

East–west gradient in ectoenzyme activities in the subarctic Pacific: Possible regulation by zinc

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Abstract

We determined depth profiles of potential activities of leucine aminopeptidase (LAPase) and β -glucosidase (BGase) in the subarctic Pacific between July and September 1997. The data revealed a systematic shift in vertical patterns of the LAPase:BGase ratios along the east-west axis of the investigated area. The ratio increased by over 10-fold with depth in the eastern region (139°W–159°W), while it varied little (<3-fold) in the central region (169°W–179°E, including the Bering Sea). It decreased with depth by about six-fold in the western region (169°E–161°E). The decrease of LAPase:BGase ratio with depth is consistent with a general pattern in the decomposition of sinking particles, i.e., protein is degraded faster than polysaccharides, but the increase of the ratio with depth suggests that export of proteinaceous materials to the mesopelagic zone is high. Cell specific LAPase activity in the upper layer (0–100 m) was positively correlated ($r = 0.92$, $P < 0.05$) with the concentration of dissolved zinc, a catalytic element of proteases. We hypothesize that the low level of dissolved zinc stresses LAPase activity in the euphotic zone, leading to high export of protein to the mesopelagic zone in the eastern subarctic Pacific. These results have important implications for modeling the carbon and nitrogen pump in open oceans where surface concentrations of trace metals are extremely low.

Ecto enzymatic hydrolysis is a critical step in the degradation of polymeric dissolved organic matter (DOM) by bacteria (Chróst et al. 1989). Several studies have examined spatial patterns of ecto enzymatic properties in marine environments and provided useful insights into the magnitude and spatial scale of organic matter dynamics (e.g., Christian and Karl 1995; Murrell et al. 1999). However, most previous studies have focused on the euphotic zone, and there are very few depth profiles of ecto enzyme activities in open oceans (Hoppe et al. 1993; Koike and Nagata 1997; Hoppe and Ullrich 1999).

The activity of ecto enzymes in the aphotic zone could be closely related to the composition of sinking particulate organic matter (POM). Previous studies have suggested that a substantial fraction of sinking POM is solubilized to DOM which may contain polymeric components and fuels bacterial production in the meso- and bathypelagic zone (Cho and Azam 1988; Nagata et al. 2000). To utilize this polymeric

DOM, free-living bacteria express the ecto enzymes, which can dynamically reflect particle compositions (Smith et al. 1992). Thus, vertical patterns of ecto enzyme activities could provide valuable information regarding diagenetic patterns of sinking POM and qualitative characteristics of the biological pump.

We report here our measurements of depth profiles of leucine aminopeptidase (LAPase) and β -glucosidase (BGase) activities as well as other bacterial variables in the upper water column (0–200 m) of the subarctic Pacific and the Bering Sea. This region is an important oceanographic province characterized by high primary productivity, high removal rate of the atmospheric CO₂ by the biological pump (Honjo 1997), and marked longitudinal variations in biogeochemical constituents and ecosystem structures. The eastern part of the region, the Gulf of Alaska, is a high nutrient-low chlorophyll environment limited by iron (Martin and Fitzwater 1988). The western part of the region, the Western and the Bering Sea gyres, is characterized by relatively high rates of atmospheric deposition of iron (Duce and Tindale 1991) and high seasonal export production by diatoms (Honjo 1997). Our purposes were to examine depth profiles of the LAPase:BGase ratio across the subarctic Pacific and to infer possible biogeochemical factors that influence compositional variability of organic substrates in the mesopelagic zone. Our results demonstrate, for the first time, a systematic, longitudinal trend in vertical pattern of the LAPase:BGase ratio, which appears to be related to zinc-stressed expression of LAPase by bacteria.

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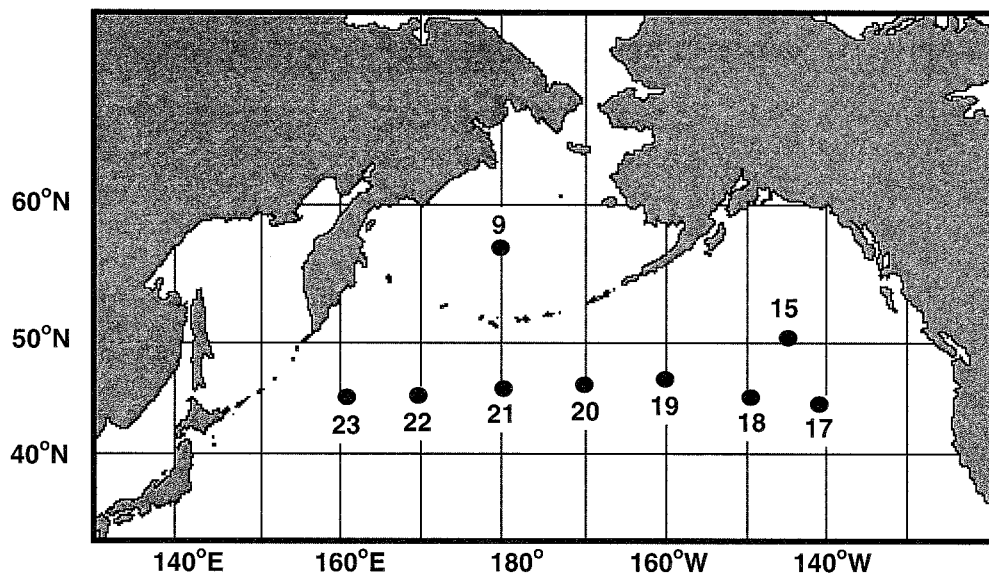


Fig. 1. Sampling stations in the subarctic Pacific and the Bering Sea during the KH-97-2 cruise of the R/V *Hakuho-maru* (25 July–2 September 1997).

