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VARIABILITY IN STEROL FLUX IN THE ICE-COVERED LAGOON SAROMA KO, HOKKAIDO, JAPAN

Hiroshi SASAKI[1]*, Toshiyasu YAMAGUCHI[1], Kentaro WATANABE[2], Atsushi TANIMURA[2] and Mitsuo FUKUCHI[2]

[1]*Faculty of Agriculture, Tohoku University, 1–1, Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai 981*
[2]*National Institute of Polar Research, 9–10, Kaga 1-chome, Itabashi-ku, Tokyo 173*

Abstract: A time-series sediment trap was deployed in a shallow lagoon, Saroma Ko, from 9 December 1986 to 2 May 1987. During the ice-covered period (mid-January to mid-April) total mass fluxes (dry weight) were lower than 2 g m^{-2} day^{-1}, while after mid-April the flux increased to more than 11 g m^{-2} day^{-1}. The dominant sterol associated with sinking particles throughout the observation period was cholesterol most of which were possibly from animal-derived sources such as scallop feces. During the ice-covered period, sterols mostly were derived from marine animals, although the sterol abundance was low in quantity. The period of phytoplankton bloom below the ice (mid-March to mid-April) did not respond to that of the maximum flux (after mid-April), but coincided with that of the occurrence of phytoplankton-derived sterols. During the ice-free periods (before mid-January and after mid-April), sinking particle sources are variable, and bottom sediment- and land-derived (mostly terrestrial plants) sterols are larger in quantity than those during the ice-covered periods, primarily because of the active water movements in the lagoon.

1. Introduction

Particulate matter plays a major role in the cycling of organic substances in the sea. In particular, sinking particulate matter collected by sediment traps are responsible for marine biological processes; produciton, transport and decomposition of organic matter. Although most of downward transport of materials are from the upper productive layers in the oceanic regions (*e.g.*, SUESS, 1981), in coastal shallow waters, there are various particle sources other than surface-derived particles, such as terrestrial or resuspended particles from the bottom.

Sterols have been used as biological markers for the sources and alterations of particulate organic matter, since the compositions are specific as to the sources (GAGOSIAN *et al.*, 1983; WAKEHAM and CANUEL, 1988) and sterols are relatively refractory against biodegradation in lipids (*e.g.*, KOYAMA *et al.*, 1979). Sterols represent one of the important membrane lipids, and act as regulators of metabolic processes in all plants and animals.

We report here the preliminary results on the variability of particulate fluxes and

* Present address: Department of Biotechnology, Senshu University of Ishinomaki, Ishinomaki 986.

the sterol compositions of a sinking particles collected using a time-series sediment trap. The field experiment was conducted in lagoon Saroma Ko throughout the winter season.

2. Meterials and Methods

Twelve samples were collected at a sampling interval of 12 days using a time-series sediment trap between 9 December 1986 and 2 May 1987 (Table 1). The trap was deployed at Station A off Toetoko in lagoon Saroma Ko (see Fig. 1 in SATOH *et al.*, 1989), and was set at a depth of 3–4 m (3 m above the bottom). The basic configuration of the trap (50 cm in mouth diameter) was described elsewhere (FUKUCHI *et al.*, 1988). To minimize losses due to decomposition of organic particles, formalin solution (*ca.* 10%) was added into the sample bottles of the trap before the deployment.

All trap samples were divided into several aliquots and used for the determinations of dry weight (DW), ash-free dry weight (AFDW), total lipids and sterols after large swimmers were removed. Each sample was filtered through a GF/C glass fiber filter. According to the procedure as described by FOLCH *et al.* (1957), an aliquot of filtered samples was extracted with chloroform: methanol (2 : 1, *v* : *v*). Lipid extracts were saponified with 2N alcoholic KOH. Non-saponifiable lipids were fractionated by silica gel thin layer chromatography developed with mixtures of hexane : diethylether : acetic acid (70 : 30 : 1, *v*:*v*:*v*). Sterols were eluted with diethylether and acetylated with acetic acid : pyridine (1 : 1, *v*:*v*) and analyzed by gas-liquid chromatography (GLC; Hitachi 163 with OV-1 silica capillary column, 25 m × 0.25 mm i.d.). Ten μl of sterol extract fraction was prepared from each sample and 1/10 aliquot was injected into GLC. The column was operated isothermally at 280°C. Sterol composition was expressed as FID (frame ionization detector) response.

