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# SHORT COMMUNICATION

# Biomass of autotrophic dinoflagellates under weak vertical stratification and contrasting chlorophyll levels in subantarctic shelf waters

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Both the biomass of autotrophic dinoflagellates and its contribution to total chlorophyll were found to increase significantly with seawater temperature and the level of stratification in southern Patagonian waters during spring and winter. The highest peak of biomass corresponded to a single species, *Prorocentrum minimum* (Pavillard) Schiller, and was detected in middle shelf waters, coinciding with the primary productivity and $CO_2$ uptake maxima reported for the area under spring conditions.

KEYWORDS: autotrophic dinoflagellates; carbon-to-chlorophyll ratio; vertical distribution; *Prorocentrum minimum*; south Patagonian waters

Changes in phytoplankton communities in temperate shelf waters in response to upper ocean temperature rise in a global warming context have been documented. These changes, probably associated to an earlier beginning of seasonal thermal stratification (Hegerl and Bindoff, 2005), involve an earlier onset of the spring bloom (usually associated with a diatom domain) and an increase in the contribution of dinoflagellates to the autotrophic community (Widdicombe *et al.*, 2010), a group well adapted to low nutrient levels and higher vertical stability, and with lower carbon sequestration efficiency compared to diatoms (Le Quéré *et al.*, 2005).

However, there are still ecologically relevant shelf areas where the carbon contribution of autotrophic dinoflagellates to total phytoplankton is unknown. In this respect, there are no data about south Patagonian waters (ca. 47–55°S) of the Argentine shelf. This region is characterized by high levels of primary production which shows a large spatial variability mainly attributed to variations in the structure, composition and

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physiological stage of the phytoplankton communities (Lutz *et al.*, 2010). It also represents one of the strongest $CO_2$ sinks per unit area with photosynthesis denoted as one of the main processes responsible (Bianchi *et al.*, 2009). In this region, two sectors of high $CO_2$ uptake from the atmosphere have been reported in early austral spring (Bianchi *et al.*, 2009): the outer shelf at 47°S and the middle shelf at 50.8°S (offshore of Grande Bay), the latter co-located with the highest primary productivity (Lutz *et al.*, 2010). Moreover, Patagonian waters show an increasing trend in satellite-derived chlorophyll (Rivas *et al.*, 2006) and a temperature rise in the Antarctic Circumpolar Current (Turner *et al.*, 2009) which enriches the outer shelf and slope waters through nutrient inputs from the Malvinas Current.

Consequently, to understand future responses of primary producers to global warming, the potential role of these organisms in productivity and $CO_2$ sequestration needs to be explored. In this context, we evaluate the role of carbon from autotrophic dinoflagellates (Cdin) in relation to total autotrophic biomass estimated as chlorophyll-*a* (Chl) along a cross-shelf gradient of south Patagonian waters during two contrasting environmental conditions (spring and winter). We hypothesize that in spring autotrophic dinoflagellates already play a key role reaching the highest values of Cdin:Chl ratios in the areas of high productivity and $CO_2$ uptake.

For this purpose, we analyzed samples collected during two oceanographic cruises carried out in south Patagonian shelf waters (47–55°S, 69–59°W; Argentine Sea) during early spring 2005 (Patagonia I, 16–24 October) and late winter 2006 (Patagonia III, 10–20 September) on board the R/V "Puerto Deseado" (Fig. 1). Vertical sampling (118 samples) was performed at 2–3 depths at 41 oceanographic stations (Sta.) using a CTD-rosette system fitted with a Seapoint fluorometer. Samples in the near-surface layer were collected at 3 m depth. In winter, the intermediate and deepest levels were set at 30 and 70 m, respectively, due to homogeneous fluorescence profiles, while in spring, samples were collected within and below the fluorescence maximum (10–50 and 20–120 m, respectively). At Sta. 10 (spring), only the near-surface layer was sampled.

