Fatty Acids of Serine, Ethanolamine, and Choline Plasmalogens in Some Marine Bivalves

Edouard Kraffe[a], Philippe Soudant[b], and Yanic Marty[a,*]

[a]Unité mixte Centre Nationale de la Recherche Scientifique (CNRS) 6521, Université de Bretagne Occidentale CS93837, 29238 Brest Cedex 3, France, and [b]Unité mixte CNRS 6539, Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, 29280 Plouzané, France

ABSTRACT: The FA composition of glycerophospholipid (GPL) classes and subclasses was investigated in whole animals of three marine bivalve mollusks: the Japanese oyster *Crassostrea gigas*, the blue mussel *Mytilus edulis*, and the Manila clam *Ruditapes philippinarum*. Individual organs (gills, mantle, foot, siphon, and muscle) of the Manila clam also were examined. The PS plasmalogen (PSplsm), PE plasmalogen (PEplsm), and PC plasmalogen (PCplsm) subclasses were isolated by HPLC, and their individual FA compositions were examined using GC. Plasmalogen forms of PS and PE, when compared to their respective diacyl forms, were found to be specifically enriched with non-methylene-interrupted (NMI) FA (7,15-22:2, 7,13-22:2, and their precursors) and 20:1n-11 FA. Such a clear specific association was not found for PCplsm. Interestingly, this trend was most apparent in PSplsm, and the above FA were found to be, respectively, the predominant PUFA and monounsaturated FA in the PSplsm isolated from the three species. This specificity was maintained in all the analyzed organs of the Manila clam but varied in proportions: The highest level of plasmalogens, NMI FA, and 20:1n-11 was measured in gills and the lowest was in muscle. These results represent the first comprehensive report on a FA composition of the PSplsm subclass isolated from mollusks. The fact that NMI FA and 20:1n-11, which are thought to be biosynthesized FA, were mainly associated with aminophospholipid plasmalogens (PE and PS) is likely to have a functional significance in bivalve membranes.

Paper no. L9385 in *Lipids 39*, 59–66 (January 2004).

Ether glycerophospholipids (GPL) are subclasses of phospholipids found in animal cells. The two predominant forms have either an alkyl or an alkenyl ether linkage at the *sn*-1 position of the glycerol moiety and an acyl linkage at the *sn*-2 position. The 1-alkenyl-2-acyl ether GPL are commonly called "plasmalogens." The biological activity of plasmalogens is not fully understood, although a number of functions have been proposed. Plasmalogens are supposed to be important in membrane dynamics, allowing the formation of inverted hexagonal structures (H_{II}) (1). Several recent studies have led to the proposal that these ether lipids also may serve as endogenous antioxidants to protect cells from oxidative stress (2–4). One other function proposed is as sinks for PUFA, to maintain high levels of these FA in some tissues (1).

It is well established that marine invertebrates are especially rich sources of plasmalogens whereas they contain only small amounts of 1-alkyl-2-acyl GPL (see Refs. 5 and 6 for reviews). Indeed, they generally have higher concentrations of plasmalogens than other well-known rich sources such as mammalian brain (7) and heart (1). In a variety of human tissues, high proportions of plasmalogen forms were found in PE and to a lesser extent in PC (see Ref. 1 for review). In winter-collected marine invertebrates, Dembitsky and Vaskovsky (8) concluded that these animals can be grouped according to the distribution of plasmalogens in their phospholipids. Plasmalogens of Coelenterata and Echinoidea exist only as PE; those of Annelida, Arthropoda, Ascidia, and most of the Echinodermata, as both PE and PC; and those of Mollusca, as PE, PC, and PS. Later, Dembitsky (9) repeated this investigation with animals collected during the summer and confirmed that, among all examined invertebrates, only mollusks contained PS plasmalogen (PSplsm).

Very few analyses of the FA composition of marine mollusk plasmalogens have been carried out. In fact, the separation of the plasmalogen subclasses from the analogous diacyl subclass in a GPL extract of molluscan tissues is quite difficult owing to the complexity of the mixture of various molecular species as well as to the similarities between their chemical structures. However, in previous investigations, the acid-catalyzed hydrolysis of the *sn*-1 position combined with a phospholipid separation by HPLC has rendered possible the determination of the FA composition, by GC, of the bulk of plasmalogen forms in the great scallop *Pecten maximus* (10–13). GC–MS identification, after enzymatic hydrolysis of phospholipid polar heads, also has been applied to describe the detailed fatty acyl chain compositions of PE plasmalogen (PEplsm) and PC plasmalogen (PCplsm) from the Japanese oyster *Crassostrea gigas* (14,15). In these studies, noticeable amounts of non-methylene-interrupted (NMI) FA were found in PEplsm. These unusual FA have been previously described in different phyla of marine invertebrates (6,16). More recently, high levels of 22:2 NMI FA were found in the polar lipids of *C. gigas* and *Ruditapes philippinarum* (17,18), but the FA composition of plasmalogen and diacyl GPL were not determined in these studies. Pathways for the biosynthesis of 20:2 NMI (5,11 and 5,13) and 22:2 NMI (7,13 and 7,15) FA have been reported in the bivalve mollusks *Scapharca broughtoni* and *Mytilus edulis* (19,20). These results indicated that mollusks have active FA elongation and desaturation systems permitting

*To whom correspondence should be addressed at Unité mixte CNRS 6521, Université de Bretagne Occidentale, CS93837, 29238 Brest Cedex 3, France. E-mail: Yanic.Marty@univ-brest.fr

Abbreviations: CL, cardiolipin, common name of diphosphatidylglycerol; GPL, glycerophospholipid(s); MUFA, monounsaturated FA; NMI, non-methylene-interrupted; ΣNMI, sum of all non-methylene-interrupted fatty acids; PCplsm, PC plasmalogen; PEplsm, PE plasmalogen; PSplsm, PS plasmalogen; SFA, saturated fatty acid(s).

the *de novo* synthesis of these NMI FA. The unknown biological roles of both plasmalogens and NMI FA in marine invertebrates have motivated investigations of the distribution patterns of plasmalogens (21) and NMI FA among different organs (22). Unfortunately, analysis of plasmalogens and NMI FA were always conducted separately, and speculations on the putative biological functions of these membrane components were never related.

Because information on the composition of FA in the *sn*-2 position of molluscan plasmalogens is very scarce, or nonexistent in the case of PS plasmalogen (PSplsm), an HPLC procedure was developed to separate the plasmalogen forms of PE, PS, and PC. In the present study, the proportions and the fatty acyl chain compositions of both 1-alkenyl-2-acyl and 1,2-diacyl analogs were investigated in whole-animal extracts of *C. gigas*, *M. edulis*, and *R. philippinarum*. For the latter, the investigation was also conducted on different organs. We demonstrate that a specific association of particular FA with plasmalogen exists in these three bivalve species.

MATERIALS AND METHODS

Chemicals. Boron trifluoride (BF_3, 10% by wt in methanol) was obtained from Supelco (St. Quentin Fallavier, France). Other reagents and solvents were purchased from Merck (Darmstadt, Germany).

