



Size diversity as an expression of phytoplankton community structure and the identification of its patterns on the scale of fjords and channels

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ABSTRACT

Large microalgae, such as diatoms can generate high levels of biomass, unlike small phytoplankton components in the austral fjords and estuarine systems of Chile. We propose a cell-size-based diversity index (H'_{size}) measured as chlorophyll-a concentrations (Chl-a) to determine if the relationship between H'_{size} versus Chl-a results in a hump-shaped distribution considering: (i) the intermediate disturbance hypothesis, (ii) phytoplankton size colonization strategies, and (iii) predictions of low H'_{size} with high or low Chl-a levels and peaks in H'_{size} with intermediate Chl-a levels. The functional responses of phytoplankton are tightly coupled to environmental conditions. Could, then, the relationship between H'_{size} and Chl-a occur on a particular temporal/spatial scale? Herein, we analyze data from three CIMAR-Fjords cruises performed between 2001 and 2002 within areas of different continental influence from 41° to 47°S. Phytoplankton samples were taken at different locations and depths, filtered to obtain Chl-a with and without size-fractionation ($> 20 \mu\text{m}$, $< 11 \mu\text{m}$), and then used to calculate H'_{size} . Total Chl-a (TChl-a) changed by two orders of magnitude between different cruises and areas. The small phytoplankton fraction ($< 11 \mu\text{m}$) dominated with low TChl-a values ($\leq 1 \text{ mg m}^{-3}$), as did the large phytoplankton fraction ($> 20 \mu\text{m}$) with high TChl-a values ($\geq 1 \text{ mg m}^{-3}$). Although other relationships can be found on smaller scales, we determined unimodal (hump-shaped) relationships through quadratic quantile regressions between the H'_{size} index and TChl-a and between H'_{size} or TChl-a and the abiotic factors when considering data from all three cruises and areas. H'_{size} versus TChl-a was driven by one variable or by different combinations of variables, and according to low–high disturbance level: high TChl-a and larger size classes predominated in deeper upper mixed layers having higher light availability, whereas shallower upper mixed layers with lower light availability presented lower TChl-a and predominantly smaller cell size classes. Thus, the larger temporal/spatial scales used here allowed us to test the hypothesis of the unimodal expression of phytoplankton patterns in these coastal ecosystems.

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1. Introduction

Phytoplankton population sizes and relative abundances of cell size classes are simultaneously determined by ecological processes, such as competition and predation, and by physiological processes, such as the efficiency of light harvesting or nutrient acquisition, both influenced by the cell size (Lewis, 1976; Reynolds, 1997). The rate of cell division and respiration scale inversely with cell size (Williams, 1964; Banse, 1976; Malone, 1980), and specifically, the nutrient acquisition and the harvesting of light are intrinsically influenced by cell size (Platt, 1981; Marañón, 2009), i.e. chlorophyll-specific photosynthetic rates decrease with an increase in cell size (Taguchi, 1976; Geider et al., 1996; Montecino and Quiroz, 2000).

The cell size structure of phytoplankton communities is important at the ecosystem level. This feature generates shifts in the carbon budget of pelagic trophic webs (González et al., 2010), affecting ecosystem properties, such as the flow of matter and energy and biogeochemical cycling (Violle et al., 2007; Litchman and Klausmeier, 2008), because the fate of phytoplankton production depends heavily of the dominant size fraction (Kahru and Leeben, 1991; Kiørboe, 1993). In terms of chlorophyll-a (Chl-a) as a proxy for phytoplankton biomass, meso-eutrophic ecosystems with Chl-a concentrations $> 5 \mu\text{g L}^{-1}$ are dominated by microphytoplankton components with a modal cell size abundance of 10–40 μm (Yentsch and Phinney, 1989; Montecino, 2001; Montecino et al., 2006, 2008). In contrast, pico and nanophytoplankton components between ≤ 2 and 4 μm dominate oligotrophic ecosystems (Malone, 1980; Chisholm, 1992). Thus, changes in environmental factors (environmental conditions, nutrient concentrations, light availability) or biological interactions, such as grazing (and mixotrophy) heavily affect the

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cell size (or taxonomic) composition of these assemblages and, hence, the biomass and Chl-a concentrations of the phytoplankton community. Here we only studied the non-biological effects.

Overall, the environmental factors can affect phytoplankton directly or indirectly. (i) Directly, light provides energy and resources supply materials for growth, reproduction, and survival of cells; and temperature regulates photosynthesis, respiration, growth, resource acquisition, and motility (Reynolds, 1997; Montagnes and Franklin, 2001; Litchman and Klausmeier, 2008). (ii) Indirectly, salinity affects the vertical stratification and, thus, the permanence of microalgae in the upper water column (Sin and Wetzel, 2002). The availability of resources, such as the amount of light as well as macronutrients, are both affected by the depth of the upper mixed layer (Z_m), the depth of euphotic layer (Z_{eu}), and the Z_m/Z_{eu} relationship; moreover, the intrusion of deeper water to the euphotic zone can cause nutrients to increase (Tremblay et al., 1997).

These abiotic changes affect phytoplankton differently depending on cell size, i.e. in oligotrophic environments, small size classes are more efficient in the acquisition of limiting nutrients (Malone, 1980; Litchman and Klausmeier, 2008). In contrast, given high nutrient concentrations, large phytoplankton generally achieves higher growth rates than small ones (Laws, 1975). One of the reasons is that the larger planktonic cells are better adapted to greater fluctuations in the light climate than the smaller cells (Bode and Fernández, 1992; Kiørboe, 1993; Gargett and Marra, 2002). However, under stratified conditions, small flagellates will persist, thanks to their swimming ability and to the sinking of larger diatoms requiring high turbulence to prevent sedimentation (Kiørboe, 1993). In contrast, flagellates require low turbulence to avoid physical damage (Cullen et al., 2002). Different resources do not have a similar limiting effect on microphytoplankton taxa because these have different parameters for characterizing the uptake and assimilation efficiencies of nutrients (Tilman et al., 1982). Therefore, conceptually, the study of microalgal communities can be approached from the cell size structure (Chl-a) of phytoplankton, considering that cell size is a functional trait based on the relationship of sizes with metabolic processes (e.g., Fenchel, 1974; Peters, 1983). For phytoplankton, functional groups are defined as groups of organisms that are related through biogeochemical processes, but not necessarily through phylogenetic relationships (Iglesias-Rodríguez et al., 2002). Different taxa can have the same or different functions in ecosystems according to specific traits (e.g., size); cell size is considered to be a morphological master trait that influences reproduction, growth, metabolism, resource acquisition, and predator avoidance (Brown and Maurer, 1986; Litchman et al., 2007; Litchman and Klausmeier, 2008).

