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Bathymetric, latitudinal and vertical distribution of protozooplankton in a cold-temperate shelf (southern Patagonian waters) during winter

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Although there have been many ecological field studies on the key components of planktonic food webs, there are still gaps in knowledge on some groups, environments and seasons. This is the first report on the spatial distribution of the density and biomass of almost all the taxonomic groups and size fractions of protozooplankton across a cold-temperate shelf during winter. Twenty-eight stations (two or three depths) were sampled on four cross-shelf transects in Patagonian waters (south-western Atlantic; 47–55°S, 60–69°W) during September 2006. Loricate ciliates, radiolarians and foraminiferans showed the lowest densities, and were distributed mainly in coastal, slope or the whole shelf waters, respectively. The density and biomass of aloricate ciliates and heterotrophic nanoflagellates and dinoflagellates were low and homogeneous both vertically and across the shelf south of 51°S, but peaked in the upper 40 m in offshore waters at 47°S. Microplanktonic aloricate ciliates, which represented 53% of the total protozooplankton biomass, reached values as high as 16 μg C L^{-1} on the last transect. Consequently, both protozooplankton biomass and its ratio to chlorophyll *a* concentration were significantly higher in the northern offshore waters. These trends were linked to higher subsurface temperature and chlorophyll *a* concentration, and lower copepod nauplii biomass. Our results probably reflect changes in both the availability of food resources and predators and the physical structure of the water column, which are a consequence of the different environmental conditions that coexist over the large latitudinal and longitudinal gradients covered during late winter.

KEYWORDS: protozooplankton; cross-shelf distribution; latitudinal gradient; winter; southern Patagonian waters

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INTRODUCTION

Protozoa are crucial in marine ecosystems due to their functions both in planktonic food webs and in biogeochemical cycles (Sherr and Sherr, 2002). Nanoflagellates, ciliates and dinoflagellates generally reach high levels of abundance and biomass, whereas sarcodines are more relevant from the biogeographical, paleoclimatic and biogeochemical points of view. Protozoa show different modes of nutrition, which vary from strict heterotrophy to mixotrophy, and even obligate phototrophy (the ciliate *Myrionecta rubra*). They are major grazers of a wide range of producers, including from bacteria to large diatoms (Sherr and Sherr, 1994, 2007). In addition, they are a significant food resource for larval and adult stages of copepods, other crustaceans and ichthyoplankton (Calbet and Saiz, 2005; Montagnes *et al.*, 2010a), thus linking the flux of carbon between the lowest and the highest trophic levels in the plankton.

Ecological field studies in temperate regions that have included the main groups of protozoa have generally focused either on shallow coastal waters (e.g. Neuer and Cowles, 1994; Putland, 2000) or on oceanic waters (e.g. Stelfox-Widdicombe *et al.*, 2000; Safi *et al.*, 2007). However, the fluctuations of the structure, density and biomass of protozooplankton across the bathymetric and productivity gradients that exist between these environments are insufficiently known. Since these organisms are the main consumers of phytoplankton in shelf waters (Verity *et al.*, 2002; Paterson *et al.*, 2008), protozooplankton abundance has been investigated mainly during the periods of high chlorophyll concentration, when both parameters decrease offshore (Verity *et al.*, 1996; Fileman *et al.*, 2002; Strom *et al.*, 2007). In contrast, only nanoflagellates have been studied on a cross-shelf gradient during the winter (Cuevas *et al.*, 2004; Granda and Anadón Álvarez, 2008), although ciliates and dinoflagellates also play a relevant role as components of the microbial food web which dominates during that season (Legendre and Rassoulzadegan, 1996). As shelf-slope ecosystems have high levels of secondary production, including most global fish catch, it is important to know the distribution of protozooplankton, since it supports higher consumers during the periods of low primary production. In addition, winter protozooplankton stocks have a fast response to fluctuations in food availability and temperature during the transition to spring, thus influencing the development and the fate of phytoplankton blooms (Strom, 2002; Irigoien *et al.*, 2005).

The aim of this study was to elucidate if the density, biomass and community structure of protozooplankton change across a cold-temperate shelf during late winter, and to assess their relationships with temperature, potential food sources (in terms of chlorophyll *a* concentration) and predators (copepod nauplii). To this end, we studied the southern Patagonian waters (Argentine shelf), which cover a large range both in longitude and in latitude (Fig. 1A), represent one of the most productive ecosystems in the World Ocean (Behrenfeld and Falkowski, 1997), and sustain important fisheries for demersal species and

