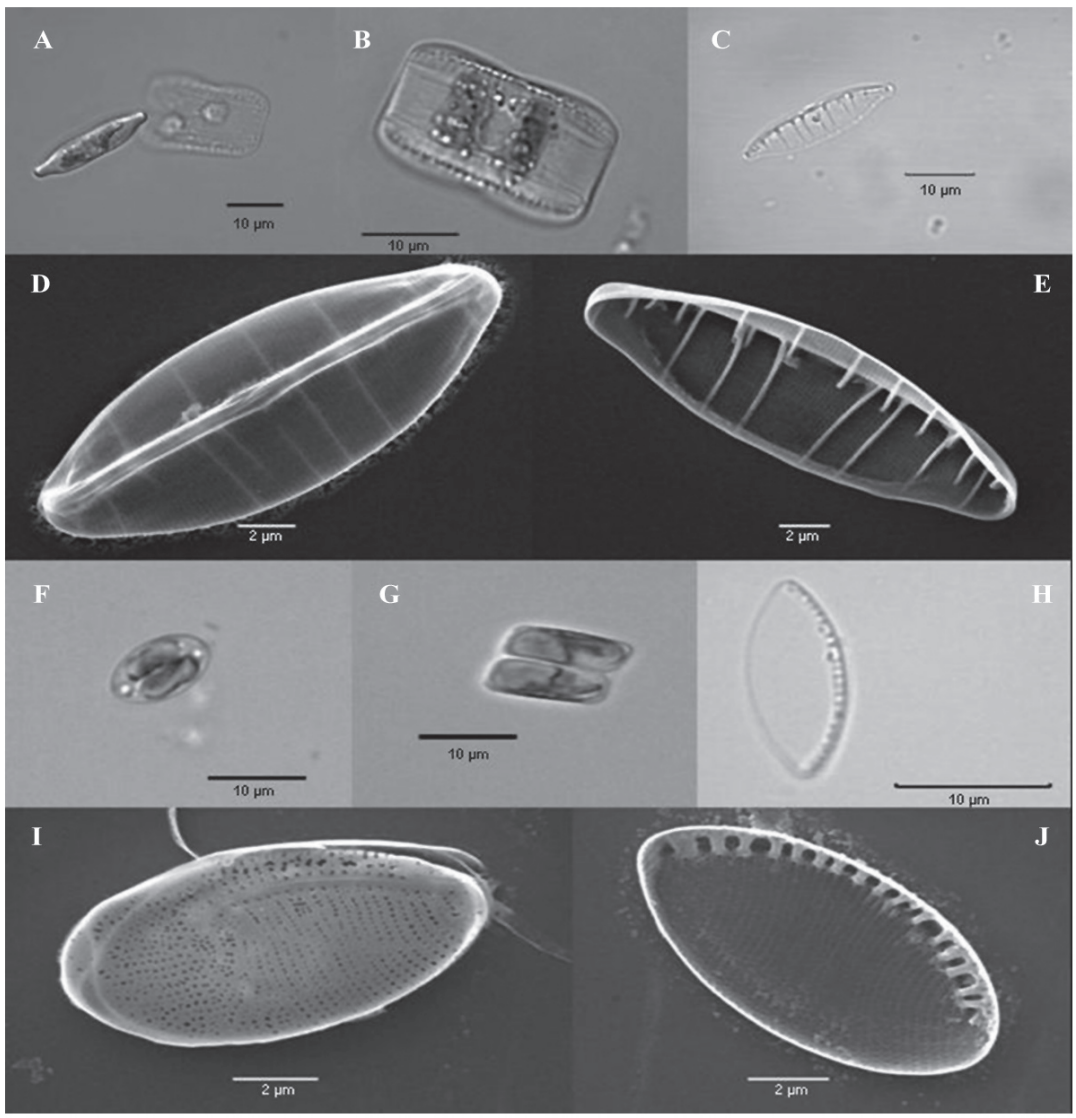

Figure 2. Cell morphology of *Nitzschia epithemioides* (CCM-UDEC 196), live cells observed in light microscope (LM). A. Valve view; B. Conectival view; C. Oxidized frustule observed in LM; D. Frustule ultrastructure observed in scanning electron microscope (SEM), external view of valve, E. Internal view of valve. Cell morphology of *Nitzschia* sp. (CCM-UDEC 297), live cells observed in LM. F. Valve view; G. Conectival view; H. Oxidized frustule observed in LM; I. Frustule ultrastructure observed in SEM, External view of Valve; J. Internal view of Valve.

