

Enzyme activities of phytoplankton in the South Shetland Islands (Antarctica) in relation to nutrients and primary production

Actividad enzimática en ensamblajes fitoplanctónicos en las Islas Shetland del Sur (Antártica) en relación a los nutrientes y producción primaria

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ABSTRACT

Given the potential significance of enzyme activities as a link between internal metabolic pathways and environmental nutrients, we investigated the relationships of nitrate reductase (NR) and alkaline phosphatase (AP) with primary production and inorganic nutrients in South Shetland Islands, Antarctica. Enzymatic activities of the phytoplankton (0.7-210 μm), primary productivity, autotrophic biomass and inorganic nutrients were studied in the upper 100 m depth at nine stations during a cruise in the northwestern area of South Shetland Islands (Antarctica), during late austral spring (December 2000). NR activities fluctuated between 0 and 42.8 $\text{nmol L}^{-1} \text{h}^{-1}$ (mean = 10.08 $\text{nmol L}^{-1} \text{h}^{-1}$, SD = 10.42 $\text{nmol L}^{-1} \text{h}^{-1}$), AP activities between 0.81 and 5.67 $\text{nmol L}^{-1} \text{h}^{-1}$ (mean = 2.68 $\text{nmol L}^{-1} \text{h}^{-1}$, SD = 0.95 $\text{nmol L}^{-1} \text{h}^{-1}$). Stations with primary productivity (PP) and chlorophyll *a* greater than 2 $\text{mg C m}^{-3} \text{h}^{-1}$ and 0.75 $\mu\text{g chlorophyll } a \text{ L}^{-1}$, respectively, presented higher enzymatic activities of nitrate reductase, alkaline phosphatase than those stations characterized by primary productivity and chlorophyll *a* less than 2 $\text{mg C m}^{-3} \text{h}^{-1}$ and 0.17 $\mu\text{g chlorophyll } a \text{ L}^{-1}$, respectively. The AP specific activity was negatively correlated with orthophosphate concentrations lower than 2.0 μM , which indicates that the microplankton were under phosphate deficient environment condition. Our results indicated that NR specific activity was positively associated with autotrophic biomass and primary productivity estimates, giving evidence of the use of nitrate by phytoplankton as external nitrogen source in surface waters. In addition, high NR activities were positively correlated with NO_3^- , suggesting the occurrence of nitrate respiration in the well oxygenated surface waters of Antarctica.

Key words: nitrate reductase, alkaline phosphatase, primary productivity, chlorophyll, Antarctica.

RESUMEN

Dada la potencial importancia de la actividad enzimática de ensamblajes fitoplanctónicos, como indicador de su metabolismo interno dominante respecto de los nutrientes, este estudio investigó las relaciones entre la nitrato reductasa (NR) y la fosfatasa alcalina (FA) con la producción primaria y nutrientes inorgánicos en las Islas Shetland del Sur, Antártica. Las variables de actividad enzimática del fitoplancton (0,7-210 μm), producción primaria, biomasa autotrófica y nutrientes inorgánicos fueron analizadas en nueve estaciones en los primeros 100 m de profundidad durante un crucero oceanográfico en el área noroeste de las Islas Shetland del Sur (Antártica), durante la primavera austral (diciembre 2000). Las actividades enzimáticas de NR fluctuaron entre 0 y 42,8 $\text{nmol L}^{-1} \text{h}^{-1}$ (promedio = 10,08 $\text{nmol L}^{-1} \text{h}^{-1}$, DE = 10,42 $\text{nmol L}^{-1} \text{h}^{-1}$) y de FA entre 0,81 y 5,67 $\text{nmol L}^{-1} \text{h}^{-1}$ (promedio = 2,68 $\text{nmol L}^{-1} \text{h}^{-1}$, DE = 0,95 $\text{nmol L}^{-1} \text{h}^{-1}$). Aquellas estaciones con estimaciones de productividad primaria (PP) y clorofila *a* mayores que $\text{mg C m}^{-3} \text{h}^{-1}$ y 0,75 $\mu\text{g clorofila } a \text{ L}^{-1}$, respectivamente, presentaron mayores actividades enzimáticas de nitrato reductasa y fosfatasa alcalina que aquellas estaciones caracterizadas por estimaciones de producción primaria y clorofila *a* menores que 2 $\text{mg C m}^{-3} \text{h}^{-1}$ y 0,17 $\mu\text{g clorofila } a \text{ L}^{-1}$, respectivamente. La actividad específica de FA fue negativamente correlacionada con ortofosfato a concentraciones menores que 2,0 μM , lo que sugiere que el

fitoplancton se encontraba en condiciones ambientales de deficiencia de fosfato. Nuestros resultados indican que la actividad específica de NR estuvo positivamente asociada a la biomasa autotrófica y producción primaria en la zona fótica, lo que evidencia el uso de nitrato como nutriente nitrogenado por el fitoplancton en aguas superficiales. Además, altas actividades de NR fueron positivamente correlacionadas con NO_3^- , lo que sugiere la ocurrencia del proceso de respiración de nitrato en aguas superficiales bien oxigenadas de la Antártica.

Palabras clave: nitrato reductasa, fosfatasa alcalina, producción primaria, clorofila, Antártica.

