Occurrence and Distribution of *cis*-7-Icosenoic Acid in the Lipids of Japanese Marine Fish, Shellfish, and Crustaceans

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Running title: Distribution of *cis*-7-Icosenoic Acid in Marine Animals

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Abstract: This paper reports the concentration and composition of the isomers of *cis*-icosenoic acid (20:1) in the fatty acids of various species of Japanese marine animals. The main purpose of this study is to reveal the distribution of a positional isomer, *cis*-7-20:1 (*c*7-20:1), in marine animals. Because this isomer overlaps with the *c*9-20:1 isomer in gas chromatography (GC) on the commonly used polar capillary columns, less information is available on its occurrence and distribution. In this study, the monounsaturated fatty acids isolated by argentation thin-layer chromatography were analyzed by GC using a highly polar capillary column, SLB-IL111 (100-m long), with the highest polarity among the commercially available GC columns. A clear separation between the *c*7- and *c*9-20:1 isomers enabled the analysis of all the isomers of 20:1 present in marine animals. The results confirmed that the *c*7 isomer was a minor component of the 20:1 in the pelagic fish, which is rich in the *c*9 and/or *c*11 isomers as reported previously. In contrast, the *c*7 isomer was one of the major isomers in flatfish, shellfish, crabs, and brittle star. In these samples, the *c*13 isomer also occurred at higher concentrations rather than the *c*9 isomer. Because such a specific pattern of the 20:1 positional isomers was generally observed in both benthic fish and its dietary animals, the *c*7 isomer in the benthic fish probably originated from its diet. These marine animals are used as food in Japan, and therefore the *c*7-20:1 isomer is consumed by humans.

Keywords: *cis*-Icosenoic acid, Fatty acid, Flatfish, Shellfish, Crab, Brittle star, Gas chromatography, SLB-IL111

Introduction

cis-Icosenoic acid (20:1) is one of the major monounsaturated fatty acids in marine organisms. The isomers of 20:1 are usually recognized as *cis*-5-, 9-, 11-, 13-, and 15-20:1 (*c*5, *c*9, *c*11, *c*13, and *c*15-20:1, respectively) [1]. In the 20:1 of the pelagic fish, the major isomer is *c*11-20:1 [2] except the northwest Pacific fish, which is rich in the *c*9-20:1 isomer rather than the *c*11 isomer [3]. Compared to these isomers, less information is available on the *cis*-7-20:1 (*c*7-20:1) isomer. Certain brittle star and flatfish were reported to be rich in this isomer. In a sample of brittle star, concentration of the *c*7 isomer was 60.9% of the total 20:1 isomers [4]. In the flathead flounder ("akagarei" in Japanese), the *c*7 isomer was 32.8–36.1% of the total 20:1 isomers [5]. Certain invertebrates and deep-sea fish were also reported to contain the *c*7-20:1 isomer [6–16]. These data indicate that the *c*7-20:1 isomer is not always rare in marine animals.

Probably, the less information on the *c*7-20:1 isomer is because of the overlapping elution with the *c*9 isomer during the fatty acid analysis by gas chromatography (GC). These isomers elute as a single peak on polar polyethylene glycol columns such as 30-m long Omegawax 320 [17], which is commonly used for the fatty acid analysis of fish oils. Even with more polar columns such as 100-m long SP-2560, the peaks of these isomers are very close to each other for quantifying the *c*7 isomer [18]. On a nonpolar 50-m long HP5 column, the *c*7-20:1 isomer co-eluted with the *c*11 isomer [4]. The compositions of the isomers of the 20:1 of the brittle star and flathead flounder were analyzed by GC-mass spectrometry (GC-MS) of the dimethyl disulfide adducts [4,5] and GC of the oxidative ozonolysis products [5] after isolating 20:1 from the total fatty acids.

Recently, we revealed that the six positional isomers of 20:1 from *c*5 to *c*15 can be almost completely resolved by GC on a highly polar ionic liquid GC column, SLB-IL100 (60-m long) [17,19]. The *c*7 and *c*9 isomers could also be separated from each other. Although the monounsaturated fatty acids should be first separated from the saturated and polyunsaturated fatty acids, this column is advantageous in that the analysis can be carried out for the methyl ester derivatives without further derivatization or degradation. This aspect is favorable for the analysis of many samples.

In this study, various species of marine animals popular in Japan were analyzed by GC using a highly polar ionic liquid column, SLB-IL111 (100-m long), with the highest polarity among the commercially available GC columns. Clear separations of the *c*5, *c*7, *c*9, *c*11, *c*13, and *c*15 isomers of 20:1 were achieved using the SLB-IL111 column as well as those using SLB-IL100 [20]. This paper reports the composition of the isomers of 20:1 as the fundamental information on the distribution of the *c*7-20:1 isomer in marine animals, particularly in the Japanese foods.

