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Role of heterotrophic dinoflagellates in the fate of diatoms released from fast ice in coastal water of Lützow-Holm Bay, East Antarctica

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ABSTRACT: To understand the fate of ice algal diatoms released from fast ice, we investigated the abundance and sinking loss of diatoms and the grazing impact on diatoms by heterotrophic dinoflagellates (HD) under the ice near Syowa Station, Antarctica, during the austral summer of 2005–2006. After a rapid increase, diatoms showed a clear declining phase. Among the diatom assemblage, *Porosira pseudodenticulata* and *Pseudo-nitzschia* cf. *turgiduloides* were abundant in the water column but low in the sinking flux as they are able to maintain their position in the surface layer after release from the fast ice. Potential grazing impact by HD was calculated to reach 233 mg C m^{-2} d^{-1}, equivalent to 48.7% d^{-1} of the diatom biomass being removed daily. Only 14.9 to 71.3 mg C m^{-2} d^{-1} (2.5 to 3.2% d^{-1}) was attributable to diatom sinking loss. This suggests that a significant fraction of the diatoms was consumed in the surface layer and the sinking loss was comparatively small. HD often had ingested diatoms in their cells, although ciliates rarely did, and the abundance of HD fecal pellets peaked after the diatom peak. In bottle incubations at *in situ* temperature, the growth rates of HD ranged from 0 to 0.19 d^{-1}, indicating almost positive growth of HD in the water column. This demonstrates that HD are major consumers of the diatoms released from the fast ice, forming a dominant trophic link between diatoms and HD in the Antarctic under-ice ecosystem.

KEY WORDS: Heterotrophic dinoflagellates · Diatoms · Ciliates · Fast ice · Antarctica

INTRODUCTION

Diatoms grow extensively on the underside of sea ice and are the dominant components of the ice algal community both in Arctic (Gosselin et al. 1990, Horner et al. 1992) and Antarctic waters (Palmisano & Garrison 1993, Ackley & Sullivan 1994, Garrison et al. 2005). During the sea ice melting season, diatoms are released from the ice, which results in high chlorophyll concentrations in the underlying water (Grossi et al. 1987, McMinn 1996, Taguchi et al. 1997). Previous studies have demonstrated that the released diatoms are an important nutritional resource for both pelagic and benthic food webs (McMahon et al. 2006, Juul-Pedersen et al. 2008).

The fate of the released diatoms is quite variable among sites, depending on whether they are consumed in the upper part of the water column or sink to the bottom (Tremblay et al. 1989). In Saroma Ko Lagoon, Japan, most organisms associated with sea ice are considered to sink and are incorporated into the benthic ecosystem due to low grazing pressure of mesozooplankton in the water column (Saito & Hattori 1997, Taguchi et al. 1997). In contrast, near Syowa Station, East Antarctica, the sinking loss to the bottom of chlorophyll (chl *a*) originating from the ice accounted for only 3.6 to 4.0% of the loss within the water column (Odate et al. 2004). In some Arctic ice-covered areas, a large fraction of the ice algal production released into

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the water column is exploited by crustacean zooplankton (Tremblay et al. 1989, Michel et al. 1993, 1996, Fortier et al. 2002).

In the water column under the fast ice near Syowa Station, abundant diatoms are observed in the austral summer (Tanimura et al. 1990, Ishikawa et al. 2001). We previously reported that *Fragilariopsis kerguelensis*, *Porosira pseudodenticulata*, *Pseudo-nitzschia* cf. *turgiduloides*, and *Thalassiosira australis*, which are recognized as ice-associated species that are found in and close to the sea ice (Palmisano & Garrison 1993, Scharek et al. 1994, Garrison et al. 2005, Scott & Thomas 2005, Roberts et al. 2007), were dominant among the diatom assemblage in the austral summer of 2005 to 2006 (Ichinomiya et al. 2008a,b). Following the predominance of these diatoms, the microalgal populations shift to phytoflagellates, e.g. autotrophic dinoflagellates and cryptophytes (Ichinomiya et al. 2007). During the course of this shift, heterotrophic dinoflagellates (HD) and ciliates increase and finally exceed autotrophs in biomass. HD have been recognized as major diatom consumers in coastal and open waters of the world oceans (Buck & Newton 1995, Strom & Strom 1996, Strom et al. 2001, Stelfox-Widdicombe et al. 2004, Horner et al. 2005, Saito et al. 2006, Sherr & Sherr 2007). In the Antarctic seas, HD have also been observed abundantly in the Weddell Sea (Nöthig et al. 1991), Ross Sea (Fonda Umani et al. 2005), and coastal waters near Davis Station (Archer et al. 1996) and Signy Island (Clarke & Leakey 1996). However, little is known about their grazing on diatoms under fast ice cover (Beaumont et al. 2002, Pearce et al. 2008). To understand the fate of the diatoms, we investigated HD grazing on diatoms, sinking loss of diatoms, and HD growth as a function of prey abundance.

MATERIALS AND METHODS

Field sampling. Sampling was conducted through ~1.8 m thick fast ice at a coastal station (69° 00′ S, 39° 37′ E) to a depth of 67 m near Syowa Station in Lützow-Holm Bay, East Antarctica, between 26 December 2005 and 2 February 2006 (Fig. 1). Water samples were collected with a 5 l Niskin bottle at depths of 2, 5, 10, 20, 30, and 50 m, and subsamples were fixed with acid Lugol's solution (1% final concentration) and glutaraldehyde (1% final concentration).

