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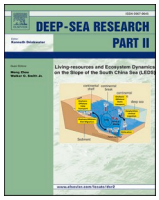

Microzooplankton and phytoplankton of Ross Sea polynya areas and potential linkage among functional traits

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ABSTRACT

The Ross Sea is characterized by a series of subsystems with different characteristics making it an extremely productive area. To understand whether species composition and functional traits of the plankton community can be used as biological tracers, we have analyzed the composition of phytoplankton and microzooplankton, and their potential relationships, in two different polynyas of the Ross Sea during the austral summer 2017. Sampling activities were carried out near Terra Nova Bay, between Cape Washington and the northern shore of the Drygalski Ice Tongue, and in the South-Central Ross Sea. We investigated the phytoplankton and microzooplankton structure using the phytoplankton body size classes and the tintinnids lorica oral diameter as functional traits, speculating on the relationship between the two plankton communities and their use as biological indicators in a changing Southern Ocean. Our data showed significant differences in terms of plankton composition and related functional traits between the two areas, suggesting the existence of distinct ecological dynamics despite the similar total carbon content. In Terra Nova Bay, heterotrophic dinoflagellates were the most abundant microzooplankton, in association with a large phytoplankton biomass mainly represented by diatoms and nano- and micro-phytoplankton. Tintinnids with large lorica oral diameters were abundant in Central Ross Sea, where phytoplankton was dominated by *Phaeocystis antarctica* and by the micro size class. Among microzooplankton, *Protoperidinium defectum, P. applanatum* and *P. incertum* were the most abundant dinoflagellates species, while *Codonellopsis gaussi, C. gaussi* forma *cylindroconica, Laackmanniella prolongata* and *Cymatocylis drygalskii* were the most abundant tintinnids. The phytoplankton was dominated by diatoms *Pseudonitzschia subcurvata*, *Fragilariopsis cylindrus*, *F. curta* and by the haptophyte *P. antarctica*. Our data indicate that beyond physical and chemical features defining distinct sectors of the Ross Sea, both species composition and functional traits of phytoplankton and microzooplankton represent a valid monitoring tool, especially with the ongoing global warming and its effects on Antarctic food webs.

1. Introduction

The Ross Sea (RS) is as a mosaic of different ecological subsystems with marked spatial-temporal variability in chemical, physical and biological properties (Smith et al., 2014; Kohut et al., 2017; Mangoni et al., 2017, 2019; Escalera et al., 2019; Bolinesi et al., 2020a; Saggiomo et al., 2021a).

Thanks to its unique features, the RS is one of the most productive regions of the Southern Ocean (Arrigo and McClain, 1994; Catalano et al., 2010; Smith et al., 2007, 2012, 2014), where large amounts of phytoplankton biomass, mainly represented by diatoms and the haptophyte *Phaeocystis antarctica*, support a diverse food web (DiTullio and Smith, 1996; Alderkamp et al., 2012; Smith et al., 2014).The dominance of different phytoplankton functional groups and the structure of their consumers play an important role in the transfer of carbon to upper trophic levels and biogeochemical cycles of the oceans (Smith et al.,

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2014; DiTullio and Smith, 1996; Saggiomo et al., 2021a; Bolinesi et al., 2020b; Misic et al., 2024).

The study of phytoplankton in the RS has been approached mainly in relation to environmental constraints, leading to the RS paradigm (Mills et al., 2010; Arrigo et al., 2010), which has recently been challenged (Mangoni et al., 2017, 2019; Escalera et al., 2019; Bolinesi et al., 2020a; Saggiomo et al., 2021b). Diatoms and *P. antarctica* have a different metabolic requirements, stoichiometric composition and trophic roles. The dominance of the two groups has been associated with different environmental conditions, in particular with the upper mixed layer (UML) depth, light availability, and nutrient concentrations. In recent decades, many studies have stated that the water column stratification favors diatoms dominance under high light conditions due to their physiological superiority in photoprotection and high tolerance to photoinhibition compared to *Phaeocystis* (Kropuenske et al., 2009; Mills et al., 2010; Arrigo et al., 2010). Diatom dominance, however, is dependent on iron bioavailability, as they require sufficient concentrations for sustaining elevated growth rates (Martin et al., 1990). Consequently, diatom populations typically dominate in stratified waters with relatively high iron concentrations, in regions near the Victoria Land Coast such as Terra Nova Bay (TNB) (DiTullio and Smith, 1996), in marginal ice zones (Smith et al., 2014), and especially near melting sea ice (Sedwick and DiTullio, 1997). In contrast, *P. antarctica*, due to their ability to photosynthesize under low irradiance and to acquire iron better than diatoms, dominates during spring in polynya areas in presence of a wide UML and intense mixing processes.

Over the last decades, the presence of intense *P. antarctica* blooms in coastal areas during summer and the high concentrations in polynya areas of biomass, parallel to the presence of diatoms in a high nutrient and low chlorophyll period (HNLC), suggests that the Antarctic paradigm does not cover all the problematic aspects of RS dynamics (Mangoni et. 2019).

In this system, with high temporal and spatial variability, changes in environmental parameters occur very rapidly, while the response of the biological communities (e.g. phytoplankton, microzooplankton) can require different time scales. The investigations which characterizeresearch in the RS has inevitably led to many unanswered questions, as demonstrated by the current Antarctic paradigm. It is possible that plankton structure could ignore the short scale of environmental fluctuations and provide important information on the existence of different subsectors of the RS, thus serving as biological tracers. In this context, it is important to consider the trophic relationships between phytoplankton and the small size upper consumers, particularly microzooplankton.

Microzooplankton represent the main phytoplankton grazers in the Antarctic trophic chain and are composed of organisms between 20 and 200 μm size. They can exert a significant top-down control on phytoplankton in the Southern Ocean (Steinberg and Landry, 2017; Christaki et al., 2021) and at the same time they represent an important food source for mesozooplankton (Irigoien et al., 2005; Sherr and Sherr, 2007, 2009). The most abundant microzooplankton groups in the Antarctic planktonic food web are heterotrophic dinoflagellates and ciliates, including tintinnids that can sometimes be the dominant group within the ciliate community (Liang et al., 2018).

Despite a high body polymorphism, tintinnids prey size is limited by their lorica oral diameter (LOD). They can, in fact, feed on prey with dimensions approximately 30% to a maximum of 45% of their LOD (Bernard and Rassoulzadegan, 1993; Dolan, 2010). The LOD is relatively constant within the same species, and considering its trophic role, is suitable as functional trait (Bernard and Rassoulzadegan, 1993; Dolan, 2010; Dolan et al., 2012).

The synergic effects of resource availability and predator-prey interactions, shape the plankton in a highly unpredictable way (Bjørnsen and Kuparinen, 1991; Kuparinen and Bjørnsen, 1992; Calbet and Landry, 2004; Schmoker et al., 2013). To this end, in the context of predictive ecology, functional traits have received increasing attention since they can provide a mechanistic foundation for understanding community structure and dynamics across environmental gradients (Edwards et al., 2013; Thomas et al., 2012).

In this study, we analyzed the structure of phytoplankton and microzooplankton in two polynyas of the Ross Sea to verify whether the two communities can be used as tracers of different subsystems. We considered the phytoplankton size classes and the tintinnid's lorica oral diameter to highlight possible interrelationships between the two assemblages and speculate on ecological perspectives for ecological research in the RS.

