NOTE

Fatty Acid Compositions of Various Tissue Lipids in the Marine Bivalves, *Megangulus venulosus* and *Megangulus zyonoensis*, from Coastal Waters of Hokkaido, Northern Japan

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Abstract: Various tissue lipids from the marine bivalves, *Megangulus venulosus* (*M. venulosus*) and *Megangulus zyonoensis* (*M. zyonoensis*), were analyzed for comparison of major fatty acids and their distribution. The main fatty acid components in this genus in all tissues were found to be 16:0 (11.0-15.2%), 18:0 (4.1-8.6%), 20:5n-3 (13.3-25.6%) and 22:6n-3 (8.1-16.7%), with n-3 polyunsaturated fatty acids most abundant. The proportion of 20:5n-3 was higher in *M. zyonoensis* than in *M. venulosus*, and 22:6n-3 was higher in *M. venulosus* than in *M. zyonoensis*. The proportions of 20:5n-3 and 22:6n-3 were highest in muscle tissue lipids from *M. venulosus* and *M. zyonoensis*, at 33.1% and 36.2%, respectively. The distribution of major fatty acid components in neutral (NL) and polar lipids (PL) was determined, with 16:0 and 20:5n-3 shown for NL, and 16:0, 20:5n-3 and 22:6n-3 for PL. The proportion of 22:6n-3 in NL and PL differed significantly, 22:6n-3 being much higher in PL. The main branched fatty acid component, 4,8,12-trimethyltridecanoic acid, was found mainly in PL of viscera tissues from either species. In addition, 2-hydroxypentadecanoic, 2-hydroxyhexadecanoic and 2-hydroxyheptadecanoic acids were also found to be present in the two species and this presence of 2-hydroxy fatty acids in the genus *Megangulus* is reported here for the first time.

Key words: fatty acid composition, tissue lipid, bivalve, *Megangulus venulosus*, *Megangulus zyonoensis*

1 Introduction

The marine bivalve, Shirogai, belonging to the genus, *Megangulus*, is a valuable food resource, owing to its soft flesh texture, rich sweetness and good taste, especially in Japan (1). However, the production of this bivalve is showing a slightly decreasing trend from year to year. Biogenic management of Shirogai is not adequate, for the reasons are unclear so far. Its reproduc-

tive cycle and environment conditions are poorly understood (2). Biochemical and physiological studies on the reproductive cycle of Shirogai have not been reported previously. But lipids have been shown to be essential to the reproductive process (3-5). Examination of lipids and fatty acids has provided some clarification of biochemical and environmental conditions for bivalve growth and distribution. Similarities and differences in fatty acids according to tissue in the leading commer-

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cial species, *Megangulus venulosus* and *Megangulus zyonoensis*, have not been reported. This paper presents for the first time an investigation for comparison of major fatty acids and their distribution. Basic clarification is made of specific roles of lipids and fatty acids in various organs as well as the reproductive cycle and natural environments of the two species.

2 Materials and Methods

The bivalves, *Megangulus venulosus* (*M. venulosus*) and *Megangulus zyonoensis* (*M. zyonoensis*), were procured from a local fish market (Tomakomai fishermen's cooperative association, Hokkaido) in March and April, 2002 and stored at -20°C until needed for analysis. All tissues were suspended in a chloroform-methanol mixture (2:1, v/v), and then homogenated for 30 sec at 11,000 rpm with an IKA ULTRA-TURRAX® T25 Basic (IKA Japan KK, Nara, Japan) for cell disruption. Lipids were extracted according to the method of Bligh and Dyer (6).

Fatty acid methyl esters (FAME) and dimethylacetals (DMA) were prepared with 14%BF₃/methanol for 1h at 90°C in a screw-capped test tube (7). FAME and DMA were purified by thin-layer chromatography (TLC) on Kieselgel 60G plates (Merck, Co., Ltd., Darmstadt, Germany) with *n*-hexane-diethyl ether (80:20, v/v) for development. Individual lipid classes were quantified by preparative TLC. The various lipid classes were separated by TLC with *n*-hexane-diethyl ether-acetic acid (80:20:1, v/v/v) or chloroform-methanol-water (65:25:4, v/v/v) and the developed TLC plates were sprayed with 50% sulfuric acid solution or Dittmer reagent (8), followed by heating at 100°C for 10 min. Neutral and polar lipids were separated with a mixture of chloroform-methanol (9:1, v/v) by TLC and then recovered from the TLC plates by scraping off the corresponding spots. Percentages of neutral and polar

lipids were expressed as amounts of total fatty acids in each lipid.

