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A comparison of microalgal fatty acids between winter and summer in Lake Saroma, Hokkaido*

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Abstract: Fatty acid compositions of planktonic and benthic microalgal assemblages, which serve essential food for such maricultured shellfish as scallops and oysters, collected from a station in Lake Saroma were analyzed. Fatty acid compositions of planktonic microalgae in winter and summer showed considerable variations. Shorter chain fatty acids such as 14:0 accounted for more than 30% of the total lipid in summer but less than 13% in winter. Unstaturated fatty acid, 16:3n-3, occupied 9.5 and 2.5% of the total lipids in winter and summer, respectively. Long chain unsaturated fatty acid, 20:5n-3, represented more than 12% of the total lipids in winter but less than 5% in summer. In winter, fatty acid profiles of planktonic and benthic microalgae were similar, except that the latter contained lower levels of 16:3n-3 and 16:3n-6 and higher levels of 16:0 and 22:6n-3. The present study demonstrates that fatty acid compositions of microalgal assemblages are significantly influenced by their habitat and/or season.

1. Introduction

Lake Saroma in Hokkaido, Japan, is a lagoon of seawater flowing in through the two channeles from the Sea of Okhotsk, and is known as the southernmost area of seasonal sea ice distribution in the northern hemisphere. Shellfish such as scallops and oysters are widely cultured in this lagoon. In midwinter, when the lake is covered by sea ice, a considerable proliferation of benthic microalgae is observed on the culture system and/or the drifting fishing net which have been set near the lake bottom. Such benthic microalgae as well as phytoplankton serve the diets for filter feeding shellfish during winter in the lake. The nutrition of maricultured shellfish might be influenced by difference in chemical compositon of their diets (LANGDON and WALDOCK, 1981).

Among organic constituents in microalgae, lipids have been regarded primarily as the source of energy and their chemical compositions have been suggested as more critical than those of protein and carbohydrate for optimal growth of herbivorous animals (HOLLAND, 1978). It is well known that most marine animals require essential fatty acid (EFA) (LANGDON and WALDOCK, 1981; WATANABE, 1982).

Composition of lipids, especially fatty acid, of microalgae is strongly influenced by their environmental conditions such as water temperature, salinity, energy source (light or organic carbon) and dissolved oxygen (e.g. ACKMAN and TOCHER, 1968; COHEN *et al.*, 1988). Among these environmental parameters, considerable seasonal difference of water temperature is most prominent in Lake Saroma (SATOH *et al.*, 1989).

From this point of view, a comparative

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Table 1. Conditions of Lake Saroma on 27 February and 28 August 1991 at the sampling site.

	27 February	28 August
Solar radiation(μE m^{-2} s^{-1})*	1421	1807
Water temperature (℃)	−1.4～+0.3	19.5～20.0
Salinity	31.2～32.0	33.6～33.9
Chl.*a* (mg m^{-3})	2.47	2.95
POC (mgC m^{-3})	230	452
Chl.*a*／(chl.*a*+pheopigments)	0.90	0.81
POC／chl. *a*	93.1	153.2

* Measured at local noon under clear sky.

study was conducted on the fatty acid composition of microalgal samples harvested both during winter and summer in Lake Saroma.

2. Materials and methods

2. 1 Samples

Samples analyzed in the present study were collected at the site off Toetoko Fisheries Port (44° 10′ N, 143° 46′ E) in Lake Saroma, Hokkaido, Japan. Benthic microalgal samples were scraped off form the drifting net, which had been set several days before, into filtered seawater in late February of 1990 and 1991. After removing macroscopic particles using forceps, suspended samples were filtered through XX13 plankton net (mesh size, 0.095 mm), and the samples retained on the plankton net were kept at −20 ℃ in a deep freezer. In late February and August of 1991, phytoplankton samples were collected by towing XX13 plankton net from a fishing boat.

2. 2 Chemical analyses

Proximate and lipid compositions of samples were analyzed by the methods described in TAKEUCHI *et al.* (1989). The content of crude protein (N × 6.25) was determined by the Kjeldahl method. Extraction of lipids was carried out using the mixture of chloroform and methanol (2:1 by volume) as described by FOLCH *et al.* (1975). Polar and neutral lipid fractions were separated by silica acid column chromatography (Sep-Pak). Each lipid classes in polar and neutral fractions were analyzed by using an Iatroscan TLC／FID (TH-10, Iatron Laboratory Inc., Japan). Fatty acid profiles of lipid were determined by gas-liquid chromatography. Crude starch was determined by the procedure of Somogi-Nelson. The values of protein, lipid and starch were expressed on a basis of dry weight of algae.