2. Materials and methods

2.1. Sampling collection and processing

Microzooplankton and phytoplankton were studied during the austral summer 2017 in the Ross Sea, within the frame of the P-Rose project (Plankton biodiversity and functioning of the Ross Sea ecosystems in a changing Southern Ocean) on board the R/V Italica. Samples were collected from 13 to 30 January 2017, at 17 stations located in two polynya areas (Fig. 1): TNB: Terra Nova Bay Area (TNB), from Cape Washington to the northern shores of the Drygalski Ice Tongue; SCR: South-Central Ross Sea (SCR), along the 175°E transect.

Supplementary Table 1 reports the sampled stations with related geographical coordinates, sampling date and depth.

At each station, water samples were collected at 3 depths (0 m, an intermediate depth between 15 and 45 m, and 100 m), depending on water column vertical profiles (i.e. fluorescence, temperature and salinity), using 12 L Niskin bottles mounted on a multisampler equipped with a CTD (9/11 Plus, Sea-Bird Electronics).

2.1.1. Microzooplankton characterization

To quantify microzooplankton, a volume between 2 L (for surface) and 11 L (for 100 m) of seawater was reverse filtered through a 10 μm mesh to reduce the volume to 250 mL, and immediately fixed with formaldehyde buffered with calcium carbonate $CaCO_3$ (1.6% final concentration). Subsamples (50 mL) were then examined in a settling chamber using an inverted microscope (magnification 200x) (Leitz Labovert, Leica DMI 300B), following the Utermöhl method (1958). The entire surface of the chamber was examined.

Although the use of formaldehyde can lead to an underestimate of naked ciliates, we chose this fixative instead of Lugol's solution because the latter can produce artifacts, such as the formation of aggregates and, as for glutaraldehyde, shrinkage in the ciliate cells (Choi and Stoecker, 1989; Leakey et al., 1994; Stoecker et al., 1994; Modigh and Castaldo, 2005). On the other hand, the results of other studies, where the aloricate ciliates represented the majority in the microzooplankton, documented that formaldehyde is a reliable fixative for Antarctic ciliates (Monti-Birkenmeier et al., 2017).

Among the microzooplankton, five main groups were considered: heterotrophic dinoflagellates, aloricate ciliates, tintinnids, micrometazoans and rare protozoans. The identification of heterotrophic dinoflagellates (Myzozoa, order Gymnodiniales and Peridiniales) was made following Balech (1976) and McMinn and Scott (2005), and for aloricate ciliates (Ciliophora, order Euplotida, Oligotrichida, Haptorida, Pleurostomatida and Chlamydodontida), on the basis of Petz et al. (1995) and Petz (2005). Tintinnids (Ciliophora, order Choreotrichida) identifications were based on Alder (1999) and Petz (2005). Empty loricae were not differentiated from filled ones because tintinnid protoplasts are attached to the lorica by a fragile strand that can easily detach during collection and fixation. Since the variation in the oral diameter of tintinnids lorica (LOD) can reflect the size spectrum of food items available (Dolan, 2010), we divided tintinnids into three classes: class 1: LOD = 20–40 μm; class 2: LOD = 41–60 μm and class 3: LOD = 61–100 μm. Micrometazoans (mainly nauplii of copepods) classification was after Larik and Westheide (2006), and the rare protozoans

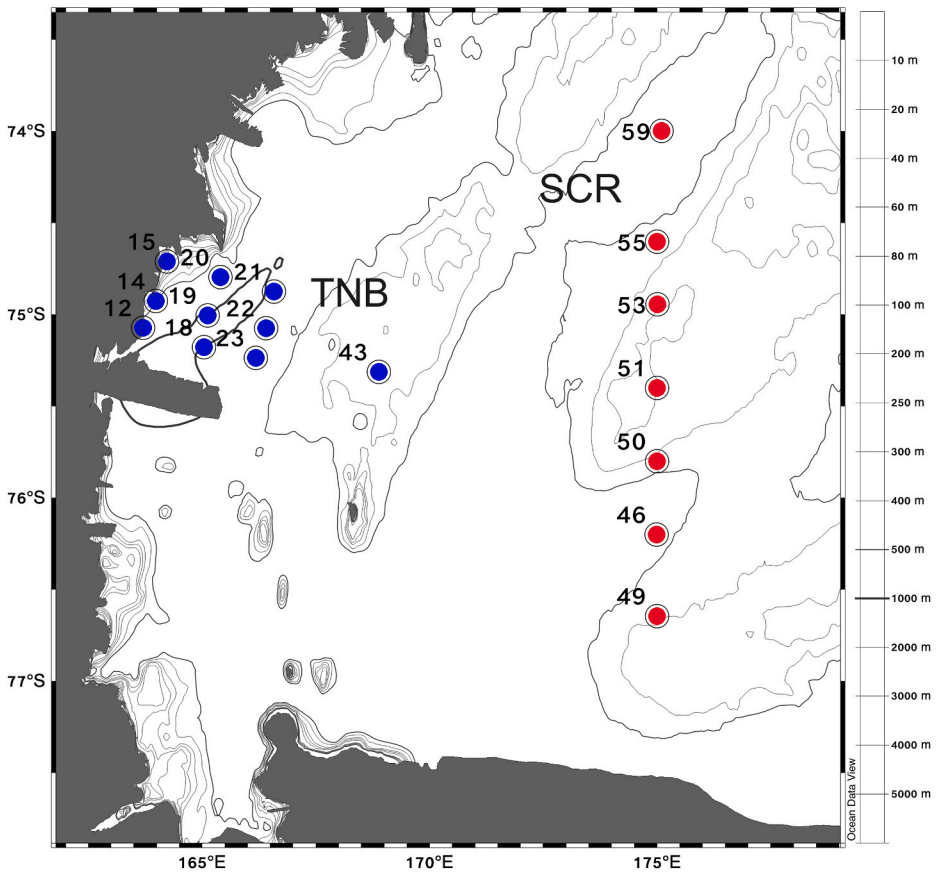

Fig. 1. Sampling stations in the two areas of the Ross Sea. Stations 18–43, in Terra Nova Bay (TNB) are indicated by blue dots; stations 49–59 in the South Central Ross Sea (SCR) and indicated by red dots. The continuous black and grey lines indicate the bathymetry of the study area (legend on the right).

(Foraminifera, order Rotalida, and Radiozoa, order Sticholonchida) were identified according to Kemle-von Mücke and Hemleben (1999) and Kling and Boltovskoy (1999).

For each taxon, biomass was estimated by measuring the linear dimensions of each organism and relating the shapes to standard geometric figures. Cell volumes were converted into carbon values using the conversion factors as follows: aloricate ciliates, pg C cell^{-1} as µm^3 x 0.14 (Putt and Stoecker, 1989); tintinnids, pg C cell^{-1} as µm^3 x 0.053 + 444.5 (Verity and Langdon, 1984); athecate heterotrophic dinoflagellates, pg C cell^{-1} as µm^3 x 0.11 (Edler, 1979); thecate heterotrophic dinoflagellates, pg C cell^{-1} as µm^3 x 0.13 (Edler, 1979); other protozoans, pg C cell^{-1} as µm^3 x 0.08 (Beers and Stewart, 1970).

2.1.2. *Phytoplankton characterization*

For the determination of phytoplankton chlorophyll *a* (chl a), 500 mL of seawater was filtered through a GF/F Whatman filters (25-mm diameter) and immediately frozen in liquid nitrogen until later analysis. To determine the phytoplankton pigmentary spectra, 2 L of seawater was filtered on GF/F Whatman filters (47-mm diameter) and the filters were cryopreserved as for Chl a. Frozen GF/F filters (25 mm diameter) were processed for the determination of Chl a and phaeopigments (phaeo) content, using a solution of 90% acetone following Holm-Hansen et al. (1965), with a spectrofluorometer (Shimadzu, Mod.RF– 6000; Shimadzu Corporation-Japan) checked daily with a Chl a standard solution (Sigma-Aldrich).

