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Characterization of Molecular Species of Fish Muscle Phosphatidylcholine*1-2

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Dorsal muscle phosphatidylcholine (PC) molecular species of sardine, mackerel, big-eyed tuna, brown sole, sand flounder, rock fish, Alaska pollack, chum salmon, blue shark, mackerel shark, carp and rainbow trout were analyzed on HPLC.

Flat fish such as brown sole and sand flounder were extremely characteristic, since these fish contained (16:0) (20:5) as the most prominent molecular species unlike the rest of the fish that had (16:0) (22:6) as the most prominant molecular species.

Principal component analysis (PCA) of the PC molecular species suggested that except for sardine dark muscle, chum salmon (feeding migration stage) and rainbow trout, a common correspondant movement against seasonal change in the content of (20:5) (20:5) was observed in the majority of these fishes.

In the previous paper,[1] the formulae that control the sequence of elution of lipid molecular species on HPLC have been demonstrated. It has been proven that the matrix model presented is invariant. And this matrix model forms the bases of molecular species identification of muscle phosphatidylcholine (PC) from fish sources that will be discussed in this paper.

Characteristics of migratory fish, bottom fish, cartilaginous fish and fresh water fish from the view point of muscle PC are demonstrated.

Experimental

Total lipids were obtained from the fish muscle tabulated in Table 1 according to the method of BLIGH & DYER. Neutral lipid composition and phospholipid composition were measured by the Iatroscan-Chromarod Method.[2-5] The developing solvents used were *n*-hexane/diethyl ether/formic acid (85:15:0.5, v/v) for the former and chloroform/methanol/ammonia/water (70:30:2:3, v/v) for the latter. Phospholipid content was determined by multiplying 25 to the phosphorus content of the lipid which had been determined by FISKE-SUBBAROW method. Preparation of pure PC, hydrolysis of PC into diglyceride, and derivation to diglyceride acetate from diglyceride were done in the same manner as shown in the previous paper.[1] HPLC fractionation and identification of molecular species of each peak on HPLC were also done in the same manner though the matrix model is available in identifying the molecular species in order to verify the results.

Results and Discussion

Characteristics of Muscle PC of Fish

The yield of total lipid and the percentage of each lipid class against total lipid are shown in Table 2 and Table 3. Fatty acid composition of diglyceride acetates which represent the fatty acid composition of PC are shown in Table 4 and Table 5. Samples shown in these tables were subjected to the PC molecular species analysis.

HPLC chromatograms of each fish are shown in Figs. 1–6. As illustrated in Fig. 1, the HPLC chromatograms of diglyceride acetate of fish muscle PC can be devided into four molecular species groups (I~IV), that is, I: molecular species com-

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Table 1. Fish examined

Samples	Mean body length and weight Locality of catch	Date of catch	
Sardine *Sardinops melanosticta*	20.0 cm, 80 g Kamiiso, Hokkaido	July 1982	A
	17.1 cm, 43 g Kamiiso, Hokkaido	Oct. 1983	
Mackerel *Scomber japonicus*	41.8 cm, 647 g Kamiiso, Hokkaido	July 1982	
	32.2 cm, 409 g Todohokke, Hokkaido	Oct. 1983	
Big-eyed tuna, Frozen *Parathunnus obeus*	110.0 cm, 20 kg from the market	—	
	100.0 cm, 24 kg Indian sea	—	
Brown sole *Limanda ferruginea*	23.7 cm, 169 g Kikonai, Hokkaido	Oct. 1982	B
	21.2 cm, 207 g Kamiiso, Hokkaido	May 1983	
Sand flounder *Linanda punctatissima*	18.3 cm, 65 g Kamiiso, Hokkaido	Dec. 1982	
	18.6 cm, 139 g Abuta, Hokkaido	May 1983	
Rock fish *Sebastes schlegeli*	19.6 cm, 187 g Toi, Hokkaido	Oct. 1982	C
	27.0 cm, 471 g Kamiiso, Hokkaido	July 1983	
Alaska pollack *Theraga chalcogramma*	44.0 cm, 610 g Uchiura bay, Hokkaido	Dec. 1981	
	41.7 g, 509 g Shikabe, Hokkaido	Jan. 1984	
Chum salmon, Male *Oncorhynchus keta*	65.0 cm, 3500 g Akkeshi, Hokkaido	June 1980	
	42.7 cm, 1367 g 45°59′–42°29′N, 167°07′–175°30′E	Aug. 1983	
Chum salmon, Female *Oncorhynchus keta*	46.8 cm, 1593 g 45°59′–49°29′N, 167°07′–175°30′E	Aug. 1983	
Blue shark *Prionace grauca*	114.5 cm, 8.5 kg 38°30′–39°30′N, 155°00′E	June 1982	D
Mackerel shark *Lamna cornubica*	88.5 cm, 10.2 kg 41°30′–43°00′N, 175°30′E	July 1982	
Carp *Cyprinus carpio*	23.0 cm, 175 g from the market	Sep. 1980	E
Rainbow trout *Salmo gairdnerii irideus*	33.8 cm, 455 g Nanae, Hokkaido	Sep. 1982	
	38.0 cm, 780 g Nanae, Hokkaido	May 1983	

A: Migratory fish. B: Bottom fish. C: Hard to classify. D: Cartilaginous fish. E: Fresh water fish.

