アラスカ湾のサケの炭化水素成分

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Hydrocarbon Components of Salmons in the Gulf of Alaska

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The hydrocarbon components in some tissues of sockeye salmon *Oncorhynchus nerka* and chum salmon *Oncorhynchus keta* caught in the Gulf of Alaska were investigated with open-tubular gas-liquid chromatography. At least 60 peaks were detected, of which 26 components were identified. The salmons had normal hydrocarbons ranging in chain length from C_{13} to C_{28} with an odd-chain predominance. The major hydrocarbons of the present samples were pristane and squalene. The total contents of these components in all samples exceeded 60% of the total hydrocarbons. In particular, pristane accounted for 85–90% of total hydrocarbons in the viscera and stomach contents of both salmons. Norphytene, phytane, and phytadiene were also detected in these samples.

The results in this study suggested that the salmons analyzed in the Gulf of Alaska were hardly polluted by crude oil.

Hydrocarbons (HCs) are minor but universal components of marine plants and animals, sea water and marine sediments. They may be biosynthesized, for instance by algae, and may also be petroleum in origin.

There have been some reviews[1,2] and a number of reports describing HC components of marine algae,[3–5] shark livers,[6,7] and sediments.[8] The HC of fishes also have been extensively, but systematically, investigated, mostly as part of marine pollution studies.[9] However, a number of papers focus on only pristane and squalene contents in marine organisms. Squalene and pristane are isoprenoid HCs widely distributed in marine organisms. Squalene accounts for a large proportion of the lipids from the livers of certain sharks.[6,7] It is a precursor of sterol biosynthesis in organisms, and has been identified as a constituent of many animal tissues. Pristane is a major HC in marine zooplankton, and is derived from phytol of dietary phytoplankton.[10]

A supertanker ran aground in the Gulf of Alaska on 24th March 1987, and about 200,000 barrels of crude oil spilled into the sea. A giant oil slick may damage the shore line and the marine environment. Since HCs are remarkably stable and not easily metabolized, HC analysis is a valuable tool for the tracing of the spread of pollutants through the marine environments.[3]

In this study, the HC components of some tissues of sockeye salmon *Oncorhynchus nerka* and chum salmon *Oncorhynchus keta* caught in the Gulf of Alaska were investigated by the analysis with open-tubular gas-liquid chromatography. We present a detailed characterization of HC components of the salmons in this paper.

Materials and Methods

Materials

The round fishes of socheye salmon and chum salmon were caught in the Gulf of Alasha by the training ship "Oshoro Maru" of Hokkaido University. The characteristics of these salmons are shown in Table 1. Both salmons were young fish (1–2 years old). The round fishes of salmons were divided into some tissues as described previously.[11] Each tissue was kept at −20°C until used.

Lipid Extraction and Analysis of Lipid Class Composition

Total lipids (TL) were extracted by the method of Bligh and Dyer.[12] The analysis of lipid class compositions of TL was carried out as described previously.[13]

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Table 1. Characteristics of samples

Sample	Sex	Body length (cm)	Body weight (g)	Collecting date and locality
Sockeye salmon *Oncorhynchus nerka*				
1	Female	33.2	515	July 1989.
2	Female	34.6	600	51°20′N, 158°01′W
3	Female	33.6	600	
Chum salmon *Oncorhynchus keta*				
1	Male	25.3	241	July 1989. 51°00′N, 160°01′W

Isolation of HCs

TL were saponified by refluxing with 1 N KOH/EtOH for 50 min, and the unsaponifables (UNS) were extracted with diethyl ether. HCs were isolated from the UNS by column or thin-layer chromatography (TLC). In column chromatography, silicic acid (Kiesel Gel 60, Merck) and *n*-hexane as developing solvent was used. TLC was carried out using Kiesel Gel 60G plates of thickness 0.5 mm and *n*-hexane/benzene (17: 1 v/v) as a solvent system.