Figura 2. Morfología celular de *Nitzschia epithemioides* (CCM-UDEC 196), células vivas observadas en microscopía fotónica (LM). A. Vista valvar; B. Vista conectival; C. Frústulo oxidado observado en LM; D. Ultraestructura del frústulo observada en microscopia electrónica de barrido (SEM), vista valvar externa; E. Vista valvar interna; F. Morfología celular de *Nitzschia* sp. (CCM-UDEC 297), células vivas observadas en LM, vista valvar; G. Vista conectival; H. Frústulo oxidado observado en LM; I. Ultraestructura del frústulo observada en SEM, vista valvar externa; J. Vista valvar interna.

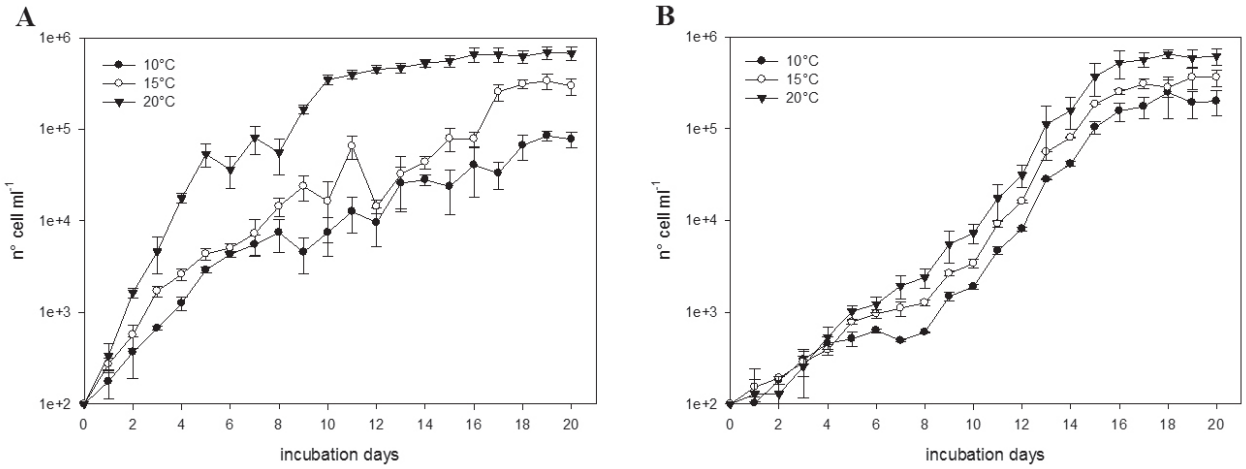

Figure 3. Growth curves for *Nitzschia epithemioides* (A) and *Nitzschia* sp. (B) derived at different temperatures and incubated in f/2 medium at 120 µmol photons m^{-2} s^{-1}, with a 18:6 h light-dark cycle.

Figura 3. Curvas de crecimiento para *Nitzschia epithemioides* (A) y *Nitzschia* sp. (B) derivadas a diferentes temperaturas e incubadas en medio f/2 a 120 µmol de fotones m^{-2} s^{-1}, con un ciclo 18:6 h luz-oscuridad.