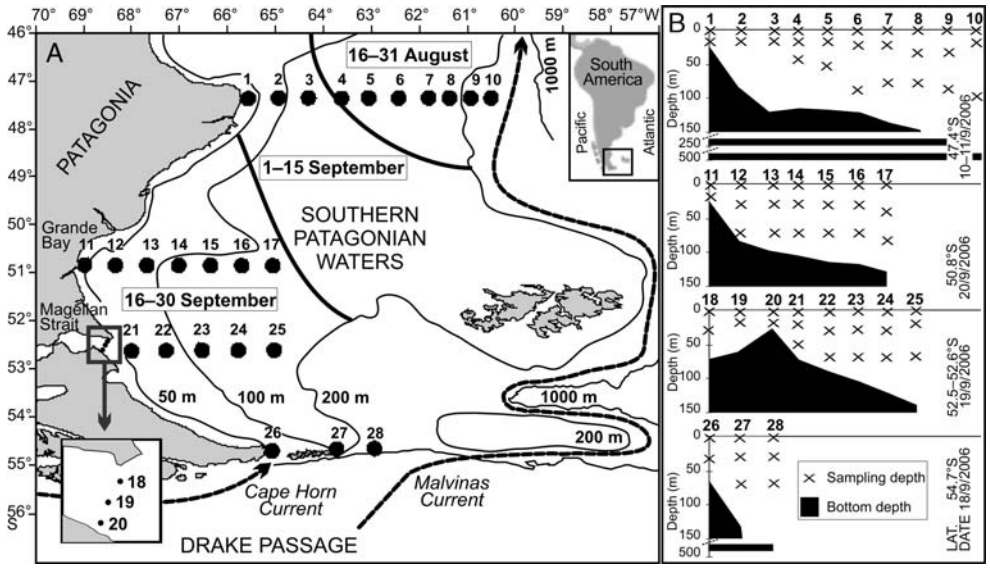

Fig. 1. Geographical location (**A**) and vertical profile (**B**) of stations sampled in southern Patagonian waters. In A, thin black lines correspond to isobaths (coastal strip, middle-outer shelf and slope: <50, 50–200 and >200 m, respectively); thick black lines delimit sectors where the beginning of the winter-to-spring sea surface warming occurs at different dates, based on satellite data on heat flux in the air-sea interface obtained between 1989 and 2000 (modified from Rivas *et al.*, 2006); arrows indicate the circulation of the main currents (modified from Piola and Rivas, 1997).

squid (Sánchez and Bezzi, 2004). Owing to the strong seasonality of this region, chlorophyll *a* levels are low in winter (<1 μg L^{-1}) and high during the rest of the year ($1-10$ μg L^{-1}) according to satellite-derived data (Romero *et al.*, 2006). The seasonal increase in chlorophyll *a* concentration begins in the northern offshore waters in late winter, and then propagates toward the SW, reaching the southern inshore waters in spring (Rivas *et al.*, 2006). This trend is a consequence of both the circulation of the nutrient-enriched Malvinas Current on the slope and the direction of propagation of the sea surface warming (Rivas *et al.*, 2006).

In this context, our hypothesis was that protozooplankton density and biomass reflect differences in chlorophyll *a* levels across and along the shelf during late winter, due to the availability of both food resources for protozoa and alternative prey for their potential predators. In addition, we predicted higher ratios of protozooplankton biomass to chlorophyll *a* concentration in northern offshore waters, which may indicate changes in the structure of the planktonic food web linked to the beginning of the warm season.

METHOD

Samples were collected in southern Patagonian waters during late austral winter (September 2006) on board the R.V. "Puerto Deseado" (cruise Patagonia III). Twenty-eight stations were sampled on four transects, which covered a bathymetric gradient from the coast to either the middle-outer shelf (at ca. 51°S and ca. 53°S) or the slope (at ca. 47°S and ca. 55°S). The comparison between the dates of our sampling (Fig. 1B) and the dates of the beginning of the sea surface warming (Fig. 1A) suggests that this process was just beginning at most of the stations (St. 11–28). In contrast, the sea surface warming had begun 25 and 10 days before our sampling in the NE and the NW sectors (St. 4–10 and St. 1–3), respectively.

At each station, two or three depths were sampled according to bottom depth (total: 76 points). The subsurface level (3 m) and the intermediate and deep levels (generally 20–30 and 70–80 m) were sampled with a centrifugal pump and Niskin bottles, respectively. Subsurface water temperature was measured continuously by two thermosalinometers (SBE 1521 and 37SI), which were calibrated with CTD measurements. To estimate chlorophyll *a* concentration, 2–4 L of seawater was filtered through GF/F filters, which were kept frozen until spectrophotometric quantification (Jeffrey and Humphrey, 1975). To investigate protozoan groups with hard structures (loricate ciliates, foraminiferans, radiolarians) and copepod nauplii, 4–20 L (generally 10 L) of seawater was concentrated through a sieve (12 μm pore size) and preserved with neutralized formaldehyde (2% final). To study aloricate ciliates and dinoflagellates, 0.6–1 L of water was preserved with Bouin's Solution (5% final), and then concentrated by settling. Samples were analyzed under an inverted microscope (Utermöhl, 1958) and cells were classified according to their morphology and size fraction (nanoplankton and microplankton). In two or three subsamples, at least 100 cells (for aloricate ciliates and dinoflagellates) or all the specimens found (for the other groups) were counted to estimate density.

To analyze the trophic mode of dinoflagellates and to quantify heterotrophic nanoflagellates, 20 mL of seawater preserved with glutaraldehyde (0.5% final) was stained with DAPI (5 μg mL^{-1}) and concentrated on black polycarbonate filters of 0.8 μm pore size (Porter and Feig, 1980). The filters were mounted on slides and kept frozen in darkness, until examination under an upright fluorescence microscope. To investigate which of the morphological types of dinoflagellates were heterotrophic, the entire filter surface was examined. In addition, to estimate the density of heterotrophic nanoflagellates, 60 optical fields ($\times 1000$) per filter were photographed and examined using an image analyzer. The cells of both groups of organisms were considered as heterotrophic when chloroplasts with red/orange autofluorescence were not detected under blue excitation (Cuevas *et al.*, 2009). The proportion of mixotrophic protists was not investigated, since its precise estimation requires experimental procedures such as incubation with fluorescently labelled prey.

