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Note

Lipid characteristics of a seep clam, *Mesolinga soliditesta*: Comparison with those of two coastal clams, *Meretrix lamarckii* and *Ruditapes philippinarum*

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ABSTRACT

The lipids and fatty acids of two coastal clams, *Meretrix lamarckii* and *Ruditapes philippinarum*, collected at 5 and 1 m of depth, and a seep clam, *Mesolinga soliditesta*, collected at 331 m of depth, were examined to assess their lipid physiology and trophic relationship with their diets. The major fatty acids of lipids in *Mer. lamarckii* and *R. philippinarum* were 14:0, 16:0, 18:0, 16:1*n*-7, 18:1*n*-9, 18:1*n*-7, 20:4*n*-6, 20:5*n*-3, and 22:6*n*-3, while those of *Mes. soliditesta* were 16:0, 18:0, 16:1*n*-7, 18:1*n*-7, 20:1*n*-7, 20:1*n*-13, 20:2*n*-7, 15 (Δ5,13-20:2), and 22:2*n*-7,15 (Δ7,15-22:2). The major polyunsaturated fatty acids (PUFAs) in the *Mer. lamarckii* and *R. philippinarum* lipids consisted of various *n*-3 and *n*-6 long-chain (LC) PUFAs, such as 20:4*n*-6, 22:4*n*-6, 20:5*n*-3, 22:5*n*-3, and 22:6*n*-3, while those in *Mes. soliditesta* muscle and viscera included various *n*-4 family PUFAs (18:3*n*-7, 18:4*n*-4, 20:2*n*-7, and 20:3*n*-7) with limited kinds of *n*-3 and *n*-6 PUFAs (20:4*n*-6 and 20:5*n*-3). These findings indicate that, like other common shallow-water clams, *Mer. lamarckii* and *R. philippinarum* ingest phytoplanktonic *n*-3 and *n*-6 LC-PUFAs, whereas *Mes. soliditesta* utilizes limited kinds of *n*-3 and *n*-6 LC-PUFAs. In contrast to the other two bivalves species, *Mes. soliditesta* yielded various *n*-4 and *n*-7 (*n*-4/*n*-7) PUFAs, which were assimilated from the chemosynthetic symbionts. The high diversity of PUFAs contained in the *Mes. soliditesta* lipids (*n*-3, *n*-4 family, and *n*-6 PUFAs) suggests that this species mixotrophically utilized both photosynthetic products and vent chemosynthetic nutrition derived from geothermal energy.

1. Introduction

Some commercially useful bivalve species (Bivalvia), particularly oysters and mussels, have been investigated in detail for fatty acids. They include the oyster (Ostreidae) *Crassostrea gigas* (Saito and Marty, 2010), the pearl oysters (Pteriidae) *Pinctada fucata* (Saito, 2004) and *Pinctada margaritifera* (Vahirua-Lechat et al., 2008), the scallops (Pectinidae) *Placopecten magellanicus* (Napolitano et al., 1992) and *Pecten maximus* (Pazos et al., 2003), the clams (Veneridae) *Ruditapes philippinarum* (Kraffe et al., 2004) and *Mactra chinensis* (Saito, 2007), and the mussels (Mytilidae) *Mytilus edulis* (Kluytmans et al., 1985) and *Mytilus galloprovincialis* (Saito, 2008). On the other hand, there is relatively little information available on the biochemical components of other mollusk species. A few reports exist on the lipid and fatty acid composition of vent bivalves; for example, the *Calyptogena* clam *C. phaseoliformis* (Saito, 2007) and the *Bathymodiolus* mussels *B. japonicus*, *B. platifrons* (Saito, 2008) and *B.* sp. (Phleger et al., 2005). There are also several reports on the fatty acid composition only of *Calyptogena magnifica* and *Bathymodiolus thermophilus* (Ben-Mlih et al., 1992), Mytilidae spp. (Fang et al., 1993; Abrajano et al., 1994), *Bathymodiolus* sp. (Jahnke et al., 1995) and *Bathymodiolus* sp. (Pond et al., 1998).