INTRODUCTION

There is a great deal of information available about the enzymatic activity of phytoplankton species under controlled conditions, as well as studies on enzymatic activity of phytoplankton assemblages performed in the field (Hung et al. 2000, Vidal et al. 2003, Lomas 2004, Sebastian et al. 2004, Iriarte et al. 2005). Eppley et al. (1969) discussed the importance of the potential use of enzymes, as nitrate reductase (NR, EC 1.6.6.1), in phytoplankton ecology studies, mainly as an indicator of external sources of nitrogen (NO_3^- or NH_4^+). Further studies examined the potential relationship between nitrate reductase activity and the rate of nitrate assimilation in marine phytoplankton (Collos & Slawyk 1976, Falkowski 1983, Blasco et al. 1984, Berges 1997, Joseph & Villareal 1998), as well as the relationship between nitrate reductase and environmental variables, such as NH_4^+ , NO_3^- , Fe and light (Collos & Lewin 1974, Collos & Slawyk 1977, Blasco & Conway 1982, Timmermans et al. 1994, Slawyk et al. 1997, Flynn & Ripkin 1999, Lomas & Glibert 2000, Lomas 2004). Nitrate Reductase (NR) is also conventionally accepted as an enzyme involved in nitrate respiration (dissimilatory process), a metabolic pathway employed by bacteria (Lloyd 1993) when oxygen is no longer present in sufficient quantity to serve as an electron acceptor for the electron transport system (Lehninger 1975).

Alkaline phosphatase (AP, EC 3.1.3.1) has been used as a biochemical indicator of phosphorus limitation on both marine (Li et al. 1998, Garde & Gustavson 1999, Stihl et al. 2001, Vidal et al. 2003, Sebastian et al. 2004) and fresh water environments (Rengefors et al. 2001, Dore & Priscu 2001). This enzyme can be found in the exterior surface of the cell, where it cleaves a variety of monoesters in surrounding water, therefore allowing

phytoplankton to use organic phosphate in limiting conditions of PO_4 (Lobban & Harrison 1994). The AP activity is also produced by other biological processes such as bacteria respiration, zooplankton excretion and phytoplankton losses (Thingstad et al. 1993, Hantke et al. 1996). Therefore, a negative relationship between AP activity and PO_4 concentrations is not always obtained (Taga & Kobori 1978, Sebastian et al. 2004). These results show that the detection of AP activity in marine environments would have alternative ecological explanations.

Given the potential significance of enzyme activities as a link between internal metabolic pathways and environmental nutrients, we investigated their relationships with primary production and inorganic nutrients in South Shetland Islands, Antarctica. In environments of fluctuating and drastic conditions, such as Antarctica (e.g., strong wind stress, high mixing rate, low light availability throughout the water column, low temperatures), biochemical adaptations can play a key role in the survival and productivity of populations and communities. Since primary productivity and biomass are correlated with nitrogen and phosphorus availability in aquatic systems, it would be expected that NR and alkaline AP activities might be valuable descriptors of the use of inorganic nitrogen and phosphorus for phytoplankton in Antarctic waters, respectively. We focused our efforts on the use and characterization on enzymatic activities and their relationship to autotrophic biomass and inorganic nutrients in South Shetland Islands (Antarctica). The purpose of the present study was to investigate the NR and AP activities of microphytoplankton in two contrasting groups of stations located offshore (south Drake Passage) and shelf waters of the Shetland Islands and to examine their relationship with nutrients and primary productivity.

MATERIAL AND METHODS

Study area and sampling

The samples were collected in the north western area of South Shetland Islands, Antarctica (14th Korea Antarctic Program) during December 2000. Primary productivity experiments were carried out at nine selected stations (k1, k4, k5, k8, k10, k14, k17, k19, k21) (Fig. 1). In order to evaluate the vertical variability in the photic layer, water samples were collected at seven depth levels at each station (0, 10, 20, 30, 50, 75, 100 m). We were focused in the water column variability since ecological parameters such as inorganic nutrients, photic layer and primary productivity

estimates exhibit pronounced vertical stratification. The samples were collected by Niskin oceanographic bottles (5 L). Nitrate reductase (NR) and alkaline phosphatase (AP) activities of the microplankton community were obtained on samples (4 to 5 L of seawater) passed through a 210 μm mesh and collected on 47 mm glass fiber GF/F filters (Whatman), which were immediately stored in liquid nitrogen for further enzymatic analysis. This study will operationally use the term micro-phytoplankton to mean autotrophic organisms of body size less than 210 μm . NR and AP activities (from two pseudo-replicates) were measured in an in vitro assay under substrate-saturated conditions and the activity was expressed as V_{max} (Berges 1997).