Table 2. Lipid composition of muscle of fish captured in 1980–1982

Sample	Lipid							
	Non-phospholipid*2					Phospholipid*2		
	Yield*1	TG	FFA	ST	NP others	PC	PS+PE	PL others
Sardine (DM)	26.8	92.5	0.4	0.4	1.7	3.7	1.8	trace
Sardine (WM)	4.3	83.1	0.5	1.8	2.0	8.6	2.6	0.2
Mackerel (DM)	5.8	72.5	0.9	0.3	0.3	4.6	8.5	2.6
Mackerel (WM)	0.9	32.0	2.7	2.5	1.2	33.2	9.4	5.6
Big-eyed tuna	0.7	13.4	0.2	1.7	trace	59.4	9.0	0.5
Brown sole	1.7	62.2	5.4	2.3	0.7	16.0	3.1	3.4
Sand flounder	1.3	64.1	0.9	2.0	4.2	18.8	1.1	0.6
Rock fish	1.4	58.8	2.5	0.5	0.3	18.5	3.8	5.0
Alaska pollack	1.0	7.3	1.9	7.8	trace	81.2	trace	1.7
Chum salmon*3	14.4	13.2	0.2	trace	trace	0.3	0.3	0.4
Blue shark	0.6	1.1	0.2	6.0	1.3	56.5	20.1	6.2
Mackerel shark	2.0	48.9	2.2	1.9	1.5	23.1	8.5	13.9
Carp	1.6	55.5	trace	6.7	trace	29.1	12.4	2.7
Rainbow trout	3.3	72.7	1.1	0.5	0.1	11.7	5.0	0.4

*1 g/100 g muscle. *2 g/100 g lipid. *3 Feeding migration, Male.
TG, triglyceride; FFA, free fatty acid; ST, sterol; NP, non-phospholipid; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; PL, phospholipid; DM, dark muscle; WM, white muscle.

Table 3. Lipid composition of muscle of fish captured in 1983–1984

Sample	Lipid							
	Non-phospholipid*2					Phospholipid*2		
	Yield*1	TG	FFA	ST	NP others	PC	PS+PE	PL others
Sardine (DM)	3.3	53.8	1.9	2.1	1.2	11.2	27.7	2.1
Sardine (WM)	0.8	29.2	0.3	4.8	6.3	12.1	44.3	5.0
Mackerel (DM)	20.0	90.9	0.2	0.4	1.3	2.4	4.5	0.3
Mackerel (WM)	8.5	92.0	trace	0.6	0.9	4.6	0.6	1.3
Big-eyed tuna	0.5	6.4	3.8	13.4	2.8	11.7	57.8	4.1
Brown sole	0.9	20.0	trace	6.0	2.1	17.5	54.0	0.4
Sand flounder	1.7	62.9	2.5	3.0	3.4	7.9	18.6	1.7
Rock fish	1.6	79.0	trace	1.0	0.2	3.5	13.4	2.9
Alaska pollack	0.7	3.6	3.0	9.8	2.6	26.7	47.0	7.3
Chum salmon*3	10.4	92.1	0.4	0.7	0.2	2.3	3.6	0.7
Chum salmon*4	12.4	94.1	trace	0.7	0.7	1.3	2.9	0.3
Rainbow trout	2.5	69.8	1.0	1.1	0.2	8.9	17.6	1.4

*1 g/100 g muscle. *2 g/100 g lipid. *3 Feeding migration, Male. *4 Feeding migration, Female.
TG, triglyceride; FFA, free fatty acid; ST, sterol; NP, non-phospholipid; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; PL, phospholipid; DM, dark muscle; WM, white muscle.

posed of highly unsaturated fatty acids such as 20: 5 or 22: 6, for instance (20: 5) (20: 5), (20: 5) (22: 6) and (22: 6) (22: 6), III: molecular species composed of generally found fatty acids such as 16: 0 or 18: 1 with combinations of 20: 5 or 22: 6, that is (20: 5) (18: 1), (18: 1) (20: 5), (22: 6) (18: 1), (18: 1) (22: 6), (20: 5) (16: 0), (16: 0) (20: 5), (22: 6) (16: 0) and (16: 0) (22: 6), and II and IV: others. As it is evident from the chromatograms (see Figs. 1–6), groups I and III accounts for at least 60% of the molecular species examined. Specifically, as shown in Fig. 1, groups I and III of the sardine white muscle accounts for about 87–88%. These two groups might control or represents the characteristics of PC of fish muscle.

The left side chromatograms in Fig. 1 are the sardines captured in summer (July, 1982) and the right side chromatograms are of those captured in fall (Oct. 1983). Though a supplementary experiment should be done to be conclusive,

Table 4. Fatty acid composition of diglyceride acetate derived from muscle phosphatidylcholine of fish captured in 1980–1982

