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Microzooplankton herbivory and community structure in the Amundsen Sea, Antarctica

Eun Jin Yang [a,*], Yong Jiang [b], SangHoon Lee [a]

[a] Division of Polar Ocean Environment, Korea Polar Research Institute, 213-3 Songdo-dong, Yeonsu-gu, Incheon 406-840, Republic of Korea
[b] College of Marine Life, Ocean University of China, Qingdao 266003, PR China

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ABSTRACT

We examined microzooplankton abundance, community structure, and grazing impact on phytoplankton in the Amundsen Sea, Western Antarctica, during the early austral summer from December 2010 to January 2011. Our study area was divided into three regions based on topography, hydrographic properties, and trophic conditions: (1) the Oceanic Zone (OZ), with free sea ice and low phytoplankton biomass dominated by diatoms; (2) the Sea Ice Zone (SIZ), covered by heavy sea ice with colder water, lower salinity, and dominated by diatoms; and (3) the Amundsen Sea Polynya (ASP), with high phytoplankton biomass dominated by *Phaeocystis antarctica*. Microzooplankton biomass and communities associated with phytoplankton biomass and composition varied among regions. Heterotrophic dinoflagellates (HDF) were the most significant grazers in the ASP and OZ, whereas ciliates co-dominated with HDF in the SIZ. Microzooplankton grazing impact is significant in our study area, particularly in the ASP, and consumed 55.4–107.6% of phytoplankton production (average 77.3%), with grazing impact increasing with prey and grazer biomass. This result implies that a significant proportion of the phytoplankton production is not removed by sinking or other grazers but grazed by microzooplankton. Compared with diatom-based systems, *Phaeocystis*-based production would be largely remineralized and/or channeled through the microbial food web through microzooplankton grazing. In these waters the major herbivorous fate of phytoplankton is likely mediated by the microzooplankton population. Our study confirms the importance of herbivorous protists in the planktonic ecosystems of high latitudes. In conclusion, microzooplankton herbivory may be a driving force controlling phytoplankton growth in early summer in the Amundsen Sea, particularly in the ASP.

1. Introduction

Microzoopankton, a key component of pelagic food webs, have long been considered as major grazers of phytoplankton in various marine environments (Archer et al., 2000; Choi et al., 2012; Sherr et al., 2013; Strom et al., 2001; Yang et al., 2012; Teixeira et al., 2012), consuming 60–70% of daily phytoplankton production worldwide (Calbet and Landry, 2004; Schmoker et al., 2013). Microzooplankton are also important food sources for consumers at higher trophic levels (Campbell et al., 2009; Saiz and Calbet, 2011), because mesozooplankton herbivory appears to be relatively low, accounting for only 10% of global primary production consumed daily (Calbet and Saiz, 2005). Thus, microzooplankton occupy an important trophic node in planktonic food webs, and their dynamics may be controlled by predation, resource availability, or hydrography (Choi et al., 2012; Safi et al., 2007). Therefore, details of microzooplankton community structure and herbivory are central to understanding carbon flow and the fate of primary production in marine ecosystem. Over the past two decades, many studies on microzooplankton have been performed in various waters worldwide (Schmoker et al., 2013). However, the quantitative role of microzooplankton in the pelagic food webs of polar oceans remains poorly understood.

Assessments of microzooplankton grazing impacts and biomass in the Southern Ocean have yielded variable results. In the Southern Ocean, microzooplankton exhibit distinct seasonality and extreme patchiness (Garzio and Steinberg, 2013; Landry et al., 2002) and the dominant species can change dramatically in different water masses (Safi et al., 2007; Selph et al., 2001; Yang et al., 2012). To date, many studies of microzooplankton in the Southern Ocean have emphasized their role as major consumers of phytoplankton (Burkill et al., 1995; Garzio et al., 2013; Landry et al., 2001; Pearce et al., 2011; Safi et al., 2007). In contrast, others have reported that microzooplankton grazing does not always significantly affect phytoplankton production

* Corresponding author. Tel.: +82 32 760 5334; fax: +82 32 760 5399.
E-mail address: ejyang@kopri.re.kr (E.J. Yang).

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(Caron et al., 2000; Froneman, 2004). Overall, previous research has suggested that the magnitude and impact of grazing by microzooplankton on phytoplankton varies greatly by place and time (Calbet and Landry, 2004; Schmoker et al., 2013), and that the quantitative role of microzooplankton in the food web is central to developing an understanding of carbon flux in the Southern Ocean.

The Amundsen Sea, which is historically known as a region of heavy ice, is currently undergoing sea ice recession (Jacobs and Comiso, 1993), and extensive phytoplankton blooms have been observed near its coasts (Smith and Comiso, 2008). Most studies in the Amundsen Sea have focused on examining the rapid retreat of the West Antarctic Ice Sheet, in relation to the upwelling of the warm Circumpolar Deep Water onto the Antarctic continental shelf (Walker et al., 2007; Wåhlin et al., 2010). Therefore, the Amundsen Sea has been described as one of the most productive and dynamic pelagic systems in the Southern Ocean (Smith et al., 2011). Rapid melting of glaciers and losses of sea ice in this area may profoundly alter ecosystem change by changing pathways of energy flow. To predict the impacts of these changes on ecosystems, it is important to understand the dynamics of the plankton community. Although oceanographic surveys have been conducted in the Amundsen Sea, ecological studies of plankton have concentrated on primary production, ciliates, mesozooplankton, and krill (Arrigo et al., 2012; Fragoso and Smith, 2012; Lee et al., 2012, 2013). Three studies on ciliates have focused on the species composition, morphology, and phylogeny of ciliates (Dolan et al., 2013; Kim et al., 2013; Jiang et al., 2014).

To our knowledge, this study represents the first step toward understanding the role of microzooplankton in the pelagic ecosystems of the Amundsen Sea. Here, we investigate spatial variation in microzooplankton assemblages and their grazing impacts on phytoplankton in various hydrographic regions within the productive Amundsen Sea. Our results emphasize the need for further research for a broader perspective on trophic linkages between the phytoplankton and microzooplankton in the different water mass during austral summer.

2. Materials and methods

2.1. Study area

The Korea Anarctic Research Program mounted a multidisciplinary survey was conducted onboard the *IBRV* Araon in the Amundsen Sea during early austral summer from 27 December 2010 to 23 January 2011 (Fig. 1). 12 Sampling stations were selected from three regions and defined as follows: (1) the Oceanic Zone (OZ), which was located in deep offshore open waters with free sea ice (stations 27, 28 and 29), (2) the Sea Ice Zone (SIZ), which was connected to both the OZ and polynya, had heavy sea ice cover (stations 6, 7, 22, and 24), and (3) the Amundsen Sea Polynya (ASP), which was open water surrounded by sea ice (stations 8, 9, 13, 18 and 21). Areas of sea ice and concentrations were based on data from the National Snow and Ice Data Center in Boulder, Colorado, that corresponded to the cruise period. The classification of three regions based on sea ice concentration, geographical features, hydrographic properties and trophic conditions follows Yager et al. (2012), Lee et al. (2012, 2013), and Jiang et al. (2014).