Sample preparation and lipid extraction. Adult *C. gigas, M. edulis,* and *R. philippinarum* were collected from the Bay of Brest during spring and summer 2001. After removing the digestive tract, whole animals of each species were pooled and homogenized with a Dangoumeau homogenizer at –180°C. Samples were analyzed in triplicates of three pooled individuals for the three species. Also, the mantle, foot, siphon, gills, and adductor muscle of *R. philippinarum* were excised, pooled, weighed, and homogenized as above (triplicate of three pooled individuals). Lipid extraction was conducted on tissue homogenates according to the method described by Folch *et al.* (23). To ensure the complete extraction of tissue lipids, a solvent-to-tissue ratio of 70:1 was used as described by Nelson (24). After removing the organic phase, the residue was washed with a mixture of $CHCl_3$/MeOH (2:1, vol/vol) to exclude any solvent retention. The final extract was stored at –20°C under nitrogen after 0.01 wt% BHT (antioxidant) was added.

Separation of polar lipids on a silica gel microcolumn. An aliquot of the lipid extracts was evaporated to dryness, and lipids were recovered with three washings of 500 µL of $CHCl_3$/MeOH (98:2, vol/vol) and deposited at the top of a silica gel microcolumn (30 × 5 mm i.d., packed with Kieselgel 60; 70–230 mesh, Merck) previously heated at 450°C and deactivated with 6 wt% H_2O (25). Neutral lipids were eluted with 10 mL of $CHCl_3$/MeOH (98:2, vol/vol). The polar lipid fraction was recovered with 20 mL of MeOH and stored at –20°C. An aliquot of this fraction was taken for the direct determination of the total GPL FA composition by GC; the rest was used for GPL class separations by HPLC and their FA composition analysis.

Separation of phospholipid classes and FA analysis. Separation of the GPL classes and subclasses was achieved using a combination of two successive HPLC separations with two different mobile phases.

(i) Non acid HPLC separation. A rapid gradient elution separation was achieved, based on a method previously described by Soudant *et al.* (26), on a Merck HPLC system (UV detection at 206 nm) equipped with a Diol phase column (OH-bound silica gel column, Lichrosorb Diol 5 µm, 250 × 4 mm i.d.; Merck). The former method was modified as follows: The initial mobile phase was an isocratic 9:1 ratio of solvent A (*n*-hexane/2-propanol/water, 40:52:1, by vol) and solvent B (*n*-hexane/2-propanol/water, 40:52:8, by vol) run for 11 min. This solvent ratio was then followed by a 1-min linear gradient to 100% solvent B. The column was maintained on solvent B for 18 min to complete the separation, then finally reactivated with 20 min of the initial 9:1 ratio of solvent A to B. The solvent flow rate was 1 mL/min during the entire elution program. This separation allowed the collection of a first fraction (within 3–19 min) containing PE (diacyl + plasmalogen form) and PC (diacyl + plasmalogen form), and a second fraction (within 19–25 min) containing cardiolipin (CL), PI, lysophosphatidylcholine (LysoPC), and PS (diacyl + plasmalogen form).

(ii) Acidic HPLC separations (Fig. 1). First and second fractions were treated separately. After evaporation to dryness under nitrogen, each fraction was recovered with two washings of 50 µL each of $CHCl_3$/MeOH (98:2, vol/vol) before being manually injected in the 200-µL loop of the second HPLC system. The separation was achieved as previously described (27). This method, based on the use of an acidic mobile phase, allows the hydrolysis of the vinyl ether bond at the *sn*-1 position of the glycerol backbone of the plasmalogen forms (10) and

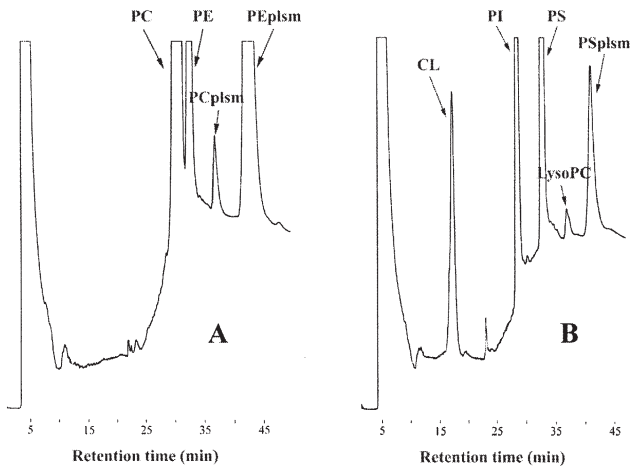

FIG. 1. Further acidic HPLC separations of the glycerophospholipid classes and subclasses. The two fractions (A) and (B) were obtained by a previous non acidic HPLC separation (not represented). Chromatographic conditions and properties for non acidic and acidic HPLC separations are indicated in the Materials and Methods section. PC and PE: phosphatidylcholine and phosphatidylethanolamine diacyl forms; PCplsm and PEplsm, phosphatidylcholine and phosphatidylethanolamine plasmalogen forms; CL: cardiolipin; PI: phosphatidylinositol; LysoPC: naturally occuring lysophosphatidylcholine; PS: phosphatidylserine diacyl form; PSplsm: phosphatidylserine plasmalogen form.

creates lyso analogs that elute later than the intact diacyl forms. This offers the possibility of analyzing separately diacyl and plasmalogen subclasses of PE and PC from the first fraction and diacyl and plasmalogen subclasses of PS from the second fraction. Each PS, PE, and PC subclass, jointly with CL, PI, and LysoPC, was collected and, after transesterification ($MeOH/BF_3$), analyzed by GC for FA composition. FA were expressed as molar percentages of the total FA content of each class or subclass. For PEplsm, PSplsm, and PCplsm subclasses, the total percentage was adjusted to 50% to take into account the alkenyl chains of the *sn*-1 position hydrolyzed by the acid mobile phase. The quantities of each class and subclass of GPL were determined from their respective quantitative spectrum of FA obtained by GC. To obtain the molar content of each analyzed fraction, a corrective factor was applied to their respective total FA molar contents: ×1 for the PSplsm, PEplsm, and PCplsm fractions and for the natural LysoPC fraction; ×1/2 for the PE-, PC-, PS-, and PI-diacyl fractions, and ×1/4 for the CL fraction.

RESULTS

Contents of plasmalogen and diacyl PE, PS, and PC in C. gigas, M. edulis, *and* R. philippinarum. PEplsm and PCdiacyl were the two major subclasses and accounted for two-thirds of the total GPL of *C. gigas*, *R. philippinarum,* and *M. edulis* (Table 1). The PSplsm, PSdiacyl, PEdiacyl, and PCplsm subclasses, together with PI, LysoPC, and CL, constituted the other third. The PEplsm subclass was more prominent than the PE-diacyl subclass, accounting for most of the PE (77.8 to 85%), whereas the PCplsm constituted only 3.1 to 10.9% of PC. The PS class contained plasmalogen and diacyl forms in similar proportions, with PSplsm accounting for 43.4, 44.8, and 66.4% of total PS in *C. gigas, R. philippinarum,* and *M. edulis,* respectively (Table 1). The same general features were also observed in the different organs isolated from the *R. philippinarum* (Table 1). Nevertheless, the proportions of plasmalogens in PS, PE, and PC varied according to the organs. PEplsm, PSplsm, and PCplsm proportions ranged, respectively, from 66.1, 28.9, and 5 in the muscle to 85.6, 67.2, and 14.6% in gills (Table 1). The sum of the plasmalogen subclasses (PSplsm + PEplsm + PCplsm) was found to reach a mean value of 41.4% of the GPL of the three species when analyzed as whole animal. Analysis of *R. philippinarum* organs indicated that the sum of plasmalogen subclasses ranged from a minimum of 28.4% in muscle to a maximum of 52.1% in gills (Table 1).