Sinking cells were collected with a cylindrical sediment trap (53 cm long, 14.5 inner diameter) with 6 sampling bottles moored at 20 m depth. The trap samples were recovered every 3 to 8 d between 29 December 2005 and 30 January 2006. The trap was filled with artificial seawater with ca. 35 g l^{-1} NaCl, but no poison or fixative was added. After recovery, the seawater in the upper part of the sampling bottles was gently removed and the trap sample in 1 of the sampling bottles was transferred to a 500 ml polyethylene bottle. The sample was made up to 500 ml with filtered seawater and fixed with formalin (2% final concentration).

Enumeration of plankton. Diatoms, autotrophic dinoflagellates, HD, and ciliates were counted in 50 to 1000 ml water samples fixed with acid Lugol's solution using the Utermöhl technique (Utermöhl 1958). The trophic states of autotrophic dinoflagellates and HD were identified by the presence of chl *a* fluorescence under blue light excitation in the samples with glutaraldehyde. Cryptophytes were identified and counted in the samples fixed with glutaraldehyde based on their autofluorescence under an epifluorescence microscope after being filtered through an Isopore membrane filter of 0.6 µm pore size (Haas 1982). HD and ciliates containing ingested diatoms inside the cells as well as HD fecal pellets containing empty diatom frustules were also counted (Fig. 2). HD and ciliates were each classified into 2 size categories, i.e. HD>20 µm, HD<20 µm, Ciliates>20 µm, and Ciliates<20 µm of equivalent spherical diameter. For the sediment trap samples, aliquots of 50 to 500 ml were examined to enumerate diatoms and HD fecal pellets using the Utermöhl technique.

Volumes of plankton were calculated from size (length and width) measured for >30 cells for each size category of each taxonomic group. Biovolume esti-

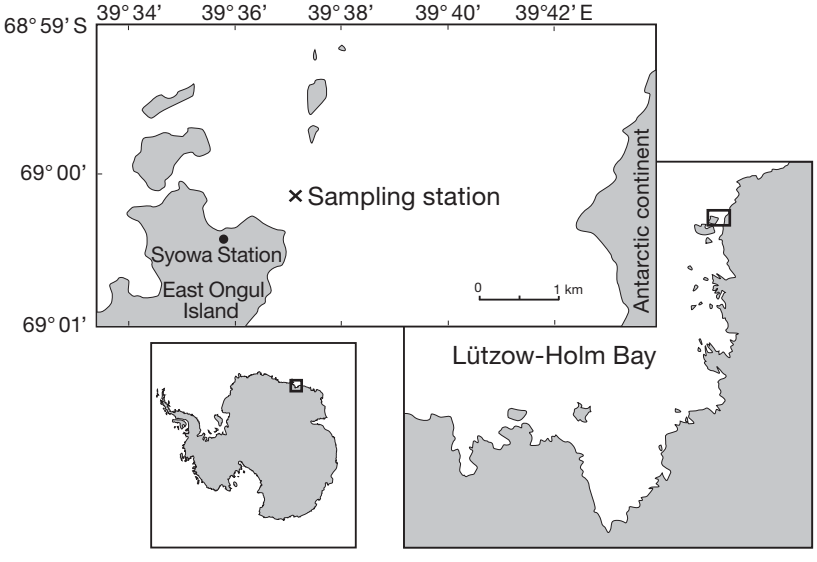

Fig. 1. Sampling station near Syowa Station, East Antarctica

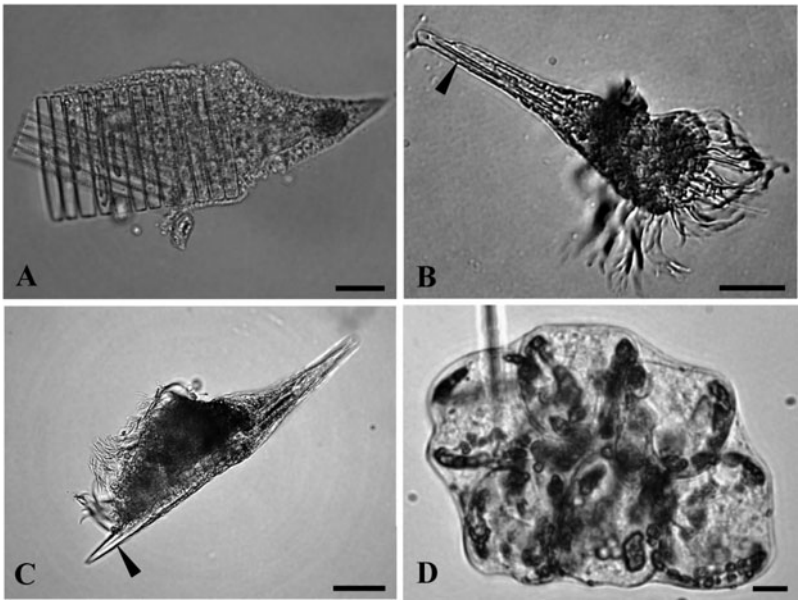

Fig. 2. Light micrographs of athecate dinoflagellates and naked ciliates ingesting diatoms, and a fecal pellet. (A) *Gyrodinium* sp. containing *Fragilariopsis kerguelensis*; (B) An oligotrich ciliate containing *Pseudo-nitzschia*-like pennate diatoms (arrow); (C) An oligotrich ciliate containing a pennate diatom (arrow); (D) A fecal pellet comprising empty frustules of *Porosira pseudodenticulata*. Scale bars = 20 µm

mates of the dominant diatoms were made from the mean linear dimensions of 300 cells in randomly selected samples (Ichinomiya et al. 2008b), since their size measurements in some samples did not reach 30 cells. The biomasses were converted to carbon weight (pg, C) from their cell or lorica volumes (µm^3, V) using the following conversion factors; cryptophytes: $\log_{10}$ C = 0.863 $\log_{10}$ V – 0.363 (Verity et al. 1992); diatoms: $\log_{10}$ C = 0.76 $\log_{10}$ V – 0.352 (Smayda 1978); dinoflagellates: C = 0.76 V$^{0.819}$ (Menden-Deuer & Lessard 2000); naked ciliates: C = 0.19 V (Putt & Stoecker 1989); and tintinnids: C = 444.5 + 0.053 V (Verity & Langdon 1984).

