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Seasonal variability of size-fractionated chlorophyll *a* in Monbetsu Harbor, Hokkaido, northern Japan

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Abstract: Size-fractionated chlorophyll-a (Chl-a) concentrations were measured weekly in the waters of Monbetsu Harbor, which lay under ice for part of the year, in order to describe seasonal variations and to gain an understanding of the physical and chemical factors that control production cycles in this neritic system. Sea ice cover first formed in February, reached a maximum on 11 March, and disappeared completely in April 1996. Averaged Chl-a concentration $(8.4 \pm 4.3 \text{ mg m}^{-2})$ in June-October (summer) was significantly higher than that $(2.5 \pm 1.8 \text{ mg m}^{-2})$ in November– May (winter), except for two values of 12 and 26 mg m⁻² observed in March. The Chl-a concentration of the >10 μ m size fraction also reached a maximum (22 mg m^{-2}) in March, contributing 92% to the total Chl-*a* biomass. Temporal variations in salinity and nutrient concentrations suggested a seasonal change in the water mass adjacent to the harbor. An alteration between dominance by the Soya Warm Current and the surface waters of the Okhotsk Sea was observed. Chlorophyll a in Monbetsu Harbor exhibited distinctive seasonal variations during the annual cycle and a peak in the >10 μ m size fraction, corresponding to sea ice coverage, was evident in March.

Key words: size-fraction, chlorophyll a, sea ice, Monbetsu

Introduction

Monbetsu Harbor is located on the northeastern coast of Hokkaido, adjacent to the Okhotsk Sea (Fig. 1). The hydrographic conditions along the northeast coast of Hokkaido are influenced by the Soya Warm Current, which runs northwest to southeast along the coast. This current is evident in summer, but during the winter it submerges below the surface waters of Okhotsk Sea (Aota 1979). Thus, the dominant surface water mass off the northeastern coast of Hokkaido shifts seasonally from Soya Warm Current derived waters to those of the Okhotsk Sea. Furthermore, latitude of the Okhotsk Sea is one of the lowest of all ice-covered waters in the northern hemisphere; ice coverage is usually observed from December through/until April (Aota 1985). Ice floes from the north reach the coast of Hokkaido in Janu-

ary and disappear in April. The annual advance and retreat of sea ice is one of the major physical factors controling the dynamics of polar pelagic systems (Legendre et al. 1992; Scott et al. 1994; Kirst & Wiencke 1995; Smith et al. 1995). Sea ice mediates several factors which regulate primary productions, e.g. light, temperature, and water column stability (Franceschini 1978; Smith & Nelson 1986). During the formation of sea ice, phytoplankton is incorporated into the ice crystals and develops into ice algal assemblages (Garrison et al. 1987). Cells can survive within the ice matrix or in brine pockets (Harrison et al. 1990). During the seasonal melting of ice, algal cells released from the ice can act as a seed stock for the spring bloom (Garrison et al. 1987; Kuosa et al. 1992). Water stability is increased by the melting of the ice and the increase in water temperature and light intensity accelerates phytoplankton growth, leading to ice edge blooms (Smith & Nelson 1985).

Few studies have incorporated the effect of sea ice on phytoplankton population dynamics in these coastal waters, with the exception of studies done in Saroma Lagoon (Satoh et al. 1989; Taguchi et al. 1997). The size classes of the phytoplankton potentially influences the response of populations to nutrients and food-chain dynamics (e.g. Martin 1970; Walsh 1976; Chervin 1978). Nitrate, ammonium and phosphate uptake is size dependent, presumably as a consequence of decreases in the surface-to-volume ratio as cell size increases (Epply et al. 1969; Friebele et al. 1978). Picophytoplankton are too small for metazoans to effectively consume, but are ingested by heterotrophic flagellates and cilliates (Campbell and Carpenter 1986; Caron et al. 1992). In this study, we describe the temporal variability in size-fractionated chlorophyll a (Chl a) in Monbetsu Harbor, Hokkaido. These data are expected to lay the foundation enabling an understanding of the physical and chemical factors that control the production cycle in this neritic system. Using the weekly data set over a one-year period, general patterns in the size structure of the phytoplankton community were also investigated.

Materials and Methods

Water samples were collected weekly from the surface and from 3-m depth at a pier in Monbetsu Harbor (Fig. 1) from 20 September 1995 to 18 September 1996 except in February when ice covered the entire harbor. The depth at the sampling site varied from 3.2 to 3.6 m depending on the tidal effect. Temperature was measured with a mercury thermometer, and salinity was determined with a CTD, model SBE-19 (Sea-Bird Electronics, Inc.). Ice cover-



Fig. 1. A map of Monbestu Harbor showing the sampling location (\bigstar) .