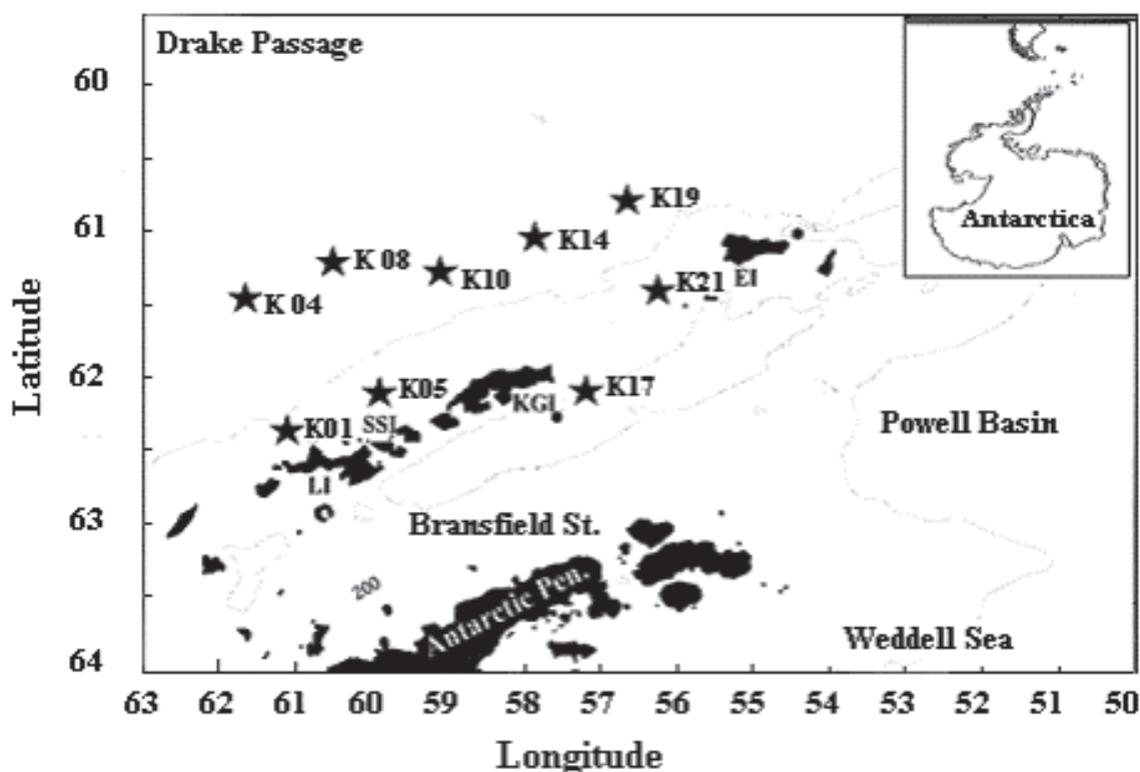


Fig. 1: Map of the sampling grid, showing the location of the selected nine stations where enzymatic activity, inorganic nutrients, primary productivity and chlorophyll *a* were sampled during 14th Korean Antarctic Program during December 2000.

Mapa del área de estudio, señalando las nueve estaciones seleccionadas y en las cuales se realizaron muestreos para actividad enzimática, nutrientes inorgánicos, productividad primaria y clorofila *a* durante el 14^o Programa Antártico Coreano en diciembre 2000.

Determination of nitrate reductase activity

The activity of this enzymatic complex was estimated through a combination of the methodologies proposed by Eppley et al. (1969), Packard et al. (1978), Timmermans et al. (1994), and Berges & Harrison (1995). The extraction medium consisted of phosphate buffer (200 mM, pH 7.9), dithiothreitol (DTT, 1 mM) and polyvinyl pyrrolidone (PVP, 0.3 % w/v). Microplankton cells and filter were homogenized in 3 mL of extraction buffer using an Ultra Turrax homogenizer. The homogenate was centrifuged at 5,500 g at 4 °C for 10 min, and the supernatant was used immediately for enzyme assays. Enzymatic assays began with the addition of NR assay (200 mM phosphate buffer, pH 7.9; 0.2 mM NADH) followed by the addition of KNO₃ (10 mM) to the supernatant and were performed in 1-cm disposable plastic cuvettes in a water-bath at 20 °C and at subdued light conditions. The reaction was stopped after 30 min with zinc acetate (550 mM). The homogenate was clarified by centrifugation (15 min at 4,000 rpm). Excess of NADH was oxidized by adding phenazine methosulphate (125 µM, PMS) and the nitrite produced was measured with sulfanilamide and N-(1-naphthyl)-ethylenediamine 2 HCl solutions. The supernatant was analyzed with a spectrophotometer at a wavelength of 543 nm. Enzyme activity was expressed in nmol nitrite formed L⁻¹ h⁻¹.

Determination of alkaline phosphatase (AP) activity

The enzymatic activity was determined by using the methodology proposed by Li et al. (1998). A nitrophenylphosphate (10 mM) solution, MgCl₂ (1 mM), and triglycine buffer (50 mM, pH 8.5) was added to the homogenized material. Microplankton cells and filter were homogenized in 3 mL of extraction buffer using an Ultra Turrax homogenizer. The homogenate was centrifuged at 5,500 g at 4 °C for 10 min, and the supernatant was used immediately for enzyme assays. After 12 h of incubation on a water-bath at 35 °C and under subdued light condition, the concentration of p-nitrophenol formed in the reaction was determined through a spectrophotometer at a wavelength of 400 nm. Enzyme activity was expressed in nmol p-nitrophenol formed L⁻¹ h⁻¹.

Primary productivity and chlorophyll a

The primary productivity experiments were carried out through the methodology proposed by Parsons et al. (1984). The water samples for the estimations of total primary productivity (0.7–210 µm) were collected at depths that corresponded to 100, 50, 30, 15, 5 and 1 % of light penetration. The extent of light (photosynthetically active radiation; PAR) penetration was determined using submersible sensor (QSP 200-D, Biospherical Instruments). The samples were incubated (on deck incubator) for 3 h (mainly between 11:00 or 12:00 and 14:00 or 15:00 h, respectively) in 100 mL polycarbonate bottles covered with perforated nickel neutral density filters (Stork Veco, Bedford, Mass.), reducing the light intensity to a similar level as the one measured at the collection depth. Each bottle was inoculated with 40 µCi of NaH¹⁴CO₃ and incubated for three hours under natural light. Temperature was regulated by running surface seawater over the incubation (-1.0 to 0 °C). After the incubation, samples were filtered (< 100 mmHg) onto 25-mm Whatman GF/F filters. The filters were washed with 0.01 N HCl and, then, 10 mL of scintillation cocktail was added (10 mL Lumagel). Radioactivity was measured in a Packard scintillation counter (Tri-Carb, model 2550).

