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Horizontal distribution of tintinnids (Ciliophora) in surface waters of the Ross Sea and polynya in the Amundsen Sea (Antarctica) during summer 2019/2020

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Abstract Information on tintinnid horizontal distribution in the Antarctic Continental Zone is scarce. During the summer of 2019/2020, tintinnid diversity and horizontal distribution in surface waters were investigated in the Ross Sea and Amundsen Sea polynya. Eight tintinnid species were found and the dominant species showed obvious horizontal distribution characteristics. In the Ross Sea, three tintinnid community groups were identified. Cymatocylis cristallina and Laackmanniella prolongata (group I) were dominant species and were mainly distributed in stations closer to the coast than were species in the other two groups. Codonellopsis gaussi (group II) and Cy. convallaria (group III) were mainly distributed in nearshore and offshore stations, respectively. In the Amundsen Sea polynya, the dominant species Cy. cristallina, L. prolongata and Salpingella faurei (group I) were mainly distributed in stations closer to the coast than were species in the other two groups. Cy. convallaria (group III) was mainly distributed in offshore stations. The distribution area where C. gaussi and C. cristallina were found in high abundance and abundance proportion of loricae with protoplasts was divided by the approximate boundary of the Antarctic Slope Front Current and Coastal Current in the Ross Sea. The highest abundance proportion in the Ross Sea was the 32-36 µm lorica oral diameter (LOD) size class (75.7%), and the 36-40 µm LOD size class (56.0%) was found in the Amundsen Sea polynya. Temperaturesalinity-plankton diagrams of the two seas revealed that temperature may be the main reason for species distribution. Our results contribute to a better understanding of horizontal distribution of the microbial food web, and serve as a baseline for future studies of pelagic community change in the Antarctic Continental Zone.

Keywords Antarctic Continental Zone, tintinnid, community structure, Ross Sea, Amundsen Sea polynya

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1 Introduction

The Southern Ocean (locations below 40°S) is made up of a complex of currents (Caccavo et al., 2018; Thompson et al., 2018). According to the positions of three fronts (the Subantarctic Front, Polar Front, and Continental Water Boundary), the Southern Ocean is divided into four distinct circular zones (Subantarctic, Polar Frontal, Antarctic and Continental Zone from north to south) around the Antarctic continent (Tomczak and Godfrey, 1994; Patmore et al., 2019). Previous studies have shown that the Subantarctic and Antarctic Zones have different zooplankton (including tintinnids) and phytoplankton compositions, divided by the Polar Front (David, 1955; Kane, 1966; Deacon, 1982; Dolan et al., 2012; Liang et al., 2020). There have, however, been few studies of tintinnid horizontal distribution in surface waters of the Continental Zone.

Tintinnids are single-cell protozoan plankton with loricae around their bodies and can live in marine and freshwater habitats. They belong to the subclass Choreotrichia, class Spirotrichea and phylum Ciliophora (Lynn, 2008). Tintinnids are primary consumers of pico-sized (0.2–2 μ m) and nano-sized (2–20 μ m) plankton, as well as important food sources of metazoans and fish larvae (Stoecker et al., 1987; Dolan et al., 1999; Gómez, 2007). They play an important role in material circulation and energy flow from the microbial food web into the traditional food chain (Azam et al., 1983; Pierce and Turner, 1992; Calbet and Saiz, 2005).

Surface dynamics in the Continental Zone are heavily influenced by the local atmosphere and ocean interactions as well as by sea ice freezing and melting processes (Fonda Umani et al., 2005). The Ross Sea ecosystem, an important component of the Continental Zone marine ecosystem, is also influenced by many currents such as the Antarctic Slope Current (ASC), Coastal Current (CC) and the Ross Sea Gyre (Williams et al., 2015). Previous studies have found several tintinnid species distributed in small areas in the western (Monti and Fonda Umani, 1995, 2000; Fonda Umani et al., 1998, 2002; Safi et al., 2012; Monti et al., 2017) and southern parts of the Ross Sea (Stoecker et al., 1995; Fonda Umani et al., 2005). The tintinnid community structure in the eastern part of the Ross Sea had not, however, been studied.