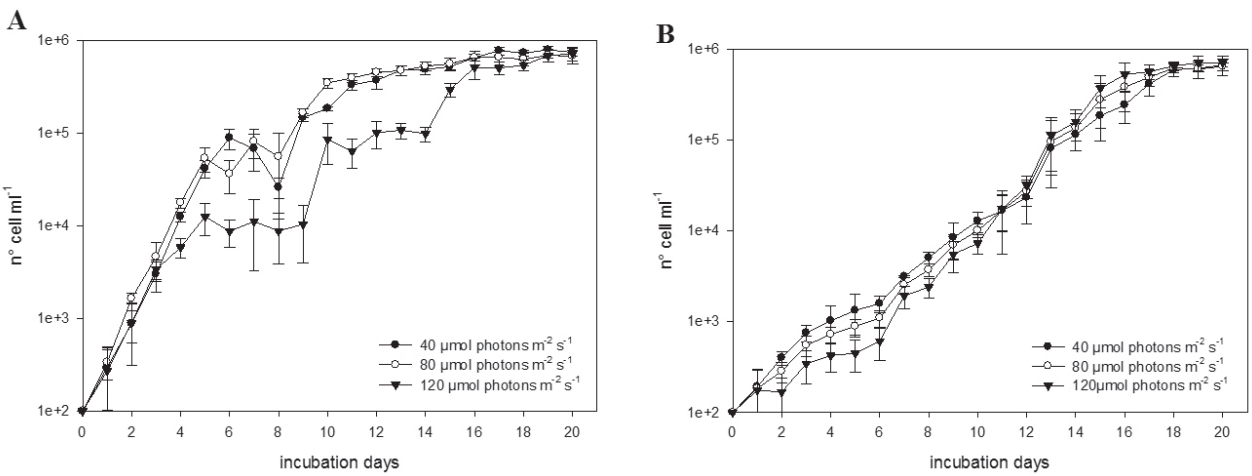

Figure 4. Growth curves for *Nitzschia epithemioides* (A) and *Nitzschia* sp. (B) derived at different light intensities and incubated in f/2 medium at 20°C, with a 18:6 h light-dark cycle.

Figura 4. Curvas de crecimiento para *Nitzschia epithemioides* (A) y *Nitzschia* sp. (B) derivadas a diferentes intensidades de luz e incubadas en medio f/2 a 20°C, con un ciclo 18:6 h luz-oscuridad.

Effects of the changes in the concentration of silica and selenium

For the experiments at different concentrations of silicate and selenium, was selected the higher temperature (20 °C) and photon flux density (120 µmol m^{-2} s^{-1}), since, in these conditions both taxa showed high cell densities.

For *Nitzschia epithemioides* the carrying capacity of the culture was reached within 16 days of incubation in normal f/2 medium and within 11 days in the f/2 medium with double silicate, f/2 medium with selenium, and f/2 medium with double silicate and selenium (Fig. 5 A). No significant differences were found in maximum cell density among normal f/2 medium (719200 ± 116895 cells ml^{-1}), f/2 medium with double silicate (1085600 ± 84639 cells ml^{-1}), f/2 medium with selenium (1133200 ± 142255 cells ml^{-1}) and f/2 medium with double silicate and selenium (1498800 ± 209599 cells ml^{-1}). Nevertheless, the multiple comparison procedures from the Tukey Test were significant ($p = 0.05$) among the f/2 medium with a double concentration of silicate and selenium and the normal f/2 medium. The exponential growth rate (k) was not significantly different among the four treatments (1.38 ± 0.31, 1.98 ± 0.44, 1.48 ± 0.54 and 1.77 ± 0.45 div d^{-1}, respectively).