Methods

Study area and sampling—Samples were collected at nine stations aboard the R/V *Hakuho-maru* during the KH 97-2 cruise conducted between 25 July and 2 September, 1997 (Fig. 1; Table 1). Sta. 9 was located in the middle of the Bering Sea. Sta. 15 was close to the Ocean Weather Station P, which is located near the southern boundary of the Gulf of Alaska gyre. Other seven stations (Sta. 17–23) transected across the Pacific between 139°W and 161°E near the latitude of 45°N. The northern North Pacific is characterized by a permanent halocline established at a depth of ca. 100 m which prevents vertical exchange of dissolved and suspended materials by wind-driven convective mixing during winter (Anderson et al. 1969). The surface (0 m and 200 m) current along 45°N is eastward (Reid 1997) with a slow rate (2 cm s^{-1} ; Fukasawa et al. 1993), suggesting that lateral transport of semilabile organic matter is probably low in the investigated region. Sample waters were collected from depths between 10 m and 200 m with 12-liter, Teflon-coated Niskin bottles mounted on a conductivity-temperature-depth (CTD)/rosette. Bottles were washed with detergent and HCl and rinsed with Milli-Q water before the cruise. Surface waters (0 m) were collected with a clean polyethylene bucket. Plastic gloves were worn and care was taken to minimize contamination during sampling.

Ectoenzyme activities—The activities of leucine aminopeptidase (LAPase) and β -glucosidase (BGase) were assayed as the hydrolysis rate of L-leucine-4-methyl-coumarinylamid hydrochloride (leu-MCA) and 4-methyl-umbelliferyl- β -D-glucopyranoside (MUF-glp) (Hoppe 1983; Chróst et al. 1989). These fluorescent substrates dissolved in 2-methoxyethanol (conc., 20 mM) were used as stock solutions. Duplicate water samples (3 ml each) were dispensed into disposable methacrylate cuvettes (prewashed with alkaline

detergent and HCl) for LAPase assay to facilitate measuring the fluorescence over time. Samples for the BGase assay were dispensed into precombusted glass vials. Dispensed samples were amended with the substrates (final conc., 0.2 mM), and incubated at in situ temperature ($\pm 2^\circ\text{C}$) in the dark. After the incubation for 4–26 h the fluorescence of hydrolytic products, 7-amino-4-methyl-coumarin (AMC; LAPase assay) or 4-methylumbelliferone (MUF; BGase assay), was measured with a spectrofluorometer (Shimadzu RF-540) at excitation/emission wave lengths (nm) of 380/440 (AMC) or 365/460 (MUF). For determination of the MUF fluorescence, the pH of reaction mixture was raised with borate buffer (0.4 M, pH 10) just before the measurement. Blank fluorescence was measured at the beginning of the incubation and the values were subtracted from those of the incubated samples. The fluorescence was calibrated with a slope of a calibration curve obtained from several concentrations of AMC and MUF. Controls were prepared with autoclaved seawater amended with substrates; the fluorescence did not increase during the incubation, indicating that non-enzymatic cleavage of leu-MCA and MUF-glp was negligible. Time course measurements were carried out on several occasions and the increase of fluorescence intensity was linear over the incubation period. The amount of substrates added was at a saturation level: The apparent half saturation concentrations (K_m) for LAPase and BGase were 48–54 μM and 20–28 μM , respectively. These K_m values were measured in samples obtained from 10 and 100 m at Sta. 23.

Bacterial abundance, production, and Chl a—Bacterial abundance was determined by epifluorescence microscopy after staining with 4',6-diamidino-2-phenyl indole (Porter and Feig 1980). Bacterial production was measured from incorporation of ^3H -leucine (Kirchman 1992). Briefly, sub-samples (10 ml) were amended with 10 nM ^3H -leucine (Amersham) and incubated for 1–12 hours at in situ tem-

Table 1. Sampling dates, positions and oceanographic properties of sampling stations.

Longitudinal zone category	Position				Surface water				Water column of 0–200 m		
	Date	Station number	Latitude	Longitude	Temp. (°C)	Sal.	NO ₃ (μM)	PO ₄ (μM)	Chl <i>a</i> (mg m ⁻²)	Bacteria (10 ¹⁴ cells m ⁻²)	Mixed layer (m)
East	24 Aug 97	17	44°60'N	139°60'W	16.8	32.59	ND	0.36	47.5	1.5	10
	8 Aug 97	15	49°53'N	144°54'W	14.4	32.53	8.6	0.88	30.9	1.1	10
	26 Aug 97	18	46°28'N	149°60'W	14.7	32.88	1.6	0.42	31.1	1.2	20
Center	27 Aug 97	19	47°01'N	159°60'W	14.4	32.96	6.1	0.72	27.3	1.0	30
	29 Aug 97	20	46°43'N	169°60'W	15.0	32.62	9.4	0.85	26.2	1.1	10
	30 Aug 97	21	45°30'N	179°60'W	14.4	32.87	7.9	0.74	26.3	1.1	10
West	25 Jul 97	9	57°24'N	179°54'E	9.1	32.90	13.3	1.27	54.4	1.8	10
	1 Sep 97	22	45°07'N	169°59'E	10.9	32.82	12.6	1.22	54.4	1.1	20
	2 Sep 97	23	44°05'N	161°44'E	13.3	33.15	6.5	0.67	57.5	1.5	20

ND, not detected (<0.13 μM).

perature ($\pm 2^\circ\text{C}$). After the incubation, samples were filtered through Sartorius filters (pore size $0.2\ \mu\text{m}$) and extracted with ice-cold 5% trichloroacetic acid (TCA) and 80% ethanol. These filters were radioassayed with a liquid scintillation counter. Duplicate samples and one TCA-killed control were prepared for each depth. Leucine incorporation was converted to bacterial production by assuming a conversion factor of 1.08×10^{17} cells mol^{-1} leucine (Kirchman 1992). Chlorophyll *a* concentrations were measured fluorometrically after solvent (N, N-dimethylformamide) extraction of suspended materials collected on Whatman GF/F filters.

Dissolved zinc concentration—Samples for dissolved zinc were collected in low-density polyethylene bottles. Samples were filtered through $0.2\ \mu\text{m}$ -pore-size Nuclepore filters, acidified to pH 2 with HCl, and kept at room temperature for more than 3 months. Zinc was concentrated by using 8-hydroxyquinoline immobilized on fluorinated metal alkoxide glass. Zinc concentration was measured by the high-resolution ICP-mass spectrometry (Sohrin et al. 1998). Clean technique was used throughout the analysis.