During cell counting, at least 75 specimens per sample were measured to estimate their biovolume and biomass. Reported conversion factors were applied for nanoflagellates and dinoflagellates (Mender-Deuer and Lessard, 2000), aloricate ciliates (Putt and Stoecker, 1989), loricate ciliates (Verity and Langdon, 1984), foraminiferans and radiolarians (Beers and Stewart, 1970) and copepod nauplii (Uye *et al.*, 1996). Total protozooplankton biomass included heterotrophic nanoflagellates and dinoflagellates, ciliates (except for *M. rubra*, which was considered separately) and sarcodines.

Nonparametric statistical tests were applied to compare biological data between two groups of stations defined according to the earlier or later start of sea surface warming (St. 4–10 and St. 11–28, respectively; Fig. 1). The intermediate condition was not considered due to the low number of stations sampled (St. 1–3). Protozooplankton biomass, chlorophyll *a* concentration, copepod nauplii biomass and ratios between these variables were contrasted between both groups of stations (Mann–Whitney's test). In addition, the first three

variables were compared between the three depth levels sampled (Friedman's test). Spearman's correlations were carried out considering all the samples and variables studied.

RESULTS

Density and biomass of protozooplankton groups

The density distribution of protozooplankton groups in the vertical, bathymetric and latitudinal gradients is shown in Fig. 2. Nanoflagellates were the most numerous organisms, with a maximum value (2×10^5 cells L^{-1}, St. 9) one order of magnitude higher than aloricate ciliates and dinoflagellates (1×10^4 cells L^{-1} for both groups, St. 4 and 6, respectively) (Fig. 2A–C). These three groups showed relatively low and homogeneous densities both in the water column and across the shelf at 51°S, 53°S and 55°S. In contrast, on the northernmost transect (47°S), their densities showed three trends: mean values were from two (nanoflagellates and aloricate ciliates) to six (dinoflagellates) times higher than on the other transects, higher values were found in the upper 40 m of the water column than at the deepest level, and there was an increase from inshore to offshore waters.

Loricate ciliates (Fig. 2D) reached their highest densities (50–400 cells L^{-1}) in the coastal zone (St. 1, 11 and 18–20) and in the subsurface and intermediate

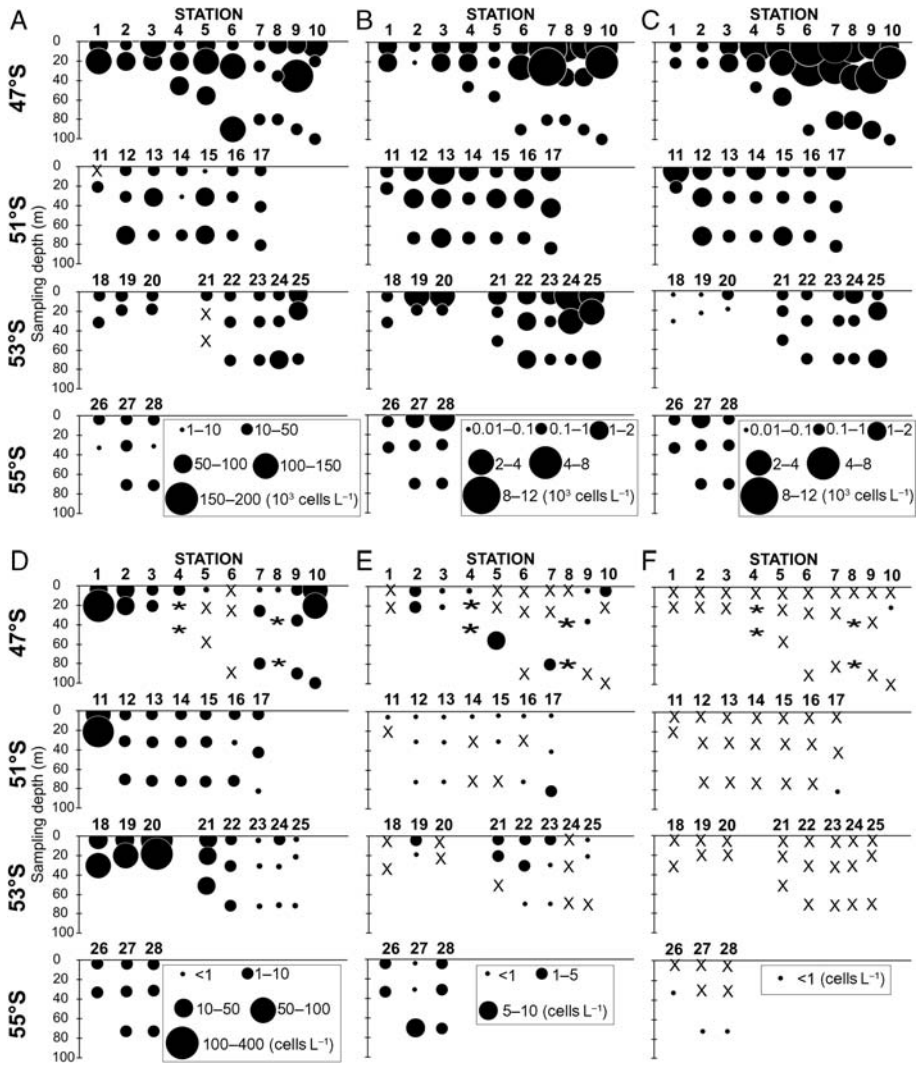

Fig. 2. Vertical distribution of the density of protozooplankton groups (**A**, heterotrophic nanoflagellates; **B**, aloricate ciliates, except *M. rubra*; **C**, heterotrophic dinoflagellates; **D**, loricate ciliates; **E**, foraminiferans; and **F**, radiolarians) from inshore to offshore waters on each transect. Cross, not detected; asterisk, no data.

levels in the northern slope (St. 10). In middle-outer shelf waters, their densities ranged from moderate (1–10 cells L^{-1}, at 51°S and 55°S) to low (<1cell L^{-1}, at 53°S) or not detectable (e.g. St. 6, 47°S). The distribution of foraminiferans (Fig. 2E) and radiolarians (Fig. 2F) overlapped in the northern and southern slope (St. 10 and 28), in the southernmost part of the shelf (55°S, St. 26–27) and at 51°S (St. 17), and both groups were more abundant in the intermediate and deep levels. Foraminiferans showed values ca. 10 times higher than radiolarians, considering both their occurrence (62 versus 7% of points sampled) and their maximum density (10 versus <1cell L^{-1}).

