Interplay between freshwater discharge and oceanic waters modulates phytoplankton size-structure in fjords and channel systems of the Chilean Patagonia

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ABSTRACT

Here, we participated in five independent research cruises that spanned almost the entire Patagonian fjords region (from 41.5 to 56.0°S) in order to determine the importance of the physical/chemical factors that influence phytoplankton size structure triggered by freshwater discharge and oceanic water intrusion. Throughout the Patagonian region phytoplankton biomass varies in association with freshwater discharge and mineral nutrient load, and to a lesser extent with surface solar radiation and photosynthesis. These correlations and the spatial domains changed depending on which size fraction was analyzed. Fresh water discharge negatively correlated with salinity and density, positively correlated with stratification and silicic acid concentration, and represents the primary influence on phytoplankton populations. More than 40% of the sites characterized as discharge-intensive locations exhibited lower total chlorophyll-a concentrations (chl-a) and phytoplankton size-structure that was dominated by small cells (< 20 µm). Oceanic nutrients (nitrate and phosphate input) are the second-most important factor that control total chl-a, favoring total chlorophyll-a concentration in the southern half of the Patagonian region. Microphytoplankton contribute to more than 75% of the total community in high productivity waters with chl-a concentrations higher than 2 µg L⁻¹ and picophytoplankton dominate when chl-a is lower than 1 µg L⁻¹. Thus, in this extensive area, the relative success of different phytoplankton size classes may be sensitive to changes in hydrological cycles, continental runoffs, and potential anthropogenic eutrophication, modifying important ecological processes and the fate of organic matter.

1. Introduction

Primary productivity and autotrophic biomass varies widely in aquatic ecosystems, where coastal and estuarine waters account for a significant fraction of the global primary productivity. Along the continental margins of South America chlorophyll-a has increased from 1998 to 2004 (Gregg et al., 2005), however, locally along the western coast of South America the concentration has decreased (Lara et al., 2016). Despite their potential role in biological productivity, carbon cycling, and nutrient remineralization, these coastal waters, including the fjords and channels of the Chilean Patagonia (references in Iriarte et al., 2007), are among the least studied ocean regions of the world. It is well known that Patagonian fjords and inner channel areas south of 41.5°S undergo strong seasonal fluctuations, mainly due to variations in
precipitations and solar radiation (Acha et al., 2004; Pickard, 1971). Since Patagonian fjords are major CO2 sink areas (Torres et al., 2011), understanding the factors that modulate the size structure of the planktonic community will help to understand the factors that control the efficiency of the biological pump. A previous study (Iriarte et al., 2012) suggested that nitrogen sources (e.g. nitrate and ammonia) could play an important role in maintaining relatively large productivity rates (3 g C m\(^{-2}\) d\(^{-1}\)) in Patagonian fjords. Additionally, water column stratification positively correlates to thin layers of high chlorophyll-a concentrations (Ríos et al., 2016), indicating the importance of mixing/ stability for autotrophic biomass in the southern part of the Patagonian fjords. Seasonal carbon fluxes varied widely in this ecosystem in terms of productivity and vertical fluxes of particulate organic carbon, reaching high values during the productive seasons (González et al., 2010, 2011, 2013, 2016). In terms of phytoplankton size structure, cells smaller than 11 µm dominate when chl-a values < 1 µg L\(^{-1}\), and cells larger than 20 µm dominate when chl-a > 1 µg L\(^{-1}\) for fjords and channels in the Aysén area from 43° to 47° South (Paredes and Montecino, 2011). This pattern was previously observed in different areas of the Patagonian fjords (Montecino and Pizarro, 2008; Pizarro et al., 2005). The area is also characterized by high precipitations (Garreaud et al., 2013), producing higher freshwater discharge into the fjord system (Kilian et al., 2013), and strong salinity gradients in the coastal zone (Dávila et al., 2002; Saldías et al., 2018). Thus, high freshwater runoff transports a large amount of energy and terrestrial carbon (Vargas et al., 2011), high rates of terrestrial nutrient input, particularly dissolved silica (Torres et al., 2011, 2014) and essential micronutrients (Iriarte et al., 2014). Consequently, it will be ideal to test whether temperature, nutrient availability, and surface radiation together with other variables affect phytoplankton size structure in a sensitive fjord systems such as those of the Chilean Patagonia.

Phytoplankton are often regulated by nutrient availability and loss processes such as mortality, grazing, viral lysises, and their importance has been studied extensively in freshwater (McQueen et al., 1989; Staehr and Sand-Jensen, 2006) and marine ecosystems (Calbet and Landry, 2004; Chen and Liu, 2010). Factors such as hydrographic conditions, water column stability, light availability, and freshwater inputs may influence the composition and productivity of phytoplankton communities in coastal and estuarine ecosystems, thus affecting biogeochemical fluxes (Chisholm, 1992). In the upper photic zone, limitation of mineral nutrients (e.g. N, P, Si, Fe) and light availability regulate autotrophic biomass and modify the structure of phytoplankton communities; mainly in high-turbidity ecosystems such as coastal zones and estuaries (Falkowski and Oliver, 2007; Marañón et al., 2012).

