

Studies on the Lipids of Coastal Waters

I. General Composition of Lipids in Sea Water, Sediments and Suspensoids

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(Figs. 1-7, Tables 1-6)

It has been known that many kinds of organic compounds are dissolved in sea water^{1)~3)}, and also recognized that the content of organic compounds increases around the eutrophicated area^{4),5)}. Since it is not necessarily obvious what kinds of organic compounds exist, and how they are decomposed in a marine environment, the studies on general composition and decomposing pathways of these compounds would be useful for the workers who engage in the water control.

In recent years, oil pollution caused by spilling of crude oil and oil products has become the serious problems especially at the coastal regions. Therefore, in order to estimate whether the environment is polluted by oil or not and to make clear the fate of lipids in general, the measurement of amounts and characterization of components of lipids, which occur in the marine environment, are an important investigation. This paper deals with the general composition of lipids extracted from the coastal sea water, sediments and suspensoids at the central part of the Seto Inland Sea in summer of 1973 and winter of 1974.

EXPERIMENTALS

Survey: The surveys were carried out during 21st - 28th, Aug., 1973 and 22nd - 24th, Jan., 1974. The samples were collected at 17 stations in Hiuchi Nada as shown in Fig. 3.

Sampling of sea water, sediments and suspensoids: Surface sea water was collected with a bucket and bottom sea water was collected by using a Van Dorn type water sampler. Both water samples were filtered immediately after sampling on board with a Millipore HA filter (pore size 0.45μ) to exclude suspensoids, and stored after adjusting their pH to 1-2 by adding 2-3 ml of 6 N HCl.

Sediments were collected with Eckman-Berge dredge from the surface of mud and stored at -20°C .

Suspensoids were collected from the surface and bottom water samples at stations 3 and 13 at low tide and high tide during the survey of 1973. Sea water was collected with a bucket or Van Dorn type water sampler as in the case of sea water, and for the first time, the water was filtered with a plankton net (129 mesh) to remove phytoplankton and zooplankton. Then the filtrate was filtered with a glass fiber filter (Whatman GF/F, previously ignited for 2 hr. at 450°C) to gather the suspensoids.

Extraction of lipids: The procedures of extraction and of analysis of lipids are shown in Fig. 1 and 2. Prior to the extraction, approximately 2 l of sea water were condensed to 0.8 l with a rotary evaporator below 45°C . The lipids were extracted from the condensed sea water with chloroform. Lipids of suspensoids and sediments were extracted with chloroform - methanol (1 : 1, v/v) directly.

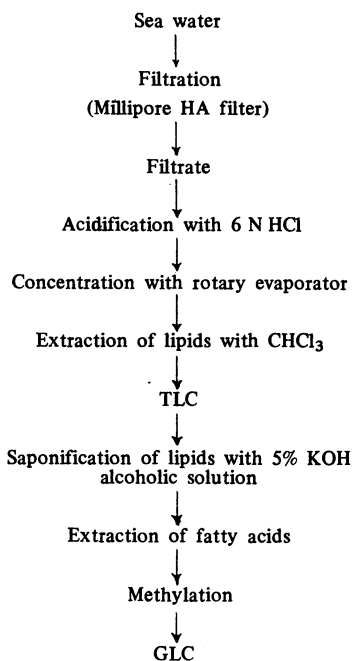


Fig. 1. The procedure for the analysis of lipids extracted from sea water.

The resulted lipids were saponified with 5 % KOH alcoholic solution, and the fatty acids were extracted three times with diethyl ether and methylated with diazomethane in ether.

Thin-layer chromatography: A silica gel G plate (0.25 mm in thickness) was used, and developed with the solvent system of petroleum ether - diethyl ether - acetic acid (87.5 : 12.5 : 1, v/v/v), and detected by charring after spraying 50 % H_2SO_4 .

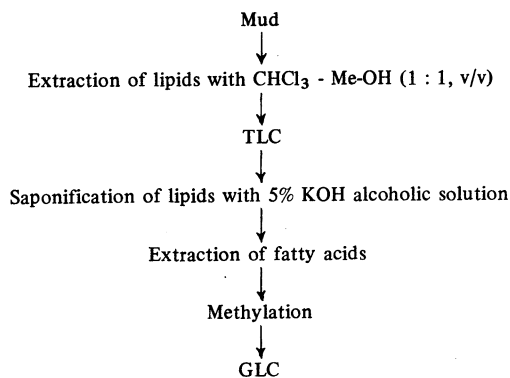


Fig. 2. The procedure for the analysis of lipids extracted from sediments.