Total chlorophyll *a* biomass (0.7–210 µm) samples were collected at standard depths similar to that of samples collected for enzymatic purposes. Chlorophyll *a* was measured fluorometrically according to the acidification method recommended by Parsons et al. (1984). Chlorophyll *a* (µg L⁻¹) size fractionation were carried out in two consecutive steps: (1) for the nanoplankton and picoplankton fractions (0.7–20 µm), seawater (100 mL) was pre-filtered using 20 µm Nytex mesh and collected on a 0.7 µm pore size GF/F Whatman glass-fiber filter, (2) the microphytoplankton fraction was obtained by subtracting the total chlorophyll from the chlorophyll estimated in step 1. Each filter was stored in a vial with acetone at 90 %, and kept at -20 °C temperature in the dark. After 15 hours, the samples were read in a TD-700 Turner Design fluorometer previously calibrated with pure chlorophyll *a* (Sigma). In this study, we used chlorophyll *a* to normalize

total enzymatic activity, despite the fact that bacteria (Thingstad et al. 1993) and zooplankton (Hantke et al. 1996) may also be the main contributor to AP activity.

Samples for phytoplankton cell-counts consisted of 125 mL subsamples, which were stored in clear plastic bottles and fixed and preserved in 1 % glutaraldehyde solution (final concentration). Sample volumes of 50 to 100 mL were filtered through Gelman (0.45 μm pore size, 25 mm diam.). The filters were mounted on microscope slides with water-soluble embedding medium (HPMA, 2-hydroxypropyl methacrylate) on board (Crumpton 1987).

Nutrients, temperature and salinity

Samples (100 mL) were collected at each of the nine stations for the analysis of nitrate and phosphate at the same discrete depth levels (0, 10, 20, 30, 50, 75, 100 m) chosen for the enzymatic protocol. For the analysis of nutrients the samples were filtered through GF/C filters following the methodology recommended by Parsons et al. (1984). Temperature and salinity measures were performed at every oceanographic station, using a CTD profiler (Seabird model SBE 19plus SEACAT) equipped with an irradiance and oxygen sensor (Seabird model SBE 43).

Statistical tests of correlation (Spearman test, Zar 1984) were performed in order to detect tendencies and relationships among the biological component (enzymatic activity) and explicative variables (nutrients, chlorophyll) using Statistica (Statsoft Inc.). A 0.05 significance level was used for all statistical analyses. We performed non-parametric test due to the use of data considered as pseudoreplicates: each enzymatic activity value corresponded to a two assayed measurements averaged from a same water sample unit (depth). Some of the samples collected during this study were missed during the enzyme extraction and assays; because of this the number of points diminished in the data analysis.

RESULTS

The horizontal distributions of temperature, salinity and density at sea surface in the study area showed southward increase in salinity,

with the lowest values (33.8 psu) located in the south of Drake Passage. Horizontal temperature and salinity gradients of about 0.3 °C and 0.3 psu, respectively, in < 50 km were found in the Drake Passage and in the eastern Bransfield Strait. Vertically, temperature and salinity values ranged between 0 to 0.8 °C at surface and -1.0 to 0.2 °C at 100 m depth, and between 33.8 to 34.2 at surface and 34.1 to 34.3 psu at 100 m depth, respectively. The vertical nitrate and phosphate profiles revealed a concentration increase of both nutrients through the first 100 m ($r_{\text{NO}_3} = 0.52$, $r_{\text{PO}_4} = 0.56$, $P < 0.001$, $n = 49$), with average (\pm SD) water column concentrations of $26.14 \pm 3.05 \mu\text{M}$ (range 22.38–36.41 μM) and $1.92 \pm 0.26 \mu\text{M}$ (range = 1.35–2.73 μM), respectively (Fig. 2A, 2B, 2C). The regression line gave a slope (Nitrate+Nitrite:Orthophosphate ratio; not shown) of 8.6, below the Redfield ratio of 15, suggesting an inorganic nitrogen deficiency relative to phosphate. However, the N:P ratio could be underestimated due to that neither ammonium nor dissolved organic nitrogen were included in estimating the ratio.