Polynyas – recurring areas of seasonally open water surrounded by sea ice – are common features in the Antarctic (Smith and Barber, 2007). The Amundsen Sea is a region characterized by a relatively narrow continental shelf, a large amount of perennial sea ice and a number of coastal polynyas adjacent to large ice shelves (Arrigo and van Dijken, 2003). Because relatively warm, salty and nutrient-rich Circumpolar Deep Water (CDW) intrudes close to the coast, the melt rate of the floating ice shelves increases, resulting in the formation of polynyas in the Amundsen Sea (Payne et al., 2007; Walker et al., 2007; Wahlin et al., 2010; Jacobs et al., 2011; Thompson et al., 2018) and subsequent phytoplankton blooms (Arrigo et al., 2003, 2012; Yang et al., 2019). There did not, however, appear to be an obvious increase in polynya tintinnid species composition and abundance around Pine Island Bay, Amundsen Sea (Dolan et al., 2013; Jiang et al., 2014, 2016a, 2016b). It was not known whether tintinnids had different horizontal distribution characteristics in the Amundsen Sea polynya.

Our study aimed to uncover: (1) the horizontal distribution of tintinnids in the Continental Zone; (2) the different tintinnid communities in the Ross Sea and the Amundsen Sea polynya; and (3) the relationship between tintinnids and environmental factors in the investigated area. These data also serve as a baseline for monitoring the planktonic zooplankton response to environmental change in the Antarctic Continental Zone.

2 Materials and methods

2.1 Study area and sample collection

Tintinnids were sampled at 43 stations from 4 to 30 January 2020, during the 36th Chinese National Antarctic Research Expedition aboard R/V *Xuelong* in two sea areas (Figure 1). In the Ross Sea, 25 stations (stations R1–R25) were sampled from 4 to 10 January 2020. In the Amundsen Sea polynya, 18 stations (stations A1–A18) were sampled from 25 to 30 January 2020 (Table 1). The sampling time only occupied 1 min from a moving ship in all stations except stations R1 and R2 (5 min). The speed of the moving ship was 10 knots·h⁻¹ (equal to 5 m·s⁻¹). Therefore, our sampling location distance was about 300 m. This distance occupied 0.30% of the distance between two stations, which was about 100 km. Ice cover data were sourced from Sea Ice Remote Sensing at the University of Bremen (https://seaice.uni-bremen.de/sea-ice-concentration/).

Tintinnid samples were collected with an onboard continuous underway sampling system at a depth of 5 m (surface water) while the ship was cruising. Between 10 L and 60 L of seawater was gently filtered through a 10- μ m mesh net. The samples (~150 mL) in the cod end of the net were transferred into sample bottles and immediately fixed with Lugol's solution (1% final concentration). Samples were kept in a cool, dark environment for preservation.

Surface water temperature (°C) and salinity were determined using a WTW Cond 3210 SET 1 portable water quality analyzer (Xylem, Munich, Germany). Chlorophyll *a* (Chl *a*) concentration was determined by filtering 500 mL of seawater through a Whatman GF/F glass fiber filter. Plankton retained on the filter was extracted in 90% ($v \cdot v^{-1}$) acetone. Fluorescence was measured according to the JGOFS protocol (Knap et al., 1996) using a Turner Trilogy fluorometer Model 10 (Turner Designs, San Jose, US).



Figure 1 Survey stations in the Ross Sea and Amundsen Sea polynya of the Antarctic. White dotted line delimits the ice edge covered by sea ice during sampling time. Arrows showed currents following Williams et al. (2015) and Thompson et al. (2018). RSG, Ross Sea Gyre; ASFC, Antarctic Slope Front Current; CC, Coastal Current. Blue triangle, group I; Red circles, group II; Cyan inverted triangle, group III. No tintinnid was found in station R23.

 Table 1
 List of stations with sampling date, sampling volume and environmental factors (temperature, salinity and Chl *a* concentration) in surface layers

Seas	Station	Longitude	Latitude	Sampling date (YYYY-MM-DD)	Sampling volume/L	Temperature /°C	Salinity	Chl <i>a</i> concentration/($\mu g \cdot L^{-1}$)
Ross Sea	R1	165°00'00''E	75°00'00''S	2020-01-04	60	0.5	34.1	3.26
	R2	166°00'00''E	75°00'00''S	2020-01-05	60	1.3	34.4	1.52
	R3	168°00'00''E	75°00'00''S	2020-01-05	15	0	33.9	0.45
	R4	170°25'01"E	75°00'00''S	2020-01-05	16	1.5	34.4	1.97
	R5	172°52'04''E	75°00'00''S	2020-01-05	17	1.4	34.5	0.61
	R6	175°16'59"E	75°00'00''S	2020-01-05	15	0.5	34.0	0.34
	R7	177°39'42''E	75°00'00''S	2020-01-06	16	0.7	34.5	0.31
	R8	179°53'39"E	75°00'00''S	2020-01-06	15	-0.4	34.0	0.24
	R9	177°45'44"W	75°00'00''S	2020-01-06	16	-0.7	34.2	0.47
	R10	179°41'42"E	75°26'07''S	2020-01-06	10	0.8	34.4	0.29
	R11	176°55'57"E	75°50'35"S	2020-01-06	11	1.0	34.4	0.38
	R12	173°37'08''E	76°18'51"S	2020-01-06	11	0.8	34.4	0.67
	R13	170°13'05"E	76°47'11"S	2020-01-07	11	1.5	34.6	0.48