FA composition of the plasmalogen and diacyl forms of PE, PS, and PC. In the three bivalve species analyzed as whole-animal extract, the total saturated FA (SFA) ranged between 34 and 47.6% in the diacyl fractions of PS, PE, and PC. SFA content was found to be very low in PEplsm and PSplsm (<2.5 and <7%, respectively; Tables 2 and 3) and slightly higher in PC-plsm (from 12.1 to 18.1% of the total FA; Table 4). The 16:0 and 18:0 acids were generally predominant in the SFA of all fractions. The MUFA content was lower in PEplsm and PC-plsm than in their respective diacyl forms for the three species (Tables 2 and 4). However, the MUFA content was lower in the PSplsm compared to the PSdiacyl only for *M. edulis* (Table 3). The 20:1n-11 FA was the main MUFA of PSplsm and of PE-plsm, accounting, respectively, for 55 to 79.2% and 41.5 to 60.9% of the total MUFA in the three species analyzed as whole animal (Tables 2 and 3). No such specific association was observed for PCplsm (Table 4). The variation of 20:1n-11 content in PS, PE, and PC subclasses followed a similar pattern in all organs analyzed from *R. philippinarum* (Fig. 2). The characteristic 20:1n-11 enrichment of the PSplsm MUFA was observed in all organs. The lowest percentage was measured in muscle and the highest percentages in mantle and gills (Fig. 2). In addition, while maintaining such a specificity, the GPL content of 20:1n-11 in organs ranged from a higher value in gills (4%) to a lower value in muscle (1.7%) (Table 5).

TABLE 1
Composition of Glycerophospholipids[a] and Plasmalogen Content of PE, PS, and PC Classes in the Whole Body of *Crassostrea gigas, Mytilus edulis,* and *Ruditapes philippinarum* and in Separated Organs from *R. philippinarum*

	Glycerophospholipid composition (mol%)[a]							
	Whole body			Organs				
	C. gigas	*M. edulis*	*R. philippinarum*	Mantle	Foot	Siphon	Gills	Muscle
PEdiacyl	5.8 ± 0.9	8.0 ± 1.0	8.1 ± 0.2	8.0 ± 1.2	8.8 ± 0.1	6.9 ± 0.5	6.1 ± 0.4	11.3 ± 0.7
PEplsm	33.6 ± 4.7	28.1 ± 1.5	31.5 ± 2.0	30.7 ± 2.2	35.1 ± 0.8	31.6 ± 1.9	36.5 ± 2.8	22.1 ± 2.6
PSdiacyl	7.2 ± 0.6	4.8 ± 1.3	8.5 ± 2.0	9.2 ± 1.1	7.2 ± 1.7	11.7 ± 2.1	5.3 ± 2.0	9.8 ± 3.0
PSplsm	5.8 ± 2.2	9.5 ± 1.8	6.9 ± 1.7	6.3 ± 2.3	5.9 ± 1.9	7.2 ± 2.5	10.6 ± 0.5	4.0 ± 1.1
PCdiacyl	36.5 ± 2.4	40.5 ± 1.7	33.9 ± 1.8	33.1 ± 0.1	33.5 ± 3.8	30.4 ± 2.5	28.9 ± 2.9	42.0 ± 5.1
PCplsm	4.7 ± 0.7	1.3 ± 0.5	2.8 ± 0.7	2.9 ± 0.7	1.9 ± 0.7	2.5 ± 0.4	5.0 ± 2.4	2.3 ± 0.8
PI	5.5 ± 1.7	5.3 ± 1.4	5.8 ± 0.9	6.9 ± 0.7	4.9 ± 1.5	6.5 ± 0.8	4.9 ± 0.3	6.6 ± 0.4
CL	1.1 ± 0.2	1.3 ± 0.8	0.9 ± 0.2	1.0 ± 0.4	1.3 ± 0.1	1.0 ± 0.2	0.8 ± 0.2	0.6 ± 0.2
LysoPC	2.2 ± 1	2.5 ± 0.9	1.7 ± 0.1	1.9 ± 0.5	1.3 ± 0.2	2.2 ± 0.1	1.9 ± 0.7	1.3 ± 0
Total plasmalogen	44.1	38.9	41.2	39.9	42.9	41.3	52.1	28.4
Plasmalogen content of the class[b]								
PE	85.0 ± 3.8	77.8 ± 1.3	79.6 ± 0.6	79.4 ± 1.2	80.0 ± 0.3	82.1 ± 2.0	85.6 ± 1.7	66.1 ± 4.1
PS	43.3 ± 11.9	66.4 ± 10.4	44.8 ± 0.3	39.9 ± 6.2	44.8 ± 2.2	37.6 ± 4.2	67.2 ± 7.6	28.9 ± 0.8
PC	10.9 ± 2.1	3.1 ± 1.3	7.5 ± 1.3	7.9 ± 1.9	5.2 ± 1.5	7.5 ± 0.3	14.6 ± 7.4	5.0 ± 1.2

[a]Expressed as mol% of total moles of glycerophospholipids, calculated as indicated in the Materials and Methods section. Results are mean ± SD (n = 3).
[b]Ratio (in %) of the plasmalogen form to the diacyl + plasmalogen forms of a class.

TABLE 2
FA Composition of PEdiacyl and PEplsm in the Whole Animals *C. gigas, M. edulis*, and *R. philippinarum*[a]