The size structure of phytoplankton determines the trophic organization of pelagic ecosystems and the efficiency by which organic matter produced by autotrophs is channeled towards upper trophic levels or exported through both the ‘classical’ and ‘microbial’ pathways. Smaller phytoplankton cells, due to higher surface-area-to-volume ratio, have a competitive advantage over large cells in nutrient-impoverished environments (Chisholm, 1992; Kiørboe, 1993). Conversely, large phytoplankton cells are capable of sustaining higher rates of biomass-specific production in nutrient-rich waters (Cermeño et al., 2005; Marañón et al., 2007), and are less strongly controlled by predators (Kiørboe, 1993). This explains why picophytoplankton (< 2µm in diameter) dominates the autotrophic biomass and primary production in oligotrophic areas, whereas microphytoplankton (> 20 µm diameter) is dominant in eutrophic areas (Agawin et al., 2000; Marañón et al., 2001). Two different scenarios can be expected depending on the prevailing phytoplankton size structure. If small cells (usually < 20 µm) are dominant, in terms of autotrophic biomass and primary production, little potential for carbon export can be expected due to the low sedimentation rates and intense recycling of matter through the microbial food web (González et al., 2010, 2013; Marañón et al., 2012). Additionally, if phytoplankton biomass is dominated by large species, organic matter can be efficiently transferred as well as enhanced, producing downward export fluxes and biological CO2 drawdown through a short classical food chain favoring the biological pump and carbon sequestration (Legendre and Le Fevre, 1995; Turner, 2002).

The paucity of regional-scale information on the relative importance of factors that may regulate the structure and productivity of phytoplankton assemblages in the Chilean Patagonia is mainly due to the extent and geographic complexity of this region, as well as the limited availability of scientific vessels. However, annual research cruises sponsored by the Chilean Navy’s CIMAR program have been conducted over the past 12 years, providing a piecemeal coverage of the entire region. Here we present a meta-analysis of data gathered from 5 such cruises (Table 1), all carried out during different but consecutive years, which span ca.14 degrees of latitude (42°–56°S). We discuss the patterns of phytoplankton size structure that emerge, and analyze their association with hydrographic conditions (temperature, salinity, density, stability of the water column), surface radiation (satellite-derived PAR), and mineral nutrients (nitrate, phosphate, silicic acid), as well as with total phytoplankton biomass and productivity rates within the euphotic zone (0–25 m depth).

Our goal is to establish whether freshwater discharges and oceanic water intrusion into the fjord modify phytoplankton size structure leading to ecological changes that may impact carbon cycling and biogeochemical fluxes in this region.

2. Materials and methods

2.1. Study area

The Chilean Patagonia constitutes one of the most important and extensive fjord and channel regions in the world, extending from the Reloncavi Fjord (41.5°S) to Cape Horn (56.0°S) (Fig. 1). It spans a linear distance of ca. 1600 km in length, and is composed of 84,000 km of coastline and 240,000 km\(^2\) of complex geomorphology and hydrography, characterized by a highly fragmented and rugged coastline with a variety of environments such as embayments, fjords, channels, straits, and estuaries (Silva and Palma, 2008). Similar to other high latitude ecosystems, Chilean fjords receive freshwater discharges from rivers – many of them of glacial origin – as well as surface runoff and ground water flows due to the strong influence of subpolar low-pressure systems associated with high rainfall (2000–5000 mm year\(^{-1}\) (Calvet and Sobarzo, 2011; Dávila et al., 2002). At the same time, these fjords and channels are strongly influenced by tidal fluctuations and by sub-surface intrusions of oceanic waters, mainly Sub-Antarctic waters (SAAW) from the South Pacific Ocean (Aiken, 2012, 2008; Palma and Silva, 2004).
2.2. Physical, chemical, and biological data

To assess the relative importance of different phytoplankton size classes across the region of interest, we compiled data on total and size-fractionated chlorophyll-a, together with CTD profiles, satellite-derived surface PAR (MODIS-Aqua, Level 3, 8-day composites), mineral nutrients (nitrate, phosphate and silicic acid), and primary production estimates from five independent research cruises (CIMAR-Fjords cruises 12–16, from 2006 to 2010, Table 1) carried out during spring between 41.5°S and 56.0°S (Fig. 1). Physical structure of the water column was recorded with a Seabird 19 CTD attached to a rosette equipped with 24 Niskin bottles. Water column stability (Brunt-Väisälä Frequency, N) was calculated as: \( N = \frac{\partial \rho}{\partial z} \), using gravity (\( g \)) and seawater density (\( \rho \)) differences among 4 layers (surface, 5, 10 and 25 m depth). Density computations were based on the atmosphere International Equation of State of Seawater (UNESCO, 1981). Surface PAR values for each station were obtained from 4-km resolution MODIS-Aqua images gathered for the 2006–2010 period from NOAA’s ERDDAP platform (http://coastwatch.pfeg.noaa.gov/erddap/griddap/erdMHpar08day.html).