For each protozooplankton group, the vertical and horizontal distribution of biomass paralleled the trends in abundance, as indicated by the positive and highly significant relationship found between these variables (Spearman's correlation, $P < 0.05$; Table I). Aloricate ciliates had a relatively low correlation coefficient due to the high variability of their cell biovolume (from 3×10^1 to 4×10^5 μm^3), which was reflected in some points of low density (<2000 cells L^{-1}) but high biomass, for example, in the intermediate depth level at St. 4 (15.8 μg C L^{-1}) and St. 12 (5.6 μg C L^{-1}). The relationship for total protozooplankton was also low, since density and biomass were dominated by nanoflagellates (88%) and aloricate ciliates (61%), respectively.

As regards the mean protozooplankton biomass in the water column, microplanktonic aloricate ciliates dominated in 86% of the stations examined (Fig. 3). This group showed a mean contribution of 53%, with values up to 85% and 92% (St. 4 and 19, respectively). Nanoplanktonic and microplanktonic dinoflagellates were most abundant between St. 5 and 10 (mean = 17%) and in coastal waters (St. 1 and 11; mean = 40%), respectively. Nanoflagellates and nanoplanktonic aloricate ciliates showed more uniform contributions in all the stations (mean = 8% and 10%, respectively), whereas loricate ciliates were only significantly abundant (20–30%) at St. 2, 10 and 11. The relative biomass of foraminiferans and radiolarians was insignificant (<0.5%) at all stations.

Protozooplankton biomass in relation to temperature, chlorophyll *a* and copepod nauplii

The mean biomass of protozooplankton in the water column showed maximum values (>5 μg C L^{-1}) at 47°S, from the middle of the shelf to the slope (St. 4, 6, 7 and 10). Intermediate averages were found at St. 3 (47°S) and St. 12 (51°S), whereas relatively low values (<2.5 μg C L^{-1}) were estimated at all the other stations (Fig. 4A). The biomass of *M. rubra*, mainly represented by the microplanktonic size fraction (91%), showed averages <0.5 μg C L^{-1} at most stations, and reached a maximum value (51°S, St. 13) one order of magnitude lower than other protozooplankton (Fig. 4B).

Chlorophyll *a* concentration varied between 0.12 μg L^{-1} (St. 9 and 10, deep level) and 2.23 μg L^{-1} (St. 4, intermediate level) when considering all the points sampled. The mean chlorophyll *a* concentration

*Table I: Correlations (Spearman's test) between the density (*D*) and biomass (*B*) of protozooplankton groups and copepod nauplii, and between such variables and bottom depth, temperature (Temp), chlorophyll* a *(Chl* a*) and copepod nauplii (Naup)*

	Between *D* and *B*		Depth	Temp	Chl *a*	Naup
Heterotrophic nanoflagellates	**0.78**	*D*	**0.30**	**0.44**	0.11	**−0.30**
		B	**0.29**	**0.43**	0.14	**−0.37**
Aloricate ciliates[a]	**0.68**	*D*	**0.25**	0.09	**0.26**	**−0.31**
		B	0.18	0.11	**0.30**	**−0.28**
Loricate ciliates	**0.95**	*D*	**−0.56**	**−0.50**	**0.36**	**0.27**
		B	**−0.54**	**−0.44**	**0.34**	**0.39**
Heterotrophic dinoflagellates	**0.89**	*D*	**0.47**	**0.61**	**0.23**	**−0.41**
		B	**0.38**	**0.63**	**0.37**	**−0.40**
Foraminiferans	**0.94**	*D*	0.00	−0.23	−0.15	0.19
		B	−0.07	−0.35	−0.06	0.19
Radiolarians	**1.00**	*D*	0.19	−0.20	−0.09	0.18
		B	0.20	−0.30	−0.08	0.10
Total protozooplankton	**0.32**	*D*	**0.33**	**0.51**	0.15	**−0.36**
		B	0.20	0.35	**0.47**	**−0.41**
Nauplii	**0.84**	*D*	−0.15	**−0.42**	−0.22	—
		B	**−0.27**	**−0.63**	−0.20	—

All the points sampled were considered ($n = 76$), except for temperature (only 3 m; $n = 28$).
Significant results ($P < 0.05$) are shown in bold.
[a]Exclude *M. rubra*.