Growth rates of HD and ciliates. Incubation experiments were conducted 5 times, on 8, 12, 18, 24, and 30 January 2006, to estimate the growth rates of HD and ciliates. Water samples for the experiments were collected from 10 m with a 5 l Niskin bottle and gently siphoned into a 20 l polycarbonate bottle through a 200 µm mesh to remove large mesozooplankton. Two 1 l aliquots of the filtered seawater were sampled to estimate the initial abundance of HD and ciliates. Experimental water was transferred into 2 polycarbonate bottles (1 l) and suspended in the water column at 10 m for 24 h. The water aliquots before (initial) and after the incubation (final) were fixed with acid Lugol's solution (final concentration 1%) for cell counts of HD and ciliates using the Utermöhl technique. Specific growth rates (µ d^{-1}) of HD and ciliates were then calculated using the equation:

$$\mu = \ln (N_t / N_0) / t \quad (1)$$

where N_0 and N_t are the initial and final abundance of HD and ciliates, and t is the incubation period (1 d).

Grazing by HD and sinking loss of diatoms. The role of grazing by HD on diatom dynamics during the second half of the investigation was examined. Grazing by HD>20 µm was estimated from their integrated biomass through the 2 to 20 m water column and by assuming a cell-specific diatom ingestion rate. Ingestion rates of HD>20 µm were calculated from the growth rates measured in the incubation experiments using a value of 0.4 for the gross growth efficiency (Bjørnsen & Kuparinen 1991). Grazing by HD<20 µm and ciliates on diatoms was not estimated, since they rarely contained diatoms. Diatom sinking flux was obtained by the sediment trap experiments. Flux from 30 January to 2 February, when the sediment trap was not deployed, was calculated to be scaled relative to the integrated diatom biomass in the 2 to 20 m water column during the previous period (27 to 30 January).

RESULTS

Environmental conditions under the fast ice. Water temperature was between –0.6 and –1.8°C in the upper 5 m and stable at –1.7°C in the deeper layer (Ichinomiya et al. 2007). Salinity at the surface dropped to <16.0 on 12 to 18 January and varied from 33.8 to 34.1 in the layer deeper than 10 m. Low sigma-t under 20 was also observed during this period (Fig. 3). In the layer deeper than 10 m, temperature and salinity were vertically more stable, and sigma-t was less variable (27.2 to 27.5).

Abundance and sinking flux of diatoms. Diatom biomass integrated through the 2 to 20 m water column increased from the beginning of January, reached a peak of 2845 mg C m^{-2} on 24 January, and decreased to 479 mg C m^{-2} on 2 February (Fig. 4A). At the beginning of the study period, sinking flux of diatoms was less than 10 mg C m^{-2} d^{-1}, suddenly increased to 86.3 mg C m^{-2} d^{-1} on 12 to 18 January, and then gradually decreased to 50.2 mg C m^{-2} d^{-1} on 27 to 30 January (Fig. 4B).

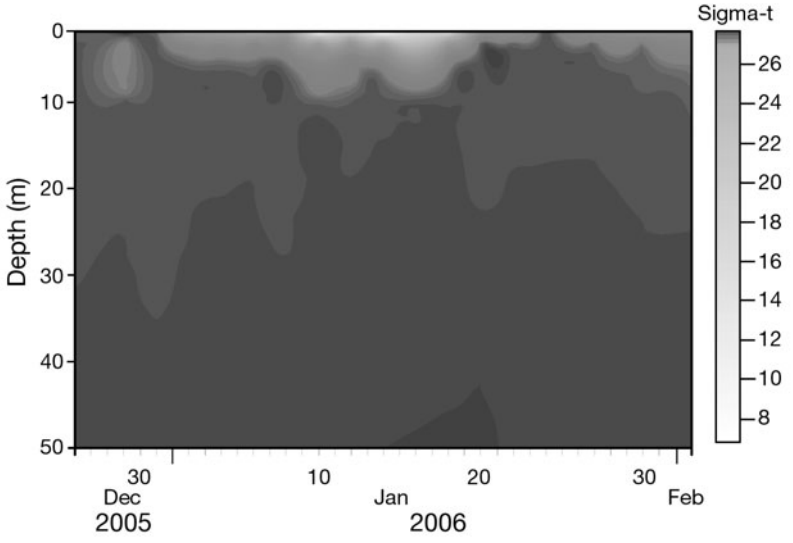

Fig. 3. Contour of sigma-t in the water column

Species composition of diatoms in the water column differed from that of sinking diatoms (Fig. 4). *Porosira pseudodenticulata* was the dominant species, accounting for 13 to 82% of the diatom biomass in the water column, but decreased to 13% on 2 February. *Pseudo-nitzschia* cf. *turgiduloides* was the second most abundant species in the water column, accounting for 26% of the diatom biomass on average. In the sinking flux, *Thalassiosira australis*, *P. pseudodenticulata*, and *Fragilariopsis kerguelensis* were abundant and accounted for 38, 23, and 19% of the total diatom sinking flux during the investigation period. *P-n.* cf. *turgiduloides* was abundant in the water column but not (7.9%) in the sinking flux.