Fatty acid	Sample													
	Sardine		Mackerel		Big-eyed tuna	Brown sole	Sand flounder	Rock fish	Alaska pollack	Chum salmon*2	Blue shark	Mackerel shark	Carp	Rainbow trout
	DM*1	WM*1	DM*1	WM*1										
12:0														0.07
14:0	1.09	1.48	0.98	1.39	0.72	2.00	1.54	1.11	2.69	3.27	1.04	1.30	1.11	0.97
15:0		0.37	0.26	0.28	0.63	0.68	0.90	0.34	0.31	0.69				0.17
16:0	18.42	32.35	30.82	24.42	25.60	22.70	29.14	26.50	41.88	35.19	22.82	34.80	32.28	29.70
16:1	0.74	1.78	2.13	0.75	0.48	1.95	1.83	6.51	0.57	0.45	5.40	3.60	2.59	3.30
17:0	0.82	0.52	0.56	0.52	1.46	1.22	1.09	1.33	0.37	1.04	0.45	trace		0.39
17:1	0.48	0.50	0.45		0.79	1.11	0.90	0.91	0.49		0.43	trace		0.32
18:0	5.99	1.11	0.90	4.29	0.92	1.93	1.41	1.63	0.82	0.78	8.93	2.80	3.08	3.46
18:1	18.69	6.80	4.22	12.09	7.24	10.24	8.27	16.49	12.32	6.26	10.04	11.30	16.71	29.75
18:2	1.09	1.84	0.45	1.34	0.56	0.21		0.66	0.46	0.33	0.77	0.60	9.06	8.60
19:1	0.45	0.63	0.21	0.32		0.33		0.21		0.35	0.35	trace		0.18
19:2		1.44	0.34			1.31			0.29	0.56				
20:0			1.26				0.97*3			0.71	1.15*3	trace	1.16*3	
20:1	1.93	0.94		1.15		1.29*3		0.56*3	1.65*3			2.00		1.31
20:2											1.45	trace		1.13
20:3		0.30											0.82	1.32
20:4	3.50	2.71	1.96	3.37	5.27	7.43	6.97	3.54	0.88	1.07	3.60	4.80	3.85	0.86
20:5	10.47	13.98	18.64	11.08	4.58	31.60	27.59	13.83	16.79	8.79	10.92	11.30	6.39	1.79
22:1												trace		0.10
22:2						0.24								0.05
22:3		0.62	0.37			0.66					0.13	trace		0.07
22:4	1.37	0.45	0.42	1.14	5.75	0.38	0.89	0.83			1.70	trace		0.31
22:5	3.39	1.76	1.11	2.16	0.44	4.51	2.73	1.34	0.55	0.89	6.01	2.50	1.17	0.83
22:6	31.57	30.42	34.90	35.70	45.56	10.69	15.77	24.21	19.93	39.62	24.56	24.90	20.67	14.69
24:2														0.56
24:3														0.06
24:6						0.30					0.24		1.11	
Unknown						0.21								

*1 DM, dark muscle; WM, white muscle.
*2 Feeding migration, Male.
*3 Ether 20:0 or 20:1.

Table 5. Fatty acid composition of diglyceride acetate derived from muscle phosphatidylcholine of fish captured in 1983–1984

Fatty acid	Sample											
	Sardine		Mackerel		Big-eyed tuna	Brown sole	Sand flounder	Rock fish	Alaska pollack	Chum salmon		Rainbow trout
	DM*	WM*	DM*	WM*						Male	Female	
14:0	1.08	1.20	0.92	0.83	0.75	2.77	1.77	10.3	2.13	4.58	3.10	1.27
15:0	0.41	0.43	0.33	0.31	0.49	1.07	0.85	0.32	0.34	0.82	0.68	0.22
16:0	24.09	36.38	21.15	28.17	33.29	34.11	31.22	33.41	29.01	29.02	32.26	36.73
16:1	1.43	1.25	1.42	1.34	1.00	7.55	2.39	3.76	1.96	1.79	1.38	3.01
17:0	0.51	0.50	0.78	0.43	1.75	1.78	1.11	0.74	0.46	1.14	0.92	0.23
17:1	0.41	0.33	0.57	0.69	0.84	0.84	0.91	0.64	0.36	0.44	0.37	0.15
18:0	0.88	0.71	5.44	3.05	2.28	2.38	1.70	1.99	0.74	1.03	1.11	1.81
18:1	5.02	2.11	15.93	10.24	23.02	11.31	10.77	18.93	13.34	8.43	8.27	12.81
18:2	0.49	0.60	1.89	0.99	0.67	0.46	0.70	1.40	1.23	0.51	0.52	5.97
19:1 or 18:4	0.17		0.51	0.47	0.31		0.33	0.37	0.45			0.63
19:2	0.17		0.70			0.28		0.23	0.49	0.69	0.56	0.25
20:0	0.11	0.33	1.30	0.23	0.12	1.05	1.86			1.01	0.77	0.34
20:1		0.12		0.72	0.98	0.07	2.24	0.75	3.02	0.10	0.10	0.15
20:2		0.11		0.14	0.33	0.08	0.06	0.21	0.14	0.11	0.09	0.30
20:3	2.99	0.12	4.26	0.13	0.13	4.64	0.17	0.18	0.06	0.65	0.58	2.99
20:4	0.15	2.35		3.74	5.96	0.19	4.70	3.87	2.21	0.79	0.67	0.29
20:5	18.85	14.20	16.41	16.78	3.74	18.53	25.12	10.02	24.46	11.49	10.18	5.90
22:1						0.11	0.27					
22:3	0.21	0.10	0.25		0.28	1.32	0.70	0.19	0.24	0.07		0.22
22:4	0.91	0.71	0.80	0.70	2.51	0.35	0.41	0.47	0.11	0.42	0.34	0.25
22:5	1.08	0.98	2.46	1.85	0.43	3.71	2.62	1.18	0.56	1.05	0.98	1.04
22:6	36.06	37.24	24.68	29.11	21.06	7.13	9.89	20.26	18.68	35.00	36.40	25.35
others	4.98	0.23	0.20	0.08	0.06	0.28	0.21	0.05	0.01	0.86	0.73	0.09

* DM, dark muscle; WM, white muscle.

molecular species composition of group III seems to change drastically in accordance with the seasonal change.