Structural identification was based on retention times obtained by GLC and mass spectral data obtained by gas chromatography-mass spectrometry (GC-MS; JEOL HX-105 with OV-1 column). The helium gas flow was 1.5 m*l*/min. The sterol acetates were injected at 160°C and heated to 280°C at a rate of 4°C/min.

Table 1. Sampling interval of time-series sediment trap experiment made in lagoon Saroma Ko.

Sample No.	Opening date	Closing date
1	9 Dec. 1986	21 Dec. 1986
2	21 Dec. 1986	2 Jan. 1987
3	2 Jan. 1987	14 Jan. 1987
4	14 Jan. 1987	26 Jan. 1987
5	26 Jan. 1987	7 Feb. 1987
6	7 Feb. 1987	19 Feb. 1987
7	19 Feb. 1987	3 Mar. 1987
8	3 Mar. 1987	15 Mar. 1987
9	15 Mar. 1987	27 Mar. 1987
10	27 Mar. 1987	8 Apr. 1987
11	8 Apr. 1987	20 Apr. 1987
12	20 Apr. 1987	2 May 1987

3. Results

There is a marked difference between the fluxes in three components (Fig. 1). The dry weight (DW) fluxes of sample Nos. 1 and 2 were 1–4 g m^{-2} day^{-1} when the lagoon was still opened. During the ice-covered period (mid-January to mid-April), DW fluxes gradually decreased to 0.215 g $m^{-2}day^{-1}$ in mid-March (No. 8) and increased again to

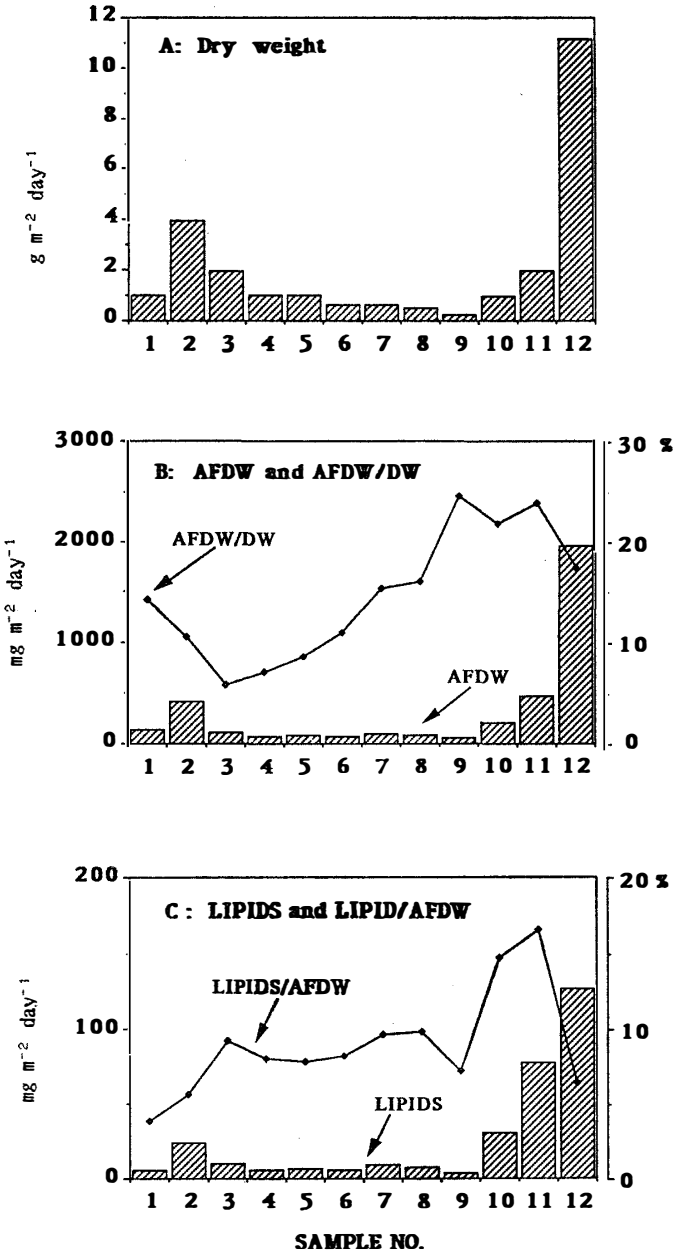

Fig. 1. Variabilities in fluxes of three sedimentary components. A: dry weight (DW) fluxes, B: ash-free dry weight (AFDW) fluxes and AFDW/DW ratios (organic matter content) expressed as percentages, C: lipid fluxes and lipid/AFDW ratios (lipid content) as percentages.