2.2. Chlorophyll-a concentration

To collect water samples for measurements of chlorophyll-*a* (chl-*a*) concentration, we installed 12 Niskin bottles (20 l each) on the CTD frame to sample waters at depths of 5, 10, 20, 30, 75, 100, 150 m and/or at the depth of the subsurface chlorophyll maximum (SCM). Water samples (500–1000 ml) for chl-*a* concentration were

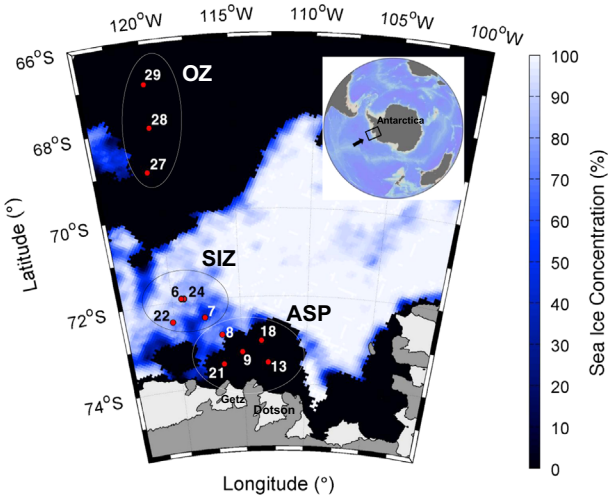

Fig. 1. Sampling stations in the Amundsen Sea from December 2010 to January 2011. Sea ice concentration was derived from SSM/I data during the survey period of January 1–12 in 2011.

taken from each depth and immediately filtered through glass fiber filter paper (47 mm; Gelman GF/F). Concentration of chl-*a* were measured onboard using a Turner design Trilogy fluorometer after extraction with 90% acetone (Parsons et al., 1984). The fluorometer had been previously calibrated against pure chl-*a* (Sigma).

2.3. Microzooplankton biomass and community composition

To determine abundances of microzooplankton by depth, we used a Niskin rosette sampler to collect water samples at 5, 10, 20, 30, 75, 100, 150 m and/or SCM. For heterotropic dinoflagellate (HDF), heterotrophic nanoflagellates (HNF) and choanoflagellates (CNF) enumeration, we preserved 300 ml samples of water with glutaraldehyde (1% final concentration), then stored them at 4 °C before staining and filtration. Subsample of 20–50 ml was filtered onto nuclepore filters (0.8 μm pore size, black) for 3–20 μm sized plankton and 50–200 ml subsample was filtered onto nuclepore filters (8 μm pore size, black) for >20 μm sized plankton. Aliquots of the preserved samples were stained with proflavin (0.33%) for an hour before filtration. During filtration, the samples were drawn down until 5 ml remained in the filtration tower. Concentrated DAPI (50 μg ml^{-1} final concentration) was then added and allowed to sit briefly (5 s) before filtering the remaining sample until dry (Taylor et al., 2011). Filters were mounted onto glass slides with immersion oil and cover slips. For HDF, HNF and CNF cells, at least 50 fields per sample were counted with an epifluorescence microscope (Olympus BX 51) at magnifications of 200–640 × using blue light excitation filter set for chlorophyll autofluorescence and UV light excitation filter set for DAPI stained cells. For ciliate enumeration, 500 ml samples of water were preserved with 4% acid Lugol's iodine solution and subsequently stored in darkness. Preserved samples were allowed to settle in the mass cylinder for at least 48 h. The upper water layer was then siphoned out, leaving 20 ml. Subsequently, 1 ml aliquot of each concentrated sample was placed in a S-R chamber and counted under a light microscope (Olympus BX51). Microzooplankton was classified as unidentified HNF, CNF, ciliates and HDF.

2.4. Phytoplankton biomass and community composition

To analysis phytoplankton community composition which have been considered as prey for microzooplankton, at the beginning of each experiment, phytoplankton sample were siphoned directly from a Niskin rosette sampler in the depth at which dilution

experiments were conducted at each station (Table 2). The method to observe the autotrophic nanoflagellates (ANF) is the same as that of HNF (see Section 2.2). Autotrophic organisms were distinguished from heterotrophs by the presence of chlorophyll, which was visualized as red fluorescence under blue light illumination. Most *Phaeocystis antarctica* cells are present as a solitary form in the water column. Solitary *P. antarctica* cells were distinguished from other autotrophic flagellates based on cell size (about 3–6 μm) and shape, chloroplast arrangement, and the presence of flagella. For picophytoplankton cells, at least 200 cells per sample were counted with an epifluorescence microscope (Olympus BX 51) at magnifications of 1000 × using blue light excitation. The method to observe diatoms is the same as that of ciliates (see Section 2.2). Phytoplankton was classified as picophytoplankton, ANF, *P. antarctica* and diatoms.

2.5. Plankton carbon biomass

To estimate the carbon biomass of plankton, cells were sized using image analysis system standardized by a calibrated ocular micrometer and calculated cell volume by measuring cell dimensions (Edler, 1979; Winberg, 1971). The following conversion factors and equations were used to transform cell volumes into carbon biomass: 0.19 μg C μm^{-3} for naked ciliates (Putt and Stoecker, 1989); 0.053 pg C μm^{-3} for loricate ciliates (Stoecker et al., 1994); carbon (pg) = 0.216 × [volume, μm^3]$^{0.939}$ for dinoflagellates and diatoms (Menden-Deuer and Lessard, 2000); 3.33 pg C $cell^{-1}$ for solitary *P. antarctica* (Mathot et al., 2000), and 220 fg C μm^{-3} for nanoflagellates and picophytoplankton (Bøsheim and Bratbak, 1987).

2.6. Grazing experiments

We estimated phytoplankton growth and microzooplankton grazing rates by the dilution method through measurements of changes in total chl-*a* concentration (Landry and Hassett, 1982). All equipment for the grazing experiments was cleaned with 10% HCl in Milli-Q water and rinsed thoroughly thrice in Milli-Q water before experiments. Plastic gloves were worn during all phases of the experiments. The stations at which dilution grazing experiments were conducted are shown in Table 2. At each station, 30 l seawater were collected in a Niskin bottle and transferred to a polycarbonate carboy. To avoid damaging delicate microzooplankton and altering phytoplankton composition, particularly in phytoplankton bloom environment, samples were not screened prior to incubation (Calbet et al., 2011). Instead, larger zooplankton such as copepods was removed using a glass pipette. Water was prepared by gravity filtration from the water bottle through an in-line filter capsule (Gelman Critcap 100, 0.2 μm pore size filter, pre-washed with 10% trace-metal grade HCl followed by Milli-Q and seawater rinses) into a clean polycarbonate bottle. The prepared water was then diluted with 0.2 μm filtered seawater to obtain duplicates containing the following proportions of prepared water: 100%, 75%, 50%, 25%, and 11%. The dilution series was established in ten 1.3 l polycarbonate bottles. Macronutrients were not added to the experimental bottles because phytoplankton growth is generally not limited by macronutrients (*e.g.* nitrate and phosphate) in the Southern Ocean. All dispensing was conducted gently to avoid cell rupture and damage. The bottles were incubated on deck for 48 h at ambient sea surface temperatures and screened to ambient light levels with neutral density screens. Subsamples were collected for each experiment at the beginning (T_0, undiluted treatment bottle) and at the end (T_{48}, each treatment bottle) of the incubation to estimate chl-*a* concentrations.

We used a linear regression model for all experiments to find the best-fit relationship between phytoplankton net growth rate and dilution level (Landry and Hassett, 1982). Phytoplankton growth rate (μ) and grazing rate (g) were estimated from the y-intercept and the negative slope of the relationship, respectively. The impacts of microzooplankton grazing on phytoplankton production (%PP) and phytoplankton standing stock (%PS) were determined following the calculation procedures of Verity et al. (1993). All statistical tests were performed using the SPSS software (ver. 9.0).

Changes in chl-*a* fluorescence per cell during incubation in the dilution experiments can provide information on phytoplankton photoadaptation that could lead to overestimation or underestimation of the phytoplankton growth rates based on chl-*a* measurements (Brown et al., 1999; Liu et al., 2002). Chl-*a* fluorescence per cell was assessed by flow cytometry (BD Accuri C6, Lee et al., 2014) from initial and final samples of the undiluted experiment bottle. Chl-*a* fluorescence per cell in picoeukaryote groups showed a minor decrease (average 5%) in the final samples compared to the initial samples (Yang et al., unpublished data). The picoeukaryotic data, which may be more comparable to the large eukaryotic cells, implies that the overall effect of photoadaptation to chl-*a* based rate estimates in this study could be insignificant.