Argentation Thin-Layer Chromatography ($AgNO_3$-TLC)

The separation of HCs based on the degree of unsaturation was carried out by preparative $AgNO_3$-TLC on silver nitrate-impregnated layer of Kiesel Gel 60G. *n*-Hexane/benzene (90: 10, v/v) was used as a solvent system.

Hydrogenation of HCs

For hydrogenation, about 10 mg palladium black (Nakalai Tesque, Kyoto) was added to 1–1.5 mg of the subfractions obtained by $AgNO_3$-TLC in methanol. The sample was stirred for 50 min under hydrogen at atmospheric pressure.

Preparation of Fatty Acid Methyl Esters (FAME)

Preparations of FAME were carried out as described previously.[14]

Open-Tubular Gas-Liquid Chromatography (GLC)

HCs analyses were also performed with a Shimadzu GC-14A (Shimadzu Seisakusho, Kyoto) equipped with a flame-ionization detector. The column was a SPELCOWAX-10 flexible fused-silica wall-coated open-tubular column (30 m × 0.25 mm i.d.; Supelco, Bellefonte, PA). The column temperature was operated by temperature programming as follows: hold at 150°C for 30 min, and then increase at 2°C per min up to 230°C and hold at the same temparature. The detector and injector were held at 250°C. The carrier gas was H_2. Peak identification was achieved by plotting retention times for *n*-alkanes, and by the comparison of the retention time with those of the standards. The standards used were 13: 0, 16: 0, 17: 0 (Nakalai Tesque., Kyoto), 14: 0, 16: 1, 18: 1, 21: 0, pristane, squalene (Kanto Chemical Co., Tokyo), and D2887 Quantitative Calibration Mix containing C_6-C_{44} *n*-alkanes (Supelco, Bellefonte, PA). The results of gas chromatography-mass spectrometry (GC-MS) were also used for identifications of some peaks.

Open-tubular GLC of FAME was carried out as described previously.[13]

GC-MS

GC-MS analyses of HCs were performed with a JEOL GCD-05-06 GC-MS system equipped with a glass column (1.5 m × 3 mm *i.d.*) packed with 3% OV-17 on 100–120 mesh Gas Chrom Q. The column temperature was programmed from 120°C to 270°C at 4°C/min. The carrier gas was He. All spectra were obtained at 70 eV ionizing electron energy and a source temperature of 300°C.

Results and Discussion

Lipid Contents and Lipid Class Compositions

Table 2 shows the contents of TL, UNS and HCs isolated from the tissues of sockeye salmon and chum salmon. The TL contents on a wet weight basis in the flesh of sockeye salmon and chum salmon were 1.4–1.9% and 1.6%, respectively. The fleshes of both salmons have lower TL contents than those reported previously.[11,13,15]

The TL contents in skin, viscera and stomach contents ranged from 6.0% to 9.7%. The livers of both salmons contained about 4.0% of TL.

Although the UNS contents in TL were less than 15% in the skin, flesh and liver of both salmons, the viscera and stomach contents con-

tained UNS at 19.5% and 48.6% of TL in sockeye salmon, and at 22.3% and 39.5% in chum salmon.

The HC contents were less than 1% of TL in some tissues except for stomach contents of sockeye salmon and liver of chum salmon. The HC contents in the stomach contents of sockeye salmon and liver of chum salmon were 5.50% and 1.57% of TL, respectively.

Table 2. Contents of total lipids, unsaponifiables, hydrocarbons, and lipid class compositions in tissues of sockeye salmon and chum salmon