Materials and methods

Materials

A total of 38 species of the pelagic fish, benthic fish, shellfish, crustaceans, and other invertebrates were obtained from the food stores in Japan (raw or frozen). The two exceptional samples were caught in the Bering Sea while fishing on a training ship "Oshoro-maru" of the Faculty of Fisheries, Hokkaido University. The Japanese common names, catching locations, and parts subjected to lipid extraction are listed in Tables 1 and 2 along with the analytical results.

Preparation of fatty acid methyl esters

The total lipids were extracted from the samples following the method of Bligh and Dyer [21]. The lipids were then converted to the corresponding fatty acid methyl esters by heating them in 7% BF_3-mathanol at 100 °C for 1 h. The methyl esters were purified by thin-layer chromatography (TLC) on Silica gel G plates (10 × 10 cm, 0.25 mm thickness; Analtech, Newark, USA) using a mixture of hexane/diethyl ether (85:15, v/v) as the eluent. For the lipids except the fish lipids, the methyl esters were further purified by TLC using toluene as the eluent to remove the dimethyl acetals obtained from the vinyl ether lipids [22].

The monounsaturated fatty acids were separated from the saturated and polyunsaturated fatty acids by argentation TLC [23]. Silica gel 60G plates (20 × 5 cm, 0.25 mm thickness; Merck, Darmstadt, Germany) were dipped in 10% $AgNO_3$-acetonitrile in dark for 30 min and then activated at 110 °C for 30 min. After spotting the fatty acid methyl esters at ~0.5 mg/cm, the plate was developed using a mixture of hexane/toluene (1:1, v/v). The monounsaturated fatty acids were extracted in diethyl ether and purified by TLC as described above.

Gas chromatography

The methyl esters of monounsaturated fatty acids were analyzed using a GC-4000 gas chromatograph (GL Sciences, Tokyo, Japan) equipped with SLB-IL111 (100 m × 0.25 mm i.d., 0.20-µm film thickness; Supelco, Bellefonte, USA) and a flame ionization detector. The oven temperature was held at 160 °C. The injector and detector temperatures were 240 °C. The carrier gas was helium at a linear velocity of 20 cm/s (345 kPa). The split ratio was 20:1. Peaks were monitored using a Shimadzu C-R3A integrator (Shimadzu Corporation, Kyoto, Japan). The peaks of the 20:1 isomers were identified by comparing the retention times with those of the *c*5- to *c*15-20:1 isomers of the flathead flounder previously confirmed by GC-MS as their dimethyl disulfide adducts [17,19].

The methyl esters of the total fatty acids were analyzed using a Shimadzu GC-18A gas chromatograph (Shimadzu Corporation) equipped with a Restek FAMEWAX column (30 m × 0.32 mm i.d., 0.25-µm film thickness; Restek, Bellefonte, USA) and a flame ionization detector. The oven temperature was programmed from 174 °C (0 min) to 240 °C at a rate of 4 °C/min and held at the final temperature for 24 min. The injector and detector temperatures were 240 °C.

The carrier gas was helium at a linear velocity of 33 cm/s at 174 °C (85 kPa). The split ratio was 25:1. The peaks were monitored using a Shimadzu C-R6A integrator (Shimadzu Corporation).

Results

GC separation of 20:1 isomers on SLB-IL111

Figure 1 shows the parts of typical gas chromatograms of the monounsaturated fatty acids, separated on a 100-m long SLB-IL111 column. Both the 60-m long SLB-IL100 column [17] and 100-m long SLB-IL111 column afforded clear separations of the six isomers of 20:1, which are different in the *cis*-olefinic bond position by two carbons from the C5 to C15 position (Figs. 1b and 1d). The elution order was: *c*5, *c*7, *c*9, *c*11, *c*13, and *c*15-20:1.

Pelagic fish

The gas chromatogram of the 20:1 from the Japanese sardine (“maiwashi”) is shown in Fig. 1a. The composition of the isomers of the 20:1 in the pelagic fish is summarized in the upper part of Table 1 along with the 20:1 content in the total fatty acids analyzed by GC using the FAMEWAX column. In general, the *c*7-20:1 isomer was low in the pelagic fish. The highest concentration of the *c*7 isomer in the 20:1was 6.7% in the Japanese surf smelt (“chika”) and 4.0% in the Japanese anchovy (“katakuchi-iwashi”), both of which had a low content of 20:1 in the total fatty acids. In the other pelagic fish, the concentrations of the *c*7 isomer were ~1% or less, whereas the *c*9 isomer was generally the highest isomer followed by the *c*11 and *c*13 isomers. The pelagic samples were also characterized by the “not detectable” level of the *c*5-20:1 isomer.