3. Results

3.1. Chlorophyll-a concentrations, and phytoplankton biomass and composition

Chl-*a* concentrations varied widely among stations and gradually increased from the OZ (average 39.7 mg m^{-2}) to the ASP (average 452.5 mg m^{-2}) (Fig. 2). The vertical distribution of chl-*a* was relatively uniform in the OZ, but decreased with depth in the ASP. We observed the highest chl-*a* concentration in the ASP, with a maximum of 12.1 μg l^{-1}. The phytoplankton assemblages varied considerably in both composition and biomass (Table 1). Phytoplankton biomass ranged from 22.8 to 448.9 μg C l^{-1}, and was highest in the ASP. The distribution of phytoplankton biomass was similar to that of chl-*a*. During the study period, diatoms and *P. antarctica* predominated in the phytoplankton composition. Phytoplankton in the SIZ was dominated by diatoms, comprising mostly single or colony *Fragilariopsis* spp. and nano-sized pennate diatoms. Likewise, phytoplankton in the OZ was also dominated by various diatoms, comprising *Fragilariopsis* spp., *Chaetoceros* spp., *Nitzshcia* spp., *Navicula* spp., and *Rhizosolenia* spp., and diatoms accounted for an average 68 ± 17% of phytoplankton biomass (Fig. 3). Diatom taxa in the ASP were also similar to those of the OZ. In the OZ, ANF and picophytoplankton were also abundant compared with other regions, and they accounted for an average of 31% of phytoplankton biomass. *P. antarctica* accounted for > 70% of phytoplankton biomass in the ASP except at stn. 8, which was co-dominated by *P. antarctica* and diatoms. Most *P. antarctica* existed as solitary cells, while the small colony type was rarely observed in the ASP.

3.2. Microzooplankton abundance, biomass, and community composition

The abundances of microzooplankton assemblages varied considerably among stations and depths (Figs. 4 and 5). The abundance of HNF ranged from 40 to 3200 cells ml^{-1}, averaging 560 ± 528 cells ml^{-1}. The abundance of CNF ranged from 0 to 1854 cells ml^{-1}. Abundances of HNF and CNF were higher in the stns. 21 and 9, respectively. The ciliate assemblage was numerically dominated by naked ciliates. Although loricate ciliates occurred in this study area, they represented only a small fraction of overall ciliate abundance. Ciliates abundance ranged from 360 to 4227 cells l^{-1}, averaging 1295 ± 1100 cells l^{-1}. Ciliates were most abundant in the upper mixed layer at stns. 9, 18 and 21. The HDF were numerically dominated by athecate HDF, which were further categorized into

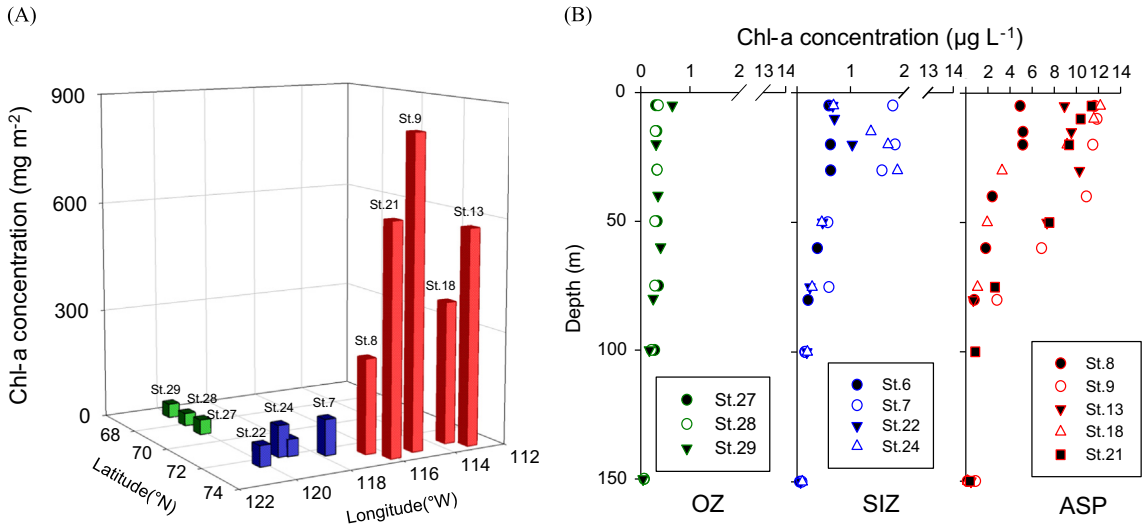

Fig. 2. Spatial and vertical distribution of chlorophyll-*a* concentration in the Amundsen Sea. (A) is for depth-integrated value from surface to 150 m and (B) is for vertical profiles. OZ – Oceanic Zone; SIZ – Sea Ice Zone; ASP – Amundsen Sea Polynya.

Table 1
Depth average biomass (μg C L^{-1}) of phytoplankton community in the Amundsen Sea.

Area division	Station	Picophytoplankton	*Phaeocystis antarctica*	Diatoms	Autotrophic nanoflagellates	Total carbon biomass
Oecanic zone (OZ)	29	2.09	0.09	11.40	9.24	22.82
	28	1.86	0.02	20.00	3.69	25.57
	27	2.41	0.95	18.03	3.00	24.38
Sea Ice Zone (SIZ)	22	0.08	0.75	64.18	5.80	70.80
	24	0.22	3.26	39.17	4.46	47.11
	6	1.03	0.39	40.28	5.87	47.57
	7	0.35	2.09	105.71	3.61	111.76
Amundsen Sea Polynya (ASP)	8	0.23	61.20	54.00	4.67	120.09
	9	9.90	246.79	141.53	15.15	413.38
	18	1.30	345.50	96.70	5.39	448.89
	21	1.60	338.77	46.90	8.71	395.98
	13	2.00	279.80	83.00	2.75	367.61
OZ	Avg. ± SD	2.12 ± 0.27	0.35 ± 0.51	16.47 ± 4.50	5.31 ± 3.42	24.25 ± 1.37
SIZ	Avg. ± SD	0.42 ± 0.42	1.62 ± 1.31	62.33 ± 31.13	4.93 ± 1.09	69.31 ± 30.38
ASP	Avg. ± SD	3.00 ± 3.91	254.41 ± 115.5	84.42 ± 37.91	7.33 ± 4.87	349.19 ± 131.4

nanoHDF (< 20 μm) and microHDF (> 20 μm), the abundances of which ranged from 1000 to 219,000 cells l^{-1} (average 45,000 ± 41,000 cells l^{-1}) and from 88 to 18,910 cells l^{-1} (average: 3510 ± 3900 cells l^{-1}), respectively. The abundance of thecate HDF ranged from 1000 to 219,000 cells l^{-1} and was highest in the ASP. The abundances of microHDF, including thecate (> 20 μm) and athecate (> 20 μm) HDF, and nanoHDF were highest at stns. 9, 18 and 21. Microzooplankton biomass ranged from 0.94 to 152.6 μg C l^{-1} (average 33.9 ± 36.2 μg C l^{-1}) and was relatively high in upper mixed layers at all stations (Fig. 6). Depth-integrated microzooplankton biomass from the surface to 150 m ranged from 1152.2 to 12,191.8 mg C m^{-2}, averaging 3722.5 mg C m^{-2} (Fig. 6). The highest microzooplankton biomass occurred in the ASP, particularly at stn. 9. HDF comprised the largest proportion of the microzooplankton assemblage. The HDF biomass contributed an average of 43% to the total microzooplankton biomass in the SIZ, and over 60% in the ASP and OZ. Of the HDF, absolute thecate HDF biomass, comprised mostly of *Protoperidinium* spp., was highest in the ASP. Athecate HDF biomass consisted mainly of *Gyrodinium* spp. and *Gymnodinium* spp., and accounted for an average of 41.2 ± 27.2% of the microzooplankton biomass. Although their absolute biomass was high in the ASP, their contribution to microzooplankton was highest in the OZ. Ciliate biomass averaged 7.8 ± 5.6 μg C l^{-1}, and accounted for an average 20.7 ± 14.1% of microzooplankton biomass. Although ciliate biomass was high in the ASP, the proportional contribution of ciliates to the microzooplankton was highest in the SIZ. Absolute HNF and CNF biomass were high in the ASP, and the proportional contributions of these organisms to microzooplankton biomass were < 20% in all three regions. Total microzooplankton biomass was positively correlated with total chl-*a* concentration (Fig. 7A). Furthermore, HDF biomass was strongly related to the proportional contribution of the *P. antarctica* to total phytoplankton biomass (Fig. 7B).