about 2 g m^{-2} day^{-1} in mid-April (No. 11). Since the lagoon was opened in mid-April (No. 12), the flux was higher than 10 g m^{-2} day^{-1}.

Flux variabilities both in particulate organic matter in terms of ash-free dry weight (AFDW) and in total lipids showed approximately same manners with that in DW (Fig. 1b and 1c). Highest values of the organic matter content (AFDW/DW) were obtained between 15 March and 20 April (Nos. 9–11). In the maximum flux (No. 12), organic matter content was about 17%, being less than the fluxes (22–25%) of three former periods (Nos. 9–11). Lipid contents (LIPID/AFDW) were higher between 27 March and 20 April (Nos. 10 and 11) than the other periods.

Typical gas chromatogram of the sterol (acetate) distributions (Nos. 5, 11 and 12) is shown in Fig. 2. The pattern of the distribution was similar to each other, but the peaks of most sterols of No. 12 were apparently higher than those of Nos. 5 and 11 (Fig. 2), suggesting the predominance in sterol abundance of No. 12. Although no quantitative determination of sterols is available, a temporal variability of total sterol

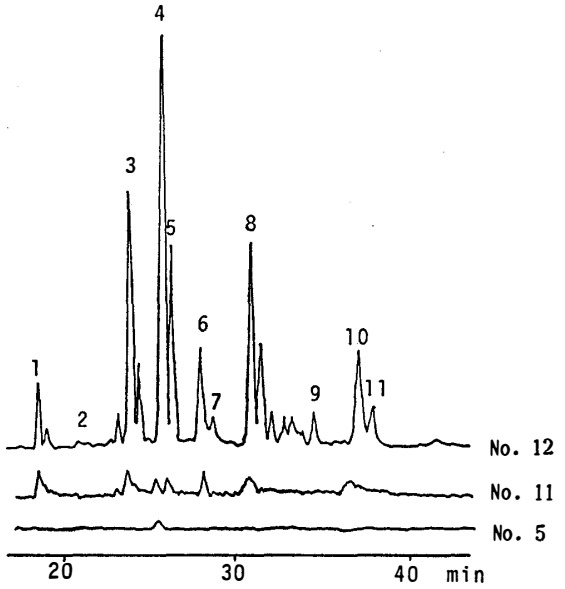

Fig. 2. Gas chromatograms of sterols (acetates) from sinking particles of sample Nos. 5, 11 and 12. Peak numbers refer to the structures in Table 2.

Table 2. Identifications of sterol acetates associated with sinking particles from sediment trap.

GC peak No.	Identification (trivial name)
1	24-norcholesta-5, 22E-dien-3β-ol (C_{26} sterol)
2	24-norcholest-22E-en-3β-ol
3	cholesta-5, 22E-dien-3β-ol (22-dehydrocholesterol)
4	cholest-5-en-3β-ol (cholesterol)
5	cholestan-3β-ol (cholestanol)
6	24-methylcholesta-5, 22E-dien-3β-ol (brassicasterol or crinosterol)
7	24-methylcholesta-22E-en-3β-ol
8	24-methylenecholest-5-en-3β-ol (24-methylenecholesterol)
9	24-ethylcholesta-5, 22E-dien-3β-ol (stigmasterol or poriferasterol)
10	24-ethylcholest-5-en-3β-ol (β-sitosterol)
11	24-ethylcholestan-3β-ol

Table 3. Major sterol distributions and relative abundances of sinking particles collected by time-series sediment trap in Saroma Ko. A trace (tr.) indicates that sterol was less than ca. 6 μg in a sample aliquot, and the value was expressed as percentage of FID response.