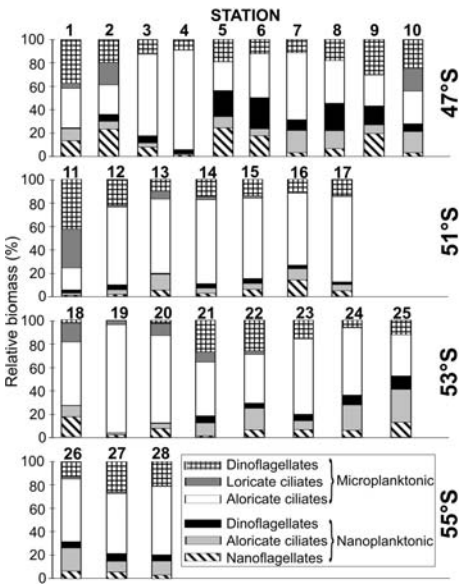

Fig. 3. Contribution of taxonomic groups and size fractions to mean protozooplankton biomass at each station.

in the water column was highest (>1 μg L^{-1}) at 47°S (St. 1, 4, 5 and 10) (Fig. 4C). At 51°S and 53°S, chlorophyll *a* values were intermediate inshore of the 100 m isobath, and lowest (<0.5 μg L^{-1}) at offshore stations, as well as on the southernmost transect (55°S).

The density and biomass of copepod nauplii fluctuated from undetectable levels (St. 6) to maximum values of 14 ind. L^{-1} and 1.3 μg C L^{-1} on the northern slope (St. 10, deep level). These parameters were significantly and positively correlated (Spearman's correlation, $P < 0.05$; Table I). Mean copepod nauplii biomass was very low at most of the stations at 47°S (<0.1 μg C L^{-1}), although it was slightly higher at St. 1 and 10, as well as between 51°S and 55°S (0.1–0.5 μg C L^{-1}) (Fig. 4D).

Mean values of biological variables showed significant differences between St. 4–10 and St. 11–28 (Mann–Whitney's test, $P < 0.05$; Fig. 5A); protozooplankton biomass and chlorophyll *a* concentration were 3- and 2-fold lower, respectively, at St. 11–28, while copepod nauplii biomass was 2-fold higher.

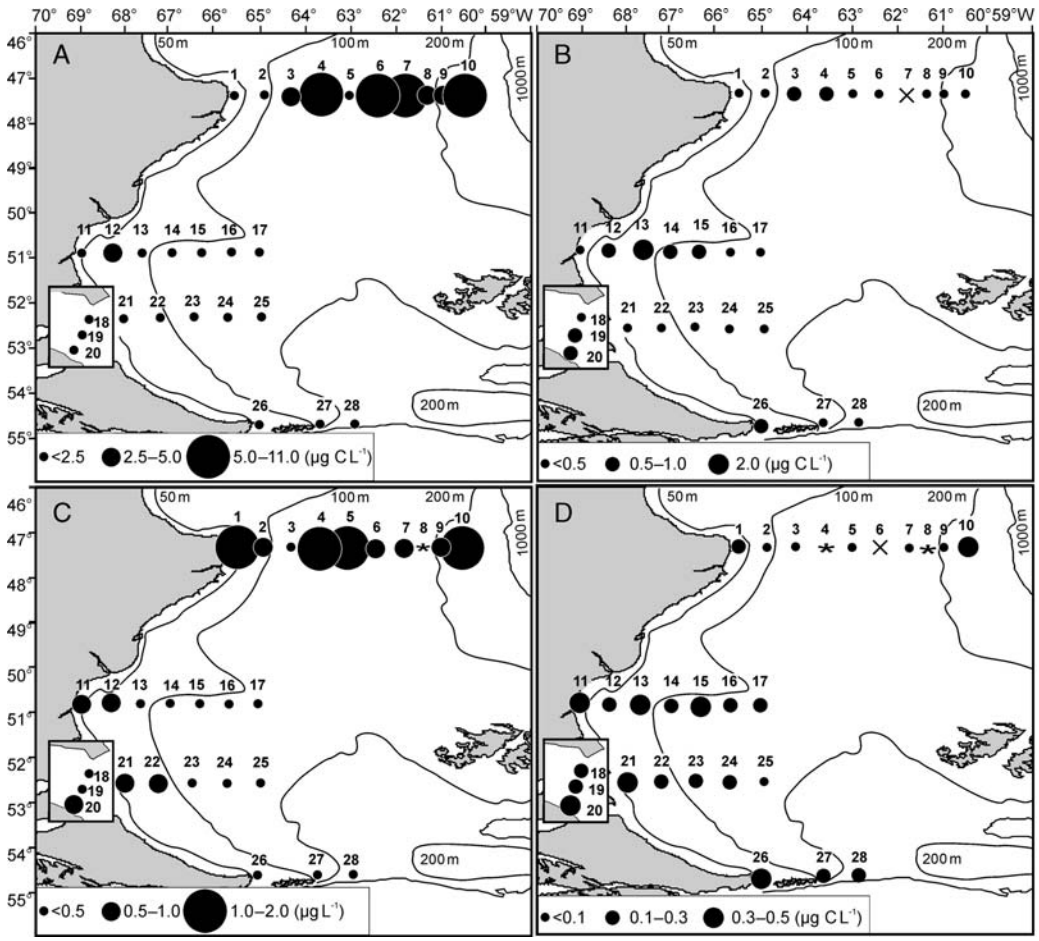

Fig. 4. Distribution of total protozooplankton biomass (**A**), *M. rubra* biomass (**B**), chlorophyll *a* concentration (**C**) and copepod nauplii biomass (**D**) from inshore to offshore waters on each transect (mean values in the water column). Cross, not detected; asterisk, no data.