Results

Data were collected during summer at nine stations which occupied a wide variety of subarctic regions (Fig. 1; Table 1). Surface water temperatures were generally high in the eastern side (max. 16.8°C at Sta. 17) and low in the western side (min. 9.1°C at Sta. 9) of the investigated area (Table 1). Similarly, temperatures in the upper mesopelagic layer (100–200 m) generally increased eastward ranging from $2.5 \pm 1.0^\circ\text{C}$ (Sta. 9) to $7.6 \pm 0.1^\circ\text{C}$ (Sta. 17). The depth of seasonal mixed layer varied in the range of 10–30 m (Table 1). The permanent halocline was at a depth of ca. 100 m (75–150 m). High concentrations of nitrate ($>1\ \mu\text{M}$, except at Sta. 17) and phosphate ($>0.3\ \mu\text{M}$) in the surface water (Table 1) suggest that primary production was not limited by N and P in most the investigated area. Chl *a* concentrations integrated over the depth of 0–200 m were generally higher in the western (max. $57.5\ \text{mg Chl } a\ \text{m}^{-2}$, at Sta. 23) than in the eastern and central (min. $26.2\ \text{mg Chl } a\ \text{m}^{-2}$, at Sta. 20) regions except for a high value in the Bering Sea ($54.4\ \text{mg Chl } a\ \text{m}^{-2}$, at Sta. 9) (Table 1).

Depth profiles of ectoenzyme activities and other bacterial variables—In general, ectoenzyme activities, bacterial abundance and production were high in the mixed layer and then decreased in deep waters with the sharpest decline between the bottom of the mixed layer (20 m) and the halocline (100 m) (Fig. 2). To evaluate the decrease of measured variables with depth, the ratios of average values in the upper mesopelagic layer (100–200 m) relative to those in the mixed layer (0–20 m) (meso/mix ratio) were calculated for each station. Results indicated that, excluding the data from the anomalous Sta. 19 profile (*see below*), the depth-dependent decrease was most pronounced for bacterial production (average meso/mix ratio = 0.03), followed by BGase (meso/mix = 0.05) and LAPase (meso/mix = 0.06) activities, and it was least pronounced for bacterial abundance (meso/mix = 0.2) (Table 2). These results suggest the deficiency of

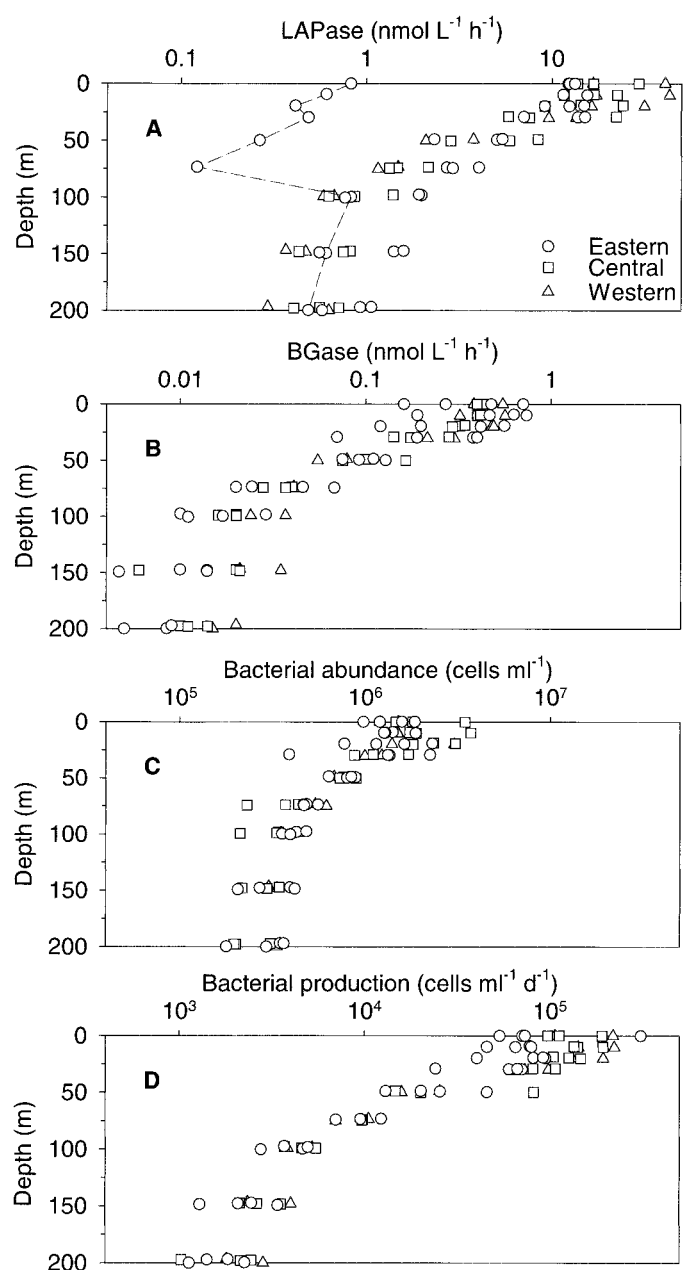


Fig. 2. Depth profiles of potential activities of LAPase (A) and BGase (B), bacterial abundance (C), and bacterial production (D) in the subarctic Pacific. Different symbols indicate different geographic locations, i.e., the eastern (circle), central (square), and western (triangle) region (see Table 1). LAPase activity at Sta. 19 was anomalous and is indicated by a dashed line (see text for explanations).

available DOM pool to sustain bacterial activities in the mesopelagic zone.