Gas-liquid chromatography: A Hitachi gas-liquid chromatograph (Model 073) was used. The column was 3 m in length, 3 mm in internal diameter, packed with 25% DEGS on Chromosorb W, 60 to 80 mesh, acid washed and siliconized. The column temperature was at 185°C, and injector and detector was at 200°C. The carrier gas (N_2) flow rate was 50 ml/min. The detector was a flame ionization detector.

RESULTS

Lipid content: The distribution of the lipid content at each station is shown in Figs. 3, 4 and 5. The lipid contents of sea water and sediments were higher at the

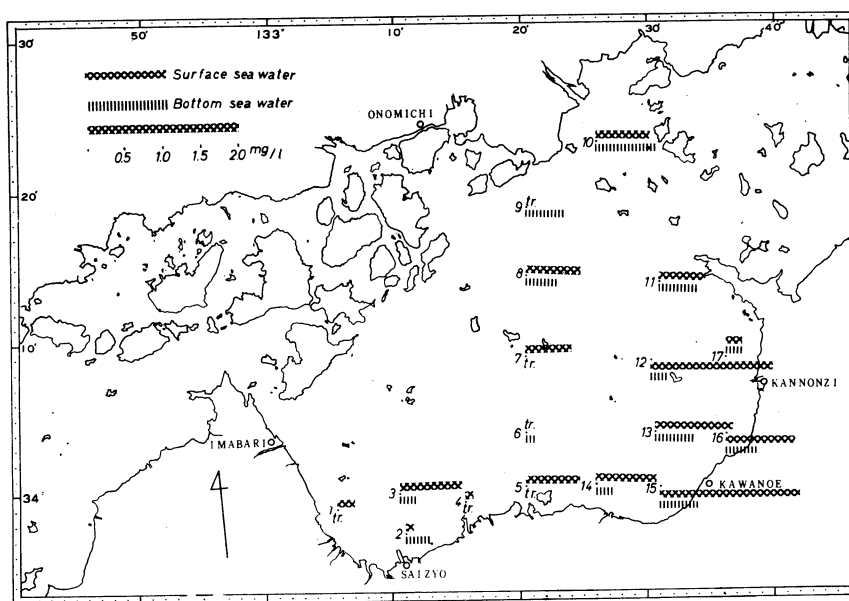


Fig. 3. The geographical distribution of lipids extracted from sea water. (Aug., 1973)

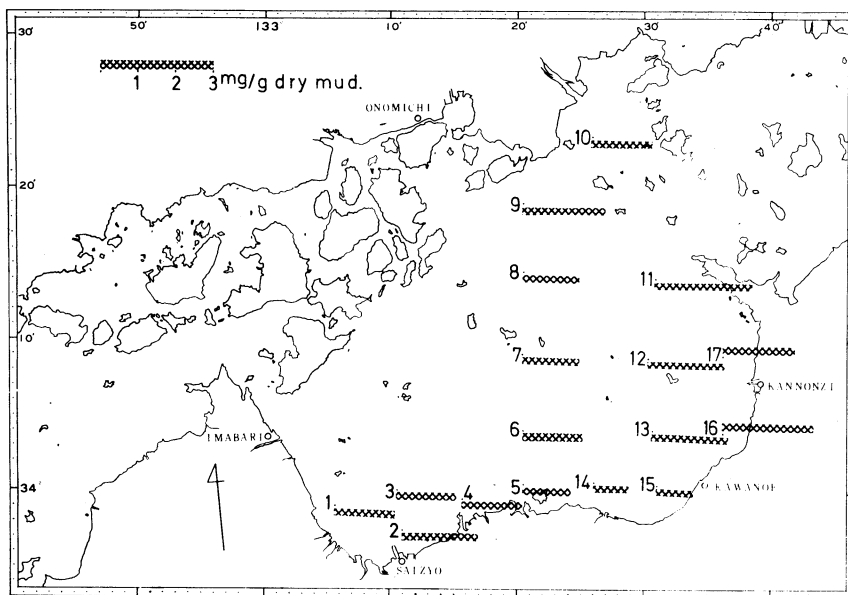


Fig. 4. The geographical distribution of lipids extracted from the sediments. (Aug., 1973)