Eight-day composite images were used because of the frequent lack of data over this region due to cloud coverage. Thus, PAR values for a given sampling station and date were extracted from the nearest pixel in the weekly image that encompassed that date. When the nearest pixel for a specific sampling site did not contain data, the monthly mean or springtime (October-November) mean were used. To measure the concentration of nitrate (NO\(^{-3}\)), phosphate (PO\(_{4}^{3-}\)), and silicic acid (Si (OH)_4), discrete water samples (50 mL) within the euphotic zone (surface, 2, 5, 10, and 25 m depth) were stored at ~20 °C in acid-cleaned, high-density polyethylene bottles and analyzed as described by Atlas et al. (1971). Size-fractionated chlorophyll-a (Chl-a) concentration was also measured within the euphotic zone (same previous depths) and for three different size classes. For the nanophytoplankton fraction (2.0–20 µm), 125 mL of seawater was pre-filtered using a 20 µm Nitex mesh and collected on a 2.0 µm Nuclepore filter. For the picophytoplankton fraction (0.7–2.0 µm), 125 mL of seawater was pre-filtered using 2.0 µm Nuclepore and collected on a 0.7 µm GF/F glass fiber filter. The microphytoplankton fraction was obtained by subtracting the previous two fractions from the total chl-a concentration (collected over 0.7 µm GF/F filters). Filters were immediately frozen (~20 °C) until later fluorometric analysis, using acetone (90% v/v) for pigment extraction (Turner Design TD-700) as described by Parsons et al. (1984). The primary production process requires the availability of nutrients and light as basic resources for phytoplankton growth. Hence, in this study, this parameter was incorporated as an indicator of the resource utilization rate by the phytoplankton (Marañón et al., 2012). All primary production estimates were obtained using the \(^{14}\)C method (Steeman-Nielsen, 1952) within the euphotic zone (above 25 m depth) during 4 h of daylight (earliest at 09:00 h and latest at 15:00 h) at the same depths described for nutrients and chl-a samples. Two replicates and two dark bottles were used for every depth. Since all cruises were carried out during different years (Table 1), satellite-based chlorophyll-a data from 2006 to 2010 were extracted. MODIS-Aqua images (NOAA’s ERDDAP platform) using eight-day composite images (http://coastwatch.pfeg.noaa.gov/erddap/griddap/erdMHCchl8day.html) were compared between years using chlorophyll-a data of every sampling site to check whether the overall conditions between cruises were comparable or not. Using average chlorophyll-a data (period of October-November) at each station and year, a one-way ANOVA analysis was performed to determine differences between years.

2.3. Data analyses

Although the samples collected on every research cruise were from the entire water column, the dataset used in this study was restricted to the top 25 m in order to avoid confounding effects of vertical variability, and to better represent the effects of surface brackish water on phytoplankton productivity and biomass. Correlation analysis was used to assess the collinearity of temperature, salinity, density, Brunt-Väisälä frequency, and nutrients. This collinearity was then used to produce statistically independent environmental axes through a principal component analysis (PCA). Depth-averaged and standardized (log transformed) values of temperature, salinity, density, Brunt-Väisälä frequency, mineral nutrients, and surface PAR for each station were used in the PCA. The association between the first two principal components of environmental variability and autotrophic biomass (total and size-fractionated) was analyzed via Pearson correlation coefficients. Given the extent, geographic complexity, and environmental heterogeneity of the Patagonian region (see Aracena et al., 2011; González et al., 2013; Jacob et al., 2014), it was assumed that any relationship between chlorophyll and environmental variables was unlikely to exist over the entire region. Therefore, a series of local-scale correlations, each centered on a sampling station, were computed using all the observations that fell within a 150 km radius around each station. The radius of 150 km for spatial neighborhoods was based on a smallest-sample...
criterion. To compute a correlation coefficient around each one of the sampling stations, neighborhoods with at least 10 pairs of values were considered in the computation of correlation coefficients. Only those producing a significant correlation (alpha = 0.05) were considered for graphical representation. A radius of 150 km was the smallest neighborhood that met these requirements. The same procedure to compute correlations was applied for both principal components and each of the four biological variables: depth-integrated total chlorophyll-α and total percentages that corresponded to micro-, nano- and pico-phytoplankton. Finally, we tested linear, logarithmic, and polynomial regressions to determine the relative importance of chlorophyll-α concentrations and primary production as (resource use rate) in the control of phytoplankton size structure. chlorophyll-α concentrations and primary production data were log_{10}-transformed prior to analysis.