Biological diversity, population size, and cell size are interdependent, and most studies have found unimodal patterns between species richness and body size or biomass, number of individuals and body size, or number of individuals and biovolume (Siemann et al., 1996; Li, 2002; Irigoien et al., 2004; Marañón, 2009). The availability of an index that includes both richness (with size classes defined *a priori*) and abundance within those classes (equivalent to evenness) would be useful. Here we applied a size diversity index (H'_{size}) with three-size classes and the Chl-a content in each class to prove the hypothesis that the relationship between size diversity (H'_{size}) and the phytoplankton TChl-a concentration reveals a unimodal (hump-shaped) distribution, with a peak of H'_{size} when Chl-a concentrations are intermediate, as the abundances of the different phytoplankton sizes are more even under an intermediate trophic status (resource availability).

In particular, one of the many theories explaining species diversity is the Connell (1978) intermediate disturbance hypothesis (IDH). Grime (1977) defines disturbances as the processes

that determine biomass removal, causing new spaces and, hence, making new resources available to other individuals (i.e., light extinction and nutrient limitation, in the case of phytoplankton). The IDH predicts unimodal biological richness and diversity in terms of the magnitude of the disruption or the time passed since the onset of the disruption (Begon et al., 1988; Sommer, 1995).

Phytoplankton colonization strategies vary among size classes, with small phytoplankters experiencing explosive reproduction and population growth; and after a disturbance, they are the first colonizers. Larger phytoplankton cells have slower reproduction and population growth, but they may outcompete the smaller phytoplankton cell sizes when resources are sufficient (Sommer, 1981; Grover, 1997; Reynolds, 1997). When the frequency, intensity, or lag of the disturbance is higher than the phytoplankton generation time or photoacclimation period, small cells predominate, but given low disturbance, large cells prevail. In both situations, H'_{size} and probably specific diversity should remain low. With intermediate levels of disturbance, H'_{size} and specific diversity should increase due to the coexistence of small, intermediate, and large microalgae. We chose this conceptual approach because the study of planktonic diversity and the causal factors that modulate it are difficult to undertake from an evolutionary perspective given a three-dimensional environment, large-scale space, small organisms, and numerous hard-to-identify species (Peters, 1983; Rodríguez, 1994). However, we can use phytoplankton cell sizes classes by applying a size index (H'_{size}) to this morphological trait in order to reduce complexity, thereby allowing us to describe the changes in these communities (Fenchel, 1974; Brown and Maurer, 1986; Bode and Fernández, 1992; Rodríguez, 1994; Brown, 1995; Montecino, 2001). Here, we also considered the IDH – originally employed to explain species diversity (Connell, 1978) – to relate the diversity of cell sizes classes (TChl-a) with disturbances generated by changes in environmental factors that directly or indirectly modifies or affects the nutrient and light availability. To estimate the unimodal relationship between H'_{size} with TChl-a and of both TChl-a and H'_{size} with the environmental factors, we used the quadratic quantile regression approach (i.e. Scharf et al., 1998, see Section 2).

Explaining the relationship between the phytoplankton structure and its environment using the functional role of microalgae in coastal ecosystems constitutes a bridge between community and ecosystem ecology. In order to detect these relationships, we considered a large database containing extensive ranges of variability in phytoplankton size classes, Chl-a, and environmental factors. We expected the patterns to change with the scale of observation as with the relationship of phytoplankton species diversity and productivity of the system (Chase and Leibold, 2002). The Patagonian fjord and channel system, with its large spatial and temporal phytoplankton variability (Montecino et al., 2006, 2008), offers the possibility to relate H'_{size} with TChl-a and these with large-scale environmental gradients. Given these scales, a macroecological analysis is appropriate for expressing phytoplankton patterns and the unimodal relationship between H'_{size} and TChl-a, including the scale on which it is statistically established. In addition, we want to determine whether a unimodal relationship exists for both TChl-a and H'_{size} with the environmental abiotic factors.

2. Methods

2.1. Sampling and ecosystem description

The samples used in this study were obtained in the large system of fjords and channels in Aysén, Chile (43°–47°S), a region characterized by low environmental stability and high spatial and temporal heterogeneity. These fjords and channels connecting

low salinity waters with oceanic waters are microbasins with unique microclimates (Silva et al., 1998; Guzmán and Silva, 2002). The input of freshwater from rivers, rain, snowfields (June–August), and glacier melting (September–November) reduces salinity and increases stratification in the adjacent water column (Pickard, 1971). These processes also provide nutrients to the water column (Silva et al., 1997), giving rise to the conditions necessary for the surface growth and accumulation of phytoplankton biomass. Increased aquaculture activities and the development of urban and industrial zones can also contribute additional nutrients in some specific areas (Soto et al., 2001; Buschman et al., 2006), thereby increasing the region's natural spatial heterogeneity. This area has high biological productivity, and the variability of the phytoplankton biomass is tightly coupled to high variability in cell sizes (Montecino and Pizarro, 2008).

Biological (Chl-a), physical (Z_m , Z_{eu} , Z_m/Z_{eu} , T , salinity), and chemical data (Si(OH)_4 , silicic acid; NO_3^- , nitrate; PO_4^- , orthophosphate;

and $\text{NO}_3^-:\text{PO}_4^-$ ratio) were obtained during one oceanographic cruise in austral winter (CIMAR-Fiordos 7–1; July 2001) and two in austral spring (CIMAR-Fiordos 7–2; November 2001 and CIMAR-Fiordos 8–2; November 2002) on board the R/V AGOR Vidal Gormaz in the Aysén Region. These cruises were organized by the Comité Oceanográfico Nacional (CONA). During the 2001 winter and spring cruises (CF7–1, CF7–2), the sampling stations were distributed in the centrally situated Moraleda Channel and the inner fjords and channels. During the 2002 spring cruise (CF8–2), the sampling stations were located in the area of the Moraleda Channel and the oceanic channels of Aysén (Fig. 1).

At each sampling station, water column temperature and salinity were obtained with an SB25 CTD. The collected data were processed by the Centro Nacional de Datos Hidrográficos y Oceanográficos de Chile (CENDHOC), part of the Chilean Navy's Servicio Hidrográfico y Oceanográfico de la Armada (SHOA).