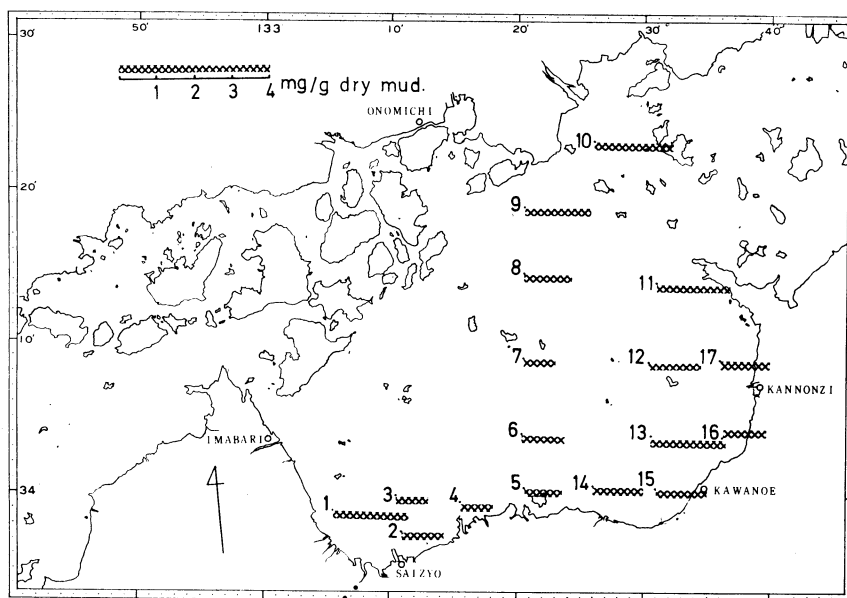


Fig. 5. The geographical distribution of lipids extracted from the sediments. (Jan., 1974)

coastal area than at the central part of this area, and especially high at the eastern part, off Kawano and Kannonzi. Although this tendency was almost the same in both summer and winter, the lipid content seemed to decrease in winter.

The lipid content of suspensoids is shown in Table 1. It is obvious from this

result that the composition of suspensoids obtained at stations 3 and 13 is different, and that the lipid content is independent of the level of tide.

Thin-layer chromatography: Thin-layer chromatogram and the percentage composition of the lipid class are shown in Fig. 6 and Table 2. Although triglycerides were a major component and sterol esters were detected in the lipids extracted from the muscle of flat fish, the former component was found in small amounts and the latter

Table 1. The lipid content of suspensoids. (Aug. 24 & 27, 1973)

St. No.	Time	Depth (m)	Weight of suspensoids (mg)	Weight of lipids (mg)	Lipid content (%)
3	13 ^h 30 ^m	0	28.5	1.2	4.21
		14	49.5	0.7	1.41
	20 ^h 40 ^m	0	50.9	2.2	4.34
		13	37.8	0.7	1.85
13	10 ^h 10 ^m	0	35.4	0.5	1.41
		21	32.6	0.7	2.15
	16 ^h 30 ^m	0	26.0	0.6	2.31
		18	30.5	0.7	2.30

Table 2. The class composition of lipids extracted from sea water, suspensoids, sediments and muscle of flat fish.

Lipids extracted from	Lipid class in %						
	Hydrocarbons	Sterol esters	Triglycerides	Free fatty acids	Free alcohols	Sterols	Polar lipids
Surface sea water	38.2		3.2	14.2	10.8	8.9	24.7
Bottom sea water	34.0		2.1	15.6	13.2	9.6	25.5
Surface suspensoids	39.0		9.2	12.9	11.2	10.2	17.6
Bottom suspensoids	38.4		8.6	20.3	9.2	10.0	13.5
Sediments	28.3		8.2	20.5	12.4	9.9	20.5
Muscle of flat fish		9.3	36.7	21.4		15.3	17.2

Thin-layer chromatoplates of Silica gel G were developed with the solvent system of petroleum ether - diethyl ether - acetic acid (87.5 : 12.5 : 1, v/v/v). The resolved lipid classes were charred and quantified by densitometry.

component was not detected in the lipids of sea water, suspensoids and sediment⁶⁾. Class comparison of lipids extracted from the organisms and its raising sea water is shown in Table 3. It is obvious that the esterified compounds are decreased, and free fatty acids and free alcohols increase in a raising sea water. Triglycerides are detected in the sea water sample obtained in summer. However, triglycerides are not detected in the winter sample and polar lipids are major components. It is also characteristic pattern for sea water, suspensoids and sediments that free fatty acids and free alcohols are found in relatively large amounts.