Benthic fish

The benthic fish in this study comprised flatfish and others, such as kichiji rockfish ("kichiji") and sailfin sandfish ("hatahata"), which contained 20:1 at 2.3–15.6% of the total fatty acids. Figure 1b shows the chromatogram obtained for brown sole ("magarei"). The lower part of Table 1 shows the composition of the isomers of the 20:1 in the benthic fish. Except four species, flatfish was rich in the *c*7-20:1 isomer. The concentrations were 21.5–46.6% of the total 20:1 isomers. The highest concentrations were found in the cresthead flounder ("kurogashiragarei") (46.6%) and flathead flounder ("akagarei") (44.7%). These *c*7-rich fishes had generally lower *c*9 isomer than the *c*11 and *c*13 isomers. In this respect, the 20:1 composition of the benthic fish was different from that of the pelagic fish as described above. Further, most of the benthic fish had up to 9.3% of the *c*5 isomer. The exceptional fishes, such as the Japanese flounder ("hirame") and Kamchatka flounder ("aburagarei"), showed the highest concentrations of the *c*9 and *c*11 isomers.

Shellfish

The bivalves and gastropods contained 2.5–12.0% of 20:1 in the total fatty acids. These levels were compared to those observed in the fish samples. All the shellfish samples showed high concentrations of the *c*7 isomer in the total 20:1 isomers (Fig. 1c and Table 2). The highest percentages were found in the brackish-water clam ("yamatoshijimi") (78.4–81.6%), Ezo-abalone ("ezoawabi") (61.1–76.6%), and spiny top-shell ("sazae") (60.2–69.7%). Other shellfish samples were also rich in the *c*7 isomer (24.0–65.1%). The high concentration of the *c*7 isomer was independent of the body parts of shellfish, i.e., the muscle and viscera. Other major isomers were the *c*11 and *c*13 isomers, whereas the *c*9 isomer was low in all the samples. This is in contrast to the results obtained for the pelagic fish. The *c*5 and *c*15 isomers were found at <1.3% level.

Crustaceans and other invertebrates

The *c*7 isomer was also found in the crabs, shrimps, and acorn barnacle ("minefujitsubo"). The concentrations were higher in the crabs and their viscera in particular (28.5–35.0%) (Fig. 1d and Table 2). The crabs were also rich in the *c*11 and *c*13 isomers, while the *c*9 isomer was low. The pattern for the composition of the isomers of 20:1 was similar to that of shellfish. The shrimp and barnacle had relatively lower *c*7 isomer than the *c*9–*c*13 isomers.

The Japanese common squid ("surumeika") contained ~4% of the *c*7 isomer in the 20:1 of their mantle and hepatopancreas. The ink sac contained higher concentrations of the *c*7 isomer (18.4%). The mantle contained the *c*11 isomer as the major isomer of 20:1, and the hepatopancreas contained the *c*9 isomer.

An unidentified brittle star ("kumohitode") was rich in the *c*7 isomer (61.1%). The brittle star also showed the highest concentration of the *c*5-20:1 isomer than all the samples examined in this study (11.2%). This level was followed by the roughscale sole ("samegarei") and flathead flounder ("akagarei").

Discussion

In general, marine fish contains the *c*11-20:1 isomer as the most abundant isomer of 20:1 [2]. In our previous research, the Atlantic herring landed on Norway was rich in this isomer (87.5% of the total 20:1 isomers) [19], which resembled the muscle lipids of other Atlantic herring, capelin, and mackerel of the Atlantic water [24]. A similar trend (*c*11, >~50%) was observed in this study for the two samples of the pelagic fish and three samples of the benthic fish. However, most of the samples of the pelagic fish showed the highest concentration of the *c*9 isomer of 20:1. Saito and Ishikawa [3] reported that the fish and squid living in the northwest Pacific are rich in the *c*9-20:1 isomer rather than the *c*11-20:1 isomer. Our results agree with their observation. Although the pelagic armorhead ("kusakaritsubodai") was rich in the *c*11 isomer, this fish was not caught in the Japanese area but in the mid-Pacific near Midway Atoll.

Compared to the pelagic fish, most of the benthic fish were rich in the *c*7 isomer. The composition of 20:1 isomers in marine fish is not independent on their diet. Almost all the 20:1 and docosenoic acid (22:1) in fish depot fat have been recognized to be of exogenous origin [25]. The high concentration of the *c*7 isomer in the benthic fish also probably originated from their diet different from that of the pelagic fish. Although the typical pelagic fish with high contents of 20:1 and 22:1 usually feed on zooplankton such as copepods [24,25], the benthic fish can feed on benthic crustaceans, shellfish, and other invertebrates. In this study, bivalves, gastropods, crabs, and brittle star were rich in the *c*7 isomer.