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Seas	Station	Longitude	Latitude	Sampling date (YYYY-MM-DD)	Sampling volume/L	Temperature /°C	Salinity	Chl <i>a</i> concentration/($\mu g \cdot L^{-1}$)
	R14	165°52'38"E	77°22'24''S	2020-01-07	14	0.9	33.8	0.2
	R15	165°46'22''E	77°33'22''S	2020-01-07	15	-1.2	33.2	0.37
	R16	165°50'42''E	77°35'36"S	2020-01-07	16	-1.5	33.0	0.33
	R17	166°57'46''E	77°05'43"S	2020-01-08	10	0.5	34.0	1.65
	R18	170°54'00''E	77°09'01''S	2020-01-08	11	0.6	34.5	0.76
Ross Sea	R19	176°13'28''E	77°16'12''S	2020-01-08	12	0.5	34.7	0.49
	R20	176°14'05''W	77°26'24''S	2020-01-09	13	-0.9	34.6	0.95
	R21	171°01'45"W	77°33'20''S	2020-01-09	13	-1.0	34.6	0.49
	R22	165°50'13"W	77°38'44"S	2020-01-09	15	-1.6	34.4	0.28
	R23	162°25'48''W	77°13'01"S	2020-01-09	12	-1.2	34.0	0.31
	R24	158°35'00''W	76°41'45"S	2020-01-09	15	-0.8	34.1	0.21
	R25	158°22'19"W	76°14'44''S	2020-01-10	12	-0.2	33.9	0.11
	A1	117°49'57''W	72°01'38"S	2020-01-25	16	-0.9	33.5	0.69
	A2	116°32'15"W	72°29'58''S	2020-01-26	15	1.5	33.7	1.45
	A3	115°00'00''W	73°00'00''S	2020-01-26	15	1.4	33.7	1.88
	A4	113°30'00''W	73°30'00''S	2020-01-27	15	1.2	34.2	3.17
	A5	112°07'00''W	74°00'00''S	2020-01-27	15	1.4	34.2	4.06
	A6	111°59'43"W	74°09'41"S	2020-01-27	15	0.3	34.1	3.74
	A7	112°06'22''W	73°42'25"S	2020-01-27	15	0.4	34.2	4.15
	A8	112°10'31"W	73°21'22''S	2020-01-27	14	0.8	34.2	2.78
	A9	112°15'25"W	73°02'00''S	2020-01-27	14	0.5	33.7	1.48
Amundsen Sea	A10	112°30'53"W	72°51'54"S	2020-01-29	15	0.8	33.4	1.24
	A11	112°44'39"W	72°42'06''S	2020-01-29	15	0.1	33.3	1.7
	A12	113°56'12''W	72°47'37''S	2020-01-29	16	1.9	34.2	1.55
	A13	116°00'00''W	73°11'02''S	2020-01-29	15	1.5	34.2	1.16
	A14	117°00'00''W	73°24'18"S	2020-01-29	14	0.9	34.1	1.36
	A15	118°00'00''W	73°36'04''S	2020-01-29	16	0.4	34.0	1.76
	A16	120°00'00''W	73°01'06''S	2020-01-30	15	0.6	33.4	2.6
	A17	120°34'26''W	72°21'28''S	2020-01-30	17	-1.2	33.1	0.93
	A18	121°07'22''W	71°48'55"S	2020-01-30	16	-1.7	33.2	1.05

2.2 Sample analysis and species identification

In the laboratory, a 25-mL subsample (or a larger volume if tintinnids were scarce) from each original sample was settled in a Utermöhl counting chamber (Utermöhl, 1958) for at least 24 h and examined using an Olympus IX 71 inverted microscope (Olympus, Tokyo, Japan) at a magnification of $\times 100$ or $\times 400$. At least 20 individuals (if possible) of each species were photographed and measured. Tintinnids have morphological plasticity and intermediate form morphologies, especially in polymorphic genera such as *Cymatocylis* and *Codonellopsis* (Dolan et al., 2013; Liang et al., 2018, 2020). To reduce deviation, tintinnid species with morphological plasticity and intermediate form morphologies were assigned to the closest species based on lorica morphology and size, according to the literature

(Laackmann, 1910; Boltovskoy et al., 1990; Zhang et al., 2012; Dolan et al., 2013; Liang et al., 2018, 2020). Because mechanical and chemical disturbance during collection and fixation can detach the tintinnid protoplasts from the loricae (Paranjape and Gold, 1982; Alder, 1999), we included empty tintinnid loricae in cell counts. Empty loricae and loricae with protoplasts for each species were counted separately. We hypothesized that the abundance proportion of loricae with protoplasts higher than 50% can reflect a suitable environment for this species.

