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NOTE

Size distribution and biomass of nanoflagellates in meso- and bathypelagic layers of the subarctic Pacific

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ABSTRACT: We examined the abundance, cell size, and biomass of nanoflagellates throughout the water column and compared the results with data on prokaryote biomass and production at 6 sampling stations in the subarctic Pacific and the Bering Sea. For the upper (0–100 m), mesopelagic (100–1000 m), and bathypelagic layers (1000–3500 m), the integrated biomass of nanoflagellates was 310 ± 290, 130 ± 56, and 31 ± 17 mg C m^{-2} (mean ± SD; n = 6), respectively, accounting for 4.8 ± 4.5, 3.2 ± 4.8, and 0.83 ± 0.67% of prokaryote biomass. The turnover time of prokaryotes (biomass/production) was significantly negatively correlated with the biomass of flagellates in the upper and bathypelagic waters, but the correlation was not significant in the mesopelagic layer. Assuming that nanoflagellates clear water 5×10^5 times greater than their own cell volume per hour, we estimated that grazing by nanoflagellates could consume 70 ± 46 and 48 ± 25% (mean ± SD; n = 6) of prokaryote production in meso- and bathypelagic layers, respectively. The above results suggest that nanoflagellates play a potentially significant role as consumers of prokaryotes in deep Pacific waters of subarctic regions.

KEY WORDS: Heterotrophic nanoflagellates · Marine bacteria · Deep Sea · Leucine incorporation · Subarctic Pacific · Bering Sea · Microbial food web · Sinking flux

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INTRODUCTION

Phagotrophic flagellates are dominant consumers of prokaryotes in marine surface waters, representing a major trophic link in the microbial loop (Strom 2000). However, in meso- and bathypelagic waters, distributions of flagellates and their role as consumers of prokaryotes have yet to be fully clarified. In a few oceanic regions, studies have examined full-depth distributions of flagellates (mid-Atlantic, Patterson et al. 1993; western North Pacific, Yamaguchi et al. 2000; northwestern [NW] Mediterranean, Tanaka & Rassoulzadegan 2002), suggesting that a general relationship between flagellate abundance and water depth can be described by a power function, with exponents being greater that those of prokaryotes. The abundance ratio of prokaryotes to flagellates tends to increase from a typical value of 1000 in surface oceans (Strom 2000) to around 5000 to 10 000 in bathypelagic layers (Yamaguchi et al. 2000). In the East Sea, Cho et al. (2000) found that flagellates graze prokaryotes in the mesopelagic layer, with cell-volume-normalized clearance rates being comparable to those in surface waters.

Here we report data on abundance, cell size distribution, and biomass of nanoflagellates in meso- and

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bathypelagic water columns of the subarctic Pacific and the Bering Sea. Six sampling stations were established to cover important biogeochemical provinces with high primary production and high export of organic carbon from surface to the ocean's interior (Longhurst 1998). Nagata et al. (2000) suggested that prokaryote production in bathypelagic water columns of this region is 3- to 7-fold greater than that in the corresponding depth layer of the subtropical gyre. Studies have also revealed that prokaryote production (Nagata et al. 2001) and ectoenzyme activity (Fukuda et al. 2000) in the mesopelagic layer vary greatly across subarctic basins. No previous studies have examined basin-scale distributions of flagellates in deep subarctic waters. Our aims were (1) to examine variations in the abundance and cell size of flagellates in meso- and bathypelagic water columns of the subarctic Pacific, and (2) to compare the results with data on prokaryote production and turnover to assess the potential importance of flagellates as consumers of prokaryotes in deep subarctic waters.

MATERIALS AND METHODS

Water sampling was carried out on board the RV 'Hakuho-Maru' during 2 cross-Pacific cruises conducted between 9 July and 8 September 1997 (KH 97-2) and 25 June and 22 July 1999 (KH 99-3). The 6 sampling stations were located in the oceanic domain of the subarctic Pacific and the Bering Sea (Table 1). Water samples were collected using 12 l Niskin bottles mounted on a conductivity, temperature, depth (CTD)/carousel sampler. Subsamples for the determination of prokaryote and nanoflagellate abundances were fixed with formaldehyde (final concentration 2%) and glutaraldehyde (1%), respectively. The fixed samples were stored at 4°C in the dark. Slides for the microscopy were prepared within 48 h after fixation and stored at –20°C.