Common shallow-water clams *Meretrix lamarckii* Deshayes (Korean hard clam, Veneroida, Veneridae) and *R. philippinarum* Adams & Reeve (Manila clam, Veneroida, Veneridae) occur in the sea off the coast of Japan and are important marine resources. These species range over the Japanese archipelago, and are also found off the Korean Peninsula, some parts of the Chinese coast, and the Philippine Islands. They filter small phytoplankton, small animals, and detrital particles as part of the grazing and detrital food chains. The diet of *Mer. lamarckii* and *R. philippinarum* consists mostly of photosynthetic products. On the other hand, the seep clam, *Mesolinga soliditesta* Okutani & Hashimoto (Veneroida, Lucinidae) occurs in the cold seep of the Nankai Trough in the western Pacific Ocean

Abbreviations: DHA, docosahexaenoic acid; DMOX, 4,4-dimethyloxazoline; E(I)PA, (e)icosapentaenoic acid; GC–MS, gas chromatography–mass spectroscopy; LC, long-chain; MUFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic acids; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA(s), polyunsaturated fatty acid(s); TAG, triacylglycerols; TFA, total fatty acids

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(Okutani and Hashimoto, 1997; Levin, 2005; Sasaki et al., 2005). *Mes. soliditesta* depends on chemosynthetic symbiotic microorganisms, such as sulfur-oxidizing bacteria, which make bacteriocytes in its gills, and may obtain lipids from the symbionts in the same way as other lucinid bivalves. Examples of the latter include *Lucinoma borealis* (Dando et al., 1994), *Myrtea spinifera*, *Lucinoma aequizonata*, and *Lucina floridana* (Anderson, 1995), *Thyasira* sp. (Southward et al., 2001), *Lucinoma aequizonata* and *Parvilucina tenuisculpta* (Duplessis et al., 2004), and *Codakia orbicularis* (Caro et al., 2009). The chemical components, such as glycerolipids, have yet to be elucidated for these clams, except in the case of *R. philippinarum* from European coastal waters (Kraffe et al., 2004; Delaporte et al., 2005; Fernández-Reiriz et al., 2006). Nutrition of lucinid bivalves mainly depends on the symbionts' products because many lucinids show a substantial reduction of the labial palps and alimentary tract (Dando et al., 1994; Anderson, 1995).

The isolation from their hosts and cultivation of symbiotic marine microorganisms is very difficult, in particular for vent organisms. However, information on the lipids of the symbionts can be obtained by comparative analysis of the lipid and fatty acids in the muscles, viscera, and gills of the host. We therefore investigated the fatty acid composition of neutral and polar lipids in three tissues of the deep-sea clam *Mes. soliditesta*, a cold-seep species with symbiotic bacteria, and compared these results with those derived from the common shallow-water clams, *Mer. lamarckii* and *R. philippinarum*.

2. Materials and methods

2.1. Materials

Seven samples of *Mer. lamarckii* (length 91 ± 3 mm; weight 141.1 ± 13.8 g) were collected at Kashima-Nada in the sea off the Japanese coast of Honshu Island in the western Pacific Ocean (35°55′N and 140°40′E) at a depth of 5 m. Seven samples of *R. philippinarum* (length 52 ± 1 mm; weight 25.7 ± 1.0 g) were collected at Lake Hamana-Ko in the sea off the Japanese coast of Honshu Island in the western Pacific Ocean (34°69′N and 137°60′E) at a depth of 1 m. Three samples of *Mes. soliditesta* (length 56 ± 1 mm; weight 65.4 ± 8.1 g) were collected at the Kanesu-no-Se Bank in the Nankai Trough of the western Pacific Ocean (34°17′N and 138°15′E) at a depth of 331 m (7.0 °C, 3.3 MPa) during dive no. 900 of the submersible "*Shinkai 2000*," belonging to the Japan Marine Science and Technology Center. After they had been measured, the specimens of *Mes. soliditesta* were immediately frozen at −80 °C in the laboratory of the mother ship "*Natsushima*."