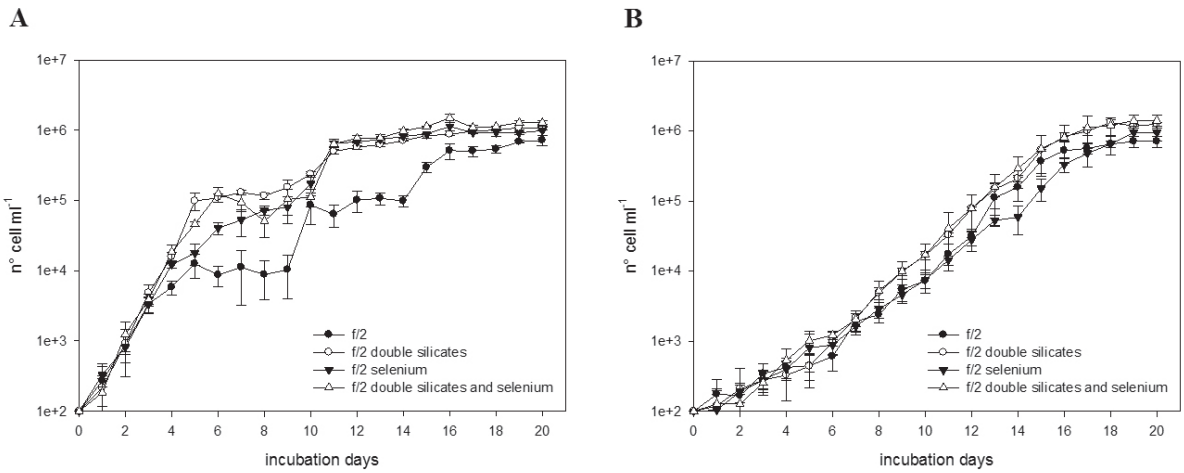

FIGURE 5. Growth curves for *Nitzschia epithemioides* (A) and *Nitzschia* sp. (B) cultures incubated in f/2 medium with diferent Na_2SiO_3 and H_2SeO_3 concentrations at 20°C and 120 µmol photons m^{-2} s^{-1}, with a 18:6 h light-dark cycle.

FIGURA 5. Curvas de crecimiento para *Nitzschia epithemioides* (A) y *Nitzschia* sp. (B) cultivos incubados en medio f/2 con diferentes concentraciones de Na_2SiO_3 y H_2SeO_3 a 20°C y 120 µmol de fotones m^{-2} s^{-1}, con un ciclo 18:6 h luz-oscuridad.

For *Nitzschia* sp., the carrying capacity of the culture was reached within 18 days of incubation for all concentrations of silicate and selenium evaluated (Fig. 5 B). No significant differences were found for maximum cell density among normal f/2 medium (7094400 ± 128640 cells ml^{-1}), f/2 medium with double silicate (1320400 ± 109038 cells ml^{-1}), f/2 medium with selenium (947520 ± 224525 cells ml^{-1}) and f/2 medium with twice the concentration of silicate and selenium (1401120 ± 296267 cells ml^{-1}), but it was observed that cells increased about twice in the medium with the highest nutrients concentration and normal f/2 medium. The exponential growth rate (k) showed no significant difference among the four treatments (0.91 ± 0.38, 0.95 ± 0.18, 0.72 ± 0.35 and 0.95 ± 0.31 div. d^{-1}, respectively).

DISCUSSION

The existing literature on the ecology, taxonomy and cultivation of microalgae of high saline ecosystems in Chile and South America is very scarce. Studies conducted in aquatic systems of the Chilean Altiplano highlight diatoms as the most important algal group in terms of abundance, since they are more tolerant to the high salinities of these systems and, therefore, being the base of the food chain, especially as diet for waterfowl (i.e. flamingos) (Vargas *et al*. 2004, Márquez-García *et al*. 2009). Diatom studies conducted in Andean bodies of water in Chile are scarce (Mühlhauser *et al*. 1995, Cruces *et al*. 2006, Rivera & Cruces 2008, 2009), due to the difficulty of sampling, the variety of morphological and hydrological characteristics, complexity of the geological patterns of their water bodies, and the wide range of chemical and biological features for these environments. The diatom taxa investigated in these studies are generally epipelics, but some are really planktonic and periphytic. They are found in freshwater, brackish water and marine habitats. *Nitzschia epithemioides*, one of the species found in lagoon Huasco is a brackish water species, found in muddy habitats (saline lakes and coastal areas), and has a wide geographic distribution. It has been reported in Europe [Romania (Caraus 2002, 2012), Great Britain (Whitton *et al*. 2003), Germany (Scholz & Liebezeit 2012), Spain (Álvarez Cobelas & Estévez García 1982, Aboal *et al.* 2003)], Asia [China (Liu 2008), Taiwan (Shao 2003-2014)], North America [New Brunswick (Thaler & Kaczmarska 2009)] and South America [Salar de Uyuni (3650 m), Altiplano, Bolivia (Rumrich *et al*. 2000, Alvial *et al*. 2008), Jujuy, Argentina (Maidana *et al*. 2009)].