2.3 Data processing

Tintinnid species richness in each station indicated the number of tintinnid species that appeared in that station. Abundance of each tintinnid species $(A_i, \text{ ind} \cdot L^{-1})$ in each station was calculated using Eq (1):

$$A_i = \frac{V \times N_i}{V_i} \,, \tag{1}$$

where V(L) is the volume of the sample, N_i is the individual number of species *i* in the subsample and V_i is the volume of the subsample (L). Total tintinnid abundance in each station was the sum of each tintinnid abundance appearing in this station. Occurrence frequency (OF, %) of each tintinnid species was calculated as the percentage of samples in which the species appeared. The number of stations where each species appeared in the Ross Sea and Amundsen Sea polynya were 25 and 18, respectively.

The dominance index (Y) of tintinnid species in one assemblage was calculated using Eq (2) (Xu and Chen, 1989):

$$Y = \frac{N_i}{\left(N \times f_i\right)},\tag{2}$$

where N_i is the number of individuals of species *i* in all samples, f_i is the occurrence frequency of species *i* in all samples and *N* is the total number of all species. Species with Y > 0.02 represented the dominant species in an assemblage.

Distributional data were presented by ODV (Ocean Data View, Version 4.7, Reiner Schlitzer, Alfred Wegener Institute, Bremerhaven, Germany), Surfer (Version 13.0, Golden Software Inc., Golden, CO, USA) and Grapher (Version 12.0, Golden Software Inc., Golden, CO, USA). Cluster analysis was performed using PRIMER (Version 5.0, PRIMER-e, Plymouth, UK) based on the abundances of different tintinnid species at each station. Group-average linkage based on the Bray-Curtis similarity matrix of the fourth root transformed from the original data was used. Correlation analysis between environmental and biological variables was performed using SPSS (Version 16, SPSS Inc., IBM Corp., Armonk, NY, USA). The significance for spatial pattern was tested by PERMANOVA analysis in PERMANOVA+ for PRIMER 6 (Anderson et al., 2008; Jiang et al., 2016c).

3 Results

3.1 Hydrographic features and tintinnid abundance

The surface water temperature ranged from -1.7° C (St. A18) to 1.9° C (St. A12) in the study areas (Figure 2; Table 1). High temperature areas were located in the western and southern sides of the Ross Sea and Amundsen Sea polynya, respectively (Figure 2). Surface salinity was high (> 34.0) in the central Ross Sea. In the Amundsen Sea polynya, salinity increased from north to south (Figure 2). In the Ross Sea, high Chl *a* concentrations (> 1.0 µg·L⁻¹) occurred on the western side near the shelf area. In the Amundsen Sea polynya, high Chl *a* concentrations occurred in stations located on the southern side of 72.5°S (Figure 2).

Total tintinnid abundance ranged from 0.0 to 816.0 ind. \cdot L⁻¹ and 4.6 to 343.9 ind. \cdot L⁻¹ in the Ross Sea and Amundsen Sea polynya, respectively. High abundance of total tintinnids (> 100 ind. \cdot L⁻¹) was found on the western and southern sides near the shelf area in the Ross Sea (Figure 2). In the Amundsen Sea polynya, high abundance of total tintinnids was found on the southern side of 72.5°S (Figure 2). The distribution of a high abundance proportion of loricae with protoplasts (> 50%) was similar to the high total abundance in the Ross Sea. In the Amundsen Sea polynya, all stations had a high abundance proportion of loricae with protoplasts (Figure 2).

3.2 Tintinnid species composition

We identified eight species from four genera (Figure 3; Table 2) from all samples. Seven species and five species were found in the Ross Sea and Amundsen Sea polynya, respectively (Table 2). Codonellopsis gaussi (Y = 0.48) and Cymatocylis cristallina (Y = 0.12) were the dominant species in the Ross Sea. In the Amundsen Sea polynya, Laackmanniella prolongata (Y = 0.47), Cy. cristallina (Y =0.27) and Salpingella faurei (Y = 0.08) were dominant species (Table 2). Cy. cristallina had the highest occurrence frequency in both the Ross Sea (68.0%) and Amundsen Sea polynya (88.9%). In the Ross Sea, C. gaussi had the highest average abundance $(60.7 \pm 162.5 \text{ ind.} \text{L}^{-1})$. In the Amundsen Sea polynya, L. prolongata had the highest average abundance $(54.0 \pm 72.2 \text{ ind.} \text{L}^{-1})$ (Table 2). Occurrence frequency of all other species was less than 36% (Table 2). All species (except Cy. vanhoeffeni, which only occurred in one station) abundance proportion of loricae with protoplast were higher than 52.2% (Table 2).

