Progress in Oceanography 174 (2019) 117–130

Contents lists available at ScienceDirect

Progress in Oceanography

journal homepage: www.elsevier.com/locate/pocean

Trophic interactions of micro- and mesozooplankton in the Amundsen Sea polynya and adjacent sea ice zone during austral late summer

Eun Jin Yang*, Youngju Lee, SangHoon Lee

Division of Polar Ocean Science, Korea Polar Research Institute, 26 Songdomirae-ro, Yeonsu-gu, Incheon 21990, Republic of Korea

ARTICLE INFO

Keywords:
Microzooplankton
Copepod
Krill larvae
Grazing
Amundsen Sea polynya

ABSTRACT

To elucidate the trophic link between micro- and mesozooplankton in the Amundsen Sea polynya (ASP) and adjacent sea ice zone, we estimated the impact of microzooplankton grazing on major phytoplankton groups, as well as the diet composition, ingestion rate, and prey selectivity of two copepods and *Euphausia crystallorophias* larvae on microbial communities during the late summer. *Phaeocystis antarctica*, which was ingested by micro- and mesozooplankton, comprised most phytoplankton biomass. Herbivorous microzooplankton consumed at least half of the phytoplankton production, but the microzooplankton grazing may not contribute strongly to the decline of the phytoplankton bloom. Three mesozooplankton species (*Calanoides acutus*, *Metridia gerlachei*, and *E. crystallorophias* larvae) preferentially grazed on ciliates and heterotrophic dinoflagellates (HDF) with sizes from 20 to > 100 μm over phytoplankton. Although microzooplankton comprised only an average of 41.7 ± 3.2% o the total carbon available in the natural prey pool, they accounted for an average of 75.4 ± 2.9% of total carbon ingested by copepods and krill larvae. Heterotrophic food sources made up a substantial proportion of mesozooplankton diets, with strong positive selection for microzooplankton at most locations regardless of phytoplankton size and type. In particular, HDF comprised the major dietary component of mesozooplankton in the study area. The presence of mesozooplankton reduced the grazing pressure on *P. antarctica* and diatoms through predation on herbivorous microzooplankton. Approximately half of the primary production capacity may have indirectly reached mesozooplankton through microzooplankton consumption. Thus, strongly selective feeding behavior and higher grazing pressure on microzooplankton indicated the importance of microheterotrophic pathways through strong trophic coupling between mesozooplankton and the microbial food web during the decline of phytoplankton bloom. In the highly productive ASP system, food web structure can be classified as multivorous, whereby herbivorous and omnivorous modes both play significant roles in carbon export, enhancing the efficiency of the pelagic food web.

1. Introduction

Resolving the trophic links of planktonic food webs is critical to understanding the carbon cycle in pelagic ecosystems. A crucial process in pelagic ecosystem dynamics is carbon transfer from primary producers to secondary producers (Fonda Umani et al., 2005; Vargas et al., 2007; Nakajima et al., 2017). As secondary producers, micro- and mesozooplankton grazing activities can affect how primary production is transferred through pelagic food webs, with implications for ecosystem function, as well as the retention and vertical export of organic carbon to deep water (Vargas and González, 2004). Thus, in order to fully understand pelagic food web dynamics, it is necessary to clarify the link between micro- and mesozooplankton, as this trophic link may explain discrepancies between phytoplankton biomass and copepod metabolic demand (Dam et al., 1995; Saiz et al., 1999; Saiz and Calbet, 2011).

Over the past three decades, it has become increasingly clear that microzooplankton play important roles of competing with mesozooplankton as phytoplankton grazers, and also as important mesozooplankton prey (Gifford, 1991; Sherr and Sherr, 2002; Calbet and Landry, 2004; Saiz and Calbet, 2011; Caron and Hutchins, 2013). Microzooplankton are capable of exerting top-down control on primary producers in pelagic ecosystems and restructuring assemblages through selective grazing, thereby influencing microbial food web dynamics (Saiz and Calbet, 2011; Teixeira et al., 2012; Schmoker et al., 2013; Yang et al., 2016). Despite the key ecological functions of microzooplankton in the carbon cycle, knowledge is limited about the amount of primary production that reaches higher trophic levels via

* Corresponding author.
E-mail address: ejyang@kopri.re.kr (E.J. Yang).

https://doi.org/10.1016/j.pocean.2018.12.003

Available online 11 December 2018

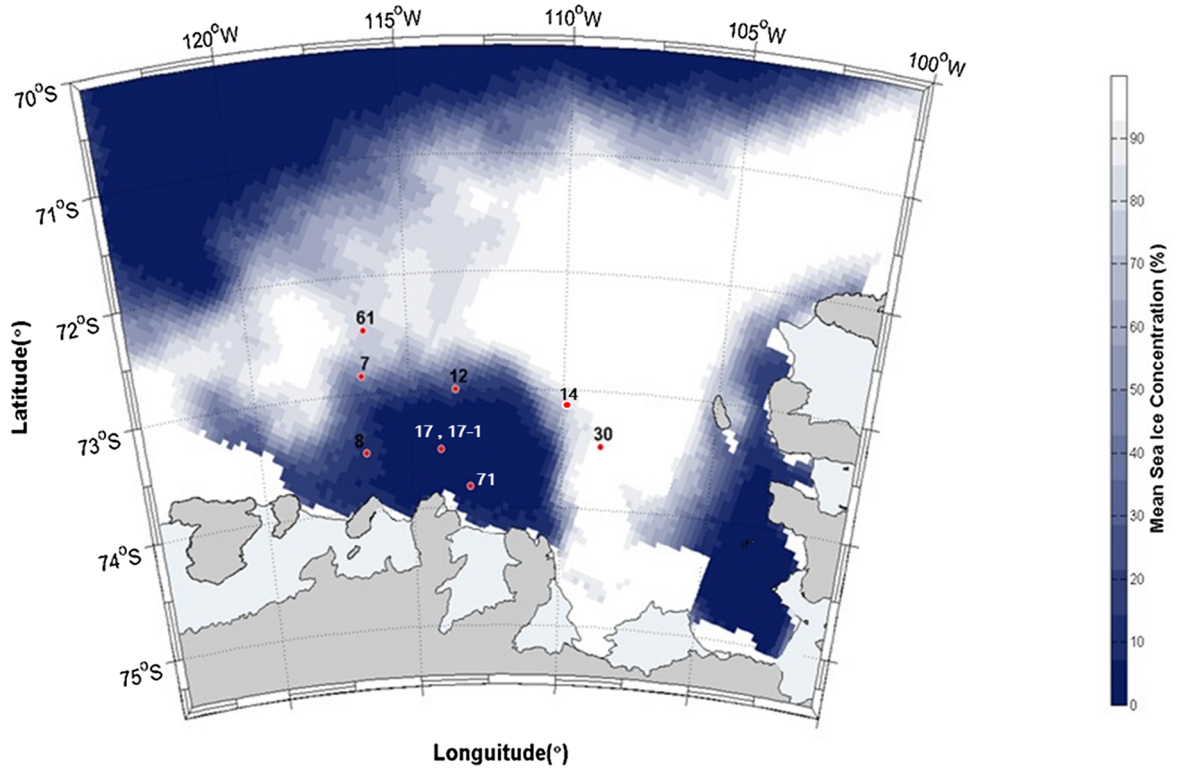

Fig. 1. Sampling stations in the Amundsen Sea from February to March 2012. Sea ice concentration was derived from SSM/I data during the survey period. St.17 and St. 17-1 are same location.

microzooplankton in cold-water systems. Although microzooplankton contribute substantially to mesozooplankton diets in various marine environments, many studies have focused on the role of mesozooplankton as phytoplankton grazers based on gut content or gut fluorescence methods in the Southern Ocean (SO) (Li et al., 2001; Pakhomov and Froneman, 2004; Lee et al., 2013; Yang et al., 2013; Gleiber et al., 2016). However, the contribution of microzooplankton to mesozooplankton diets in the SO remains poorly quantified. A global analysis of copepod diets showed that ciliates comprise an average of about 30% of ciliate plus phytoplankton consumption (Calbet and Saiz, 2005). Many mesozooplankton feed selectively on microzooplankton when available, and algal ingestion alone is often insufficient to meet metabolic demands in the ocean (Kleppel, 1993; Atkinson, 1996; Saiz and Calbet, 2011). Mesozooplankton grazing on microzooplankton and selection of specific prey can affect microzooplankton community composition and can impact the biomass and structure of populations at lower trophic levels via trophic cascades (Broglio et al., 2004; Vargas et al., 2008; Yang et al., 2009; Campbell et al., 2016). Thus, mesozooplankton selectivity for specific prey can be an important factor driving biogeochemical cycles by affecting the direction and efficiency of carbon flow (Castellani et al., 2008). Clarifying the feeding selectivity of a copepod species when presented with multiple food types is critical to understanding carbon flow in the pelagic ecosystem.

The Amundsen Sea, one of the most productive and dynamic pelagic systems in the SO (Smith et al., 2011), is a climate-sensitive region in the Antarctic, where glaciers and ice cover have been declining rapidly over the past several decades (Walker et al., 2007). Rapid environmental change caused by warming may affect food web dynamics, causing significant changes at all trophic levels. To predict the impacts of these changes on ecosystems, it is important to understand planktonic food web dynamics. The Amundsen Sea polynya (ASP) is one of the most productive polynyas in the SO (Arrigo and van Dijken, 2003), and phytoplankton is predominant by *Phaeocystis antarctica* (Lee et al., 2016). A recent study in the ASP reported that microzooplankton consumed 55.4–107.6% of daily phytoplankton production and that the major herbivorous fate of phytoplankton appears to be driven by the microzooplankton population, at least in the early summer (Yang et al., 2016). By contrast, the grazing impacts of the dominant copepods (*Rhincalanus gigas*, *Calanoides acutus*, and *Metridia gerlachei*) on phytoplankton are low, with 4% of the daily primary production consumed (Lee et al., 2013). These results suggest that heterotrophic food sources (i.e., ciliates and heterotrophic dinoflagellates) are important for sustaining mesozooplankton populations in the Amundsen Sea. Several studies have demonstrated that copepods have higher clearance rates of microzooplankton than those of phytoplankton, indicating positive selection for microzooplankton in the SO (Atkinson, 1996; Calbet et al., 2006; Yang et al., 2013). However, no information is available on the relative importance of microzooplankton and phytoplankton as food sources for mesozooplankton in the Amundsen Sea. Due to the limited information available on the grazing activities of different grazer groups in the ASP, assessing their quantitative and ecological importance in this ecosystem and determining their trophic position and influence on planktonic food web dynamics remain challenging.

Here, we present a study that aimed to improve our understanding of the trophic link between micro- and mesozooplankton in the Amundsen Sea during late summer. This study was guided by three hypotheses: (i) microzooplankton gazing is expected to play a significant role in the decline of the phytoplankton bloom, (ii) microzooplankton are an important component of three mesozooplankton diets in the highly productive ASP, and (iii) the relative contributions of phyto- and microzooplankton to mesozooplankton diets can vary according to the prey type, size spectrum, and trophic status. To evaluate these hypotheses, we employed *Calanoides acutus*, *Metridia gerlachei*, and *Euphausia crystallorophias* larvae as grazers in grazing experiments and conducted incubation experiments with mesozooplankton grazing on phytoplankton and microzooplankton under diverse natural prey assemblages. These experiments were conducted in parallel with dilution experiments to estimate the impacts of microzooplankton grazing

on major phytoplankton groups to gain a better understanding of the planktonic food web. We analyzed the diet composition, ingestion rate, and prey selectivity of the two copepod species and *E. crystallorophias* larvae (hereafter referred as 'krill larvae').

