Fatty acids of Antarctic gastropods: distribution and comparison with Mediterranean species

Ácidos grasos en gasterópodos antárticos: distribución y comparación con especies mediterráneas

Conxita ÁVILA*[1], Angelo FONTANA**, Mauro ESPOSITO***, Maria Letizia CIAVATTA** and Guido CIMINO**

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ABSTRACT

Fatty acids of three different lipid pools: free fatty acids (FFA), storage lipids (triglycerides and wax esters, SL) and phospholipids (PL) of mantle and viscera of Antarctic gastropods were analyzed and compared to species from the Mediterranean Sea. We analyzed specimens of the Antarctic species: *Bathydoris clavigera* Thiele, 1912, *Tritonia challengeriana* Bergh, 1884 and *Marseniopsis mollis* (Smith, 1902), and the Mediterranean species: *Hypselodoris picta* (Schultz, 1836) and *Dendrodoris limbata* (Cuvier, 1804). Fatty acid composition was very different between viscera and mantle of the same individuals, and the amounts of polyunsaturated fatty acids were significantly higher in the mantle. There were higher levels of polyunsaturated fatty acids in mantle phospholipids of Antarctic molluscs than in Mediterranean molluscs. Arachidonic and eicosapentaenoic acids were the dominant species of phospholipids in Antarctic molluscs, whereas octadecaenoic acid was the most abundant species in the phospholipid pools of Mediterranean animals. A comparison of the SFA/PUFA (saturated vs. polyunsaturated fatty acids) and MUFA/PUFA (monounsaturated vs. polyunsaturated fatty acids) indexes in SL and PL of Antarctic and Mediterranean specimens showed statistically significant differences among them, thus suggesting a relationship with environmental temperature.

RESUMEN

Se analizan los ácidos grasos de tres tipos de lípidos, ácidos grasos libres (FFA), lípidos de reserva (triglicéridos y ésteres de ceras, SL) y fosfolípidos (PL), en el manto y las vísceras de gasterópodos antarcticos, y se comparan con especies mediterráneas. Las especies estudiadas fueron las antarcticas *Bathydoris clavigera* Thiele, 1912, *Tritonia challengeriana* Bergh, 1884 y *Marseniopsis mollis* (Smith, 1902), y las mediterráneas *Hypselodoris picta* (Schultz, 1836) y *Dendrodoris limbata* (Cuvier, 1804). La composición de ácidos grasos difirió mucho entre vísceras y manto de algunos especímenes, y la cantidad de acidos grasos poliinsaturados fue significativamente más elevada en el manto. Se encontraron mayores niveles de éstos últimos en el manto de las especies antarcticas que en el de las mediterráneas. Los ácidos araquidónico y eicosapentanoico fueron los dominantes en los PL de las especies antarcticas, en los PL de las especies mediterráneas lo fue el ácido

* Centre d'Estudis Avançats de Blanes (CEAB), C.S.I.C., c/ Accés a la Cala Sant Francesc 14, 17300 Blanes, Girona, Spain.

** Istituto per la Chimica di Molecole di Interesse Biologico (ICMIB) del CNR. Via Campi Flegrei, 34, Comprensorio Olivetti, 80078 Pozzuoli (NA), Italy.

*** Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM). Via Salute 2, 80055 Portici (NA), Italy.

[1] Corresponding author. E-mail: conxita@ceab.csic.es. Fax number: 34-972-337806.

octadecanoico. La comparación entre los índices SFA/PUFA (ácidos grasos saturados vs. poliinsaturados) y MUFA/PUFA (ácidos grasos monoinsaturados vs. poliinsaturados) de los SL y PL de los dos grupos de especies mostró diferencias estadísticamente significativas entre ellos, lo que sugiere una relación con la temperatura ambiental.

KEY WORDS: fatty acids, Antarctic, gastropods, opisthobranch molluscs, chemical ecology.
PALABRAS CLAVE: ácidos grasos. Antártica, gasterópodos, moluscos opistobranquios, ecología química.

INTRODUCTION

Fatty acids in invertebrates are known to have broad biological roles, including lipid energy reserves, components of cellular structures such as biomembranes, and regulation of biosynthesis of eicosanoids, among others. Environmental conditions, such as diet or temperature, are closely related to lipid metabolism and may modulate the activities of the membrane (VOOGT, 1983; CULLIS AND HOPE, 1991; URICH, 1994; NELSON, LEIGHTON, PHLEGER AND NICHOLS, 2002). In fact, the physical properties of cell membranes are affected by even minor variations in the proportions of phospholipids, glycolipids, sterols and fatty acids (CULLIS AND HOPE, 1991; URICH, 1994). Also, it is well known that poikilotherms alter their membrane lipid composition in response to varying environmental temperature (URICH, 1994; MOON, HIGASHI, ZOLTAN AND MURATA, 1995; CHAKKODABYLU AND THOMPSON, 1984; DEY, BUDA, WIIK, HALVER AND FARKAS, 1993; HALL, THOMPSON AND PARRISH, 2000; FARKAS, FODOR, KITAJKA AND HALVER, 2001). The most common changes involve re-tailoring of phospholipid heads, sterol content of membranes and unsaturation of phospholipid fatty acids (URICH, 1994; CHAKKODABYLU AND THOMPSON, 1984; DEY *ET AL.*, 1993). One rational explanation of these variations is that cells compensate the temperature decrease with the increase of the membrane fluidity (*e.g.* the content of unsaturated fatty acids in cell membranes becomes higher at lower temperatures). For marine organisms, the temperature-dependent composition of membrane lipids has been reported in some bacteria (ROTERT, TOSTE AND STEIER, 1993; SAKAMOTO, HIGASHI, MURATA AND BRYANT, 1997; QUOC AND DUBACQ, 1997; RUSSELL, 1998; RUSSELL AND NICHOLS, 1999), unicellular algae (SATO, MURATA, MIURA AND UETA, 1979; THOMPSON, GUO, HARRISON AND WHYTE, 1992; LEHMAL, 1999), cnidarians (e.g. CARBALLEIRA, MIRANDA AND RODRÍGUEZ, 2002), molluscs (KATTNER, HAGEN, GRAEVE AND ALBERS, 1998; GILLIS AND BALLANTYNE, 1999; FREITES, LABARTA AND FERNÁNDEZ-REIRIZ, 2002), and fish (HAZEL, 1984; DEY *ET AL.*, 1993).