Fig. 2 shows the chromatograms of mackerels captured in the same day with sardine. The characteristic of this fish is the very complicated composition in group IV especially in dark muscle. Molecular species of (16: 0) (22: 5), (16: 0) (20: 4), (17: 1) (22: 6), (20: 5) (18: 0), (18: 0) (20: 5), (22: 6) (18: 0) and (18: 0) (22: 6) can be examplify in this group.

Fig. 3 shows the chromatograms of bottom fish such as brown sole and sand flounder captured in October, December and May. These fish are extremely outstanding since the most predominant component of these fish is (16: 0) (20: 5) instead of (16: 0) (22: 6) unlike other fish examined.

The chromatograms of big-eyed tuna, Alaska pollack, carp and rainbow trout are shown in Fig. 4, and the chromatograms of rock fish, blue shark, mackerel shark and rainbow trout are shown in Fig. 5. Throughout the chromatograms in Figs. 4 and 5, (16: 0) (22: 6) is the most predominant peak though in case of Alaska pollack, it contains (16: 0) (22: 6) and (16: 0) (20: 5) almost equally (shown by arrows in Fig. 4). The similarity among fresh water fish and cartilaginous fish is the very few content of group I *i.e.* the molecular species composed of highly unsaturated fatty acids such as (20: 5) (20: 5), (20: 5) (22: 6) and (22: 6) (22: 6), although the former contains less amount of molecular species that contains 20: 5.

The content of group I between a and b differs significantly in the case of big-eyed tuna. This is seemed to be the result of differences in days of frozen storage since other freshly prepared migratory fish examined contains group I abundantly.

Fig. 6 shows the chromatograms of chum salmon. As it is evident from this Fig. 6, chum salmons under feeding migration are extremely rich in (16: 0) (22: 6).

As it is observed throughout the figures, predominant molecular species are usually composed of 16: 0, 18: 1, 20: 5 and 22: 6 such as (20: 5)

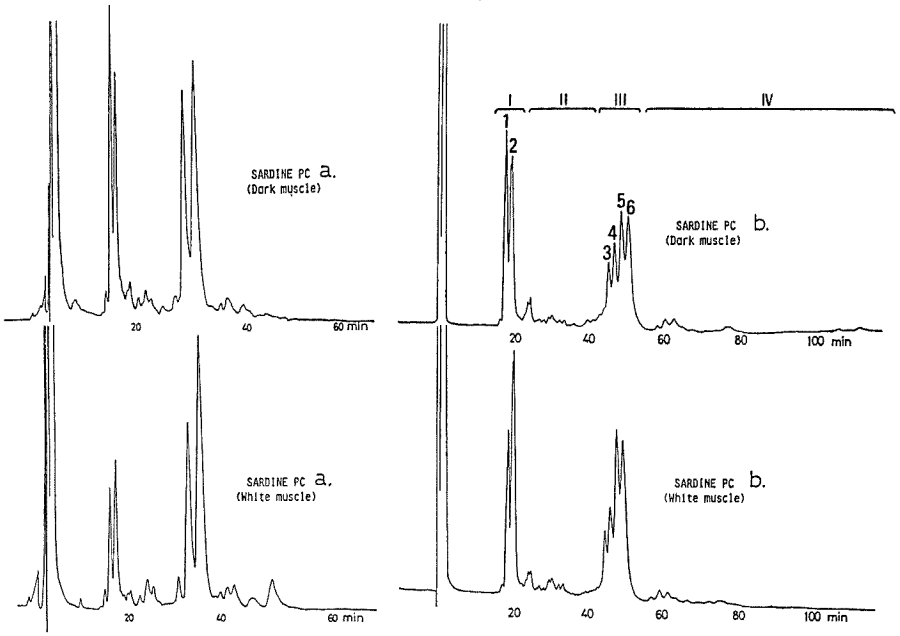

Fig. 1. HPLC chromatograms of sardine muscle PC. PC is converted to diglyceride acetate for thorough separation on HPLC.

a: Captured in 1982. b: Captured in 1983.

I: Groups composed of highly unsaturated fatty acids, that is, 1: (20: 5) (22: 6), 2: (22: 6) (22: 6).

III: Groups composed of highly unsaturated fatty acids with the combinations of generally found fatty acids, that is, 3: (20: 5) (16: 0), 4: (16: 0) (20: 5), 5: (22: 6) (16: 0) and 6: (16: 0) (22: 6).

II & IV: Others.

Condition

Instrument: Hitachi 638-50
Column: LiChrosorb RP-18 (8×250 mm, tandem)
Flow rate: 1.5 ml/min
Detector: RI, 8×10-20
Eluent: isopropanol/acetone/methanol/acetonitrile (1: 1: 3: 4, v/v)
Temperature: Ambient

(20: 5), (20: 5) (22: 6), (22: 6) (22: 6), (18: 1) (20: 5), (18: 1) (22: 6), (16: 0) (20: 5), (16: 0) (22: 6) and (16: 0) (18: 1). Fig. 7 illustrates the amount of (20: 5) (20: 5), (20: 5) (22: 6), (22: 6) (22: 6), (20: 5) (18: 1)+(18: 1) (20: 5), (22: 6) (18: 1)+(18: 1) (22: 6), (20: 5) (16: 0)+(16: 0) (20: 5), (22: 6) (16: 0)+(16: 0) (22: 6) and (18: 1) (16: 0)+(16: 0) (18: 1) by radar charts. These charts are shown in sequence of elution (clockwise) and the original data have been employed from the area of each peak in Figs. 1-6. As it is evident from this Fig. 7, the predominant peaks composed of 16: 0, 18: 1, 20: 5 and 22: 6 well show the characteristics of each fish. Bottom fish such as brown sole and sand flounder is outstanding owing to the ratio of (16: 0) (20: 5) and (16: 0) (22: 6). All other fish except Alaska pollack contains (16: 0) (22: 6) as the most predominant component while Alaska pollack contains both component almost equally. From the view point of seasonal variation, rainbow trout shows an extraordinary change. It is considered that the closed environment of this fish forced the changes of PC molecular species for adaptation. Fatty fish such as sardine and mackerel also show considerable changes compared with other marine fish examined.