2. Methods

2.1. Study area and sampling

This study was conducted during a Korean Amundsen Sea expedition on board the icebreaker R/V Araon during the late austral summer from February 9 to March 10, 2012 (Fig. 1). For the grazing experiment, 8 sampling stations were selected during February. We revisited the center of the polynya (St. 17) at the beginning of March to examine the phytoplankton bloom process, and named St. 17-1. Sampling area was classified the Amundsen Sea Polynya (ASP), which was open water surrounded by sea ice (St. 7, 8, 12, 17 and 71) and marginal sea ice zone (SIZ) (St. 14, 30, 61). Sea ice concentrations were based on data from the National Snow and Ice Data Center in Boulder, Colorado, that corresponded to the cruise period. Seawater samples for biological analyses and grazing experiments were collected using Niskin bottles attached to a rosette sampler.

2.2. Microzooplankton grazing experiments

We estimated phytoplankton growth and microzooplankton grazing rates using the dilution method by measuring changes in the total chlorophyll-*a* (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 twice in Milli-Q water before experiments. Plastic gloves were worn during all phases of the experiments. At each station, 30 l seawater was collected in a Niskin bottle and transferred to a polycarbonate carboy. To avoid damaging delicate microzooplankton and altering phytoplankton composition, particularly in the phytoplankton bloom samples were not screened prior to incubation (Calbet et al., 2011). Instead, larger zooplankton, such as copepods, were 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 end (T_{48}, each treatment bottle) of the incubation to estimate chl-*a* concentration. 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 (μ) and grazing (g) rates 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).

2.3. Mesozooplankton grazing experiments

Water samples were collected from surface and subsurface chlorophyll maximum (SCM) depth at some stations (Table 1) using Niskin bottles, and were gently transferred to a 20 l carboy. Live zooplankton were collected with a Bongo net (mesh apertures 330 and 505 μm) via vertical towing from a depth of 200 m to the surface. The average speed of all tows was 60 m/min. Within 1 h after collection, we selected the numerically dominant species, krill larvae, and the major copepods, *C. acutus* and *M. gerlachei* under a dissecting microscope (Lee et al., 2013). Undamaged individuals of each copepod species and krill larvae were immediately sorted and transferred into 2.3 l polycarbonate bottles without the addition of nutrients. The numbers of zooplankton selected during each experiment are presented in Table 1. Three control bottles without copepods and three experimental bottles with copepods of each. The experimental bottles were incubated in a flow system incubator and maintained at a temperature within ± 0.5 °C of the ambient surface temperature and manually rotated every 3–4 h during incubations. The experimental bottle was incubated in the same incubator with microzooplankton grazing experiments. At the end of the experiment, no dead copepods were found. At the beginning and end of each incubation period, we collected subsamples for assessing the plankton abundance and composition, and the chl-*a* level. At the end of each experiment, the dry weight of each animal was measured using a microbalance (MC5; Sartorius AG, Göttingen, Germany) after drying at 60 °C for 24–48 h.

2.4. Chlorophyll-a, phytoplankton, and microzooplankton enumeration

Water samples for chl-*a*, phytoplankton, and microzooplankton analyses were taken from stations for dilution experiments (Table 2). Chlorophyll-*a* concentrations 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). To determine the abundance of plankton, except ciliates, we preserved 300 ml samples of water with glutaraldehyde (1% final concentration), then stored them at 4 °C before staining and filtration. Subsamples of 50–100 ml were filtered onto nucleopore filters (0.8 μm pore size, black). Aliquots of the preserved samples were stained with proflavin (0.33%) for 1 h 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 phytoplankton and microzooplankton cells, at least 100 fields per sample were counted with an epifluorescence microscope (Olympus BX 51) at magnifications of 200–640× using a blue light excitation filter set for chlorophyll autofluorescence and UV light excitation filter set for DAPI stained cells. Autotrophic organisms were distinguished from heterotrophs by the presence of chlorophyll, which was visualized as red fluorescence under blue light illumination. Most *P. antarctica* cells were distinguished from other autotrophic flagellates based on cell size and shape, chloroplast arrangement, and the presence of flagella. For picophytoplankton cells, at least 200 cells per sample were counted at magnifications of 1000× using blue light excitation. For ciliates, 500 ml water samples were preserved with 4% acid Lugol's iodine solution and subsequently stored in darkness. Preserved samples were allowed to settle in mass cylinders for at least 48 h. The upper water layer was then siphoned out, leaving 20 ml. Subsequently, a 1 ml aliquot of each concentrated sample was placed in an S-R chamber and counted using light microscope (Olympus BX51). To estimate the plankton carbon biomass, cells were sized using an image analysis system standardized using a calibrated ocular micrometer and the cell volume calculations were based on measured cell dimensions and the closest geometric shapes for individual cells (Winberg and Duncan, 1971; Edler, 1979). Microzooplankton was classified as heterotrophic nanoflagellates (HNF), ciliates, and heterotrophic dinoflagellates (HDF). Phytoplankton was classified as autotrophic picophytoplankton (APP), autotrophic nanoflagellates (ANF), autotrophic dinoflagellates (ADF), *P. antarctica*, and diatoms. The

Table 1
Initial conditions for mesozooplankton grazing experiments.

Exp. No	Station (Depth)	Predator	Number per bottle	Incubation Time (h)	Body weight (μgC ind.$^{-1}$)
Z1a	8 (5 m)	*Calanoides acutus (VI)*	4	32	175 ± 87
Z1b	8 (5 m)	*Euphausia crystallorophias* (larvae)	2	32	252.1 ± 23
Z1c	8 (30 m)	*Calanoides acutus (VI)*	4	35	168.3 ± 26
Z2	12 (5 m)	*Metridia gerlachei (VI)*	4	35	128.2 ± 19
Z3a	71 (5 m)	*Metridia gerlachei (VI)*	3	30	129.3 ± 21
Z3b	71 (5 m)	*Euphausia crystallorophias* (larvae)	2	30	198.2 ± 23
Z3c	71 (30 m)	*Calanoides acutus (VI)*	4	32	179.8 ± 45
Z6	30 (5 m)	*Calanoides acutus (VI)*	3	40	172 ± 21
Z7	14 (30 m)	*Metridia gerlachei (VI)*	3	38	102.2 ± 31
Z8	61 (5 m)	*Calanoides acutus(VI)*	3	44	170.5 ± 23

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 (Børsheim and Bratbak, 1987).

2.5. Data analysis

Ingestion rates of mesozooplankton on phyto- and microzooplankton were calculated using Frost's equation (Frost, 1972), corrected for reduced microzooplankton grazing because of predation by mesozooplankton, according to the formula given by Nejstgaard et al. (2001). In experiments, the results from all replicates or triplicates were averaged. Ingestion rates were calculated only when the difference in prey concentration between the control and experimental bottles proved significant ($p < 0.05$). Mesozooplankton prey selectivity was determined using Chesson's index of selectivity (α), which relates ingestion rates of the different food types with their availability (Chesson, 1983). The parameter α calculated the capture probability based on the probability of prey encounters:

$$\alpha = (r_i/p_i)/\Sigma(r_i/p_i)$$

where r_i is the proportion of the prey *i* in the diet, p_i is the proportion of the prey *i* in the environment, and $\Sigma\,\alpha = 1$. If the total number of prey species is *n*, and $\alpha > 1/n$, selective copepod predation may have occurred. Alternatively, if $\alpha < 1/n$, prey avoidance may have occurred. This index is density independent and determines whether prey items were ingested in higher or lower proportions than that expected owing to their relative biomass in the field (Vargas et al., 2008). Using these data, we examined whether certain size ranges or groups were selected preferentially by the mesozooplankton.

3. Results

3.1. Initial grazing experiment conditions, plankton biomass, and composition

The water temperatures ranged from −1.67 to −1.05 °C between 5 and 30 m depth (Table 2). The initial carbon biomass of the phytoplankton ranged from 14.5 to 109.0 μg C L^{-1} and was highest at St.17 (Fig. 2). Phytoplankton was generally predominated by *P. antarctica* and diatoms. Phytoplankton in the SIZ was dominated by diatoms, comprising mostly *Fragilariopsis cylindrus*, *F. nana* and nano-sized pennate diatoms (data not shown), that accounted for an average of 59.9% of the phytoplankton biomass. *Phaeocystis antarctica* accounted for > 60% of the phytoplankton biomass in the ASP. The size-fractionated phytoplankton showed that < 10 μm was the most dominant size in the ASP, and accounted for 62.1% of the total phytoplankton biomass (Fig. 2). Phytoplankton in the SIZ was dominated by cells with sizes of 20–50 and 50–100 μm on average. The carbon biomass of the microzooplankton ranged from 10.1 to 72.3 μg C L^{-1}, and the highest microzooplankton biomass occurred in the ASP. HDF comprised the largest proportion of the microzooplankton assemblage, contributing an average of 47.7% to the total microzooplankton biomass. Of the HDF, athecate HDF biomass consisted mainly of *Gyrodinium* spp., *Gymnodinium* spp., and accounted for an average of 33.3% of the microzooplankton biomass. The ciliate was mainly dominated by naked ciliates, and their biomass accounted for an average of 40.1% of the microzooplankton biomass. The microzooplankton was dominated by the > 50–100 μm size groups, accounting for > 60% of the total microzooplankton biomass. Microzooplankton biomass comprised an average of 41.7% of the total phytoplankton and microzooplankton biomass (Fig. 2).

Table 2
Summary parameters and results of microzooplankton grazing impact on chlorophyll-a concentration 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: the correlation coefficient of the linear regression between phytoplankton growth and dilution factor, $p < 0.05$, NS: not significant.

Station	Depth(m)	Initial Chl-a (μg l^{-1})	μ (d^{-1})	g (d^{-1})	PP (%)	PS (%)	r^2	Temperature (°C)	Nitrate (μM)
7	5	3.39	0.23	0.11	50.6	10.4	0.89	−1.67	21.2
8	5	3.32	0.28	0.14	53.5	13.1	0.92	−1.57	15.2
8	30	4.28	0.31	0.18	61.8	16.5	0.72	−1.39	14.8
12	5	3.86	0.34	0.2	62.9	18.1	NS	−1.62	20.0
71	5	4.63	0.39	0.21	58.7	18.9	0.68	−1.34	12.2
71	30	4.86	0.37	0.23	66.4	20.5	NS	−1.37	13.0
17	5	5.20	0.41	0.21	56.3	37.5	0.79	−1.24	9.4
30	5	0.95	0.23	0.09	41.9	8.6	0.85	−1.05	12.2
14	30	0.86	0.20	0.05	26.9	4.9	NS	−1.14	29.2
61	5	2.50	0.24	0.11	48.8	10.4	0.81	−1.78	21.2
Avg. ± SD		3.38 ± 1.53	0.30 ± 0.07	0.15 ± 0.06	52.7 ± 11.7	15.9 ± 9.1		−1.42 ± 0.24	16.8 ± 5.98