3. Results and Discussion

3. 1 Oceanographic conditions and the dominant species of microalgae

Oceanographic conditions at the sampling site are shown in Table 1. Although Lake Saroma is usually iced over from mid-January to early April, the ice cover did not extend to the whole lake surface in winters of 1990 and 1991. Water temperature and salinity ranged −1.4 ～+0.3℃ and 31.2 ～32.0 in late February and 19.5 ～20℃ and 33.6 ～ 33.9 in late August, respectively. The difference of water temperature between winter and summer was considerably large. The solar radiation at the lake surface at around noon on clear day was about 1400 μE m^{-2} s^{-1} in winter and 1800 μE m^{-2} s^{-1} in summer. The light level at the lake bottom, where benthic microalgal bloom was formed, was less than 20 % of the incident solar radiation. Thus, the proliferation of benthic microalgae occurred under relatively weak light.

The dominant species of benthic microalgae in winter was the pennate diatom *Nitzschia*

frigida and the centric diatom *Odontella* sp. *Nitzschia frigida* with cell size as long as approximately 300 μm formed stellate colonies. The dominant species of planktonic microalgae in winter and summer was *Nitzschia* spp. and *Thalassiosira* spp., respectively.

3. 2 Proximate and lipid compositions of benthic microalgae

The proximate compositons of benthic microalgae are indicated in Table 2. In organic basis, crude protein, crude starch (containing polysaccharide) and crude lipid were 13.5, 69.5 and 17.0%, respectively. The values for crude starch and lipid were almost the same as those of the ice alga, *Navicula glaciei*, in the Antarctic region (WHITAKER and RICHARDSON, 1980). The content of crude protein, 13.5% in the present study, was lower than those in diatoms (30~52%, MYKLESTAD, 1974). The chemical composition of microalgae is controlled largely by interplay between the supply of energy through photosynthesis and the synthetic capacity of cells (SHUTER, 1979). The benthic microalgae living under low temperature condition (e.g. in ice-covered lakes) display lower metabolic rates simply as a consequence of physical effects of low temperature on enzymes and metabolic reactions (MCCONVILLE *et al.*, 1985), which will result in compositions of crude starch and lipid similar to those of ice algae. Compositons of neutral lipids, polar lipids and other lipids in benthic microalgae are shown in Table 3. The neutral lipids occupied more than 60 % of the total lipids and this fraction was dominated by free fatty acid, triglyceride s and cholesterol esters. The percentages of lipid components obtained were at the same levels as those in diatoms (ORCUTT and PATTERSON, 1975).

Compositions of the total lipids, free fatty acids and polar lipids are summarized in Table 4. In the total lipids, such fatty acids as 14:0, 16:1n-7, 16:3n-6, 16:3n-3 and 20:5n-3 dominated (more than 10%). In free fatty acids 16:0, 16:1-7 and 20:5n-3 were dominant. Contrarily, in polar lipids, 14:0, 16:0 and 16:3n-3 were dominant. Comparing the fatty acid compositions of total lipids in benthic microalgae to theose in ice algae reported by WHITAKER and RICHARDSON (1980), the percentages of 14:0 and 16:3n-3 were rather high in benthic microalgae. The predominance of 22:6n-3 was similar to that typically found in other diatom species (CHUECAS and RILEY 1969, ORCUTT and PATTERSON 1975). The sum of n-3 fatty acids in the present study was higher than those in cultured diatoms (OLSEN, 1989).

Table 2. Proximate compositions of benthic microalgae in late February 1990.

	Dry basis(%)	Organic basis(%)*
Crude protein	5.3	13.5
Crude starch	27.2	69.5
Crude lipid	6.7	17.0
Crude ash	60.8	–

* Except for crude ash.

Table 3. Content of lipid classes in benthic microalgae (on crude lipid basis).

	Lipids(%)
Neutral lipids	62.7
Cholesterol esters	3.8
Triglycerides	4.9
Free fatty acids	51.0
Free sterols	1.0
Diglycerides	1.0
Monoglycerides	1.0
Polar lipids	20.6
Phosphatidyl ethanolamine*	20.6
Sphingomyelin	tr**
Others	16.7

* Lysophosphatidyl ethanolamine is included.
** Trace.

3.3 Fatty acid compositions of phytoplankton in winter and summer

The fatty acid compositions of phytoplanckton in late February and in late August 1991 are shown in Table 5. The major fatty acids were saturated(14:0, 16:0), monounsaturated (16:1n-7) and polyunsaturated (16 : 3n-3, 20 : 5n-3) ones (more than 60% of the total fatty acids) in both seasons. The content of n-3

Table 4. Fatty acid compositions of benthic microalgae in February 1990 (area %).