3. Results

3.1. Latitudinal variability of physical-chemical data and chlorophyll-α

Annual satellite-based median chlorophyll-α showed similar values between 2006–2007 and 2008–2009, with a slight decrease with latitude (Fig. 2). No significant differences between years were observed (F (4670) = 1.7, p = 0.149), which is a sign of low interannual variability in the region during the observed years (from 2006 to 2010). Upper and lower quartiles overlapped from 2006 to 2010, where only values from 2010 showed the shortest range. The total range of satellite-based chlorophyll-α overlapped from 2006 to 2008, but showed a lower range only during the spring of 2009 (Fig. 2). Euphotic zone depth-averaged temperatures decreased from north to south Patagonia during the spring, from 12.3 to 4.7 °C (Fig. 3A), with a stronger latitudinal gradient than the northern section of the Patagonia (59.0–58.5°S, and also at the inner stations of glacial fjords in the Southern Strait of Magellan (~54.5°S). Stations located within fjords and channels exhibited the lowest salinity values, which is consistent with a stronger influence of freshwater discharges by local rivers (Fig. 3B). Average salinity values below 20 were observed at the Reloncaví fjord (41.5°S), Aysén fjord (45.5°S), Baker channel (48.0°S), and Ultima Esperanza Sound (51.5°S), whereas oceanic waters were dominant at Boca del Guapo (44.0°S), Penas Gulf (47.5°S), and south of the Beagle channel (~54.5°S). Brunt-Väisälä frequency exhibited a latitudinal pattern that mirrored patterns of surface salinity north of ~52°S, and revealed strong horizontal gradients in vertical stratification, with the highest values at fjords Reloncaví (middle and head of the fjord), Puyuhuapi (45°S), Aysén, and Baker (Fig. 3C). No latitudinal variability in phosphate and nitrate concentrations was observed (Fig. 3D and E), which did not strongly correlate with salinity or density (results not shown), although higher concentrations of both nutrients were observed at oceanic sites (Fig. 3D and E). Average phosphate and nitrate above 25 m of depth varied from 0.2 to 1.6 μM, and from 0.1 to 16.5 μM, respectively, resulting in the euphotic-zone average inorganic N/P ratio of 9.0 (standard error = 0.3) for the entire Patagonian ecosystem. Silicic acid concentrations varied widely north of 48°S, and showed a strong relationship with water column stratification (r = 0.68, p < 0.001) as evidence of freshwater discharges, where average values ranged from 1.3 to 55.2 μM (Fig. 3F). South of 48°S, silicic acid varied over a much narrower range (0–9.9 μM) and was negatively correlated with salinity values (r = −0.63, p < 0.001). High values of depth-integrated chl-a (>200 mg m⁻²) were estimated for the Reloncaví fjord, and the gulfs of Ancud and Corcovado in northern Patagonia. In contrast, low chl-a values (4.0–81.1 mg m⁻²) were observed from 44 to 53°S regardless of mineral nutrient concentrations, water-column stability, and hydrographic conditions (Fig. 3G). Depth-integrated chl-a increased again south of 53.0°S, from the northern section of the Magellan Strait to Cape Horn, with values ranging from 25.7 to 821.8 mg m⁻² (Fig. 3G). Phytoplankton size structure varied largely across the region under spring conditions, with a clear dominance of microphytoplankton (>80%) from 41.5 to 44.0°S, and a more variable composition from 44.0 to 53.0°S, where picophytoplankton appeared in higher proportion at sites Puyuhuapi Channel, Bakker Channel, and Ultima Esperanza, with low average salinity (i.e. higher fresh water discharges) (Fig. 3H–J). South of 53.0°S, the picophytoplankton contribution was consistently low (<6%), whereas the nanophytoplankton fraction was highly variable (20–72.5%), and the microphytoplankton fraction had the highest contribution of total chl-a as in the northern section of the Patagonia (59.0–98.0%) although with greater spatial variability (Fig. 3H–J). Mean surface PAR for the period 2006–2010 showed a latitudinal as well as a longitudinal gradient in surface light conditions (Fig. 4A), with dramatic differences between inner channels and fjords (<20 Einstein m⁻² s⁻¹) and areas such as the Inner Sea of Chiloe in northern Patagonia and the east of the Magellan Strait in the south (>25 Einstein m⁻² s⁻¹). This pattern appeared even clearer when surface PAR estimates corresponding to the specific sampling sites and dates were examined (Fig. 4B). High values (>34 Einstein m⁻² s⁻¹) were observed for stations located in the northern (42–45°S) and southern sections (53–56°S), whereas lower values (<34 Einstein m⁻² s⁻¹) were observed in fjords such as Puyuhuapi and Aysén (45.0 and 45.5°S, respectively). The lowest surface PAR values (<25 Einstein m⁻² s⁻¹) were found at stations located between 49.0 and 52.5°S, on the western side of the Southern Ice Field (Fig. 4B).

3.2. Main control factors on phytoplankton size structure

The first two principal components obtained from a PCA on depth-averaged hydrographic data, nutrient concentrations, and surface PAR explained 63% of the total variance (Table 2). The first principal component (PC1) explained 36.0% of total variability and accounted mostly for the influence of low salinity, low density, and silicic acid rich waters that are discharged from Patagonian rivers. The second principal component (PC2) accounted for 27.3% of total variability, and represented the zonal gradient in nutrient concentrations, which in this region is manifested mostly through the intrusion of nitrate- and phosphate-rich oceanic waters (Table 2).