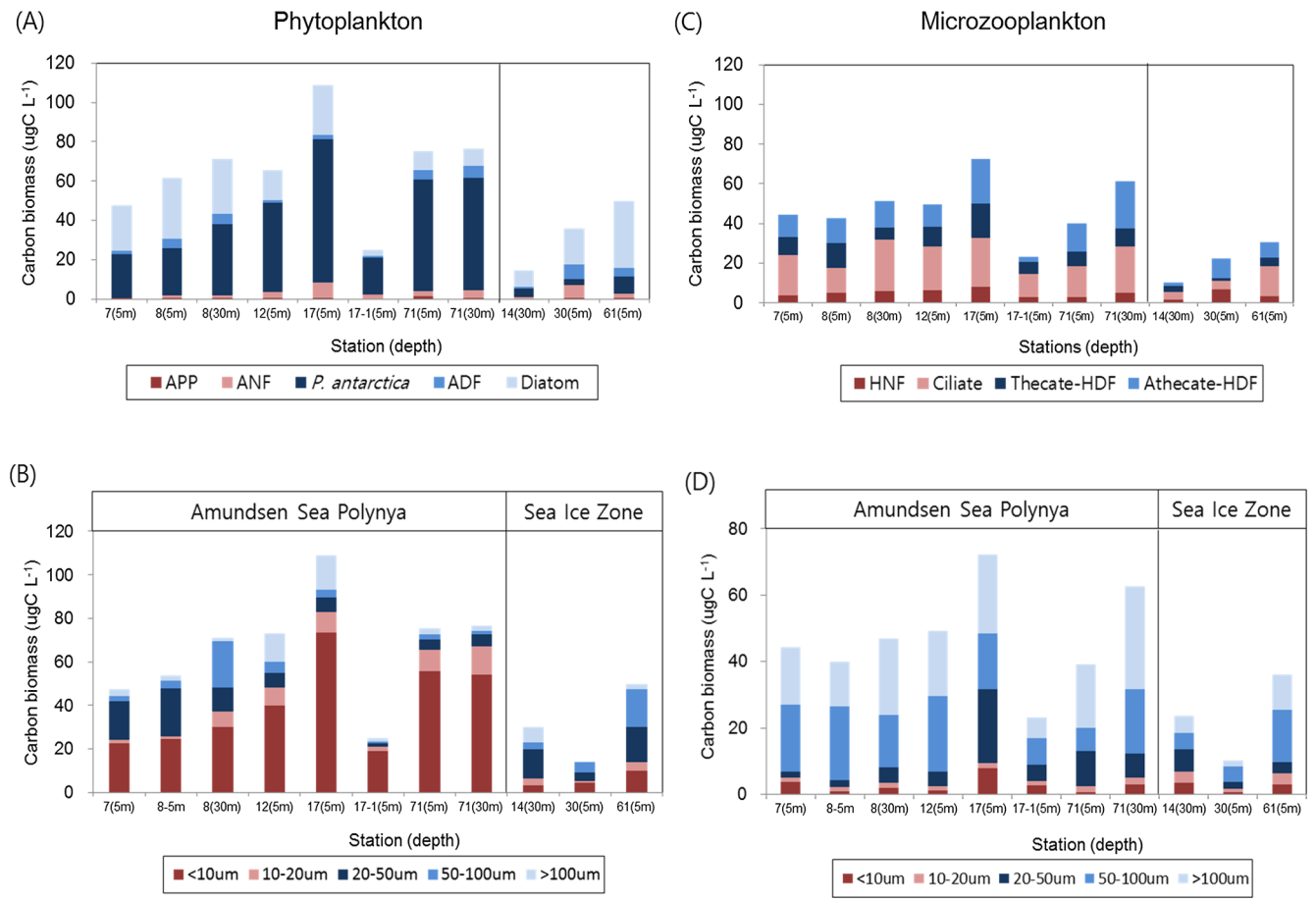

Fig. 2. Initial taxon specific (A and C) and size specific (B and D) carbon biomass of phyto- and microzooplankton in this study area. APP, autotrophic picoplankton; ANF, autotrophic nanoflagellate; ADF, autotrophic dinoflagellate; HNF, heterotrophic nanoflagellate; HDF, heterotrophic dinoflagellate.

3.2. Microzooplankton grazing impact on major phytoplankton groups

Details of phytoplankton growth and microzooplankton grazing rates for all dilution experiments are summarized in Tables 2 and 3. Total phytoplankton growth rates and microzooplankton grazing rate based on chlorophyll-a concentration ranged 0.20–0.41 d^{-1} (average, 0.33 d^{-1}) and 0.05–0.23 d^{-1} (average, 0.18 d^{-1}), respectively (Table 2). Of the phytoplankton, diatoms had relatively lower growth rates (average, 0.25 ± 0.04 d^{-1}) and APP recorded the highest growth rate (average, 0.43 ± 0.13 d^{-1}) (Table 3). Microzooplankton grazing rates were relatively lower for diatoms and the highest grazing rate was recorded for APP. Based on chl-a concentration, microzooplankton removed 4.9–37.5% (average, 15.9%) of chl-*a* standing stocks daily, and 26.6–66.4% (average, 52.7%) of the daily phytoplankton production (Table 2). Grazing impact of microzooplankton on phytoplankton were relatively high in the ASP (58.6% d^{-1}) than that in the SIZ (39.2% d^{-1}). For major phytoplankton groups, the percentages of production grazed were 90.3–117.7% (average, 100.3%) for the APP, 42.6–68.9% (average, 60.8%) for *P. antarctica*, and 34.4–57.2% (average, 45.7%) for the diatoms, respectively (Table 3). On average, more than half of daily phytoplankton production (average, 52.7%) was consumed by microzooplankton, but there was wide variation over the study period.

3.3. Mesozooplankton grazing rates on phytoplankton and microzooplankton

Grazing rate by mesozooplankton on the phyto- and microzooplankton differed according to prey type and size (Figs. 3 and 4). Phytoplankton and microzooplankton were consumed at rates of 1.45–8.33 μg C ind.$^{-1}$ d^{-1} and 3.45–27.05 μg C ind.$^{-1}$ d^{-1} by mesozooplankton, respectively (Table 4). The average daily grazing rate of *C. acutus* and *M. gerlachei* on phytoplankton and microzooplankton fell within the range of values reported in previous studies (Froneman et al., 1996; Dubischar and Bathmann, 1997; Mayzaud et al., 2002; Gleiber et al., 2016). Microzooplankton was consumed at a higher rate than phytoplankton by mesozooplankton. Grazing rates on both phytoplankton and microzooplankton taxa were always higher for krill larvae than the two copepods, with the exception of HNF and ANF. Among the phytoplankton, *P. antarctica* was consumed at a higher rate (average 1.67 μg C ind.$^{-1}$ d^{-1}) than that of other phytoplankton taxa, and accounted for > 50% of the phytoplankton carbon ingested by mesozooplankton. Diatoms and ADF accounted for an average of 28.7% and 21.1% of the phytoplankton carbon ingested by mesozooplankton. The mesozooplankton diet mostly comprised HDF in all experiments. Of the HDF, the athecate HDF was ingested at high rates (average 4.83 μg C ind.$^{-1}$ d^{-1}) by both copepods and krill larvae, and accounted for an average of 43.2% of the microzooplankton carbon ingested by the mesozooplankton. The ciliates accounted for an average of 36.9% of the total microzooplankton carbon ingested by mesozooplankton. Microzooplankton was mostly a constant component of mesozooplankton diets, contributing 34.6–48.2% of the total prey carbon available and 70.4–79.3% of the total carbon ingested by the mesozooplankton. Among the total prey components (phytoplankton and microzooplankton), athecate HDF and ciliates were the most important prey items in mesozooplankton diets (Fig. 5), accounting for an average of 32.6% and 28.1% of the total daily summed carbon rations of the total prey, respectively. Although *P. antarctica* and diatoms were the

Table 3
Summary parameters and results of grazing impact on biomass of major phytoplankton taxa derived from dilution experiments. μ: phytoplankton growth rate, g: microzooplankton grazing rates, PS (%): daily phytoplankton standing stocks grazed, PP (%): daily phytoplankton production grazed, ND: not determined, NS: not significant, *p < 0.05, **p < 0.01, ***p < 0.001.

Station	Depth (m)	Taxa	Initial biomass (μg l^{-1})	μ (d^{-1})	g (d^{-1})	PP (%)	PS (%)	*p*
8	5	*P. antarctica*	23.86	0.31	0.19	64.9	17.3	**
		Diatom	30.82	0.24	0.11	48.8	10.4	*
		ADF	4.97	ND	ND	ND	ND	ND
		ANF	1.53	0.34	0.22	68.5	22.9	NS
		APP	0.28	0.56	0.49	90.3	38.7	**
8	30	*P. antarctica*	36.14	0.35	0.22	64.1	25.2	**
		Diatom	27.93	0.29	0.18	65.4	16.4	*
		ADF	5.43	0.23	0.11	50.6	10.4	NS
		ANF	1.62	0.61	0.45	79.3	36.2	NS
		APP	0.28	0.63	0.65	102.2	47.7	***
12	5	*P. antarctica*	45.29	0.37	0.24	68.9	21.3	*
		Diatom	15.56	0.24	0.13	57.1	12.2	**
		ADF	1.37	0.37	0.14	42.2	13.1	*
		ANF	3.20	0.39	0.40	102.1	33.0	NS
		APP	0.33	0.30	0.34	111.2	28.8	*
71	5	*P. antarctica*	56.50	0.36	0.23	67.9	20.5	*
		Diatom	9.50	0.22	0.07	34.3	6.7	NS
		ADF	4.95	0.29	0.16	58.7	14.7	**
		ANF	2.67	0.36	0.24	70.5	21.3	NS
		APP	1.44	0.45	0.34	79.9	28.8	*
71	30	*P. antarctica*	57.00	0.34	0.22	68.5	19.7	**
		Diatom	8.90	0.25	0.09	38.9	8.6	*
		ADF	6.10	0.34	0.17	54.3	15.6	*
		ANF	4.02	ND	ND	ND	ND	NS
		ANP	0.57	0.55	0.49	91.5	38.7	**
30	5	*P. antarctica*	3.00	0.22	0.12	57.2	11.3	NS
		Diatom	18.20	0.28	0.1	38.9	9.5	*
		ADF	7.58	0.25	0.09	38.9	8.6	**
		ANF	6.51	0.34	0.16	51.3	14.8	NS
		ANP	0.41	0.26	0.27	103.3	23.7	*
14	30	*P. antarctica*	4.48	0.28	0.11	42.7	10.4	NS
		Diatom	8.59	0.17	0.06	37.3	5.8	**
		ADF	0.59	ND	ND	ND	ND	NS
		ANF	0.70	0.21	0.06	30.7	5.8	NS
		APP	0.15	0.38	0.41	106.4	33.6	*
61	5	*P. antarctica*	8.84	0.24	0.12	52.9	11.3	*
		Diatom	44.07	0.31	0.13	45.7	12.2	**
		ADF	4.21	0.22	0.08	38.9	7.7	*
		ANF	2.35	0.25	0.12	47.0	10.4	NS
		APP	0.25	0.36	0.44	117.7	35.6	**

dominant prey groups among all prey components, they contributed an average of < 10% of the total carbon consumed by mesozooplankton. Among the phytoplankton, phytoplankton in the < 10 and 20–50 μm size classes were consumed preferentially, and 35.9% and 30.2% of the phytoplankton carbon consumed by the mesozooplankton, respectively (Figs. 2, 4 and 5). Microzooplankton in 50–100 μm and > 100 μm size classes were ingested at a relatively high rate throughout all the experiments, and accounted for an average of 43.2% and 31.4% of the microzooplankton carbon consumed by mesozooplankton. Particularly, krill larvae ingested the highest rates of microzooplankton in the > 100 μm size class, accounting for an average of 57.0% of the microzooplankton carbon consumed by krill larvae. Among the total prey components, the 50–100 μm size taxa was the most important prey item in mesozooplankton diets (Fig. 5), accounting for an average of 39.1% of the total carbon summed of the phyto-and microzooplankton. Mesozooplankton did not consume, or consumed at a low rate, < 20 μm total plankton, with the exception of those in the ASP, in which *P. antarctica* was the dominant phytoplankton.

Grazing rates by mesozooplankton were positively correlated with the initial concentrations of total prey and body carbon biomass ($r^2 = 0.8$, $p < 0.01$). The daily grazing of total prey biomass was 5.1–9.6% of body carbon for *C. acutus*, 4.7–8.3% of body carbon for *M. gerlachei*, and 11.8–14.0% of body carbon for krill larvae (Table 4).