When a principal component analysis was carried out using the concentrations of the *c*7–*c*13 isomers, this probability seemed to be true from the plots of PC1 *vs*. PC2 (Fig. 2). Roughscale sole ("samegarei") preferentially feed on brittle star [26,27]. The flathead flounder ("akagarei") was also reported to feed brittle star, even though regional or seasonal difference was observed [28]. As shown in Fig. 2, the plot for the flathead flounder fell near that of brittle star, and roughscale also showed the plot relatively near brittle star rather than benthic crustaceans. In contrast, most of the flatfish afforded the plots near the benthic crustaceans. These flatfish probably had a similar dietary habit, i.e., particularly benthic crustaceans and crab. The Japanese flounder ("hirame") and pointhead flounder ("sohachi") that showed the plots far from the other flounders may have resulted from dietary small fish characteristic of these species[29,30]. The Kamchatka flounder ("aburagarei") was caught in the Bering Sea. Habitat also seems to affect the composition of the isomers of dietary 20:1. This flounder living in the Bering Sea were reported to preferentially take walleye pollock [31].

This study also showed that the *c*7 isomer was mainly present in shellfish and brittle star. In a recent study on the trophic relationship of benthic invertebrate fauna from the continental slope in the Sea of Japan, approximately half of the studied animals such as bivalve, brittle star, and starfish contained significant amounts of the *c*7-20:1 isomer [15]. The authors suggested the importance of the benthic microbial loop in the animals' diets [15]. It is unclear whether the *c*7-20:1 isomer originally occurred in shellfish. However, a high concentration of the *c*7 isomer was found in filter-feeding bivalves, herbivorous Ezo-abalone ("ezoawabi"), and carnivorous Ezo-neptune ("ezobora"). The *c*7-20:1 acid seems to be synthesized in shellfish rather than its diets.

Because no evidence for Δ7-desaturase in animals has been reported so far, possible biosynthesis pathway of the *c*7-20:1 isomer seemed limited to the chain elongation of *c*5-18:1 or β-oxidation of *c*9-22:1, which can be synthesized from saturated fatty acids by Δ5- and Δ9-desaturases, respectively [4]. In the GC on the SLB-IL111 column, the *c*5-18:1 isomer was detected along with the *c*5-20:1 isomer in the shellfish (data not shown), which supports the desaturation at the Δ5 position and pathway *via* the chain elongation of the *c*5-18:1 isomer.

As a function of 20:1, a recent study elucidated the effect of saury oil monounsaturated fatty acids containing large amounts of 20:1 and 22:1 isomers on metabolic disorders in mice [32]. Dietary monounsaturated fatty acids improved insulin resistance and alleviated metabolic syndrome risk factors by reducing blood glucose and lipids. These favorable changes were attributed to an improved adipocytokin profile [32]. The monounsaturated fatty acid concentrate of saury oil contains the *c*11- and *c*13-20:1 isomers at the concentrations of 22.73% and 5.44%, respectively. The major isomer, *c*11, was different from that of saury analyzed in this study (*c*9 isomer; Table 2), and occurrence of the *c*7 isomer was unknown. It seems important to precisely determine the composition of the isomers of the 20:1 in edible marine lipids to determine bioactive fatty acids.

In conclusion, this is the first report on the details of the composition of a positional isomer of 20:1, in particular, the concentrations of the *c*7-20:1 isomer in various species of marine animals. The result shows that the *c*7 isomer was low in the pelagic fish, whereas most of the benthic animals contain this isomer at significantly high concentrations in the total 20:1 isomers. Except brittle star, the animals analyzed in this study are consumed as foods in Japan.

Therefore, humans consume the *c*7-20:1 isomer through marine foods.