Principal Component Analysis of Fish Muscle PC Molecular Species

The computer program used for principal com-

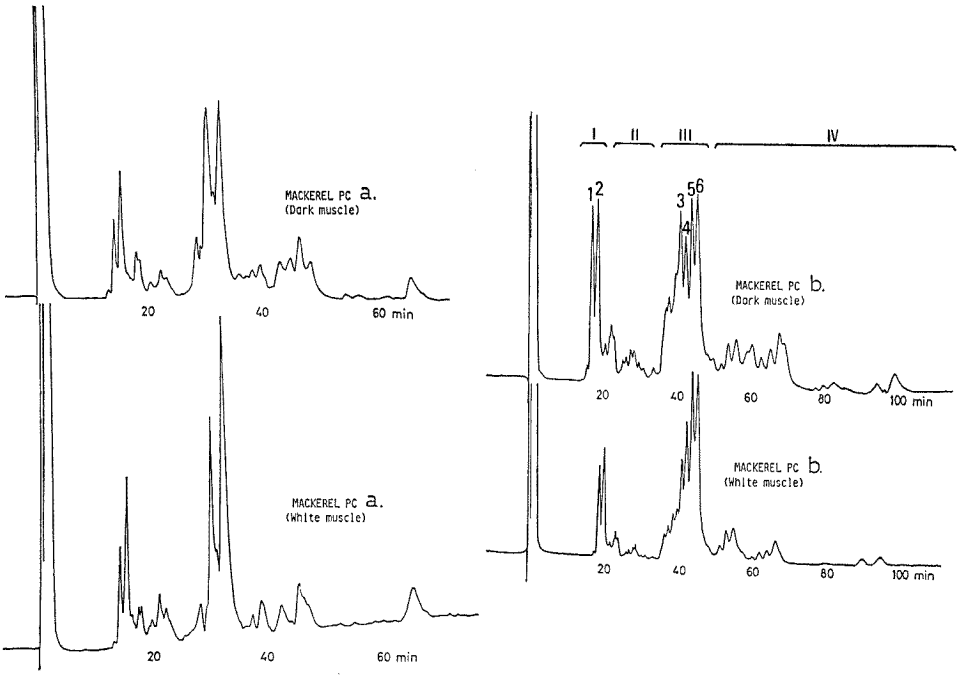

Fig. 2. HPLC chromatograms of mackerel muscle PC.
a: Captured in 1982. b: Captured in 1983.
Numbers and conditions are the same as in Fig. 1.

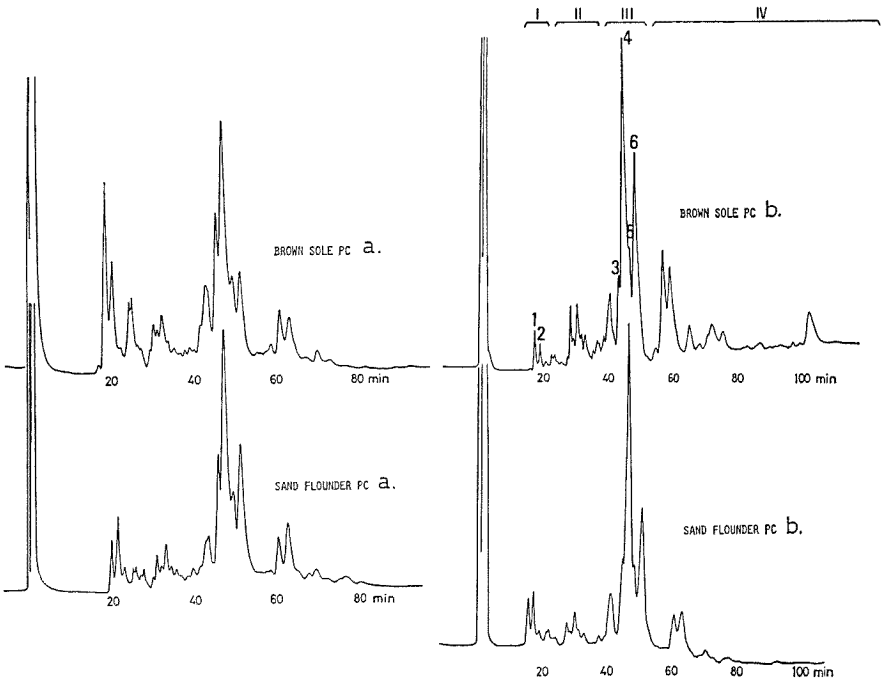

Fig. 3. HPLC chromatograms of brown sole and sand flounder muscle PC.
a: Captured in 1982. b: Captured in 1983.
Numbers and conditions are the same as in Fig. 1.