3.3 Tintinnid community classification

We identified three and two distinctive tintinnid community groups in surface waters of the Ross Sea and Amundsen Sea polynya, respectively, based on cluster analysis using tintinnid species data. Among them, Group II only occurred in the Ross Sea (Figures 4 and 5). PERMANOVA tests showed significant differences between the two groups irrespective of the environment (pseudo-F = 11.686, P = 0.001) or abundance (pseudo-F = 8.9262, P = 0.001) data, which showed that the grouping was reasonable (Tables 3 and 4).

In the Ross Sea, Group I was dominated by *Cy. cristallina* and *L. prolongata* (abundance proportion > 50%). Group II was dominated by *C. gaussi* except in Sts R15–R17. Group III was dominant by *Cy. convallaria* (Figure 4). Among them, Group II had both the maximum abundance (816.0 ind.·L⁻¹, St. R19) and average abundance (114.4 ± 199.8 ind.·L⁻¹). This was followed by Groups I (34.3 ± 54.1 ind.·L⁻¹) and III (1.3 ± 0.3 ind.·L⁻¹). The abundance proportion of other species at most stations was less than 13.1% (except in St. R3, where the abundance proportion was 50%) (Figure 4).



Figure 2 Horizontal distribution of temperature, salinity, Chl *a* concentration, total tintinnid abundance and abundance proportion of loricae with protoplast in the Ross Sea and Amundsen Sea polynya.



Figure 3 Photomicrographs of tintinnid species in survey stations. **a**, *Cymatocylis vanhoeffeni*; **b**, *Cy. drygalskii*; **c**, *Cy. cristallina*; **d**, *Cy. convallaria*; **e**, *Laackmanniella prolongata*; **f**, *Codonellopsis gaussi*; **g**, *C. glacialis*; **h**, *Salpingella faurei*.

Table 2	Tintinnid species dimensions (LL: lorica length; LOD: lorica oral diameter), maximum abundance (A_{max}), average abundance
	(AA), abundance proportion of loricae with protoplasts (AP), dominance index (Y), occurrence frequency (OF), and station with
	maximum abundance (St.) in the Ross Sea and Amundsen Sea polynya

~ .			Ross Sea				Amundsen Sea polynya							
Species	LL/µm	LOD/µm	$A_{max}/$ (ind.·L ⁻¹)	AA/ (ind.·L ⁻¹)	AP/%	Y	OF/%	St.	$A_{max}/$ (ind.·L ⁻¹)	AA/ (ind.·L ⁻¹)	AP/%	Y	OF/%	St.
Codonellopsis gaussi	155.1 ± 21.2	36.3 ± 2.1	806.3	60.7 ± 162.5	87.8 ± 11.0	0.48	64.0	R19	8.3	0.9 ± 2.1	90.9 ± 18.1	< 0.01	22.2	A10
C. glacialis	42.68 ± 0.6	31.7 ± 0.4	0.6	0.1 ± 0.2	100.0 ± 0.0	< 0.01	16.0	R3	-	-	-	-	-	-
Cymatocylis convallaria	131.3 ± 12.7	86.7 ± 3.5	2.5	0.4 ± 0.7	100.0 ± 0.0	< 0.01	32.0	R7	23.5	2.1 ± 5.6	68.1 ± 27.9	< 0.01	33.3	A1
Cy. cristallina	213.5 ± 10.0	90.1 ± 4.5	152.0	13.7 ± 32.8	76.3 ± 21.8	0.12	68.0	R16	96.25	29.7 ± 30.5	52.2 ± 24.6	0.27	88.9	A15
Cy. drygalskii	286.2 ± 30.3	99.3 ± 5.7	16.1	0.8 ± 3.2	70.7 ± 47.8	< 0.01	16.0	R1	-	-	-	-	-	-
Cy. vanhoeffeni	297.9 ± 30.3	100.8 ± 0.5	1.6	0.1 ± 0.3	0.0 ± 0.0	< 0.01	4.0	R10	-	-	-	-	-	-
Laackmanniella prolongata	260.1 ± 3.0	40.0 ± 3.0	84.2	4.4 ± 16.8	94.0 ± 7.5	< 0.02	36.0	R1	239.9	54.0 ± 72.2	81.2 ± 8.2	0.47	83.3	A14
Salpingella faurei	87.7 ± 12.2	12.0 ± 0.8	-	-	-	-	-	-	30.7	9.7 ± 9.6	99.7 ± 1.0	0.08	83.3	A14
Total species			816.0	80.2 ± 166.4	86.2 ± 14.8	0.96	96.0	R19	343.9	96.4 ± 103.7	77.0 ± 11.0	1.00	100	A14