	C. gigas		*M. edulis*		*R. philippinarum*	
FA	Diacyl[b]	Plsm[c]	Diacyl	Plsm	Diacyl	Plsm
Branched[d]	2.2 ± 0.3	0.2 ± 0.1	1.2 ± 0.1	0.1 ± 0.0	7.4 ± 0.8	1.0 ± 0.2
14:0	0.8 ± 0.4	0.1 ± 0.1	0.8 ± 0.1	0.1 ± 0.1	0.6 ± 0.0	0.0 ± 0.0
15:0	0.6 ± 0.2	0.1 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.0 ± 0.0
16:0	13.6 ± 0.2	1.2 ± 0.2	12.5 ± 0.5	0.6 ± 0.2	8.0 ± 0.0	0.7 ± 0.1
17:0	3.2 ± 0.7	0.2 ± 0.0	2.8 ± 0.2	0.1 ± 0.0	2.5 ± 0.1	0.2 ± 0.0
18:0	19.4 ± 3.5	0.8 ± 0.1	17.6 ± 1.0	0.6 ± 0.1	15.0 ± 2.4	0.4 ± 0.0
16:1n-7	0.7 ± 0.1	0.1 ± 0.1	1.2 ± 0.8	0.5 ± 0.2	3.0 ± 0.1	0.8 ± 0.1
18:1n-9	2.2 ± 1.7	0.3 ± 0.2	1.2 ± 0.2	0.2 ± 0.0	4.8 ± 0.7	1.8 ± 0.4
18:1n-7	3.3 ± 1.2	0.3 ± 0.2	3.4 ± 0.6	0.2 ± 0.0	1.8 ± 0.1	0.2 ± 0.0
20:1n-11	1.5 ± 0.0	2.8 ± 0.1	0.8 ± 0.1	1.7 ± 0.0	3.6 ± 0.6	3.1 ± 0.3
20:1n-9	0.8 ± 0.1	0.3 ± 0.0	4.0 ± 0.3	1.2 ± 0.0	2.6 ± 0.3	0.7 ± 0.0
20:1n-7	4.4 ± 0.1	0.6 ± 0.1	1.3 ± 0.1	0.3 ± 0.0	5.2 ± 1.0	0.4 ± 0.0
18:3n-3	0.7 ± 0.1	0.2 ± 0.0	1.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.2	0.1 ± 0.0
18:4n-3	0.6 ± 0.2	0.3 ± 0.0	1.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	0.1 ± 0.1
20:2NMI(5,11)	0.0 ± 0.0	0.2 ± 0.1	1.4 ± 0.2	7.0 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
20:2NMI(5,13)	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	2.9 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
20:4n-6	5.6 ± 0.5	1.3 ± 0.1	2.6 ± 0.3	2.5 ± 0.2	5.0 ± 0.1	1.0 ± 0.0
20:5n-3	23.9 ± 0.6	9.3 ± 0.1	22.9 ± 0.6	17.2 ± 0.6	5.3 ± 0.4	2.1 ± 0.2
22:2NMI(7,13)	0.6 ± 0.0	1.9 ± 0.0	0.2 ± 0.0	0.9 ± 0.2	1.4 ± 0.1	3.7 ± 0.3
22:2NMI(7,15)	4.6 ± 0.5	9.2 ± 0.2	1.0 ± 0.0	3.5 ± 0.4	3.8 ± 0.2	10.2 ± 0.3
22:3NMI(7,13.16)	0.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	1.4 ± 0.1	0.5 ± 0.1	1.6 ± 0.2
22:4n-6	0.4 ± 0.1	0.9 ± 0.0	0.3 ± 0.1	0.7 ± 0.1	2.2 ± 0.1	4.3 ± 0.1
22:5n-6	0.3 ± 0.0	0.8 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	1.1 ± 0.0	1.4 ± 0.1
22:5n-3	1.1 ± 0.2	2.9 ± 0.1	0.9 ± 0.1	1.6 ± 0.0	3.0 ± 0.0	4.4 ± 0.1
22:6n-3	6.9 ± 0.6	14.4 ± 0.6	18.5 ± 0.8	5.3 ± 0.4	17.1 ± 0.8	8.9 ± 0.6
Others[e]	1.4 ± 0.8	0.8 ± 0.2	1.5 ± 0.6	0.5 ± 0.4	4.2 ± 0.7	2.4 ± 0.3
Total NMI	5.7 ± 0.5	12.1 ± 0.3	3.3 ± 0.1	15.7 ± 0.4	6.0 ± 0.0	15.8 ± 0.3
Total SFA	40.3 ± 3.3	2.5 ± 0.6	35.3 ± 0.9	1.4 ± 0.0	34.0 ± 1.7	2.3 ± 0.3
Total MUFA	13.0 ± 3.2	4.6 ± 0.5	12.0 ± 1.3	4.1 ± 0.3	22.4 ± 2.9	7.3 ± 0.8
Total PUFA	46.7 ± 0.1	42.9 ± 1.0	52.7 ± 0.5	44.5 ± 0.4	43.6 ± 1.2	40.3 ± 1.1

[a]Results expressed as mol% are mean ± SD (n = 3). For abbreviations see Table 1.
[b]"Diacyl" refers to both *sn*-1 and *sn*-2 fatty acyl chains of the diacyl form.
[c]"Plsm" refers to *sn*-2 fatty acyl chains of the plasmalogen form; the total percentage was adjusted to 50% to take into account the alkenyl chains of the *sn*-1 position hydrolyzed by the acid mobile phase as described in Materials and Methods section.
[d]Branched-chain FA (mainly 15:0 and 17:0 iso and anteiso).
[e]Total of 11 FA detectable (16:1n-5, 18:1n-5, 18:2n-6, 18:2n-4, 18:3n-6, 18:3n-4, 20:2n-6, 20:3n-6, 20:4n-3, 21:4n-6, and 21:5n-3, none of which was more than 1.0% in any subclasses).

Long-chain PUFA with 20 and 22 carbons were found to be the dominant PUFA in the plasmalogen and diacyl fractions. They were principally constituted by NMI FA as well as 22:6n-3, 20:5n-3, and 20:4n-6. The different NMI FA were the 20:2 NMI (5,13-20:2 and 5,11-20:2) and the 22:2 NMI (7,15-22:2 and 7,13-22:2). Low levels of 22:3 NMI (7,13,16-22:3) were also detected in the FA of the three bivalves. Considering the sum of these NMI FA (ΣNMI), *C. gigas, M. edulis*, and *R. philippinarum* showed a high percentage of ΣNMI in PSplsm (12.7, 20.2, and 20.3% of the total FA, respectively) and in PEplsm (12.1, 15.7, and 15.8% of the total FA, respectively). ΣNMI in PSplsm and PEplsm were generally higher than those measured, respectively, in PSdiacyl and PEdiacyl, except in the PS of *C. gigas,* where it reached 13.9% of total FA in PSdiacyl (Tables 2 and 3). The predominant NMI FA was the 7,15-22:2 isomer, which was the major PUFA of PSplsm in the three species. *Mytilus edulis* plasmalogens also contained a high proportion of 20:2 NMI, these isomers being predominantly localized in PEplsm (Table 2). In *R. philippinarum* organs, as in the whole body of the three bivalves, the three plasmalogen subclasses showed a specific association with ΣNMI when compared to their diacyl analogs (Fig 3). This specificity was especially important in PSplsm and PEplsm and to a lesser extent in PCplsm. Such specificity was maintained, whereas the content of ΣNMI in GPL ranged from a higher value in gills (16.1%) to a lower value in muscle (4.3%). As in the whole body, the predominant NMI of *R. philippinarum* organs was the 7,15-22:2 isomer, along with minor amounts of 7,13-22:2; the ratio of these two FA was unchanged whatever the organ (data not shown).

DISCUSSION

Importance of plasmalogen subclasses. Plasmalogens constituted a high proportion of GPL, in *C. gigas*, *R. philippinarum,*

TABLE 3
FA Composition of PSdiacyl and PSplsm in the Whole Animals *C. gigas, M. edulis*, and *R. philippinarum*[a]