The spatial distribution of PCA scores included a large spatial heterogeneity at the different fjords and channels (Fig. 5A and B). Thus, along the PC1 axis (Fig. 5A), freshwater discharges directly affected sites at the eastern continental margin (e.g. Reloncaví fjord, Puyuhuapi Channel, Aysén fjord, and Baker Channel). Along the PC2 axis, the PCA ordination of physical-chemical conditions indicated a separation between stations located north and south of 48°S (Fig. 5B and C). Non-glacial rivers with high silicon inputs and strong density stratification near the surface, and also oceanic waters rich in nitrate and phosphate influenced fjords and channels located in the northern section of...
Patagonia (Fig. 5B and C). Pearson correlations computed over the 150 km spatial neighborhoods (Fig. 6) indicated that total and size-fractionated chl-$a$ are significantly correlated with both river influence (PC1) and oceanic nutrients (PC2) at a number of sub-regions. However, the extent of these sub-regions and the sign of significant correlations change depending on which pair of biological variables and environmental features (i.e. principal component) are compared. For instance, while the correlation between total chl-$a$ and PC1 was significantly negative for 43% of the sites in influenced by the Southern Ice Field (48–54°S), as well as along the Gulf of Corcovado and Moraleda Channel (~ 44°S), at the northernmost stations, located along the Reloncaví fjord and the northern section of the Chiloé Inner Sea it was positive (Fig. 6A). A very similar distribution was found for the microphytoplankton fraction in PC1 (Fig. 6B), which is consistent with the fact that the largest contribution to phytoplankton biomass at 40% of the stations corresponded to large-cell taxa. The nanophytoplankton fraction positively correlated with PC1 at only one site near the Boca del Guafo area (Fig. 6C). Correlations for picophytoplankton and PC1 were almost a mirror image of the correlations found for the microphytoplankton fraction. Positive correlations were found for the stations near Boca del Guafo (~ 44°S) and those in influenced by Southern Ice Field glaciers (50–53°S); correlations were negative for stations located inside the fjords Reloncaví, Puyuhuapi, Aysén, and Quirralco (Fig. 6D). Spatial correlation patterns between depth-integrated chl-$a$ and the influence of oceanic nutrients (i.e. nitrate and phosphate) represented by PC2 (Fig. 5) were clearly different from those found for PC1. There was a latitudinal change in the significant correlations between total chl-$a$ and PC2 (Fig. 6E), suggesting that phytoplankton biomass responds negatively to the influence of oceanic nutrients in channels and fjords located in northern Patagonia (north of 48°S), but...
Fig. 5. Principal component analysis (PCA) of the N = 135 stations according to hydrographic and nutrient conditions as well as surface PAR measured at each station. Maps in (a) and (b) show the spatial distribution of PCA loadings for PC1 and PC2, respectively. The colored bar in (b) applies to both maps. (c) Ordination of stations according to PC1 and PC2, which jointly explained 63% of the total variance (see Table 1), with symbols corresponding to the research cruises specified in Fig. 1, and vector labels indicating the individual variables (Temp: temperature, sali: salinity, sigm: sigma-theta (density), BVF: Brunt-Väisälä frequency, par: photosynthetically active radiation, Si: silicic acid, N: nitrate, P: phosphate, N/P: nitrate:phosphate ratio, Si/N: silicic acid:nitrate ratio). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 6. Spatial variability of Pearson correlation between total chlorophyll-a (A, E) and size-fractionated (microphytoplankton: B, F; nanophytoplankton: C, G; picophytoplankton: D, H) and the first two principal components obtained from a PCA of hydrographic and chemical variables in the water column (see Fig. 5c). PC1: A, B, C and D. PC2: E, F, G and H) Red and blue symbols indicate positive and negative correlations, respectively. Only significant correlations are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
positively in those located south of 48°S. Only few sites correlated significantly with the microphytoplankton fraction with PC2 (Fig. 6F), whereas the nanophytoplankton fraction positively correlated with oceanic nutrients east of the southern Patagonian Ice Fields and at some sites of the Strait of Magellan (Fig. 6G). Finally, the picophytoplankton fraction was positively correlated with oceanic nutrients at the Reloncaví Fjord (middle and head of the fjord), northern section of the Chiloé Inner Sea, and negatively correlated at sites near the Beagle Channel (Fig. 6H).

3.3. Resource supply rate and phytoplankton size structure

The extensive fjord ecosystem that was studied presented highly complex hydrographic conditions formed by the interaction of marine and fresh water, where we expected a large horizontal variation in the concentration of nutrients. Thus, it is possible to observe sharp latitudinal and longitudinal gradients in nutrient concentrations even after depth-averaging nutrient data from 0 to 25 m in depth (Fig. 3D–F). Some of this spatial variability in mineral nutrients can also be observed in the relative contribution of different size fractions to total autotrophic biomass. The microphytoplankton fraction increases rapidly with increasing total chl-a, reaching values > 75% when autotrophic biomass is above 10 µg L−1 (Fig. 7A). A concomitant drop in the contribution of total biomass was observed in the nanophytoplankton fraction, with < 40% when total chl-a levels were above 10 µg L−1 (Fig. 7B). A larger drop in total chl-a was observed in the picophytoplankton portion, which tended to dominate when total chlorophyll-a was < 2 µg L−1 but contributed < 10% of total biomass when total chl-a concentrations were above 5 µg L−1 (Fig. 7C). These patterns appeared to be a consequence of a strong association between size partitioning and primary production rates (Fig. 8). Primary production is an indicator of the resource utilization rate since phytoplankton requires a supply of nutrients and light to grow. The contribution of microphytoplankton increases rapidly with primary production reaching values above 50% at rates of 50 µg C L−1 d−1, following a logarithmic relationship (Fig. 8A, y = 6.712 ln(x) + 26.819, R² = 0.16, p < 0.01, n = 136). In contrast, nano- and picophytoplankton decrease at higher productivity rates. At low productivity rates, nano-phytoplankton showed a large variation in its relative contribution (0–85%) and its contribution varies from 5 to 45% when primary production increases above 200 µg C L−1 d−1 (Fig. 8B). Picophytoplankton contribution decreases rapidly through the productivity range (Fig. 8C). At production rates above 100 µg C L−1 d−1 picophytoplankton contributes only with 2–40% of the total autotrophic biomass.
4. Discussion