3.4. Prey selectivity

The selectivity index α was used to test the degrees of mesozooplankton selectivity for specific plankton groups. The selectivity index showed obvious differences between microzooplankton and phytoplankton assemblages; size-dependent patterns of prey selection were also evident (Figs. 6 and 7). The two copepods and krill larvae showed a strong preference for microzooplankton over phytoplankton. In all experiments, the α-values for ciliates, HDF, and ADF were consistently higher than the threshold (α = 011) for selective feeding, indicating that mesozooplankton had a positive preference for these three plankton groups, especially athecate HDF (i.e., most values were above the 1:1 line). However, α-values in major phytoplankton groups were generally much lower than the selection threshold, indicating that mesozooplankton did not feed effectively on diatoms and *P. antarctica*. Feeding selectivity patterns on each prey components was similar for the two copepods and krill larvae, but the size-dependent selective feeding pattern was significantly different for *M. gerlachei* than that of *C. acutus* and krill larvae. The α-values for the phyto-and microzooplankton in the small size range (< 20 μm) were below the selective

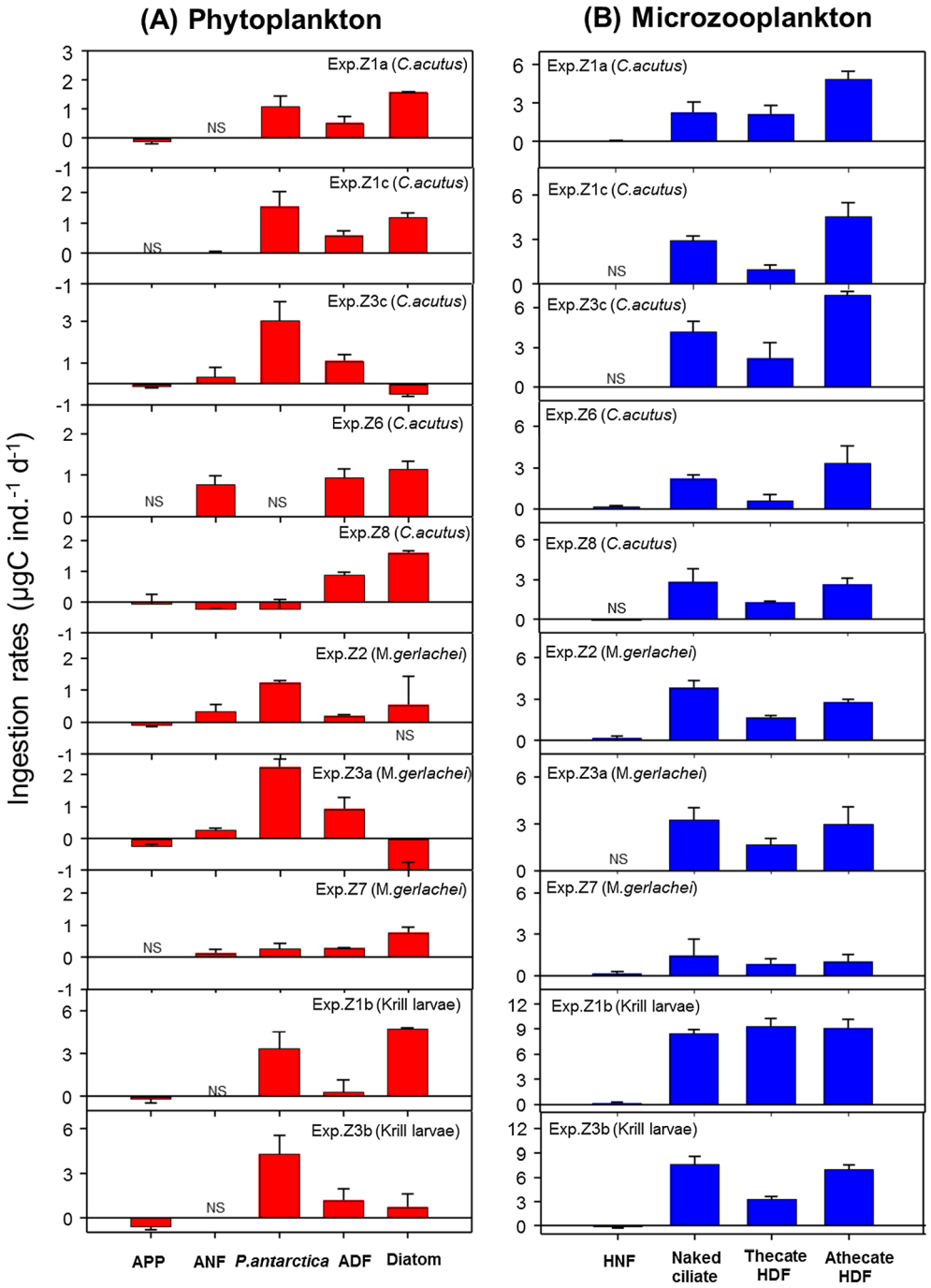

Fig. 3. Mean (± SD) ingestion rates of the two copepods and Krill larvae on phytoplankton (A) and microzooplankton (B) based on prey taxa. Ingestion rates were significant between control and experimental prey concentration ($P < 0.05$). NS, not significant. Note that y-axis has different scale for different species. See Fig. 2 for label identification.

threshold in all experiments. *Calanoides acutus* and krill larvae positively selected phyto- and microzooplankton in the > 50 µm size range, whereas *M. gerlachei* positively selected phyto- and microzooplankton in the 20–50 µm size range. Particularly, krill larvae showed a strong preference for > 100 µm-sized prey.

4. Discussion

4.1. Plankton distribution and microzooplankton grazing on phytoplankton

In the ASP, the phytoplankton bloom peaked in late December (2010) and began to decline in early summer (January 2011) (Yager et al., 2016; Yang et al., 2016). Phytoplankton biomass quickly declined during the later stage (February 2012) and the end of the bloom (March 2012) in late summer (Fig. 8). *Phaeocystis antarctica* was the dominant phytoplankton species in the ASP, comprising > 70% of total phytoplankton during the summer season (Yager et al., 2016; Yang et al., 2016). The decline of *P. antarctica* blooms in the ASP is affected by the microzooplankton assemblage and its grazing activity (Yager et al., 2016; Yang et al., 2016; Swalethorp et al., 2018). In late summer, microzooplankton biomass was lower than that reported in early summer, and the decline of microzooplankton biomass corresponded to the decline in the phytoplankton biomass (Fig. 8). HDF, comprising large *Gyrodinium* spp. of > 50 µm, was the numerically dominant component

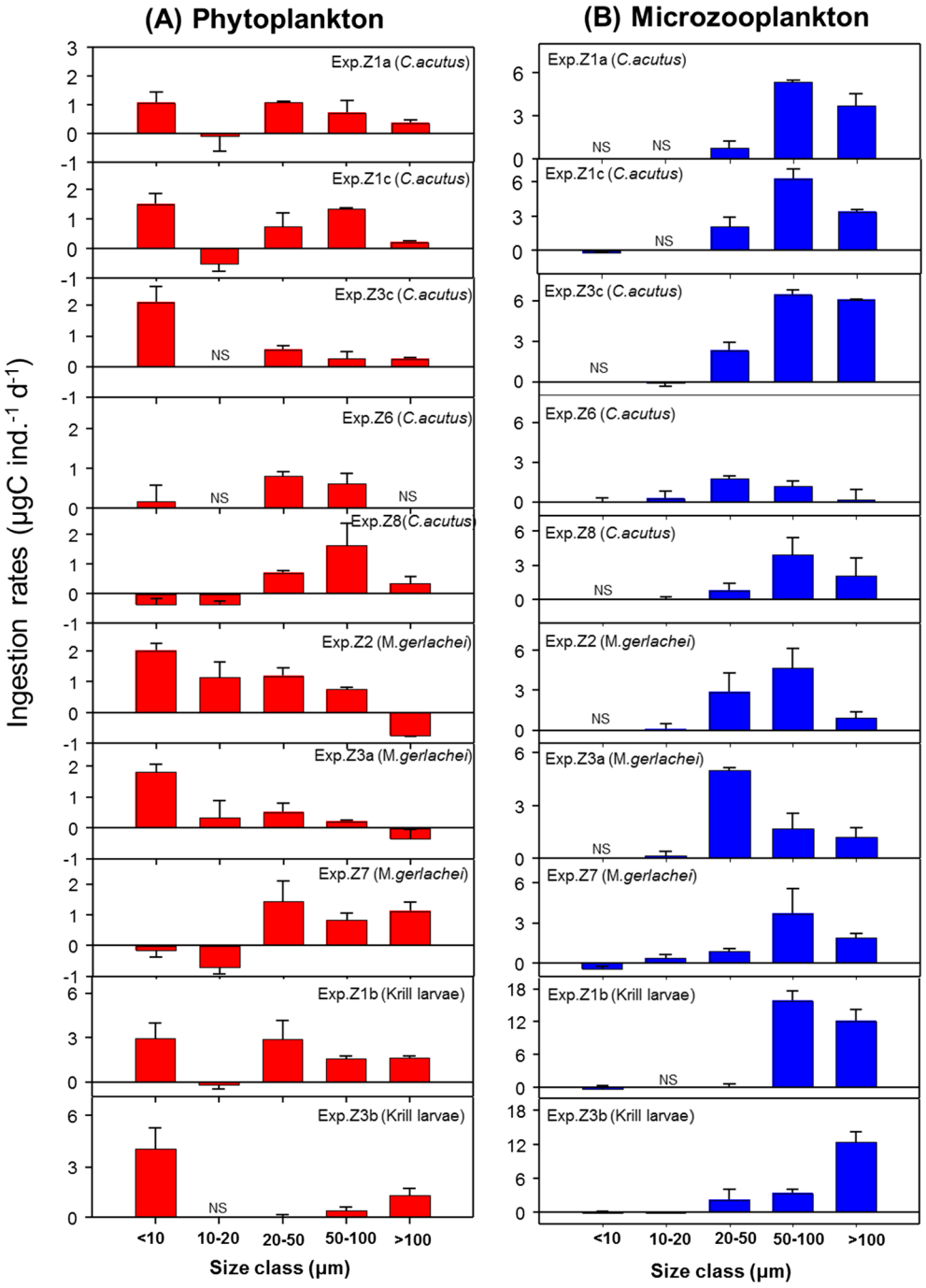

Fig. 4. Mean (± SD) ingestion rates of the two copepods and Krill larvae on phytoplankton (A) and microzooplankton (B) based on size class. Ingestion rates were significant between control and experimental prey concentration ($P < 0.05$). NS, not significant. Note that y-axis has different scale for different species.

of microzooplankton biomass in summer (Fig. 2). The importance of HDF in colonies of and/or single-celled *Phaeocystis* and diatom bloom systems has been reported in other locations and ASP (Stelfox-Widdicombe et al., 2004; Sherr and Sherr, 2007; Grattepanche et al., 2011; Swalethorp et al., 2018). Although HDF was dominant during the phytoplankton bloom, the contribution of HDF to microzooplankton biomass gradually decreased toward late summer (Fig. 8). During the decline of the bloom, a shift from HDF to ciliate dominance was observed, with the ciliate:HDF biomass ratio increasing from 0.3 to 1.3. Thus, the decline of HDF, the main grazer of *P. antarctica* and diatoms, would influence microzooplankton grazing on phytoplankton.

Microzooplankton did not selectiverly ingest all particles, but instead consumed primarily highly concentrated particles of small size. Microzooplankton preferentially grazed on APP (100.3% d^{-1}), rather than on diatoms (45.7% d^{-1}) and *P. antarctica* (60.8% d^{-1}), which was dominant in this study area (Table 3). The marked impact of grazing on APP demonstrated that microzooplankton grazing might control the growth of small phytoplankton that they can readily feed on during the late summer. Microzooplankton grazing pressure on *Phaeocystis* depends on whether *Phaeocystis* occurs in its single-cell or colonial form (Caron et al., 2000; Grattepanche et al., 2011). In the ASP, *P. antarctica* was not well controlled by microzooplankton when in the colonial stage at the bloom peak (December 2010); however, immediately after the peak of the *P. antarctica* bloom, when colonies break up into single cells, microzooplankton herbivory increased significantly (Yang et al., 2016; Swalethorp et al., 2018). We observed both small colonies and single

Table 4
Daily ingestion rate (SD) and body ration (SD) of the two copepods and Krill larvae on phytoplankton and microzooplankton. Relative contribution of microzooplankton to total ingeston is shown in bold.