In order to find a spatial distribution of AP and NR activities in the study region, we grouped the stations according to their vertical distribution of primary productivity (PP) estimates and depth of the photic layer (Fig. 3A, 3B). Two distinct groups of stations emerged based on those properties (from here on referred as Group I and II). Group I included stations with high PP estimates located at the surface layer and a mean photic layer of 45 m (stations 1, 4, 5, 8) (Fig. 3A). They were located northwest off Shetland Islands. Group II corresponded to stations 10, 14, 17, 19 and 21, located northeast off Shetland Islands, with PP estimates relatively high at subsurface layer and a mean photic layer of 100 m depth. We examined the vertical distribution of NR and AP activities related to primary productivity and nutrient variables within the photic layer. Here we assumed that biomass of the microphytoplankton could be the main biological component of the total enzymatic activity observed. Vertical distributions showed that the depths with highest NR and NR specific activities (ratio between net activity and chlorophyll *a*; i.e., $\text{nmol substrate } \mu\text{g chlorophyll}^{-1} \text{ time}^{-1}$) were located between

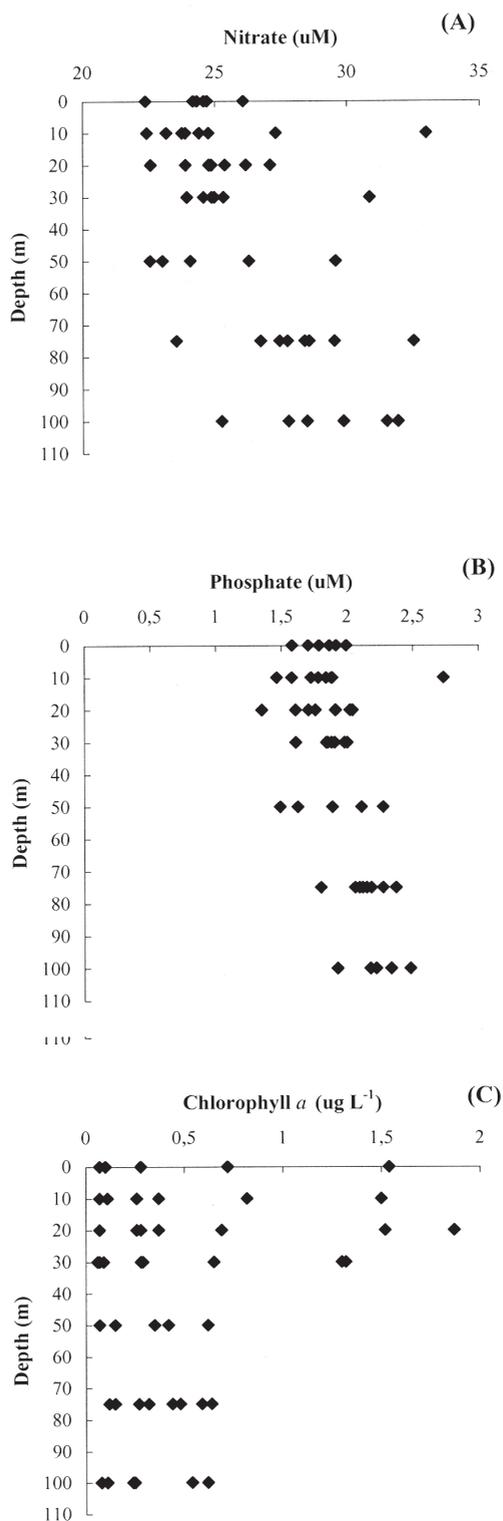


Fig. 2: Vertical distribution of (A) nitrate (μM), (B) orthophosphate (μM) and (C) chlorophyll a ($\mu\text{g chlorophyll } a \text{ L}^{-1}$) at the nine stations in the northwestern area of the South Shetland Islands, Antarctica.

Distribución vertical de (A) nitrato (μM), (B) ortofosfato (μM) y (C) clorofila a ($\mu\text{g clorofila } a \text{ L}^{-1}$) en las nueve estaciones en el área noroeste de las Islas Shetland del Sur, Antártica.