For the determination of chemiofunctional groups through pigmentary spectra composition, frozen GF/F filters (47 mm diameter) filters were homogenized and resuspended in 100% methanol and analyzed by High-Performance Liquid Chromatography (HPLC) (Hewlett Packard, 1100 Series) using a reverse phase C8 column (3 µm Hyperloop MOS), as described by Vidussi et al. (1996). Chlorophylls and carotenoids were determined using a diode array detector set at 440 nm, making it possible to determine the absorption spectrum of the 350−750 nm interval for each peak to check the purity of single pigments. The column was calibrated using different pigment standards provided by the International Agency for ^{14}C Determination (VKI Water Quality Institute, Copenhagen, Denmark).

Quantification was based on the absorbance at 440 nm and the factor response (peak area/pigment concentration) for each pigment (Mantoura and Repeta, 1997). The use of HPLC in the study of phytoplankton communities has been widely demonstrated to be a useful tool in estimating the phytoplankton community composition through the analyses of photosynthetic pigments (Jeffery and Vesk, 1997; Wright et al., 1996). This technique enables detecting and identifying microscopically overlooked or undetermined ultraphytoplankton species (Ansotegui et al., 2003; Antajan et al., 2004; Garibotti et al., 2003; Saggiomo et al., 2023) providing reproducible results. The contribution of phytoplankton groups to the total Chl a was estimated by CHEMTAX 1.95 software (Latasa, 2007) using an iterative process to find the optimal pigment:chl-a ratios. The groups identified were cyanophytes (Cyano), chlorophytes (Chloro), prasinophytes (Prasino), euglenophytes (Eugleno), cryptophytes (Crypto), diatoms (Diato), pelagophytes (Pelago), haptophytes (Hapto6HF), dinoflagellates (Dino) and xanthophytes (Xanto).

For the identification and enumeration of phytoplankton, 500 mL were collected at surface and intermediate depths and preserved in formaldehyde (4% final concentration) buffered with $CaCO_3$. Phytoplankton were identified to the lowest possible taxonomic rank (Medlin and Priddle, 1990; Hasle and Syvertsen, 1997; Scott and Thomas, 2005; Saggiomo et al., 2021b). In case of doubtful identifications, specimens were classified at a higher rank. Cells were counted using an inverted light microscope (Zeiss Axiophot) at 400 × magnification with phase

contrast optics (Zingone et al., 2010).

2.2. Statistical analysis

Statistical analysis was carried out using PAST 4.04 software. Biological data were analyzed by a non-metric multidimensional scaling based on a distance matrix computed with the Bray-Curtis dissimilarity (Bray and Curtis, 1957), with all data points represented in a two-dimensional coordinate system (Taguchi and Oono, 2005). Cluster analysis of microzooplankton and phytoplankton was completed considering composition, stations and sampled depths, with clusters joined based on the average distance between all members in the two groups computed by the Bray-Curtis index. Assuming that the chemofunctional groups defined the size structure of phytoplankton and including the role of the oral diameter of tintinnids lorica, a linear Spearman correlation was performed between phytoplankton size classes, LOD (class 1, 2 and 3) and chemofunctional groups to investigate the relationship between functional traits. To assess if composition was primarily responsible for difference between TNB (Area 1) and SCR (Area 2), a SIMPER test was performed using the Bray-Curtis similarity measure (Clarke, 1993). The overall significance of the difference was assessed by ANOSIM (analyses of similarity) (Clarke, 1993).

3. Results

3.1. Microzooplankton abundance and carbon content

The maximum microzooplankton abundance was 9080 ind. L^{-1} at St. 14 (20 m), while the minimum was 13 ind. L^{-1} at St. 23 (100 m) (Table 1). A similar pattern was found for carbon content with a maximum of 38 μg C L^{-1} at St. 14 (20 m) and minimum at St. 23 (100 m) with 0.1 μg C L^{-1}. Heterotrophic dinoflagellates were the most abundant group, with an average of 1292 ± 1719 ind. L^{-1}, followed by tintinnids with 650 ± 596 ind. L^{-1} that represented the highest carbon content (7.0 ± 6.4 μg C L^{-1}) within the microzooplankton (Table 1).

Abundances were higher at the surface compared to 100 m, with an average of 3000 ± 2130 ind. L^{-1} and 237 ± 247 ind. L^{-1}, respectively, and the carbon content being 17.2 ± 8.4 μg C L^{-1} in the upper layers and 3.3 ± 4.4 μg C L^{-1} at 100 m. The microzooplankton average abundance differed between the two areas, with 2977 ± 2400 ind. L^{-1} in TNB, and 796 ± 766 ind. L^{-1} in SCR. In contrast, the average carbon content in the two areas was very similar: 12.5 ± 9.6 μg C L^{-1} in TNB and 12.5 ± 9.8 μg C L^{-1} in SCR.

Heterotrophic dinoflagellates (67% of total) and ciliates (aloricate and tintinnids, 6% and 27% respectively) were present in all the samples; in contrast foraminiferans and radiozoans were infrequently encountered (<1%). In TNB, heterotrophic dinoflagellates represented 72% of the total and tintinnids 22%; in SCR, the former represented 11% and the latter 84%. Tintinnids accounted for 56% of total carbon biomass, dinoflagellates for 28%, aloricate ciliates for 10% and micrometazoans 6%. In TNB, dinoflagellates contributed 40% of the total biomass and ciliates 55% (aloricate and tintinnids), while in SCR the ciliates contribution to the total carbon reached 82%.

3.2. Microzooplankton composition

A total of 48 taxa, corresponding to 27 species and 20 genera, were identified (Supplementary Table 2).

The most common dinoflagellates were *Protoperidinum defectum* (Fig. 2a), *P. applanatum* (Fig. 2b) and *P. incertum* (Fig. 2c), while *Codonellopsis gaussi* (Fig. 2d), *C. gaussi* forma *cylindroconica* (Fig. 2e),

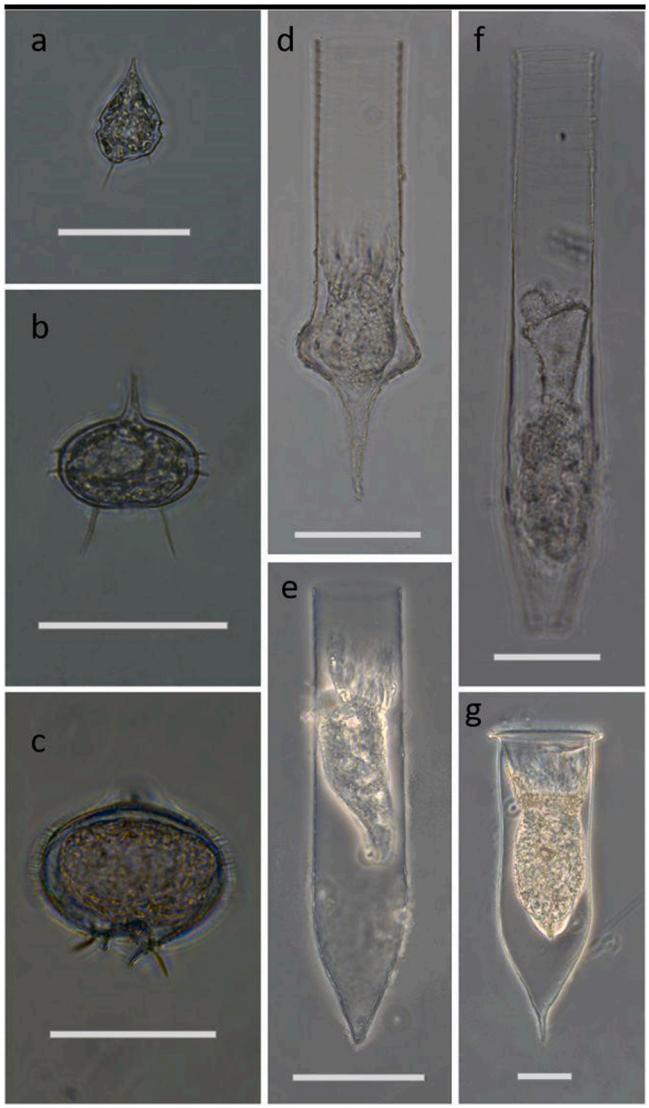

Fig. 2. The most representative species detected in the study area. *Protoperidinium defectum* (**a**), *Protoperidinium applanatum* (**b**), *Protoperidinium incertum* (**c**), *Codonellopsis gaussi* (**d**), *Codonellopsis gaussi* forma *cylindroconica* (**e**), *Laackmanniella prolongata* (**f**), *Cymatocylis drygalskii* (**g**). All scale bars are 50 μm in length.