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age data were obtained from the Monbetsu Radar Station at the Sea Ice Research Laboratory, Low Temperature Institute, Hokkaido University. Ice coverage was calculated as the percentage cover over a circular area with a radius of ca. 50 km from the sampling station, and was measured at 0900 h every day.

Water samples were pre-filtered with 333 μ m mesh net and kept frozen at -20° C until analysis. Duplicate subsamples of 100 ml were filtered sequentially through 10 μ m, 2.0 μ m and 0.2 μ m polycarbonate filters to enable size fractionation. Additional subsamples of 100 ml were filtered onto Whatman GF/F glass fiber filters to determine the total Chl-*a* concentration. A filtration vacuum of <100 mmHg was used. Chlorophyll *a* was extracted from each filter with N,N-dimethyl formamide (DMF) at 4°C for 24 h (Suzuki & Ishimaru 1990) and was determined fluorometrically on a Turner Designs fluorometer Model–10AU using procedures described in Holm-Hansen et al. (1965). Nitrate, nitrite, phosphate and silicate were measured using a Bran and Lubbe Model AACS–II Autoanalyzer.

Results

Over the study period, water temperature varied from -1.3 to 22°C. Salinity varied from 23 to 34 PSU (Fig. 2a). The sudden decrease in the surface salinity in April and May was probably due to a run off of melted snow and ice from the watershed. Sea ice first formed in Monbetsu Harbor in February and reached maximum coverage (ca. 100%) on 11 March, as determined with the radar scope. Following this maximal period, ice coverage decreased and sea ice disappeared completely on 23 April (Fig. 2b). Nutrient concentrations increased from October to December and remained at high concentrations until May (Fig. 3). Nitrate plus nitrite concentration varied from 0.10 to $17 \,\mu$ M (Fig. 3a). Phosphate concentration varied from 0.16 to $1.6 \,\mu$ M (Fig. 3b). Silicate concentration varied from 0.37 to $86 \,\mu$ M (Fig. 3c). High silicate concentrations occuring only at the surface in April and May were associated with the low salinity water, through to be terrestrial run off.



Fig. 2. a. Temporal variations of salinity (*circle*) and temperature (*triangle*). Solid and broken lines indicate values at the surface and 3-m depth, respectively. b. Temporal variation of percentage ice-coverage in the area monitored by the Monbetsu Radar Station.



Fig. 3. Temporal variation of nitrate plus nitrite (a), phosphate (b) and silicate (c) concentrations. *Solid* and *broken lines* indicate values at the surface and 3-m depth, respectively.



Fig. 4. a. Temporal variation of total Chl-*a* biomass in Monbetsu Harbor. b. Temporal variation of size-fractionated Chl-*a* biomasses. *Closed circles with solid line* indicate the >10 μ m fraction. *Closed triangles with broken line* indicate the 2.0–10 μ m fraction. *Open circles with dotted line* indicate the 0.2–2.0 μ m fraction. c. Temporal variation in the percentage contribution of each size fraction to total Chl-*a* concentration.

Total Chl-*a* concentration filtered on GF/F was proportional to the sum of the size-fractionated Chl-*a* concentrations. The regression line exhibited a significant correlation (R=0.906) with a slope of 1.0. Temporal variations in Chl-*a* concentration at the surface and at 3-m depth exhibited a similar pattern, and the averages of total Chl-*a* concentration were $2.2\pm$ 2.1 mg m⁻³ at the surface and 2.1 ± 1.9 mg m⁻³ at 3-m depth. Correlation of size-fractionated Chl-*a* concentration between the two depths (0 m vs 3 m) were statistically significant, with correlation coefficients of 0.758 for the >10 µm fraction, 0.736 for the 2.0–10 µm fraction and 0.651 for the 0.2–2.0 µm fraction. Chlorophyll-*a* concentrations at both depths were integrated to express the biomass in the water column under a square meter of surface area. Total Chl-*a* biomass varied from 1.0 to 26 mg m⁻² (Fig. 4a). Temporal variation in size-fractionated Chl-*a* biomass was characterized by a distinctive spring peak in the >10 µm fraction with a moderately high concentration in this fraction from June to September (Fig. 4b). The maximum Chl-a biomass of the >10 μ m fraction was 22 mg m⁻², recorded in March. Percentage contribution of each size fraction to the sum of size-fractionated Chl-a biomass was calculated (Fig. 4c). The >10 μ m size fraction was dominant during most of the year, averaging 71±15% over the study period. The other size fractions (0.2–2.0 and 2.0–10 μ m) predominated in November and December when total Chl-a biomass was low (<2.0 mg m⁻²). The maximum contributions of the 2.0–10 μ m and 0.2–2.0 μ m fractions reached 43 % and 32 % respectively, in November, and averaged 23±11 % (2.0–10 μ m) and 5.9±6.0 % (0.2–2.0 μ m) over the study period.