Sample	TL*1 (wt%)	UNS*2 (%)	HC*3 (%)	Lipids*4 (% in TL)					
				HC+WE	TG	FFA	ST	DG	PL
Sockeye salmon 1									
Skin	9.7	3.2	0.10	0.8	64.7	5.9	4.8	5.7	18.1
Flesh	1.4	2.2	0.03	1.1	47.0	9.1	5.2	5.9	31.7
Liver	4.0	8.2	0.48	3.2	2.9	27.7	7.6	4.1	54.5
Viscera	8.6	19.5	0.32	26.6	26.2	19.1	6.4	7.2	14.5
Stomach contents	6.0	48.6	5.50	84.6	—	5.6	2.4	2.7	4.7
Sockeye salmon 2									
Flesh	1.5	5.4	0.10	—	28.7	11.9	6.4	5.7	47.3
Liver	3.3	10.5	0.31	1.2	—	65.3	8.7	—	24.8
Sockeye salmon 3									
Flesh	1.9	5.3	0.09	0.4	40.8	15.8	3.4	1.7	37.9
Liver	4.0	14.3	0.37	2.2	0.4	68.5	8.3	—	20.6
Chum salmon 1									
Skin	7.4	4.6	0.15	2.5	53.8	8.2	4.5	4.6	26.4
Flesh	1.6	6.7	0.22	3.2	13.6	12.3	5.5	5.5	59.9
Liver	3.7	9.7	1.57	4.2	1.7	31.6	8.8	4.3	49.4
Viscera	8.2	22.3	0.41	37.3	15.0	21.0	6.5	7.4	12.8
Stomach contents	8.1	39.5	0.88	75.0	—	5.9	7.0	3.0	9.1

*1 Total lipids.
*2 Unsaponifiables (% in TL).
*3 Hydrocarbons (% in TL).
*4 HC+WE, Hydrocarbons+Wax esters; TG, Triacylglycerols; ST, Sterols; DG, 1,2-diacylglycerols; PL, Polar lipids.

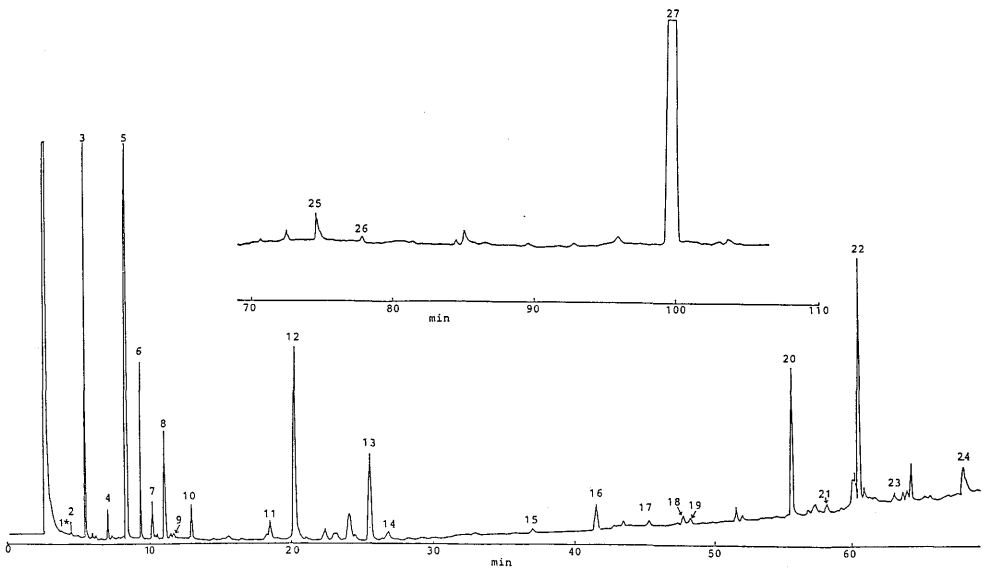

Fig. 1. Gas chromatogram of hydrocarbons in the flesh of sockeye salmon.
GLC: SUPELCOWAX-10 (30 m×0.25 mm i.d.), hold at 150°C for 30 min, and then increase at 2°C per min up to 230°C.
* See Table 4.