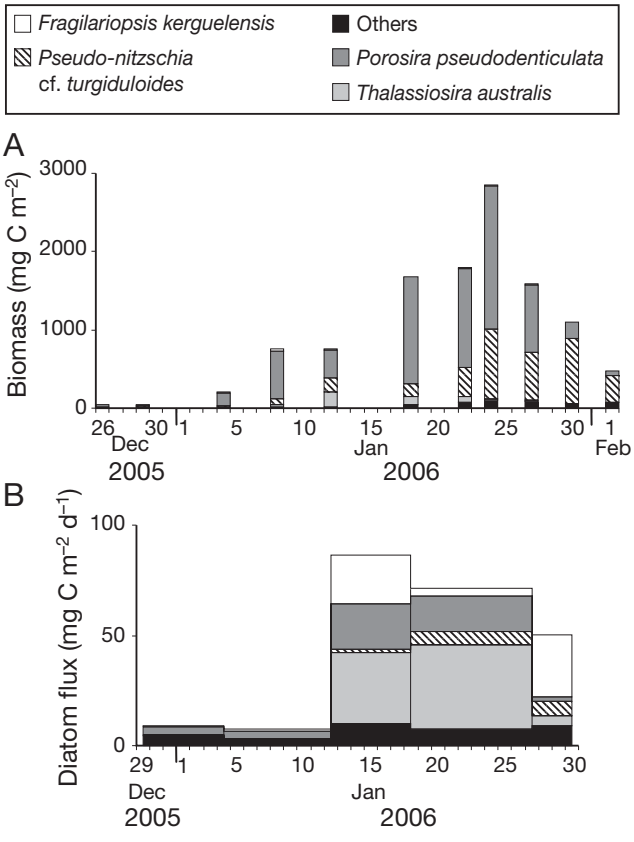

Fig. 4. Temporal variations in (A) integrated values through the water column from 2 to 20 m and (B) sinking fluxes of the diatom assemblage at 20 m (redrawn from Ichinomiya et al. 2008b)

Abundance and vertical distributions of HD, ciliates, and their prey organisms. Among both HD>20 µm and <20 µm, athecate dinoflagellates *Gyrodinium* spp. were most abundant, with an average abundance of 1.3×10^2 and 3.5×10^3 cells l^{-1}, respectively (Table 1). *Katodinium* spp., *Protoperidinium* spp., and unidentified species (probably *Amphidinium*) were minor components (32.5 to 2.7×10^2 cells l^{-1} on average). Oligotrich ciliates (*Laboea*, *Strombidium*, *Tontonia*) dominated both Ciliates>20 µm and <20 µm (1.2×10^3 and 1.4×10^3 cells l^{-1} on average, respectively), and other species, including tintinnids, were less abundant (26.6 to 1.2×10^2 cells l^{-1}). Unidentified HD and ciliates were almost limited to 2 m, with a maximum abundance of $>10^4$ cells l^{-1}. *Gymnodinium* spp. were abundant and showed autofluorescence of chloroplasts, so that this taxon was included in the autotrophic dinoflagellates.

Among the heterotrophic protists, HD>20 µm and Ciliates>20 µm were the major components, and their mean biomasses were almost equal to each other at 8.2 and 8.8 µg C l^{-1}, respectively (Table 2). Biomasses of HD<20 µm and Ciliates<20 µm were minor and comprised <1.0 µg C l^{-1}. Mean abundance of HD>20 µm that retained ingested diatoms in their cells was 1.6×10^2 cells l^{-1} and those of HD<20 µm, Ciliates>20 µm, and Ciliates<20 µm were as low as 0.03 to 15.1 cells l^{-1}.

Biomass of HD>20 µm gradually increased from mid-January and reached its maximum of 60.6 µg C l^{-1} at 10 m on 2 February (Fig. 5A). Ciliates>20 µm occurred from the end of January in the 2 to 10 m layer, and the maximum of 69.2 µg C l^{-1} was observed at 2 m on 2 February (Fig. 5B). Diatoms were mainly distributed at 5 to 20 m during the period of 18 to 27 January (over 100 µg C l^{-1}), with a maximum of 203 µg C l^{-1} at 10 m on 24 January (Fig. 5C). A dense bloom of phytoflagellates (autotrophic dinoflagellates and cryptophytes) was observed in the surface layer (2 to 10 m), with a peak of 101 µg C l^{-1} at 2 m on 30 January (Fig. 5D).

Table 1. Taxa of heterotrophic dinoflagellates (HD) and ciliates, and their mean and range of abundance throughout the investigation period

Group	Type	Taxon	Abundance (cells l^{-1}) Mean	Range
HD>20 µm	Athecate	*Gyrodinium*	1.3×10^3	31–9600
		Katodinium	32.5	~3000
	Thecate	*Protoperidinium*	1.0×10^2	5–400
HD<20 µm	Athecate	*Gyrodinium*	3.5×10^3	100–25 000
		Katodinium	1.1×10^2	~960
		Unidentified	2.7×10^2	~18000
Ciliates>20 µm	Naked	*Didinium*	1.2×10^2	~1700
		Mesodinium	52.2	~2900
		Euplotid	30.2	~770
		Oligotrich	1.2×10^3	~12 000
		Unidentified	26.6	~240
	Tintinnid	*Salpingella*	90.0	~2000
Ciliates<20 µm	Naked	*Mesodinium*	93.0	~710
		Oligotrich	1.4×10^3	3–27 000
		Unidentified	1.0×10^2	~26 000

Distribution and sinking flux of HD fecal pellets. The abundance of HD fecal pellets was almost consistently highest at 10 m (Fig. 6A). Abundance started to increase from the beginning of January, reached a peak of 9.6×10^2 pellets l^{-1} at 10 m on 27 January, and decreased thereafter until 2 February. After the peak at 10 m, the abundance at 20 m showed a peak on 30 January and gradually increased at 30 and 50 m. HD fecal pellet flux increased to 6.0×10^5 pellets m^{-2} d^{-1} on 12 to 18 January, and then further increased to a maximum of 2.2×10^6 pellets m^{-2} d^{-1} on 27 to 30 January (Fig. 6A).