Discussion

Long-term storage of phytoplankton at -20° C leads to a decrease in pigment recovery. Humphrey and Wooton (1966) found that, when spectrophotometrically measured, the Chl *a* of phytoplankton stayed stable for up to 8 weeks. However, the results of the SCOR Working Group storage experiment indicate that the recovery of Chl *a*, as analyzed by HPLC after 60-d storage, was 82% for mixed microalgae and 85% for natural phytoplankton (Mantoura et al 1997). In this study, we may therefore underestimate the real Chl-*a* concentrations.

Abiotic conditions in the harbor oscillate between two distinctive periods during the annual cycle, June–October (summer) and November–May (winter). When temperatures and salinities during these two periods were plotted on a T-S diagram, most of the data could be separated into two groups, with limits at ca. 10°C and 33 PSU (Fig. 5). Water masses in the summer period were of higher salinity and temperature than those in the winter, although surface water, which was strongly affected by run off, occasionally exhibited salinities lower than 33 PSU even during the summer period. Macronutrient concentrations were correrated with these water masses, with higher concentrations in the winter period than in the summer. Differences in the salinity and nutrient concentrations between the two periods were statistically significant (p < 0.001, in *t*-test). Salinity and nutrient concentrations observed in Monbetsu Harbor

		Monbetsu Harbor		0	
	Depth	Summer June–Oct.	Winter Nov.–May	Soya warm Current	Surface Water
Salinity	0 m	32.9±0.68	30.0±3.0	≥33.6‰ ¹	≤32.5‰ ¹
(PSU)	3 m	33.3 ± 0.42	31.7 ± 1.1		
Nitrate+Nitrite	0 m	1.5 ± 1.5	8.6 ± 4.2	$0.36 \pm 0.25^{*2}$	$3.1 \pm 3.5^{*2}$
(µM)	3 m	1.4 ± 1.1	6.2 ± 3.8		
Phosphate	0 m	0.37 ± 0.16	0.96 ± 0.41	0.21 ± 0.07^2	0.47 ± 0.20^2
(μM)	3 m	0.40 ± 0.14	0.87 ± 0.35		
Silicate	0 m	5.5 ± 3.6	29 ± 22	3.1 ± 2.7^{2}	7.3 ± 2.5^2
(µM)	3 m	6.1 ± 2.1	17 ± 10		
Chlorophyll a	0 m	3.0 ± 1.9	0.83 ± 0.99		
$(mg m^{-3})$	3 m	2.6 ± 1.5	0.83 ± 0.79		
$(mg m^{-2})$	integrated	8.4±4.3	4.3±6.0		

 Table 1. Average salinities, nutrient and Chl-a concentrations in Monbetsu Harbor, the Soya Warm Current and the surface waters of the Okhotsk Sea.

¹ Aota (1985). ² Maita (1985).

* Nitrate concentration.

suggest a change in water masses between summer and winter. Surface water from the Okhotsk Sea is characterized by low salinities ($\leq 32.5\%$), low temperatures and high nutrient concentrations, whereas water from the Soya Warm Current is characterized by a salinity of $\geq 33.6\%$ (Aota 1985). Furthermore, nutrient concentrations in the Soya Warm Current are significantly lower than those of the surface waters of the Okhotsk Sea (Maita 1985). Hydrographic conditions in Monbetsu Harbor appear to be seasonally influenced by the waters of the Soya Warm Current during the summer period and the Okhotsk Sea during the winter period. Abiotic factors and Chl-*a* concentrations during each period are summarized in Table 1.

Size-fractionated chlorophyll data from various oceanographic provinces have revealed an unequivocal fact about phytoplankton size that the fractional contribution of small cells to the total standing crop increases as total chlorophyll decreases (Chisholm 1992). A data set from the Mediterranean Sea indicated a general pattern in the size structure of phytoplankton communities, in which the concentration of Chl *a* in each fraction has an upper limit, corresponding to roughly 0.5, 1, and $2 \mu g 1^{-1}$ for the <1, <3, and <10 μ m size fractions, respectively



Fig. 5. Temperature-salinity diagram during the period from 20 September 1995 to 18 September 1996 in Monbetsu Harbor. *Trian*gles and circles indicate values at the surface and 3-m depth, respectively. Closed and open symbols indicate values from June to October and from November to May, respectively.