Table 3. Hydrocarbon compositions in tissues of sockeye salmon and chum salmon (wt%)

Peak No.	Hydrocarbon	Skin		Flesh				Liver				Viscera		Stomach contents	
		S*1	C*2	S			C	S			C	S	C	S	C
		1	1	1	2	3	1	1	2	3	1	1	1	1	1
1	13:0	0.05	0.10	0.01	0.14	0.08	0.03	0.01	0.06	0.04	0.01	0.01	0.01	—	0.01
2	14:0	0.11	0.15	0.07	0.22	0.14	—	0.05	0.26	0.12	0.01	0.02	0.01	0.05	0.01
3	15:0	5.82	2.80	6.40	7.75	5.94	1.63	0.48	2.32	3.12	0.97	0.75	0.55	0.30	0.36
4	16:0	0.21	0.19	0.23	0.31	0.24	0.17	0.21	0.53	0.30	0.57	0.03	0.08	0.06	—
5	pristane	19.96	50.54	20.19	30.30	38.30	54.67	19.68	10.25	38.80	39.46	85.99	84.91	84.42	90.77
6	17:0	1.64	1.19	1.65	1.75	1.21	0.81	0.53	1.15	0.82	1.06	0.13	0.15	0.10	0.09
7	17:1	0.48	0.29	0.45	0.32	0.36	0.17	0.06	0.14	0.23	0.11	0.09	0.05	—	0.05
8	norphytene	1.69	1.84	1.42	1.83	1.88	1.27	0.31	0.55	0.73	0.80	0.85	0.83	0.84	0.85
9	phytane	—	0.16	0.11	0.14	0.11	0.12	0.13	0.28	0.29	0.44	—	—	—	—
10	18:0	0.43	0.37	0.43	0.48	0.34	0.34	0.42	1.13	0.52	0.99	0.05	0.74	—	0.11
11	19:0	0.32	0.28	0.33	0.39	0.27	0.23	0.22	0.48	0.34	0.51	0.03	0.06	—	0.03
12	19:1	3.75	2.25	4.60	0.81	0.64	1.49	4.66	3.48	1.52	2.78	0.39	0.63	0.29	0.03
13	phytadiene	1.87	1.76	2.09	3.12	2.01	1.90	0.70	0.61	1.19	1.22	1.36	1.46	1.46	1.65
14	20:0	—	0.23	0.23	0.30	0.19	0.19	0.29	0.89	0.32	0.51	0.02	—	—	0.03
15	21:0	—	0.09	0.11	—	0.07	0.09	0.11	0.21	0.09	0.18	0.02	—	—	—
16	21:1	0.67	1.09	0.54	0.43	0.14	0.63	0.22	0.35	0.37	0.45	0.03	0.03	—	—
17	22:0	—	0.09	0.06	0.17	0.02	0.10	0.17	0.24	0.10	0.21	—	—	—	—
18	21:5	0.40	0.25	0.25	0.06	0.02	0.18	0.80	0.14	0.36	0.14	0.13	0.14	0.15	0.10
19	23:0	0.17	0.06	0.13	—	0.01	0.06	0.15	0.42	0.06	0.19	0.01	0.08	—	—
20	21:6	3.70	3.33	2.67	1.50	2.22	2.74	0.66	0.22	1.23	1.36	3.93	3.23	4.27	2.84
21	24:0	—	—	0.15	0.07	0.01	0.08	0.12	0.62	0.12	0.20	0.02	—	—	—
22	Unknown	4.18	2.71	3.94	4.42	2.68	1.97	2.36	3.56	1.98	2.73	0.11	0.16	0.09	0.10
23	25:0	0.29	0.11	0.16	0.10	0.11	0.07	0.15	0.25	0.43	0.08	0.02	—	—	—
24	26:0	0.63	0.55	0.61	0.59	0.16	0.51	—	0.55	—	0.11	0.09	0.05	—	—
25	27:0	0.93	0.38	0.26	0.71	0.28	0.40	1.37	4.41	1.99	2.30	0.06	0.12	0.46	—
26	28:0	—	0.08	0.16	0.04	0.07	0.08	0.12	0.08	0.16	0.07	0.02	—	—	—
27	squalene	41.22	21.98	43.46	34.62	34.17	25.19	49.35	44.10	31.97	24.57	3.55	2.73	1.92	0.60
Others		11.48	7.13	9.29	9.43	8.33	4.88	16.71	23.36	13.53	17.86	2.29	3.98	5.59	2.37
Total n-alkanes		10.60	6.67	10.99	13.02	9.14	4.79	4.40	13.60	8.53	8.08	1.28	1.85	0.97	0.64
Total n-alkenes		8.83	7.15	8.51	3.12	3.38	5.15	6.40	4.33	3.71	4.84	4.56	4.00	4.71	3.02
Total isoprenoids		64.74	76.28	67.27	70.01	76.47	83.15	70.13	55.79	72.98	66.49	91.75	89.93	88.64	93.87
CPI*3		4.40	3.96	5.60	4.65	5.97	3.12	3.68	2.20	3.83	2.24	9.91	2.19	16.88	8.78