For dinoflagellates, density and biomass estimations and trophic mode determination were made under inverted and fluorescence microscopes, respectively (Sherr and Sherr, 2007). Depending on natural abundance, an average of 70–100 and 30 cells were counted and measured, respectively, per sample scanning half of three 25 mL sedimentation chambers under the inverted microscope from samples preserved in Bouin solution (6% f.c.). The number of cells measured increased in samples with higher morphological variability reaching up to 70 cells. The species *Prorocentrum minimun* was identified during the procedure, due to the high abundances observed in some samples. Glutaraldehyde fixed samples (0.5% f.c.) were concentrated onto black polycarbonate filters and examined under fluorescence microscopy to estimate the proportion of chlorophyll and non-chlorophyll containing cells under blue (450–480 nm) light (considered autotroph and heterotroph, respectively). This ratio was then applied to the inverted microscope estimates of dinoflagellates distinct to *P. minimum*. To obtain total autotrophic biomass for the Grande Bay spring transect, all other main phytoplankton groups were estimated under inverted (diatoms, silicoflagellates) or fluorescence (nanoflagellates, cyanobacteria, picoeukaryotic algae) microscope. Carbon biomass for cyanobacteria and the other groups was obtained by applying the conversion factor of Bertilsson *et al.* (Bertilsson *et al.*, 2003) and Menden-Deuer and Lessard (Menden-Deuer and Lessard, 2000), respectively.

Chlorophyll-*a* was extracted overnight in 90% acetone and estimated by spectrophotometry (Jeffrey and Humprey, 1975). Samples for nutrient analysis were preserved with $HgCl_2$, stored at 4°C and measured with an autoanalyzer (Evolution III, Alliance Instruments) following standard procedures (Kattner and Becker, 1991). The ratio between organic carbon of dinoflagellates and total chlorophyll (Cdin:Chl) was calculated and treated as ranges of values analyzed in a comparative context since it involves known biases related to the taxonomic composition of the community and the time of the year, among other factors. Simpson parameter (a measure of vertical stratification) was calculated for all stations (except Sta. 10). Paired Wilcoxon's tests were performed to compare near-surface physical and biological variables between 10 sites with similar location between periods (Fig. 1A) and autotrophic dinoflagellate biomass between different depths.

The comparison of the Cdin:Chl ratio along the inshore–offshore gradient revealed an uneven spatial and temporal distribution. In spring, the ratio showed low (<1) to intermediate (1–10) values at 53 and 29% of the sites, respectively, with the low ones mostly in coastal and middle shelf and the intermediate ones in middle-outer shelf waters at 47°S (Fig. 1B). This indicates that at 47°S, autotrophic dinoflagellates were neither major contributors to the spring chlorophyll increase (Sta. 4, Fig. 1A) nor to the associated $CO_2$ sink (Fig. 1B). In addition, it explains the lack of significant correlations between chlorophyll and Cdin:Chl ratio and Cdin (Table I).