Our data set was built from observations collected during five research cruises (CIMAR 12, 13, 14, 15 and 16) conducted during consecutive spring seasons (Table 1). A logical concern with this kind of dataset is how much of the spatial variability we present here is due to temporal changes in the physical-chemical conditions of the euphotic zone, rather than to persistent spatial patterns in hydrography and nutrient supply (Fig. 3). Comparison of satellite-based chlorophyll-a from 2006 to 2010 (Fig. 2) showed an overlap in the overall conditions related to autotrophic biomass, displaying low temporal variability between years (from 2006 to 2010). Thus our temporally-fragmented observations can be directed to address questions that pertain to the large Patagonian ecosystem, representing mainly three aspects: (i) the contrasting hydrographic conditions along this large estuarine region, (ii) the inputs of mineral nutrients (marine versus terrestrial origin), and (iii) the entire range of productivity/resources available. The wide range of physical-chemical conditions (Fig. 3A–C) highlighted the strong influence of freshwater discharges (e.g. Reloncavi fjord, Puyuhuapi channel, Aysén fjord, Baker channel, and channels near Puerto Natales) on the water column at different latitudes, adding the estuarine variability to our analysis. The entire region also exhibits strong gradients in mineral nutrient concentrations, such as the latitudinal decrease in nitrate (1 order of magnitude) and silicate (2 orders of magnitude) from northern to southern Patagonia (Fig. 3D–F). In the southern section (south of the Patagonian Ice Fields) the fluor- uscence is considerably lower compared to the glacial influence, affecting the total input of nutrients and suspended sediment load into the fjord ecosystem (Aracena et al., 2011; Dávila et al., 2002), where surface fjord waters contain low silicic acid levels (Fig. 2F, Torres et al., 2011). Furthermore, a latitudinal trend towards lower primary production rates in southern Patagonia was also reported (Aracena et al., 2011). At the ecosystem level, the results of this analysis stress the importance of two main estuarine features (river discharges and nutrients from oceanic waters), rather than other physical control factors that are common in modulating the phytoplankton size structure of other coastal systems.

4.1. Impact of fresh water discharges

A clear signal is obtained primarily for the influence of freshwater inputs (low salinity, low density, strong stratification, and high silicon waters), and secondarily for the influence of oceanic waters with high concentrations of nitrate and phosphate (Table 2). The effects of river discharges on hydrographic conditions (i.e. stratification of the water column) and silicon availability appear to limit mainly large autotrophic cells (Fig. 6A), and to favor the small size fraction at sites south of 48°S, and also at Puyuhuapi Channel and Aysén fjord (Fig. 6D). In this respect, increased abundance of pico- and nanophytoplankton was reported for coastal waters strongly influenced by rivers (Iriarte et al., 2018; Jochem, 2003; Liu et al., 2004; Vargas et al., 2012). The central Patagonia transition at ~48°S was described as an area with elevated freshwater discharges from large rivers such as the Baker and Pascua (1133 and 753 m³s⁻¹, respectively; National Water Directorate, www.dga.cl). Additionally, the glaciars associated to the Northern and Southern Patagonian Ice Fields influence the content of inorganic matter (glacial silt) and light attenuation in this region (Montecino and Pizarro, 2008; Pizarro et al., 2005). Together with glacial sediments, river-borne suspended material reduce light availability, hence surface PAR values, south of 48°S (Fig. 4B), and in fjords such as Puyuhuapi and Aysén (~45°S), and rivers such as Baker with a high concentration of suspended sediments (González et al., 2013). Consistently, primary production rates that are low compared to areas not influenced by glaciers were reported for this section of the Patagonia (e.g. 91 mg C m⁻² d⁻¹ at Caleta Tortel, (Aracena et al., 2011); 422 mg C m⁻² d⁻¹ near Baker river mouth, (González et al., 2013). Our results suggest that autotrophic biomass and its composition are strongly impacted by river discharges at latitudes south of 48°S, and also at Puyuhuapi Channel and Aysén fjord, with a substantial reduction in depth-integrated chl-a and a mixed phytoplankton size distribution, with cells < 20 µm (nano- and picophytoplankont) representing > 50% of total chl-a. A slightly different condition was observed at the Sub-Antarctic system of the Magellan Strait, Cape Horn, and the Beagle Channel where river discharges did not have a significant negative correlation with the chlorophyll-a concentration (Fig. 6A and B), and chl-a ranges were at the same magnitude compared to northern Patagonian channels and fjords (i.e. Reloncavi fjord, Ancud Gulf, Corcovado Gulf, Fig. 3G). Although there are numerous glaciers nearby, salinity at the observed sites (surface waters above 25 m) was similar to other estuarine sites and did not decrease compared to sites where river discharges have a strong influence (e.g. Puyuhuapi channel, Baker channel, Ultima Esperanza, Fig. 3B). Additionally, low silicate/nitrate ratio characterized this area (Fig. 3), and light availability was not limited in this area (Fig. 4B), resulting in a chl-a concentration similar to previous studies (González et al., 2016; Iriarte et al., 2001).

Hydrographic variability (i.e. temperature and salinity) modulates autotrophic production by way of seasonal changes in stratification (strongly stratified in summer and weakly stratified in spring and autumn), and the balance between fluvial and marine influences on nutrient conditions. Changes in these two conditions can lead to contrasting phytoplankton community structures, depending on the availability of blooming species and turbulence conditions (Smayda, 2002). Stratification strongly affects light penetration and the availability of nutrients in surface waters (Goebel et al., 2005). In the stratified condition, flagellate forms (i.e. dinoflagellates) or small diatoms might be favored, however they may be controlled by nutrient inputs such as nitrate and phosphate (Guo et al., 2014). In our analysis, stratified waters (high Brunt-Väisälä frequency, Fig. 2) favored autotrophic cells in the low-size range including mainly picophytoplankton cells (e.g. cyanobacteria, picocyanobacteria). A previous study in the region demonstrated that stratified conditions and high silicate levels positively correlated with cells with low surface/volume ratios (Alves-de-Souza et al., 2008). In southern Patagonian fjords, areas with high freshwater input and strong layering processes favored the formation of thin layers with high chl-a concentration (Ríos et al., 2016). Thus, during spring conditions, a greater contribution of autotrophic nanoflagellates versus dinoflagellates was observed (González et al., 2011, 2010). When stratification weakens, the microphytoplankton contribution reaches its highest values. Under this hydrographic and nutrient scenario, chain-forming diatoms from the genera Thalassiosira, Chaetoceros, and Skeletonema dominates the microphytoplankton biomass (González et al., 2011, 2010).