Downwelling spectral radiation $E_d(\lambda)$ was measured each day around noon at different wavelengths of the visible light spectrum

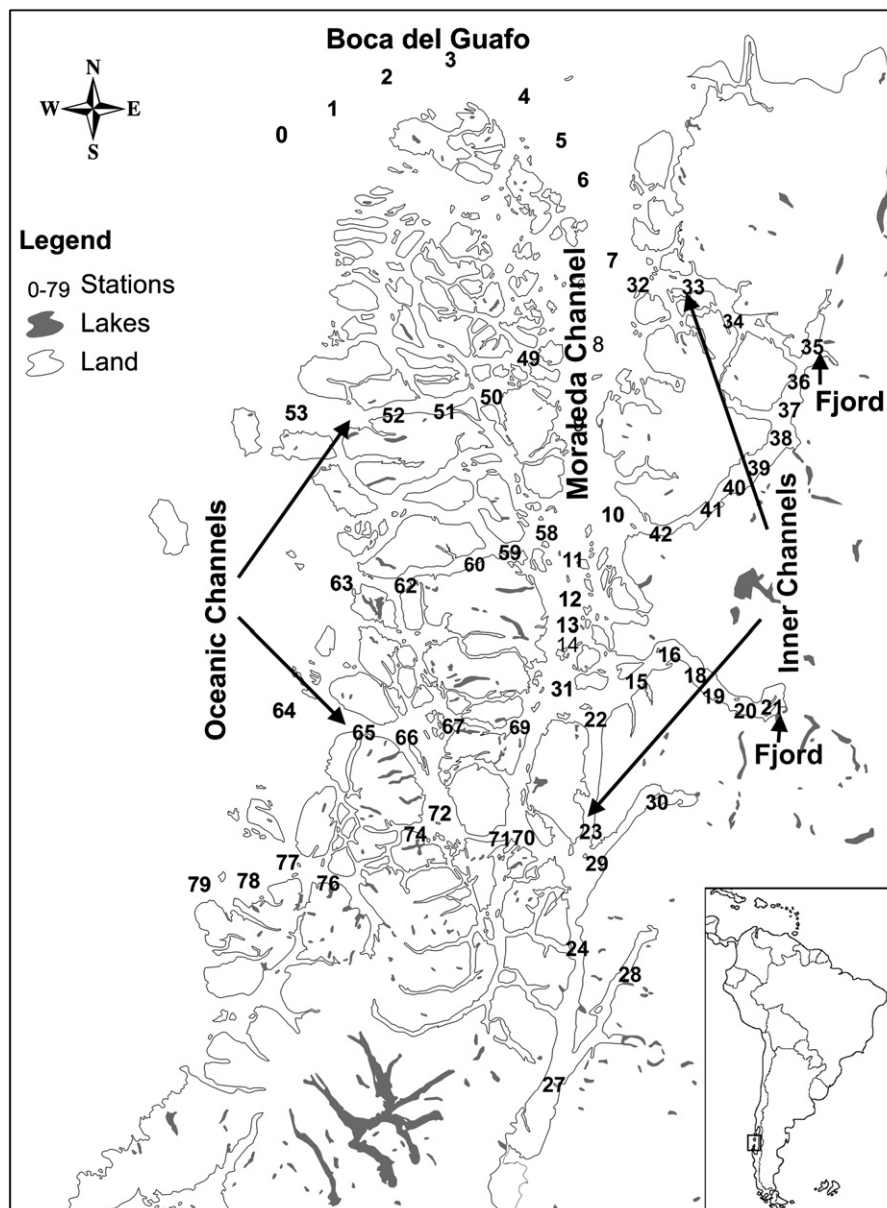


Fig. 1. Distribution of sampling stations (bold numbers) from all three CIMAR-Fiordos cruises showing the large spatial scale, different terrestrial influences, and geographical diversity of these stations. The different geographic groups are shown with arrows as examples for fjords, oceanic and inner channels, Moraleda Channel, and Boca del Guafo.

(411, 442, 489, 555 nm) using a submersible OCI, OCR-200 spectroradiometer (Satlantic®). The extinction coefficients $K_d(\lambda)$ were calculated for the most penetrating wavelengths (489 or 555 nm) (Kirk, 1994) in order to estimate the depth of the euphotic zone ($Z_{eu}(\lambda)$).

The upper mixed layer (I_m) was determined with the 0.01 psu m^{-1} (or 0.1 psu $10 m^{-1}$) differences because, in this region, homothermal water columns are not homogeneous in density due to abrupt changes in salinity caused by terrigenous fluvial input, precipitation, and glacial melting (Nelson Silva, pers. comm.). Also, in simple terms, the ratio between Z_m and $Z_{eu}(\lambda)$ is indicative of the amount of light received by phytoplankton in Z_m (Riley et al., 1949; Sverdrup, 1953). For a cell in the water column with a small $Z_{eu}(\lambda)$ compared to Z_m , light is limiting (high level of disturbance), whereas the opposite is true when Z_{eu} is large (but then nutrient resources would also represent a high level of disturbance). When the depths of Z_m and $Z_{eu}(\lambda)$ coincide, the level of disturbance for the cells is intermediate. Therefore, the value of $Z_m/Z_{eu}(\lambda)$ ranges from 1 if the depths match, to > 1 if the $Z_{eu}(\lambda)$ is less than Z_m , and < 1 if the $Z_{eu}(\lambda)$ is greater than Z_m .

2.2. Chlorophyll-*a* fractions and size diversity

Water samples were taken at three or five depths between the surface and 50 m by using Niskin bottles (3 L) arranged in a rosette and considering the thermal structure and the depth of $Z_{eu}(\lambda)$ at each station. Each discrete sample was prefiltered through a 200 μm nylon mesh, and three water subsamples (200–500 mL) were filtered separately using a manifold and a vacuum pump to obtain (i) the total Chl-*a* concentration (TChl-*a*) was only filtered through a 0.7 μm filter and (ii) the fractions < 20 and $< 11 \mu m$ were first sieved by gravity through the corresponding 20 or 11 μm nylon meshes on top of glass fiber filters (GF/F Whatman) with a 0.7 μm pore nominal size (Montecino and Quiroz, 2000). All the filters were stored immediately in liquid nitrogen until further laboratory analysis (Parsons et al., 1991; Jeffrey et al., 1997). Pigment determination was done after solvent extraction (90% acetone) and then quantified in a fluorometer (10 AU Turner Design). Calibration was done with standard chlorophyll-*a* (Sigma, USA). The Chl-*a* concentration in the large microphytoplankton fraction (20–200 μm) present in each sample was obtained by TChl-*a* minus $< 20 \mu m$. The Chl-*a* concentrations of the small phytoplankton fraction, including small nanophytoplankton and picophytoplankton, are the result of TChl-*a* minus $< 11 \mu m$. The arbitrary Chl-*a* concentrations for the intermediate fraction corresponding to large nanophytoplankton (11–20 μm) were calculated taking the difference of $< 20 \mu m$ minus $< 11 \mu m$. The statistical analysis for each sample was considered using the results of TChl-*a* concentrations and of the environmental factors for data of the same time, place, and depth.