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Figure legends

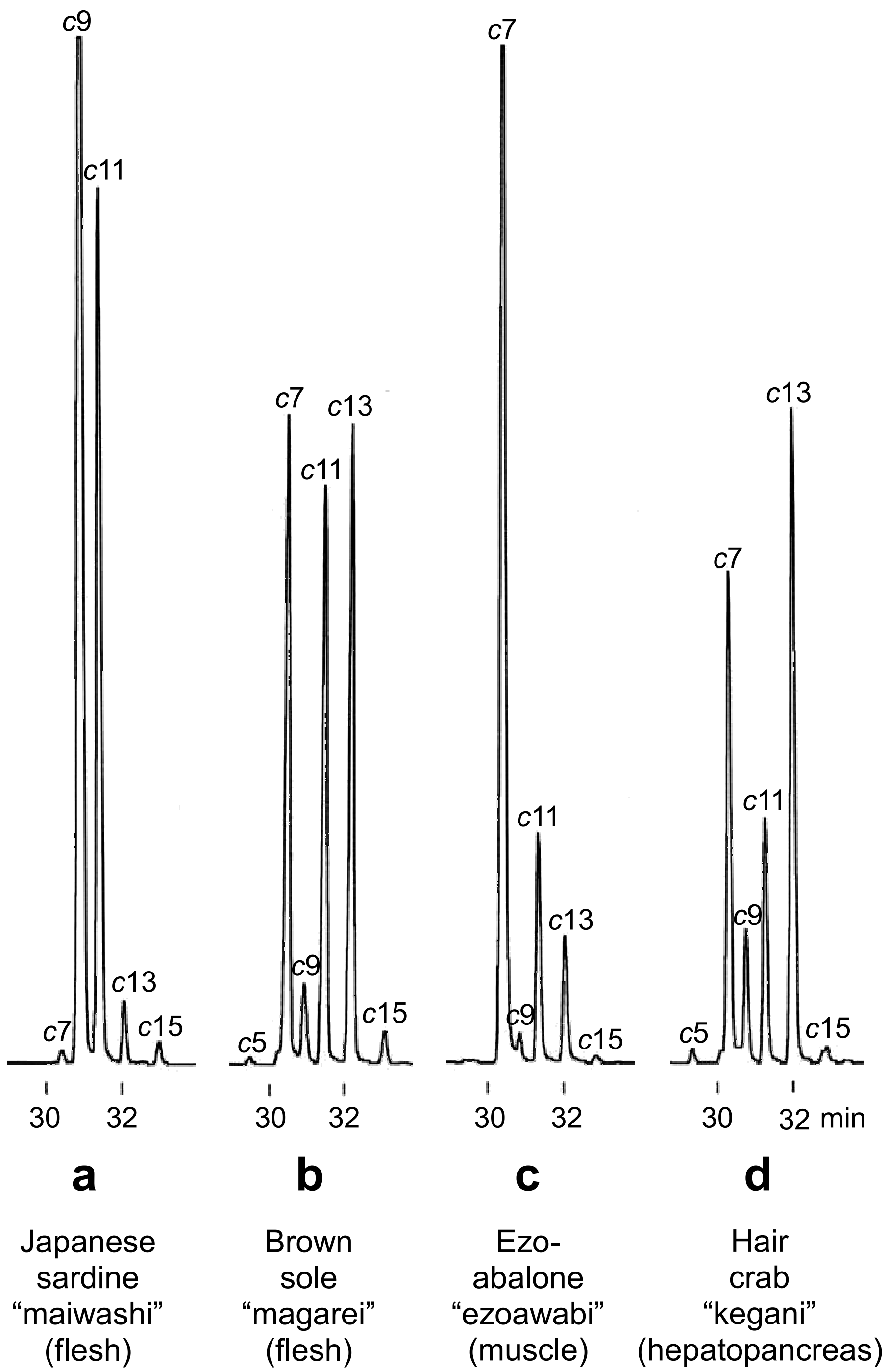
Fig. 1 Typical gas chromatograms of 20:1 methyl esters, prepared from the Japanese marine animals, on the ionic liquid column SLB-IL111 (100 m × 0.25 mm id) at isothermal 160 °C.

Fig. 2 Scores plot of first and second principal components (PC1 and PC2) derived from the concentrations of the major *c*7-, *c*9-, *c*11-, and *c*13-20:1 isomers in the total 20:1 isomers of the individual samples. PC1 and PC2 were calculated from the following equations:

$$PC1 = 0.56\ C_{c7\text{-}20:1} - 0.56\ C_{c9\text{-}20:1} - 0.43\ C_{c11\text{-}20:1} + 0.43\ C_{c13\text{-}20:1} \quad (1)$$

$$PC2 = 0.39\ C_{c7\text{-}20:1} + 0.38\ C_{c9\text{-}20:1} - 0.58\ C_{c11\text{-}20:1} - 0.60\ C_{c13\text{-}20:1} \quad (2)$$