The temperature in Andean ecosystems, shows strong daily fluctuations and low average temperature of the water, particularly Lagoon Huasco which exhibited an average temperature of 5 °C (Risacher *et al*. 2003), *in situ* water temperature was 12.5 °C (November, summer 2012). Therefore, for laboratory cultures was evaluated as lower temperature, 10 °C, and to increased cell densities and growth rates 15 and 20 °C. Both taxa (*N. epithemioides* and *Nitzschia* sp.) exhibited growth rates and cell densities higher at the highest tested temperature (20 °C). These results are consistent with the trend found by other researchers in other benthic diatoms under culture conditions. Admiraal (1977) conducted unialgal cultures of estuarine benthic diatoms, *Amphiprora* cf. *paludosa*, *Nitzschia* cf. *dissipata*, *Navicula arenaria* and *Nitzschia sigma* at different temperatures, obtaining a higher growth rate from 16 to 20 °C for *N. arenaria*, the other three species obtained high growth rates at 25 °C or above this value. Grinten *et al*. (2005)

studied the growth of the diatom *Nitzschia perminuta* at different temperatures (7, 15 and 20 °C), finding the highest maximum growth rate at 25 °C (0.65 ± 0.14 div. d^{-1}), followed by 15 °C (0.30 ± 0.10 div. d^{-1}) and 7 °C (0.26 ± 0.03 div. d^{-1}). Scholz & Liebezeit (2012) evaluated the effect of different temperatures in the cultures of 25 species of marine benthic diatoms. Most species isolated reached maximum growth rates at higher temperatures (10 to 35 °C), with a low growth significant and decrease in cell density at temperatures ≥ 30 °C and ≤ 4 °C.

The results reported in this study suggest a good tolerance capacity of diatoms for a range of light among 40 and 120 µmol photons m^{-2} s^{-1}, since, cell density and exponential growth rate were not affected by light intensity to which the cultures were exposed. Although there were no significant differences ($p \leq 0.05$) in the exponential growth rate among irradiances evaluated, *N. ephitemioides* presented a trend to higher growth rates at the lower photon flux densities (1.73 ± 0.38 and 1.80 ± 0.56 div. d^{-1}, at 40 and 80 µmol photons m^{-2} s^{-1}, respectively). Instead, *Nitzschia* sp. exhibited the highest growth rate at 120 µmol photons m^{-2} s^{-1} (0.96 ± 0.39 div. d^{-1}). Van der Grinten *et al*. (2005) reported results with a similar tendency to those found for *N. ephitemioides* in the diatom *Nitzschia perminuta* that presented a low increase in cell density at the higher light intensity (200 µmol m^{-2} s^{-1}), compared to the lower light intensities (40 and 5 µmol m^{-2} s^{-1}) and a highest maximum growth rate at 40 µmol m^{-2} s^{-1} (0.39 ± 0.04 d-1) than at 5 and 200 µmol m^{-2} s^{-1} (0.28 ± 0.03 and 0.29 ± 0.02 d^{-1}, respectively). These differences in growth could be attributable to that benthic diatoms are efficient in the use of low irradiances for inorganic assimilation (Rivkin & Putt 1987). On the other hand, Correa-Reyes *et al*. (2001), isolated several strains of benthic diatoms of the *Nitzschia* gender in batch cultures under different light conditions and suggest that some strains of bentic diatoms could be cultured under high light irradiance without photoinhibition, which could explain the behavior that we reported in this study for *Nitzschia* sp. The available scientific information suggests that the saturation light intensity may vary among benthic diatoms species, several authors have speculated about an inherently greater resistance to irradiance stress of microphytobenthic algae. Diatoms intertidal microphytobenthos often are exposed to high radiation, disrupting the ability of the cells to balance the fluctuating supply of light energy with the demands of cellular metabolism and growth, thus exposing the photosynthetic machinery to the risks of over –excitation and oxidative damage. It is therefore likely that benthic microalgal species possess several mechanical and physiological adaptations that enable survival in a highly variable environment (Blanchard & Montagna 1992, Peletier *et al.* 1996, Kromkamp *et al.* 1998, Barranguet *et al*. 1998).