		μgC ind.$^{-1}$ day^{-1}		% body C day^{-1}		Ingestion
Exp. No	Predator	Phytoplankton	Microzooplankton	Phytoplankton	Microzooplankton	(% Microzooplankton)
Z1a	*Calanoides acutus (VI)*	3.09 (1.19)	9.3 (2.06)	1.77	5.31	**75.0**
Z1c	*Calanoides acutus (VI)*	3.24 (1.15)	8.55 (1.21)	1.93	5.09	**72.5**
Z3c	*Calanoides acutus (VI)*	3.91 (0.90)	13.4 (1.60)	2.18	7.45	**77.4**
Z6	*Calanoides acutus (VI)*	2.56 (1.11)	6.44 (1.97)	1.49	3.75	**71.5**
Z8	*Calanoides acutus (VI)*	1.97 (0.98)	6.71 (0.90)	1.15	3.93	**77.3**
Z2	*Metridia gerlachei (VI)*	2.21 (1.29)	8.5 (1.59)	1.73	6.64	**79.3**
Z3a	*Metridia gerlachei (VI)*	2.23 (0.64)	7.89 (2.12)	1.73	6.12	**77.9**
Z7	*Metridia gerlachei (VI)*	1.45 (0.69)	3.45 (0.99)	1.42	3.37	**70.4**
Z1b	*Euphausia crystallorophias* (larvae)	8.33 (1.71)	27.05 (3.28)	3.30	10.70	**76.4**
Z3b	*Euphausia crystallorophias* (larvae)	5.67 (2.25)	17.9 (1.81)	2.86	9.03	**75.9**

cells of *P. antarctica* at the time of sampling. The colony of *P. antarctica* may affect the grazing rate and composition of microzooplankton. During the later stage of the bloom, microzooplankton consumption never exceeded phytoplankton production, accounting for an average of 52.7% of phytoplankton production (Table 2). Thus, at least half of phytoplankton production may be available for vertical carbon export or direct consumption by metazoan zooplankton such as copepods or krill. In the ASP, microzooplankton consumed an average of 46% and 71% of primary production at the bloom peak and immediately after the bloom, respectively (Yager et al., 2016; Yang et al., 2016). The role of microzooplankton was fundamental to the decline of phytoplankton immediately after the *P. antarctica* bloom, whereas during the later stage of the bloom, the declines of microzooplankton biomass and grazing indicated that microzooplankton may not be essential to the decline of the *P. antarctica* bloom. This result does not concur with our first hypothesis. Several factors may explain the decline of microzooplankton herbivory and biomass even when phytoplankton biomass remained high. First, decreasing microzooplankton herbivory may be attributed to the quality and quantity of prey. Colony formation of plankton and changing physiological cell condition may reduce microzooplankton grazing on these species. When a phytoplankton bloom is senescent, grazers may strongly reduce their grazing rate, even if they are abundant (Sherr and Sherr, 2009; Calbet et al., 2011). In late summer, phytoplankton blooms might decrease accompanied by cell

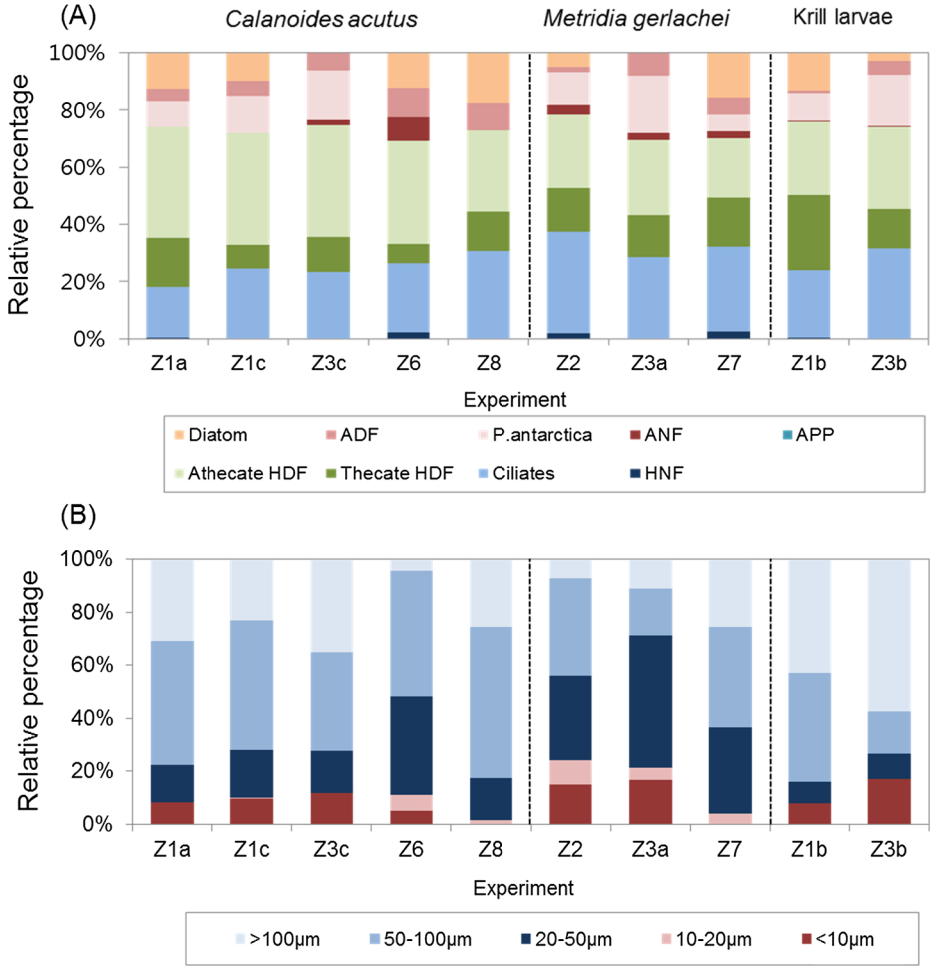

Fig. 5. Relative significance of each prey taxa (A) and size class (B) to the total diets of two copepods and krill larvae. See Fig. 2 for label identification.

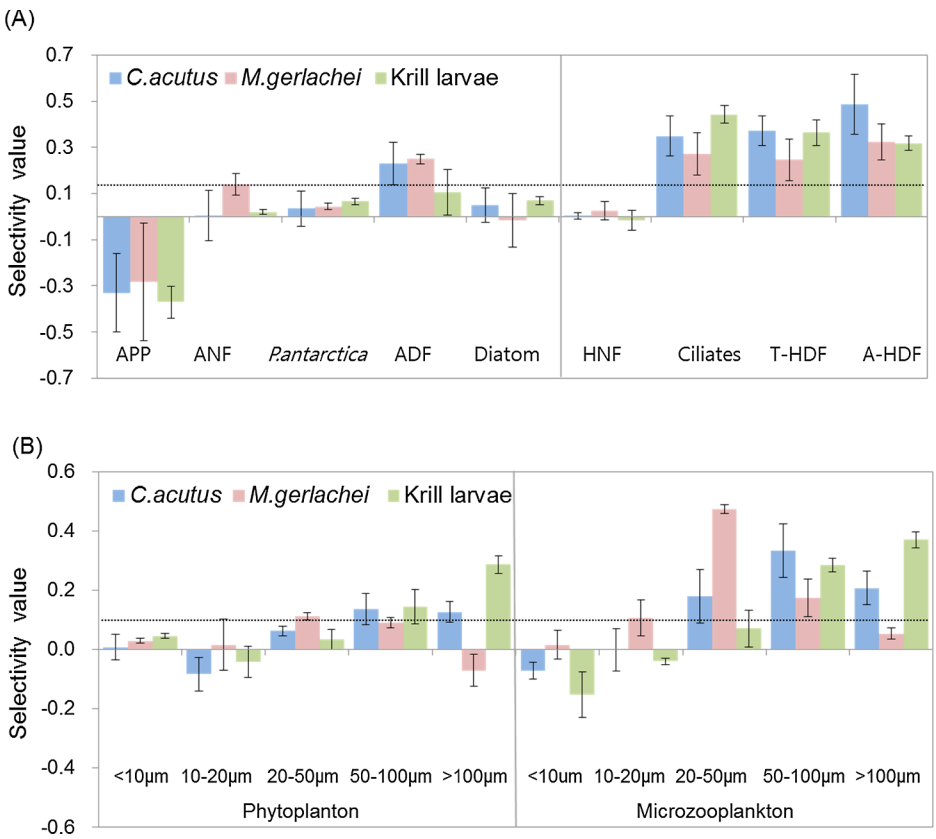

Fig. 6. Chesson's index values calculated for selectivity patterns of the two copepods and krill larvae with respect to prey composition (A) and size-class of prey (B). Value above 0.11 ($=1/n$ classes, $n = 9$, n is number of prey classes) for prey groups and 0.10 ($n = 10$) for size of prey indicate positive prey selection for the particular prey, while values below 0.11 and 0.10 indicate negative prey selection. T-HDF and A-HDF indicate thecate heterotrophic dinoflagellate and athecate heterotropic dinoflagellate. See Fig. 2 for label identification.

senescence and lysis due to decreased light intensity or nutrient limitation, which may affect microzooplankton production. Second, the decline of microzooplankton herbivory may be attributable to variations in the microzooplankton community composition and its low biomass. In the APS, vigorous microzooplankton herbivory was stimulated by increasing biomass of prey and grazers during the early summer (Yang et al., 2016). In late summer, decreasing microzooplankton biomass and the decline of HDF as the dominant grazer of phytoplankton might affect microzooplankton grazing. Another possible explanation for the decline of microzooplankton herbivory may be a top-down impact from mesozooplankton. During the summer, the mesozooplankton and krill abundance increased from the early to late summer, suggesting top-down control of mesozooplankton on microzooplankton (Lee et al., 2013; La et al., 2015).