where C represents the concentration of each 20:1 isomer in the total 20:1 acids. PC1 + PC2 explained 55.8 and 25.6% of the variability, respectively.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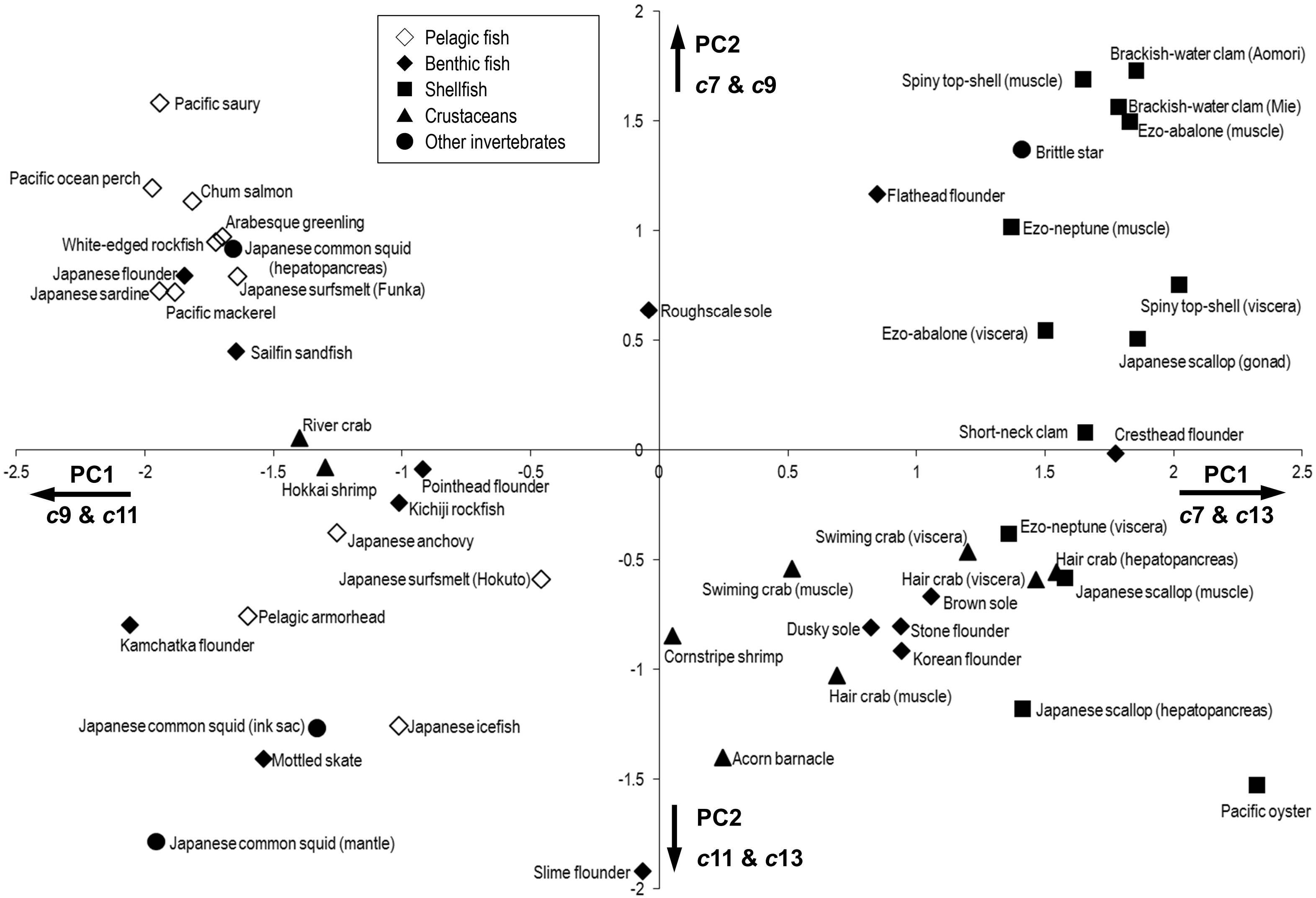
Japanese sardine "maiwashi" (flesh)
Brown sole "magarei" (flesh)
Ezo-abalone "ezoawabi" (muscle)
Hair crab "kegani" (hepatopancreas)

Fig. 1



Pelagic fish
Benthic fish
Shellfish
Crustaceans
Other invertebrates
PC2
c7 & c9
PC2
c11 & c13
PC1
c9 & c11
PC1
c7 & c13
Pacific saury
Pacific ocean perch
Chum salmon
Arabesque greenling
White-edged rockfish
Japanese common squid (hepatopancreas)
Japanese flounder
Japanese sardine
Japanese surfsmelt (Funka)
Pacific mackerel
Sailfin sandfish
Roughscale sole
River crab
Hokkai shrimp
Pointhead flounder
Kichiji rockfish
Japanese anchovy
Japanese surfsmelt (Hokuto)
Pelagic armorhead
Kamchatka flounder
Japanese common squid (ink sac)
Japanese icefish
Mottled skate
Japanese common squid (mantle)
Slime flounder
Brackish-water clam (Aomori)
Spiny top-shell (muscle)
Brackish-water clam (Mie)
Ezo-abalone (muscle)
Brittle star
Flathead flounder
Ezo-neptune (muscle)
Spiny top-shell (viscera)
Ezo-abalone (viscera)
Japanese scallop (gonad)
Short-neck clam
Cresthead flounder
Ezo-neptune (viscera)
Swiming crab (viscera)
Hair crab (hepatopancreas)
Hair crab (viscera)
Swiming crab (muscle)
Japanese scallop (muscle)
Brown sole
Dusky sole
Stone flounder
Korean flounder
Cornstripe shrimp
Hair crab (muscle)
Japanese scallop (hepatopancreas)
Acorn barnacle
Pacific oyster