We qualitatively examined the dominant diatom species in 350 fecal pellets in the water column. The empty frustules of *Fragilariopsis kerguelensis* (Fig. 2A) and *Thalassiosira australis* (Ichinomiya et al. 2008a) in the pellets were hardly damaged, and of the 350 pellets examined, 21 contained *F. kerguelensis* and 11 contained *T. australis*. *Porosira pseudodenticulata* and *Pseudo-nitzschia* cf. *turgiduloides* were often difficult to identify to species, being compressed and compacted in the pellets (Fig. 2D, Ichinomiya et al. 2007). However, *Porosira*-like large centric and *Pseudo-nitzschia*-like elongated pennate diatom frustules were abundantly observed in 121 and 238 pellets, respectively. Few pellets (2 to 9 pellets) contained *Entomoneis* spp. and the cysts of *Polarella glacialis* (dinoflagellate), which are members of the sea ice community (Montresor et al. 1999).

Growth rates of HD and ciliates. During the incubation of HD and ciliates in the bottles suspended *in situ*, abundance of prey organisms such as diatoms (9.5 to 203 µg C l^{-1}) and phytoflagellates (0.37 to 50.0 µg C l^{-1}) in the ambient water was widely variable, while water temperature (–1.8 to –1.5°C) and salinity (33.9 to 34.0) were relatively constant (Table 3).

Temporal change in growth rates differed between HD and ciliates (Table 4). Mean growth rates of HD>20 µm and HD<20 µm were positive (0.06 to 0.19 and 0 to 0.16 d^{-1}) except for 0 d^{-1} in HD<20 µm on 18 January, but no clear relationship with the abundance or composition of ambient prey organisms was

Table 2. Mean (SD) cell size, abundance, and biomass of heterotrophic dinoflagellates (HD), naked ciliates, and 4 dominant diatom species throughout the investigation period. Length: diameter (centric diatoms) or length in apical axis. Width was measured along pervalar axis

	Length (µm)	Width (µm)	Cell volume ($\times 10^3$ µm^3)	Abundance ($\times 10^3$ cells l^{-1})	Biomass (µg C l^{-1})	Ingested diatoms (cells l^{-1})
Heterotrophic protists						
HD>20 µm	65.5 (9.7)	32.0 (6.7)	53.6 (4.5)	1.4 (1.9)	8.2 (11.5)	160.0 (250.0)
HD<20 µm	18.7 (3.0)	8.8 (0.9)	0.8 (0.3)	3.6 (4.7)	0.7 (0.9)	7.3 (21.4)
Ciliates>20 µm	39.3 (8.9)	29.2 (3.9)	32.4 (2.6)	1.5 (2.6)	8.8 (15.4)	15.1 (26.2)
Ciliates<20 µm	16.7 (1.1)	14.9 (1.3)	2.1 (0.4)	2.5 (1.3)	0.8 (2.8)	0.03 (1.3)
Diatoms[a]						
Fragilariopsis kerguelensis	58.8 (14.0)	6.5 (0.9)	20.3 (8.9)	10.2 (14.5)	0.38 (0.53)	
Porosira pseudodenticulata	53.7 (10.7)	44.5 (8.5)	85.7 (27.5)	9.8 (13.0)	24.6 (32.7)	
Pseudo-nitzschia cf. *turgiduloides*	92.8 (13.2)	2.8 (0.5)	3.0 (1.3)	340.0 (494.0)	11.5 (16.9)	
Thalassiosira australis	37.4 (7.9)	33.3 (7.9)	34.4 (23.9)	7.5 (14.3)	1.4 (3.1)	

[a]Data from Ichinomiya et al. (2008b)