H'_{size} was estimated using the Shannon–Wiener index (Spellerberg and Fedor, 2003), in which the TChl-*a* of each fraction was used as its abundances according to:

$$H'_{size} = - \sum p_i \ln p_i^S, \quad i = 1$$

where p_i is the Chl-*a* proportion of the size class i of the same sample and S (taking values = 1–3) is the total number of the size fractions obtained from the same sample.

2.3. Statistical analyses

Due to heterocedasticity, a non-parametric rank analysis Kruskal–Wallis test for multiple independent samples was computed for TChl-*a* concentrations between cruises (Sokal and Rohlf, 1981; Statistica, 1997) in order to initially characterize the temporal heterogeneity of the TChl-*a* and to be sure that we used

an appropriate time scale to test the hypothesis of unimodality between H'_{size} and TChl-*a*. This was necessary because we had observed homogeneity in mean chlorophyll concentrations or between measurements of the same station between seasons in another investigation within the same area (Montecino et al., 2008). The Pearson correlation was used to test the relationship between Chl-*a* of each size fraction and TChl-*a*. Further, we used the non-parametric rank analysis Kruskal–Wallis test to obtain the spatial variation in TChl-*a* between three different geographical areas: (1) the eastern fjords and inner channels, (2) Boca del Guafo and the large, northern Moraleda Channel, and (3) the western oceanic channels of the archipelago. These areas were separated according to continental influence, river runoff, and stratification (Silva et al., 1995; Guzmán and Silva, 2002). Furthermore, the Meninea Constriction (45°30'S) has been described as a transition between terrestrial and oceanic influences that prevents the passage of deep, cold, saltier waters into the channels and estuarine area (Silva et al., 1995, 1997).

As above, the temporal and horizontal spatial variability of H'_{size} was determined by applying the non-parametric rank analysis Kruskal–Wallis test. The relationship of H'_{size} versus TChl-*a* was analyzed separately for each cruise and then for all cruises together using the Pearson correlation coefficient after \log_{10} data standardization.

To test the hypothesis about the expected unimodal distribution, we used quadratic algorithms to estimate the peak of the relationship between H'_{size} with TChl-*a* and of both TChl-*a* and H'_{size} with the environmental factors, since this approach has been used with several important ecological matters that involve correlations between variables resulting in polygonal shapes (Li, 2002; Schröder et al., 2005). Moreover, this is appropriate when all factors that could be affecting the dependent variable are not measured (Koenker, 1978; Cade et al., 1999; Knight and Ackerly, 2002; Schröder et al., 2005), i.e. this would be the case for the effect of grazing on phytoplankton H'_{size} . Under these circumstances, highly dispersed data are obtained, generally with a lot of outliers that may seriously affect the results of regressions based on the ordinary least squares tests. Furthermore, the upper and lower boundaries of response variables for the scatter diagrams often change at different rates due to changes in the independent variables, whereas quantile regression analyses provide consistent estimates of the upper limits of the dependent variable (Scharf et al., 1998).

The maximum H'_{size} at intermediate TChl-*a* was tested for significance first separately for each cruise and then using the data from all three cruises through quantile regression analysis with a quadratic fit for the 10–95 percentiles (Scharf et al., 1998; Cade et al., 1999; Knight and Ackerly, 2002) with bootstrapping using the “quantreg” R library software (freely available at <http://www.R-project.org/>).

The correlation curves for these relationships obtained from the quadratic equation coefficients and the constants given for the quantile regression analysis were plotted over the data distributions. This analysis was also used to test the unimodal relationship hypothesis of H'_{size} and TChl-*a* with environmental factors, as obtained by the fit of the quadratic least squares (QLS). To reject a better fit of the data to monotonically increasing or decreasing lines, we analyzed the respective determination coefficients using the Line Fitting program with the Table Curve 2D.

3. Results

3.1. Total chlorophyll-*a* variability

The differences of the mean TChl-*a* values across all three cruises CF7–1, CF7–2, and CF8–2 were significant ($H=281.96$,

$df=2$, $N=357$, $p < 0.0001$). Mean TChl-a was higher in November 2002 than in November and July 2001 (Table 1), and the maximum TChl-a values increased by one order of magnitude between successive cruises and years (0.4 and 4.4 mg Chl-a m^{-3} in 2001 and 48.5 mg Chl-a m^{-3} in 2002). In terms of spatial variability, the mean TChl-a changed significantly between the three geographical areas ($H=165.01$, $df=2$, $N=357$, $p < 0.0001$; Table 1) and was highest in the oceanic channels and lowest at stations with more continental influence. However, the mean TChl-a was highest in spring 2002, both in the Moraleda Channel zone and in the oceanic channels.

3.2. Total and size-fractionated chlorophyll-a relationship

The relationship between the size-fractionated Chl-a and TChl-a showed that, during the CF7-1 winter cruise, the TChl-a was always < 1.0 mg m^{-3} and the small phytoplankton fraction ($< 11 \mu m$) was directly and significantly correlated with TChl-a (Fig. 2). When the TChl-a increased to > 1.0 mg m^{-3} , the large phytoplankton fraction ($> 20 \mu m$) was correlated with the TChl-a during both spring cruises, indicating that this size fraction is the main component of TChl-a (Fig. 2). The small phytoplankton fraction ($< 11 \mu m$) was dominant at stations with a stronger continental influence and the large phytoplankton fraction ($> 20 \mu m$) was dominant at oceanic stations (not shown).

3.3. Size diversity variability

In this study, the H'_{size} index ranged from 0 to 1.08 and maximum H'_{size} values were similar during the three cruises (1.06–1.08), with the lowest average H'_{size} found for the 2002 spring cruise (CF8-2) and significantly different from the 2001 winter and spring cruises ($H=30.74$, $df=2$, $N=302$, $p < 0.001$; Table 1). In space, H'_{size} differed significantly among the three geographical areas ($H=25.01$, $df=2$, $N=302$, $p < 0.001$; Table 1), being lowest in the oceanic channels.