4.2. Mesozooplankton diet composition and selective feeding

Mesozooplankton generally take advantage of the most common prey (phytoplankton) available to them during phytoplankton blooms (Fessenden and Cowles, 1994; Yang et al., 2010; Campbell et al., 2016). However, when phytoplankton biomass is low and dominated by small cells, microzooplankton contribute significantly to mesozooplankton diets (Batten et al., 2001; Calbet and Saiz, 2005; Dutz and Peters, 2008). Contrary to previous results, the present study revealed that less-abundant microzooplankton constituted a substantial proportion of the diet of both copepods and krill larvae, despite the phytoplankton biomass being consistently high (Figs. 3 and 4; Table 4). Microzooplankton comprised an average of 41.7% of available carbon and 75.4% of the carbon consumed by two copepod species and krill larvae (Fig. 5; Table 4). Although the two copepods and krill larvae have different body sizes and feeding strategies, their grazing activity on microzooplankton communities was similar. Among all prey, HDF was an important dietary item for mesozooplankton, even though the HDF biomass was significantly less than that of phytoplankton (Fig. 5). Moreover, copepods and krill larvae showed a strong selective preference for microzooplankton over phytoplankton, and showed a specific preference for HDF. Many previous studies have focused on the link between copepods and ciliates (Calbet and Saiz, 2005), and HDF has often been excluded from such studies despite the importance of HDF as a key component of the microzooplankton. Several studies have reported that dinoflagellates could be as important as ciliates in copepod diets in oligotrophic systems or when HDF biomass is high (Schnetzer and Caron, 2005; Fileman et al., 2010; Saiz and Calbet, 2011). In productive polynya ecosystems, high grazing rates and strong selectivity for HDF in mesozooplankton diets is attributable to the greater initial biomass and larger cell size of HDF compared to ciliates. Ciliates were dominant among cells with sizes of 20–50 μm, whereas HDF were 50–100 μm in this study area (data not shown). The larger HDF encountered by mesozooplankton in this study area might have been more suitable as prey than smaller ciliates. Interestingly, mesozooplankton also strongly selected for ADF, which were present at low prey concentrations. Both ADF and HDF comprised 52.8% of the mesozooplankton diet in this study area, indicating that the dinoflagellate-mesozooplankton relationship might be more important when dinoflagellate biomass is relatively high. Thus, the HDF-mesozooplankton relationship must be considered in further planktonic food web analyses. Additionally, the low grazing rate of mesozooplankton on phytoplankton during the study period may be attributed to the dominance of *P. antarctica* and diatoms, which mesozooplankton may find unpalatable (Turner et al., 2002; Nejstgaard et al., 2007; Saiz and Calbet, 2011). *Phaeocystis antarctica* and diatoms made up an average of 11.7% and 7.9% of mesozooplankton diets, respectively. Several studies have reviewed various negative effects of *P. antarctica* on zooplankton, particularly during the colonial stage (Gasparini et al., 2000; Irigoien et al., 2003), which has been supported by biochemical analysis (Claustre et al., 1990). The low grazing rate on diatom concurs with the results of Saiz and Calbet (2011) that globally, diatoms contribute only 8% of copepod diets, even in highly productive systems. These results indicate

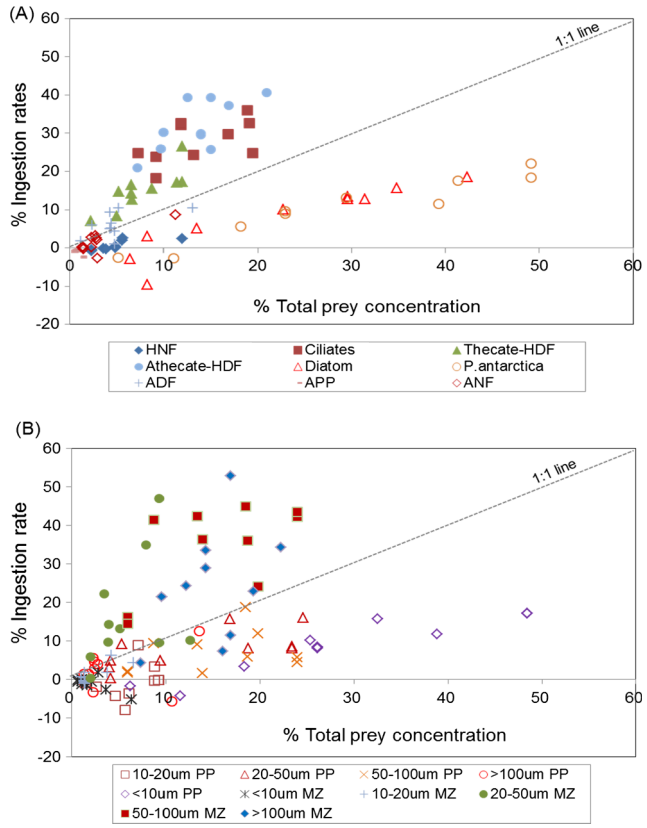

Fig. 7. Relative presence of total prey concentration in the diet of two copepods and krill larvae as a function of its relative concentration in the water. Data above the 1:1 line indicates positive grazing selection for that particular prey. (A) is prey compositions and (B) is prey size classes. PP is phytoplankton, MZ is microzooplankton.

that *P. antarctica* and diatoms are generally the most abundant food sources in the Amundsen Sea, but that species-specific characteristics such as cell size, prey quality, and palatability may prevent mesozooplankton from consuming them under certain conditions.

Three mesozooplankton species exhibited omnivorous feeding during the late summer. *Calanoides acutus*, which is widely considered herbivorous (Atkinson, 1995; Pasternak and Schnack-Schiel, 2001; Urban-Rich et al., 2001), mainly fed on ciliates and HDF rather than phytoplankton in the late summer (Figs. 3 and 4). Previous studies have reported that *C. acutus* could shift from herbivory to omnivory to meet its energy requirements under conditions of limited food availability (Calbet et al., 2006; Yang et al., 2013). Our results demonstrated that *C. acutus* might be omnivorous in *Phaeocystis*-dominant systems based on prey quality and size availability. The omnivorous behavior of *M. gerlachei* and krill larvae in this study area was supported by the fact that *E. crystallorophias* and *M. gerlachei* are mainly carnivorous during winter when food is scarce and omnivorous during summer when phytoplankton bloom (Hagen and Auel, 2001; Ju and Harvey, 2004). Specifically, *E. crystallorophias* diets range from more carnivorous during early spring to omnivorous at the onset of the phytoplankton bloom (Pakhomov and Perissinotto, 1996). Most mesozooplankton are omnivorous with flexible feeding behavior, and the degree of omnivory depends primarily on the food environment, and also varies among mesozooplankton taxa (Metz and Schnack-Schiel, 1995; Froneman et al., 1996; Calbet et al., 2006). Thus, the omnivorous feeding activity of mesozooplankton during the late summer may have a fundamental effect on the structures of phyto- and microzooplankton communities in this study area.

Most mesozooplankton exhibit prey selectivity as a strategy to maximize their survival and reproductive capacity. During this study, copepods and krill larvae positively selected ciliates and dinoflagellates as food, and specifically selected > 20 μm-sized microzooplankton as prey in all experiments (Fig. 6). This result clearly showed that mesozooplankton preferentially ingest larger microzooplankton at greater rates over similarly-sized phytoplankton. This selective feeding behavior was independent of the phytoplankton biomass and taxa. The selective feeding patterns of individual mesozooplankton were similar in terms of prey composition, but mesozooplankton showed differing prey size preferences. A strong preference was shown by mesozooplankton grazing on microzooplankton, with 50–100 μm-sized prey preferred by *C. acutus*, 20–50 μm-sized prey by *M. gerlachei*, and > 100 μm-sized prey by krill larvae (Fig. 5). These differences in size selectivity among mesozooplankton may be attributable to mesozooplankton body size. Relatively large krill larvae showed a strong preference for larger prey. In contrast, *M. gerlachei*, which is considered a small-particle grazer (Hopkins and Torres, 1989; Perissinotto, 1992), showed a strong selective preference for relatively small prey. Patterns of selectivity among copepods mostly depend on prey size, motility, food quality, and the specific type of predator (Cowles et al., 1988; Atkinson, 1995; Rollwagen Bollens and Penry, 2003; Castellani et al., 2008). A combination of prey motility and size has been suggested to be the main factor in the selective feeding patterns of Antarctic copepods (Atkinson, 1995). Food quality (composition) and size are both reasonable possibilities for the prey selection factors used by mesozooplankton in the study area. The effects of selective feeding on the composition and size of food particles indicated that larger ciliates and HDF might experience stronger top-down regulation than smaller species. Therefore, selective feeding behavior by mesozooplankton may affect the size structure and diversity of plankton communities, especially those of microzooplankton, which in turn could affect the efficiency of carbon flux in the ASP ecosystem.

During the sampling periods, the average grazing rate of krill larvae was twice as high as that of the two copepods, possibly as a result of greater krill body mass. Individual grazing rates showed a positive correlation with carbon biomass and initial food concentration (Table 4). This result was supported by a previous study showing that food availability and carbon biomass were major factors in shaping natural copepod-feeding rates (Saiz and Calbet, 2011). Mesozooplankton daily rations of phytoplankton as a percentage of body carbon ranged from 1.1% to 3.1%, and fell within the ranges observed in previous summer studies (Lee et al., 2013; Gleiber et al., 2016), but were barely sufficient to meet metabolic demands. This result is common for SO copepods (Atkinson et al., 1996; Mayzaud et al., 2002; Calbet et al., 2006), indicating that microzooplankton may provide an important food source. In addition, carbon biomass rations from microzooplankton averaged 5.1% for the two copepods, and 9.6% for krill larvae. However, the total daily rations from phytoplankton and microzooplankton combined were still low (< 14% body carbon per day). Thus, food availability in all experiments was insufficient to meet the daily requirements of krill larvae and copepods. This finding could help to explain why low mesozooplankton abundance was observed in this study (Lee et al., 2013; Wilson et al., 2015). Despite selective feeding by microheterotrophs, mesozooplankton probably also ingest organic detritus or small metazoans in food-limited environments in order to meet their metabolic needs. For example, *M. gerlachei* exhibited positive selection for *Oithona similis*, and krill larvae ingested *O. similis* and ciliates (Wickham and Berninger, 2007). These assumptions suggest that zooplankton must be strongly omnivorous or carnivorous to survive in this region.

4.3. Trophic link of micro- and mesozooplankton in the planktonic food web

The micro-mesozooplankton link is an important pathway for energy and material flux, and eventually connects to the classic fish food chain (Calbet and Saiz, 2005; Saiz and Calbet, 2011). Nevertheless, few studies have reported higher grazing rates of mesozooplankton on