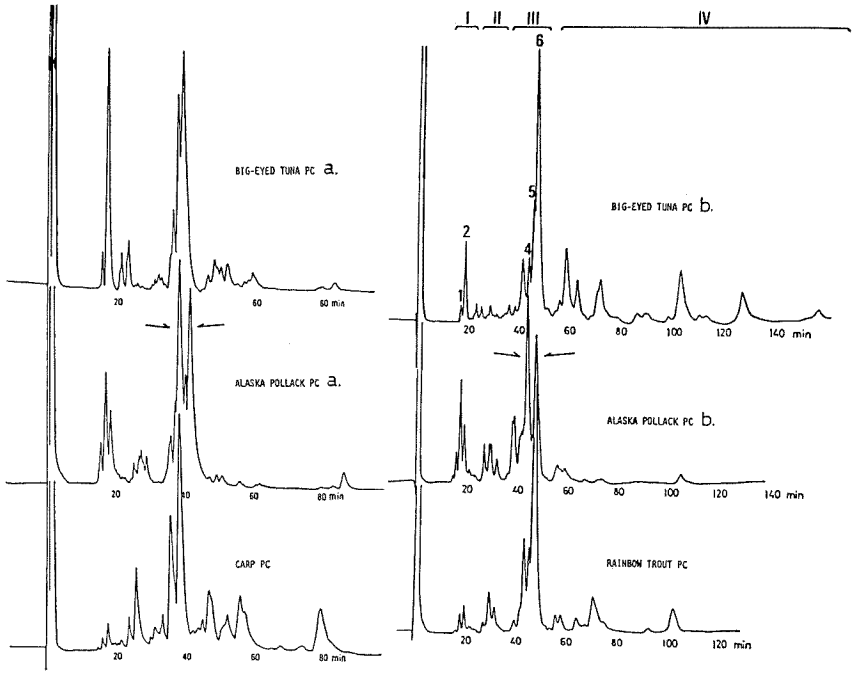

Fig. 4. HPLC chromatograms of big-eyed tuna, Alaska pollack, carp and rainbow trout muscle PC.

a: Captured in 1981. b: Captured in 1984 for Alaska pollack and a: Frozen 1981, b: Frozen 1983 for big-eyed tuna.
Carp is the sample of 1980 and rainbow trout is the sample of 1983.
Numbers and conditions are the same as in Fig. 1.

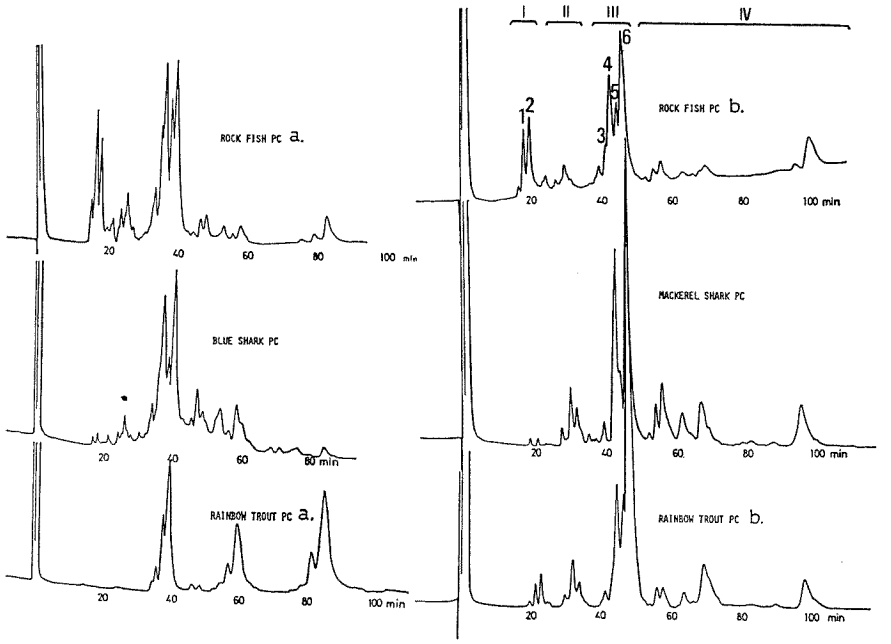

Fig. 5. HPLC chromatograms of rock fish, blue shark, mackerel shark and rainbow trout muscle PC.

a: Captured in 1982. b: Captured in 1983.
Blue shark and mackerel shark are the samples of 1982.
Numbers and conditions are the same as in Fig. 1.

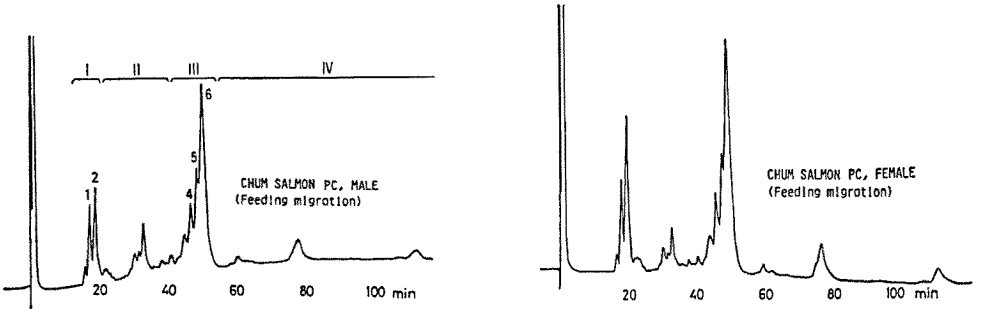

Fig. 6. HPLC chromatograms of chum salmon muscle PC.
Both are the samples of 1983.
Numbers and conditions are the same as in Fig. 1.