3.3. Grazing impact of microzooplankton on phytoplankton

Phytoplankton growth rates and microzooplankton grazing rates for all 25 dilution experiments are summarized in Table 2. Negative phytoplankton growth was observed in 1 of the 25 dilution experiments due to high negative intrinsic growth rates, and significant slopes were observed in 20 of the 24 experiments. To avoid biasing the data, all coefficients were included, whether or not they were significant, in our estimates of average rates. Growth rates of phytoplankton ranged from 0.25 to 0.43 d^{-1}, averaging 0.33 ± 0.04 d^{-1}. Grazing rates ranged from 0.15 to 0.41 d^{-1}, averaging 0.24 ± 0.07 d^{-1}. Growth and grazing rates were relatively low in the SIZ compared to the ASP and OZ, with statistically significant differences among regions (one-way ANOVA, $P < 0.001$). The microzooplankton grazing rate exceeded the phytoplankton growth rate at two sites in the ASP (Table 2). Grazing rate was significantly correlated with phytoplankton

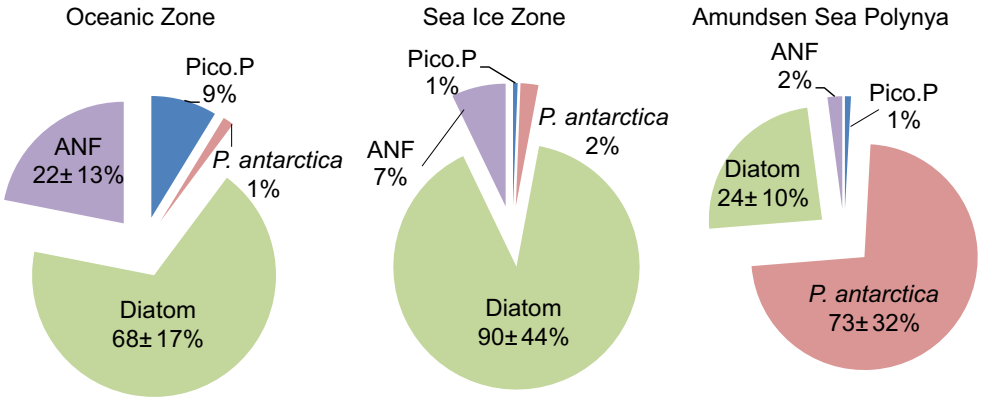

Fig. 3. Average relative contribution of phytoplankton community to total phytoplankton biomass in the Amundsen Sea. ANF – autotrophic nanoflagellate; PicoP. – picophytoplankton.

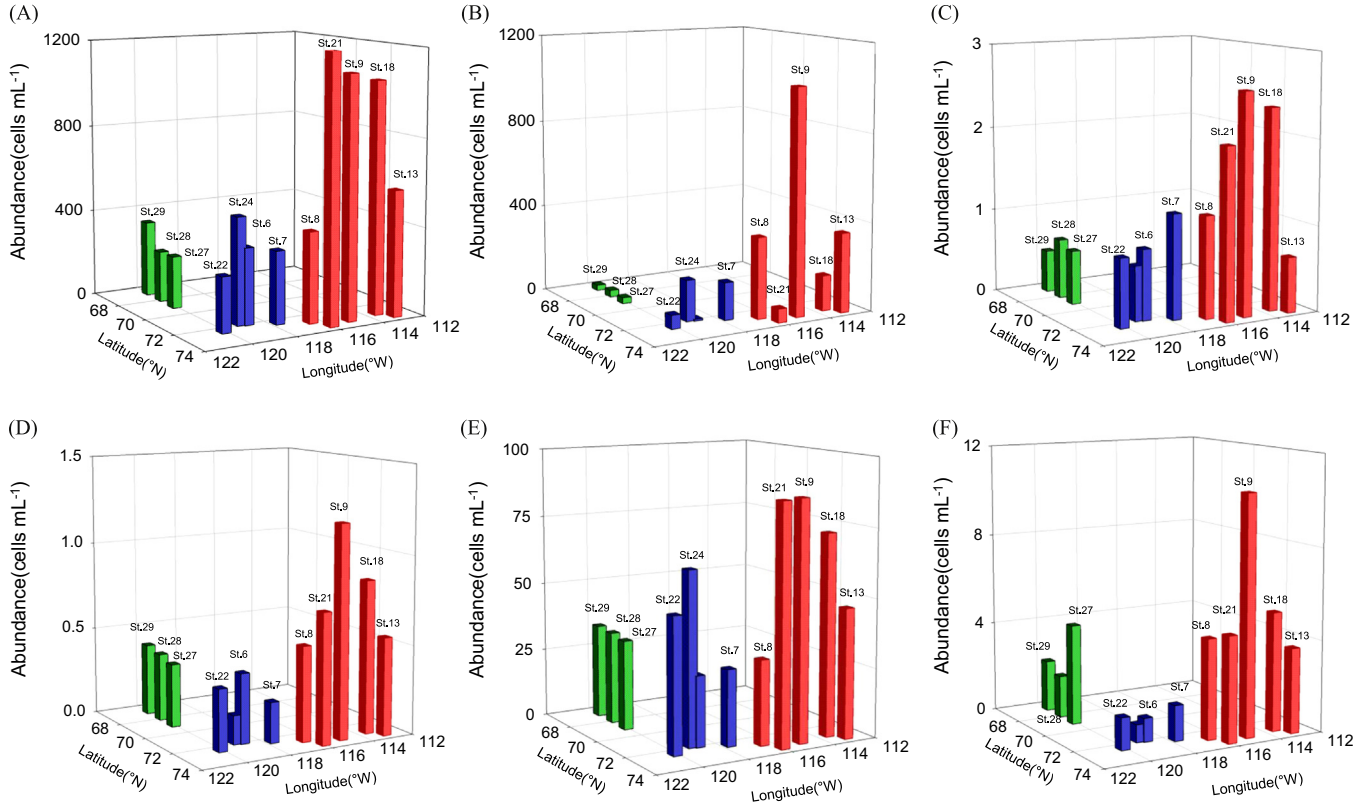

Fig. 4. Spatial distribution of microzooplankton composition in the Amudnsen Sea. (A) Heterotrophic nanoflagellate (HNF), (B) choanoflagellate (CNF), (C) ciliates, (D) thecate-heterotrophic dinoflagellate (> 20 μm) (thecate HDF, > 20 μm), (E) athecate-heterotrophic dinoflagellate (< 20 μm) (athecate HDF, < 20 μm) and (F) athecate-heterotrophic dinoflagellate (> 20 μm) (athecate HDF, > 20 μm).

growth rate ($P < 0.01$) and initial chl-*a* concentration with statistically significant ($P < 0.01$) (data not shown). The daily proportion of chl-*a* standing stock consumed by microzooplankton ranged from 13.9% to 33.6% (average 21.5 ± 5.3%). Microzooplankton grazing consumed 55.4–107.6% of daily phytoplankton production. Phytoplankton were most heavily grazed in the ASP, with an average of 89.7% of daily production removed. On average, more than half of daily phytoplankton production (average 77.3 ± 13.1%) was consumed by microzooplankton, but there was wide variation over the study period. Grazing impact increased with increasing prey (initial chl-*a* concentration) and grazer biomass (Fig. 8). It was also positively correlated with the HDF biomass and the ratio of *P. antarctica* to phytoplankton biomass (Fig. 8).