	C. gigas		*M. edulis*		*R. philippinarum*	
FA	Diacyl[b]	Plsm[c]	Diacyl	Plsm	Diacyl	Plsm
Branched[d]	1.1 ± 0.3	0.2 ± 0.1	1.2 ± 0.0	0.3 ± 0.0	2.3 ± 0.2	0.5 ± 0.1
14:0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.0 ± 0.1
15:0	0.2 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16:0	5.2 ± 0.4	2.8 ± 0.8	8.7 ± 2.9	2.7 ± 0.6	5.2 ± 0.9	1.5 ± 0.2
17:0	2.6 ± 0.5	0.6 ± 0.2	4.1 ± 0.3	0.2 ± 0.0	3.6 ± 0.2	0.3 ± 0.0
18:0	32.0 ± 5.0	3.1 ± 0.3	26.4 ± 0.8	1.8 ± 1.1	36.1 ± 0.6	2.3 ± 0.3
16:1n-7	0.1 ± 0.1	0.1 ± 0.1	0.9 ± 0.0	0.4 ± 0.0	0.8 ± 0.0	0.2 ± 0.0
18:1n-9	0.8 ± 0.7	0.3 ± 0.2	0.7 ± 0.0	0.1 ± 0.0	1.8 ± 0.4	0.5 ± 0.1
18:1n-7	1.3 ± 0.8	0.5 ± 0.5	1.9 ± 0.0	0.2 ± 0.0	0.7 ± 0.1	0.1 ± 0.0
20:1n-11	4.6 ± 0.4	12.5 ± 1.9	0.7 ± 0.1	2.5 ± 0.1	2.2 ± 0.1	8.4 ± 0.2
20:1n-9	0.8 ± 0.1	1.5 ± 1.4	5.8 ± 1.6	1.0 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
20:1n-7	7.2 ± 1.8	1.3 ± 0.6	1.0 ± 0.5	0.2 ± 0.0	1.0 ± 0.1	0.3 ± 0.0
18:3n-3	0.3 ± 0.0	0.0 ± 0.1	1.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.2	0.0 ± 0.0
18:4n-3	0.1 ± 0.2	0.1 ± 0.1	0.5 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
20:2NMI(5,11)	0.1 ± 0.1	0.1 ± 0.0	1.0 ± 0.0	2.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
20:2NMI(5,13)	0.3 ± 0.2	0.2 ± 0.1	0.5 ± 0.0	0.9 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
20:4n-6	5.7 ± 0.3	1.2 ± 0.3	2.3 ± 0.6	1.2 ± 0.0	5.5 ± 0.4	0.9 ± 0.1
20:5n-3	20.1 ± 1.4	4.7 ± 0.2	8.1 ± 0.6	6.2 ± 0.6	5.7 ± 0.0	1.0 ± 0.1
22:2NMI(7,13)	1.8 ± 0.1	2.2 ± 0.0	0.4 ± 0.0	1.2 ± 0.1	1.5 ± 0.0	6.1 ± 0.2
22:2NMI(7,15)	11.6 ± 1.2	10.1 ± 2.6	6.0 ± 0.0	14.9 ± 4.0	4.6 ± 0.3	13.6 ± 0.8
22:3NMI(7,13,16)	0.1 ± 0.0	0.2 ± 0.1	0.5 ± 0.0	1.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.2
22:4n-6	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.7 ± 0.1	2.0 ± 0.0	1.9 ± 0.0
22:5n-6	0.2 ± 0.0	0.5 ± 0.2	0.6 ± 0.0	0.7 ± 0.1	1.2 ± 0.0	1.5 ± 0.0
22:5n-3	0.4 ± 0.2	1.1 ± 0.1	1.5 ± 0.2	1.7 ± 0.2	3.5 ± 0.1	1.6 ± 0.1
22:6n-3	2.4 ± 0.7	5.7 ± 1.1	23.9 ± 0.1	8.6 ± 2.1	18.3 ± 0.6	6.4 ± 0.2
Others[e]	0.3 ± 0.3	0.2 ± 0.1	1.5 ± 0.1	0.4 ± 0.0	2.0 ± 0.5	1.1 ± 0.2
Total NMI	13.9 ± 1.1	12.7 ± 2.7	8.4 ± 0.1	20.2 ± 4.2	6.5 ± 0.3	20.3 ± 0.8
Total SFA	41.8 ± 5.3	7.0 ± 0.4	40.8 ± 3.9	5.5 ± 1.6	47.6 ± 0.2	4.7 ± 0.6
Total MUFA	14.8 ± 3.2	16.4 ± 1.9	11.0 ± 2.3	4.5 ± 0.1	7.5 ± 0.9	10.6 ± 0.0
Total PUFA	43.4 ± 2.1	26.6 ± 1.5	48.1 ± 1.6	40.0 ± 1.5	44.9 ± 0.7	34.6 ± 0.6

[a–f]For footnotes see Table 2.

and *M. edulis*. Plasmalogens were found in especially high proportions in PE (>78%) and in fairly low proportions in PC (<11%). Although PSplsm was a minor GPL component (about 7% of the total GPL), it represented an important part of PS, ranging from 45% in *C. gigas* and *R. philippinarum* to 67% in *M. edulis.* These results were in good agreement with those first reported on 14 bivalve species by Dembitsky and Vaskovsky (8) and those reported on *C. gigas* (59.8% of PEplsm in PE and 7.2% of PCplsm in PC) by Koizumi *et al.* (14).

FA composition of plasmalogen subclasses. A new combination of two HPLC separations allowed the analysis of the FA profile of the different GPL classes and subclasses in three bivalve species. To date, very little information is available on the FA composition of plasmalogen subclasses from marine bivalves. Only two studies described the FA composition of PEplsm and PCplsm of *C. gigas* (14,15), but the FA composition of the PSplsm has never been studied. To the best of our knowledge, the present study represents the first comprehensive report on the FA composition of the PS plasmalogen subclass in marine bivalves and, in fact, in marine mollusks as well. The FA pattern of PSplsm clearly showed a high specificity for NMI FA and 20:1n-11, and this characteristic was very consistent in the three bivalve species analyzed. The 7,15-22:2 isomer, a NMI FA, was the main PUFA encountered in the PSplsm subclass. Although present in a smaller proportion than the 7,15-22:2, the 7,13-22:2 isomer also showed also a specific association with the PSplsm compared to the PSdiacyl form. The other noticeable point was the very high percentage of 20:1n-11, accounting for more than half of the MUFA in PSplsm. Compared to the FA composition of PSplsm, the composition of PEplsm presents a lower but significant specificity for NMI FA and 20:1n-11, whereas in PCplsm these FA are present only as traces. Koizumi *et al*. (14) also indicated that, in *C. gigas*, most of the 22:2 NMI occurred in PEplsm. Although their results clearly showed the specific presence of 20:1n-11 in the PEplsm, this characteristic had not been discussed. In the erythrocyte membrane of the marine bivalve *S. broughtoni* (28), taking into account the corrections published later (29), the high concentrations of 22:2 NMI found in PE and PS were both demonstrated to be constituted mainly by the plasmalogen form. In mollusk and mammalian erythrocytes, the aminoglycerophospholipids (PE and PS) are primarily localized in the inner layer of the membrane (28,30). Since additional evidence is available for such asymmetry of phospholipids in other biomembranes (30), one may conclude that NMI and 20:1n-11

TABLE 4
FA Composition of PCdiacyl and PCplsm in the Whole Animals *C. gigas, M. edulis,* and *R. philippinarum*[a]