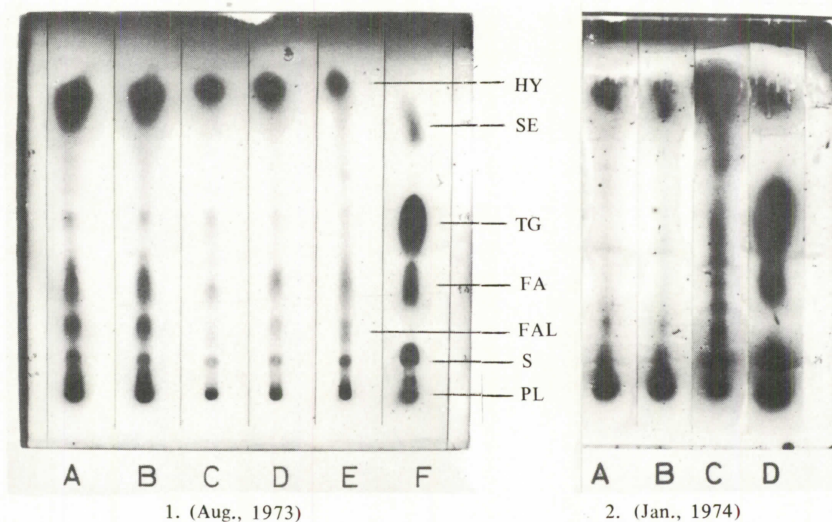


Fig. 6. The thin-layer chromatograms of the lipids extracted from sea water, sediments, suspensoids and muscle of flat fish.
 1-A, Surface sea water; 1-B, Bottom sea water; 1-C, Surface suspensoids; 1-D, Bottom suspensoids; 1-E, Sediments; 1-F, Muscle of flat fish; 2-A, Surface sea water; 2-B, Bottom sea water; 2-C, Sediments; 2-D, Muscle of flat fish.
 HY, Hydrocarbons; SE, Sterol esters; TG, Triglycerides; FA, Free fatty acids; FAL, Free alcohols; S, Sterols; PL, Polar lipids.

Table 3. Class comparison of lipids extracted from the organisms and its raising sea water.

Specimen	Sea anemone <i>Anthopleura asiatica</i>		Starfish <i>Asterias amurensis</i>		Laver <i>Porphyra</i> sp.	
	Organism 3.3 % (wet)	Water 1.9 mg/l	Organism 6.0 % (wet)	Water 22 mg/l	Organism 0.7 % (wet)	Water 15.3 mg/l
Raising day	20 (living)		6 (dead)		10 (living) Org., dark	
Lipid content						
Lipid class in %						
Hydrocarbons	1.4	11.0	tr.		10.2	12.9
Sterol esters	} 34.3	} 7.3	} 7.3	} 12.7	} 5.4	} 8.6
Wax esters						
Diacyl glyceryl ethers	13.3	2.2	12.8	tr.	-	-
Triglycerides	21.5	10.6	50.0	tr.	21.2	} 36.4
Free fatty acids	13.4	12.8	13.5	21.4	23.5	
Free alcohols	2.1	5.1	6.3	42.1	6.6	9.3
Free sterols	9.0	28.5	4.3	10.3	7.2	12.6
Partial glycerides	1.7	9.1	2.8	10.3	15.1	10.0
Phospholipids (Polar lipids)	3.5	13.5	3.0	3.2	10.8	10.2

Thin-layer chromatoplates of Silica gel G were developed with the solvent system of petroleum ether - diethyl ether - acetic acid (87.5 : 12.5 : 1, v/v/v). The resolved lipid classes were charred and quantified by densitometry.

Table 4. The percentage composition of fatty acids of sea water, sediments and muscle of flat fish (Aug., 1973).