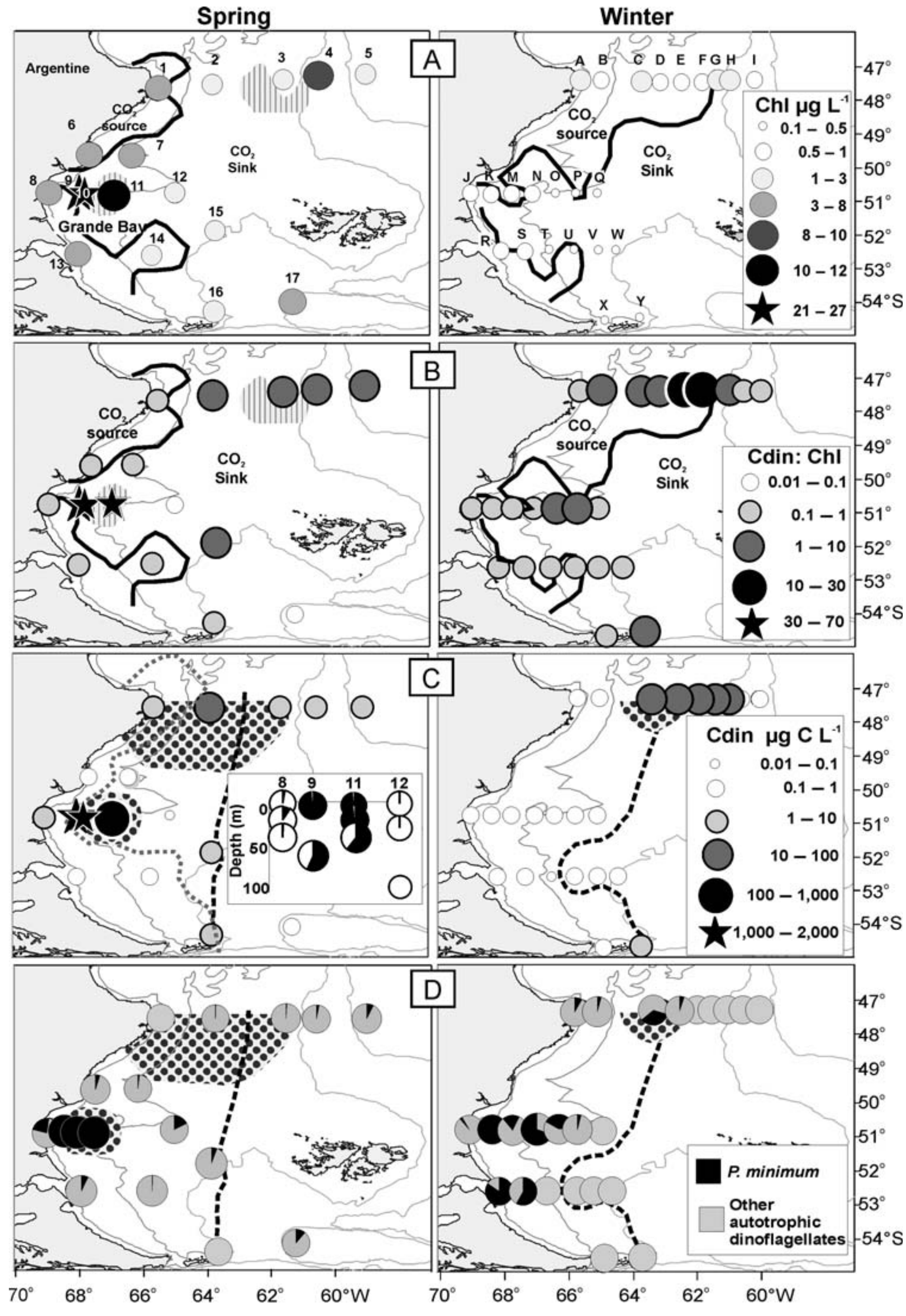


**Fig. 1.** Spatial distribution of chlorophyll-*a* (Chl, **A**), Cdin:Chl ratio (**B**), Cdin (**C**), and *Prorocentrum minimum* contribution to autotrophic dinoflagellate biomass (**D**) in near-surface waters (3 m) of the south Patagonian shelf during spring and winter (stations indicated by numbers and letters, respectively). Solid thin grey lines (from coast to slope): 50, 100, 200 and 1000 m isobaths. Solid black lines: sea-air delta $pCO_2 = 0$ μatm (modified from Bianchi *et al.*, 2009) that separates the $CO_2$ sink and source areas. Dashed black lines: 33.4 isohaline. Dotted grey line: in panel C (spring): critical Simpson parameter ($50\ J\ m^{-3}$) according to Bianchi *et al.* (2005). Dashed areas: strongest $CO_2$ sinks (delta pCO2 $< -120$ μatm) (Bianchi *et al.*, 2009). Dotted areas: local increase in surface temperature to 7.1°C. Cake diagrams in panel C (spring): contribution of *P. minimum* to total autotrophic carbon in the water column of the Grande Bay transect.

*Table I: Near surface (3 m depth) mean (and ranges) of physical and chemical parameters from south Patagonian waters during spring and winter and Spearman's correlation coefficients of total autotrophic dinoflagellate carbon (Cdin) and Cdin:Chl ratio versus temperature (Temp), chlorophyll (Chl) and Simpson parameter (SP)*