Table 1
Minimum, maximum and average abundance and carbon content of microzooplankton. *s.d* is the standard deviation.

Groups	Abundance (ind. L^{-1})				Carbon content (μg C L^{-1})			
	min	max	average	*s.d.*	min	max	average	*s.d.*
Dinoflagellates	0	7114	1292.2	1719.5	0	15.9	3.6	3.9
Aloricate Ciliates	0	792	121.9	178.2	0	7.2	1.2	1.8
Tintinnid Ciliates	2	2635	650.3	596.0	0.1	31.4	7.0	6.4
Micrometazoans	0	60	12.2	13.9	0	9.1	0.7	1.4
Others	0	23	2.3	4.9	0	0.2	0	0
Total	13	9080	2078.9	2181.9	0.1	38	12.5	9.6

Laackmanniella prolongata (Fig. 2f) and *Cymatocylis drygalskii* (Fig. 2g), were the most abundant tintinnids.

As concerns heterotrophic dinoflagellates, *P. defectum*, together with the other *Protoperidinium* species, significantly contributed the high biomass found in TNB.

Protoperidinium defectum (20–30 μm long), with characteristic antapical horns of different length (Fig. 2a), showed the highest abundance (4815 ind. L^{-1}) and was the smallest species in the group. *Protoperidinium applanatum* and *P. incertum* maximum abundances were 433 and 440 ind. L^{-1}, respectively. The first had an ellipsoidal cell from 20 to 40 μm long (excluding horns) (Fig. 2b), while *P. incertum* was an almost pentagonal cell around 50 μm long (excluding horns) and a hypotheca with divergent antapical spine (Fig. 2c). *P. antarcticum* (maximum 150 ind. L^{-1}) was the largest organism in the group, 100–150 μm long and roughly 100 μm in diameter.

Aloricate ciliates were abundant in the upper layers of TNB. In particular, the species *Gymnozoum sympagicum* (maximum abundance 550 ind. L^{-1}) was found in almost all coastal samples. This species is ~50 μm long with an ellipsoid shape and was distinguished from *G. viviparum* mainly by its smaller size.

Among tintinnids, *Codonellopsis* genus and *L. prolongata* were most abundant in SCR. *Codonellopsis gaussi* was the most common, reaching the maximum of 1187 ind. L^{-1} at St. 49 (0 m). *Codonellopsis gaussi* had a short lorica around 130 μm long and 40 μm wide (Fig. 2d), and showed different morphotypes, as *C. gaussi* forma *cylindroconica* with a similar dimension but without an expanded posterior bowl (Fig. 2e). The latter was more abundant in the southern SCR (St. 46, 49, 50 and 51), reaching the highest value (390 ind. L^{-1}) at St. 51 (100 m). *Laackmanniella prolongata* (maximum abundance 765 ind. L^{-1}) had a cylindrical annulated hyaline lorica, longer than 200 μm and with a constant open diameter of ~45 μm (Fig. 2f). This species was present in both areas with a similar average abundance (about 120 ind. L^{-1}). *Laackmanniella naviculifaera*, with shorter dimensions and similar oral diameter, was present at a lower abundance (maximum 136 ind. L^{-1}) especially in TNB. *Cymatocylis drygalskii* was the most common species (maximum of 150 ind. L^{-1}) especially in the southern SCR. This species had a cylindrical shape hyaline wall with different lorica length (180–300 μm), mainly due to the antapical horn (Fig. 2g). The diameter of the bowl was constant (around 90 μm) and poorly correlated with lorica length. *Amphorides laackmanni* was mainly detected in TNB. This species had a hyaline wall with few longitudinal fins, very short body sized around 60 μm long and with a 20 μm opening diameter. The oral dimensions of *A. laackmanni*