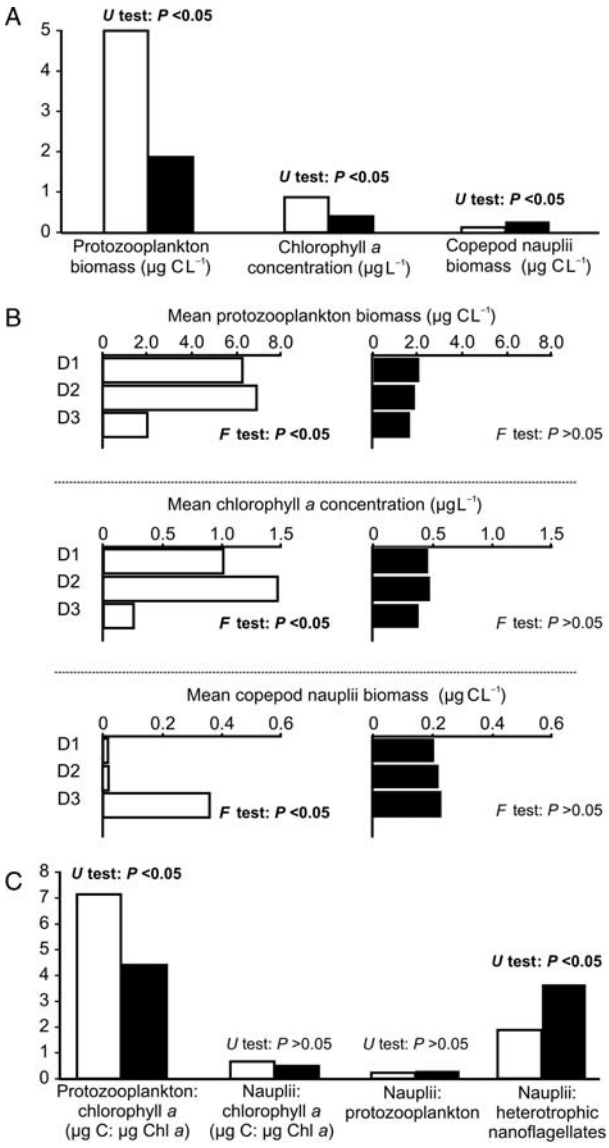

Fig. 5. Comparison of protozooplankton biomass, chlorophyll *a* concentration and copepod nauplii biomass between the stations 4–10 (white bars) and 11–28 (black bars). Mean values (**A**), vertical distribution (**B**) and ratios (**C**). In C, the ratio of nauplii biomass to heterotrophic nanoflagellate biomass was also included. In A and C, results of the Mann–Whitney's test (*U*) between St. 4–10 and St. 11–28 are shown. In B, results of the Friedman's test (*F*) between depth levels (subsurface, intermediate and deep = D1, D2 and D3, respectively) both at St. 4–10 and St. 11–28 are shown. Total number of samples = 21 (St. 4–10) and 49 (St. 11–28).

Between depth levels, significant differences were found only at St. 4–10 (Friedman's test, $P < 0.05$; Fig. 5B), where protozooplankton biomass and chlorophyll *a* concentration were 3- to 5-fold higher in the subsurface and the intermediate levels, while copepod nauplii biomass was one order of magnitude higher in the deep level.

The ratio of protozooplankton biomass to chlorophyll *a* concentration varied from 1 (St. 18) to 15 µg C: µg Chl *a* (St. 7). Its mean value was significantly (ca. 2-fold) higher at St. 4–10 than at St. 11–28 (Mann–Whitney's test, $P < 0.05$; Fig. 5C). The ratio of nauplii biomass was lower than 1 in relation to both chlorophyll *a* concentration and total protozooplankton biomass. In contrast, the ratio of nauplii biomass to nanoflagellate biomass was as high as 50 (St. 21), and its mean value was significantly higher at St. 11–28 than at St. 4–11 (Mann–Whitney's test, $P < 0.05$; Fig. 5C).

The density and biomass of almost all protozooplankton groups correlated significantly and positively with bottom depth, temperature and chlorophyll *a* concentration, but negatively with copepod nauplii density and biomass (Spearman's test, $P < 0.05$; Table I). These trends were only disrupted by non-significant correlations between nanoflagellates and chlorophyll *a*, and between aloricate ciliates and either temperature or bottom depth. Total protozooplankton followed the tendencies of nanoflagellates and aloricate ciliates in terms of density and biomass, respectively. Loricate ciliates and copepod nauplii covaried positively with each other, and negatively with bottom depth and temperature. Neither foraminiferans nor radiolarians showed significant correlations with any of the variables analyzed.

DISCUSSION

General trends of protozooplankton groups

Field studies on community structure, density and biomass of planktonic protozoa across temperate shelves are scarce at regional and global scales, and have generally focused only on some of the most abundant groups during spring and summer (Table II). To our knowledge, this is the first study on the spatial distribution of protozooplankton, including almost all groups, in the water column from a cold-temperate shelf during the winter.

Although sampling with a pump could have led to a slight underestimation of fragile planktonic cells in subsurface waters, we do not expect an influence on the main trends found in this study, since this kind of sampler provides comparable estimations to that obtained with bottles (Sutor, 2004).