| | Spring | Winter |
|---|---|---|
| Variable/parameter | | |
| Temperature (°C) | 6.49 (4.80–7.64) | 6.07 (4.39–7.21) |
| Salinity | 33.20 (32.27–34.01) | 33.26 (32.49–33.95) |
| Wind speed (knots) | 12.56 (2.00–24.00) | 12.07 (**2.00**–25.00) |
| Nitrate (μM) | 13.17 (**8.12**–19.83) | 14.25 (12.12–16.57) |
| Nitrite (μM) | 0.33 (0.16–0.64) | 0.26 (0.14–0.68) |
| Silicate (μM) | 2.01 (**0.72**–5.90) | 2.48 (**1.03**–4.41) |
| Phosphate (μM) | 1.10 (**0.37**–1.49) | 1.33 (1.21–1.43) |
| N:Si | 8.14 (3.41–**13.42**) | 6.59 (3.58–**12.50**) |
| N:P | 13.76 (8.79–**26.48**) | 10.94 (8.99–12.64) |
| Spearman's correlations | | |
| Chl versus Cdin | 0.32 | 0.39 |
| Chl versus Cdin:Chl | 0.19 | −0.01 |
| Temp versus Cdin | 0.50[a] | 0.52[a] |
| Temp versus Cdin:Chl | 0.49[a] | 0.49[a] |
| SP versus Cdin | 0.69[a] | 0.56[a] |
| SP versus Cdin:Chl | 0.71[a] | 0.55[a] |

Bold: data corresponding to Grande Bay Stations (9 and 11, spring; O–M, winter).
[a]Significant results of Spearman's correlations ($P < 0.05$).

Higher Cdin:Chl ratios (30–70) in spring were found only at three contiguous sites in the middle-shelf waters offshore of Grande Bay (Sta. 9–11, 50.8°S). These stations also showed the highest chlorophyll (21–27 μg L$^{-1}$) and Cdin (max. 2 $10^3$ μg C L$^{-1}$) levels (Fig. 1A and C). This can be exclusively attributed to the presence of the potentially harmful species *Prorocentrum minimum* (Fig. 1D), a mixotrophic dinoflagellate which represents the first bloom of this taxon recorded in this region with a density of $8 \times 10^6$ cells L$^{-1}$. Moreover, all these parameters coincided with: (i) the highest primary productivity (2899 mg C m$^{-2}$ day$^{-1}$; Lutz *et al.*, 2010) and the highest $CO_2$ uptake (with delta $pCO_2 < -120$ μatm; Bianchi *et al.*, 2009) according to the data gathered during the same cruise (Patagonia I); (ii) a large chlorophyll patch located 70–100 km offshore, extending 50 km from ca. 50.5 to 51°S and 100 km from 66.9 to 69°W, according to satellite images from the sampling day at Sta. 9–11 (data not shown). In the remaining area, the species density did not exceed $9.7 \times 10^3$ cells L$^{-1}$ and its contribution to Cdin averaged 25% (Fig. 1D). These three stations were also characterized by a local increase in near-surface temperature (Fig. 1C and D) of ca. 0.5°C relative to the adjacent stations (Sta. 8 and 12: 6.5 and 6.3°C, respectively), the highest nitrite concentration considering all samples (0.94 μM, Sta. 11, 10 m depth) and the highest N:Si and N:P ratios, as well as the lowest nitrate, phosphate, silicate and wind speed values at subsurface waters for the whole area studied (Table I).

Following the pattern of chlorophyll concentration, which drastically decreased towards the extremes of the Grande Bay transect (Fig. 1A and Lutz *et al.*, 2010), biomass of *P. mimimum* diminished more than three orders of magnitude towards Sta. 8 and 12 (Fig. 1C). This, together with the absolute domain of the species in terms of autotrophic carbon biomass at bloom stations (Fig. 1C), confirms *P. minimum* as being most likely responsible for such chlorophyll increase.

The winter period, compared to spring, was characterized by significantly lower levels of chlorophyll (Wilcoxon's test, $P = 0.003$, Fig. 1A) and Cdin ($P = 0.007$; Fig. 1C), but no differences were found in the Cdin:Chl ratio ($P = 0.87$; Fig. 1B). The highest values of these three parameters occurred on the northern transect (47°S) between the $CO_2$ sink and source regions (Fig. 1), but the largest contribution of *P. minimum* to Cdin (Fig. 1D) was found again offshore of Grande Bay under environmental conditions similar to those of spring: the lowest wind speed and Si concentration, and the highest N:Si ratio of the area studied (Table I). Considering the inter-annual persistence of the high chlorophyll patch offshore of Grande Bay (Romero *et al.*, 2006), our winter results revealed that this high productivity sector has a stable potential for spring bloom developments of *P. minimum*.