Nanoflagellates were double-stained with DAPI and FITC, collected on black-stained, 0.8 µm pore size filters (Nuclepore), and counted using epifluorescence microscopy (Zeiss Axioplan; filter set 48 79 02 for DAPI fluorescence and 48 79 09 for FITC fluorescence) according to Sherr & Sherr (1983). We filtered 20 to 100 ml of water using filtration funnels with an effective filtering area of 9.6×10^6 or 2.0×10^8 µm^2. We counted at least 150 cells or 50 fields to estimate cell abundance. Errors associated with counting (coefficients of variation, CVs) were 12 ± 5% (mean ± SD; n = 6), 16 ± 3% (n = 12), and 30 ± 11% (n = 6) for the upper (<100 m), mesopelagic (100–1000 m) and bathypelagic layers (>1000 m), respectively. The abundance of prokaryotes was determined using the DAPI method (Porter & Feig 1980). At least 400 cells were counted to estimate cell abundance. Cell images of nanoflagellates (FITC) and prokaryotes (DAPI) were captured using a cooled charge-coupled device (CCD) camera (C4880-7 controlled by Argus 50, Hamamatsu). The Marr-Hildreth algorithm was used to detect cell edges (Viles & Sieracki 1992). Digitized images (1 pixel = 0.12 µm) were used to determine the length and width of each cell. Cell volumes were calculated by assuming that cells were cylindrical rods with hemispherical caps (prokaryotes) or spheres (nanoflagellates). For each sample, at least 50 (prokaryotes) or 250 cells (nanoflagellates) were measured to derive geometric means. Bacterial production was estimated from the incorporation rate of ^{3}H-leucine assuming a conversion factor of 1.55 kg C mol Leu^{-1} as described by Nagata et al. (2000). Nanoflagellate biomass was estimated by assuming a carbon to volume ratio of 0.22 pg C µm^{-3} (Børsheim & Bratbak 1987). Bacterial biomass was estimated assuming that each cell contains 15 fg C (Caron et al. 1995).

Table 1. Locations, dates of surveys and oceanographical properties of sampling stations. Data are from cruise reports and are owned by the Ocean Research Institute (ORI)

Location	Stn	Date of survey	Position	Water depth (m)	Sampling depth (m)	Surface water temperature (°C)	Water column of 0–100 m NO$_3$ (µM)	Water column of 0–100 m Chl *a* (mg m^{-2})
Western	6(97)	21 Jul 97	48° 00′ N, 177° 04′ E	4822	10, 100, 500, 3000	8.9	16.6	29.7
	1(99)	28 Jun 99	44° 01′ N, 155° 01′ E	5350	10, 100, 400, 3000	8.1	11.8	36.5
Easter)	15(97)	8 Aug 97	49° 53′ N, 144° 54′ W	4263	10, 100, 500, 2000	14.4	8.65	26.3
	13(99)	17 Jul 99	50° 00′ N, 145° 01′ W	4265	10, 100, 400, 3000	10.1	11.1	34.1
Bering Sea	9(97)	25 Jul 97	57° 24′ N, 179° 53′ E	3807	10, 100, 500, 2000, 3750	9.1	13.3	52.8
	5(99)	3 Jul 99	55° 00′ N, 180° 00′ E	3833	10, 100, 400, 3000	6.4	11.2	62.8

RESULTS AND DISCUSSION

Mean (±SD) cell volume of nanoflagellates in the mesopelagic layer (7.11 ± 1.14 μm^3, n = 12; 100–1000 m) did not differ significantly ($p > 0.05$, ANOVA) from that in the bathypelagic layer (6.57 ± 0.90 μm^3, n = 7; >1000 m), whereas the corresponding value in the upper layer (10.5 ± 3.4 μm^3, n = 6; 10 m) was significantly ($p < 0.05$) greater than those in meso- and bathypelagic layers, a tendency primarily due to the decrease in the frequency of large cells (>30 μm^3 cell^{-1}) with increasing depth (Fig. 1). The mean cell volume of prokaryotes varied little with depth: 0.0200 ± 0.0034 (n = 6), 0.0179 ± 0.0018 (n = 12), and 0.0189 ± 0.0024 (n = 7) μm^3 in the surface, meso-, and bathypelagic layers, respectively. In the NW Mediterranean, Tanaka & Rassoulzadegan (2002) found that the nanoflagellate cell volume at a depth of 30 m (26.2 μm^3 cell^{-1}) was greater than that in deeper (300 and 2000 m) layers (15.2 μm^3 cell^{-1}), a depth trend consistent with what we found in the subarctic Pacific. However, for both surface and deep layers, the mean cell volumes of nanoflagellates in the Mediterranean were about 2-fold greater than the cell volumes in subarctic regions. Factors that might affect mean cell volume of nanoflagellates include size-selective grazing by predators (e.g. ciliates; Fenchel 1980), prey concentrations (Fenchel 1982), temperature, and hydrostatic pressure. We speculate that smaller cell volumes may have some advantage in cold and deep (high static pressure) waters where higher water viscosity results in increasing drag force on motile nanoflagellates, a hypothesis that might explain larger cell size of nanoflagellates in warmer waters of the Mediterranean (temperature range, 10–18°C even in deeper layers, Tanaka & Rassoulzadegan 2002) than in colder waters of the subarctic Pacific (2–14°C; Table 1). However, we note that decompression during sampling and fixation might affect cell structure and cell size distributions of fragile cells of nanoflagellates in a taxon-specific fashion (e.g. Choi & Stoecker 1989), a possibility that should be investigated in future studies.