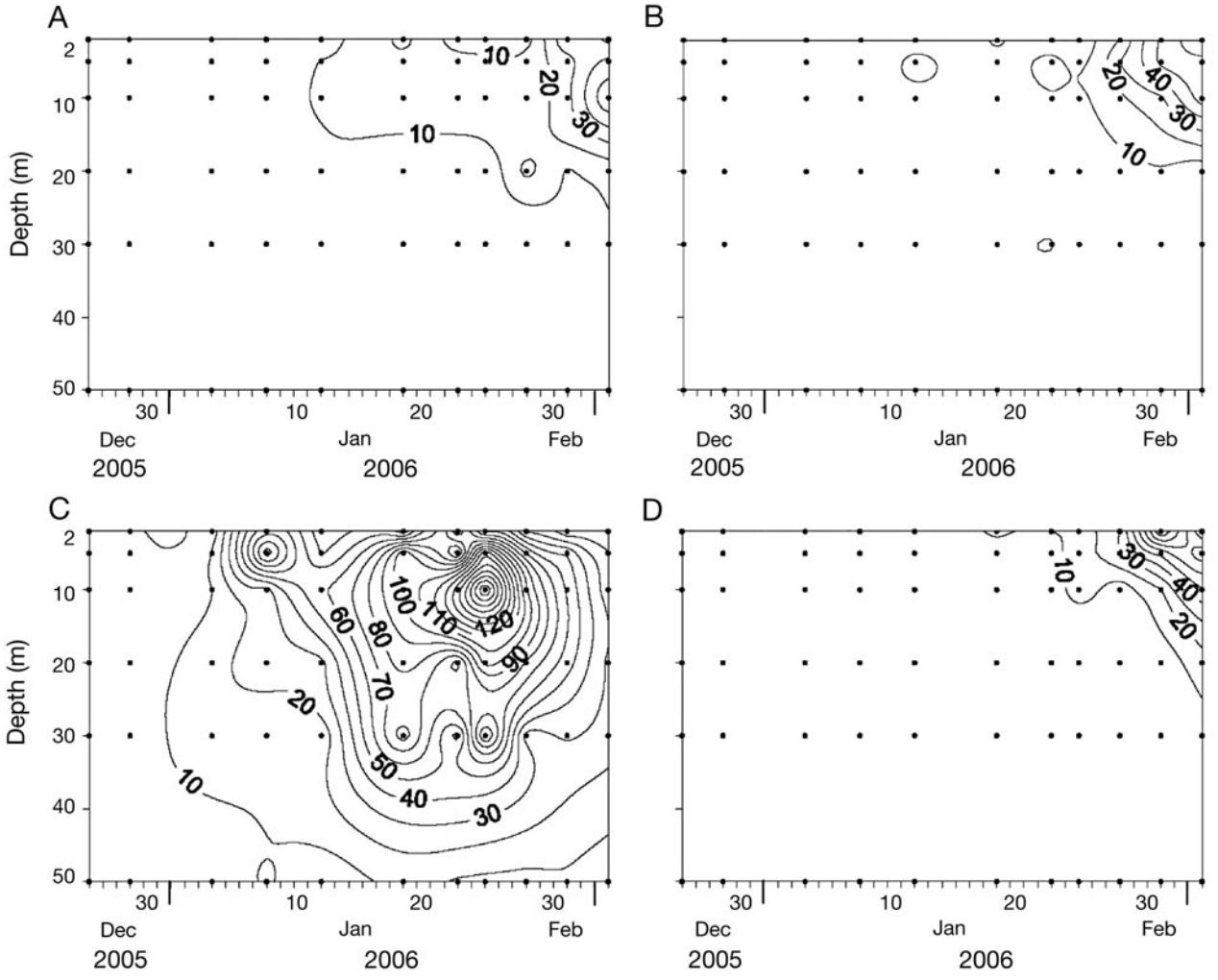

Fig. 5. Contours of the biomasses (µg C l^{-1}) of (A) heterotrophic dinoflagellates (HD)>20 µm, (B) ciliates>20 µm, (C) diatoms, and (D) phytoflagellates

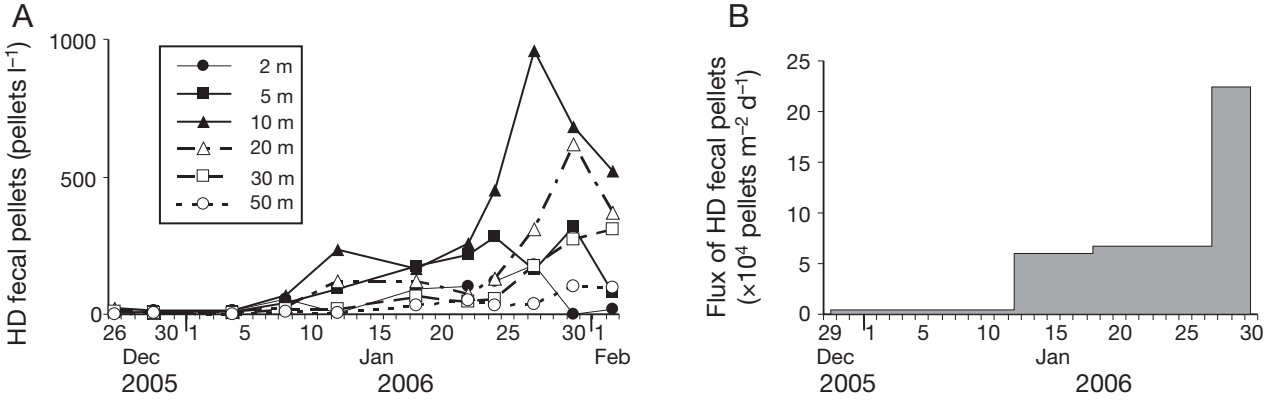

Fig. 6. Temporal variations in (A) abundance and (B) sinking flux of heterotrophic dinoflagellate (HD) fecal pellets

detected. On the other hand, Ciliates>20 µm and Ciliates<20 µm achieved high growth rates of 0.17 to 0.38 d^{-1} on 24 and 30 January when phytoflagellates were abundant (15.7 to 50.0 µg C l^{-1}), but their growth rates were low or negative (–0.26 to 0.12 d^{-1}) on 8 to 18 January when phytoflagellates were scarce (0.37 to 3.5 µg C l^{-1}).