Temperatures in spring were significantly higher than in winter (Wilcoxon's test, $P < 0.01$), while salinity did not show clear seasonal variations ($P = 0.37$). *Prorocentrum minimum* had a dissimilar spatial distribution between both periods, being numerically more relevant in middle shelf waters within the low salinity (<33.4) water band mainly in winter (Fig. 1D). This suggests that the combination of high salinity and low temperature is less favorable for the development of this species, while under higher spring temperatures the species can tolerate higher salinities. In agreement, the highest near-surface abundance of *P. minimum* in winter ($3 \times 10^3$ cells L$^{-1}$) coincided with a local temperature increase to 7.2°C (Sta. C., Fig. 1D). Furthermore, both Cdin and the Cdin:Chl ratio increased with temperature during both periods (Table I). These findings suggest that a moderate upper ocean temperature increase in south Patagonian waters could favor the development of *P. minimum*, thus increasing its absolute abundance and contribution to the autotrophic dinoflagellate biomass. This prediction is supported by recent reports of

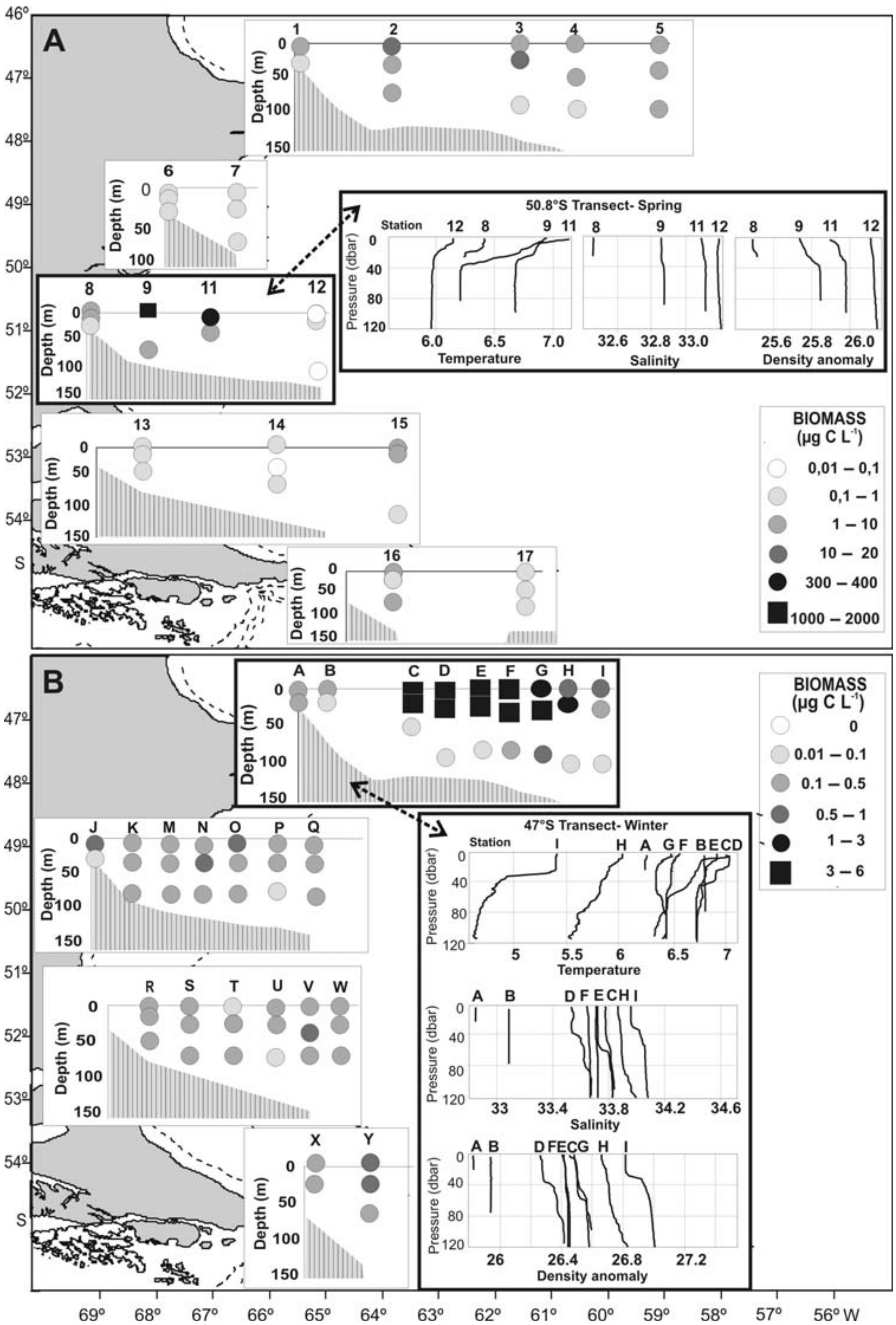

**Fig. 2.** Vertical distribution of autotrophic dinoflagellate biomass in south Patagonian waters during spring (**A**) and winter (**B**) and profiles of temperature (°C), salinity and density anomaly ($\sigma_0$, kg m$^{-3}$) at the 50.8°S (spring) and 47°S (winter) transects.

increased autotrophic dinoflagellate abundances (including *P. minimum*) associated with a temperature rise of 0.5°C in a 15-year time scale (Widdicombe *et al.*, 2010).

In general, both seasons showed high levels of vertical mixing (Simpson parameter max.: 58 J m$^{-3}$) compared to summer (Simpson max. > 200 J m$^{-3}$; Bianchi *et al.*, 2005). Despite the weak stratification, the deepest layer hosted significantly lower values of Cdin than the upper (Wilcoxon's test, $P = 0.02$) and intermediate ($P = 0.002$) levels. The slightly more stratified stations with the highest near-surface abundances (spring: Sta. 9 and 11; winter: Sta. C–F) showed the lowest deep carbon values (Fig. 2A and B). This observation, together with the positive relation between Simpson parameter and Cdin and the Cdin:Chl ratio (Table I) indicates that even a weak stratification contributes to cell retention in the upper water column and enhances the proliferation of dinoflagellates. In less-stratified regions, the deepest layer comprised on average 60 and 50% of the carbon (spring and winter) relative to those values estimated in the upper layer, suggesting that substantial amounts of dinoflagellate carbon could reach the sediments. As the mean near bottom flow is presumably onshore (Palma *et al.