Silicon is an essential element for diatoms, both as structural material and as metabolic regulators, affecting a wide range of cellular processes. The results suggest that an increase in silicates concentrations on the culture medium could promote the growth of *N. epithemioides* and *Nitzschia* sp. since a tendency was observed to higher cell densities (1.08 x 10^6 ± 84.639 and 1.32 x 10^6 ± 109.038 cells ml^{-1}, respectively) and exponential growth rates (1.98 ± 0.44 and 0.95 ± 0.18 div. d^{-1}, respectively) with respect at the normal f/2 medium and f/2 medium plus selenium. Raniello *et al*. (2007) conducted experiments to evaluate the effect of different silicate and selenium concentrations on diatom *Cocconeis neothumensis*, reporting that one of the highest exponential growth rates was observed in f/2 medium with double the amount of silicate (0.85 ± 0.11 day^{-1}). These experimental conditions also provided the highest cell density at saturation (ca. 2,700 cells mm^{-2}).

The results on the effect of selenium in cultures of *N. epitemioides* and *Nitzschia* sp. were not significant, but these suggest an increase in the maximum cell density (1133200 ± 142255 and 947520 ± 224525 cells ml^{-1}, respectively) compared to the maximum cell density observed in cultures using normal f/2 medium (ca. 720,000 cells ml^{-1} for both strains). However, exponential growth rates of both strains did not show a positive increase relative to other nutrient concentrations evaluated. This could be attributed to that despite the importance of selenium for normal development and growth of diatoms, intraspecific and interspecific differences exist for the requirement of selenium in microalgaes. Doblin *et al.* (1999) reported that there was no change in growth or biomass yields in *Chaetoceros* cf. *tenuissimus* cultures after approximately 60 generations, indicating that this diatom species has no selenium requirement, or that its selenium requirement was met by the background selenium levels in the culture medium. This adds to the findings of Harrison *et al*. (1988) who demonstrated the variable Se requirement within the *Chaetoceros* genus.

As for the simultaneous addition of silicate and selenium to the culture medium. We observed an increase in the maximum cell density for the two strains under study, although this was only significant (Tukey test) for *N. epithemioides* and among the normal f/2 medium (719200 ± 116895 cells ml^{-1}) and the f/2 medium with double the amount of silicate and selenium (1498800 ± 209599 cells ml^{-1}). The exponential growth rates were not significantly different in any treatment in both strains. Raniello *et al*. (2007) report different results for benthic diatom *Cocconeis neothumensis* indicating that a simultaneous increase of silicate concentration and the presence of selenium in the culture medium results in a loss of the positive effects detected with single nutrient. They argued that a higher SiO_3^{2-} amount in the culture medium, lead to a decreased uptake of SeO_3^{2-} by *C. neothumensis* cells and viceversa. Or occurring a negative interaction between selenium and

silicates inside the cell, in the context of diatom physiology, although very limited data has been reported in the literature regarding the metabolic interference between selenium and silicates (Müller *et al*. 2005). These differences with our results could be attributed to that diatoms have different physiological and metabolic behavior, and to that culture conditions tested by Raniello were not the same of the present study.

In conclusion both *N. epithemioides* and *Nitzschia* sp. shown an increased cell density and exponential growth rate at 20 °C. The different light intensities not significantly influenced the growth of both taxa. An increase in the concentration of silicates or simultaneous increase in silicates and selenium in the culture medium can be considered as possible strategy to improve cell density of benthic diatom *N. epithemioides* in batch cultures.

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