Grazing by HD and sinking loss of diatoms. On 24 January, HD>20 µm biomass through the 2 to 20 m water column (184 mg C m^{-2}) and grazing (78.0 mg C

Table 3. Ambient environment conditions and biomass of diatoms and phytoflagellates (at 10 m depth) in the incubation experiments

Date in 2006	Water temperature (°C)	Salinity	Chl *a* (µg l^{-1})	Diatoms (µg C l^{-1})	Phytoflagellates (µg C l^{-1})
4 January	–1.8	34.0	1.0	9.5	0.37
12 January	–1.7	33.9	4.8	59.2	3.5
18 January	–1.7	33.9	4.5	102	2.6
24 January	–1.6	33.9	6.0	203	15.7
30 January	–1.5	33.9	4.2	58.3	50.0

Table 4. Mean growth rates (range of observed rates) of heterotrophic dinoflagellates (HD) and ciliates

Date	Growth rates (d^{-1})			
	HD>20 µm	HD<20 µm	Ciliates>20 µm	Ciliates<20 µm
4 January	0.07 (±0.01)	0.14 (±0.05)	–0.26 (±0.03)	–0.09 (±0.09)
12 January	0.19 (±0.03)	0.10 (±0.08)	0.12 (±0.03)	0.02 (±0.07)
18 January	0.06 (±0.02)	0 (±0.03)	–0.19 (±0.09)	0.04 (±0.03)
24 January	0.17 (±0.02)	0.07 (±0.06)	0.20 (±0.04)	0.17 (±0.01)
30 January	0.12 (±0.01)	0.16 (±0.03)	0.38 (±0.04)	0.31 (±0.06)

oligotrich ciliates were dominant taxa (Table 1). HD and ciliate communities were similar to those in other Antarctic coastal areas (Archer et al. 1996, Beaumont et al. 2002), but differed from the predominance of thecate dinoflagellates *Protoperidinium* spp. at a nearby site reported in a previous study (Ishikawa et al. 2001). In the present study, Lugol's fixation allowed the preservation of fragile athecate dinoflagellates, since the range of abundance of *Protoperidinium* spp. (5 to 400 cells l^{-1}, Table 1) was similar to that in the previous study (0 to 10^3 cells l^{-1}, Ishikawa et al. 2001). Unidentified HD and ciliates with high abundances of over 10^4 cells l^{-1} may be due to the input from sea ice habitats, since these taxa occurred mostly at 2 m where low sigma-t was observed (Fig. 3).

m^{-2} d^{-1}) were low relative to diatom biomass (2845 mg C m^{-2}, Table 5). The percent of diatom biomass removed daily by HD>20 µm grazing was only 2.7 % d^{-1}. However, grazing by HD>20 µm reached a maximum of 220 mg C m^{-2} d^{-1} on 2 February, equivalent to 45.8 % d^{-1} of the diatom biomass, corresponding with the decrease of diatom biomass (479 mg C m^{-2}) and the increase of HD>20 µm biomass (732 mg C m^{-2}). Diatom sinking flux was always smaller than grazing by HD>20 µm (14.9 to 71.3 mg C m^{-2} d^{-1}) and contributed only 2.5 to 3.1 % d^{-1} of the diatom biomass removed daily.

DISCUSSION

Our data provide information on temporal variations in biomass, fecal pellets, growth rates, and grazing on diatoms by HD and ciliates under the fast ice at an Antarctic coastal water site. Athecate *Gyrodinium* spp. and

HD as major diatom grazers under fast ice

During the study period, HD>20 µm gradually increased in the upper 20 m from mid-January during the diatom decline (Fig. 5A,C). HD fecal pellets peaked after the diatom peak and contained the dominant diatoms (Fig. 6, Table 2). This indicates a predator–prey relationship between HD and diatoms, and that the pellets were produced through ingestion of diatoms by HD in the water column.

Among the diatom assemblage, *Porosira pseudodenticulata* and *Pseudo-nitzschia* cf. *turgiduloides* were predominant in the water column, and *Fragilariopsis kerguelensis* and *Thalassiosira australis* were abundant in the sinking flux (Fig. 4, Table 2). The daily sinking flux of *P. pseudodenticulata* and *P.-n.* cf. *turgiduloides* contributed a relatively small fraction of their standing stocks in the upper 20 m (Ichinomiya et al. 2008b). On the other hand, the standing stocks of *F. kerguelensis*

Table 5. Grazing on diatoms by heterotrophic dinoflagellates (HD)>20 µm and diatom sinking flux during diatom decline, and percent of diatom biomass removed daily by HD grazing and sinking. All values were integrated through the 2 to 20 m water column. Specific ingestion of HD>20 µm was calculated using the growth rates measured in the present study and assuming 0.4 for growth efficiency. Ingestion on 27 January and 2 February 2006 was assumed to be the same as that on the previous day. The sinking flux from 30 January to 2 February was calculated to be scaled relative to the diatom biomass on 27 to 30 January

Sampling date in 2006	Diatom biomass (mg C m^{-2})	HD>20 µm			% diatom biomass removed		
		Biomass (mg C m^{-2})	Specific ingestion (d^{-1})	Grazing (mg C m^{-2} d^{-1})	Diatom sinking flux (mg C m^{-2} d^{-1})	Grazing (% d^{-1})	Sinking (% d^{-1})
24 January	2845	184	0.463	85.0	71.3	3.0	2.5
27 January	1584	270	0.463	125	50.2	7.9	3.2
30 January	1096	410	0.318	131	34.0	11.9	3.2
2 February	479	732	0.318	233	14.9	48.7	3.2

and *T. australis* contributed largely to the sinking flux. The former 2 species can maintain their populations in the water column, whereas the latter 2 species sink shortly after release from the sea ice. To maintain their position in the surface water column would be advantageous for optimizing photosynthesis after the ice melt (Michel et al. 1993). However, such behavior of *P. pseudodenticulata* and *P.-n.* cf. *turgiduloides* would increase grazing mortality by pelagic grazers.