Additionally in our extensive study area, river discharges are usually defined as oligotrophic carrying low nitrate and phosphate loads (Fig. 3D and E). Theoretical and experimental evidence demonstrate that small cells have higher rates of nutrient uptake per unit biomass and lower half-saturation constants due to their higher surface to volume ratio (Aksnes and Egge, 1991). Furthermore, smaller cells have a lower minimum cellular metabolic requirement that selectively allows them to survive at lower resource concentrations than larger cells (Grover, 1991). Thus lower resource requirements by pico- and nanophytoplankton size cells may support higher population densities of these size classes. Consequently, pico- and nano-phytoplankton appear to have advantages over microphytoplankton under higher river discharges that produce nutrient-limited conditions, giving them apparent benefits over larger cells, modifying the community size distribution during the productive season analyzed.

4.2. Nutrient rich oceanic waters and secondary control factors

Opposite to the river discharges, the intrusion of oceanic waters rich in mineral nutrients (i.e. nitrate and phosphate) promoted higher chl-a
concentrations at some sites in the area southern than 48°S (Fig. 6E). As we discussed previously, when nutrients are in low supply, phytoplankton biomass and production rates are low and the community is dominated by small cells, which due to their large surface-area-to-volume ratio are better prepared to withstand nutrient limitations (Aksnes and Egge, 1991; Kierboe, 1993). Additionally, limitation of specific nutrients may lead to different autotrophic communities. Fjord waters rich in silicic acid and nitrate lead to a major contribution of large cells, where chain-forming diatoms are typically dominant (González et al., 2010). Silicic acid is important in the formation of diatom silica shells, hence their ratio with nitrate has been hypothesized to affect diatoms (Rabalais et al., 1996). Furthermore, nitrate can be stored in diatom vacuoles giving a high vacuole/cytoplasm ratio (Stolte and Riegman, 1995) leading to large phytoplankton cells. The influence of riverine and glacier freshwaters substantially increase dissolved silicon, but not nitrate or phosphate, in the surface waters of these fjord ecosystems (Vargas et al., 2011). This nutrient ratio supports the high proportion of small cells (pico- and nano-phytoplankton fractions) that may be dominated by small autotrophic flagellates and dinoflagellates, together with picoeukaryotes.

Other factors, such as temperature did not show an important influence on phytoplankton size structure as some previous studies had predicted. Warmer temperatures were expected to shift phytoplankton size structure towards a larger contribution of small cells to total autotrophic biomass. For the eastern and western North Atlantic Ocean, (Morán et al., 2010) temperature alone explains 73% of the variability of picophytoplankton regardless of the trophic status or nutrient load. This pattern is partly explained by the temperature-size rule for protist (Atkinson et al., 2003), which predicts a decrease in the mean cell size as temperature increases. Contrasting, a meta-analysis of temperature, phytoplankton size structure, and productivity rates concluded that resource availability rather than temperature is the main factor controlling the observed phytoplankton size structure (Marchán et al., 2012). Thus, the effects of water temperature and resource availability (e.g. mineral nutrients and productivity rates) must be evaluated separately. Our analysis uses a sufficiently large data set making it possible to reject the hypothesis that temperature plays a direct role in controlling phytoplankton size structure (Table 2, Fig. 5), at least for this extensive fjord ecosystem.

In addition to estuarine features, our analysis indicated that surface radiation limits autotrophic size structure, but to a lesser degree than river discharges and oceanic nutrients (Table 2). Unfortunately, few PAR data were available thus the effect of light in the euphotic zone (above 25 m in depth) was poorly evaluated as a controlling factor for phytoplankton size structure compared to other physical and chemical variables. However, assuming that our extracted MODIS-Aqua images are good estimates of surface PAR values and represent a large part of the entire fjord ecosystem studied, the satellite-derived PAR values were used as our best estimates of the effect of light on phytoplankton structure size. As an example, the mean surface PAR for the 2006–2010 period (Fig. 4A) includes not only the latitudinal variability, it also includes the large effect of suspended material from rivers and inorganic matter and sediments from glaciers as elements of light attenuation, which has also been observed in previous studies (Marín et al., 2013; Pizarro et al., 2000, 2005). The use of mineral nutrients and their transformation into algal biomass requires solar radiation as an energy source to drive photosynthesis, thus the importance of mineral nutrient loads can be constrained by the availability of sunlight for phytoplankton (Cloern, 1999). The efficient capture of photons by small cells becomes an advantage when light is a limiting factor (Sunda and Huntsman, 1997). It seems that, at least during the spring months when our data were collected, light was a limiting factor in some estuarine regions (mainly at glacier-derived freshwater discharges), and the input of mineral nutrients exerted a stronger controlling effect on phytoplankton. Going one step further into our PCA results, a third principal component that accounts for 13% of total variability (76% of the total accumulative variability) revealed an important control over surface PAR along with N-P ratios (Table 2). Thus, the combined effect of light limitation and mineral nutrient ratios potentially play a role in determining the size distribution of the autotrophic community in this type of ecosystem. A likely example might be the Strait of Magellan area (south of 53°S), where the river influence and nutrient rich oceanic waters are expected to limit chl-a concentration and microphytoplankton abundance. However, the high depth-integrated chl-a concentration and the fraction of microphytoplankton are surprising (Fig. 3G). Previous studies (e.g. Iriarte et al., 2001) showed that springtime phytoplankton in the Strait of Magellan are dominated by large autotrophic cells (chain forming diatoms and thecate dinoflagellates). A potential explanation for the observed phytoplankton size structure in this area is the low silicate/nitrate ratio (Fig. 3) and high surface PAR observed at this latitude (Fig. 4B). Jacob et al. (2014) also reported light limitation in Patagonian fjords, especially for semi-enclosed coastal waters (influenced by glacial melting) but not for continental waters.