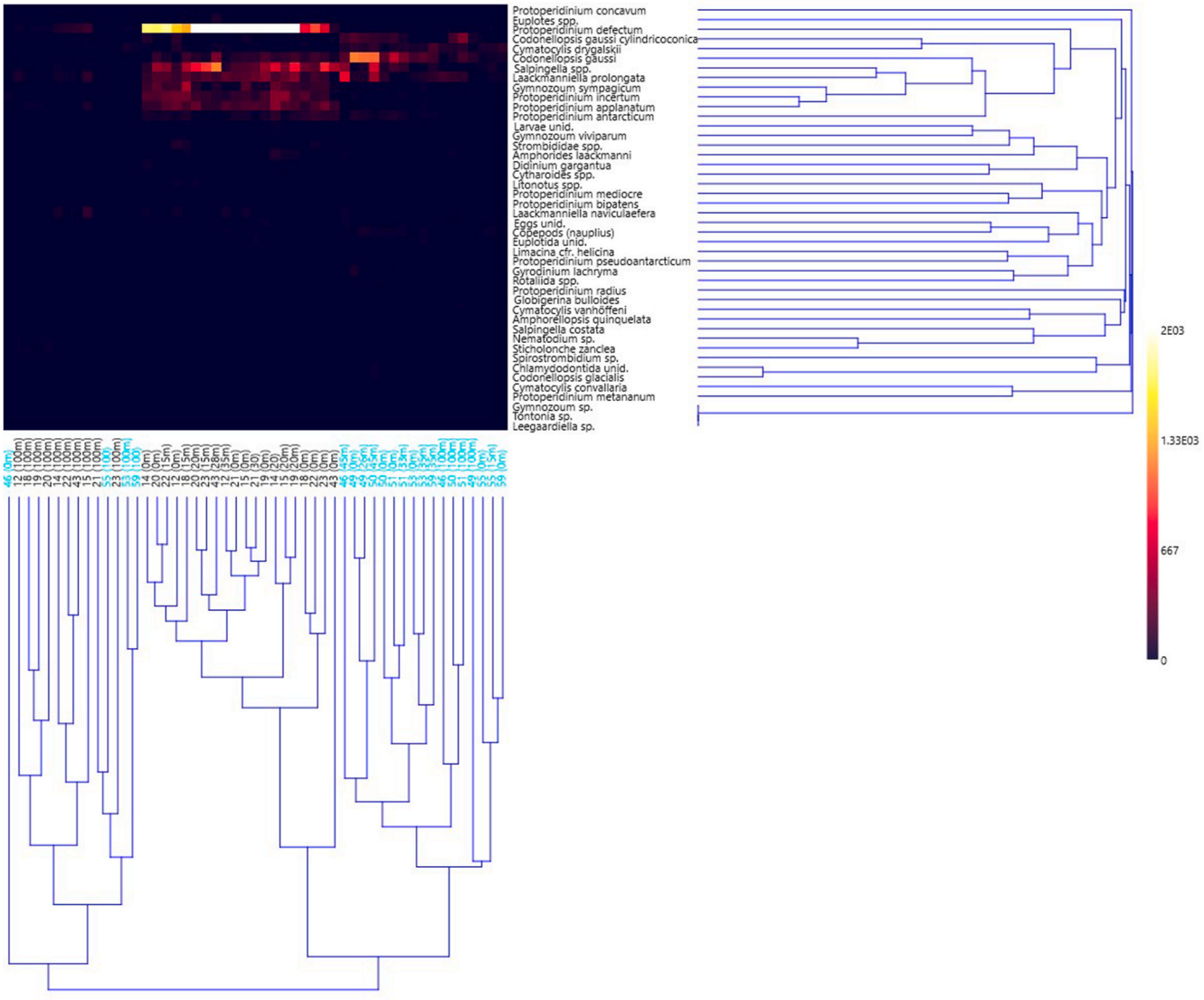

Fig. 3. Joined clustering analyses based on Bray-Curtis analyses. Stations and related depths are grouped along the X axis and microzooplankton species along the Z axis. Colors indicate the species abundances (number of individuals). Stations TNB, black numbers; stations SCR, aqua numbers.

were similar to that of *Salpingella* genus and co-occurred in TNB.

Copepod nauplia were present in almost all samples, mainly at intermediate depths, while *Sticholonche zanclea* was generally found at 100 m. Foraminiferans were infrequently encountered (max 10 ind. L^{-1} at St. 51, 33 m), but were found at the surface and intermediate depths of SCR.

Considering the tintinnids LOD, genera with small LOD dimensions were mainly present in TNB, such as *Amphorides* and *Salpingella*, while in SCR tintinnids with larger LOD occurred. In the northern part of the transect of SCR, 56% of the tintinnids (e.g. *Codonellopsis* and *Laackmanniella*), had LOD 2 (41–60 μm), while the south was dominated by *Cymatocylis* species with LOD 3 (61–100 μm). In the outside transect, only 17% of the tintinnids had LOD 1 (20–40 μm).

A cluster analysis based on the average distance between all members in the two groups was computed using the Bray-Curtis index (Fig. 3). The stations of TNB at shallow and intermediate depths were characterized by *P. defectum*, and by the absence of *C. gaussi* forma *cylindroconica* and *C. drygalskii*, both of which were present in SCR. The SCR instead had greater concentrations of *C. gaussi* especially in the upper 50 m of stations 49–50, showing a distribution of species which, unlike TNB, did not show marked differences between the surface and deeper layers. This leads to a clear separation of the stations in TNB and the shallow groups.

To assess which microzooplankton taxa were primarily responsible for the differences between TNB and SCR, a similarity percentage test was completed (Table 2). Results indicated that *P. defectum* accounts for 32.6% of average dissimilarity with all taxa explaining 87.7% of the overall dissimilarity. The analysis of similarity (ANOSIM) indicated the existence of a significant net dissimilarity between TNB and SCR ($R = 0.395$; $p = 0.001$).

3.3. Phytoplankton distribution

Different phytoplankton abundance and composition were observed in the two areas (Fig. 4). A maximum of up to $32.6x10^6$ cells L^{-1} was found in TNB. Diatoms were the most abundant, contributing more than 70% at all stations. *Fragilariopsis* spp. and *Pseudo-nitzschia* spp. were dominant at both surface and intermediate depths. *Pseudo-nitzschia subcurvata* was most abundant with $12.7x10^6$ cells L^{-1} while *Fragilariopsis curta* and *F. cylindrus* had $2x10^6$ cells L^{-1} and $4x10^6$ cells L^{-1}, respectively. Other *Fragilariopsis* species (*F. kerguelensis*, *F. rhombica*, *F. obliquecostata* and *F. sublinearis*) showed low abundances. *Cylindrotheca closterium* was relatively abundant ($1.8x10^6$ cells L^{-1}), while *Chaetoceros* and *Dactyliosolen* did not exceed 10^5 cells L^{-1}. Dinoflagellates reached a maximum of $0.47x10^6$ cells L^{-1}, while *P. antarctica* and other flagellates reached a maximum of $2.94x10^6$ cells L^{-1} and $3.61x10^6$ cells L^{-1}, respectively.

Phytoplankton abundance, in the SCR, was half that of TNB, but was more heterogeneous. *P. antarctica* and other small flagellates dominated. *Phaeocystis*, with a maximum of $5.47x10^6$ cells L^{-1}, represented up to 50% of the total composition. Other flagellates had $1.67x10^6$ cells L^{-1}, while dinoflagellates abundance was $0.24x10^6$ cells L^{-1}.

A similarity test was completed to assess differences between the two areas (Table 3). *Phaeocystis* accounted for 18.51% of the average dissimilarity with all taxa explaining 61.3% of the overall dissimilarity. The analysis of similarity indicated a weak but significant dissimilarity between the two areas ($R = 0.105$; $p = 0.001$).

Table 2
ANOSIM and SIMPER test to evaluate the significant difference between TNB and SCR and assess which microzooplankton taxa are responsible for the observed difference between the areas. Overall dissimilarity 87.8%.

SIMPER TEST	Overal avg dissimilarity: 87.8%				
Taxon	Av. dissim	Contrib. %	Cumulative %	Mean 1	Mean 2
Protoperidinium defectum	32.57	37.09	37.09	1.39E+03	3.97
Codonellopsis gaussi	11.88	13.53	50.62	83.8	294
Salpingella spp.	9.84	11.20	61.82	344	89.1
Laackmanniella prolongata	6.95	7.91	69.73	169	112
Cymatocylis drygalskii	4.82	5.49	75.22	11.4	76.7
Protoperidinium applanatum	4.63	5.28	80.5	158	2.89
Codonellopsis gaussi cylindroconica	4.02	4.57	85.07	7.42	85
Protoperidinium incertum	3.71	4.22	89.29	144	5.48
Gymnozoum sympagicum	2.60	2.96	92.25	106	2.2

ANOSIM		**p-values uncorrected significance**		
			TNB	SCR
Permutation N:	9999	TNB		**0.001**
Mean rank within:	513.7	SCR	**0.001**	
Mean rank between:	765.2			
R:	0.3946			
p (same):	0.0001			

3.4. Relationship between microzooplankton and phytoplankton

The Gaussian distribution model of the microzooplankton along the phytoplankton biomass gradient (Chl a), highlights how the tintinnids present a different trend compared to the other groups (Fig. 5). An increase between 2 and 3 μg L^{-1} Chl a of total microzooplankton and heterotrophic dinoflagellates was observed, reaching the maximum near 3.75 μg L^{-1} Chl a. Aloricate ciliates showed the same pattern, although abundances were significantly lower. Among all groups, only tintinnids showed a Gaussian distribution, with a maximum abundance near 2.5 μg L^{-1} Chl a. On one hand, this recalls the concept of standing culture when considering the interactions between primary producers and primary consumers, while on the other, it gives us the possibility to verify whether the trend of tintinnids can be explained by taking functional traits into consideration. After dividing the tintinnids into classes 1, 2 and 3, depending on the LOD, we found significant correlations ($p < 0.05$) between tintinnids, the micro-, nano and pico-phytoplankton classes, as well as chemiofunctional groups (Fig. 6).