Ciliates>20 µm were not a potential competitor for diatoms, since Ciliates>20 µm hardly ingested diatoms (Table 2). This is due to the fact that *Porosira pseudodenticulata*, the most abundant diatom in biomass (Table 2), could not be a prey item of Ciliates>20 µm. While an optimal prey size for a ciliate is ca. 1/10 of its own size (Hansen 1992, Hansen et al. 1994), *P. pseudodenticulata* was larger in size (85.6×10^3 µm^3, Table 2) than Ciliates >20 µm (32.4×10^3 µm^3). Euplotid ciliates are known to ingest large diatoms (Gowing et al. 2001), but their abundance was low (Table 2). Oligotrich ciliates occasionally ingested elongated pennate diatoms (Fig. 2). Ciliates>20 µm were mainly distributed near the surface at 2 to 5 m, where phytoflagellates were abundant but diatoms were sparse (Fig. 5B–D). On the other hand, HD can ingest such large prey due to the ability of gymnodinoid dinoflagellates to ingest prey larger than themselves (Buck et al. 2005, Saito et al. 2006) and of thecate dinoflagellates to feed extracellularly (Jacobson 1999). This suggests that the main prey items of ciliates are phytoflagellates and that, among the heterotrophic protists, only HD>20 µm could utilize larger-sized *P. pseudodenticulata*.

Growth of HD

Little is known about growth rates of HD and ciliates in Antarctic coastal areas (Archer et al. 1996). Unfortunately, we conducted only duplicate incubations in each experiment and were thus unable to calculate differences between the experiments. However, growth of HD in the water column was always positive except for 18 January, where a value of 0 d^{-1} for HD<20 µm indicated that HD were reproducing in the water column (Table 4), while some of the HD found in the water column may have originated from sea ice (Buck et al. 1990, Garrison et al. 2005, Roberts et al. 2007). The growth rates of HD>20 µm and <20 µm may also have been underestimated, since their mortality was not considered. The present growth rates of HD>20 µm (0.06 to 0.19 d^{-1}) are similar to those observed in the coastal areas around Davis Station (0.014 to 0.15 d^{-1}, Archer et al. 1996), but the growth rates of HD<20 µm (0 to 0.16 d^{-1}) were lower than those in the Weddell Sea (0.31 to 0.32 d^{-1} maximum growth rate, Bjørnsen & Kuparinen 1991). Considerable grazing on HD<20 µm by copepod nauplii that passed through 200 µm mesh (0 to 40 animals l^{-1}), HD>20 µm and Ciliates>20 µm, which are known to ingest small dinoflagellates (Jeong et al. 2007), may have taken place during the incubation.

The growth rates of HD seem to be uncorrelated with changes in prey concentrations (Table 3). On the other hand, the growth rates of both Ciliates>20 µm and <20 µm were high when phytoflagellates were abundant, but low or negative when phytoflagellates were scarce. Potential growth rates and efficiency of HD are lower than those of ciliates (Hansen 1992, Hansen et al. 1997, Strom & Morello 1998). Therefore, HD might be less responsive to episodic increases in food supply than ciliates.

Grazing by HD and diatom sinking loss

Grazing by HD>20 µm was estimated to be a significant factor in the diatom decline, reaching a maximum of 45.8% d^{-1} of the diatom biomass removed daily (Table 5). High grazing impact by microzooplankton accounting for 33% of the phytoplankton standing stock d^{-1} has also been observed around the Australian Davis Station in late summer (Pearce et al. 2008). The assumed growth efficiency of 0.4 is within the range of literature values (Hansen et al. 1997). Therefore, the high grazing impact on diatoms in the present study was due to the high abundance of HD, assuming that HD>20 µm ingest only diatoms. We have no direct evidence of phytoflagellate ingestion by HD. However, HD increased even after diatoms decreased and phytoflagellates increased (Fig. 5), suggesting that HD ingest not only diatoms but also phytoflagellates when diatom abundance becomes low. This trophic pathway from phytoflagellates to HD should be characterized to understand fast ice ecosystems.

Diatom sinking flux accounted for only 2.5 to 3.2% d^{-1} of the diatom biomass removed daily, which was less than removal by HD>20 µm grazing (Table 5). Odate et al. (2004) also reported a low contribution (3.6 to 4.0%) of sinking to the total decline of chlorophyll in the water column at 2 different sites near Syowa Station in 1992. The low estimated contribution to the sinking loss is considered to be due to the longer retention capability of *Porosira pseudodenticulata* and *Pseudo-nitzschia* cf. *turgiduloides* within the upper water column (Ichinomiya et al. 2008b). Low sinking rates of diatoms released from sea ice have been observed in the Arctic ice-covered areas of Hudson Bay, Canada (Tremblay et al. 1989, Michel et al. 1993). This suggests that sinking loss is not always the main factor in the decline of diatom abundance.

CONCLUSIONS

This study demonstrates that the diatoms released from sea ice showed different dynamics, whereby some maintain their populations in the water column while others sink to the bottom near Syowa Station, Antarctica. A significant part of the former remains in the water column and is subjected to grazing by HD. After the diatom decline, phytoflagellates developed and formed a principle prey item of ciliates (Ichinomiya et al. 2007). Although HD and ciliates play important roles in linking primary production to higher trophic levels (Calbet & Saiz 2005, Schmidt et al. 2006, Sherr & Sherr 2007), their functions are different. Under the fast ice, HD ingest diatoms released from the ice and ciliates ingest phytoflagellates that grow underneath the ice. By this process, HD largely control the fate of the released diatoms.

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