Fatty acid	Surface sea water			Botton sea water			Sediments			Muscle of flat fish
	St.5	St.8	St.15	St.5	St.8	St.15	St.5	St.8	St.15	
C _{12:0}	1.75	4.79	2.01	7.24	4.86	8.21	2.20	2.40	2.98	tr.*
C _{12:1}	0.94	0.98	0.60	2.28	1.57	2.23				
C _{13:0}	0.25	0.27	0.20	0.54	0.43	0.46	0.98	1.69	1.93	
C _{13:1}	1.13	1.31	1.40	1.61	2.14	2.11	1.61	1.18	1.72	
C _{14:0}	6.39	9.00	9.02	9.23	10.95	11.50	8.89	8.04	8.67	1.89
C _{14:1}	0.75	2.00	2.51	2.68	2.29	2.50	10.20	7.21	8.78	
C _{15:0}	2.26	2.36	2.01	2.58	4.28	3.15	4.23	5.34	4.93	0.95
C _{15:1}	0.50	0.98	1.00	1.18	1.33	1.39				
C _{16:0}	27.08	25.94	24.67	23.99	32.33	28.39	20.73	20.72	20.29	22.09
C _{16:1}	6.14	7.55	8.82	8.66	7.71	7.14	22.11	8.55	16.37	5.06
C _{17:0}	2.26	1.16	2.13	2.15	tr.	1.77	1.76	2.85	1.34	1.27
C _{17:1}	4.01	1.81	2.51	1.45	3.14	1.46	2.28	4.02	2.03	0.73
C _{18:0}	9.40	11.89	11.58	6.98	12.00	7.89	2.42	4.27	3.34	7.06
C _{18:1}	11.85	14.99	11.73	8.86	6.67	6.63	13.50	17.34	14.77	13.77
C _{18:2}	3.38	1.29	2.28				1.11	1.34	2.55	1.38
C _{18:4}	0.94						0.77			
C _{19:0}	1.25	0.15								
C _{19:1}								0.53		
C _{20:0}	4.51	2.32	6.87	8.64	3.64	5.28	0.73	1.36	1.02	
C _{20:1}								1.47	0.79	2.67
C _{20:2}							0.56	1.56	tr.	tr.
C _{20:3}	3.38	4.06	2.81	2.33	2.74	3.34		1.29	2.49	
C _{20:4}			1.88				1.47	3.62	1.36	
C _{20:5}	4.14	2.90	4.51	5.13	tr.	1.15				14.27
C _{22:0}							1.25			
C _{22:1}										2.61
C _{22:3}							1.75	2.54	4.22	
C _{22:5}										3.08
C _{22:6}										23.16
N.I.P.**	7.68	4.25	2.46	4.46	3.93	5.39	1.47	2.68	0.71	
S.F.A.*	55.15	57.88	57.49	61.35	68.49	66.65	43.19	46.67	44.50	33.26
U.F.A.**	37.16	37.87	40.05	34.18	27.59	27.95	55.36	50.65	55.08	66.73

* tr.: Trace

** N.I.P.: Not identified peak

* S.F.A.: Saturated fatty acids

** U.F.A.: Unsaturated fatty acids

The composition of fatty acids: The fatty acid compositions of sea water and sediment are shown in Table 4 and 6. Myristic and palmitic acids were major components in all samples, and palmitoleic acid was found in large amounts in the sediments. Oleic acid was also a main component in all samples except bottom sea water obtained in January. Stearic acid was not a dominant component in the sediments, but its amount was relatively large in sea water. The amount of palmitoleic acid extracted from sea water was larger in August than in January. The fatty acid composition of suspensoids

(Table 5) was characterized by a high percentage of palmitic acid (22–46%). Myristic, palmitic, stearic and oleic acids were also detected as major components, and arachidic acid was found in relatively large amounts.

Comparing the fatty acids extracted from sea water, sediments and suspensoids with that of flat fish muscle, it is obvious that the polyunsaturated fatty acids are not detected abundantly in the former, and that the amount of short chain fatty acids of the former is greater than that of the latter⁷⁾.

Table 5. The percentage composition of fatty acids extracted from suspensoids.