	C. gigas		*M. edulis*		*R. philippinarum*	
FA	Diacyl[b]	Plsm[c]	Diacyl	Plsm	Diacyl	Plsm
Branched[d]	1.6 ± 0.1	1.2 ± 0.0	1.5 ± 0.4	0.9 ± 0.5	4.1 ± 0.1	2.3 ± 0.1
14:0	2.7 ± 0.4	0.7 ± 0.1	2.4 ± 0.2	1.5 ± 1.2	1.1 ± 0.2	0.2 ± 0.0
15:0	1.9 ± 0.2	0.7 ± 0.1	1.1 ± 0.1	0.2 ± 0.2	1.0 ± 0.0	0.2 ± 0.0
16:0	24.0 ± 2.3	7.4 ± 0.7	25.0 ± 1.0	9.8 ± 4.7	22.1 ± 0.8	5.1 ± 0.7
17:0	1.6 ± 0.2	0.8 ± 0.0	1.1 ± 0.0	1.1 ± 0.6	1.8 ± 0.1	0.6 ± 0.0
18:0	3.8 ± 0.0	2.7 ± 0.0	3.1 ± 0.1	4.6 ± 1.7	4.4 ± 0.0	3.5 ± 0.3
16:1n-7	2.2 ± 0.1	0.7 ± 0.0	3.1 ± 0.8	0.7 ± 1.0	3.3 ± 0.4	0.8 ± 0.0
18:1n-9	3.7 ± 0.1	1.8 ± 0.0	1.7 ± 0.1	0.9 ± 0.3	5.0 ± 0.3	2.0 ± 0.0
18:1n-7	8.1 ± 0.5	2.2 ± 0.3	3.4 ± 0.4	0.5 ± 0.8	1.9 ± 0.1	0.4 ± 0.0
20:1n-11	0.8 ± 0.1	0.9 ± 0.1	0.3 ± 0.0	0.6 ± 0.2	0.6 ± 0.1	1.3 ± 0.2
20:1n-9	0.6 ± 0.0	0.2 ± 0.0	2.7 ± 0.4	1.0 ± 0.0	0.6 ± 0.1	0.4 ± 0.1
20:1n-7	3.1 ± 0.5	1.1 ± 0.1	0.9 ± 0.2	0.5 ± 0.1	0.9 ± 0.2	1.0 ± 0.1
18:3n-3	1.5 ± 0.1	0.8 ± 0.1	1.6 ± 0.1	0.3 ± 0.4	0.2 ± 0.0	0.0 ± 0.0
18:4n-3	2.0 ± 0.2	1.0 ± 0.2	2.5 ± 0.2	0.5 ± 0.6	0.9 ± 0.1	0.4 ± 0.2
20:2NMI(5.11)	0.1 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	1.1 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
20:2NMI(5.13)	0.6 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.2	0.1 ± 0.0	0.1 ± 0.1
20:4n-6	4.7 ± 0.2	2.4 ± 0.0	2.2 ± 0.4	1.8 ± 0.2	3.9 ± 0.1	1.5 ± 0.0
20:5n-3	17.9 ± 1.2	10.1 ± 0.5	19.3 ± 0.1	11.3 ± 1.7	8.5 ± 0.4	2.5 ± 0.2
22:2NMI(7,13)	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	1.0 ± 0.0
22:2NMI(7,15)	1.9 ± 0.1	2.0 ± 0.1	0.6 ± 0.0	0.8 ± 0.2	0.7 ± 0.0	2.5 ± 0.1
22:3NMI(7,13,16)	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
22:4n-6	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	2.8 ± 0.3	2.5 ± 0.4
22:5n-6	0.4 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.2	1.5 ± 0.0	1.1 ± 0.1
22:5n-3	1.4 ± 0.0	1.2 ± 0.1	1.9 ± 0.0	1.2 ± 0.3	5.0 ± 0.2	3.3 ± 0.3
22:6n-3	10.3 ± 0.1	8.9 ± 0.6	20.3 ± 1.3	9.4 ± 1.1	24.7 ± 0.7	14.5 ± 0.1
Others[e]	3.5 ± 0.6	2.2 ± 0.3	3.1 ± 0.3	0.4 ± 0.6	3.9 ± 0.5	1.7 ± 0.6
Total NMI	2.9 ± 0.1	2.4 ± 0.1	1.9 ± 0.1	2.7 ± 0.7	1.4 ± 0.1	3.8 ± 0.2
Total SFA	35.9 ± 3.3	13.6 ± 0.5	34.1 ± 0.8	18.1 ± 1.0	34.6 ± 0.5	12.1 ± 0.5
Total MUFA	19.4 ± 1.5	6.9 ± 0.1	12.9 ± 1.7	4.2 ± 1.2	13.2 ± 0.5	6.7 ± 0.7
Total PUFA	44.7 ± 1.8	29.5 ± 0.4	52.9 ± 1.0	27.7 ± 2.2	52.3 ± 1.0	31.2 ± 0.2

[a–f]For footnotes see Table 2.

FA also are asymmetrically distributed in the lipid bilayer and involved in specific cellular functions of the inner membrane.

The most striking results were that plasmalogen and diacyl subclasses of PS and PE can be distinguished according to the origin of their unsaturated FA. The 22:6n-3, 20:5n-3, and 20:4n-6, which were the predominant FA of the PSdiacyl and PEdiacyl, originate mainly from the phytoplanktonic diet of bivalves. Indeed, bivalves have a limited or total inability to synthesize 20–22-carbon PUFA with more than three double bonds (31,32). On the other hand, the NMI FA, which were the predominant PUFA of the PSplsm, are synthesized *de novo* by bivalves, as first proposed by Ackman and Hooper (16) and demonstrated by Zhukova (19,20). The pathway consists of a Δ9 desaturation and elongation of 16:0 and 18:0, respectively, into 20:1n-7 and 20:1n-9. A C_{20}Δ5 desaturation converts them, respectively, into 5,13-20:2 and 5,11-20:2, which are finally elongated, respectively, into 7,15-22:2 and 7,13-22:2. Similarly, 20:1n-11, found only as traces in microalgae (33), could also be of endogenous origin, although there is no demonstration of that in the literature. Only a few studies have indicated the presence of a high concentration of 20:1n-11 in bivalves (34,35). It might be possible that a Δ9 desaturase acting on 20:0 produces 20:1n-11 in the same way as Δ9 desaturase(s) acting on 16:0 and 18:0 produce the two precursors 20:1n-7 and 20:1n-9. Unlike these NMI precursors, 20:1n-11 did not appear to be subjected to further Δ5 desaturation, suggesting the C_{20}Δ5 desaturase may be "Δ13 and Δ11 specific."

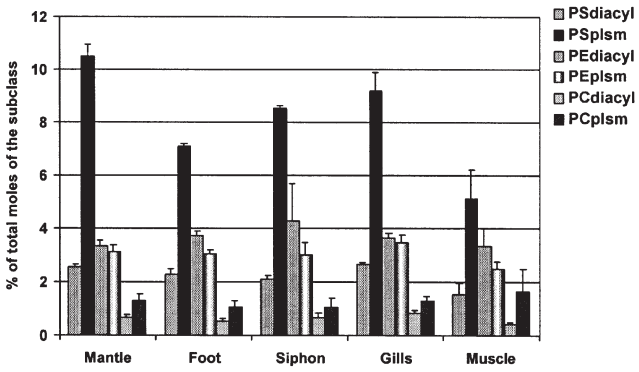

FIG. 2. Percent composition (in mol%, mean ± SD, $n = 3$) of 20:1n-11 from diacyl and plasmalogen forms of glycerophospholipid PS, PE, and PC of *Ruditapes philippinarum* organs.

TABLE 5
ΣNMI and 20:1n-11 Compositions in Organs of *R. philippinarum* (in mol% of the total FA of glycerophospholipids)

	Mantle	Foot	Siphon	Gills	Muscle
ΣNMI[a]	10.6 ± 0.2	10.9 ± 0.5	9.3 ± 0.1	16.1 ± 1.1	4.3 ± 0.8
20:1n-11	3 ± 0.1	2.8 ± 0.1	3 ± 0.2	4 ± 0.3	1.7 ± 0.1

[a]Sum of all non-methylene-interrupted FA [20:2NMI(5.11), 20:2NMI(5.13), 22:2NMI(7,13), 22:2NMI(7,15), and 22:3NMI (7.13.16)].