*, 2008), part of this carbon may return to the upper layers near-shore, where intense tidal currents fully mix the water column. Such a process would contribute to the carbon transfer to the atmosphere observed in the coastal region (Bianchi *et al.*, 2005, 2009).

The bloom of *P. minimum* was associated with a permanent salinity front (Fig. 1C; Bianchi *et al.*, 2005), which favors the accumulation of autotrophic biomass in general. The increase in water column stability together with the closed circulation in this region (e.g. Palma *et al.*, 2008) and calm wind conditions contribute to dinoflagellates blooms (e.g. Jäger *et al.*, 2008). Morover N:P ratios between 20 and 30, only reached at Grande Bay in the present study (Table I), seem to be optimal for *Prorocentrum* development (Li *et al.*, 2009). This set of physical and chemical conditions supports the idea that the linkage between turbulent mixing and nutrient availability regulates the structure of phytoplankton communities.

The high chlorophyll subsurface accumulation at bloom stations (Lutz *et al.*, 2010, their Fig. 4) is attributed to *P. minimum*, whose biomass, though it comprises half of the autotrophic carbon towards the deepest waters (Fig. 1C), decreased more than two orders of magnitude (Fig. 2A). The non-limiting nitrate concentrations reported here (Table I) and also for summer and fall (Paparazzo *et al.*, 2010), as well as the high swimming speed and positive phototaxis of *P. minimum*, would prevent the need for vertical displacements (Tyler and Seliger, 1981) and contribute to compensating for the sedimentation effect. All these features may explain the near-surface (3 m) accumulation observed at Sta. 9 and 11, sampled during hours of darkness and high light intensity (21 h and 15 h local time, respectively). A series of mechanisms that prevent UVR-induced damage (Carreto *et al.*, 1990) in dinoflagellates would allow this surface accumulation even during hours of high light intensity.

The relevance of dinoflagellates in the area suggests future studies aimed to evaluate the responses of these organisms to changes in stratification and wind forcing and the consequent impact on productivity levels and $CO_2$ uptake in south Patagonian waters.

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