FAME were analyzed by gas-liquid chromatography (GC) with a Shimadzu GC-8A instrument (Shimadzu Seisakusho Co., Kyoto, Japan) equipped with a flame ionization detector and a SUPELCOWAX™-10 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Supelco Inc., Bellefonte, PA, USA). The column temperature was isothermal at 210°C. The injector and detector temperatures were 250 and 260°C, respectively. The carrier gas was helium at a flow rate of 1.0 mL/min. FAME and DMA were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard Agilent 5973N MSD and Agilent 6890N GC (Hewlett-Packard, Palo Alto, CA, USA) equipped with an OMEGAWAX™-320 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness; Supelco Inc., Bellefonte, PA, USA). Column temperature was programmed as follows: isothermal at 150°C for 1 min, increased from 150 to 250°C (10°C/min) where maintained for 5 min. All spectra were obtained at an ionization energy of 70 eV (electron impact ionization). Analysis in all cases was conducted in duplicate with the results expressed as mean values. Most standards and reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

3 Results and Discussion

Total lipids (TL) of mantle, muscle and viscera as wet weight basis of percentage were 0.7, 0.5 and 0.8% in *M. venulosus*, and 0.6, 0.6 and 1.6% in *M. zyonoensis*, respectively (shown in **Table 1**). The mantle and muscle lipids for either species were mainly polar lipids (PL), and constituted 77-78% of TL. Viscera tissue from *M. zyonoensis* contained 80% neutral lipids (NL), the amount of which was greater than from

Table 1 Total Lipid Content and Proportion (%) of Neutral and Polar Lipids in Various Tissues from *Megangulus venulosus* and *Megangulus zyonoensis*.

	Mantle		Muscle		Viscera	
	<i>M. venulosus</i>	<i>M. zyonoensis</i>	<i>M. venulosus</i>	<i>M. zyonoensis</i>	<i>M. venulosus</i>	<i>M. zyonoensis</i>
Total lipid (wet wt)	0.7	0.6	0.5	0.6	0.8	1.6
Neutral lipids*	21.7	22.9	24.7	23.3	22.7	80.2
Polar lipids*	78.3	77.1	75.3	76.7	77.3	19.8

* Value expressed as amounts of total fatty acids.

Table 2 Fatty Acid Composition (%) of Total Lipids in Various Tissues of *Megangulus venulosus* and *Megangulus zyonoensis*.

Fatty Acid	Mantle		Muscle		Viscera	
	<i>M. venulosus</i>	<i>M. zyonoensis</i>	<i>M. venulosus</i>	<i>M. zyonoensis</i>	<i>M. venulosus</i>	<i>M. zyonoensis</i>
TMTD ¹⁾	<0.1	<0.1	<0.1	<0.1	1.9	2.2
phytanic acid ²⁾	0.3	0.7	0.3	0.4	0.3	1.1
2-hydroxy 15:0	0.6	<0.1	<0.1	<0.1	nd ⁵⁾	nd
2-hydroxy 16:0	2.6	2.1	1.7	1.7	1.6	0.4
2-hydroxy 17:0	0.7	<0.1	0.5	<0.1	<0.1	nd
iso 17:0	0.4	0.4	0.3	<0.1	0.4	<0.1
iso 18:0	0.9	0.8	0.7	0.6	0.7	0.5
iso 19:0	0.6	0.4	0.3	0.4	0.3	<0.1
14:0	0.7	1.3	0.9	1.3	1.2	4.0
15:0	1.9	1.6	1.9	1.5	1.8	0.6
16:0	11.0	14.0	12.5	15.2	11.0	12.9
17:0	0.9	0.7	0.8	0.6	0.8	0.2
18:0	8.2	8.4	8.6	8.4	8.5	4.1
19:0	0.7	0.6	<0.1	nd	<0.1	nd
20:0	0.3	0.3	0.2	<0.1	0.3	<0.1
16:1n-7	3.0	4.0	3.6	4.7	3.3	10.8
16:1n-4 and/or n-5	0.2	0.2	0.2	0.3	0.7	0.3
17:1	<0.1	0.6	<0.1	0.4	<0.1	<0.1
18:1n-9	2.5	2.0	2.2	1.6	1.9	1.0
18:1n-7	2.3	3.1	2.1	2.6	2.7	4.3
20:1n-13	3.6	2.4	3.3	1.6	2.8	0.8
20:1n-9	2.1	2.2	1.6	2.5	2.1	1.3
20:1n-7	3.7	4.5	3.4	3.8	4.2	2.6
18:2n-6	0.5	0.5	0.2	0.5	0.7	0.5
20:2n-6	0.8	1.1	0.7	0.8	0.8	0.8
20:2	0.3	0.4	0.3	<0.1	0.3	0.5
7,13-22:2	1.2	0.8	0.6	<0.1	0.9	<0.1
7,15-22:2	6.3	4.3	3.3	2.4	4.7	1.0
16:3	<0.1	<0.1	<0.1	<0.1	<0.1	0.8
18:3	<0.1	<0.1	<0.1	<0.1	<0.1	0.6
20:3	0.4	0.3	0.3	<0.1	0.3	<0.1
16:4	nd	<0.1	nd	nd	nd	1.8
18:4n-3	0.5	0.8	0.7	0.8	<0.1	3.7
20:4n-6	6.2	4.3	4.5	2.6	4.4	1.3
20:4n-3	0.5	0.7	0.5	0.5	0.7	1.2
22:4n-6	1.7	1.0	1.2	0.7	1.3	<0.1
20:5n-3	13.3	17.8	16.4	22.3	17.3	25.6
21:5n-3	1.0	1.2	1.6	1.6	1.7	1.7
22:5n-6	1.4	0.9	1.5	0.8	1.2	<0.1
22:5n-3	4.5	3.7	5.2	5.0	4.1	1.3
22:6n-3	11.9	10.2	16.7	13.9	11.5	8.1
Unidentified ³⁾	2.3	1.7	1.2	0.5	3.6	4.0
Saturated ⁴⁾	29.5	30.6	28.4	29.7	28.5	24.9
Monoenes	17.4	19.0	16.4	17.5	17.7	21.1
n-6 PUFA	10.6	7.8	8.1	5.4	8.4	2.6
n-3 PUFA	31.7	34.4	41.1	44.1	35.3	41.6
Others	10.8	8.2	6.0	3.3	10.1	9.8