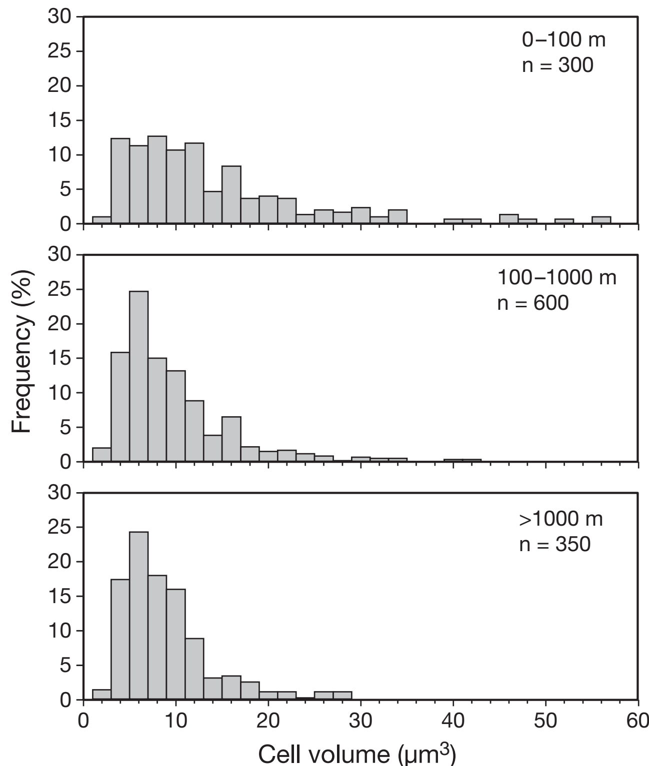

Fig. 1. Size distribution of the cell volume of nanoflagellates in the surface (0–100 m), mesopelagic (100–1000 m), and bathypelagic (>1000 m) layers in pooled data obtained from 6 sampling stations in the subarctic Pacific

Median values (ranges) of the abundance of nanoflagellates in the upper, meso-, and bathypelagic layers were 1400 (180–3300), 60 (5.7–260), and 6.6 (1.4–12) cells ml^{-1}, respectively; the abundance decreased by 3 orders of magnitude with depth. These results are consistent with data reported in previous studies conducted in other oceanic regions, including the NW Mediterranean (Tanaka & Rassaoulzadegan 2002) and western North Pacific (Yamaguchi et al. 2000).

The integrated biomass of nanoflagellates in the surface (0–100 m), mesopelagic (100–1000 m), and bathypelagic (100–3500 m) water columns was 310 ± 290, 130 ± 56, and 31 ± 17 mg C m^{-2} (mean ± SD), respectively; the corresponding values for prokaryotes were 1800 ± 930, 3500 ± 2500, and 1400 ± 650 mg C m^{-2} (Table 2). The biomass of nanoflagellates in each layer accounts for on average 4.8, 3.2, and 0.83% of prokaryote biomass. The integrated prokaryote production in the upper, meso-, and bathypelagic layers was 180 ± 110, 47 ± 33, and 1.3 ± 0.55 mg C m^{-2} d^{-1}, respectively; the turnover time (biomass/production) of prokaryotes in each layer was estimated to be 12 ± 6.2, 97 ± 53, and 1100 ± 320 d. We found that the turnover time of prokaryotes tended to decrease with increasing biomass of flagellates in the upper and bathypelagic layers; Pearson's correlation coefficients were –0.957 ($p = 0.0028$, n = 6) and –0.914 ($p = 0.0108$, n = 6), respectively. There was no significant correlation between prokaryote turnover time and flagellate biomass in the mesopelagic layer ($p > 0.05$). Although data are limited, the negative correlation between prokaryote turnover time and flagellate biomass is consistent with the hypothesis that nanoflagellates exert a significant grazing pressure on prokaryotes in the bathypelagic layer.