*1 Sockeye salmon.
*2 Chum salmon.
*3 Carbon preference index.

Table 2 shows lipid class compositions of TL in the tissues of sockeye salmon and chum salmon. TL in the skin and flesh of both salmons consisted of mainly triacylglycerols (TG), free fatty acids (FFA) and polar lipids (PL). Sterols (ST) and 1,2-diacylglycerols (DG) were comprised about 5% of TL. The FFA and PL constituted 80% of TL in the liver of both salmons. TG were minor constituents in the liver. The main constituents in the viscera and stomach contents of both salmon were HCs+WE accounting for more than 25% and 70% of TL, respectively. HCs+WE may consists of mainly WE originating from the copepods as their diets.

Analysis of HCs with Open-Tubular GLC

Fig. 1 shows the gas chromatogram of HCs in the flesh of sockeye salmon. At least 60 peaks were detected of which 26 components were identified. The GLC analysis of the sub-fractions fractionated according to the degree of unsaturation after hydrogenation supported the identification of some peaks. The sum of 26 components was more than 80% of total HCs in all samples.

The chain lengths range for *n*-alkane was 13 to 28, and the dominant *n*-alkanes were 15: 0 and 17: 0. Major *n*-alkenes were 19: 1 and 21: 6. These components are important in marine algae,[1] Many marine algae contain 21: 6 accounting for 80–90% of total HCs.[5] A useful indicator of odd/even selectivity is the Carbon Preference Index (CPI).[1] As the CPI values of the present samples ranged from 2.19 to 16.88 as shown in Table 3, the salmons analyzed in this study are characterized by an odd-chain preference. Most marine organisms contain HCs series ranging in chain length from 13 to 33 with an odd-chain predominance.[2] Reinhardt and Vanvleet reported that even-chain *n*-alkanes were preponderant in 80% of 22 species of Antarctica mid-water zooplankton and fish.[16] Pristane and squalene were predominant constituents of HC in salmons. Norphytene, phytane and phytadiene were also detected in these samples.

HC Compositions in the Tissues of Sockeye Salmon and Chum Salmon

Table 3 shows the HC compositions in the tissues of sockeye salmon and chum salmon. Although the skin and flesh contained 15: 0 accounting for about 6% of total HCs in sockeye salmon, 2% in chum salmon, this component accounted for less than 1% in the viscera and stomach contents of both salmons. In the skin, flesh and liver, the proportions of 19: 1 ranged from 0.81% to 4.66% in sockeye salmon, from 1.49% to 2.78% in chum salmon. However, 19: 1 contents in the viscera and stomach contents of both salmons were less than 1% of total HCs. Although the contents of 21: 6 in the tissues except the liver of both salmons ranged from 1.50% to 4.27% of total HCs, the liver of sockeye salmon and chum salmon contained 0.22–1.23% and 1.36%. The sum of pristane and squalene in all tissues of both salmons ranged from 54.35% to 91.37% of total HCs. Pristane accounted for more than 84% of total HCs in the viscera and stomach contents of both salmons. Inoue *et al.* reported on the basis of the GLC patterns of HCs that pristane, which was a major component in the oceanic salmon, was a minor component in rainbow trout and in the kokanee (the land-locked form of socheye salmon).[17] The HC compositions were not presented in their paper. In this study, pristane is a major component in all tissues, particularly the viscera and stomach contents, of both salmons. Pristane is the principal HC in marine copepods.[1] Avigan and Blumer demonstrated the synthesis of pristane from dietary phytol by copepods,[10] and postulated that the herbivorous zooplankton might be "the most significant source of this HC" for all marine organisms and sediments.