4.3. Consequences of small-cell versus large-cell dominance

The relation between increased resource utilization rate (i.e. primary production) and a greater dominance of large cells is a common feature in our analysis (Figs. 7 and 8). Thus, large cells contribute to more than 75% of the autotrophic biomass in high productive waters (Fig. 7A). Irgoien et al. (2005) showed the importance of the relation between cell size and growth rates or growing conditions, thus a combination of controlling factors might modulate the resulting dominant phytoplankton size distribution. Stratified waters with non-limiting conditions of silicic acid, nitrate, and phosphate lead to high autotrophic biomass dominated by large cells, as observed from the Reloncaví fjord to Boca del Guafo (Fig. 3), resulting in high primary productivity rates (González et al., 2010), and increasing the potential for carbon sequestration. Our results also reveal that not only large autotrophic cells (> 20 μm) dominate the euphotic zone during the productive season (i.e. spring), but also small cells represent a high proportion of the total phytoplankton biomass (Fig. 3A and J), with low productivity rates (Fig. 8B and C). The dominance of small nano- and picophytoplanktonic groups leads to a microbial-type food web that partitions the autotrophic autochthonous organic matter, which cannot be transferred to higher trophic levels, supporting the recycling of organic matter. The efficiency of the biological pump, i.e. how the organic matter is sequestered in the ocean if microbial or the classical food web type dominate (Legendre and Le Fevre, 1995), can present large variability in Patagonian fjords. Thus, the shifting balance between hydrography and resource control phytoplankton size structure may have important implications in the region.

Other factors such as grazing (i.e. top-down control, not included in our analysis), may have a major role in structuring the phytoplankton community in aquatic ecosystems such the Patagonian fjords. Nevertheless, previous studies, which were carried out during the same research cruises used in this analysis (Table 1), have estimated that a low proportion of the primary productivity can be consumed by heterotrophic plankton in this study area. In the northern area (41.5–44.0°S) less than 2% of the primary productivity can be consumed by euphausiids and calanoid copepods (González et al., 2010). Similar evidence found in the Aysén Fjord (45.3°S) indicates that heterotrophic nanoplankton consumes less than 2% of the total primary productivity, where heterotrophic flagellates consume 1.1% and other heterotrophs (i.e. ciliates, copepods and euphausiids) reach even lower values (González et al., 2011). In river-influenced areas, such as Baker and Pascua rivers (47.0–48.0°S), heterotrophs consume 33% of the primary productivity, where heterotrophic flagellates and small calanoid copepods each have an impact of 14% in relation to primary productivity (González et al., 2013). Far south (52–56°S), Euphausia vallentini consumed 3% of the gross primary production in sites near the
Magellan Strait and Beagle Channel (González et al., 2016). Mixotrophic flagellates can also be important in fjord ecosystems (Havskum and Riemann, 1996) and may represent an important control factor on small autotrophs. In fact, in northern Patagonian fjords, mixotrophic flagellates were reported as a dominant trophic group showing a strong control during winter conditions (Czyzynka et al., 2011), but not during the productive season. Even though other potential grazers (e.g. larval invertebrates, picoheterotrophs and others) were not considered in those estimates, top-down control does not seem to exceed more than 50% of the autotrophic primary production, contrary to previous revisions from different ecosystems (e.g. Calbet and Landry, 2004). Therefore, the previous evidence seems to indicate that hydrography and resource supply variables (i.e. inorganic nutrients, light availability) are significant controlling factors for phytoplankton productivity and community structure in the Patagonian fjord ecosystem. 

Evaluating the respective roles of control factors such as hydrographic variability and resource supplies (e.g. mineral nutrient loads and light availability in the euphotic zone) are important for predicting the response of pelagic ecosystems to local and global changes. High latitudes, ecosystems, such as fjords and channels in the Chilean Patagonia are projected to experience some of the largest climate changes in the coming decades (IPCC, 2014). Thus environmental impact from climate change (i.e. warming, ice melting, and glacial retreat) and local/anthropogenic stressors (e.g. growing human population, fish/mussels farming) expected for this area (Iriarte et al., 2010; Iriarte, 2018) will modify the hydrography, nutrient rates and light availability affecting phytoplankton and the subsequent biotic productivity. It is expected that environmental changes will affect the basic functionality of coastal ecosystems, thus understanding the effects of changes in resource availability will be central to predicting future alterations in the phytoplankton size structure and its related processes.

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