Table 3	Results of	of PERM	[ANO	VA based on Euc	lidean distance
	matrices	derived	from	log-transformed	environmental
	1 4 1 4				

data D				
Source	df	MS	Pseudo-F	Р
Groups	1	27.947	11.686	0.001
Residual	41	2.3915		
Total	42			

In the Amundsen Sea polynya, Group I was dominated by *Cy. cristallina*, *L. prolongata* and *S. faurei*. Group III was dominated by *Cy. convallaria* (Figure 5). The average

 Table 4
 Results of PERMANOVA based on Bray Curtis similarity matrices derived from Square root

transformed abundance data between two groups								
Source	df	MS	Pseudo-F	Р				
Groups	1	22525	8.9262	0.001				
Residual	41	2523.5						
Total	42							

abundance of Group I (106.6 \pm 105.7 ind. L^{-1}) was higher than that of Group III (14.7 \pm 12.5 ind. L^{-1}). No other species occurred in the Amundsen Sea polynya (Figure 5).



Figure 4 Three tintinnid community groups were classified by cluster analysis and its tintinnid abundance and abundance proportion at each station in the Ross Sea. Group I (red dotted box) was dominated by *Cymatocylis cristallina* and *Laackmanniella prolongata*; Group II (black dotted box) were dominated by *Codonellopsis gaussi*; Group III (blue dotted box) was dominated by *Cy. convallaria*.

3.4 Horizontal distribution of dominant species and *Cymatocylis convallaria*

The horizontal distribution of dominant species and *Cy. convallaria* in different seas showed distinctive characteristics (Figures 6 and 7). In the Ross Sea, high abundance of *C. gaussi* and *Cy. convallaria* was mainly distributed in the southern and central sea areas, respectively. The high abundance proportion of loricae with protoplasts (> 60%) of these two species was mainly distributed in the central Ross Sea (Figure 6). Both high

abundance and abundance proportion of loricae with protoplasts of *Cy. cristallina* were mainly distributed in the western Ross Sea near shelf areas (Figure 6). In the Amundsen Sea polynya, both high abundance and abundance proportion of loricae with protoplasts of *L. prolongata, Cy. cristallina* and *S. faurei* were mainly distributed on the southern side of 72.5°S, while *Cy. convallaria* was mainly distributed in opposite areas (Figure 7).

With respect to the distribution patterns of each species in surface waters of the two seas, *Cy. cristallina*,



Figure 5 Two tintinnid community groups were classified by cluster analysis and its tintinnid abundance and abundance proportion at each station in the Amundsen Sea polynya. Group I (red dotted box) was dominated by *Cymatocylis cristallina*, *Laackmanniella prolongata* and *Salpingella faurei*; Group III (blue dotted box) was dominated by *Cy. convallaria*.

L. prolongata, S. faurei and *Cy. convallaria* showed a similar characteristic (Figure 8). The distribution pattern of *C. cristallina* and *C. gaussi*, however, was inverse (Figure 8), especially in the Ross Sea (Figure 6).

3.5 Species richness and abundance proportion in tintinnid lorica oral diameter size class

The numbers of species richness and high abundance proportion in tintinnid lorica oral diameter (LOD) size classes were not consistent in the Ross Sea and Amundsen Sea polynya (Figure 9). Although the number of species richness in > 76 μ m LOD size-classes was highest in both the Ross Sea (4) and Amundsen Sea polynya (2), the highest abundance proportions were 32–36 μ m (75.7%) and 36–40 μ m (56.0%) LOD size class, respectively (Figure 8). *C. gaussi* and *L. prolongata* were the main contributors to these two LOD size classes, respectively. The 12–16 μ m LOD size class only occurred in the Amundsen Sea polynya, and *S. faurei* was the sole species found (Figure 9).



Figure 6 Abundance and abundance proportion of loricae with protoplast of *Codonellopsis gaussi* (**a**), *Cymatocylis cristallina* (**b**) and *Cy. convallaria* (**c**) in the Ross Sea.