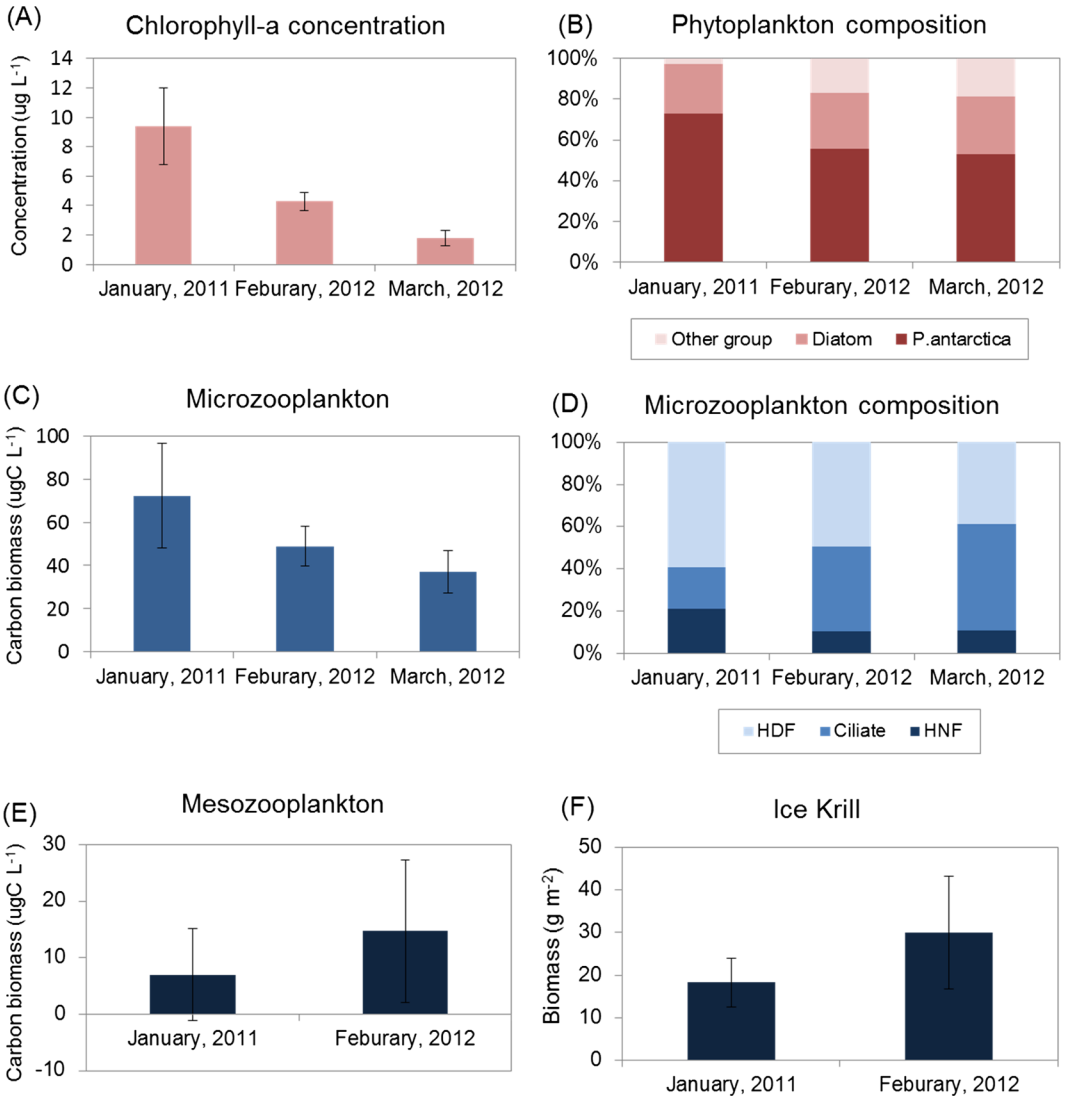

Fig. 8. Annual comparison of average chlorophyll-a concentration (A), phytoplankton composition (B), microzooplankton biomass (C), microzooplankton composition (D), mesozooplankton biomass (E) and Ice krill (F). Phytoplankton and microzooplankton are quoted from the data in Yang et al. (2016) for January 2011. Mesozooplankton is quoted from the data in Lee et al. (2013) for January 2011, and the that of February 2012 is unpublished. Ice krill is quoted from the data in La et al. (2015).

microzooplankton than on phytoplankton in the SO (Atkinson, 1996; Calbet et al., 2006; Wickham and Berninger, 2007; Yang et al., 2013). The dearth of information available on the mesozooplankton grazing rates on microzooplankton precludes any speculation about the potential role of the primary grazers in the pelagic food web of the SO. In this study area, estimates of micro- and mesozooplankton grazing have important implications for carbon fluxes and ecosystem functions. Although the evidence from our experiments is restricted due to the limited types of organisms tested, three conclusions can be drawn to clarify ASP ecosystem dynamics during late summer. First, microzooplankton consumed at least half of the phytoplankton production, but the grazing pressure of microzooplankton may not contribute strongly to the decline of the bloom due to low food quality, declining microzooplankton biomass, shifts of the dominant species, and grazing pressure from mesozooplankton. Second, copepods and krill larvae preferentially ingested microzooplankton over phytoplankton, and positively selected for large microzooplankton at most locations, regardless of the phytoplankton size and composition. Third, mesozooplankton could reduce the grazing pressure of microzooplankton on phytoplankton, thus controlling the populations of HDF and ciliates > 20 μm in size. As a result, about half of the primary production may indirectly reach copepods through their consumption of ciliates and HDF. Ciliates and HDF may thereby provide a direct trophic link between primary producers and mesozooplankton in the Amundse Sea.

Together, the results of this study confirmed the important role played by microzooplankton as a trophic intermediary in the food web of a productive polynya ecosystem. In the APS, microzooplankton were more efficient than mesozooplankton in the decline of the phytoplankton bloom during the bloom peak, and microzooplankton grazing accounted for about 34% of primary production (Yager et al., 2016). Immediately after the bloom peak (Januray 2011), microzooplankton herbivory became increasingly important, with microzooplankton removing > 80% of the primary production (Yang et al., 2016), whereas < 3% of primary production was grazed by mesozooplankton (Lee et al., 2013). In contrast, during the late summer, microzooplankton biomass and herbivory gradually decreased toward the end of bloom, and microzooplankton removed about half of daily primary production. At that time, mesozooplankton and krill biomass increased more strongly than during the early summer (Fig. 8). These results indicate that the microheterotrophic pathway, which may become more important for mesozooplankton during the decline phase of blooms, transfers photosynthetically fixed carbon to higher trophic

levels, and thereby enhancing the efficiency of the pelagic food web. Although the two copepods and krill larvae used in this study do not represent the entire mesozooplankton community in the study area, their selective feeding behavior and higher grazing pressure on large microzooplankton over phytoplankton indicate the importance of the microheterotrophic pathway via strong trophic coupling between mesozooplankton and the microbial food web in the highly productive Amundsen Sea. To better understand ASP ecosystem dynamics, the study reported herein emphasizes the need for further research to gain a broader perspective on the trophic link between the microbial community and mesozooplankton in polynya ecosystems of the high-latitude SO.

Acknowledgments

The authors thank the captain and crew of the *IBRV* ARAON who were most helpful in all shipboard operations. This research was supported by the Korea Polar Research Institute, South Korea (KOPRI grants; PE18060).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pocean.2018.12.003.

References

Arrigo, K.R., van Dijken, G.L., 2003. Phytoplankton dynamics within 37 Antarctic coastal polynya systems. J. Geophys. Res. 108 (C8), 3271. https://doi.org/10.1029/2002JC001739.
Atkinson, A., 1995. Omnivory and feeding selectivity in five copepod species during spring in the Bellingshausen Sea, Antarctica. ICES J. Mar. Sci. 52, 385–396.
Atkinson, A., 1996. Subantarctic copepods in an oceanic, low chlorophyll environment: ciliate predation, food selectivity and impact on prey populations. Mar. Ecol. Prog. Ser. 187, 85–96.
Atkinson, A., Shreeve, R.S., Pakhomov, E.A., Priddle, J., Blight, S.P., Ward, P., 1996. Zooplankton response to a phytoplankton bloom near South Georgia, Antarctica. Mar. Ecol. Prog. Ser. 144, 195–210.
Batten, S.D., Fileman, E.S., Halvorsen, E., 2001. The contribution of microzooplankton to the mesozooplankton diet in an upwelling filament off Galicia. Prog. Oceanogr. 51, 385–398.
Børsheim, K.Y., Bratbak, G., 1987. Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar. Ecol. Prog. Ser. 36, 171–175.
Broglio, E., Saiz, E., Calbet, A., Trepat, I., Alcaraz, M., 2004. Trophic impact and prey selection by crustacean zooplankton on the microbial communities of an oligotrophic coastal area (NW Mediterranean Sea). Aquat. Microb. Ecol. 35, 65–78.
Calbet, A., Landry, M.R., 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol. Oceanogr. 49, 51–57.
Calbet, A., Saiz, E., 2005. The ciliate-copepod link in marine ecosystems. Aquat. Microb. Ecol. 38, 157–167.
Calbet, A., Atienza, D., Broglio, E., Alcaraz, M., Vaque, D., 2006. Trophic ecology of *Calanoides acutus* in Gerlache Strait and Bellingshausen Sea waters (Antarctica, December 2002). Polar Biol. 29, 510–518.
Calbet, A., Saiz, E., Almeda, R., Movilla, J.I., Alcaraz, M., 2011. Low microzooplankton grazing rates in the Arctic Ocean during a *Phaeocystis pouchetii* bloom (Summer 2007): fact or artifact of the dilution technique? J. Plankton Res. 33, 687–701.
Campbell, R.G., Ashjian, C.J., Sherr, E.B., Sherr, B.F., Lomas, M.W., Ross, C., Alatalo, P., Gelfman, C., Van Keuren, D., 2016. Mesozooplankton grazing during spring sea-ice conditions in the eastern Bering Sea. Deep Sea Res. Part II Top. Stud. Oceanogr. 134, 157–172.
Caron, D.A., Dennett, M.R., Lonsdale, D.J., Moran, D.M., Shalapyonok, L., 2000. Microzooplankton herbivory in the Ross Sea, Antarctica. Deep Sea Res. Part II Top. Stud. Oceanogr. 47, 3249–3272.
Caron, D.A., Hutchins, D.A., 2013. The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. J. Plankton Res. 35, 235–252.
Castellani, C., Irigoien, X., Mayor, D.J., Harris, R.P., Wilson, D., 2008. Feeding of *Calanus finmarchicus* and *Oithona similis* on the microplankton assemblage in the Irminger Sea, North Atlantic. J. Plankton Res. 30, 1095–1116.
Chesson, J., 1983. The estimation and analysis of preference and its relationship to foraging models. Ecology 64, 1297–1304.
Claustre, H., Poulet, S., Williams, R., Marty, J.-C., Coombs, S., Mlih, F.B., Hapette, A., Jezequel-Martin, V., 1990. A biochemical investigation of a *Phaeocystis* sp. bloom in the Irish Sea. J. Mar. Biol. Assoc. U.K. 70, 197–207.
Cowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100, 41–49.
Dam, H.G., Zhang, X., Butler, M., Roman, M.R., 1995. Mesozooplankton grazing and metabolism at the equator in the central Pacific: implications for carbon and nitrogen fluxes. Deep Sea Res. Part II Top. Stud. Oceanogr. 42, 735–756.
Dubischar, C.D., Bathmann, U.V., 1997. Grazing impact of copepods and salps on phytoplankton in the Atlantic sector of the Southern Ocean. Deep Sea Res. Part II Top. Stud. Oceanogr. 44, 415–433.
Dutz, J., Peters, J., 2008. Importance and nutritional value of large ciliates for the reproduction of *Acartia clausi* during the post spring-bloom period in the North Sea. Aquat. Microb. Ecol. 50, 261–277.
Edler, L., 1979. Phytoplankton and chlorophyll: recommendations on methods for marine biological studies in the Baltic Sea. Balt. Mar. Biolog. Publ. 5, 1–38.
Fessenden, L., Cowles, T.J., 1994. Copepod predation on phagotrophic ciliates in Oregon coastal waters. Mar. Ecol. Prog. Ser. 107, 103–111.
Fileman, E., Petropavlovsky, A., Harris, R., 2010. Grazing by the copepods *Calanus helgolandicus* and *Acartia clausi* on the protozooplankton community at station L4 in the Western English Channel. J. Plankton Res. 32, 709–724.
Fonda Umani, S., Tirelli, V., Beran, A., Guardiani, B., 2005. Relationships between microzooplankton and mesozooplankton: competition versus predation on natural assemblages of the Gulf of Trieste (northern Adriatic Sea). J. Plankton Res. 27, 973–986.
Froneman, P.W., Pakhomov, E., Perissinotto, R., McQuaid, C., 1996. Role of microplankton in the diet and daily ration of Antarctic zooplankton species during austral summer. Mar. Ecol. Prog. Ser. 143, 15–23.
Frost, B.W., 1972. Effects of size and concentration of food particles on feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 17, 805–815.
Gasparini, S., Daro, M.H., Antajan, E., Tackx, M., Rousseau, V., Parent, J.-Y., Lancelot, C., 2000. Mesozooplankton grazing during the *Phaeocystis globosa* bloom in the southern bight of the North Sea. J. Sea Res. 43, 345–356.
Gifford, D.J., 1991. The protozoan-metazoan trophic link in pelagic ecosystems. J. Eukaryot. Microbiol. 38, 81–86.
Gleiber, M.R., Steinberg, D.K., Schofield, O.M., 2016. Copepod summer grazing and fecal pellet production along the Western Antarctic Peninsula. J. Plankton Res. 38, 732–750.
Grattepanche, J.-D., Vincent, D., Breton, E., Christaki, U., 2011. Microzooplankton herbivory during the diatom–*Phaeocystis* spring succession in the eastern English Channel. J. Exp. Mar. Biol. Ecol. 404, 87–97.
Hagen, W., Auel, H., 2001. Seasonal adaptations and the role of lipids in oceanic zooplankton. Zoology 104, 313–326.
Hopkins, T.L., Torres, J.J., 1989. Midwater food web in the vicinity of a marginal ice zone in the western Weddell Sea. Deep Sea Res. Part I Oceanogr. Res. 36, 543–560.
Irigoien, X., Titelman, J., Harris, R.P., Harbour, D., Castellani, C., 2003. Feeding of *Calanus finmarchicus* nauplii in the Irminger Sea. Mar. Ecol. Prog. Ser. 262, 193–200.
Ju, S.J., Harvey, H.R., 2004. Lipids as markers of nutritional condition and diet in the Antarctic krill *Euphausia superba* and *Euphausia crystallorophias* during austral winter. Deep Sea Res. Part II Top. Stud. Oceanogr. 51, 2199–2214.
Kleppel, G., 1993. On the diets of calanoid copepods. Mar. Ecol. Prog. Ser. 99, 183–195.
La, H.S., Lee, H., Fielding, S., Kang, D., Ha, H.K., Atkinson, A., Park, J., Siegel, V., Lee, S.H., Shin, H.C., 2015. High density of ice krill (*Euphausia crystallorophias*) in the Amundsen sea coastal polynya, Antarctica. Deep Sea Res. Part I Oceanogr. Res. 95, 75–84.
Landry, M., Hassett, R., 1982. Estimating the grazing impact of marine microzooplankton. Mar. Biol. 67, 283–288.
Lee, D.B., Choi, K.H., Ha, H.K., Yang, E.J., Lee, S.H., Lee, S., Shin, H.C., 2013. Mesozooplankton distribution patterns and grazing impacts of copepods and *Euphausia crystallorophias* in the Amundsen Sea, West Antarctica, during austral summer. Polar Biol. 36, 1215–1230.
Lee, Y., Yang, E.J., Park, J., Jung, J., Kim, T.W., Lee, S., 2016. Physical-biological coupling in the Amundsen Sea, Antarctica: influence of physical factors on phytoplankton community structure and biomass. Deep Sea Res. Part I Oceanogr. Res. Pap. 117, 51–60.
Li, C., Sun, S., Zhang, G., Ji, P., 2001. Summer feeding activities of zooplankton in Prydz Bay, Antarctica. Polar Biol. 24, 892–900.
Mathot, S., Smith, W.O., Carlson, C.A., Garrison, D.L., Gowing, M.M., Vickers, C.L., 2000. Carbon partitioning within *Phaeocystis antarctica* (Prymnesiophyceae) colonies in the Ross Sea, Antarctica. J. Phycol. 36, 1049–1056.
Mayzaud, P., Tirelli, V., Errhif, A., Labat, J., Razouls, S., Perissinotto, R., 2002. Carbon intake by zooplankton. Importance and role of zooplankton grazing in the Indian sector of the Southern Ocean. Deep Sea Res. Part II Top. Stud. Oceanogr. 49, 3169–3187.
Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr. 45, 569–579.
Metz, C., Schnack-Schiel, S., 1995. Observations on carnivorous feeding in Antarctic calanoid copepods. Mar. Ecol. Prog. Ser. 129, 71–75.
Nakajima, R., Yamazaki, H., Lewis, L.S., Khen, A., Smith, J.E., Nakatomi, N., Kurihara, H., 2017. Planktonic trophic structure in a coral reef ecosystem–Grazing versus microbial food webs and the production of mesozooplankton. Prog. Oceanogr. 156, 104–120.
Nejstgaard, J.C., Naustvoll, L.J., Sazhin, A., 2001. Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton. Mar. Ecol. Prog. Ser. 221, 59–75.
Nejstgaard, J.C., Tang, K.W., Steinke, M., Dutz, J., Koski, M., Antajan, E., Long, J.D., 2007. Zooplankton grazing on *Phaeocystis*: a quantitative review and future challenges. Biogeochemistry 83, 147–172.
Pakhomov, E., Froneman, P.W., 2004. Zooplankton dynamics in the eastern Atlantic sector of the Southern Ocean during the austral summer 1997/1998—Part 2: grazing impact. Deep Sea Res. Part II Top. Stud. Oceanogr. 51, 2617–2631.
Pakhomov, E., Perissinotto, R., 1996. Antarctic neritic krill *Euphausia crystallorophias*: spatio-temporal distribution, growth and grazing rates. Deep Sea Res. Part I