2.2. Lipid extraction and analysis of lipid classes

After 1 month of storage at −40 °C in the laboratory, samples of *Mes. soliditesta* were dissected into 3 groups of organs; gill, muscles (foot and adductor), and other organs (viscera). Three organs of *Mes. soliditesta* and whole soft parts of *Mer. lamarckii* and *R. philippinarum* were individually dissected (Table 1). Each sample was homogenized in a mixture of chloroform and methanol (2:1, vol/vol) and the lipids of the homogenized sample were extracted according to the Folch procedure (1957). The crude lipids of *Mer. lamarckii*, *R. philippinarum*, and *Mes. soliditesta* were separated into classes on silicic acid columns (Merck and Co. Ltd., Kieselgel 60, 70–230 mesh), and a quantitative analysis of the lipid constituents was performed using gravimetric analysis of fractions collected from column chromatography (Saito and Hashimoto, 2010). The first eluate (dichloromethane/*n*-hexane, 2:3, v/v) was collected by as steryl esters, wax esters, and diacyl glyceryl ethers fractions (Table 1). This was followed by eluting triacylglycerols (TAG) with dichloromethane and eluting the sterols with dichloromethane/ether (35:1, v/v); eluting the diacylglycerols

Table 1
Lipid contents and lipid classes of three species of Japanese clams.[a]

Organs	Sample no.	Replications for lipid extraction	Lipid contents[b]	Replications for separation	Wax esters[c]	Steryl esters[c]	Diacylglycerylethers[c]	TAG[c,d]	Sterols[c]	Diacyl-glycerols[c]	Free fatty acids[c]	PE[c, d]	Phospholipids[c,e]	PC[c,d]	Total glycerides
Meretrix lamarckii															
Whole	1	7	0.9 ± 0.1	7	0.0 ± 0.0	3.1 ± 0.3	1.9 ± 0.2	53.5 ± 3.9	10.4 ± 1.1	1.4 ± 0.1	3.4 ± 0.3	10.8 ± 0.9	6.6 ± 0.9	8.9 ± 1.6	79.9
Ruditapes philippinarum															
Whole	2	7	0.5 ± 0.0	7	0.0 ± 0.0	1.1 ± 0.3	3.8 ± 0.2	21.0 ± 3.0	23.1 ± 1.7	1.3 ± 0.4	8.4 ± 0.7	21.1 ± 1.4	6.3 ± 1.3	13.8 ± 1.6	
Mesolinga soliditesta															
Muscle	3	3	0.5 ± 0.0	3	0.5 ± 0.5	3.2 ± 2.5	1.0 ± 1.0	20.6 ± 5.7	9.5 ± 0.8	0.3 ± 0.3	9.4 ± 0.2	27.1 ± 2.5	11.9 ± 3.7	16.5 ± 3.6	74.9
Gill	3	3	1.3 ± 0.3	3	0.8 ± 0.4	0.1 ± 0.1	0.9 ± 0.4	20.7 ± 4.7	5.8 ± 0.7	4.2 ± 1.1	11.1 ± 2.6	30.3 ± 12.2	15.5 ± 5.8	10.6 ± 3.0	77.8
Viscera	3	3	0.7 ± 0.0	3	0.7 ± 0.4	0.3 ± 0.0	1.0 ± 0.5	37.8 ± 12.3	8.6 ± 5.1	2.5 ± 2.2	8.6 ± 4.4	17.5 ± 3.0	11.3 ± 8.1	11.5 ± 6.1	78.9

[a] Data are mean ± standard errors (n = 3–7).
[b] Results are expressed as weight per cent of wet tissues.
[c] Results are expressed as weight per cent of total lipids.
[d] TAG, PE, and PC means triacylglycerols, phosphatidylethanolamine, and phosphatidylcholine, respectively. TAG and sterols were the major components in the neutral lipids of all samples, while medium levels of PE and PC were observed in polar lipids.
[e] Phospholipids fraction contained noticeable levels of ceramide aminoethylphosphonate and other minor phospholipids.