Sterols (%)	Sample No.											
	1	2	3	4	5	6	7	8	9	10	11	12
1	2.1	tr.									12.3	3.1
3	2.8	4.4						14.0			14.9	16.8
4	78.7	70.6	100.0	tr.	100.0	100.0	100.0	37.6	tr.	48.3	7.3	34.9
5	4.0	7.8	tr.				tr.	8.1		51.6	7.5	12.1
6	4.6	5.0						10.9		tr.	11.2	7.3
8	4.7	5.1						17.3		tr.	12.6	17.2
10	3.1	7.1						12.1		tr.	34.1	8.0

abundance associated with sinking particles is similar to that of total lipids.

The dominant sterols throughout the observation period were C_{26} sterol, 22 dehydrocholesterol, cholesterol, cholestanol, 24-methylcholesta-5, 22E-dien-3β-ol (brassicasterol or crinosterol), 24-methylenecholesterol and β-sitosterol (summarized in Table 2). Percentage compositions of the dominant sterols are shown in Table 3.

Before the lagoon was covered with ice (Nos. 1 and 2), cholesterol was the dominant sterol (>70%) and the other sterols were also detected. During the lagoon was covered with ice (Nos. 3–9), cholesterol was the only component except for No. 8, because trace amounts of sterols other than cholesterol were detected. In the stage of ice melting (Nos. 10 and 11), relative abundances of cholestanol (No. 10) and β-sitosterol (No. 11) increased. After the lagoon was opened, all kinds of sterols were obtained and cholesterol was most abundant (>30%).

4. Discussion

Time-series hydrographic and fluorescence data for the same station can be found in FUKUCHI *et al.* (1989). Lagoon surface was covered with ice on January 16, 1987, and opened on April 17, 1987. Chlorophyll *a* concentration in the water column below the ice was less than 1 mg m^{-3} under cold water temperature (−1.5°C) untill mid-March, while the pigment concentration increased to 4–5 mg m^{-3} in warmer waters (>0°C) during the spring bloom (mid-March to mid-April).

According to the mass flux data (Fig. 1), the downward particulate fluxes below the fast ice were low as compared with those in the periods without ice at the lagoon surface. On the other hand, the high relative contributions of organic matter and lipids in sinking particles were observed in the stage of ice melting, approximately coinciding with that of the phytoplankton bloom. The highest flux (No. 12) occurred after the bloom. This inconsistency between the maximum flux and the phytoplankton bloom suggests that the sedimentation of organic particles in the waters is responsible not only for marine biological activities but also for water movements (advection and mixing).

The major possible sources of organic material for sinking particles in lagoon Saroma Ko were fast-sinking large particles (*e.g.* fecal pellets of animals), slowly sinking

small particles (*e.g.* phytoplankton), resuspended sediment particles from the bottom, and land-derived particles.

Preliminary microscopic observation of sediment trap samples showed that there were denatured fece-like particles possibly derived from scallops which is known as one of the aquaculture products in lagoon Saroma Ko. The relative quantitative contribution of scallop feces in each sample appeared to be high in Nos. 10, 11 and 12.

GAGOSIAN *et al.* (1983) obtained sediment trap samples off the coast of Peru and revealed that an important feature of the sterol distribution of copepod fecal pellets, molts and carcasses is the predominance of cholesterol and 22-dehydro-cholesterol. In the present trap sample, a significant part of these sterols would come from animal-derived sources (*e.g.* zooplankton fecal pellets, carcasses, scallop feces).

C_{26} sterol obtained from the present trap samples (Nos. 1, 11 and 12) has been found in water samples taken in the euphotic zone as described by VOLKMAN *et al.* (1980) and GAGOSIAN *et al.* (1983). They suggested that the source of the sterol appeared to be phytoplakton and zooplankton.

YAMAGUCHI *et al.* (1986) reported that dominant sterols of three diatoms, *Skeletonema costatum*, *Chaetoceros gracilis* and *Thalassiosira decipience*, were brassicasterol, cholesterol, and 24-methylencholesterol, respectively. Among them, brassicasterol is known to be typical of diatom origin (*e.g.*, KANAZAWA *et al.*, 1971). Diatom sterols were observed in Nos. 11 and 12, reflecting notable imprint of diatom contribution.