3.4. Size diversity and total chlorophyll-a relationships

During the 2001 winter cruise (CF7-1), TChl-a was always very low and no correlation was found with H'_{size} . In the spring of the same year (CF7-2), no correlation was obtained even though H'_{size} tended to increase with greater TChl-a. On the contrary, in spring 2002 (CF8-2), when maximum TChl-a was highest, a significant linear negative relationship was found between H'_{size} and TChl-a (Fig. 3). Several fittings were used to test our hypothesis and non-linear adjustments were found. Once all the data were plotted together, the relationship between H'_{size} and TChl-a was significant and unimodal in all percentiles analyzed (Fig. 3; Table 2) when quantile regressions analysis was applied.

Table 1

Total Chl-a (mg Chl-a m^{-3}) and H'_{size} values separated in time for the 2001 and 2002 cruises and in space for three different geographical areas of the Aysén Region. Average, standard deviation, and number of samples (N) are indicated for the 2001 cruises (CF7-1, CF7-2) and the 2002 (CF8-2) cruise. For TChl-a, the Kruskal–Wallis test showed temporal significant differences among the three cruises ($p < 0.0001$) and spatial significant differences among fjords and channels with oceanic channel areas ($p < 0.0001$). For H'_{size} , the temporal and spatial differences were significant for three cruises ($p < 0.0001$).

		Total Chl-a			H'_{size}		
		Average	St. dev.	N	Average	St. dev.	N
Time	July 2001; C7-1	0.10	0.06	118	0.56	0.27	112
	November 2001; C7-2	0.63	0.67	140	0.59	0.25	109
	November 2002; C8-2	9.63	8.52	107	0.46	0.21	89
Space	Fjords and inner channels	0.42	0.66	158	0.55	0.25	134
	Boca del Guafo and Moraleda Channel	3.27	8.49	124	0.59	0.27	107
	Oceanic channels	7.93	5.22	83	0.45	0.19	69

3.5. Relationships between total chlorophyll-a and size diversity with abiotic factors

Fig. 4 shows the TChl-a unimodal relationship with the abiotic factors (specifically nitrate, orthophosphate, the $NO_3:PO_4$ ratio; Fig. 4a–c) and with the light proportion in the upper mixed layer

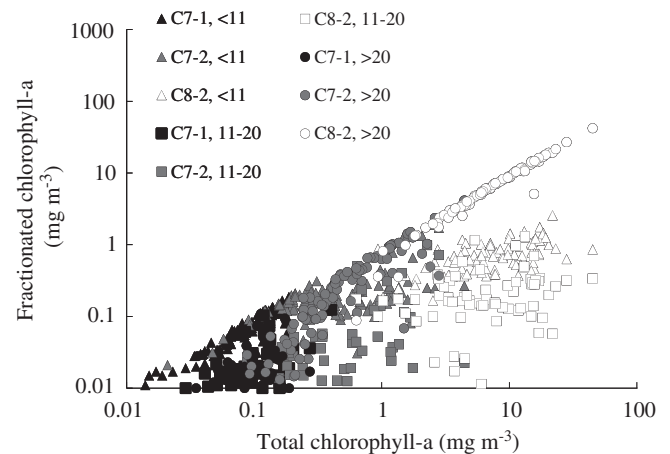


Fig. 2. Fractionated versus total chlorophyll-a (mg Chl-a m^{-3} , in log scales). Results of the Pearson correlations of the different size-fractions with TChl-a: $< 11 \mu m$ during CF7-1 cruise ($r^2=0.822$, $N=106$, $p < 0.0001$), $> 20 \mu m$ and TChl-a during CF7-2 cruise ($r^2=0.732$, $N=97$, $p < 0.0001$), and $> 20 \mu m$ during CF8-2 cruise ($r^2=0.865$, $N=88$, $p < 0.0001$).

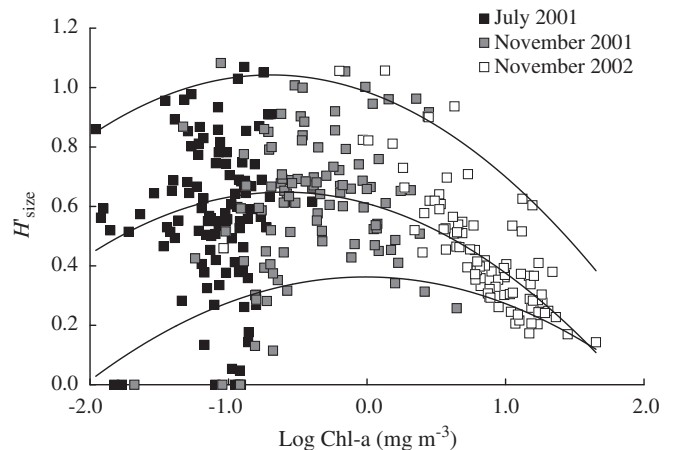


Fig. 3. Phytoplankton size diversity (H'_{size}) versus log total chlorophyll-a (mg Chl-a m^{-3}) relationships. July 2001 winter cruise (black squares), November 2001 spring cruise (grey squares) ($r^2=0.0324$, $N=107$, $p=0.740$), November 2002 spring cruise (white squares) ($r^2=0.819$, $N=88$, $p < 0.0001$). Plotted curves are for selected regression quantile estimates (0.95, 0.50, and 0.10).

Table 2

The significant p -values for the quadratic regressions (from quantile 0.95–0.1) for H'_{size} versus log TChl-a; TChl-a versus orthophosphate, nitrate, $\text{NO}_3^-:\text{PO}_4^-$, and $Z_m/Z_{eu}(\lambda)$, and for H'_{size} versus environmental factors: temperature (T) and silicic acid (Si). $p < 0.05$ is the lowest significance considered.

Quantile	p -values						
	$H'_{size}/\text{TChl-a}$	TChl-a/ PO_4^-	TChl-a/ NO_3^-	TChl-a/ $\text{NO}_3^-:\text{PO}_4^-$	TChl-a/ $Z_m/Z_{eu}(\lambda)$	H'_{size}/T°	$H'_{size}/\text{Si}(\text{OH})_4$
0.95	0.005	< 0.001	0.001	0.023	0.080	0.166	0.907
0.90	0.015	< 0.001	0.003	< 0.001	0.002	0.107	0.968
0.85	0.013	< 0.001	0.016	< 0.001	< 0.001	0.104	0.001
0.80	0.001	< 0.001	0.008	< 0.001	< 0.001	0.239	0.002
0.75	< 0.001	< 0.001	0.043	< 0.001	< 0.001	0.917	0.178
0.70	< 0.001	< 0.001	0.071	< 0.001	< 0.001	0.652	0.120
0.65	< 0.001	0.002	0.221	< 0.001	< 0.001	0.847	0.134
0.60	< 0.001	0.003	0.219	< 0.001	< 0.001	0.996	0.074
0.55	< 0.001	0.008	0.328	< 0.001	< 0.001	0.691	0.122
0.50	< 0.001	0.001	0.294	< 0.001	< 0.001	0.683	0.011
0.45	< 0.001	< 0.001	0.105	< 0.001	< 0.001	0.668	0.067
0.40	< 0.001	< 0.001	0.039	< 0.001	< 0.001	0.513	0.009
0.35	< 0.001	0.001	0.054	< 0.001	< 0.001	0.582	0.595
0.30	< 0.001	0.002	0.007	< 0.001	< 0.001	0.409	0.578
0.25	< 0.001	0.005	0.013	< 0.001	0.002	0.275	0.315
0.20	< 0.001	0.108	0.023	< 0.001	0.092	0.100	0.715
0.15	< 0.001	0.015	0.045	< 0.001	0.059	0.096	0.942
0.10	0.005	< 0.001	0.036	0.001	0.032	0.318	0.590