Fig. 2

Table 1 Positional isomer composition of icosenoic acid (20:1) in marine fish samples

Common name	Scientific name	Japanese name	Location	Part	Total 20:1 content (wt%)[c]	Isomer composition of 20:1 (%)					
						c 5	*c* 7	*c* 9	*c* 11	*c* 13	*c* 15
Pelagic fish											
Arabesque greenling	*Pleurogrammus azonus*	hokke	Esan[a]	flesh[b]	8.1	-	1.6	66.8	24.8	5.7	1.1
Chum salmon*	*Oncorhynchus kata*	shirozake	Funka Bay[a]	flesh[b]	16.5	-	0.8	71.0	23.4	4.2	0.6
Japanese anchovy*	*Engraulis japonicus*	katakuchi-iwashi	Sawara[a]	whole	1.8	-	4.0	37.3	43.1	12.8	2.9
Japanese icefish	*Salangichthys microdon*	shirauo	Funka Bay[a]	whole	5.1	-	2.4	22.9	52.8	20.1	1.9
Japanese sardine	*Sardinops melanostictus*	maiwashi	Kushiro[a]	flesh[b]	6.4	-	0.6	62.8	33.2	2.4	1.0
Japanese surfsmelt	*Hypomesus japonicus*	chika	Hokuto[a]	whole	1.4	-	6.7	33.3	33.3	26.7	-
			Funka Bay[a]	whole	6.7	-	1.2	63.8	26.7	7.3	1.1
Pacific mackerel	*Scomber japonicus*	masaba	Unknown (Japan)	flesh[b]	9.3	-	0.6	62.7	32.2	3.4	1.1
Pacific ocean perch	*Sebastes alutus*	arasukamenuke	USA	flesh[b]	11.5	-	-	72.6	24.7	1.8	0.9
Pacific saury*	*Cololabis saira*	sanma	Akkeshi[a]	flesh[b]	14.2	-	0.7	79.0	17.8	1.0	1.5
Pelagic armorhead	*Pentaceros richardosoni*	kusakaritsubodai	Midway Atoll	flesh	0.9	-	1.1	32.6	54.2	9.4	2.8
White-edged rockfish	*Sebastes taczanowskii*	ezomebaru	Esan[a]	flesh[b]	11.1	-	0.2	67.2	24.7	6.1	1.8
Benthic fish											
Brown sole	*Pleuronectes herzensteini*	magarei	Funka Bay[a]	flesh[b]	3.9	0.4	32.7	4.4	28.7	32.0	1.9
Cresthead flounder	*Pleuronectes schrenki*	kurogashoiragarei	Nemuro[a]	flesh[b]	5.8	0.5	46.6	3.5	15.1	33.0	1.3
Dusky sole	*Pleuronectes mochigarei*	asabagarei	Funka Bay[a]	flesh[b]	4.4	1.8	25.3	8.1	29.5	33.3	2.1
Flathead flounder	*Hippoglossoides dubius*	akagarei	Kushiro[a]	flesh[b]	5.2	6.8	44.7	23.1	11.0	13.4	1.1
Japanese flounder	*Paralichthys olivaceus*	hirame	Hakodate[a]	flesh[b]	9.1	-	1.0	63.7	30.4	3.7	1.2
Kamchatka flounder	*Atheresthes evermanni*	aburagarei	Bering Sea	flesh[b]	15.6	-	0.3	33.6	62.9	2.5	0.7
Korean flounder	*Glyptocephalus stelleri*	hireguro	Muroran[a]	flesh[b]	5.1	0.3	22.6	10.3	26.4	38.7	1.8
Pointhead flounder	*Cleisthenes pinetorum*	souhachi	Muroran[a]	flesh[b]	5.4	0.7	7.1	40.6	33.9	16.4	1.3
Roughscale sole	*Clidoderma asperimum*	samegarei	Hidaka[a]	flesh	9.5	9.3	31.7	23.3	27.0	7.3	1.4
Slime flounder	*Microstomus achne*	babagarei	Hidaka[a]	flesh[b]	5.7	0.3	6.2	6.8	49.8	35.0	2.0
Stone flounder	*Kareius bicoloratus*	ishigarei	Kikonai[a]	flesh[b]	3.2	0.5	21.5	12.8	23.7	38.9	2.7
Kichiji rockfish	*Sebastolobus macrochir*	kichiji	Miyagi pref.	flesh[b]	8.5	1.2	5.4	38.7	37.2	15.9	1.7
Mottled skate	*Raja pulchra*	meganekasube	Shari[a]	flesh	2.3	-	-	23.2	63.4	13.4	-
Sailfin sandfish	*Arctoscopus japonicus*	hatahata	Hiroo[a]	flesh[b]	4.8	-	1.4	56.7	33.2	7.4	1.3

* The isomer compositions were previously obtained by GC on SLB-IL100 [11].