3.6 Relationship between five tintinnids and environmental factors

Temperature–salinity–plankton diagrams showed that the high abundance of four abundant species and *Cy. convallaria* in the Ross Sea and Amundsen Sea polynya had different temperature and salinity ranges. The salinity range of tintinnid species in the Ross Sea (32.9–34.7) was relatively wider than in the Amundsen Sea polynya (33.1–34.3) (Figure 10). In the Ross Sea, *C. gaussi* and *Cy. cristallina* had a wider temperature range (-1.7° C to 1.5° C) than *L. prolongata* (-1.2° C to 1.2° C). In the Amundsen Sea polynya, *Cy. cristallina*, *L. prolongata* and *S. faurei* were found in high abundance and at temperatures > 0°C, whereas *Cy. convallaria* was found at temperatures < 1°C (Figure 10).

The abundance of each tintinnid species had a different correlation with environmental factors (depth, temperature, salinity and Chl *a*) (Table 5). *Cy. convallaria* and *S. faurei* had negative and positive correlations with temperature in the Amundsen Sea polynya, respectively. *C. gaussi* had a negative correlation with salinity in the Amundsen Sea

polynya. Cy. cristallina had a positive correlation with Chl a in both the Ross Sea and Amundsen Sea polynya (Table There was less correlation between 5). environmental and factors convallaria Cy. and L. prolongata.

4 Discussion

Dolan et al. (2012) examined 56 publications and found that 192 tintinnid species could be divided into endemic Southern Ocean species and widespread species. The assemblage of Southern Ocean endemics is found mostly within the Antarctic Zone, and delimited by the average location of the Polar Front (Dolan et al., 2012). The south side of the Polar Front, however, contains a huge sea area. There were few studies of tintinnid horizontal distribution in areas located on the southern side of the Polar Front. Our study found eight tintinnids in the Ross Sea and Amundsen Sea polynya, and several dominant species showed horizontal distribution characteristics according to their high abundance and abundance proportion of loricae with protoplasts.



Figure 7 Abundance and abundance proportion of loricae with protoplast of *Laackmanniella prolongata* (**a**), *Cymatocylis cristallina* (**b**), *Salpingella faurei* (**c**) and *C. convallaria* (**d**) in the Amundsen Sea polynya.

4.1 Tintinnid distribution characteristics in the Antarctic

Spatial distribution information is the key to understanding tintinnid function in the marine ecosystem of the Antarctic.

Horizontally, *C. glacialis* is mainly distributed in the Polar Front Zone. *C. gaussi* and *Cy. convallaria* are mainly distributed in the Polar Front and Antarctic Zones, while *L. naviculaefera* (a synonym of *L. prolongata*), *Cy. cristallina*, *Cy. drygalskii*, *Cy. vanhoeffeni* and *S. faurei*



Figure 8 Relationship between abundance of *C. cristallina* and *L. prolongata* (**a**), *Salpingella faurei* (**b**), *Codonellopsis gaussi* (**c**) and *Cy. convallaria* (**d**).



Figure 9 Species richness and abundance proportion of tintinnid LOD size-classes in the Ross Sea and Amundsen Sea polynya.

are mainly distributed in the Antarctic Zone (close to the coast) (Liang et al., 2020). Our results in the Ross Sea and Amundsen Sea polynya were similar to those of Liang et al. (2020).

Vertically, *C. gaussi* and *Cy. convallaria* were mainly distributed in summer surface water ranging from the surface to a depth of 100 m. *L. naviculaefera*, *Cy. cristallina*, *Cy. drygalskii*, *Cy. vanhoeffeni* and *S. faurei* were mainly distributed in winter water. A high abundance of *L. naviculaefera* and *Cy. cristallina* occurred in the upper 300 m depth. *Cy. drygalskii*, *Cy. vanhoeffeni* and *S. faurei* were mainly distributed at depths deeper than 50 m (Liang

et al., 2018). These findings may be the reason why *Cy. drygalskii*, *Cy. vanhoeffeni* and *S. faurei* were found in low abundance in surface waters of the Ross Sea and Amundsen Sea polynya in our results. Further studies into vertical distribution of these species are needed.

4.2 Horizontal distribution of tintinnids in the Ross Sea and Amundsen Sea polynya

Previous studies have shown that *Codonellopsis gaussi*, *C. glacialis* and *Laackmanniella prolongata* were dominant species in western and southern coastal areas of the Ross



Figure 10 Temperature-salinity-plankton diagrams for dominant tintinnid and *Cy. convallaria* in the Ross Sea and Amundsen Sea polynya. Black dots represent sampling points where no species occurred.