An exception to this general pattern was a depth profile of LAPase at Sta. 19; the activities in the mixed layer were low (0.41–0.83 nmol L⁻¹ h⁻¹) and differed little from the values in the upper mesopelagic layer (0.49–0.83 nmol L⁻¹ h⁻¹); there was a minimum of 0.12 nmol L⁻¹ h⁻¹ at 75 m

Table 2. Bacterial ectoenzymatic activity, abundance and production in the mixed layer (0–20 m; Mix) and the upper part of the mesopelagic zone (100–200 m; Meso) of the subarctic Pacific.*

Station	LAPase (nmol L ⁻¹ h ⁻¹)			BGase (nmol L ⁻¹ h ⁻¹)			Abundance (10 ⁶ cells ml ⁻¹)			Production (10 ⁴ cells ml ⁻¹ d ⁻¹)		
	Mix	Meso	Ratio†	Mix	Meso	Ratio	Mix	Meso	Ratio	Mix	Meso	Ratio
Eastern												
Sta. 17	14.6±1.1	0.64±0.12	0.04	0.67±0.09	0.010±0.005	0.01	2.01±0.26	0.37±0.07	15.6±13.4	0.17±0.09	0.01	
Sta. 15	11.1±1.7	1.48±0.50	0.13	0.19±0.08	0.018	0.09	1.37±0.23	0.42±0.06	4.7±0.7	0.25±0.11	0.05	
Sta. 18	12.1±0.5	1.49±0.46	0.12	0.45±0.03	0.017±0.010	0.04	1.14±0.15	0.35±0.08	8.3±1.1	0.30±0.17	0.04	
Sta. 19	0.6±0.2	0.64±0.17	1.04	0.33±0.26	0.010±0.006	0.03	1.25±0.42	0.25±0.10	7.7±0.5	0.34±0.12	0.04	
Central												
Sta. 20	15.9±1.4	0.78±0.08	0.05	0.39±0.04	0.018±0.004	0.05	1.85±0.45	0.24±0.05	11.7±1.6	0.38±0.15	0.03	
Sta. 21	11.5±2.3	0.49±0.12	0.04	0.38±0.08	0.011±0.005	0.03	1.84±0.05	0.25±0.07	17.7±2.6	0.31±0.13	0.02	
Sta. 9	25.3±3.6	0.92±0.42	0.04	0.37±0.05	0.017±0.006	0.04	3.42±0.33	0.33±0.02	12.2±2.2	0.23±0.14	0.02	
Western												
Sta. 22	16.8±0.5	0.42±0.15	0.02	0.40±0.09	0.022±0.002	0.05	1.37±0.15	0.33	12.7±1.8	0.27±0.11	0.02	
Sta. 23	38.5±6.2	0.59±0.10	0.02	0.53±0.05	0.029±0.012	0.05	2.02±0.88	0.37±0.06	21.2±1.6	0.40±0.12	0.02	
Average‡	16.3	0.83	0.06	0.41	0.017	0.05	1.8	0.32	12.4	0.30	0.03	
(CV, %)	(52)	(50)		(32)	(34)		(38)	(18)	(40)	(27)		

* Values are means ± SD (n = 2 or 3).
 † Ratio of values in the mesopelagic layer to that in the mixed layer (meso/mix ratio).
 ‡ Mean and coefficient of variation (CV) for values obtained from eight stations excluding Sta. 19.

(Fig. 2). The data from this station are not included in the following analysis unless noted.

For the LAPase activity, there was a regional pattern in the meso/mix ratio. The average meso/mix ratio of LAPase in the eastern stations (0.10 ± 0.05 , $n = 3$) was significantly greater than the average ratio in the central and western stations (0.03 ± 0.01 , $n = 5$) (two-tailed Student's *t*-test, $P = 0.03$). For other bacterial variables (BGase activity, bacterial abundance and production), no significant regional differences in meso/mix ratios were detected ($P > 0.06$).

Longitudinal trend in LAPase:BGase ratio and other bacterial variables—We calculated ratios of LAPase to BGase activities (both are potential activities at a saturated substrate level) to examine their vertical and geographic variations. The LAPase:BGase ratio has been used as an index of bacterial utilization patterns of organic polymers; high ratios indicate the dominant use of protein-rich polymers, whereas low ratios indicate the dominant use of polysaccharide-rich polymers (Christian and Karl 1995; Murrell et al. 1999). We found that the LAPase:BGase ratios in the upper mesopelagic layer varied greatly; the lowest ratio of 19 was at Sta. 22 and the highest ratio of 176 was at Sta. 15 (Fig. 3). Notably, there was a highly systematic, longitudinal gradient in the LAPase:BGase ratios in the mesopelagic layer: The ratios continuously increased eastward (Fig. 3). The pattern was described well by the following equation: $\log(\text{LAPase:BGase}) = 0.012 \times \epsilon - 0.63$ ($n = 9$, $r^2 = 0.76$, $P < 0.005$, including the data from Sta. 19), where ϵ is the east longitude ($^{\circ}\text{E}$) (the west longitude [ω , $^{\circ}\text{W}$] was converted to ϵ as follows; $\epsilon = 360 - \omega$).

In the surface water, the LAPase:BGase ratio also varied significantly, although the range of the variation (19–74) was not as great as that in the mesopelagic layer (Fig. 3). In contrast to the mesopelagic layer, the LAPase:BGase ratio in the surface water tended to decrease eastward: $\log(\text{LAPase:BGase}) = -0.007 \times \epsilon + 2.9$, $n = 8$, $r^2 = 0.48$, $P = 0.06$ (Fig. 3). This longitudinal pattern of LAPase:BGase ratio in the surface water was more closely related to the variability of LAPase activity than that of BGase activity: LAPase activity tended to decrease eastward [$\log[\text{LAPase}] = -0.006 \times \epsilon + 2.5$ [$n = 8$, $r^2 = 0.55$, $P < 0.05$]], whereas BGase activity did not exhibit any longitudinal trend. Bacterial abundance and bacterial production were not correlated with the east longitude in either surface or mesopelagic layers.