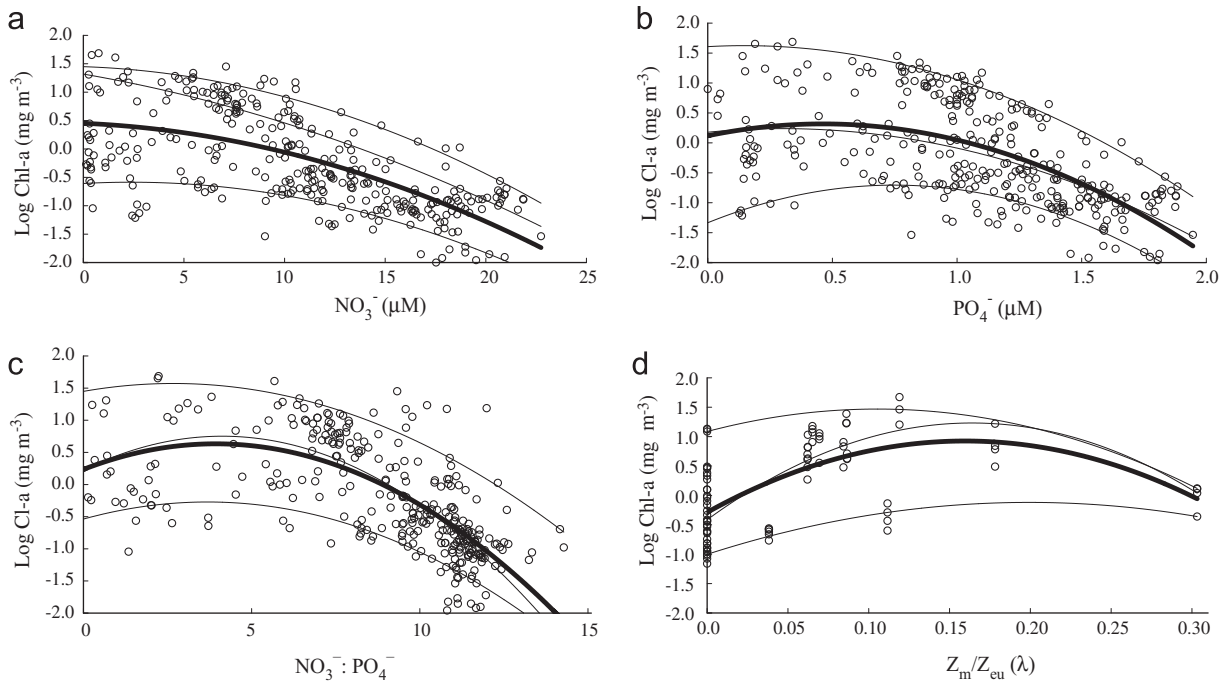


Fig. 4. Phytoplankton total chlorophyll-a (mg Chl-a m^{-3}) versus nutrient variability with the quadratic curves of the selected regression quantile estimates (0.95, 0.75, and 0.10) and the quadratic least squares fit (QLS) corresponding to 50% (solid line): (a) nitrate ($y = -0.0036x^2 - 0.0154x + 0.452$; $r^2 = 0.4338$), (b) orthophosphate ($y = -0.9265x^2 + 0.8569x + 0.1185$; $r^2 = 0.3407$), (c) $\text{NO}_3^-:\text{PO}_4^-$ ($y = -0.0257x^2 + 0.2024x + 0.2355$; $r^2 = 0.476$), and (d) $Z_m/Z_{eu}(\lambda)$ ($y = -47.641x^2 + 15.206x - 0.288$; $r^2 = 0.3585$).

(Fig. 4d). The maximum value of the Z_m/Z_{eu} ratio was < 1 because the euphotic layer was incidentally always larger than the mixed layer. Even so, TChl-a was greatest when this ratio, which ranged from 0 to 0.3, showed intermediate values (Fig. 4d).

The distribution of phytoplankton TChl-a (log scale) values was greatest at intermediate temperatures (around 11°C , range: $4.7\text{--}12.9^\circ\text{C}$). Values higher than 1.0 mg m^{-3} TChl-a were distributed from 9.7 to 12.5°C . Salinity indicated only low levels of TChl-a at minimum values (range: $2.05\text{--}33.35$ psu). TChl-a was $> 1.0 \text{ mg m}^{-3}$ at salinities from 14.24 to 33.35 psu (not shown).

An analysis of H'_{size} relationships with abiotic factors revealed a unimodal relationship with silicic acid in the 80–85 percentiles

(Table 2) and in the 85 and 95 percentiles for temperature (Fig. 5; Table 2).

4. Discussion

In pelagic, coastal upwelling and in estuarine austral ecosystems, a relationship between phytoplankton H'_{size} and TChl-a has strong implications for food webs, carbon transfer pathways, and energy flows. In environments dominated by small phytoplankton, the microbial web is dominant, whereas the classical trophic chain prevails in areas dominated by large phytoplankton

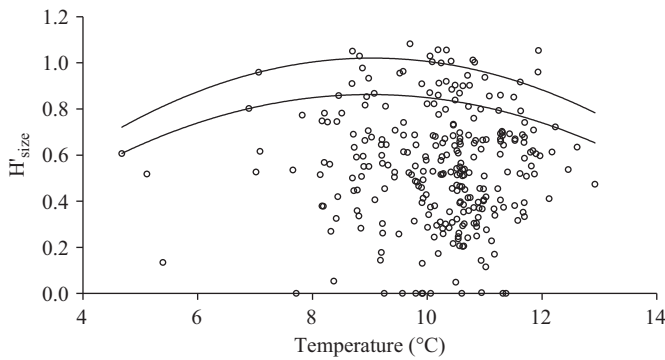


Fig. 5. Relationship of phytoplankton size diversity (H'_{size}) with temperature ($^{\circ}\text{C}$), showing the quadratic curves of the selected regression quantile estimates (0.95 and 0.85).