Maximum magnitudes of density and biomass reached by aloricate ciliates and heterotrophic nanoflagellates and dinoflagellates in southern Patagonian waters during late winter were comparable to those found in other shelves during spring and summer (Table II). However, one order of magnitude higher densities have been reported for nanoflagellates (Cuevas

Table II: Ecological studies on protozooplankton across temperate shelves, including ranges of density and biomass of heterotrophic nanoflagellates (NF), heterotrophic dinoflagellates (D), aloricate ciliates (AC) and loricate ciliates (LC)

		Density (cells L^{-1})				Biomass (μg C L^{-1})				
Region	Season	NF (10^5)	D (10^3)	AC[c]	LC	NF	D	AC[c]	LC	Reference
Gulf of Alaska (58–60°N, 148–150°W)	Spring	—	≈2–37	*≈2–17*		—	≈1–21	*≈2–24*		Strom *et al.* (2001)
	Spring–summer	2–5	3–17[f]	4–8	<0.1	—	—	—	—	Liu *et al.* (2005)
	Spring–summer	—	<700	*5–36*[e]		—	≈1–60	≈10–40[e]	<5	Strom *et al.* (2007)
Irish Sea (53–54°N, 4–6°W)	Spring–summer	—	<34	*<24*[d]		—		*1–41*[d]		Edwards and Burkill (1995)
	Summer	—	—	≈0–60	0–1	—	—	*≈0–80*		Montagnes *et al.* (1999)
Off England (50–50.5°N, 4°W)	Summer	—	13–56[a]	7–15[a]	0.2–3[a]	—		*<178*		Fileman *et al.* (2002)
Celtic Sea (48–50°N, 5–10°W)	Spring	—		*2.5–20*		—	<1–5	≈2–22	—	Fileman *et al.* (2007)
Cantabrian Sea (43.6–43.8°N, 6°W)	Spring–autumn	1–9[b]	—	*1–14*[b,e]		—	—	*3.5–54*[b,e]		Ganda and Anadón Álvarez (2008)
	Winter	0.4–9[b]	—	—	—	—	—	—	—	Ganda and Anadón Álvarez (2008)
Georges Bank (40–42°N, 66–68°W)	Summer	—	—	0.3–11	<0.4	—	—	—	—	Stoecker *et al.* (1989)
Cape Hateras (35–37°N, 74–76°W)	Spring	10–30	10–80	*1–3*		—	—	—	—	Verity *et al.* (1996)
	Spring–summer	≈1–60	—	—	—	—	—	—	—	Sherr *et al.* (2002)
Off Chile (36°S, 73–74°W)	Spring	0.7–30	—	—	—	—	—	—	—	Cuevas *et al.* (2004)
	Winter	0.6–40	—	—	—	—	—	—	—	Cuevas *et al.* (2004)
Argentine Shelf (35–55°S, 56–67°W)	Summer–autumn	—	—	0.04–16	0–3	—	—	<0.1–20	0–6	Santoferrara and Alder (2009)
(47–55°S, 60–69°W)	Winter	0–2	0.02–11	0.06–12	0–0.4	0–2	<0.1–3	<0.1–16	0–2	Present study

Values that include more than one group are italicized.
[a] and [b] are mean values in the water column (0–25 and 0–70 m, respectively); all the other values correspond to discrete depths.
[c]Exclude *M. rubra*, except when [d] included; [e] not specified by the authors.
[f]Include autotrophic dinoflagellates.

et al., 2004) and dinoflagellates (Strom *et al.*, 2007). The abundance of these three protozooplankton groups increased offshore at 47°S (Fig. 2A–C; Table I), although they frequently have an inverse cross-shelf trend (Verity *et al.*, 1996; Fileman *et al.*, 2002; Sherr *et al.*, 2002; Strom *et al.*, 2007). In contrast, loricate ciliates peaked all along the coastal zone (Fig. 2D), showing densities similar to those found in other coastal environments during winter (e.g. Modigh and Castaldo, 2002; Barría de Cao *et al.*, 2005). Quantitative information about sarcodines in neritic waters is rarer, since they usually are poorly sampled or ignored in plankton surveys (Boltovskoy, 1999; Calbet, 2008). Nevertheless, their density (Fig. 2E–F) was two orders of magnitude lower than that reported in waters off England (Fileman *et al.*, 2002).

Relationship between protozooplankton biomass and environmental conditions during late winter

The distribution of protozooplankton biomass was clearly linked to the different environmental conditions that coexist in southern Patagonian waters during late winter. Both protozooplankton biomass and chlorophyll *a* concentration showed lower and homogeneous values south of 51°S (St. 11–28), whereas they peaked in the upper 40 m from the middle of shelf to the slope at 47°S (St. 4–10) (Figs 4A and C and 5A and B). These trends are probably related to the differences in both the seawater temperature (4–6°C and 6–7°C in St. 11–28 and St. 4–10, respectively) (ANTARES network http://www.antares.ws, original data from NASA) and the physical structure of the water column. Although the winter vertical mixing would have prevailed in the southern sector, a weak stratification would have started in the northernmost transect (Fig. 1A) (Rivas *et al.*, 2006).

These findings agree with the general concept that low levels of both food resources (in terms of chlorophyll *a* concentration) and temperature keep protozooplankton biomass low during winter. Both factors have a tight and direct relationship with protozooplankton density and biomass (e.g. Leakey *et al.*, 2002; Levinsen and Nielsen, 2002; Montagnes *et al.*, 2010b), as we found in this study (Table I). In this context, ca. 1 month after the start of the sea surface warming (St. 4–10, Fig. 1A), aloricate ciliates and dinoflagellates reached high abundances (Fig. 2B and C), resulting in a significantly higher ratio of protozooplankton biomass to chlorophyll *a* concentration (Fig. 5C). In agreement with our results, a 2-fold change in this ratio (but only considering ciliates) has been reported along a productivity gradient in the Mediterranean Sea (Dolan *et al.*, 1999). In the region under study, such a change may reflect a seasonal succession in the structure of the planktonic food web.