Fatty acid	St. 3				St. 13			
	13 ^h 30 ^m		20 ^h 40 ^m		10 ^h 10 ^m		16 ^h 30 ^m	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
C _{12:0}	0.85	0.96	0.91	1.79	1.10	1.15	1.87	1.92
C _{12:1}	0.28	0.70	0.77	1.74	0.44	1.15	1.31	1.44
C _{12:2}			0.43	0.80		0.96	0.94	
C _{13:0}	tr.*	tr.	0.21	tr.	0.03	0.16	0.28	tr.
C _{13:1}	0.71	0.64	0.77	1.35	0.81	tr.	2.19	2.40
C _{13:2}			0.51	1.50			1.12	
C _{14:0}	8.24	7.50	7.80	6.63	11.85	8.43	17.74	8.81
C _{14:1}	1.52	2.00	1.29	2.49	0.70	2.74	2.81	3.08
C _{15:0}	1.30	1.61	1.50	1.99	1.19	3.01	1.87	2.20
C _{15:1}		0.32	0.43	2.29	tr.	2.19	tr.	1.12
C _{16:0}	45.77	40.26	38.97	22.13	40.03	30.23	23.61	29.73
C _{16:1}	12.37	11.15	10.61	12.46	10.68	11.83	9.28	10.94
C _{17:0}			1.03	2.69	tr.	1.73	1.69	tr.
C _{17:1}	1.18	1.18	2.57	3.49	1.47	3.50	3.00	2.80
C _{18:0}	9.10	8.79	7.54	10.14	9.15	13.55	10.87	13.54
C _{18:1}	6.40	7.43	6.19	7.98	7.05	9.86	8.49	10.58
C _{18:2}	0.71		1.09		2.57	1.86	1.62	
C _{19:0}		tr.	1.29					tr.
C _{20:0}	5.64	10.29	4.63	16.04	7.33	5.72	6.18	6.13
C _{20:3}		1.79	4.50					
C _{20:5}	2.13	1.98	1.29	4.36	1.88	1.92	2.62	2.40
N.I.P.**	3.79	3.40	5.66		3.74		2.50	2.88
S.F.A. ⁺	70.90	69.41	63.88	61.41	70.68	63.98	64.11	62.33
U.F.A. ⁺⁺	25.30	27.19	30.45	38.46	25.60	36.01	33.38	34.76

* tr: Trace

** N.I.P.: Not identified peak

⁺ S.F.A.: Saturated fatty acids

⁺⁺ U.F.A.: Unsaturated fatty acids

DISCUSSION

The distribution of lipid content shown in Fig. 3, 4 and 5 is coincident with that of dissolved organic carbon reported by Okaichi⁴⁾, namely the concentration of dissolved organic carbon is greater at the area off Kawano and Kannonzi. According to

Table 6. The percentage composition of fatty acids of sea water, sediments and muscle of flat fish (Jan., 1974).

Fatty acid	Surface sea water			Bottom sea water			Sediments			Muscle of flat fish
	St.5	St.8	St.10	St.5	St.8	St.10	St.5	St.8	St.10	
C _{12:0}	1.55	1.68	2.52	1.70	1.32	1.76	1.86	1.14	1.75	tr.*
C _{12:1}	0.23	0.30	0.66	2.21	0.44	0.14	0.77	0.31	0.28	
C _{12:2}	1.29	1.43	1.48	2.04	1.54	1.53	1.72	1.49	1.23	
C _{13:0}	0.46	0.30	0.33	0.34	0.29	0.56	0.86	0.56	0.74	
C _{13:1}			0.82							
C _{14:0}	8.25	8.46	8.38	10.86	9.24	11.02	8.71	8.42	9.56	1.89
C _{14:1}	1.32	1.07	1.53	1.36	1.65	1.30	10.81	13.08	9.46	
C _{14:2}	0.52	0.46	0.66	tr.	0.44	0.56	2.79	2.42	1.58	
C _{15:0}	4.01	3.81	3.94	3.73	3.63	5.42	5.50	5.20	5.25	0.95
C _{16:0}	27.73	27.81	32.24	35.13	35.77	37.32	18.96	18.36	19.58	22.09
C _{16:1}	4.21	1.98	3.07	2.04	1.66	5.56	22.88	25.05	25.01	5.06
C _{17:0}	1.72	1.24	1.10	1.02	0.61	1.39	1.72	1.98	1.40	1.27
C _{17:1}	2.29	1.43	2.63		1.92	1.67	2.99	2.99	3.31	0.73
C _{18:0}	9.45	10.50	11.50	13.17	13.10	10.44	1.54	1.49	1.40	7.06
C _{18:1}	7.43	8.20	4.57	2.62	3.38	6.25	12.01	12.21	12.60	13.77
C _{18:2}							2.06	1.42	2.80	1.38
C _{18:3}	0.70	3.00	2.85	2.73	1.93	1.01				
C _{19:0}	1.77	2.40	1.75	2.23	0.95	1.22				
C _{19:1}	0.51	0.69	0.77			0.35				
C _{19:3}							tr.	0.20	tr.	
C _{20:0}	2.99	3.62	1.97	2.82	2.34	1.76	1.34	0.87	0.39	
C _{20:1}							0.49	0.30	0.53	2.67
C _{20:2}										tr.
C _{20:5}							1.21	0.79	1.00	14.27
C _{22:0}	4.19	6.61	6.57	6.79	7.26	4.13	0.55	0.41	0.35	
C _{22:1}							1.15	1.30	1.78	2.61
C _{22:2}	9.76	15.02	10.67	9.16	12.52	6.64				
C _{22:3}	9.63									
C _{22:5}										3.07
C _{22:6}										23.16
S.F.A. ⁺	62.12	66.43	70.30	77.79	74.51	75.02	41.04	38.43	40.42	33.26
U.F.A. ⁺⁺	37.89	33.58	29.71	22.16	25.48	25.01	59.03	61.56	59.58	66.73