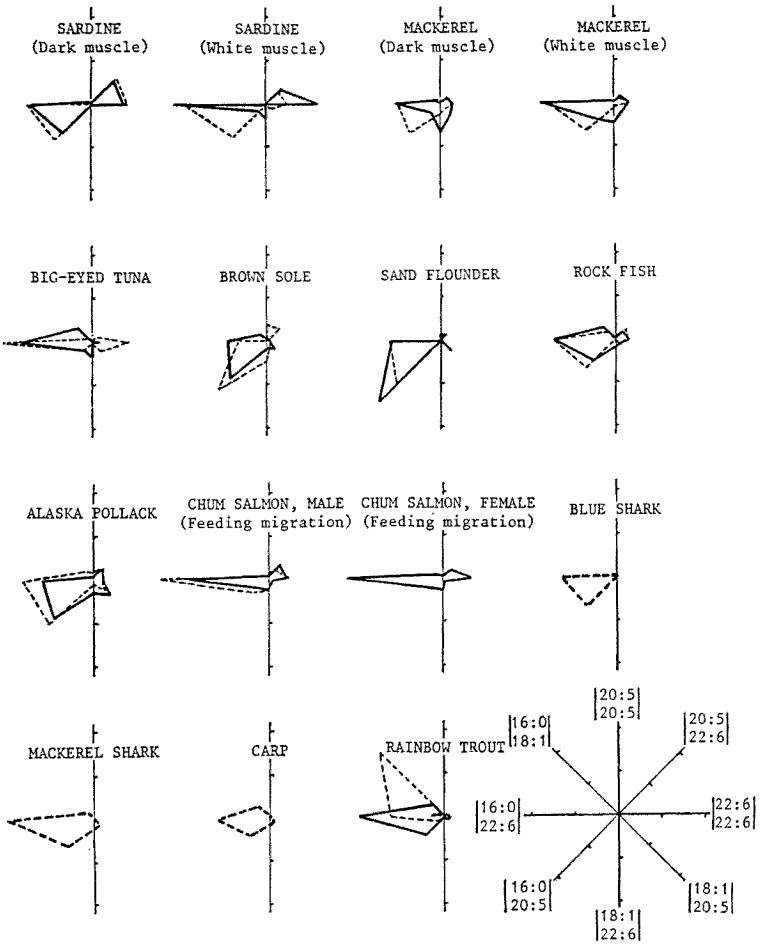

Fig. 7. Radar charts of the main molecular species of muscle PC, in relative %.
Dotted lines are the ones captured in 1980–1982.
Solid lines are the ones captured in 1983–1984.

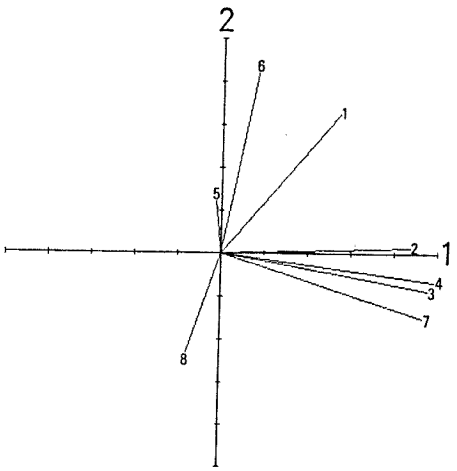

Fig. 8. Eigenvectors of major molecular species on the first and second principal component plane on PCA.
1. (20: 5)(20: 5) 2. (20: 5)(22: 6)
3. (22: 6)(22: 6)
4. (20: 5)(18: 1)+(18: 1)(20: 5)
5. (22: 6)(18: 1)+(18: 1)(22: 6)
6. (20: 5)(16: 0)+(16: 0)(20: 5)
7. (22: 6)(16: 0)+(16: 0)(22: 6)
8. (18: 1)(16: 0)+(16: 0)(18: 1)

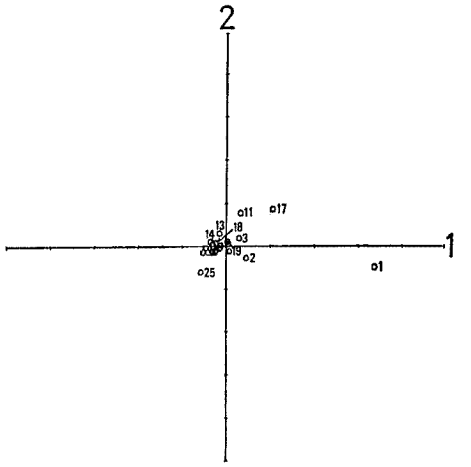

Fig. 9. Plots of principal loading on the first and second principal component plane on PCA. Refer to the eigenvectors in Fig. 8 as background of this plane.
1. Sardine PC (Dark muscle), July 1982
2. Sardine PC (Dark muscle), Oct. 1983
3. Sardine PC (White muscle), July 1982
4. Sardine PC (White muscle), Oct. 1983
5. Mackerel PC (Dark muscle), July 1982
6. Mackerel PC (Dark muscle), Oct. 1983
7. Mackerel PC (White muscle), July 1982
8. Mackerel PC (White muscle), Oct. 1983
9. Big-eyed tuna PC, Frozen 1981
10. Big-eyed tuna PC, Frozen 1983
11. Brown sole PC, Oct. 1982
12. Brown sole PC, May 1983
13. Sand flounder PC, Dec. 1982
14. Sand flounder PC, May 1983
15. Rock fish PC, Oct. 1982
16. Rock fish PC, July 1983
17. Alaska pollack PC, Dec. 1981
18. Alaska pollack PC, Jan. 1984
19. Chum salmon PC, Male, June 1980
20. Chum salmon PC, Male, Aug. 1983
21. Chum salmon PC, Female, Aug. 1983
22. Blue shark PC, June 1982
23. Mackerel shark PC, July 1982
24. Carp PC, Sep. 1980
25. Rainbow trout PC, Sep. 1982
26. Rainbow trout PC, May 1983

ponent analysis (PCA) was the modified program of "Personal Computer Library, Vol. 3".[8] The original program was written for NEC PC-8001 personal computer. But it was modified for NEC PC-8801 mkII personal computer, for instance, the arrangement of the program was changed into N-88 BASIC, and the character mode was changed into graphic mode.