In contrast to aloricate ciliates and dinoflagellates, nanoflagellates reached only moderate abundances (Fig. 2A), probably due to a low availability of their picoplanktonic prey (Sherr and Sherr, 1994). The low proportion of the <2 μm chlorophyll fraction in shelf waters during the beginning of the seasonal phytoplankton increase (Rodrigues and Williams, 2002; Calvo-Díaz *et al.*, 2008) could explain the lack of a significant correlation between total chlorophyll *a* and nanoflagellates (Table I). Even if the growth of larger phytoplankters is generally coupled with bacterial production (Gasol and Duarte, 2000), there may be a delay of at least 2 weeks before an increase in the density of heterotrophic nanoflagellates (Kuuppo *et al.*, 1998).

The relatively low abundance of nanoflagellates could also be explained by an increased grazing by ciliates and dinoflagellates (McManus and Fuhrman, 1990; Sherr and Sherr, 2002). Furthermore, nanoflagellates may have been an important food resource for copepod nauplii, since the widths that we measured in such larvae (72–108 μm) suggest the preference for prey in a size range of 3–5 μm (Hansen *et al.*, 1994). The top-down pressure exerted by copepod nauplii probably was higher south of 51°S, where both their biomass and the ratio to nanoflagellate biomass increased significantly (Fig. 5A and C). In this context of scarcity of alternative food resources for copepod nauplii, their consumption of heterotrophic nanoflagellates is suggested also by the negative relationship found between them (Table I).

Quantitative relevance of microplanktonic aloricate ciliates

Protozooplankton biomass was clearly dominated by microplanktonic aloricate ciliates under almost all the environmental conditions examined (Fig. 3), in agreement with reports from other shelves and seasons (e.g. Fileman *et al.*, 2007; Strom *et al.*, 2007; Granda and Anadón Álvarez, 2008). An unfavorable condition for ciliate dominance coincides with high abundances of diatoms large enough to prevent consumption by these organisms, thus leading the main grazers of such phytoplankters, the heterotrophic dinoflagellates, to prevail (Jeong, 1999; Sherr and Sherr, 2007). While ciliates usually feed at a predator–prey size ratio of 8:1, heterotrophic dinoflagellates can consume cells as large as themselves, or even larger (Hansen *et al.*, 1994). In fact, we found the highest proportions of dinoflagellates under two conditions known to favor diatom growth, in mixed coastal waters (Margalef,

1978) and under weak stratification, which is usually linked to the spring bloom in temperate environments (Cushing, 1989). The diatoms we observed support this suggestion. However, we found large numbers of microplanktonic diatoms coincident with the highest biomass of microplanktonic aloricate ciliates (St. 4), which was attributed to a large species (50×60 μm) of the family Strobilidiidae (Lynn and Small, 2002). Ciliates of the same family and similar size dominated in an experimental bloom simulation and proved to be important consumers of large diatoms (Aberle *et al.*, 2007), thus indicating that these protozoa can occasionally be important in such a role.

If we had included *M. rubra*, the contribution of microplanktonic aloricate ciliates to protozooplankton biomass would have increased only 6%, since this species occurred at relatively low abundances (10^2–10^3cells L^{-1}), typical of winter (Sanders, 1995) or oceanic waters (Montagnes *et al.*, 2008). However, *M. rubra* was quantitatively relevant at a restricted site of the shelf (St. 13; Fig. 4B), where its biomass was 2- and 7-fold higher than the maximum values found in southern Patagonian waters during summer (1.2 μg C L^{-1}) and autumn (0.3 μg C L^{-1}), respectively (Santoferrara and Alder, 2009). At this site, *M. rubra* contributed 65% of microplanktonic aloricate ciliate biomass and 15% of chlorophyll *a* concentration, if we consider a maximum cellular content of chlorophyll *a* of 60 pg $cell^{-1}$ (Johnson and Stoecker, 2005). This site was located in Grande Bay, where the maximum biomass of copepodids and adult copepods is found during the winter (Sabatini and Álvarez Colombo, 2001).

Although phytoplankton represents a far larger biomass in marine ecosystems, ciliate biomass comprises 30% of the copepod daily carbon ration, and such contribution is higher (49%) when low production levels result from small algae (Calbet and Saiz, 2005), as it occurs under typical winter conditions (Legendre and Rassoulzadegan, 1996). In addition, when heterotrophic dinoflagellates reach high abundances, they are as significant as ciliates in the diet of copepods (Fileman *et al.*, 2010). Consequently, the carbon contribution of microplanktonic aloricate ciliates in southern Patagonian waters, including *M. rubra* and dinoflagellates at some sites, suggests a significant role of these organisms in sustaining copepod populations in southern Patagonian waters during late winter.

CONCLUSION

Although field studies that have emphasized the spatial distribution of total protozooplankton in temperate neritic environments are scarce, we conclude that both their maximum magnitudes in density and biomass and their vertical distribution are relatively constant between shelves, and that instead, their bathymetric distribution depends on individual properties of each shelf. In this study, the protozooplankton biomass was clearly linked to different chlorophyll *a* levels, which were attributed to dissimilar conditions in both temperature and the physical structure of the water column that coexist in southern Patagonian waters during late winter. Experimental work is still needed to confirm the function of protozoa both as consumers and as prey under natural conditions, but our *in situ* data suggest that they reflect changes in food availability and that protozooplankton, mainly microplanktonic aloricate ciliates, are important in the transfer of carbon to higher consumers across a cold-temperate shelf during winter.

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