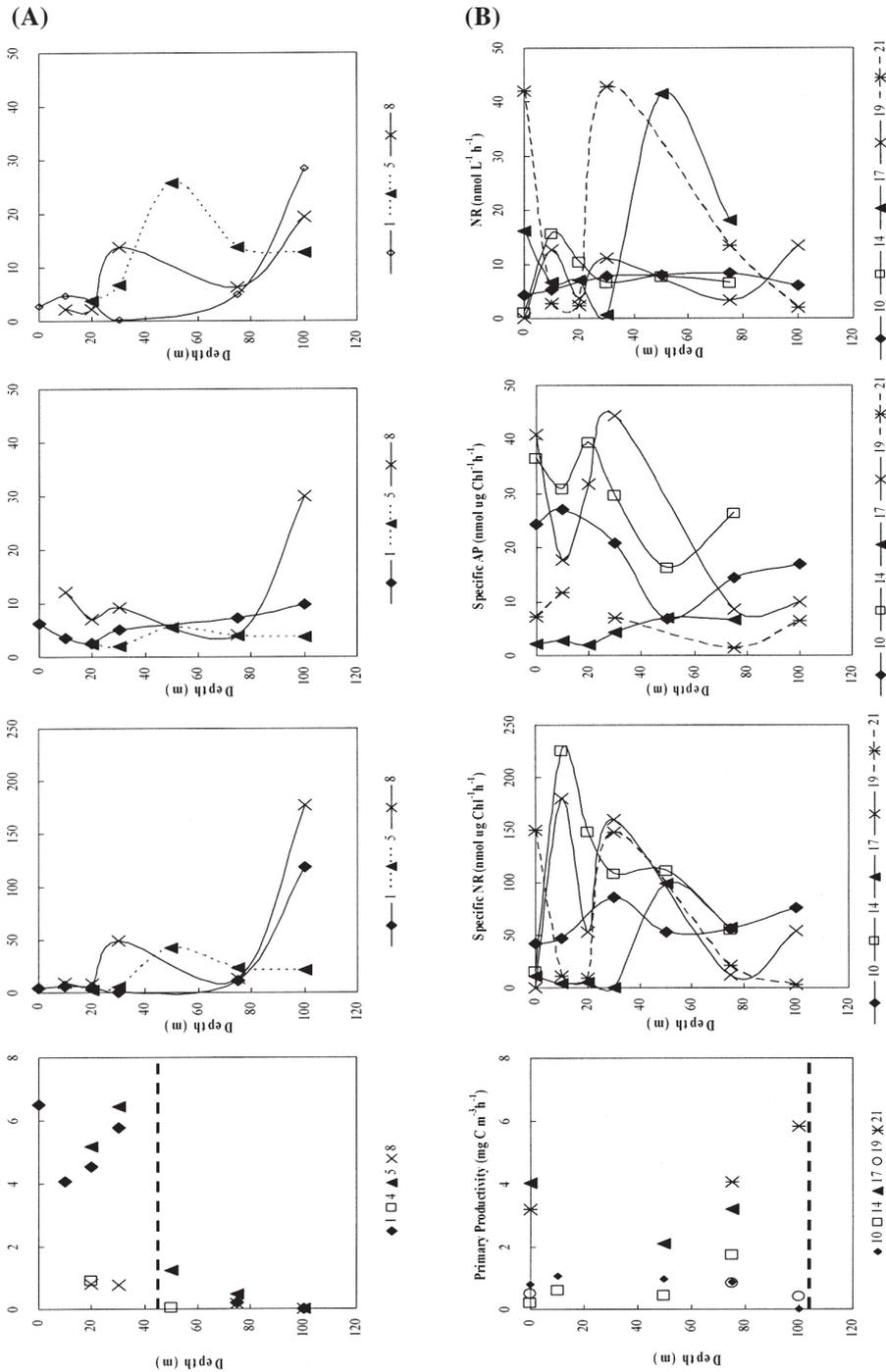


Fig. 3: Vertical distribution of primary productivity (mg C m⁻³ h⁻¹), nitrate reductase (nmol L⁻¹ h⁻¹), specific enzymatic activities of nitrate reductase (nmol µg chlorophyll a h⁻¹) and alkaline phosphatase (nmol µg chlorophyll a h⁻¹) at stations (A) 1, 4, 5, 8 and stations (B) 10, 14, 17, 19, 21 in the northwestern area of the South Shetland Islands, Antarctica. Dotted lines indicate the 1 % photic layer.

Distribución vertical de productividad primaria (mg C m⁻³ h⁻¹), nitrato reductasa (nmol L⁻¹ h⁻¹), actividad enzimática específica de nitrato reductasa (nmol µg clorofila a h⁻¹) y fosfatasa alcalina (nmol µg clorofila a h⁻¹) en las estaciones (A) 1, 4, 5, 8 y las estaciones (B) 10, 14, 17, 19, 21 en el área in noroeste de las Islas Shetland del Sur, Antártica. Línea segmentada indica el 1 % de penetración de luz.

50 and 100 m depth, well below the photic zone (45 m) at Group I (Fig. 3A). In contrast, at Group II high NR and NR specific activities were detected in the upper 50 m depth above the photic zone (approximately 100 m) (Fig. 3B). In the case of AP, high AP activities were observed in the surface layer (30 m depth) at orthophosphate concentrations below 2 μM . The vertical distribution of AP specific activity showed maximum values in the upper surface layer (0–30 m depth) at Group II (Fig. 3B). When all the data set is pooled together,

there were two interesting patterns: (i) when AP was chlorophyll *a* normalized, an inverse hyperbolic trend was obtained (Fig. 4A). In this case, the AP specific activity was negatively correlated with phosphate concentrations lower than 2.0 μM (mostly AP values at surface layer), and above 2.0 μM the AP specific activity was positively correlated with phosphate (mostly AP values at greater depths) and, (ii) NR activities were positively correlated with NO_3^- (Fig. 4B), both variables increasing their values with depth.