Squalene accounted for about 40% in the skin, flesh and liver of sockeye salmon, 25% in those tissues of chum salmon, while less than 4% of total HCs in the viscera and stomach contents of both salmons. In teleost fishes, as in other animals, the *de novo* biosynthesis of HCs is limited to squalene.[1] It became apparent that there was a difference of the HC composition depending on the tissues. The HC compositions of viscera and stomach contents seemed to be influenced by their diets. Further work is in progress to confirm the differences of HC compositions depending on species and localities.

Fatty Acid Compositions of TL in the Tissues of Sockeye Salmon and Chum Salmon

Table 4 shows fatty acid compositions of TL in the tissues of sockeye salmon and chum salmon. The major components were 14: 0, 16: 0, 18: 1 (n-9), 18: 4 (n-3), 20: 1 (n-11), 20: 1 (n-9), 20: 5 (n-3), 22: 1 (n-11 and n-13) and 22: 6 (n-3). These components were detected at a level above 3% for the total fatty acids in one or more samples.

Table 4. Fatty acid compositions in tissues of sockeye salmon and chum salmon (wt%)

Fatty acid	Skin		Flesh				Liver				Viscera		Stomach contents	
	S*	C*	S			C	S			C	S	C	S	C
	1	1	1	2	3	1	1	2	3	1	1	1	1	1
14: 0	5.2	3.7	5.4	3.5	3.2	2.6	2.3	2.7	2.1	2.7	6.2	4.8	9.7	9.3
15: 0	0.9	0.6	0.4	0.6	0.5	0.5	—	0.7	0.7	0.7	1.0	0.9	1.3	1.3
16: 0	11.3	11.1	14.8	16.4	15.1	15.4	11.9	16.7	14.0	13.7	8.6	9.4	8.0	9.3
16: 1 n-7	2.4	2.0	2.1	1.7	2.3	1.3	0.9	1.3	1.0	0.9	1.2	1.1	0.7	0.7
iso-17: 0	0.9	0.7	0.9	0.6	0.7	0.7	1.1	0.9	0.7	1.0	0.8	0.8	0.8	0.8
16: 2 n-4	0.7	0.6	1.2	0.8	0.9	0.6	1.1	1.4	1.8	1.6	1.1	1.1	1.4	1.4
18: 0	1.4	1.5	1.4	1.8	1.9	1.5	2.6	4.5	4.4	2.8	0.9	1.1	0.6	0.8
18: 1 n-11	1.2	1.0	0.1	0.6	0.7	0.7	1.9	3.4	1.8	2.4	0.5	0.5	0.1	0.2
18: 1 n-9	7.0	6.4	6.2	8.5	9.2	4.7	5.3	11.1	6.6	4.8	2.7	2.9	1.2	1.5
18: 1 n-7	1.2	1.4	1.3	1.5	1.4	1.3	0.9	1.7	1.3	1.1	0.6	0.8	0.4	0.5
18: 1 n-5	1.3	1.1	1.3	0.8	1.3	0.9	1.2	1.9	1.5	1.4	0.8	0.8	0.9	0.9
18: 2 n-6	1.5	1.5	1.5	1.3	1.2	1.0	0.7	0.8	0.7	0.8	0.9	0.8	0.6	0.5
18: 3 n-3	1.2	1.1	1.1	1.1	1.0	0.8	0.5	0.6	0.5	0.6	0.8	0.7	0.7	0.5
18: 4 n-3	5.3	6.0	5.1	4.0	3.0	4.1	1.0	0.9	1.1	2.1	7.0	5.9	11.9	6.8
20: 1 n-11	9.6	9.2	8.7	3.9	5.8	5.7	4.3	2.9	3.2	3.8	6.7	5.8	2.9	3.2
20: 1 n-9	4.8	3.7	4.8	2.2	2.3	2.6	2.5	2.2	2.2	2.2	5.1	4.3	3.5	4.1
20: 4 n-6	0.3	0.4	0.3	0.5	0.4	0.6	2.0	2.6	3.1	2.5	0.4	0.6	0.3	0.3
20: 4 n-3	1.6	1.7	1.5	1.6	1.6	1.4	2.2	1.9	1.8	1.8	1.5	1.4	1.5	1.3
20: 5 n-3	5.7	8.4	5.3	9.0	8.0	10.3	14.8	8.9	11.9	13.6	8.5	9.1	13.5	9.1
22: 1 n-11, n-13	16.5	12.4	15.1	6.6	7.8	8.3	3.6	1.9	2.8	3.8	21.2	19.1	15.3	22.0
22: 1 n-9	1.8	1.3	1.7	0.7	0.9	0.9	0.4	0.3	0.4	0.7	2.0	1.9	1.3	1.9
22: 5 n-3	0.8	1.3	0.8	1.2	1.4	1.3	1.8	2.1	2.2	1.6	0.7	1.1	0.6	0.6
22: 6 n-3	5.1	13.4	5.4	23.8	21.2	25.6	27.3	18.2	25.3	25.1	10.0	13.3	12.1	11.5
24: 1 n-9	1.4	0.1	1.2	0.8	1.2	0.9	0.3	0.7	0.9	0.9	1.3	0.0	0.8	1.5
Others	10.9	9.4	12.4	6.5	7.0	6.3	9.4	9.7	8.0	7.4	9.5	11.8	9.9	10.0