FA composition of plasmalogen subclasses in R. philippinarum *organs*. In marine bivalves, quantities of plasmalogens and of NMI FA vary according to organs (21,22). The FA pattern of GPL classes and subclasses of different organs of *R. philippinarum* was thus examined to assess whether the relationship between *de novo* synthesized FA and plasmalogens exists in all the organs. The specific location of NMI FA and 20:1n-11 in aminoglycerophospholipid plasmalogens was evidenced in all the organs analyzed. However, the proportions of these compounds varied according to the organ. In other words, the greater the plasmalogen content was in the organ, the higher the proportion of the biosynthesized FA was among the total FA of GPL. The highest proportions of plasmalogens, NMI FA, and 20:1n-11 were found in gills, but mantle, foot, and siphon also contained significant quantities of these compounds. The lowest proportion was found in the muscle. A previous study indicated large concentrations of 22:2 NMI FA in polar lipids isolated from gills, mantle, and foot of the hardshell clam *Mercenaria mercenaria* (22). Also, particularly high levels of 20:2 and 22:2 NMI were found in polar lipids of the gills of the mussel *M. edulis* (20). The gills and the mantle of the sea scallop *Placopecten magellanicus* (a pectinidae) exhibited consistently higher 20:1n-11 content than the muscle (35). An earlier review (21) concluded that gills have rather important proportions of plasmalogens, whereas muscle contains the lowest quantities of plasmalogens.

Although not clearly established, a number of functions already have been proposed for NMI FA and plasmalogens in marine organisms. NMI FA were supposed to confer resistance in tissues exposed most often to environmental physicochemical variations (22) or to attack by microbial lipases (37). This could explain the selectivity for NMI FA encountered in the gills, mantle, foot, and siphon of *R. philippinarum*. The high quantities of NMI FA reported in polar lipids of marine organisms also are regularly linked to a structural role in the membrane. They could be important for membrane properties such as phase transition temperature, membrane fluidity, or activity of membrane-bound proteins. However, based on conformational analysis, Rabinovich and Ripatti (38) concluded that the acyl chains with NMI double bonds are principally involved in adjusting membrane fluidity of these poikilothermic animals. The same role was proposed for acyl chains that have one *cis* double bond, as in 20:1n-11. In parallel, Chapelle (21) speculated that plasmalogens in marine animals may be metabolized to maintain cell integrity in response to environmental stresses such as temperature, pH, or salinity. One other function proposed for plasmalogens is that these ether lipids may act as antioxidative components in mammalian cells (2–4,39). In mollusks, in taking into account the asymmetry of their distribution, plasmalogens may function as scavengers of cell reactive oxygen species to protect other components in membranes from oxidative stress.

The present results provide the first evidence that aminoplasmalogens, PSplsm and PEplsm, reflect a higher selectivity for the biosynthesized FA ΣNMI and 20:1n-11 than for dietary PUFA such as 20:5n-3 and 22:6n-3. Moreover, 20:1n-11 can now be considered to be associated with or functionally equivalent to NMI FA in bivalves. The selective incorporation of ΣNMI and 20:1n-11 in PSplsm and PEplsm as well as the parallel variations of NMI FA and 20:1n-11 levels and plasmalogen contents in the different organs of *R. philippinarum* suggests the existence of possible synergistic properties of these compounds that should implicate them in the biological membrane functions of mollusks.

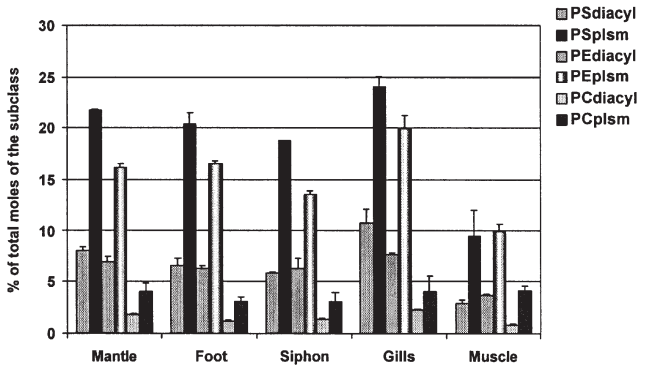

FIG. 3. Percent composition (in mol%, mean ± SD, $n = 3$) of ΣNMI from diacyl and plasmalogen forms of glycerophospholipid PS, PE, and PC of *Ruditapes philippinarum* organs. ΣNMI: sum of all non-methylene-interrupted FA, mainly 22:2NMI(7,15) with minor amounts of 22:2NMI-(7,13), 20:2NMI(5,11), 20:2NMI(5,13), and 22:3NMI(7,13,16).

ACKNOWLEDGMENTS

The authors would like to thank Dr. Aswani K. Volety, Florida Gulf Coast University (Fort Myers, FL), for English revisions. This work was supported by a grant from the Ministère de l'Education Nationale de la Recherche et de la Technologie (MENRT, Paris, France).

REFERENCES

1. Nagan, N., and Zoeller, R.A. (2001) Plasmalogens: Biosynthesis and Functions, *Prog. Lipid Res. 40*, 199–229.
2. Leray, C., Cazenave, J.-P., and Gachet, C. (2002) Platelet Phospholipids Are Differentially Protected Against Oxidative Degradation