* tr: Trace

+ S.F.A.: Saturated fatty acids

++ U.F.A.: Unsaturated fatty acids

the results presented by Okaichi⁸⁾, the amino acid content of suspensoids was high at the area near the coast. As Hirota⁹⁾ reported that the number of zooplankton was large at the eastern part of Hiuchi Nada, it is considered from these results that the concentration of dissolved lipids may be influenced by high productivity or fast metabolic rate in marine organisms at this region of Hiuchi Nada. It is also considered that the distributing pattern of lipids extracted from sediments depends on the tidal current or the rate of sedimentation, because the tidal current is weak at the eastern part of Hiuchi Nada.

Considering the column of water, lipid content is generally greater in the surface than in the bottom of both seasons. It is recognized that dissolved organic compounds are condensed at the microlayer of surface ($100\text{--}150\ \mu$)¹⁰. Therefore, it is supposed that lipids are also concentrated at the surface layer, because the lipids have low specific gravity and have a surface active nature.

The lipid content of suspensoids is ranged between 1.4% to 4.3%. As sea water was filtered with glass fiber filter and suspensoids were gathered on it, a part of dissolved lipids may be absorbed on the filter. Nemoto *et al.*¹¹) also found small lipid granules in the suspended materials by the histochemical staining method in the East China Sea and its adjacent waters. Although the lipid content of suspensoids is greater in the surface than in the bottom at St. 3, the vertical distribution of lipids extracted from suspensoids seems to be uniform at St. 13. Considering this result, it can be also suggested that the quality of suspensoids is almost same at high tide and low tide. This may explain that lipid content of suspensoids is independent of the tidal current at these stations.

The thin-layer chromatogram and the class composition of lipids are shown in Fig. 6 and Table 2. It is interesting from these results that sterol esters are not detected and triglycerides are decreased in all the lipids of sea water, sediment and suspensoids. From these results (Table 2 and Table 3), it is obvious that the esterified compounds are decomposed in the marine environment, and then free fatty acids, free alcohols and sterols are derived⁶). It is also unclear whether free alcohols are derived from free fatty acids or n-alkanes as described by Van Der Linden¹²). It is considered from the low content of triglycerides that the lipids are highly decomposed in sea water and successively in sediments and suspensoids. Comparing the thin-layer chromatogram of lipids collected in summer with that in winter, triglycerides decreased more in winter. As the lipid content is generally less in winter, it is suggested that the supply of lipids in a marine environment is larger in summer than in winter.

The fatty acid composition of sea water and sediments is similar to the results reported by Williams¹³), Jeffrey¹⁴), and Stauffer *et al.*¹⁵) The long chain polyunsaturated fatty acids are not detected and the shorter chain fatty acids are contained in a large amounts. Although Schultz¹⁶) reported the existence of the long chain polyunsaturated fatty acids in the surface particulate matter collected in the North Atlantic and the absence of them in the particulate matter obtained at coastal waters, the long chain polyunsaturated fatty acids were also not detected in the suspensoids in Hiuchi Nada. This may be characteristic of the coastal water, or depends on the filtration of sea water with a plankton net (129 mesh) to remove the phytoplankton, because Schultz did not filtrate the sea water with the plankton net. However, the results obtained in Hiuchi Nada are similar to the results of Schultz in the point of the high percentage of short chain fatty acids.

The percentage of unsaturated fatty acids increases successively in the order of

bottom sea water, surface sea water and sediments (Fig. 7). If it is postulated that the unsaturated fatty acids are decomposed readily, the bottom sea water decomposes them greatly, then the surface sea water and the sediments follow. The biodeterioration of marine lipids by bacteria will be reported later.