(Thingstad and Sakshaug, 1990; Lenz, 1992; Thingstad et al., 1997; Vargas et al., 2007; Pavés and González, 2008; González et al., 2010). TChl-a concentrations are commonly used for estimating the abundance of phytoplankton assemblages based on the 0.74 allometric coefficient between the Chl-a concentration of unicellular algal cells and their biomass (Niklas, 1994). Small cells have generally less pigment than large cells due to biophysical constraints (Li, 1997). In addition, conservative carbon-to-chlorophyll-a ratios have been reported for different taxonomic groups (Sathyendranath et al., 2009), each having its characteristic size range.

In the study area, although different taxa play very different roles, and their size ranges may overlap, the dominant size fraction was significantly correlated with the total phytoplankton: at low TChl-a values ($< 1.0 \text{ mg m}^{-3}$), the smaller phytoplankton fraction was dominant, whereas at $> 1.0 \text{ mg TChl-a m}^{-3}$, the microphytoplanktonic fraction was dominant. These results are consistent with different marine environments and further confirm that the abundance of TChl-a depends on the dominant size fraction (Malone, 1980; Agustí et al., 1987; Harris et al., 1987; Yentsch and Phinney, 1989; Chisholm, 1992; Iriarte et al., 1993, 2007; Sin et al., 2000; Irigoien et al., 2004). Moreover, the proportion of small cells diminishes with increments in the total phytoplankton biomass, although constant values are maintained throughout the year (González et al., 1989; Montecino and Quiroz, 2000; Marañón, 2009).

Due to the unusually low levels of TChl-a measured in the 2001 winter cruise and the extremely elevated concentrations of TChl-a in the 2002 spring cruise, we encountered low H'_{size} values in both periods. However, in winter 2001, the low mean H'_{size} values were higher than in spring 2002, probably due to a different representation of the abundance of the intermediate size class during the first cruise. Anyhow, in both cases, H'_{size} was lower on average than in spring 2001. Of the three geographic areas studied that of Boca del Guafo and Moraleda Channel had the highest mean H'_{size} , indicating that this intermediate zone was characterized by the coexistence of all three size components with their various and different competitive strategies. Distinct terrigenous influences could modulate in favor of small fraction cells (probably flagellates) adapted to stratified water columns with small $Z_{eu}(\lambda)$ or large diatoms thriving in oceanic areas with large $Z_{eu}(\lambda)$ and Z_m , including facultative mixotrophy of some large flagellates (Czypionka et al., this issue) or grazing. Stratified conditions, high irradiance, and enrichment processes all lead towards favorable conditions for the proliferation of intermediate-sized flagellates (Margalef, 1978; Smayda, 1997), and the development of harmful algal blooms (HABs). In this system the impact of HABs should also have an effect on H'_{size} .

In ecosystems, different processes operate on different (and possibly multiple) spatial and temporal scales to create or maintain variations in biota and/or habitat (Chapman et al., 2010). To our knowledge, no records exist for a relationship between H'_{size} with TChl-a or H'_{size} with environmental variables. Although reasonable hypotheses about important scales can be identified for many habitats based on previous studies and local knowledge, in studies involving sampling, the scale of observation must be known in advance and should not 'emerge' from the data being measured (Chapman et al., 2010). Thus, prior to evaluating the consequences of the processes investigated, it is necessary to determine: (i) the scale on which there is large variability and (ii) how common patterns are across space and time. In the particular case of patterns that reflect the phytoplankton abundances and diversity, we must consider that, although the temporal scales of the life spans of phytoplankton are on the order of hours to days (Reynolds, 1988), annual cycles regulate the successional changes through climatic and hydrological factors (Sommer et al., 1993).

In this study, the relationship between H'_{size} and TChl-a varied as a result of differences in Chl-a and in the dominant size fractions between the three cruises. However, when analyzing the whole data set, a unimodal distribution was obtained. Because TChl-a and H'_{size} variability depended on the changes in the environmental conditions (biotic and abiotic) and resources between different areas and seasons, we considered several spatial and temporal scales (in this case, more than three degrees in latitude and extreme seasons) to analyze the data in order to discover the underlying patterns. These patterns should not change when adding more data from other areas or times. It is also possible that a single cruise include all three orders of magnitude of Chl-a, depending on the intrinsic variability. Nevertheless, in this study, we were able to define the appropriate scale for this empirical hump-shaped distribution pattern, suggesting that the relationship between H'_{size} and TChl-a was determined for an interseasonal time scale, although other relationships exist on seasonal or intraseasonal scales, i.e. when the TChl-a reached high values, H'_{size} decreased linearly with Chl-a (spring 2002, C8-2, Fig. 3).

These results are consistent with data from the North Sea, where high phytoplankton abundances occurred with intermediate biomass (as measured by cell sizes and Chl-a content) and maximum diversity was found with intermediate phytoplankton densities (Li and Dickie, 2001; Li, 2002). This indicates that it is possible for the unimodal pattern to occur in a variety of marine systems including austral estuaries, fjords, and channels, but only when samples are collected across the widest possible range of environmental variability or on smaller scales when the ecosystem evidences large variability over time.

The IDH model of Connell (1978) proposes a hump-shaped relationship between species richness and the frequency, intensity, and time elapsed after an environmental disturbance. In ecology, it is common to test this relationship using quadratic curves describing unimodal relationships (i.e. Molies et al., 2003). Nonetheless, some relationships are not given exactly this way, since the mid-point between high and low disturbances is, of course, an assumption and the responses may be biased in some cases (Elliott et al., 2001). Although opinions differ as to the use of theories developed originally for terrestrial ecosystems (Wilson, 1994), these theories have been successfully adapted to phytoplankton succession (Reynolds, 1988) and other applications (i.e. Hewes et al., 2009). We believe that it is possible to use IDH because we can describe the intensity of the disturbance for the various phytoplankton size classes considering factors (such as Z_m) that alter the availability of light and nutrients, eventually causing the removal of phytoplankton. Moreover, it is not highly important to determine whether the source of the factors is

internal or external (Sommer et al., 1993). It is sometimes easy to get confused with the concept of disturbance, using it as an event that completely destroys life in certain places. However, Connell (1978) showed that the number of species remaining in coral populations following a hurricane over an eleven-year period depended on the intensity of the disturbance. Here, we used the Shannon–Wiener diversity index because it measures both the presence and absence of a class and the representation of each class, and size-fractionated Chl-a because diversity can be measured as everything that can be classified, including biomass and chlorophyll concentrations (Margalef, 1958).