The outstanding longitudinal shift of the vertical pattern of the LAPase:BGase ratio is illustrated in Fig. 4 which shows three typical profiles obtained at western (Sta. 22, 170°E), central (Sta. 20, 170°W) and eastern (Sta. 18, 150°W) stations. Table 3 summarizes the statistical parameters from the linear regression analysis of the LAPase:BGase ratio versus depth. These results clearly indicate the highly systematic, longitudinal trend in the vertical pattern of the LAPase:BGase ratio. That is, the ratios increased significantly over depth (positive slope, $P < 0.05$) in the eastern region (Sta. 15, 17, 18, 19), while they varied little with depth ($P > 0.1$) in the central region (Sta. 9, 20, 21), and the ratios decreased significantly with depth (negative slope, $P < 0.05$) in the western region (Sta. 22, 23). The vertical

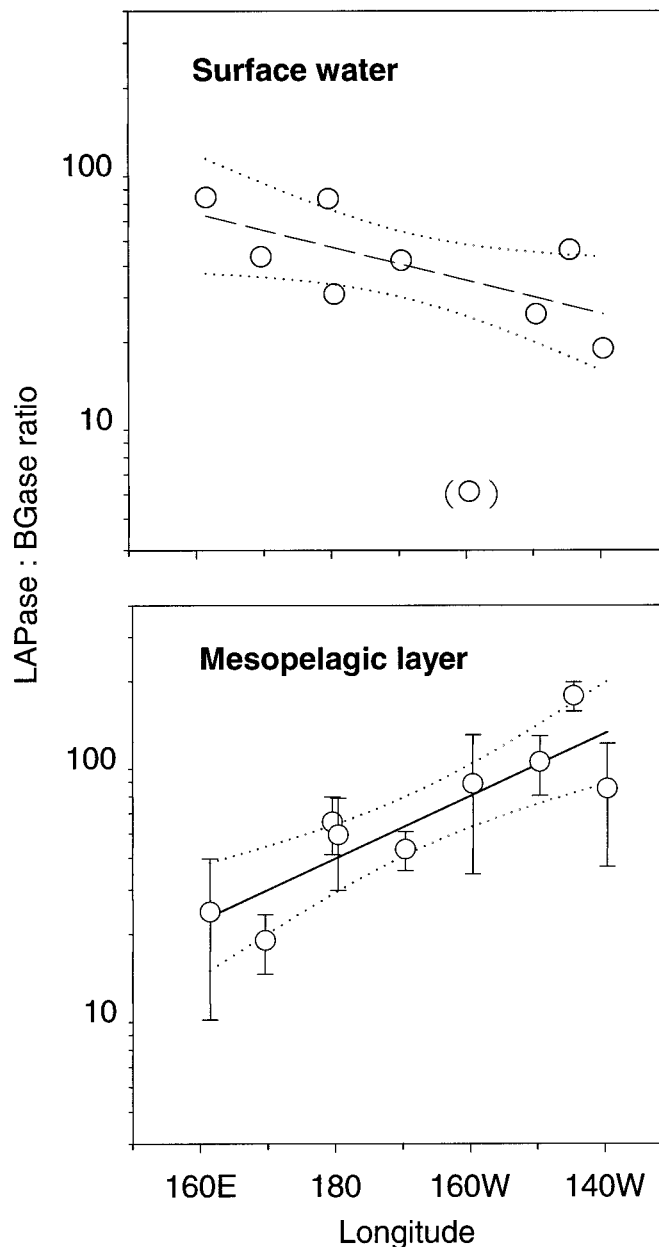


Fig. 3. Longitudinal tendency of LAPase:BGase ratios (mean ± 1 SD) in the surface water (0 m) and the upper part of the mesopelagic zone (100–200 m) in the subarctic Pacific. The linear regression lines are provided for the surface water (dashed line; a datum from Sta. 19, which is indicated with parenthesis, was excluded) and the mesopelagic layer (solid line) with 95% confidential limits (dotted lines). The relations between the east longitude (ϵ ; see test text for explanations) and enzyme ratio (y) are described as follows: $y = -0.007 \times \epsilon + 2.9$ ($n = 8$, $r^2 = 0.48$, $P = 0.06$) in the surface water and $y = 0.012 \times \epsilon - 0.63$ ($n = 9$, $r^2 = 0.76$, $P < 0.005$) in the mesopelagic layer.

slope was significantly correlated with the east longitude [Pearson's r between the linear slope of the depth profile (m^{-1}) and the east longitude of the station ($^{\circ}\text{E}$) = 0.88, $n = 9$, $P < 0.01$, including the data from Sta. 19], suggesting that the vertical trend of the LAPase:BGase ratio was influ-

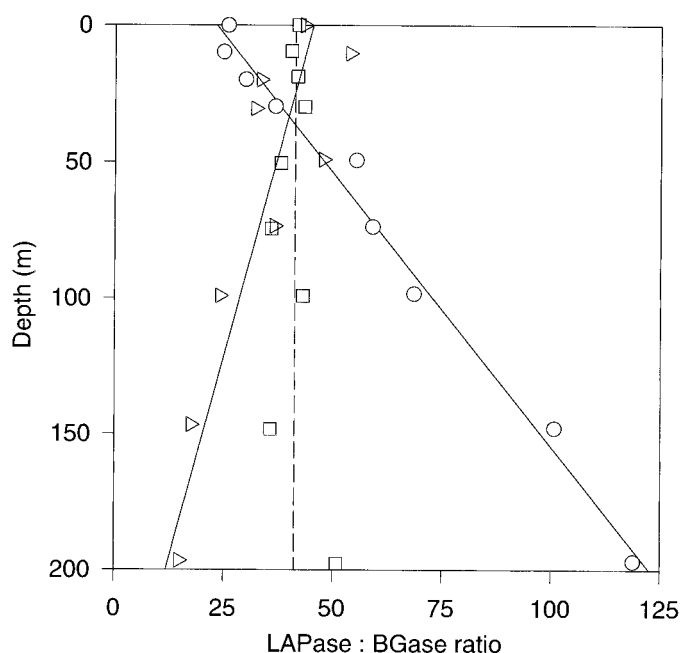


Fig. 4. Depth profiles of LAPase:BGase ratio at Sta. 18 (circle), Sta. 20 (square) and Sta. 22 (triangle). Each profile represents the pattern in the eastern, central, and western region of the subarctic Pacific, respectively. The linear regression lines of the LAPase:BGase ratio versus depth are also given (solid line). The dashed line indicates the mean of the ratios at Sta. 20, where the correlation was not significant between the ratio and depth. See Table 3 for parameters of the regression.

enced by a factor (or factors) that continuously and systematically changed over longitude.