"EIGENVALUE" was calculated by the Jacobi method from correlation matrix. The contribution up to the second principal component was 67.4%.

In Fig. 8, eigenvectors of major molecular species are shown as small numbers on the first and second principal component plane. Principal loadings of all fish examined were plotted on the first and second principal component plane as shown in Fig. 9.

As shown in Fig. 9, sardine dark muscle has a drastic seasonal change (point number 1 and number 2). And among the white flesh fish, Alaska pollack has a large seasonal change (point number 17 and number 18). The movement of point number 1 to point number 2 is the direction of eigenvector number 4 that shows the direction of (20: 5) : 1)+(18:(18 1) (20: 5) combinations. So we might say that sardine dark muscle has a large seasonal variation in the molecular species composed of 20: 5 and 18: 1.

Fig. 10 is the magnified figure of Fig. 9. From Fig. 10, generally speaking, there seems to be a direction on the axis of oval shape shown in the movement between the same fish except in the

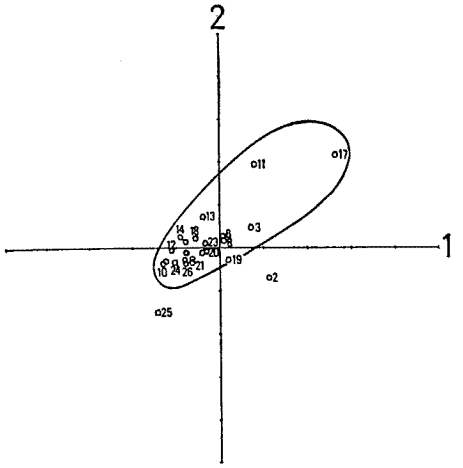

Fig. 10. Plots of principal loading on the magnified first and second principal component plane on PCA. Refer to the eigenvectors in Fig. 8 as background of this plane.
Sample numbers are the same as in Fig. 9.
Number 1 is out of range.

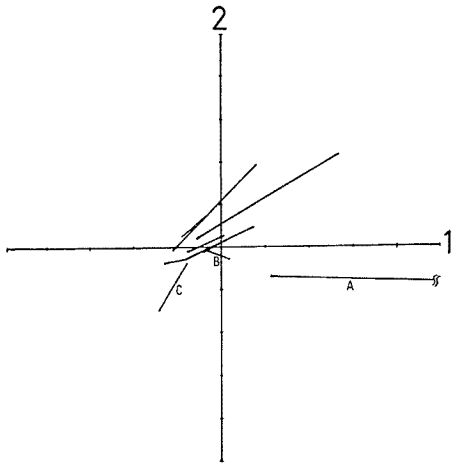

Fig. 11. Movement of the plots between the two different seasons on the same plane in Fig. 10.
Line A: Sardine PC (Dark muscle)
Line B: Chum salmon PC, Male
Line C: Rainbow trout PC

case of sardine dark muscle, chum salmon and rainbow trout. This direction coincide with eigenvector of (20: 5) (20: 5). Though further supplementary studies, should be done to get a conclusion, it is assumed that (20: 5) (20: 5) is the most reflectable molecular species against seasonal

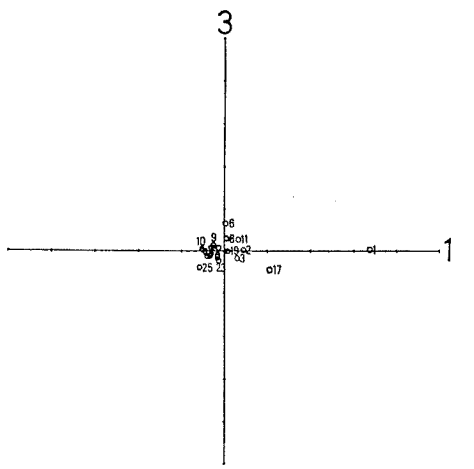

Fig. 12. Plots of principal loading on the first and third principal component plane on PCA. Sample numbers are the same as in Fig. 9.

variations among the majority of fish. This is more evidently shown in Fig. 11, and only sardine dark muscle (shown as A), chum salmon (shown as B) and rainbow trout (shown as C) have different directions. As it is well known, the general characteristics of these three fish is as follows:

Sardine dark muscle:	Drastic change in lipid content.
Chum salmon:	Migration from sea to river.
Rainbow trout:	Fresh water fish.

So, these characteristics might affect the molecular species of muscle PC.

Fig. 12 shows the distribution of each fish on the first and third principal component plane. The outstandingly deviated points are those of sardine dark muscle (point number 1) and Alaska pollack (point number 17) as it is seen in the first and second principal component plane. Those of rainbow trout and mackerel dark muscle also have a deviation in this plane, but not so large as it is in the case of sardine dark muscle and Alaska pollack.

Though supplementary studies should be done to be conclusive, molecular species composition of fish muscle PC seem to vary in some kind of fish in considerable degrees while they keep their principal characteristics.

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