4. Discussion

4.1. Environmental conditions, and phytoplankton biomass and composition

The plankton ecosystem across the Southern Ocean has been directly influenced by changes in physical forcing and hydrographic conditions (Garzio and Steinberg, 2013; Landry et al., 2001; Safi et al., 2007; Yang et al., 2012). Over the study period, we encountered diverse environmental conditions from heavy sea-ice cover (100%) to oceanic water (< 10% cover), and a concomitant range of trophic conditions ranging from low chl-*a* ($< 0.5\ \mu g\ l^{-1}$) in oceanic water to high chl-*a* concentration ($12.1\ \mu g\ l^{-1}$) in the ASP (Table 1; Fig. 2). Based on physical parameters, location, sea ice concentration, and trophic conditions, the study area was divided into three

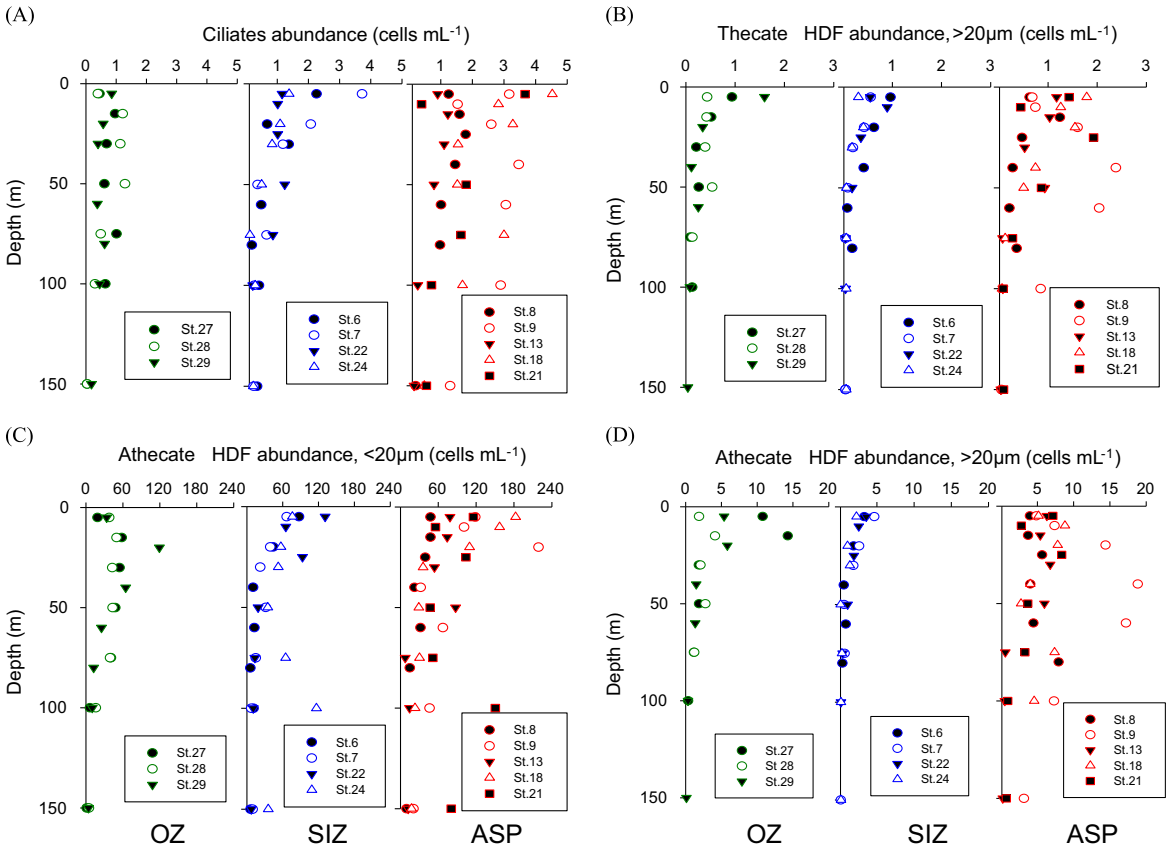

Fig. 5. Vertical profiles of microzooplankton composition in the Amudnsen Sea. (A) Ciliates, (B) thecate-heterotrophic dinoflagellate (> 20 μm), (C) athecate-heterotrophic dinoflagellate (< 20 μm) and (D) athecate-heterotrophic dinoflagellate (> 20 μm). HDF, heterotrophic dinoflagellate.

regions: OZ, SIZ and ASP (Dolan et al., 2013; Hahm et al., 2014; Lee et al., 2012, 2013; Jiang et al., 2014). The OZ was in deep offshore open waters with free sea ice and low primary productivity. The SIZ, which was connected to both the OZ and ASP, had heavy sea ice cover, colder sea water, lower salinity, and intermediate chl-*a*. The ASP was characterized by phytoplankton blooms and higher seawater temperatures (Figs. 2 and 3). Phytoplankton blooms in the ASP were initiated by a combination of increased light availability and increased seawater temperatures in early summer (Hahm et al., 2014). Phytoplankton in the ASP were dominated by *P. antarctica*, although we cannot ignore the presence of diatoms, which constituted an average 24 ± 10% of phytoplankton biomass. Diatoms in the SIZ and OZ accounted for an average of 90 ± 44% and 68 ± 17% of phytoplankton biomass, respectively, which means that diatoms were the most important component in the SIZ. The diatoms in the SIZ were dominated by *Fragilariopsis* spp. reported as pack ice algae (Lizotte, 2001). A similar pattern of *P. antarctica* bloom in the ASP and diatom domination in the SIZ was described previously in the Amundsen and Ross Seas (Alderkamp et al., 2012; Smith et al., 2000). Diatoms and *P. antarctica* have different nutrient utilization characteristics and support very different higher trophic level communities (Arrigo et al., 1999). Therefore, the relative contributions of these two phytoplankton taxa influence the biogeochemistry and ecology of the region, and may be climate sensitive (*e.g.*, Alderkamp et al., 2012; Arrigo et al., 1999; Fragoso and Smith, 2012). Furthermore, differences in phytoplankton composition and biomass according to water mass may be influenced by the dynamics of microzooplankton and/or higher-trophic-level organisms.

4.2. Microzooplankton biomass and community structure

Combined studies of ciliates, HDF, and HNF (including CNF) biomass in the Southern Ocean are rare (Becquevort et al., 1992; Garrison and Buck, 1989; Safi et al., 2007; Stoecker et al., 1995; Yang et al., 2012). This study is the first comprehensive description of microzooplankton communities in the Amundsen Sea. During our study period, microzooplankton abundance and biomass were in the same range or higher than those reported previously from the Southern Ocean (Garzio and Steinberg, 2013; Pearce et al., 2008; Stoecker et al., 1995). Microzooplankton biomass varied strongly among regions and depth, and was associated with the distribution of phytoplankton biomass (Fig. 7). That is, the resulting high phytoplankton biomass led to high microzooplankton biomass (Archer et al., 1996; Burkill et al., 1995), as evidenced by the significant positive correlation between microzooplankton and chl-*a* (Fig. 7). These results suggest that microzooplankton populations can increase rapidly in response to elevations in phytoplankton biomass; this rapid response in microzooplankton is probably attributable to high rates of population growth, which can match those of phytoplankton (Banse, 1992; Frost, 1993).