Dissolved zinc concentration—Concentrations of dissolved Zn were measured at six stations representing western (Sta. 23), central (Sta. 9, 21) and eastern (Sta. 15, 17, 19) regions. The depth profile of dissolved Zn was a nutrient-type; low concentration (below the detection limit, <0.4 nM) in surface waters with increasing concentration over depth

(Fig. 5). The depth at which the concentration exceeds 0.4 nM ($D_{>0.4}$) varied with longitude. $D_{>0.4}$ was much greater at eastern stations (100–150 m, Sta. 15, 17, 19) than at western and central stations (20–75 m, Sta. 9, 21, 23) (Fig. 5).

Discussion

The most striking finding of this study was a large scale, systematic variation in the vertical pattern of LAPase:BGase ratio over longitude in the subarctic region (Figs. 3–4; Table 3). It is notable that the variation of LAPase:BGase ratio (0.97–192) that we observed in a single climate zone (i.e., subarctic region) covers almost the entire range of activity ratios reported for other various aquatic systems (Table 4), indicating high variability in organic substrate regimes in the subarctic Pacific. Based on measurement of LAPase:BGase ratios in open oceans, Christian and Karl (1995) suggested a large-scale shift in the mode of DOM-bacteria coupling in surface waters from a protein-based regime in the subtropical Pacific and the Antarctic to a polysaccharide-based system in the equatorial Pacific. In western Pacific coastal environments, Murrell et al. (1999) found that LAPase:BGase ratios tended to increase with salinity, suggesting that polysaccharides are large component of riverine organic material. These data support the hypothesis that the qualitative nature of DOM-bacteria fluxes varies substantially in aquatic systems and that ectoenzyme ratios sensitively reflect the pattern of DOM utilization by bacteria. Note that the potential activities of LAPase and BGase may not adequately model the actual hydrolysis rate of substrates in situ, but they must represent bacterial responses to compositional changes in polymeric substrates. Below we develop hypotheses to explain the observed pattern of the LAPase:BGase ratio in the subarctic Pacific and discuss its implications for carbon and nitrogen cycling.

It has been observed that the amino acid:carbohydrate ratio of sinking POM decreases with depth (Haake et al. 1993), indicating that proteinaceous components of sinking POM are more rapidly degraded than polysaccharide fraction. Experiments have consistently shown that bacteria de-

Table 3. The linear regression results of the relationship between LAPase:BGase ratio and water depth. The model used is $Y = a \cdot Z + b$, where Y is LAPase:BGase ratio and Z is depth (m). SE, standard error.

Station	$a (\pm SE)$	$b (\pm SE)$	n	r^2	P^*
Eastern					
Sta. 17	0.39 ± 0.09	18 ± 8	9	0.73	<0.005
Sta. 15	0.97 ± 0.34	59 ± 24	8	0.58	<0.05
Sta. 18	0.50 ± 0.02	23 ± 2	9	0.99	<0.001
Sta. 19	0.50 ± 0.16	-6.8 ± 15	9	0.60	<0.05
Central					
Sta. 20	0.02 ± 0.02	40 ± 2	9	0.10	NS
Sta. 21	0.11 ± 0.06	34 ± 6	9	0.28	NS
Sta. 9	-0.12 ± 0.07	74 ± 6	9	0.34	NS
Western					
Sta. 22	-0.17 ± 0.04	45 ± 4	9	0.71	<0.005
Sta. 23	-0.26 ± 0.10	64 ± 9	9	0.50	<0.05

* Two-tailed Student's t -test of the null hypothesis; $a = 0$. NS, not significant ($P > 0.1$).

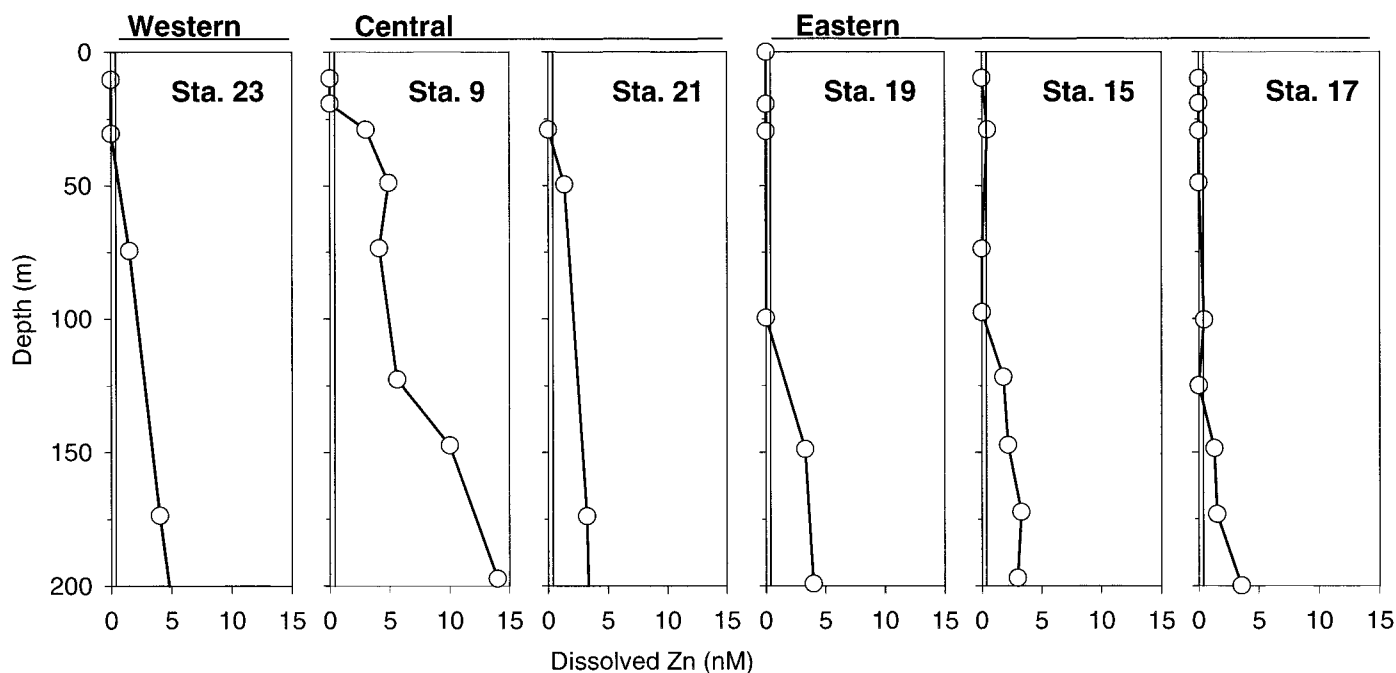


Fig. 5. Depth profiles of dissolved zinc concentration in the subarctic Pacific. The detection limit was 0.4 nM, which is indicated by a vertical line in each graph. When zinc concentrations are below the detection limit, symbols are places on the y-axis.