by Plasmalogens, *Lipids 37*, 285–290.
3. Maeba, R., Sawada, Y., Shimasaki, H., Takahashi, I., and Ueta, N. (2002) Ethanolamine Plasmalogens Protect Cholesterol-Rich Liposomal Membranes from Oxidation Caused by Free Radicals, *Chem. Phys. Lipids 120*, 145–151.
4. Maeba, R., and Ueta, N. (2003) Ethanolamine Plasmalogen and Cholesterol Reduce the Total Membrane Oxidizability Measured by the Oxygen Uptake Method, *Biochem. Biophys. Commun. Res. 302*, 265–270.
5. Sargent, J.R. (1987) Ether-Linked Glycerides in Marine Animals, in *Marine Biogenic Lipids, Fats, and Oils* (Ackman, R.G., ed.), pp. 176–193, CRC Press, Boca Raton, FL.
6. Joseph, J.D. (1982) Lipid Composition of Marine and Estuarine Invertebrates. Part II: Mollusca, *Prog. Lipid Res. 21*, 109–153.
7. Schrakamp, G., Schutgens, R.B.H., Schalkwijk, C.G., and Van Den Bosch, H. (1991) Comparative Studies on Plasmalogen Biosynthesis in Rat Brain Homogenates and Isolated Oligodendroglial Cells During Development, *Brain Dysfunct. 4*, 217–227.
8. Dembitsky, V.M., and Vaskovsky, V.E. (1976) Distribution of Plasmalogens in Different Classes of Phospholipids of Marine Invertebrates, *Biol. Morya (Kiev) 5*, 68–72.
9. Dembitsky, V.M. (1979) Plasmalogens in Phospholipids of Marine Invertebrates, *Biol. Morya (Kiev) 5*, 86–90.
10. Soudant, P., Marty, Y., Moal, J., and Samain, J.F. (1995) Separation of Major Polar Lipids in *Pecten maximus* by High-Performance Liquid Chromatography and Subsequent Determination of Their Fatty Acids Using Gas Chromatography, *J. Chromatogr. B 673*, 15–26.
11. Soudant, P., Moal, J., Marty, Y., and Samain, J.F. (1996) Impact of the Quality of Dietary Fatty Acids on Metabolism and the Composition of Polar Lipid Classes in Female Gonads of *Pecten maximus* (L.), *J. Exp. Mar. Biol. Ecol. 205*, 149–163.
12. Soudant, P., Moal, J., Marty, Y., and Samain, J.F. (1997) Composition of Polar Lipid Classes in Male Gonads of *Pecten maximus* (L.). Effect of Nutrition, *J. Exp. Mar. Biol. Ecol. 215*, 103–114.
13. Soudant, P., Marty, Y., Moal, J., Masski, H., and Samain, J.F. (1998) Fatty Acid Composition of Polar Lipid Classes During Larval Development of Scallop *Pecten maximus* (L.), *Comp. Biochem. Phys. A 121*, 279–288.
14. Koizumi, C., Jeong, B.Y., and Ohshima, T. (1990) Fatty Chain Composition of Ether and Ester Glycerophospholipids in the Japanese Oyster *Crassostrea gigas, Lipids 25*, 363–370.
15. Jeong, B.Y., Ohshima, T., and Koizumi, C. (1990) Molecular Species of 1-*O*-Alkyl-1′-enyl-2-acyl-, 1-*O*-Alkyl-2-acyl- and 1,2-Diacyl Glycerophospholipids in Japanese Oyster *Crassostrea gigas* (Thunberg), *Lipids 25*, 624–632.
16. Ackman, R.G., and Hooper, S.N. (1973) Non-Methylene-Interrupted Fatty Acids in Lipids of Shallow-Water Marine Invertebrates: A Comparison of Two Molluscs (*Littorina littorea* and *Lunatia triserita*) with the Sand Shrimp (*Crangon septumspinosus*), *Comp. Biochem. Phys. 46B*, 153–165.
17. Dunstan, G.A., Volkman, J.K., and Barrett, S.M. (1993) The Effect of Lyophilization on the Solvent Extraction of Lipid Classes, Fatty Acids and Sterols from the Oyster *Crassostrea Gigas, Lipids 28*, 937–944.
18. Caers, M., Coutteau, P., and Sorgeloos, P. (1999) Dietary Impact of Algal and Artificial Diets, Fed at Different Feeding Rations, on the Growth and Fatty Acid Composition of *Tapes philippinarum* (L.) Spat, *Aquaculture 170*, 307–322.
19. Zhukova, N.V. (1986) Biosynthesis of Non-Methylene-Interrupted Fatty Acids from [^{14}C]Acetate in Molluscs, *Biochim. Biophys. Acta 878*, 131–133.
20. Zhukova, N.V. (1991) The Pathway of the Biosynthesis of Non-Methylene-Interrupted Dienoic Fatty Acids in Molluscs, *Comp. Biochem. Phys. 100B*, 801–804.
21. Chapelle, S. (1987) Plasmalogens and *O*-Alkylglycerophospholipids in Aquatic Animals, *Comp. Biochem. Phys. 88B*, 1–6.
22. Klingensmith, J.S. (1982) Distribution of Methylene and Non-Methylene-Interrupted Dienoic Fatty Acids in Polar Lipids and Triacylglycerols of Selected Tissues of the Hardshell Clam (*Mercenaria mercenaria*), *Lipids 17*, 976–981.
23. Folch, J., Lees, M. and Sloane Stanley, G.H. (1957) A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*, 497–509.
24. Nelson, G.J. (1993) Isolation and Purification of Lipids from Biological Matrices, in *Analyses of Fats, Oils, and Derivatives* (Perkins, E.G., ed.), pp. 20–59, AOCS Press, Champaign.
25. Marty, Y., Delaunay, F., Moal, J., and Samain, J.F. (1992) Changes in the Fatty Acid Composition of the Scallop *Pecten maximus* (L.) During Larval Development, *J. Exp. Mar. Biol. Ecol. 163*, 221–234.
26. Soudant, P., Chu, F.L.E., and Marty, Y. (2000) Lipid Class Composition of the Protozoan *Perkinsus marinus*, an Oyster Parasite, and Its Metabolism of a Fluorescent Phosphatidylcholine Analog, *Lipids 35*, 1387–1395.
27. Kraffe, E., Soudant, P., Marty, Y., Kervarec, N., and Jehan, P. (2002) Evidence of a Tetradocosahexaenoic Cardiolipin in Some Marine Bivalves, *Lipids 37*, 507–514.
28. Chelomin, V.P., and Zhukova, N.V. (1981) Lipid Composition and Some Aspects of Aminophospholipid Organization in Erythrocyte Membrane of the Marine Bivalve Mollusc *Scapharca broughtoni* (Shrenck), *Comp. Biochem. Phys. 69B*, 599–604.
29. Zhukova, N.V., and Svetashev, V.I. (1986) Non-Methylene-Interrupted Dienoic Fatty Acids in Molluscs from the Sea of Japan, *Comp. Biochem. Phys. 83B*, 643–646.
30. Yorek, M.A. (1993) Biological Distribution, in *Phospholipids Handbook* (Cevc, G., ed.), pp. 745–775, Marcel Dekker, New York.
31. De Moreno, J.E.A., Moreno, V.J., and Brenner, R.R. (1976) Lipid Metabolism of the Yellow Clam, *Mesodesma mactroïdes*: 2–Polyunsaturated Fatty Acid Metabolism, *Lipids 11*, 561–566.
32. Waldock, M.J., and Holland, D.L. (1984) Fatty Acid Metabolism in Young Oysters, *Crassostrea gigas*: Polyunsaturated Fatty Acids, *Lipids 19*, 332–336.
33. Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., and Garland, C.D. (1989) Fatty Acid and Lipid Composition of 10 Species of Microalgae Used in Mariculture, *J. Exp. Mar. Biol. Ecol. 128*, 219–240.
34. Stefanov, K., Seizova, K., Brechany, E., and Christie, W.W. (1992) An Unusual Fatty Acid Composition for a Fresh-Water Mussel, *Unio tumidus*, from Bulgaria, *J. Nat. Prod. 55*, 979–981.
35. Napolitano, G.E., and Ackman, R.G. (1993) Fatty Acid Dynamics in Sea Scallops *Placopecten magellanicus* (Gmelin, 1791) from Georges Bank, Nova Scotia, *J. Shellfish Res. 12*, 267–277.
36. Cook, H.W. (1996) Fatty Acid Desaturation and Chain Elongation in Eukaryotes, in *Biochemistry of Lipids, Lipoproteins and Membranes* (Vance, D.E., and Vance, J.E.), pp. 129–152, Elsevier Science, Amsterdam.
37. Paradis, M., and Ackman, R.G. (1977) Potential for Employing the Distribution of Anomalous NMI Dienoic Fatty Acids in Several Marine Invertebrates as Part of Food Web Studies, *Lipids 12*, 170–176.
38. Rabinovich, A.L., and Ripatti, P.O. (1991) The Flexibility of Natural Hydrocarbon Chains with Non-Methylene-Interrupted Double Bonds, *Chem. Phys. Lipids 58*, 185–192.
39. Morand, O.H., Zoeller, R.A., and Raetz, C.R.H. (1988) Disappearance of Plasmalogens from Membranes of Animal Cells Subjected to Photosensitized Oxidation, *J. Biol. Chem. 263*, 11597–11606.

[Received September 18, 2003; accepted November 22, 2003]