Fig. 6. Relationship between total Chl-*a* concentrations and Chl-*a* concentrations in the 2.0-0.2 μ m (*closed circles*), 10-2.0 μ m (*squares*) (a) and >10 μ m (open circles) (b) fractions.

(Raimbault et al. 1988). In nutrient rich and high chlorophyll areas, there is a maximum "potential biomass" (i.e. $0.50 \ \mu g \ l^{-1}$) of picoplankton chlorophyll, which under these conditions is a relatively small percentage of the total phytoplankton chlorophyll. Our size-fractionation data agree with the general patterns indicated by previous workers, although the maximum "potential biomass" is supposed to be lower than $0.50 \ \mu g \ l^{-1}$ for the picoplankton and the upper limits of the amount of Chl *a* are roughly 0.25 and $1.7 \ \mu g \ l^{-1}$ for the 0.2–2.0 and 2.0– $10 \ \mu m$ size fractions, respectively (Fig. 6a). In contrast, the Chl-*a* concentrations (Fig. 6b). Thus, variability in total Chl *a* reflects the amount of cells >10 $\ \mu m$. These results support the supposition that, beyond certain thresholds, chlorophyll can only be added to a system by adding a larger size class of cells (Chisholm 1992).

In Monbetsu Harbor, the contribution of the >10 μ m size fraction reached 94–96% of the total Chl-*a* biomass, with the most drastic increase in its biomass in March (Fig. 4b, c). Interestingly, this change corresponded to the annual peak in ice coverage (Fig. 2b). Diatoms are one of the dominant groups in Antarctic microalgal assemblages in ice-covered waters (i.e. Bunt & Wood 1963; Garrison et al. 1987; Ligowski & Kopczynska 1991). Garrison et al. (1987) demonstrated a high degree of similarity between ice and water column assemblages at a receding ice-edge in the Weddell Sea during the austral spring. Seeding from the ice was experimentally demonstrated as a factor in the increase of algal biomass (Kuosa et al. 1992). However, at present it is not known whether ice-algae were responsible for this bloom-like event in Monbetsu Harbor.

Weekly sampling and size-fractionated Chl-*a* measurements revealed the form of seasonal variability in phytoplankton biomass in Monbetsu Harbor. A change in water masses is proposed to have occurred between summer and winter. Chlorophyll-*a* regimes in Monbetsu Harbor can be classified into three periods: summer (June–Oct.), winter (Nov.–Feb., April and May) and the ice-covered period (March). Averaged Chl-*a* concentrations were calculated for the winter period, excluding data from the ice-covered period in March, and the difference between summer ($8.4\pm4.3 \text{ mg m}^{-2}$) and winter ($2.5\pm1.8 \text{ mg m}^{-2}$) concentrations was statistically significant (p<0.001 in *t*-test). Further studies should be directed to elucidate the contribution of ice algae and the effect of sea ice on the overall community structure in the harbor.

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Literature Cited

Aota, M. 1979. Variation of hydrographic conditions along the coast of Okhotsk Sea in Hokkaido. Bull. Coast. Oceanogr. 17: 1-11.

Aota, M. 1985. Coastal water of Okhotsk Sea, p. 1105. In Coastal Oceanography of Japanese Islands (ed. Oceanogr. Soc. Jpn). Tokai Univ. Press, Tokyo. (In Japanese.)

Bunt, J. S. & E. J. F. Wood 1963. Microalgae and sea ice. Nature 199: 1254-1255.

Campbell, L. & E. J. Carpenter 1986. Estimating the grazing pressure of heterotrophic nanoplankton on Synechococcus spp. using the sea water dilution and selective inhibitor techniques. Mar. Ecol. Prog. Ser. 33: 121–129.

Caron, D. A., E. L. Lim, G. Miceli, J. B. Waterbury & F. W. Valois 1992. Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. Mar. Ecol. Prog. Ser. 76: 205-217.