 Table 5
 Spearman's rank correlation between abundant tintinnid abundance and temperature, salinity and Chl a concentration

Species	Amundsen Sea					
Species	Temperature	Salinity	Chl a concentration			
Codonellopsis gaussi	-0.320	-0.559^{*}	-0.405			
Cymatocylis convallaria	-0.676**	-0.085	-0.188			
Cy. cristallina	0.331	0.263	0.506^{*}			
Laackmanniella prolongata	0.414	0.467	0.365			
Salpingella faurei	0.534^{*}	0.445	0.154			

Sea (Monti and Fonda Umani, 1995; Fonda Umani et al., 2002, 2005). Our study areas covered the central, eastern, western and southern Ross Sea, and can provide a comprehensive understanding of tintinnid community structure in surface waters. During the summer of 1987/1988 and 1989/1990, Monti and Fonda Umani (1995) found that the genus Cymatocylis was abundant in the offshore stations, while the species C. gaussi and L. naviculaefera (equal to L. prolongata) were abundant in stations closer to the coast. Our results also showed that L. prolongata was abundant in stations closer to the coast. Other species, however, showed a different distribution tendency in our results, where Codonellopsis gaussi and Cy. convallaria were abundant in offshore stations, and Cy. cristallina was abundant in the stations closer to the coast. We speculate that the limited co-occurrence of these species in previous studies might have been due to the small sampling scales employed in previous studies.

The Amundsen Sea is one of the most productive areas in the Southern Ocean (Smith et al., 2011; Arrigo and Alderkamp, 2012; Fragoso and Smith, 2012) and many coastal polynyas are found there. Our study area of Amundsen Sea polynya was similar to that of Dolan et al. (2013) and Jiang et al. (2014, 2016a, 2016b), who also studied a region of high productivity. A total of seven species was found during summer 2010/2011 and 2011/2012 (Dolan et al., 2013; Jiang et al., 2014, 2016a, 2016b). Of these studies, Dolan et al. (2013) defined Cy. convallaria and C. gaussi as polymorphic species and co-occurrence was found. Our study, however, found Cy. convallaria distributed in offshore stations, and this species had no co-occurring relationship with C. gaussi in the Amundsen Sea polynya. We also found that the co-occurrence of L. prolongata and Cy. cristallina appeared to be similar to that reported by Liang et al. (2018, 2020). This phenomenon was similar to the "species group" proposed by Fager and McGowan (1963). Their positive correlation might have been because they had similar reactions to environmental properties.

4.3 Tintinnid assemblage variation

The prey size of tintinnids is related to their LOD and their preferred food particle size is approximately 25% of their LOD (Dolan et al., 2002; Dolan, 2010). The LOD is both a key ecological and taxonomic characteristic for tintinnids (Dolan and Pierce, 2012). No previous reports, however, have discussed tintinnid LOD distribution on a large scale in surface waters of the Ross Sea. In the present study, the dominant LOD size class in the Ross Sea was found to be $32-36 \mu m$, which clearly differed from LOD composition in Amundsen Sea polynya (36–40 μm).

Previous studies have shown that Antarctic endemic species with larger LOD (> 100 μ m) size classes had a higher abundance proportion on the southern side of the Polar Front (Dolan et al., 2012; Liang et al., 2020). Our

results showed a similar phenomenon. Lee et al. (2012) reported that large-celled phytoplankton (> 20 μ m, 64.1%) were dominant in the polynya near the Antarctic continent, and small-celled phytoplankton were mainly distributed northwards to the non-polynya area in the Amundsen Sea. Therefore, the higher abundance proportion of the large tintinnid LOD size class in the Amundsen Sea polynya may relate to the greater amount of large phytoplankton in this area.

Because of their widespread distribution, identifiable morphology and outer lorica protection, the use of tintinnid species as a valuable bioindicator of various oceanographic conditions has been suggested, such as a bioindicator for different water masses (Balech, 1972; Taniguchi, 1983; Kato and Taniguchi, 1993; Rakshit et al., 2017; Wang et al., 2021). Previous studies showed that three main currents occurred in surface waters of the Ross Sea (Williams et al., 2015; Thompson et al., 2018). Our results of high abundance and abundance proportion of loricae with protoplast between *C. gaussi, Cy. convallaria* and *Cy. cristallina* might also indicate the boundary between the Antarctic Slope Front Current and Coastal Current in surface waters.

5 Conclusions

We report tintinnid species richness, horizontal distribution and relationship with environmental factors in the Ross Sea and Amundsen Sea polynya, Antarctic, during summer 2019/2020. Three distinctive tintinnid groups were classified according to cluster analysis. Among them, dominant species showed distinctive horizontal distribution characteristics. High abundance and abundance proportion of loricae with protoplast distribution area of Cy. cristallina and C. gaussi might can reveal approximate boundary of the Antarctic Slope Front Current and Coastal Current. Temperature - one element in the relationship between tintinnids and environmental factors - may be the main reason for tintinnid species distribution in surface waters. Our findings provide basic data and an approach that helps to explain increasing climate changes in Antarctic ecosystems.

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