On a macroecological scale, orthophosphate, nitrate, and the $\text{NO}_3^-:\text{PO}_4^-$ ratio were associated with TChl-a, also unimodal in shape, since the highest TChl-a values occurred at some intermediate level within these nutrient values (0.4–7.0 μM for nitrate, 0.2–0.8 μM for orthophosphate) and the $\text{NO}_3^-:\text{PO}_4^-$ ratio (2.5–5.0) during the three CIMAR-Fiordos cruises. Temporally, the causal relationship between TChl-a and nutrients is complex because the state of the phytoplankton assemblages is a consequence of the nutrient concentrations prior to sampling (Alves de Souza et al., 2008).

H'_{size} presented the highest values with intermediate levels of silicic acid (data not shown); these were not exactly core values for the gradients. Again, this is not inconsistent with the IDH (see earlier). The explanation could be that given high silicic acid availability through soil erosion into estuaries and coastal areas, we expected the typical spring diatom bloom (mostly from the larger size fraction), so that the sequence of events of a large diatom-dominated bloom from late spring/early summer to late summer would end in a flagellate-dominated bloom (intermediate-small fraction) as in temperate coastal waters (Margalef, 1978; Officer and Ryther, 1980). Furthermore, in most fjords areas, good indicators of terrigenous input include non-limiting silicic acid levels that vary little (Silva and Guzmán (2006); Vargas et al., this issue). Yet exceptions occur, such as in the Ballena Fjord in Patagonia located much further south, where insufficient silicic acid inputs into the fjord prevent diatom proliferations. Consequently, other types of phytoplankton blooms (e.g., small dinoflagellates) appear in these nitrate-enriched waters (Torres et al., this issue).

The effects of some controlling factors were not measured in this study. These include zooplankton grazing pressure (Calbet and Landry, 2004), which, according to González et al. (2010), changes between spring and summer in austral Chile. Therefore, the interpretation of the curves should focus on the upper percentiles of the response variable, which denote the restrictions imposed by the evaluated controlling variable (Cade et al., 1999). In this case, the higher size diversity occurred at intermediate silicic acid levels, but close to the lowest values, probably because at that time there was higher grazing pressure on the largest fraction.

Among the abiotic factors, temperature was significantly associated with phytoplankton H'_{size} , showing the highest values at intermediate temperatures (8–9 °C). Nevertheless, the statistically significant relationship was unimodal only for the 95 and 85 percentiles of the data set. This could be explained by the fact that below the 85 percentile, H'_{size} is not controlled by temperature alone.

Stratification strongly affects the fields of light and nutrients in ocean surface waters.

In the North Sea, the maximum phytoplankton size diversity was reported at intermediate levels of water column stratification (Li, 2002). Here, most of sampling stations showed highly stratified water columns and only the oceanic stations were well mixed. However, spatially, locations with greater depths of Z_m did not present the highest TChl-a, and those without upper mixed

layers reached only low concentrations of TChl-a with low H'_{size} . Intermediate values of Z_m originated the highest values of TChl-a and H'_{size} (data not shown). Temporally, the sites with low Z_m in winter 2001 were mostly in areas with a major continental influence and low TChl-a, presenting low H'_{size} (< 0.6 on average). Those places with deep Z_m (Moraleda and oceanic channels) and high TChl-a also had low H'_{size} . This supports the unimodal distributions of Z_m with TChl-a and H'_{size} , with maximum values at intermediate depths of Z_m .

Although light is limiting and shows significant seasonal changes at these latitudes, its biological effectiveness is generally controlled by upper ocean stability (Gargett and Marra, 2002). Therefore, the $Z_m/Z_{\text{eu}}(\lambda)$ relationship determines and is indicative of the amount of light available in the mixing zone and, for this reason, it is a measure of the variability in environmental disturbances between different places. Because many coastal stations do not have an upper mixing zone, the $Z_m/Z_{\text{eu}}(\lambda)$ index was only positive for the oceanic stations, inner channels, and Moraleda Channel. The latter also presented deeper euphotic and mixed zones, both favorable conditions for the proliferation of large diatoms with high biomasses and, therefore, low H'_{size} . However, the attenuation coefficient of light depends on the absorption of particulate matter (dead and alive) and also of colored dissolved organic matter (CDOM) (Kirk, 1994; Retamal et al., 2007, 2008). At stations with major terrestrial and freshwater influences and in winter, the absorption of radiation is given mainly by CDOM, unlike at oceanic stations and those sampled in spring (Pizarro et al., 2005; Montecino et al., 2006). This would explain why the shallow depth of $Z_{\text{eu}}(\lambda)$ coupled to the shallow high stratification resulted in low phytoplankton TChl-a and a competitive disadvantage for the large size classes. In contrast, the latter conditions were optimal for the predominance of the smaller classes (i.e. phytoflagellates) and, therefore, resulted in low H'_{size} values.

5. Conclusions

1. The H'_{size} index expressed the phytoplankton structure and revealed a robust phytoplankton pattern on the scale of austral fjords and channels.
2. In the austral fjords and channels of Aysén, the dominant cell size fraction (size class) was related to TChl-a. Thus, smaller cells in winter or at stations with continental influence were predominant at low TChl-a (< 1.0 mg m^{-3}), whereas the larger microphytoplankton fraction was dominant at TChl-a > 1.0 mg m^{-3} in spring and at the more oceanic stations and oceanic channels. Subsequently, the size composition of phytoplankton changed both in time and space in relation to TChl-a.
3. In the austral fjords and channels of Aysén, the relationship between H'_{size} and TChl-a changed seasonally as a result of differences in the Chl-a concentrations and the dominant size fractions between cruises. However, this generated a unimodal distribution only on large spatial and temporal scales given ample ranges of environmental factors.
4. H'_{size} and TChl-a were related to low–high disturbance levels: high TChl-a and larger size classes predominated in deeper Z_m columns, but with higher light availability, whereas water columns with shallow Z_m and low light availability presented lower TChl-a and predominantly smaller size classes. The patterns obtained in these austral fjords and channels of Chile were related to the high variability of Chl-a, and they responded to the intrinsic variability of the system on different scales of observation. Lastly, large temporal/spatial scales covering the entire range of H'_{size} and TChl-a variability

allowed unimodal expressions of phytoplankton patterns according to the IDH model in this system.

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