1) 4,8,12-Trimethyltridecanoic acid.

2) Including 16:2n-4.

3) Each unidentified component less than 1% of total fatty acids.

4) Phytanic acid omitted from saturated fatty acids.

5) nd: not detected.

M. venulosus. The reason for this could not be determined in the present study. The difference in which lipid and/or fatty acid composition is related to the environmental conditions. Therefore, it may be clarified only through additional study. Major NL and PL were found to be triacylglycerol and sterol, and phosphatidylethanolamine (PE) and phosphatidylcholine (PC) for both species, based on TLC comparison of authentic standards.

Predominant fatty acids in TL of various tissues were 16:0 (11.0-15.2%), 18:0 (4.1-8.6%), 20:5n-3 (13.3-25.6%) and 22:6n-3 (8.1-16.7%) as enumerated in **Table 2**, as was also observed by Caers *et al.* and Kochi in the scallop *Argopecten purpuratus* (9) and the venus shell *Phacosoma japonica* (10). Major fatty acid compositions were essentially the same for all these species. Many marine species of molluscs are rich in 20:5n-3, with less 22:6n-3 (11). The proportions of 20:5n-3 and 22:6n-3 of TL for *M. venulosus* and *M. zyonensis* differed significantly. In all tissues, *M. venulosus* was a higher in 22:6n-3 than in *M. zyonensis*, whereas *M. zyonensis* had more 20:5n-3 than *M. venulosus*. The proportion of 20:5n-3 was more abundant in viscera than other tissues in these two species. The proportions of n-3 polyunsaturated fatty acids (PUFA) were particularly high in muscle tissue for *M. venulosus* and *M. zyonensis*, at 41.1 and 44.1%, respectively. The proportions of n-3 and n-6 PUFA were richly present in muscle tissue, constituting nearly 50% of total fatty acids. In viscera from *M. zyonensis*, 16:1n-7 was much greater than in any other tissue with, the minimum amount being 18:0.

Minor branched saturated fatty acids found were 4,8,12-methyltridecanoic (TMTD), phytanic (PA), 15-methylhexadecanoic (iso 17:0), 16-methylheptadecanoic (iso 18:0) and 17-methyloctadecanoic acids (iso 19:0). Odd-chain saturated fatty acids were pentadecanoic (15:0), heptadecanoic (17:0) and nonadecanoic acids (19:0). PA was present in all tissues, but TMTD could be found only in the viscera tissue of either species in small amounts (*M. venulosus* and *M. zyonensis*, at 1.9 and 2.2%, respectively). PA and TMTD were produced via the degradation of dietary chlorophyll, as already reported for various bivalve molluscs (11, 12).

2-Hydroxy fatty acids were identified by GC-MS, as 2-hydroxypentadecanoic (key fragment ions of the methyl ester at m/z 272 $[M]^+$ (8%), 213 $[M-59]^+$ (86%),

103 (18%), and 90 (81%)), 2-hydroxyhexadecanoic (key fragment ions at m/z 286 $[M]^+$ (12%), 227 $[M-59]^+$ (89%), 103 (26%), and 90 (65%)) and 2-hydroxyheptadecanoic acids (key fragment at m/z 300 $[M]^+$ (17%), 241 $[M-59]^+$ (95%), 103 (27%), and 90 (70%)). No other 2-hydroxy fatty acids, possibly owing to having a chain-length greater than C_{20} , could be detected by GC. 2-Hydroxy fatty acids generally derive from sphingolipids which are nervous functions and the structural components of membranes. So far 2-hydroxyhexadecanoic and 2-hydroxyheptadecanoic acids have been shown to be present in some species of mussels according to the literature (13, 14). To the best of authors' knowledge, 2-hydroxy fatty acids have been identified in the genus *Megangulus* for the first time in the present study.