grade protein faster than polysaccharides during decomposition of phytoplankton-derived detritus (Skopintsev 1981) and that LAPase:BGase ratios decrease during phytoplankton decay (Middelboe et al. 1995). These general trends suggest that the relative abundance of polysaccharides in sinking POM should increase with depth, resulting in an intensive supply of polysaccharide-rich material to deeper

waters and a decrease in the LAPase:BGase ratio over depth. This hypothesis is consistent with our results in the western region of the subarctic Pacific in which LAPase:BGase ratio decreased with depth (Fig. 4; Table 3). However, in the eastern region, we found a significant increase of LAPase:BGase ratio with depth (Fig. 4; Table 3), a pattern which clearly contradicts the hypothesis that polysac-

Table 4. LAPase:BGase ratios in various aquatic environments.

Habitat *	LAPase:BGase ratio †	Source
River and lake		
Frederiksborg Slotsso (Lake)	12–32	Middelboe et al. (1995)
Ottawa River	6.5	Sinsabaugh et al. (1997)
Maumee River	35	Sinsabaugh et al. (1997)
Sacramento River	0.79–17	Murrell et al. (1999)
Coast and estuary		
Fjord, Germany	7.9–31	Hoppe (1983)
Southern California Bight (0–25 m)	8–41	Smith et al. (1992)
Central Bay, CA	30–56	Murrell et al. (1999)
Suisun Bay, CA	10–24	Murrell et al. (1999)
Belgian coast	10	Sinsabaugh et al. (1997)
Sagami Bay, Japan (0–1,000 m)	1.60–61.7	Fukuda et al. (unpubl.)
Otsuchi Bay, Japan (0–80 m)	15.9–156	Fukuda et al. (unpubl.)
Open ocean		
Equatorial Pacific	0.276	Christian and Karl (1995)
Subtropical Pacific	213	Christian and Karl (1995)
Antarctic	339–1052	Christian and Karl (1995)
Transitional North Pacific (0–1,000 m)	14.1–61.7	Fukuda et al. (unpubl.)
Subarctic Pacific (0–200 m)	0.97–192	This study

* The data were collected for surface water samples unless noted with a depth range in the parenthesis.

† Ratios of potential activities.

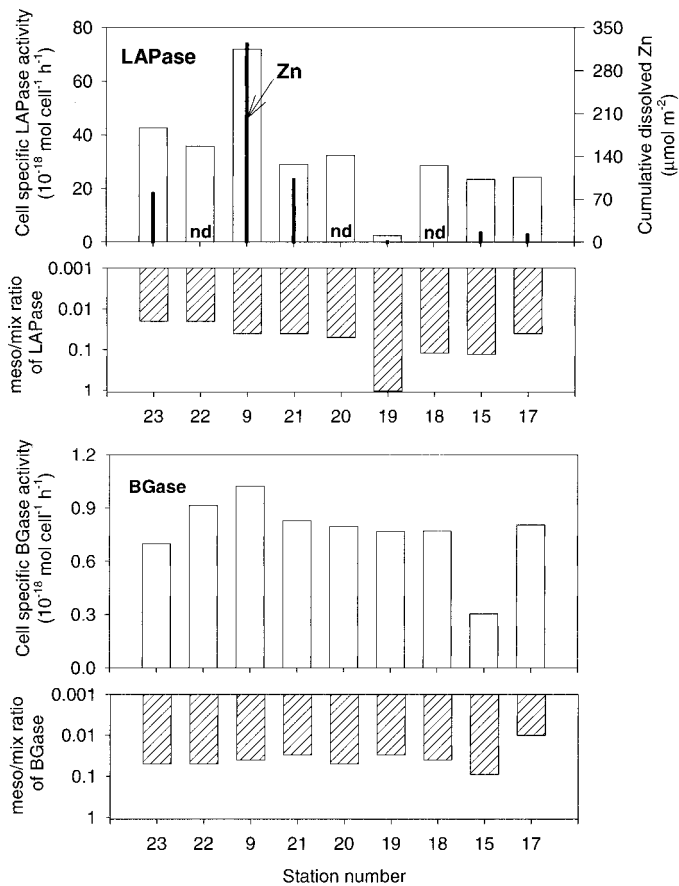


Fig. 6. Comparison of the integrated dissolved zinc (closed bars) and cell specific activities of ectoenzymes (ratio of integrated activity to integrated bacterial abundance; open bars) in the water column of 0–100 m. The ratios of the average enzyme activities in the upper mesopelagic layer (100–200 m) relative to those in the mixed layer (0–20 m) (meso/mix ratio) are also shown (shaded bars; logarithmic scale). The enzyme activities are corrected to the values measured at 15°C with assumed Q_{10} of 2.2 (Rosso and Azam 1987). Stations are placed along the longitudinal positions. **nd**, zinc concentration not determined.

charide-rich material is predominantly exported to the mesopelagic zone. Importantly, this “depth trend-reversal” of the LAPase:BGase ratio occurred successively along the longitudinal axis of the investigated area (Table 3). Thus, there appears to be a strong east-west gradient in environmental or ecological factors that substantially influence the mode and vertical pattern of organic matter-bacteria interactions in the subarctic Pacific.