Of the microzooplankton, HDF were numerically the most important component, ranging from 20.1% to 80.1% of microzooplankton biomass (Fig. 6). Large HDF; *e.g.*, *Gyrodinium* > 100 μm in length and *Protoperidinium* spp., were especially abundant in the ASP where *P. antarctica* were the dominant primary producers. *Protoperidinium* spp. were uniquely important components in the ASP, although they are often associated with diatom blooms in the Southern Ocean (Archer et al., 1996). In contrast, athecate HDF accounted for > 50% of microzooplankton in the OZ, which was dominated by diatoms. The importance of HDF in colonial and/or single-celled *Phaeocystis* blooms and diatom blooming systems has

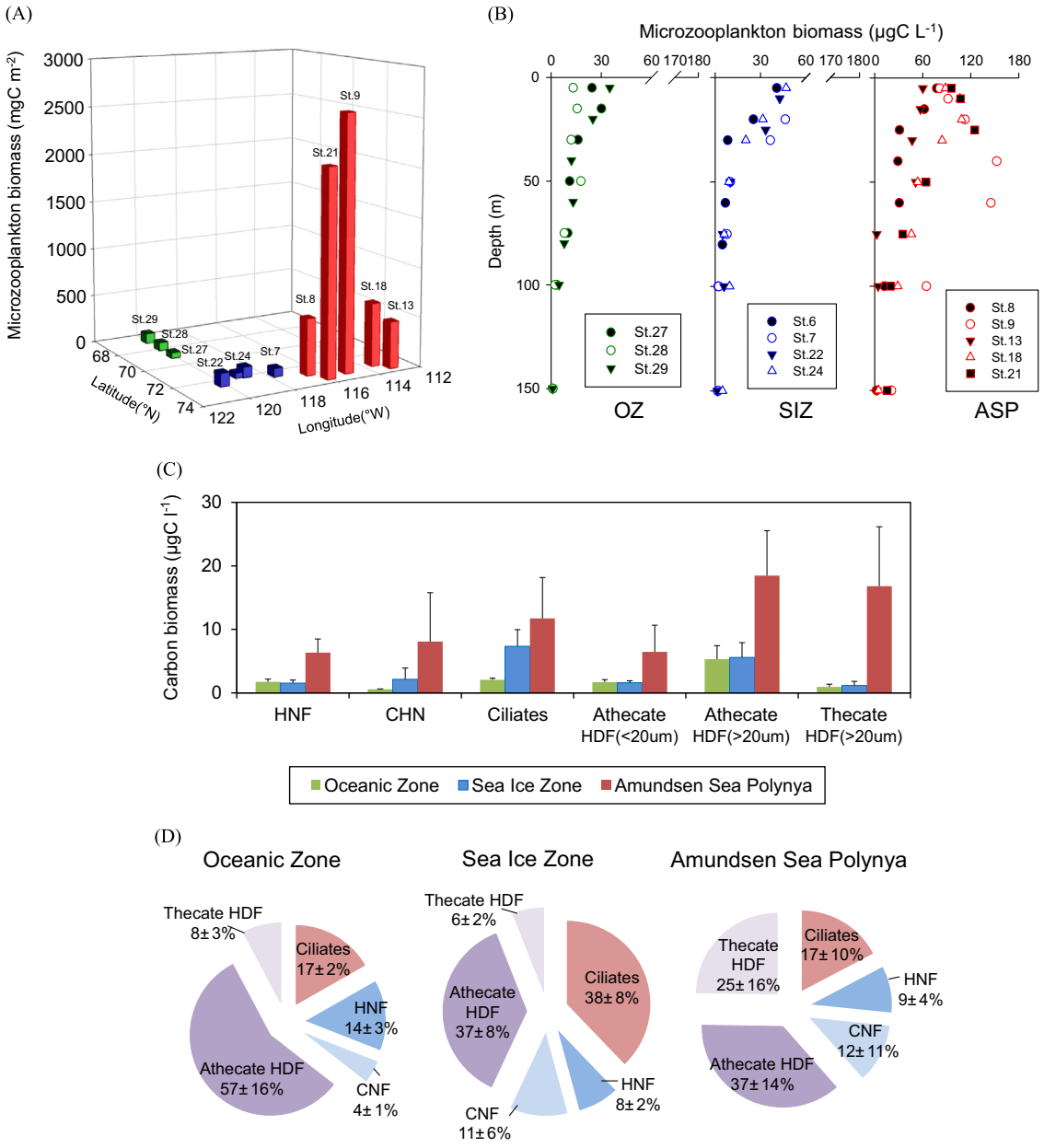

Fig. 6. Carbon biomass of microzooplankton in the Amundsen Sea: (A) is for depth-integrated carbon biomass from surface to 150 m, (B) is for vertical profile, (C) is average biomass for microzooplankton community in different regions, and (D) is for average relative contribution of microzooplankton community. HNF, heterotrophic nanoflagellate; CNF, choanoflagellate; HDF, heterotrophic dinoflagellate.

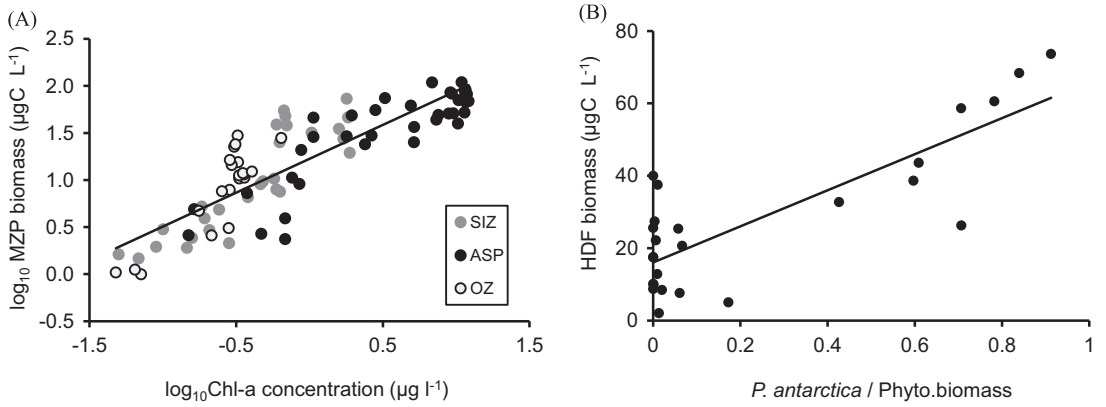

Fig. 7. Log–log relationship between microzooplankton (MZP) biomass and chlorophyll-*a* concentration (A), and heterotrophic dinoflagellate (HDF) biomass and *Phaeocystis antarctica*/ phytoplankton biomass ratio (B) in the Amundsen Sea. Regression equation: (A) $y=0.72x+1.22$ ($R^2=0.74$, $p<0.001$) and (B) $y=49.80x+16.0$ ($R^2=0.66$, $p<0.01$). OZ – Oceanic Zone; SIZ – Sea Ice Zone; ASP – Amundsen Sea Polynya.

Table 2
Summary parameters and results of grazing impact by microzooplankton derived from dilution experiments. μ: phytoplankton growth rate, g: microzooplankton grazing rates, PS (%): daily phytoplankton standing stocks grazed, PP (%): daily phytoplankton production grazed, r^2: correlation coefficient, $p < 0.05$, ns: not significant, SD: standard deviation.