Fatty acid	Total lipids	Free fatty acids	Polar lipids
14:0	13.36	9.14	16.25
15:0	0.30	0.39	0.49
16:0	6.29	12.52	13.13
16:1n-7	14.85	17.26	10.48
16:1n-5	0.27	0.28	tr*
16:2n-7	6.08	3.73	5.83
16:3n-6	16.11	7.92	9.65
16:3n-3	21.64	8.07	15.19
17:0	0.13	0.12	0.63
18:0	0.21	0.69	1.78
18:1n-(9+7)	1.35	3.35	2.84
18:1n-5	tr*	0.65	0.11
18:2n-4	0.13	0.05	tr*
18:2n-6	0.29	0.79	0.67
18:3n-6	0.22	0.30	0.53
18:3n-3	0.18	0.21	0.11
18:4n-3	1.68	1.10	2.24
20:0	0.08	tr*	0.42
20:2n-9	0.12	0.12	0.21
20:4n-6	0.11	0.22	0.62
20:4n-3	0.03	0.18	0.29
20:5n-3	11.80	24.42	7.69
22:1n-9	tr*	0.14	0.17
22:5n-3	tr*	tr*	0.24
22:6n-3	0.43	1.80	2.56
Σn-3	35.79	35.78	28.32
Σn-6	16.73	9.23	11.47
Σn-3HUFA**	12.29	26.40	10.78

* Trace.
** Highly unsaturated fatty acids, containing more than C_{20} with n-3 fatty acids.

group in winter was remarkably high compared with that in summer. In n-3 group, 20:5n-3 accounted for more than 12% of the total in winter, while less than 5% in summer. According to ACKMAN and TOCHER (1968), the content of 20:5n-3 was higher under low temperature (10℃) than under higher temperature (20℃) in the cultured chrysophycean alga, *Monochrysis lutheri*. Most species of diatoms contained the greatest proportion of 20:5n-3 under low levels of irradiance (THOMPSON *et al.*, 1990). OLSEN (1989) found an increase in n-3 highly unsaturated fatty acids (n-3HUFA) content (20:5n-3 and 22:6n-3) in *Isochrysis galbana* with decreasing light intensity in this culture. Considering these facts, it is most likely that the difference of 20:5n-3 between winter and summer phytoplankton cells is due to the differences of water temperature and light condition in their habitat. Biosynthesis of n-3HUFA is considered to occur only in cells of marine algae (POHL, 1982). Although

Table 5. Fatty acid compositions of the total lipid in planktonic microalgae in late February and late August 1991 (area %).

Fatty acid	Winter	Summer
14:0	12.99	34.05
14:1	0.22	0.10
15:1	0.88	1.01
16:0	12.67	15.89
16:1n-7	17.31	20.42
16:2n-7	4.25	tr*
16:3n-6	4.30	2.14
16:3n-3	9.47	2.45
17:0	0.17	0.12
18:0	1.76	1.00
18:1n-(9+7)	4.86	2.02
18:1n-5	0.13	tr*
18:2n-6	1.64	0.92
18:3n-6	0.14	0.11
18:3n-3	0.85	0.98
18:4n-3	3.97	1.88
20:0	1.05	tr*
20:1	0.30	0.16
20:2n-6	1.73	0.70
20:3n-3	tr*	0.92
20:4n-3	0.23	0.13
20:5n-3	12.29	4.19
22:1	0.23	tr*
22:5n-3	0.25	tr*
22:6n-3	1.55	1.59
Σn-3	28.61	11.94
Σn-6	7.96	4.05
Σmonoenes	23.10	22.70
Σn-3HUFA**	14.32	6.83

* Trace.
** Highly unsaturated fatty acids, containing more than C_{20} with n-3 fatty acids.

the mechanism by which n-3HUFA are formed in marine algae is not yet clear, the importance of n-3HUFA for human health and for aquacultured fish or shellfish has recently been recognized (e.g. WATANABE, 1982; LEAF and WEBER, 1988). Thus, the supply of n-3HUFA to maricultured shellfish during winter by benthic and planktonic microalgae is thought to be very important. High content of n-3HUFA in the total lipids analyzed in scallops and oysters from Lake Saroma (SATOH *et al.*, unpubl. data) could be originated from their diets, i.e. planktonic and benthic microalgae which contained high n-3HUFA in winter.

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サロマ湖における冬季および夏季の微細藻類の脂肪酸の比較

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要旨：サロマ湖ではホタテガイやカキが広範囲に養殖されている。これら貝類の餌料として，冬季には植物プランクトンや底生微細藻類が，また夏季には植物プランクトンが重要な供給源となっていると考えられる。これら微細藻類の一般組成および脂肪酸組成を調べて餌料価値を検討したところ，冬季と夏季の植物プランクトンの脂肪酸組成に大きな違いが認められた。すなわち，夏季には 14:0 が脂質含量の 30%以上を占めるが，冬季には 13%以下であったのに対して，不飽和脂肪酸である 16:3n−3 および 20:5n−3 の含有率はそれぞれ冬季には9.5 および12%，夏季には2.5および4.2%であった。また，冬季の底生微細藻類および植物プランクトンの脂肪酸組成の比較から，底生微細藻類では 16:3n−3 および 16:3n−6 の含有率が高く，植物プランクトンでは 16:0 および 22:6n−3 の含有率が高かいことが明らかとなった。これら脂肪酸組成の相違は，生息環境や季節に大きく依存していることが示唆された。