To examine whether flagellates can play a significant role as grazers of prokaryotes in deep waters, we used our data on the flagellate cell volume to estimate the clearance rate, assuming that a flagellate clears a

Table 2. Depth integrated prokaryote production (PP) (mg C m^{-2} d^{-1}), biomass of prokaryotes (Pro) or nanoflagellates (NF) (mg C m^{-2}), biomass ratio (Pro/NF) and prokaryote turnover time (Pro/PP, d) in surface (0–100 m), mesopelagic (100–1000 m) and bathypelagic (1000 m–bottom) layers

Location	Stn	Surface					Mesopelagic					Bathypelagic				
		PP	Pro	NF	Pro/NF	Pro/PP	PP	Pro	NF	Pro/NF	Pro/PP	PP	Pro	NF	Pro/NF	Pro/PP
Western	6(97)	160	2700	57	47	17	51	7600	120	66	150	0.75	1200	6.1	200	1600
	1(99)	160	890	700	1.3	5.5	8.1	1300	144	9.0	160	0.60	600	22	28	1000
Eastern	15(97)	68	1200	30	42	18	26	2600	68	39	101	0.78	1000	21	47	1300
	13(99)	110	1400	370	3.7	12	93	5500	200	27	59.7	1.0	710	36	20	680
Bering Sea	9(97)	210	3200	95	33	15	27	2400	68	36	91.7	1.2	2000	4.9	400	1100
	5(99)	380	1200	600	2.0	3.1	80	1600	180	9.1	20.4	0.70	640	43	15	910
	Mean	180	1800	310	21	12	47	3500	130	31	97	1.3	1000	31	120	1100
	SD	110	930	290	22	6.2	33	2500	56	21	53	0.55	520	17	150	320
	CV (%)	60.0	53.3	94.8	100	52.3	70.4	70.7	43.5	69.0	54.6	42.6	50.3	52.9	130	29.5

volume of water 5×10^5 times greater than its own cell volume h^{-1}, which is an average value for nanoflagellates having similar cell size in the euphotic and mesopelagic layers in the East Sea (5–48, 6–11 µm^3, respectively; Cho et al. 2000). With this assumption, we estimated that the fraction of water cleared by flagellate assemblages is $(1.7 \pm 1.6) \times 10^{-1}$, $(7.9 \pm 3.4) \times 10^{-3}$, and $(4.8 \pm 3.4) \times 10^{-4}$ d^{-1} for the upper, meso- and bathypelagic layers, respectively. By multiplying the clearance rate with the prokaryote biomass, grazing rates by flagellates on prokaryotes are estimated to be 2.1 ± 1.5, $(3.2 \pm 2.8) \times 10^{-2}$, and $(1.5 \pm 0.7) \times 10^{-4}$ µg C l^{-1} d^{-1}. These values account for a significant fraction (120, 70, and 48% for the upper, meso-, and bathypelagic layers) of prokaryote production, suggesting that flagellates could be major consumers of prokaryotes not only in the upper ocean but also in meso- and bathypelagic layers.

The above estimates are associated with uncertainties due to inadequacies in our knowledge on microbial metabolism in deep waters. Prokaryote production in decompressed samples collected from below 1000 m may be significantly underestimated (Bianchi et al. 1999), suggesting that the turnover time of prokaryotes that we determined with decompressed samples would be too low. Prokaryote turnover time might be overestimated due to the inclusion of dead cells in DAPI counts (Zweifel & Hagström 1995). Future studies should test whether the cell-volume-normalized clearance rates of flagellates (5×10^5–10^6 h^{-1}), which Cho et al. (2000) obtained in the mesopelagic water of the East Sea, generally apply to deeper layers and other regions. Despite the above uncertainties, our first order estimates of the grazing rates of nanoflagellates on prokaryotes and a negative correlation between the turnover time of prokaryotes and flagellate biomass in the bathypelagic layer are consistent with the notion that flagellates play a significant role as grazers of prokaryotes in deep oceanic waters. Other factors such as virus-induced mortality (Weinbauer et al. 2003) or scavenging by sinking particles (Stolzenbach & Elimelech 1994) might also explain a significant fraction of the mortality or loss of prokaryotes in meso- and bathypelagic waters. Given the major role of carbon consumption by prokaryotes in carbon cycling in the ocean's interior (Cho & Azam 1988, Nagata et al. 2000), future studies should examine in greater detail the fate of prokaryote production in deep oceans.

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