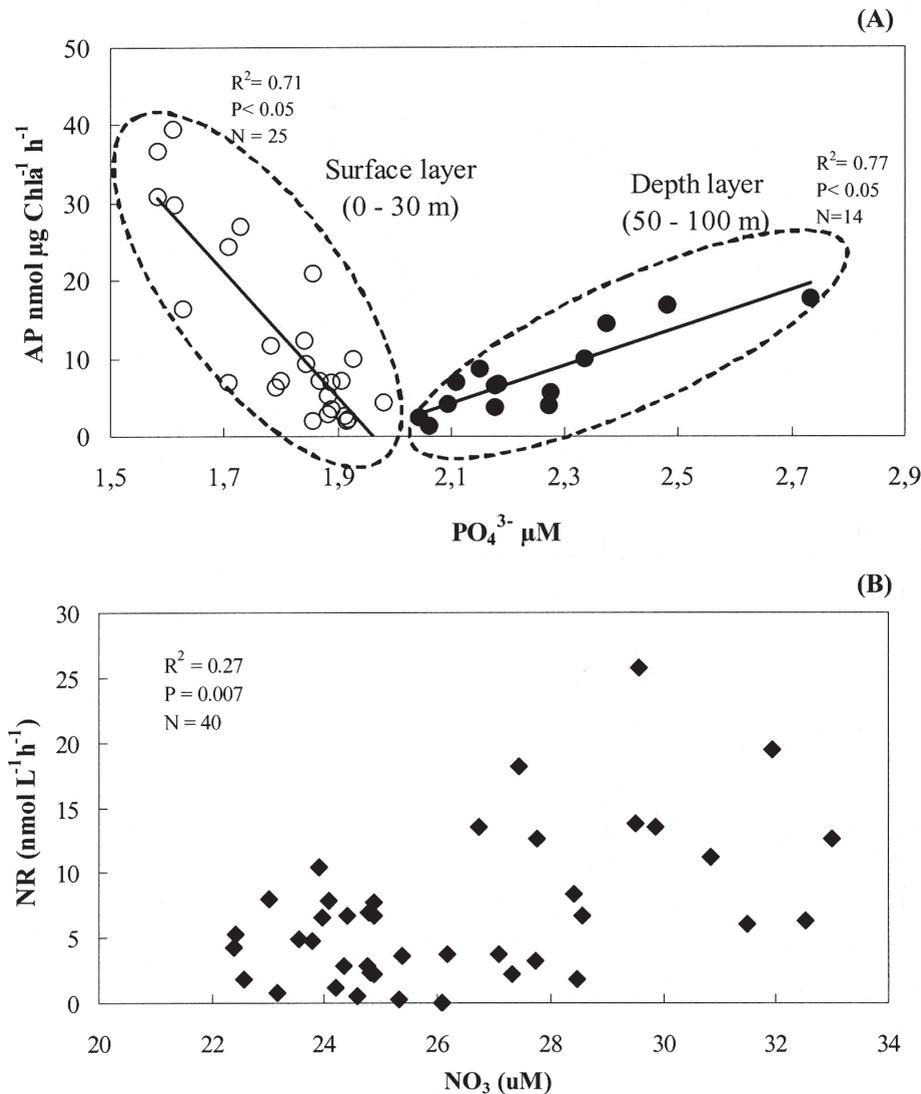


Fig. 4: Relationship between: (A) specific alkaline phosphatase and orthophosphate, and (B) nitrate concentrations versus nitrate reductase activities, considering stations sampled in the northwestern area of the South Shetland Islands, Antarctica.

Relación entre: (A) fosfatasa alcalina específica y ortofosfato, y (B) concentración de nitrato versus actividad de nitrato reductasa, considerando las estaciones muestreadas en el área noroeste de las Islas Shetland del Sur, Antártica.

The autotrophic biomass of pico- and nanoplankton fractions was dominant through the upper 100 m depth at all nine stations. The relative contribution of the small size classes to the chlorophyll *a* was greater than 55 % in all stations (mean = 78 %, SD = 10.58 %), with an average concentration of $0.36 \pm 0.41 \mu\text{g}$ chlorophyll *a* L⁻¹ compared to the microphytoplankton fraction (22 ± 10.58 %), with an average concentration of $0.09 \pm 0.07 \mu\text{g}$ chlorophyll *a* L⁻¹. Autotrophic flagellates such as naked *Gymnodinium* spp., *Cryptomonas* sp. and *Phaeocystis antarctica* in motile stage were major contributors to the phytoplankton biomass at all stations, accounting, as a whole, for more than 75 % of the total phytoplankton particulate carbon.

DISCUSSION

Biochemical adaptations of phytoplankton species are crucial in the modulation of autotrophic biomass and primary productivity, especially in highly climatic-oceanographic fluctuating marine environments, such as Antarctica. Our findings showed detectable enzymatic activities of NR and AP at depths within and below the photic zone in Antarctic waters around South Shetland Islands area. In general, AP and NR values fluctuated with the same magnitude as those reported for other marine areas: AP = 0–30 nmol MF-P L⁻¹ h⁻¹, central Atlantic Ocean (Vidal et al. 2003, Sebastian et al. 2004) and NR = 0–20 nM h⁻¹, Portuguese upwelling area (Slawyk et al. 1997), east China Sea (Hung et al. 2000) and southern coastal area of Chile (Iriarte et al. 2005). The incubation temperature (20–25 °C) of performed enzyme assays, compare to “in situ” temperature (-1 to 1 °C), as well as the non-limiting conditions of substrate concentration may explain some of the highest enzyme activity values observed in Antarctic cold waters. Despite that, our approach revealed two important findings relating enzymatic activity: (i) the AP specific activity was negatively correlated with phosphate concentrations lower than 2.0 μM , which indicates that the phytoplankton were under P-deficient condition and, (ii) NR activities were correlated with high NO₃⁻ values below the photic zone suggesting the occurrence of nitrate

respiration in the well oxygenated surface waters of Antarctica.

Regarding our first result, usually AP specific activity has been inversely associated with inorganic phosphate levels mainly in oligotrophic systems (Nausch 1998, Li et al. 1998, Vidal et al. 2003). Despite the fact that high concentrations of inorganic phosphate may repress AP synthesis (Loban & Harrison 1994), the finding of detected AP activities in Antarctica, suggest to us that AP may also be used as a descriptor of phosphate deficiency. Although in this study a low N:P ratio suggests nitrogen deficiency in the upper water column, the observed surface phosphate concentrations were within the range of values (mean = 1.34 μM) considered as limiting primary productivity in Antarctica (Jennings et al. 1984). The observed high AP specific activities in Antarctica (> 10 nmol μg chlorophyll *a*⁻¹ h⁻¹) were associated to concentrations lower than 2 μM of orthophosphate in the surface layer (upper 50 m depth), suggesting a phosphate threshold of 2 μM for the regulatory algae metabolism of AP synthesis in this ecosystem. This threshold may vary among ecosystem: of 0.1 μM in the north-western Africa (Sebastian et al. 2004), of 1 μM in the Baltic Sea (Nausch 1998).