Class 3 tintinnids (LOD 61–100 μm), showed a positive correlation with diatoms, and were negatively correlated to chlorophytes and haptophytes, as both are usually represented by small species. Class 2 tintinnids (LOD 41–60 μm), were positively correlated to haptophytes, and a negatively correlated to micro-phytoplankton. Class 1 tintinnids (LOD 20–40 μm) were positively correlated to micro-phytoplankton, chlorophytes and haptophytes, but inversely correlated to nano-phytoplankton and diatoms. The pico-phytoplankton were positively correlated to cryptophytes. Other classes did not show statistically significant correlations with the other groups, likely resulting from the broad size spectrum within the same phytoplankton group and the formation of colonies (e.g. *P. antarctica*).

The distribution of variables by non-metric multidimensional scale showed a net separation between stations of TNB and SCR (Fig. 7).

Many of the samples of TNB grouped on the left side of the plot, near 20–30 m depth, while other points showed a greater dispersion, with differences between the two areas becoming slightly lower at 100 m. The global distributions of points gave rise to 5 different convex hulls with the largest ones including deeper samples.

4. Discussion

The Ross Sea is among the most studied regions of Antarctica and is the most productive area of the entire Southern Ocean. Plankton dynamics have been widely described in relation to environmental factors, with less information on the relationship between phytoplankton and

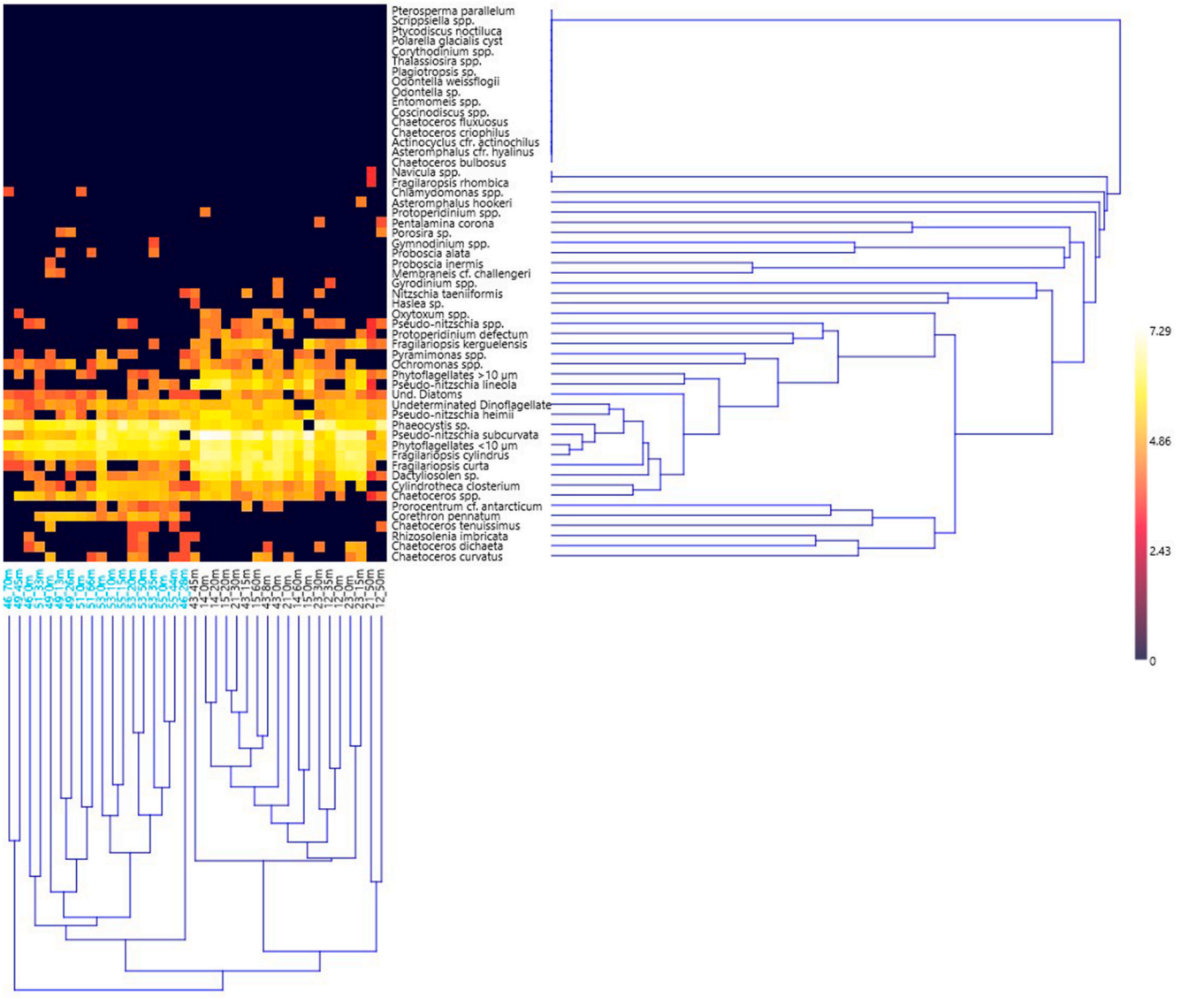

Fig. 4. Joined clustering analyses based on Bray-Curtis analyses. Stations and related depth are grouped along the X axis and phytoplankton species along the Z axis. Colors indicate the species abundances (number of individuals). Stations TNB, black numbers; stations SCR, aqua numbers.

microzooplankton.

We investigated the composition and structure of phyto and microzooplankton in two areas of the RS, addressing aspects related to the interrelationships between the two compartments and speculating how they can be used as biological tracers. Beyond the specific composition of the phytoplankton and microzooplankton in the two areas, our objective was to verify the existence of possible interrelationships between the functional traits of the two groups. Since the body size of microbial communities plays an important role in the trophodynamics of the oceans (Beaugrad et al., 2010), we considered the size classes of the phytoplankton (micro-, nano- and pico-) and the tintinnids LOD (based on its role in determining the size class of the prey) as functional traits.

Since phytoplankton bloom dynamics in the RS have been considered in relation to new evidence emerging from different context (Smith et al., 2014; Mangoni et al., 2017, 2019; Kouth et al., 2017; Park et al., 2019; Escalera et al., 2019; Bolinesi et al., 2020a; Saggiomo et al., 2021b), this approach can contribute to improve the scientific information on these ecological aspects. Many studies, in fact, have speculated that an increase of melting processes and changes in sea-ice coverage in Antarctic waters will favor water column stratification, CO_2 fluxes and light availability, affecting the phytoplankton bloom dynamics and structure. Although today we are still far from understanding exactly how these changes will affect the RS trophodynamics, recent studies have predicted an increase in the overall abundance of nanoflagellates (2–20 µm) in RS waters, together with evidenced reporting the presence of under ice blooms dominated by small phytoflagellates, some of which of fresh-water origin, in coastal areas affected by continental melting processes (Saggiomo et al., 2021b). Considering that the Antarctic food web is notoriously based on large diatoms, an increase in smaller sized phytoplankton can have dramatic effects Antarctic trophodynamics, directly affecting the microzooplankton abundance and composition (Hall and Safi, 2001). In this context, the role of microzooplankton can become more important as predators of smaller size phytoplankton and prey of mesozooplankton (Chen et al., 2012).

A major uncertainty on RS microbial trophodynamics concerns the trophic fate of *P. antarctica* (Caron et al., 2000; Grattepanche et al., 2011a, 2011b). The decline of *Phaeocystis* is based on iron limitation, with the breakup of colonies into single-cells that are more vulnerable to predation (Yang et al., 2016; Zhang et al., 2023). The colonies of *Phaeocystis*, protected by a mucous skin, are too large for microzooplankton ingestion, while single cells are a suitable food source (Peperzak et al., 1998; Stelfox-Widdicombe et al., 2004). Some

Table 3
ANOSIM and SIMPER test to evaluate significant differences between TNB and SCR and assess which phytoplankton taxa are responsible for the observed difference between the areas. Overall average dissimilarity 63.34%.