- Chervin, M. 1978. Assimilation of particulate organic carbon by estuarine and coastal copepods. *Mar. Biol.* **49**: 265–275.
- Chisholm, S. W. 1992. Phytoplankton size, p. 213–237. In *Primary Productivity* and *Biogeochemical Cycles* in the Sea (eds. Falkowski, P. G. & A. D. Woodhead). Plenum Press, New York.
- Eppley, R. W., J. N. Rogers & J. J. McCarthy 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. *Limnol. Oceanogr.* 14: 912–920.
- Franceschini, G. A. 1978. Solar radiation over the Weddell and Ross Seas. Antarctic J.U.S. 13: 172-173.
- Friebele, E. S., D. L. Correll & M. A. Faust 1978. Relationship between phytoplankton cell size and the rate of orthophosphate uptake: in situ observations of an estuarine population. *Mar. Biol.* 45: 39–52.
- Garrison, D. L., K. R. Buck & G. A. Fryxell 1987. Algal assemblages in antarctic pack ice and in ice-edge plankton. J. Phycol. 23: 564-572.
- Harrison, W. G., G. F. Cota & R. E. H. Smith 1990. Nitrogen utilization in ice algal communities of Barrow Strait, Northwest Territories, Canada. Mar. Ecol. Prog. Ser. 67: 275–283.
- Holm-Hansen, O., C. J. Lorenzen, R. N. Holmes & J. D. H. Strickland 1965. Fluorometric determination of chlorophyll. J. Cons. Perm. Int. Expl. Mer. 30: 3-15.
- Humphrey, G. F. & M. Wooton 1966. Comparison of the techniques used in the determination of phytoplankton pigments, p. 37-63. In *Determination of Photosynthetic Pigments in Seawater*. UNESCO monograph on oceanographic methodology No.1, UNESCO, Paris.
- Kirst, G. O. & C. Wiencke 1995. Ecophysiology of polar algae. J. Phycol. 31: 181-199.
- Kuosa, H., B. Norrman, K. Kivi & F. Brandini 1992. Effects of antarctic sea ice biota on seeding as studied in aquarium experiments. *Polar Biol.* 12: 333–339.
- Legendre, L., S. F. Ackley, G. S. Dickmann, B. Gulliksen, R. Horner, T. Hoshiai, I. A. Melnikov, W. S. Reeburgh, M. Spindler & C. W. Sullivan 1992. Ecology of sea ice biota. 2. Global significance. *Polar Biol.* 12: 429–444.
- Ligowski, R. & E. Kopczynska 1991. Distribution of net phytoplankton in the sea-ice zone between Elephant Island and the South Orkney Islands (December 1988–January 1989). Polish Polar Res. 12: 529–546.
- Maita, Y. 1985. Coastal water of Okhotsk Sea. Chemistry, p. 23-33. In *Coastal Oceanography of Japanese Islands* (ed. Oceanogr. Soc. Jpn). Tokai Univ. Press, Tokyo. (In Japanese.)
- Mantoura, R. F. C., S. W. Wright, S. W. Jeffery, R. G. Barlow & D. E. Cummings 1997. Filtration and storage of pigments from microalgae, p. 283–305. In *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*, (eds. Jeffery, S. W., R. F. C. Mantoura & S. W. Wright). UNESCO Publishing, Paris.
- Martin, J. H. 1970. Phytoplankton-zooplankton relationships in Narragensett Bay. IV. The seasonal importance of grazing. *Limnol. Oceanogr.* 15: 413–418.
- Raimbault, P, M. Rodier & I. Taupier-Letage 1988. Size fractionation of phytoplankton in the Ligurian Sea and the Algerian Basin (Mediterranean Sea): Size distribution versus total concentration. Mar. Microb. Food Webs 3: 1-7.
- Satoh, H., Y. Yamaguchi, K. Watanabe & Y. Aruga 1989. Light conditions and photosynthetic productivity of ice algal assemblages in Lake Saroma, Hokkaido. Jpn. J. Phycol. 37: 274–278.
- Scott, P., A. McMinn & G. Hosie 1994. Physical parameters influencing diatom community structure in eastern Antarctic sea ice. *Polar Biol.*14: 507–517.
- Smith, W. O. & D. M. Nelson 1985. Phytoplankton bloom produced by a receding ice edge in the Ross Sea: spacial coherence with the density field. *Science* 227: 163–166.
- Smith, R. C., K. S. Baker, W. R. Fraser, E. E. Hofmann, D. M. Karl, J. M. Klinck, L. B. Quetin, B. B. Prezelin, R. M. Ross, W. Z. Trivelpicce & M. Vernet 1995. The Palmer LTER: A long-term ecological research program at Palmer station, Antarctica. *Oceanography* 8: 77–86.
- Suzuki, R. & T. Ishimaru 1990. An improved method for the determination of phytoplankton chlorophyll using N,N-dimethylformamide. J. Oceanogr. Soc. Jpn 46: 190–194.
- Taguchi, S., H. Saito, H. Hattori & K. Shirasawa 1997. Vertical flux of ice algal cells during the ice melting and breaking periods in Saroma Ko Lagoon, Hokkaido, Japan. Proc. NIPR Symp. Polar Biol. 10: 56–65.
- Walsh, J. J. 1976. Herbivory as a factor in patterns of nutrient utilization in the sea. *Limnol. Oceanogr.* 21: 1–13.