Oceanogr. Res. Pap. 43, 59–87.
Pasternak, A.F., Schnack-Schiel, S.B., 2001. Feeding patterns of dominant Antarctic copepods: an interplay of diapause, selectivity, and availability of food. Hydrobiologia 453, 25–36.
Perissinotto, R., 1992. Mesozooplankton size-selectivity and grazing impact on the phytoplankton community of the Prince Edward Archipelago (Southern Ocean). Mar. Ecol. Prog. Ser. 79, 243–258.
Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford, pp. 173.
Putt, M., Stoecker, D.K., 1989. An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol. Oceanogr. 34, 1097–1103.
Rollwagen Bollens, G., Penry, D.L., 2003. Feeding dynamics of *Acartia* spp. copepods in a large, temperature estuary (San Francisco Bay, CA). Mar. Ecol. Prog. Ser. 257, 139–158.
Saiz, E., Calbet, A., Irigoien, X., Alcaraz, M., 1999. Copepod egg production in the western Mediterranean: response to food availability in oligotrophic environments. Mar. Ecol. Prog. Ser. 187, 179–189.
Saiz, E., Calbet, A., 2011. Copepod feeding in the ocean: scaling patterns, composition of their diet and the bias of estimates due to microzooplankton grazing during incubations. Hydrobiologia 666, 181–196.
Schmoker, C., Hernández-León, S., Calbet, A., 2013. Microzooplankton grazing in the oceans: impacts, data variability, knowledge gaps and future directions. J. Plankton Res. 35, 691–706.
Schnetzer, A., Caron, D.A., 2005. Copepod grazing impact on the trophic structure of the microbial assemblage of the San Pedro Channel, California. J. Plankton Res. 27, 959–971.
Sherr, E.B., Sherr, B.F., 2002. Significance of predation by protists in aquatic microbial food webs. Antonie Van Leeuwenhoek 81, 293–308.
Sherr, E.B., Sherr, B.F., 2007. Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. Mar. Ecol. Prog. Ser. 352, 187–197.
Sherr, E.B., Sherr, B.F., 2009. Capacity of herbivorous protists to control initiation and development of mass phytoplankton blooms. Aquat. Microb. Ecol. 57, 253–262.
Smith, J.A., Hillenbrand, C.-D., Kuhn, G., Larter, R.D., Graham, A.G., Ehrmann, W., Moreton, S.G., Forwick, M., 2011. Deglacial history of the West Antarctic Ice Sheet in the western Amundsen Sea embayment. Quat. Sci. Rev. 30, 488–505.
Stelfox-Widdicombe, C., Archer, S., Burkill, P., Stefels, J., 2004. Microzooplankton grazing in *Phaeocystis* and diatom-dominated waters in the southern North Sea in spring. J. Sea Res. 51, 37–51.
Stoecker, D.K., Gifford, D.J., Putt, M., 1994. Preservation of marine planktonic ciliates: losses and cell shrinkage during fixation. Mar. Ecol. Prog. Ser. 110, 239–299.
Swalethorp, R., Dinasquet, J., Logares, R., Bertilsson, S., Kjellerup, S., Krabberød, A.K., Moksnes, P., Nielsen, T.G., Riemann, L., 2018. Microzooplankton distribution in the Amundsen Sea Polynya (Antarctica) during an extensive *Phaeocystis antarctica* bloom. Prog. Oceanogr. 170, 1–10.
Taylor, A.G., Landry, M.R., Selph, K.E., Yang, E.J., 2011. Biomass, size structure and depth distributions of the microbial community in the eastern equatorial Pacific. Deep Sea Res. Part II Top. Stud. Oceanogr. 58, 342–357.
Teixeira, I., Crespo, B., Nielsen, T.G., Figueiras, F., 2012. Role of microzooplankton during a *Phaeocystis* sp. bloom in the Oosterschelde (SW Netherlands). J. Mar. Syst. 94, 97–106.
Turner, J.T., Ianora, A., Esposito, F., Carotenuto, Y., Miralto, A., 2002. Zooplankton feeding ecology: does a diet of *Phaeocystis* support good copepod grazing, survival, egg production and egg hatching success? J. Plankton Res. 24, 1185–1195.
Urban-Rich, J., Dagg, M., Peterson, J., 2001. Copepod grazing on phytoplankton in the Pacific sector of the Antarctic Polar Front. Deep Sea Res. Part II Top. Stud. Oceanogr. 48, 4223–4246.
Vargas, C.A., González, H.E., 2004. Plankton community structure and carbon cycling in a coastal upwelling system II. Microheterotrophic pathway. Aquat. Microb. Ecol. 34, 165–180.
Vargas, C.A., Martínez, R.A., Cuevas, L.A., Pavez, M.A., Cartes, C., González, H.E., Escribano, R., Daneri, G., 2007. The relative importance of microbial and classical food webs in a highly productive coastal upwelling area. Limnol. Oceanogr. 52, 1495–1510.
Vargas, C.A., Martínez, R.A., González, H.E., Silva, N., 2008. Contrasting trophic interactions of microbial and copepod communities in a fjord ecosystem, Chilean Patagonia. Aquat. Microb. Ecol. 53, 227–242.
Verity, P.G., Stoecker, D.K., Sieracki, M.E., Nelson, J.R., 1993. Grazing, growth and mortality of microzooplankton during the 1989 North Atlantic spring bloom at 47 N, 18 W. Deep Sea Res. Part I Oceanogr. Res. Pap. 40, 1793–1814.
Walker, D.P., Brandon, M.A., Jenkins, A., Allen, J.T., Dowdeswell, J.A., Evans, J., 2007. Oceanic heat transport onto the Amundsen Sea shelf through a submarine glacial trough. Geophys. Res. Lett. 34, 1–4.
Wickham, S.A., Berninger, U.-G., 2007. Krill larvae, copepods and the microbial food web: interactions during the Antarctic fall. Aquat. Microb. Ecol. 46, 1–13.
Wilson, S.E., Swalethorp, R., Kjellerup, S., Wolverton, M.A., Ducklow, H.W., Yager, P.L., 2015. Meso-and macro-zooplankton community structure of the Amundsen Sea Polynya, Antarctica (Summer 2010–2011). Elem. Sci. Anth. 3, 000033.
Winberg, G., Duncan, A., 1971. Methods for the estimation of production of aquatic animals. Academic Press, New York.
Yager, P.L., Sherrell, R., Stammerjohn, S., Ducklow, H., Schofield, O., Ingall, E., Wilson, S., Lowry, K., Williams, C., Riemann, L., 2016. A carbon budget for the Amundsen Sea Polynya, Antarctica: estimating net community production and export in a highly productive polar ecosystem. Elem. Sci. Anth. 4, 000140.
Yang, E.J., Jiang, Y., Lee, S., 2016. Microzooplankton herbivory and community structure in the Amundsen Sea, Antarctica. Deep Sea Res.Part II Top. Stud. Oceanogr. 123, 58–68.
Yang, E.J., Ju, S.-J., Choi, J.-K., 2010. Feeding activity of the copepod *Acartia hongi* on phytoplankton and micro-zooplankton in Gyeonggi Bay, Yellow Sea. Estuar. Coast. Shelf Sci. 88, 292–301.
Yang, E.J., Kang, H.-K., Yoo, S., Hyun, J.-H., 2009. Contribution of auto-and heterotrophic protozoa to the diet of copepods in the Ulleung Basin, East Sea/Japan Sea. J. Plankton Res. 31, 647–659.
Yang, G., Li, C., Sun, S., Zhang, C., He, Q., 2013. Feeding of dominant zooplankton in Prydz Bay, Antarctica, during austral spring/summer: food availability and species responses. Polar Biol. 36, 1701–1707.