Typical non-methylene interrupted (NMI) fatty acids were detected and determined as 7,15-22:2 and 7,13-22:2, the former being present at a high concentration (1.0-6.3%) in all tissues. These fatty acids are usually present in lipids of many marine invertebrates, such as mussels, oysters and gastropods (11, 16, 17). The functions of NMI fatty acids are not fully understood; possibly, they may compete with PUFA for the uptake into tissue lipids in marine organisms and act as inhibitors of lipid peroxidation processes in the membranes of marine bivalves (11, 18). No other NMI fatty acids could be identified here, owing to the amounts being too small to permit detection.

Aldehydes, which originate from plasmalogens (1-*O*-alk-1'-enyl-2-acyl glycerophospholipids), mainly present in PL, with chain-lengths of C_{16} , C_{17} , C_{18} and C_{20} were always found to be present in all tissues (shown in **Table 3**). Aldehydes were determined and characterized as dimethylacetals (DMAs) by GC-MS. Predominant DMAs were 18:0 DMA and 20:1 DMA, at 50-56% and 25-34%, respectively. The content of 18:0 DMA was highest in viscera. Except for minor DMA components, the present findings accord well with data for scallop and oyster species, *Argopecten purpuratus* and *Crassostrea gigas*, respectively (9, 15). In contrast to other marine organisms, the composition of DMA in bivalves is very poorly documented. The components and distribution of DMA in various bivalve tissues as well as its functions may offer clues to understand the response of marine bivalves to environmental stresses (19).

For clarification of the main fatty acid component distribution in NL and PL (mainly PC and PE), fatty

Table 3 Proportion (%) of Dimethylacetals Detected in Methanolyzates of Total Lipids in Various Tissues of *Megangulus venulosus* and *Megangulus zyonensis*.

Dimethylacetal	Mantle		Muscle		Viscera	
	<i>M. venulosus</i>	<i>M. zyonensis</i>	<i>M. venulosus</i>	<i>M. zyonensis</i>	<i>M. venulosus</i>	<i>M. zyonensis</i>
16:0	4.4	6.3	6.5	9.2	4.3	6.2
17:0	6.3	5.1	6.5	4.2	6.0	<0.1
18:0	51.6	53.8	50.0	50.0	56.4	56.3
18:1	5.7	3.2	3.3	2.5	3.4	12.5
20:0	3.8	4.4	<0.1	<0.1	<0.1	<0.1
20:1	28.2	27.2	33.7	34.1	29.9	25.0

Fig. 1 Comparison of Fatty Acid Components in Neutral and Polar Lipids of Mantle, Muscle and Viscera.

acids in each lipid fractionated were analyzed for comparison with other tissue lipids, and these results are shown in **Fig. 1**. TMTD in PL was found to be higher than in NL in viscera of the two species in this study. One isolated PL in *M. zonoensis* was found to contain a very high level of TMTD, as much as 7% of total fatty acids. TMTD has been observed in considerable amounts in the scallop *Argopecten purpuratus* and *Placopecten magellanicus*, mainly in PL and NL (mainly triacylglycerol), respectively, from digestive glands (9, 20) and therefore would appear to be a biological marker of food web effects. This is also supported based on determinations of NL and PL fractions from various bivalve tissues. Major fatty acids of PL in either species were 16:0, 20:5n-3 and 22:6n-3, and for NL 16:0 and 20:5n-3. The proportion of 22:6n-3 in NL and PL differed significantly, 22:6n-3 being much higher in PL. The content of 20:5n-3 considerably exceeded that of 22:6n-3 in NL and PL, and 20:5n-3 was highest in *M. zonoensis* in both fractions. Differences in proportions of n-3, n-6 PUFA and TMTD of TL in various tissues would therefore appear to be due to difference in NL and PL, as noted in this study.

Fatty acids of *M. venulosus* and *M. zonoensis* were noted here to be similar to those observed in scallop, oyster and mussel species, though various branched saturated fatty (9, 15, 18) and 2-hydroxy fatty acids (13, 14) as minor components have also been shown to be present in some marine bivalves. Fatty acid compositions in the two species were virtually the same. The present study provides basic data on the distribution of major fatty acids in the two species and consequently may lead to a better understanding of the functions of lipids and fatty acids in various organs of these bivalves. The lipid classes and fatty acid compositions of *M. venulosus* and *M. zonoensis*, as determined by seasonal variation and the reproductive cycle, should be clarified more precisely through additional research.

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