SIMPER TEST	Overal avg dissimilarity: 63.34%				
Taxon	Av. dissim	Contrib. %	Cumulative %	Mean 1	Mean 2
Phaeocystis sp.	18.51	30.18	30.18	1.87E+06	2.44E+06
Pseudo-nitzschia subcurvata	15.74	25.67	55.85	3.03E+06	6.32E + O5
Fragilariopsis cylindrus	6.30	10.26	66.11	1.19E+06	2.42E + D5
Phytoflagellates <10 µm	5.65	9.21	75.31	9.03E+05	8.98E+05
Fragilariopsis curta	3.94	6.42	81.74	5.85E+05	2.55E + D4
Dactyliosolen sp.	1.70	2.76	84.50	2.77E+05	2.15E+04
Pseudo-nitzschia heimii	1.60	2.61	87.12	2.30E+05	3.30E+04
Chaetoceros spp.	1.40	2.27	89.39	8.73E+04	1.65E+05
Pseudo-nitzschia lineola	1.29	2.10	91.49	2.76E+05	1.00E+04

ANOSIM		**p-values uncorrected significance**		
			TNB	SCR
Permutation N:	9999	TNB		**0.003**
Mean rank within:	2171	SCR	**0.003**	
Mean rank between:	2411			
R:	0.1055			
p (same):	0.00032			

microzooplankton, such as dinoflagellates *Gyrodinium* spp., *Gymnodinium* spp. and tintinnids, have the capacity to graze on single-cells and small colonies of *Phaeocystis* (Admiraal and Venekamp, 1986; Weisse and Scheffel-Möser, 1990; Bjørnsen and Kuparinen, 1991; Stoecker et al., 1995; Nejstgaard et al., 2007). Due to the unpalatability of the colonies of *P. antarctica*, they can sink from the photic zone and lysed by bacteria. In this respect, the role of small consumers, as heterotrophic nanoflagellates, feeding on bacteria was re-evaluated where the presence of senescence and/or lysis of algal assemblages represented an important source of food for heterotrops (Caron et al., 2000), but it remains unclear how microzooplankton respond to phytoplankton blooms. Several studies have demonstrated that microzooplankton can graze on over half the daily primary production, exerting a significant top-down control on phytoplankton assemblages in the Southern Ocean (Bjørnsen and Kuparinen, 1991; Kuparinen and Bjørnsen, 1992; Calbet and Landry, 2004; Schmoker et al., 2013).

The most abundant microzooplanktonic organisms recorded in this study were heterotrophic dinoflagellates, and tintinnids. The former can grow in a wide range of environmental conditions due to their ability to feed on a large spectrum of prey size including planktonic organisms even bigger than themselves (Gaines and Taylor, 1984; Jacobson and Anderson, 1986; Jeong et al., 2010). In particular, thecate heterotrophic dinoflagellates, such as *Protoperidinium* genus, are pallium feeders with a preference for diatoms and a maximum prey size up to approximately four times their body diameter (Naustvoll, 2000). In contrast, tintinnids prefer prey between 2 and 30 µm and are unable to ingest diatoms having cellular extensions (Verity and Villareal, 1986; Dolan, 2010). In Antarctica, dinoflagellates and aloricate ciliates can be the most abundant microzooplanktonic groups, followed by tintinnids (Alder and Boltovskoy, 1991; Calbet et al., 2005; Garcia et al., 2016).

At coastal stations of TNB, where melting and related nutrient enrichment sustain high phytoplankton biomass (Bolinesi et al., 2020a), the microzooplankton was dominated by heterotrophic dinoflagellates, ciliates aloricate and, to a less extent, by tintinnids of small dimensions. The presence of heterotrophic dinoflagellates and aloricate ciliates can be related to the high abundance of diatoms (Petz et al., 1995; Monti-Birkenmeier et al., 2017; Garcia-Oliva and Wirtz, 2022). Heterotrophic dinoflagellates were mainly represented by the pallium feeders *Protoperidinium* genus, while aloricate ciliates mainly belong to *Gymnozoum* genus suggests the influence of melting ice processes on the community (Petz et al., 1995). Indeed, *G. sympagicum* is typically found in multiyear land-fast ice or in waters affected by ice melt (Petz et al., 1995; Garzio and Steinberg, 2013; Monti-Birkenmeier et al., 2017). Tintinnids represented only 22% of the microzooplankton community in TNB, with the dominance of *Salpingella* and *Amphorides* genera, characterized by a small oral diameter (LOD 1, around 20 µm). This aspect indicates the availability of small-sized prey in the area, as indirectly suggested by the positive correlation between tintinnids Class 1 and micro-phytoplankton (i.e. the inability of the group to feed on large phytoplankton cells), and the negative correlation between Class 1 and nano-phytoplankton (i.e. the standing crop). The lack of tintinnids with larger LOD may be explained by a competitive interaction with heterotrophic dinoflagellates feeding on the same prey size (20–30 µm).

The microzooplankton community in SCR was mainly dominated by tintinnids with medium-large lorica oral dimension (LOD = 41–60 and 61–100 µm), representing 84% of the assemblage. This could be related to the presence of colonies of *P. antarctica* in the area. Tintinnids, in fact, are reported to be among the grazers of both small colonial and single cells of *P. antarctica* (Nejstgaard et al., 2007), and the increase of large LOD in SCR was probably related to the right diameter of food particles and to the absence of prey competitors. Heterotrophic dinoflagellates, in fact, require relatively high prey concentration (diatoms and aloricate ciliates) to support a rapid growth (Hansen, 1992). The high percentage

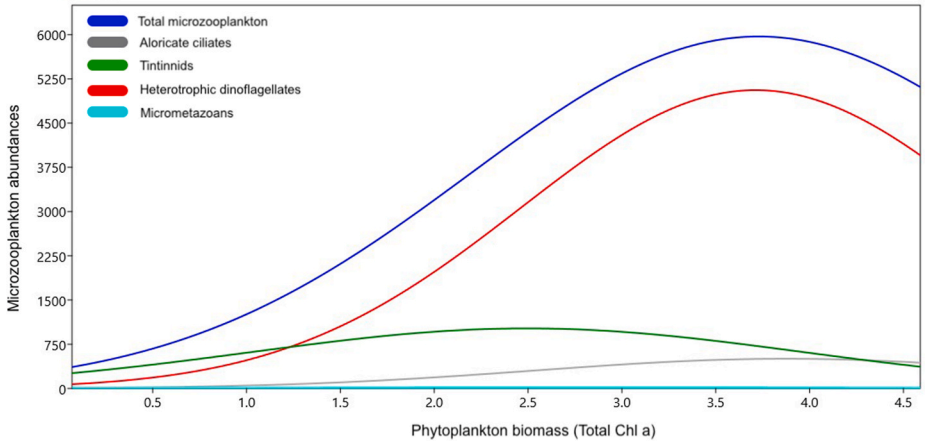

Fig. 5. Species packing (Gaussian) models with Gaussian response of functional microzooplankton abundances along the gradient of total phytoplankton biomass (Total Chl a).