[a] Location in Hokkaido prefecture.

[b] Including skin.

[c] Concentration of 20:1 in total fatty acids (wt%).

Table 2 Positional isomer composition of icosenoic acid (20:1) in shellfish, crustaceans, and other invertebrate samples

Common name	Scientific name	Japanese name	Location	Part	Total 20:1 content (wt%)[c]	Isomer composition of 20:1 (%)					
						c 5	*c* 7	*c* 9	*c* 11	*c* 13	*c* 15
Shellfish											
Brackish-water clam	*Corbicula japonica*	yamatoshijimi	Aomori pref.	whole	6.8	0.3	81.6	1.5	12.8	3.8	-
			Mie pref.	whole	9.4	0.2	78.4	1.7	14.3	5.4	-
Japanese scallop	*Mizuhopecten yessoensis*	hotategai	Shiriuchi[a]	muscle	7.2	-	38.0	2.2	21.5	37.8	0.6
				hepatopancreas	2.5	-	28.9	0.5	27.3	43.4	-
				gonad	10.7	-	60.2	0.3	15.9	23.1	0.5
Pacific oyster	*Crassostrea gigas*	magaki	Konbumori[a]	whole	7.4	-	24.0	-	11.4	64.6	-
Short-neck clam	*Ruditapes philippinarum*	asari	Hokkaido pref.	whole	6.6	-	51.8	1.0	20.0	27.2	-
Ezo-abalone	*Hariotis discus hannai*	ezoawabi	Hakodate[a]	muscle	5.2	-	76.6	2.0	13.1	7.7	0.6
				viscera	7.4	-	61.1	-	22.9	16.0	-
Ezo-neptune	*Neptunea polycostata*	ezobora	Funka Bay[a]	muscle	12.0	-	65.1	4.7	20.6	9.6	-
				viscera	11.5	0.3	36.4	7.7	20.6	34.9	0.3
Spiny top-shell	*Turbo cornutus*	sazae	Chiba pref.	muscle	3.0	-	69.7	13.3	6.9	10.1	-
				viscera	5.6	1.3	60.2	4.7	8.4	25.5	-
Crustacean											
Hair crab	*Erimacrus isenbeckii*	kegani	Erimo[a]	muscle	1.5	-	8.0	4.3	24.6	34.9	28.2
				hepatopancreas	7.8	1.0	30.1	9.4	14.9	42.7	2.0
				viscera	5.7	1.0	28.5	8.7	16.1	41.6	4.1
River crab	*Geothelphusadehaani*	sawagani	Unknown (Japan)	whole	0.6	-	13.1	37.9	45.8	3.3	-
Swiming crab	*Portunus trituberculatus*	gazami	Hokkaido pref.	muscle	1.4	2.4	24.8	11.4	30.6	26.5	4.3
				viscera	6.0	1.7	35.0	5.9	24.3	32.3	0.8
Cornstripe shrimp	*Pandalus hypsinotus*	toyamaebi	Shakotan[a]	whole	2.6	1.3	11.9	19.0	33.7	29.4	4.7
Hokkai shrimp	*Pandalus latirostris*	hokkaiebi	Kaminokuni[a]	whole	4.1	2.1	5.7	40.4	40.3	9.7	1.9
Acorn barnacle	*Balanus rostratus*	minefujitubo	Aomori pref.	whole	1.3	0.5	2.5	15.2	32.8	39.6	9.5
Invertebrates											
Japanese common squid	*Todarodes pacificus*	surumeika	Hiyama[a]	mantle[b]	2.9	-	4.7	9.6	82.8	1.9	1.0
				hepatopancreas	5.6	-	4.3	62.4	27.6	3.9	1.8
				ink sac	6.4	-	18.4	6.4	73.3	1.5	0.4
Brittle star	(unidentified)	kumohitode	Bering Sea	whole	7.5	11.2	61.1	7.1	11.7	7.9	1.0

[a] Location in Hokkaido prefecture.

[b] Including skin.

[c] Concentration of 20:1 in total fatty acids (wt%).