Area division	Station	Mixed layer depth (m)	Sampling depth (m)	Initial Chl-*a* ($\mu g\ l^{-1}$)	μ (d^{-1})	g (d^{-1})	PS (%)	PP (%)	r^2	Temperature (°C)	Nitrate (μM)
Oecanic zone	29	12	5	0.69	0.43	0.28	24.4	69.8	0.91	−0.93	25.07
(OZ)	29		15	0.76	0.39	0.24	21.3	66.1	0.92	−1.30	27.36
	29		25	0.42	0.32	0.30	25.9	94.6	0.84	−1.30	27.36
	28	12	5	0.30	0.36	0.21	18.9	62.6	0.96	−1.18	26.57
	27	20	5	0.26	0.29	0.19	17.3	68.7	ns	−0.82	25.93
	27		15	0.35	0.39	0.32	27.4	84.8	0.88	−1.23	26.57
	27		40	0.38	0.29	0.18	16.5	65.4	0.74	−1.49	27.36
Sea Ice Zone	24	20	5	0.85	0.30	0.19	17.3	66.7	ns	−1.34	26.00
(SIZ)	24		30	1.47	0.31	0.16	14.7	55.4	0.73	−1.59	25.43
	24		50	0.27	0.25	0.15	13.9	63.0	0.7	−1.80	28.21
	22	16	15	1.18	0.29	0.23	20.5	81.6	0.69	−1.64	27.36
	6	18	5	0.60	0.29	0.18	16.4	65.4	0.83	−1.56	18.36
	6		35	0.62	0.29	0.19	17.3	68.7	0.73	−1.69	20.21
	7	15	5	1.73	0.29	0.21	18.9	75.2	ns	−1.29	22.07
	7		15	1.60	0.33	0.23	20.5	73.1	0.71	−1.63	22.64
	7		35	0.79	0.27	0.20	18.1	76.6	0.68	−1.76	24.07
Amundsen Sea	9	54	10	11.20	–	0.36	–	–	ns	−0.79	9.50
Polynya	9		20	11.48	0.40	0.41	33.6	102.0	0.82	−0.81	10.29
(ASP)	21	25	5	9.47	0.38	0.34	30.2	91.2	ns	−0.44	6.93
	21		25	9.34	0.33	0.36	30.2	107.6	0.92	−0.67	10.36
	18	20	5	12.17	0.34	0.28	22.6	84.7	0.69	−0.45	6.50
	18		20	10.88	0.37	0.33	28.8	90.9	0.91	−0.99	15.71
	18		35	3.43	0.32	0.25	22.1	80.8	0.83	−1.65	23.93
	13	63	5	8.92	0.30	0.22	19.7	79.2	0.91	−0.36	14.50
	13		15	9.48	0.33	0.26	22.8	81.5	0.86	−0.44	14.00
OZ	Avg. ± SD			0.45 ± 0.19	0.35 ± 0.05	0.25 ± 0.06	21.67 ± 4.32	73.15 ± 11.9		−1.17 ± 0.23	26.60 ± 0.86
SIZ	Avg. ± SD			1.01 ± 0.51	0.29 ± 0.02	0.19 ± 0.03	17.52 ± 2.31	69.52 ± 7.98		−1.58 ± 0.17	23.82 ± 3.28
ASP	Avg. ± SD			9.39 ± 2.67	0.35 ± 0.03	0.29 ± 0.06	26.26 ± 5.04	89.72 ± 10.4		−0.72 ± 0.43	12.78 ± 5.64

been reported in other locations (Archer et al., 1996; Grattepanche et al., 2011; Stelfox-Widdicombe et al., 2004; Sherr and Sherr, 2007). In the ASP, the significant positive correlation between HDF biomass and ratio of *P. antarctica* to phytoplankton biomass supported the previous findings (Fig. 6). Ciliates were the second most abundant group of microzooplankotn in this study area. Although ciliate biomass was lower than that of HDF in the Amundsen Sea, the proportional contribution of ciliates to microzooplankton biomass was highest in the SIZ. Planktonic ciliates occurred in pockets in the melting sea ice in late spring and early summer (Stoecker et al., 1993), and their distribution might be affected by exchanges between the sea ice and the water column (Stoecker et al., 1995; Garzio and Steinber, 2013). Since large naked ciliates (> 100 μm in length) were also observed occasionally in the SIZ, we cannot rule out the possibility that large ciliates may be important grazers of diatoms in the SIZ, as demonstrated in the Arctic Ocean (Sherr et al., 2013). Based on previous studies and observations in this study, the high contribution of ciliates to microzooplankton biomass in the SIZ is likely related to the sea ice condition and/or food type.

HNF and/or CNF have been reported as dominant components of microzooplankton under land-fast ice in winter and spring (Vaqué et al., 2008), and in the marginal ice zone of the Weddell Sea (Becquevort et al., 1992). Although the abundance of HNF (including CNF) was high in the ASP, they comprised an average of 19% of the microzooplankton biomass in all regions. Thus, we could not ignore the presence of HNF and CNF in microzooplankton biomass. HNF has been documented as potentially important grazers of heterotrophic bacteria and picophytoplankton (Guillou et al., 2001; Pearce et al., 2010, 2011; Safi et al., 2007; Shinada et al., 2003). However, we would expect them to have a significant role as grazers of bacteria as well as picophytoplankton, as the contribution of picophytoplankton to total phytoplankton was less than 2% in the study area, except in the OZ.

During this study, microzooplankton biomass and composition varied both among sites and with depth, and their distribution usually reflected their available potential prey biomass and composition, although the sea ice condition likely also played a role. This result implies that food supply is among the most important factors controlling the spatial dynamics of microzooplankton assemblages.

4.3. Microzooplankton herbivory on phytoplankton

The most pronounced result from this study is that microzooplankton remove a substantial portion of phytoplankton production in cold water regions with high primary productivity. Microzooplankton herbivory accounted for between 55.4% and 107.6% (average 77.3 ± 13.1%) of daily phytoplankton production in the Amundsen Sea. Clearly, microzooplakton grazing consumes the majority of phytoplankton production and that secondary production by microzooplakton is important. However, this result disagrees with that of Rose and Caron (2007) who reported that extremely low seawater temperature exert a strong constraint on the microzooplankton growth rate during phytoplankton blooms in high latitudes. Caron et al. (2000) also assessed microzooplankton grazing in the Ross Sea Polynya, which has similar environmental characteristics to the ASP, and found that most of the low grazing rates were due to very low water temperatures. However, our observation of high microzooplankton herbivory in the cold temperature region coincident with phytoplankton blooms does not support their finding, while our results supported a previous study in the Bering Sea, Arctic Ocean (Sherr et al., 2013). Although some studies have reported locally extremely low grazing by microzooplankton in the Southern Ocean (Caron et al., 2000; Froneman, 2004), our result was higher than microzooplankton herbivory (average 53.0% of daily primay production)