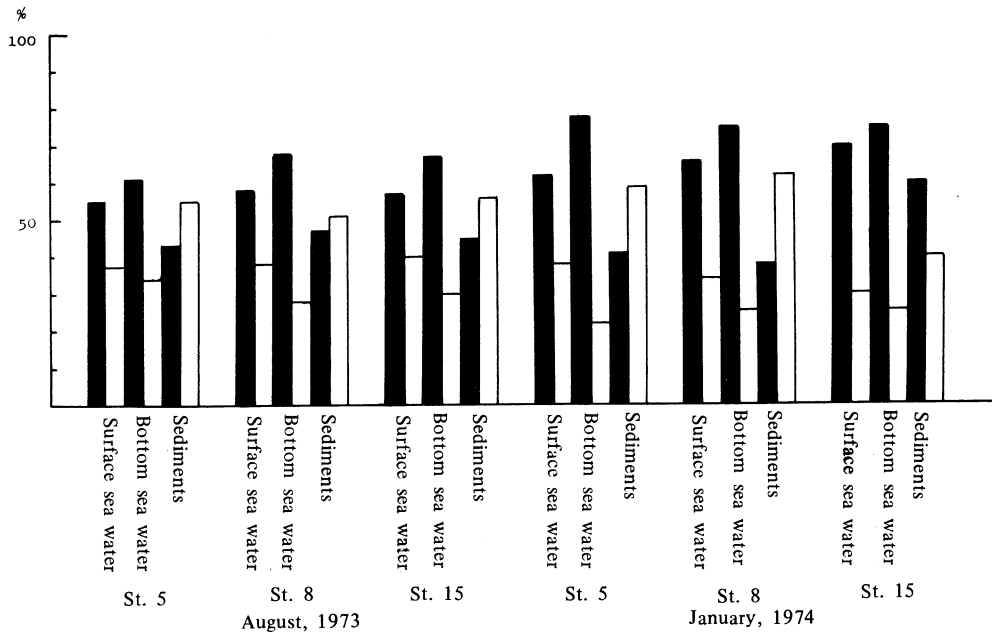


Fig. 7. The percentage composition of the saturated and unsaturated fatty acids of the lipids extracted from surface and bottom sea water and sediments.

■ Saturated fatty acids
□ Unsaturated fatty acids

SUMMARY

1. The lipid content of sea water and the sediment ranged from trace to 1.8 mg/l and from 0.9 mg/g to 2.6 mg/g, respectively. The lipid content was greater in the coastal waters than in the central area of Hiuchi Nada, and especially high at the area off Kawano and Kannonzi.

2. As the ester type of sterols was not detected and since the percentage of triglycerides was small in the lipids extracted from sea water, sediments and suspensoids, it is considered that the esterified compounds are hydrolyzed in the marine environment. The triglycerides were fewer in winter than in summer.

3. Considering the lipid class and the fatty acid composition, it is suggested that the decomposition of lipids was comparatively high in the bottom sea water, then in the surface sea water and sediments.

4. It is recognized that the suspensoids contain the lipids, however, the quality of the lipids is very different from that of marine organisms.

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沿岸水域の脂質に関する研究

I. 海水・底土・懸濁物中に含有される脂質の一般組成

鹿山 光 ・ 山田 久

海水は多くの有機物を包含し、脂質も例外ではない。本報は、燧灘の17測点における1973年夏期と1974年冬期の海水・底土・懸濁物に含まれる脂質を分析した結果を報告する。

- (1) 海水および底土の脂質含量は、それぞれ痕跡～1.8 mg/l, 0.9～2.6 mg/g の範囲にあった。燧灘の沿岸と中央部の水域を比較し、脂質含量は前者の方が多く、とくに川之江および観音寺沿岸水域で高い結果を得た。
- (2) いずれの試料の脂質においても、ステロールのエステル型成分はみられず、トリグリセリドも少量しか認められないことから、エステル型化合物は海の環境下に加水分解され易いと考えられる。トリグリセリドの含有割合は、夏よりも冬において少ない結果を示した。
- (3) 脂質のクラスと脂肪酸の組成を検討して、脂質の分解は底層水で比較的高く、表層水および底土がこれに次ぐものと示唆される。
- (4) 懸濁物も脂質を含有する。しかし、その組成は海洋生物のそれとは非常に異なっていることを認めた。