We propose that one of these factors could be the availability of Zn in the upper waters. Studies have suggested that the LAPase from marine bacteria is mostly a metallo-enzyme (Fontigny et al. 1987) which contains Zn as an essential catalytic element (e.g., Stensvåg et al. 1993). In contrast, BGase doesn’t contain metallic cofactors (e.g., Han and Srinivasan 1969). Because dissolved Zn concentrations can be extremely low (<0.1 nM) in oceanic surface waters (Bruland et al. 1994), we hypothesize that expression of LAPase can be limited by Zn in surface waters. Note that a dominant

source of dissolved Zn to North Pacific surface waters is atmospheric deposition (Bruland 1980), which exhibits a strong longitudinal pattern in the subarctic Pacific: High fluxes in the west and low fluxes in the east (Duce and Tindale 1991). Thus, we further hypothesize that the longitudinal gradient in the magnitude of Zn deposition affects the geographic distribution of surface LAPase activity but not that of BGase activity in the investigated area.

In order to test the above hypotheses, we compare our ectoenzyme data with concentrations of dissolved Zn. Although Zn data are available for only a few stations and depths, we found that the depth at which the concentration exceeds 0.4 nM ($D_{>0.4}$) was much greater at eastern stations (100–150 m, Sta. 15, 17, 19) than at western stations (20–75 m, Sta. 9, 21, 23; Fig. 5). The vertical distribution of dissolved Zn concentration could be influenced by several factors (atmospheric deposition, biological uptake/regeneration, and water column physics; Bruland et al. 1994). Regardless of the mechanism, the geographic tendency of $D_{>0.4}$ seems to be consistent with the hypothesis that Zn is more depleted in the east than in the west of the subarctic Pacific.

Figure 6 shows the distributions of integrated dissolved Zn concentration and cell specific activities of ectoenzymes (ratio of integrated activity to integrated bacterial abundance) in the upper water layer (0–100 m) above the permanent halocline. To evaluate the distribution of total amount of enzymes (Christian and Karl 1995), the enzyme activities (both LAPase and BGase) are corrected to the values measured at 15°C with assumed Q_{10} of 2.2 (Rosso and Azam 1987). Although Q_{10} might vary depending on enzymes and environments (Christian and Karl 1995), we note that the assumption of the Q_{10} value (1.7–2.6, a range reported for the Antarctic and the subtropical Pacific by Christian and Karl 1995) affects little our general conclusion about the activity-Zn relationship. Integrated Zn is positively correlated with LAPase activities (Pearson’s $r = 0.92$ and 0.85 , $n = 6$, $P < 0.05$, for cell specific activity and integrated activity, respectively). In contrast, there was no significant correlation between Zn concentrations and BGase activities ($P > 0.1$ for both cell specific and integrated BGase). Neither bacterial growth rate (integrated production/integrated cell), integrated bacterial abundance nor integrated Chl *a* concentrations was correlated with the integrated Zn concentrations ($P > 0.2$), suggesting that rates of organic substrate supply were not directly related to Zn concentrations. These results are consistent with the hypothesis that bacterial expression of LAPase was limited by Zn in the eastern subarctic Pacific. Note that the above correlation analysis is based on a quite limited data set from the same-bottle determinations of LAPase activity and Zn concentrations in open oceans. We emphasize that more data are needed to verify these conclusions.

The possible repression of LAPase activity due to Zn deficiency in the eastern region could result in insufficient degradation of protein in the upper waters, leading to intensive export of protein to the mesopelagic layer. High Zn concentrations (1–14 nM) in the upper mesopelagic layer (Fig. 5) would relieve LAPase repression, allowing attached and free-living bacteria to hydrolyze protein actively. Thus, in the eastern region, one would expect relatively high LAPase

activity in the upper mesopelagic layer. In support of this notion, our data showed that the average meso/mix ratio of LAPase in the eastern stations was significantly greater than the ratio in the central and western stations (Table 2; Fig. 6). In contrast, the vertical pattern of BGase activity was relatively uniform; the meso/mix ratio did not vary systematically over the regions (Table 2; Fig. 6). These differential responses of LAPase and BGase to the surface deficiency of Zn and their propagative effects on organic matter metabolism in the upper mesopelagic layer could explain the systematic, longitudinal variation in depth profiles and horizontal distribution of LAPase:BGase ratio that we found in the subarctic Pacific (Table 3; Fig. 3, 4).

Besides Zn availability, other factors could also affect vertical patterns of LAPase and BGase activities in subarctic waters. Honjo (1997) found differences in the qualitative nature of sinking POM between the Gulf of Alaska and the Bering Sea. He suggested that the taxonomic composition of dominant phytoplankton could affect the quality of sinking POM. Vertical migration of zooplankton may also affect the quality of organic substrates utilized by mesopelagic bacteria. For example, salp fecal pellets include a large amount of protein (Madin 1982) and can account for a substantial fraction of the sinking flux in the Gulf of Alaska (Iseki 1981). Whether or not these factors can explain the highly systematic longitudinal pattern of LAPase:BGase ratio in the subarctic Pacific is open for future research.

In summary, our study demonstrated a systematic, geographic shift of vertical patterns of LAPase:BGase ratios in the subarctic Pacific. These results are crucial to understanding carbon and nitrogen cycling in the water column, because bacteria are the major consumer of organic matter in ocean's interior (Cho and Azam 1988; Nagata et al. 2000). Especially, our findings of vertical patterns of protein hydrolysis can provide clues to the qualitative characteristics of biological pump, because protein accounts for a substantial fraction (18–91%) of particulate organic carbon in surface waters (Wakeham et al. 1984). We suggest that Zn deficiency may result in repression of LAPase synthesis in surface waters. The inhibition of bacterial degradation of protein in surface layers may result in high inputs of proteinaceous materials to the mesopelagic zone. Previous work indicates that Zn deficiency can repress the synthesis of carbonic anhydrase in phytoplankton (Morel et al. 1994), which may suppress the biological carbon pump driven by photosynthetic production. In contrast, our results suggest that Zn deficiency can accelerate the carbon and nitrogen pump via repression of bacterial LAPase and degradation of protein in the euphotic zone. Clearly, future studies should examine in greater detail relationships between Zn availability and protein degradation, and possible competition between bacteria and phytoplankton for trace metals in open oceans.

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