Cholestanol is a transformed phase of cholesterol by microorganisms under anoxic conditions (reduction products by hydrogenation), and thus sediments contain relatively larger amount of cholestanol than fresh organisms and suspended particles (EYSSEN *et al.*, 1973; CRANWELL, 1988; SALIOT *et al.*, 1988). The cholestanol/cholesterol ratios of Nos. 1 and 2 (0.05–0.11) were similar to those of surface sediments in ice-covered Antarctic waters (0.09–0.12 from SMITH *et al.*, 1989), but were smaller than those of Nos. 10–12 (0.35–1.06). It is possibly considered that water movements in the periods of Nos. 10–12 were more active than those of Nos. 1 and 2, and thus cholestanol-rich deep sediments from the bottom were resuspended, because the ratio tends to increase with depth in sediments (NISHIMURA and KOYAMA, 1977).

The origin of β-sitosterol usually found in the ice-free periods is generally assigned to terrestrial higher plants (HEFTMAN, 1971; GOODWIN, 1981). Terrestrial sources including plant materials were easily transported into the lagoon with land waters during the ice-free period. It is also possible that waters from the land might also transport land-derived sources other than plants in this period. However, the present study did not show clear identifications of land-derived sterols such as coprostanol which was used as an indicator of man and domestic animal feces (MURTAUGH and BUNCH, 1967).

Before the lagoon froze in mid-January (Nos. 1 and 2), sterols in the traps were variable, and cholesterol dominated. All particle sources (phytoplankton-, animal-, resuspended sediment- and land-derived particles) would be included.

During the ice-covered period (Nos. 3–9), particulate fluxes in terms of DW, AFDW, total lipids and possibly total sterols remained low. Sterols other than cholesterol were rarely detected except for No. 8, primarily due to low abundances of total sterols. The ice would prevent the water movements in the water column from wind stress during the winter. Under these conditions, small amounts of bottom sediment-

or land-derived sterols were obtained. Phytoplankton would not be induced to grow under low water temperature during this period. Thus animal-derived sources, represented by cholesterol, were most likely the prevailing component of sinking particles.

As to the apparent occurrence of sterols at No. 8 (3 March–15 March), there should be various particle sources, regardless of flux. In particular, phytoplankton-derived sterols (brassicasterol, crinosterol) and a terrestrial plant-derived sterol (β-sitosterol) occurred below the ice, nevertheless they were not found in both the former and latter periods (Nos. 7 and 9–10). Part of sinking particles of No. 8 possibly came from the Sea of Okhotsk through small paths, although no marked increase of phytoplankton in the same location during this period was noticed (FUKUCHI *et al.*, 1989). Another possible source material for the sudden occurrence of the sterols is fouling algae which were found at the trap (FUKUCHI *et al.*, 1989). The algae caused possible contaminations to the *in situ* fluorometer. The fouling algae might contribute to the particulate flux of No. 8 as contaminants, although we cannot determine the amount of the fouling algae to be trapped.

From mid-March to mid-April (Nos. 10 and 11), the phytoplankton density began to increase in the water column (SATO *et al.*, 1989; FUKUCHI *et al.*, 1989), the period of which coincided with that of the occurrence of phytoplankton-derived sterols associated with sinking particles (No. 11). In this period, variable particles were also collected primarily due to active water movements which would gradually be intensified as the ice melted.

Since the lagoon was opened, the increase of the particulate flux (No. 12) was substantially attributed to every pariticle source. A combination of phytoplankton and animal activities and active water movements resulted in the highest particle flux in this period.

The present result gives a preliminary information on particle sources and the temporal variabilities. The discussions described above are only on a relative basis. It is difficult to quantify the amount of autochthonous (phytoplankton- or animal-derived) *vs.* allochthonous (resuspended or land-derived) materials. Although sterols represented a minor component of organic particles, some of possible particle sources were identified. For example, sterol compositions showed the occurrence of algal particles during the ice-covered period (Nos. 8, 10 and 11), when no marked flux increase was found. Thus sterols will be useful as biological markers of sediment trap samples.

To expand sample coverage of particles in and around Saroma Ko is clearly needed to understand more fully the relationships between the various sinking particle sources such as various organisms, bottom sediments, suspended particles and various terrestrial sources.

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