* Abbreviations of samples are the same as shown in Table 3.

These major components except for 18: 4 (n-3) were almost similar to those of previous reports.[11,13,15] The proportions of 18: 4 (n-3) in the skin, flesh and viscera of both salmons were about 6% of total fatty acids. The stomach contents of sockeye salmon contained 18: 4 (n-3) accounting for 11.9% of the total fatty acids. As 18: 4 (n-3) is normal component of herbivorous copepods,[18] this fatty acid may originate from copepods as their diets. It is reported that *n*-alkane is converted to fatty acid through an oxidation.[2] If this metabolic pathway is being used, odd-chain fatty acids should be present in the samples. As shown in Table 4, odd-chain fatty acids were hardly detected in this study.

Contaminations of Salmons with Crude Oil

The HCs of marine organisms contaminated with crude oil have been studied by a number of investigators.[19] Motohiro and Inoue analyzed HCs by GLC in the muscle of chum salmon contaminated with crude oil.[20] However, the gas chromatogram presented in their paper is indistinct owing to the low packed column resolution. The sensitivities of their method are probably too low to judge the oil pollution of fish. Paradis and Ackman analyzed HCs in lobster meat by open-tubular GLC, and reported that the ratio of 17: 0 to pristane in polluted lobster meat exceeded 1.0, while this ratio was less than 1.0 in unpolluted lobster meat.[21] Analysis of petroleum-derived HCs invariably showed very complex patterns of aliphatic components, which in fresh crude oil extend to C_{40} and beyond, with little or no odd-chain preference.[1] In this paper, we obtained a satisfactory gas chromatographic separation of major components by open-tubular GLC. The chain length of HCs in the present samples ranged from 13 to 28, and no HC having chain length exceeded 40 was detected. The straight chain HCs in all samples have an odd-chain preference. Moreover, major HCs were pristane and squalene, and the proportions of pristane were much higher than those of 17: 0 in

all samples. Therefore, it is suggested that the salmons analyzed in this study were hardly contaminated with crude oil.

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