We suggested that small sized phytoplankton, with AP activities > 10 nmol μg chlorophyll *a*⁻¹ h⁻¹, could be indication of that phytoplankton populations was possibly P-deficient regime. Since the pico-and nanoplankton size-classes contributed substantially to the chlorophyll *a* biomass (78 %), it is highly likely that it is responsible for the observed AP activity. This is coherent with observations conducted in marine systems limited by phosphorus (e.g., northern Red Sea), where AP activity was found to be strongly associated with nanoplankton (62–92 % of total AP activity) and picoplankton (50–71 %) size classes (Li et al. 1998, Stihl et al. 2001). Otherwise, the relatively high AP may express also other biological processes such as bacteria respiration and zooplankton excretion and phytoplankton losses occurring in the upper layer (Thingstad et al. 1993, Hantke et al. 1996) processes no studied here. On the other hand, above 2.0 μM the AP specific activity was positively related with orthophosphate, however there are no clear explanations for this

relationship in the context of phosphate limitation. Probably, the observed high AP activities at high orthophosphate concentrations may reflect physiological status of different organisms as well as the influence of environmental conditions (i.e., light), thus independent of ambient phosphate concentrations.

Nitrate may be important to the dynamics of phytoplankton in Antarctica, since it is a major nitrogen source for phytoplankton. In this study, the N:P ratio below Redfield (near 9.0), suggest nitrogen deficiency in the upper water column. Our results indicated that at some stations nitrate reductase specific activity was positively associated with primary productivity estimates, giving evidence of the use of nitrate by phytoplankton as external nitrogen source at surface waters (Eppley et al. 1969, Blasco et al. 1984, Slawyk et al. 1997, Campbell 1999, Hung et al. 2000, Lomas 2004). This relationship could have physiological implications for Antarctic phytoplankton since nitrate reductase synthesis is regulated by carbon metabolism and nitrogen sources (Campbell 1999) as well as with ambient iron levels (Timmermans et al. 1994). Based on the low ambient iron (< 0.1 nM) and high nitrate concentrations (~25 μM) observed in Antarctic waters (Cochlan et al. 2002), it seemed that surface phytoplankton was adapted to low iron concentrations, suggesting that factors other than low iron concentrations (i.e., species composition, light; Hung et al. 2000) are affecting NR activity and hence higher values of phytoplankton biomass and primary production in the Antarctic region. Lomas & Glibert (2000) found a negative relationship between NO_3^- uptake and temperature. The authors hypothesized that under low temperature and high NO_3^- conditions, phytoplankton uptake and store high intracellular NO_3^- concentrations, enhancing the activity of NR.

On the other hand, the simultaneously increase of NR activity and NO_3^- concentrations by depth, suggest us that non-autotrophic organisms are responsible for the high NR activity observed at some stations. It is surprising that those NR activities occurred under well oxygenated deep conditions, and associated to chlorophyll *a* less than 0.05 $\mu\text{g L}^{-1}$ and zero primary productivity estimates.

Dissimilar NR activity has been observed below the photic zone in the oxygen minimum zone of the Humboldt Current System which is consistent with the presence of a secondary nitrite maximum associated with low oxygen waters (Packard et al. 1978). However, independent of the oxygen levels, nitrate respiration can take place at the same time than oxygen respiration (co-respiration), allowing the use of nitrate as an auxiliary oxidant (Berks et al. 1995). In fact, the NR method could be measuring both the dissimilatory and assimilatory reduction of NO_3^- (Packard et al. 1978, Berges & Harrison 1995). There have been several studies confirming the process of corespiration of nitrate and oxygen in aquatic sediments and bottom waters (Lloyd 1993, Carter et al. 1995, Wolgast et al. 1998, Kostka et al. 1999). The ecological implication for the hypothesis that dissimilatory NR process could take place in the cold and oxygenated waters of Antarctica is that corespiration of oxygen and nitrate by microplankton and bacteria may be particularly important in environments or portions of the water column rich in dissolved/particulate organic carbon. We can not restrain ourselves from mentioning the possibility that carbon availability through the water column may play a crucial role in controlling the dynamics of nitrogen in HNLC regions. Carbon enrichment stimulate microorganism growth, leading to high respiration and, therefore, to an increment in the demand for nitrate (via nitrate reduction catalysed by nitrate reductase). This demand increases the flux of nitrate to nitrite in oxic and micro-oxic environments (Wolgast et al. 1998). The understanding of the dynamics of the dissimilatory reduction of nitrate during the spring phytoplankton bloom is a major step forward towards the proper assessment of the regenerative capacity of microplankton community in Antarctic waters.

In this study, in the Antarctic, an enzymatic approach was used to explore the relationship between nutrient availability and autotrophic biomass, and it may be important to understand ecological processes in marine areas. This study was conducted during early spring conditions (pre-summer bloom), thus seasonal variability in enzymatic activity is expected due to changes in the phytoplankton assemblages. From our results we suggest that, phytoplankton assemblages could be living

under limiting phosphate conditions (hyperbolic relationship) and give evidence of the occurrence of nitrate respiration in the well oxygenated surface waters of Antarctica.

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