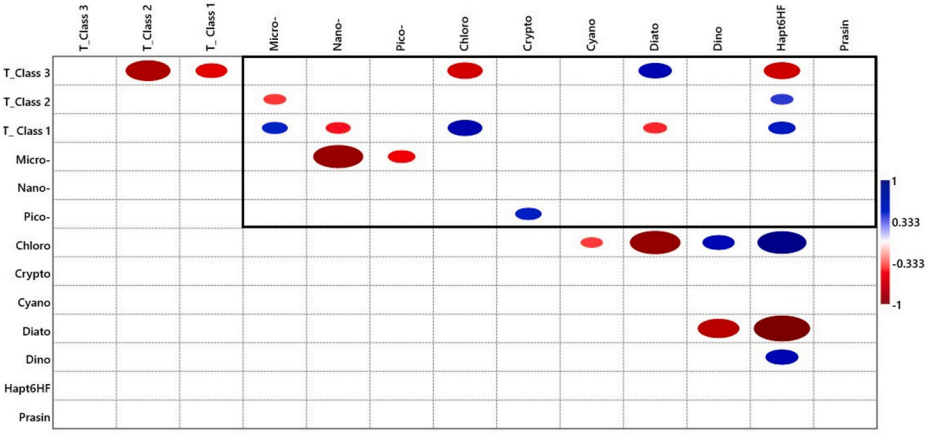

Fig. 6. Univariate correlation (Pearson's index) between all the functional variables. All values have $p < 0.05$ with colour and diameters of dots indicating the extent of correlation. Blue indicates a positive and red a negative correlation. The black square indicates the relationship between the considered functional traits and phytoplankton chemofunctional groups. T_Class 3: tintinnids class 3; T_Class 2: tintinnids class 2; T_Class 1: tintinnids class 1; Micro-: microphytoplankton; Nano-: nanophytoplankton; Pico-: picophytoplankton; Chloro: chlorophytes; Crypto: cryptophytes; Cyano: cyanophytes; Diato: diatoms; Dino: autotrophic dinoflagellates; Hapt6HF: haptophytes; Prasin: prasinophytes.

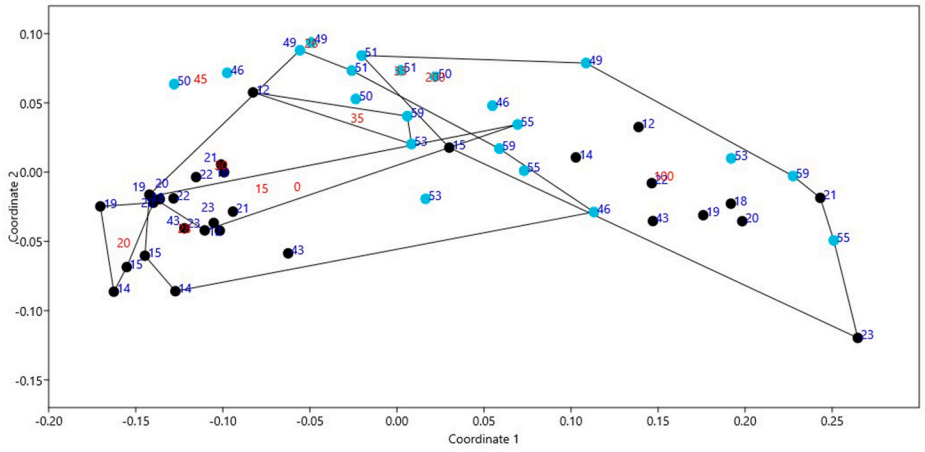

Fig. 7. Non-metric multidimensional scaling based on a distance matrix computed with the Bray-Curtis index. Stations of TNB are represented by black dots and stations of SCR are represented by light-blue dots. Different sampling depths are reported in red, and the depth's groups are shown by convex hulls.

of tintinnids relative to dinoflagellates could also be related to a later stage of microzooplankton succession, as described in previous studies, where tintinnids became more abundant after the decrease of heterotrophic dinoflagellates (Fonda Umani et al., 2002; Monti-Birkenmeier et al., 2021). In this respect, the presence of choanoflagellate blooms reported by Escalera et al. (2019) underlines the presence of a large amounts of organic matter in this area.

Despite the lower microzooplankton abundance in SCR, the biomass values in the two areas were comparable, confirming the dominance and importance of larger tintinnids in the Antarctic carbon cycle. Tintinnids with larger dimensions are common and endemic in the Antarctic areas (Dolan et al., 2012; Liang et al., 2020). Conversely, tintinnids with smaller dimensions have increased in the last years in the RS, with the first recorded of the genus *Amphorides* in the area in 2014 (Monti-Birkenmeier et al., 2022). The increasing trend of tintinnids with small dimensions in TNB resembles the recent increase of cells with smaller size in the phytoplankton community (Saggiomo et al., 2021b).

Considering the significant relationship emerging between phytoplankton and microzooplankton functional traits, we speculate that what is observed in Fig. 6 can reflect changes within the phytoplankton community structure. The lorica oral diameter of tintinnids determines the size of the phytoplankton on which these organisms can graze (Fig. 6) and suggest that the pico-class (<2 μm, poorly represented in the Ross Sea) has no relation with tintinnids classes. At the same time, nano- and micro-phytoplankton show a different correlation with tintinnids of class 1 and 2, depending on LOD. Class 1 is unable to feed on microphytoplankton, but can feed on nano-phytoplankton (which decreases as consequence of grazing pressure); in the same way, class 2 can feed on micro-phytopankton determining the negative correlations with the group. Tintinnid class 3 has a broad mouth spectrum, with no significant correlation with phytoplankton size, although the class showed a positive correlation with diatoms and a negative correlation with haptophytes and chlorophytes.

The representation of plankton (Fig. 7) is consistent with results that have included physical and chemical properties in comparing different areas of the Ross Sea (Smith et al., 2014; Bolinesi et al., 2020a; Mangoni et al., 2017). We believe that the greater similarity between the deep samples compared to the surface ones is linked to a lower variability of this environment compared to the surface layer, where temperature, salinity and continental contributions linked to the melting of sea ice exert an influence on the different subsystems (Bolinesi et al. (2020a). The difference between the samples of the superficial layer and the deeper ones confirms the response of the analyzed communities to the complex of environmental variations that characterize different

environments and subsystems (coastal system compared to the offshore one, or superficial environment compared to the deep one).

Considering the overall dataset, we speculate that phytoplankton structure can play a pivotal role in shaping the microzooplankton community of the RS, acting both as ecological descriptor of environmental conditions and precursor for tintinnids succession. Thus, the plankton communities' functional traits can contribute to recognizing the ongoing changes in Antarctic coastal waters, where small groups play an increasingly important role in an area historically considered dominated by large cells.

5. Conclusion

Our results emphasize a functional approach that underlines the existence of distinct ecological subsystems in the Ross Sea. The differences observed between Terra Nova Bay (TNB) and the South Central Ross Sea (SCR) makes plankton communities, particularly their functional traits, a valid and poorly used monitoring tool in studying the response of polar systems to climate change. In this respect, the presence of few tintinnids with small lorica oral diameter (LOD) in TNB, and the large amount of tintinnids with medium-large LOD in SCR, relates to the different phytoplankton structure in the two areas. Heterotrophic dinoflagellates dominated in TNB, where the phytoplankton biomass was represented by diatoms and nano- and microplankton. Tintinnids dominated in SCR where *P. antarctica* (mainly as small colonies) and diatoms showed similar percentage.

Despite differences in composition, the total carbon content between TNB and SCR was similar, suggesting the importance of group partitioning in defining the ecological value of the carbon transfer to upper trophic levels. This type of approach can open new perspectives in polar research, especially considering the role of plankton in carbon transfer and ocean-atmosphere interactions in a changing Southern Ocean.

CRediT authorship contribution statement

Marina Monti-Birkenmeier: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **Tommaso Diociaiuti:** Data curation, Formal analysis. **Francesco Bolinesi:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Maria Saggiomo:** Data curation, Formal analysis, Methodology. **Olga Mangoni:** Conceptualization, Data curation, Funding acquisition, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr2.2024.105393.

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