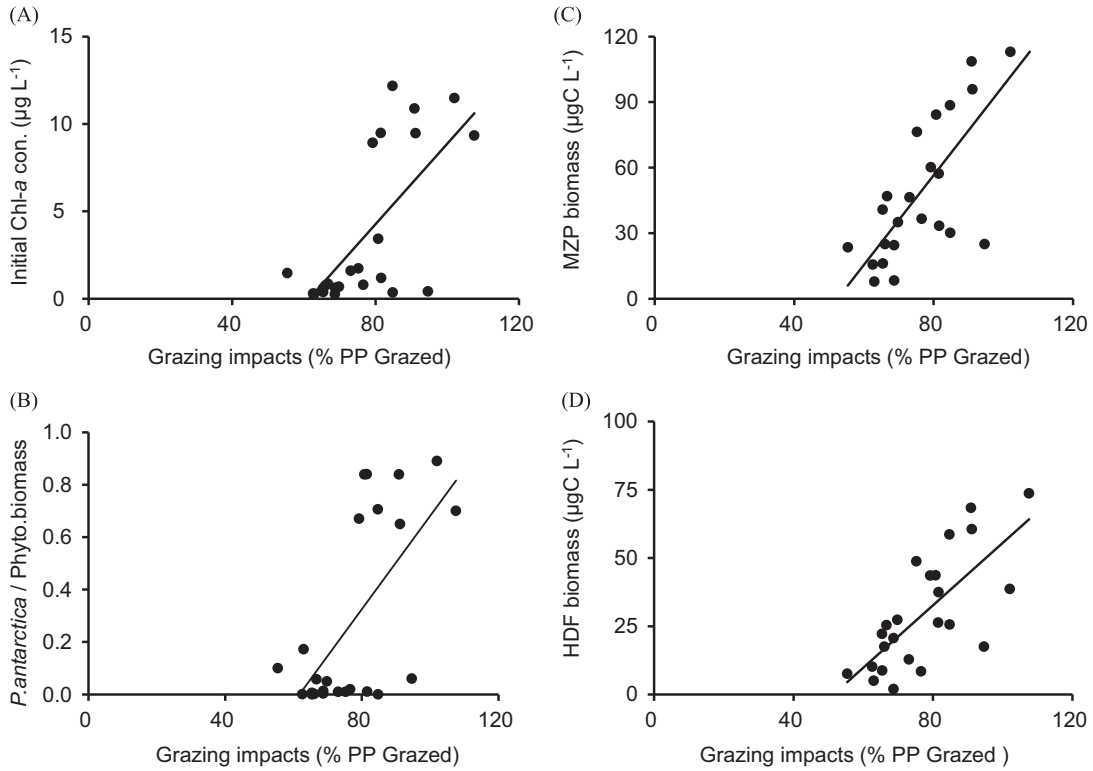

Fig. 8. Relationship between grazing impacts of microzooplankton and (A) initial Chl-*a* concentration, (B) *Phaeocystis antarctica*/ phytoplankton biomass ratio, (C) microzooplankton (MZP) biomass, and (D) heterotrophic dinoflagellate (HDF) biomass. Linear regression equations; (A) $y=2.05x-107.6$ ($R^2=0.59$, $p<0.001$), (B) $y=0.23x-14.16$ ($R^2=0.47$, $p<0.001$), (C) $y=1.14x-58.90$, ($R^2=0.52$, $p<0.001$), and (D) $y=0.02x-1.10$ ($R^2=0.42$, $p<0.001$).

reported previously from the Southern Ocean (Schmoker et al., 2013). Therefore, vigorous microzooplankton herbivory indicates that cold temperatures may not be the main factor modulating microzooplankton herbivory in the Amundsen Sea at least in early summer.

The grazing activity of microzooplankton was correlated with phytoplankton and microzooplankton biomass over entire survey, indicating that the grazing capacity is stimulated by increasing prey and grazer biomass (Fig. 8). Surprisingly, the marked microzooplankton herbivory occurring in the ASP, along with higher phytoplankton biomass and *P. antarctica* blooming, coincided with high microzooplankton biomass, especially for HDF biomass (Fig. 6). One possible explanation for high microzooplankton herbivory in blooming systems is the phytoplankton composition and size that can be considered as suitable prey of microzooplankton. The ability of microzooplankton to ingest *Phaeocystis* spp. has been widely discussed in the literature (see reviews by Whipple et al., 2005; Nejstgaard et al., 2007). It is generally accepted that microzooplankton exert pressure mostly on single *Phaeocystis* cells, while the colonial form and the physiological condition of the *Phaeocystis* cell can reduce microzooplankton grazing (Calbet et al., 2011). In the Ross Sea Polynya, Caron et al. (2000) reported that microzooplankton were unable to control the growth of colonial *P. antarctica*, but they observed significant grazing activity in the austral spring when single *P. antarctica* cells would be expected to dominate. During our study, *P. antarctica* mostly comprised single cells and the small-colony form was uncommon in the samples we collected, hence microzooplankton herbivory would not have decreased in our study area. Based on previous studies on *Phaeocystis* grazing activity, microzooplankton might play a significant role in controlling single-celled *P. antarctica* blooming in the ASP. Alternatively, high microzooplankton herbivory was also attributable to microzooplankton composition and high biomass, particularly predominant HDF. HDF appeared to be the major consumers of phytoplankton, including *Phaeocystis* colonies and diatoms (Grattepanche et al., 2011; Selph et al., 2001; Sherr and Sherr, 2007), which are considered unsuitable prey for most grazers (Nejstgaard et al., 2007; Tang et al., 2001). Furthermore, ciliates have previously been shown to be active grazers of single *Phaeocystis* cells (Archer et al., 2000; Tang et al., 2001). The large number of ciliates observed in the ASP, mostly 20–100 μm in cell size, implies that they may have been grazing small plankton, including the abundant single *Phaeocystis* cells. Therefore, vigorous microzooplankton herbivory in the ASP may be interpreted as the combined result of high microzooplankton biomass, particularly HDF, and predominant single *P. antarctica* cells as suitable prey of microzooplankton, as suggested by the significant correlation between grazing impact and ratio of *P. antarctica* to phytoplankton biomass, and between grazing impact and HDF biomass (Fig. 8).

During this study, microzooplankton herbivory accounted for an average of $71.1 \pm 9.7\%$ of phytoplankton production in diatom-dominated regions, and $89.7 \pm 10.4\%$ in the *P. antarctica*-dominated region, implying that only a small amount of primary production may be available for vertical carbon export and for direct consumption by metazoan zooplankton (such as copepods or krill) in both regions. Our results are in agreement with those of Ducklow et al. (2015), who conducted particle flux studies from sediment traps in the ASP and confirmed that the ASP system appears to export only a small fraction of its primary production to the deep ocean. Since diatoms are also likely to be more prone to vertical export than *P. antarctica* (Reigstad and Wassmann, 2007), carbon export efficiency might be higher in the SIZ than in the ASP. Therefore, compared to diatom-based systems, *Phaeocystis*-based production would be largely remineralized and/or channeled through the microbial food web rather than contributing to vertical carbon flux (Reigstad and Wassmann, 2007). We also compared microzooplankton to mesozooplankton grazing impact in the same study area. The major copepods removed about 4% of daily primary production (Lee et al., 2013), considerably lower than that removed

by microzooplankton. These results demonstrate that the microzooplankton and their herbivorous activity provide the major route for the herbivorous fate of phytoplankton. Clearly, a large amount of primary production was consumed by microzooplankton compared to all other biological (mesozooplankton consumption) and physical (sinking) loss processes. Therefore, vigorous microzooplankton herbivory might be one of the leading causes of low carbon export to the deep ocean in the pelagic ecosystem of the Amundsen Sea.

5. Conclusion

Our study suggests that microzooplankton are significant consumers of phytoplankton in the highly productive Amundsen Sea during the early summer, and confirmed the importance of microzooplankton herbivory in the plankton food web of cold temperature regions. In the Amundsen Sea, *P. antarctica* and diatoms co-dominated, resulting in a large and active microzooplankton community and posing a substantial threat to prey species. Vigorous microzooplankton herbivory measured in this study is likely due to high grazer biomass, grazer composition and presence of a suitable food source. Microzooplankton herbivory accounted for between 55.4% and 107.6% (average 77.3 $\pm$ 13.1%) of daily phytoplankton production, suggesting that the majority of phytoplankton in the Amundsen Sea was not removed by sinking or other grazers, but was grazed by microzooplankton, at least in early summer. Clearly, in these waters, the major herbivorous fate of phytoplankton was mediated by the microzooplankton population. Therefore, a significant amount of phytoplankton production might pass through the microzooplankton node before becoming available to copepods and krill in early summer. Our study suggests that the duration and magnitude of the phytoplankton bloom might be controlled by grazing pressure exerted by microzooplankton that develop at the same time as the phytoplankton. The ASP is located in one of the most rapidly and profoundly changing regions in the Southern Ocean (Ducklow et al., 2015). Because of ongoing changes in the Amundsen Sea, there is an urgent need for better understanding of the food web structures in a region of rapid climate change. This study will provide a reference point to better understand trophic interactions in the planktonic food web in the Amundsen Sea.

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