Gastropods are one of the most diverse animal groups, both in form, behavior and habitats (PONDER AND LINDBERG, 1997), and their evolutionary success could be related, among others, to their extraordinary ability to become adapted to different environments. This makes these animals particularly suitable for investigating the temperature effect on the cellular homeostasis. Gastropods have often been chemically studied for their ability to produce secondary metabolites with ecological significance or potential use as drugs or pharmacological tools (IRELAND, COPP, FOSTER, MCDONALD, RADISKY AND SWERSEY, 1993; ÁVILA, 1995; SHU, 1998), but surprisingly, they have received little attention regarding the effect of the environmental conditions on their lipid levels (URICH, 1994; KATTNER *ET AL.*, 1998; ISAY AND BUSAROVA, 1984). Actually, there are many descriptive studies on the primary lipids of prosobranchs (see VOOGT, 1983), but there have been almost no investigations on opisthobranchs. In fact, only some very early studies are reported on *Aplysia kurodai*

Table I. Data on the specimens studied quantitatively.
Tabla I. Datos de los especímenes estudiados cuantitativamente.

Species	No. of specimens analyzed	Depth (m)	Geographic area	Size (cm)	Wet (w) or Dry (d) weight (g)
Bathydoris clavigera	1	462	Weddell Sea	9.5	45 w
Tritonia challengeriana	1	446	Weddell Sea	5	3.5 w
Marseniopsis mollis	1	227	Weddell Sea	4.5	22.6 w
Hypselodoris picta	3	2-12	Mediterranean Sea	7.5-10	1.8-2.8 d
Dendrodoris limbata	2	2-12	Mediterranean Sea	5-8	0.8-1.2 d

(Baba, 1937) (TANAKA AND TOYAMA, 1959), *Aplysia fasciata* Poiret, 1789 and *Pleurobranchaea meckeli* Meckel in Leue, 1813 (TIBALDI, 1966), and more recently on the pteropod *Clione limacina* (Phipps, 1744) (KATTNER *ET AL.*, 1998).

During our ongoing research on natural products of marine invertebrates, we repeatedly observed high contents of fatty acids in extracts of Antarctic organisms, mainly opisthobranch molluscs and sponges. Therefore, we decided to further investigate this fact by carrying out a comparative study on the fatty acid content in Antarctic and Mediterranean gastropods. We selected two opisthobranch species and a single prosobranch species from Antarctica, and two Mediterranean opisthobranch species. We report here the composition and tissue distribution of their fatty acids in three different lipid pools: phospholipids (PL), storage lipids (SL, i.e. triglycerides and wax esters), and free fatty acids (FFA).

MATERIALS AND METHODS

Materials

The species studied here were the opisthobranchs *Bathydoris clavigera* Thiele, 1912 and *Tritonia challengeriana* Bergh, 1884 and the prosobranch *Marseniopsis mollis* (Smith, 1902), from Antarctica, and the opisthobranchs *Hypselodoris picta* (Schultz, 1836) and *Dendrodoris limbata* (Cuvier, 1804) from the Mediterranean (Table I). The species *T. challengeriana* had been named *Marionia cucullata* (Couthouy *in* Gould, 1852) in the past, but its taxonomy has been recently revised by MUNIAÍN AND SCHRÖDL (1999). Mediterranean species were selected due to our previous knowledge of their chemical ecology and their availability, while Antarctic species were selected because of their availability and their size (which should be large enough to allow for chemical analysis).

Preliminary qualitative studies were carried out with several specimens of these and other species not reported here, in order to test and improve the chemical methodology. Antarctic specimens used in this quantitative study were collected during the German expedition ANT XIII/3 (EASIZ I) to the Eastern Weddell Sea in January 1996 (Table I) (ARNTZ AND GUTT, 1997). The Mediterranean specimens were collected by scuba-diving in the Gulf of Naples (Italy) in June 1996 (Table I). All biological samples were immediately frozen and kept at –30°C until the chemical analyses were performed.

Dissection and extraction of molluscs

The specimens were carefully dissected in order to separate mantle and viscera. Since only a limited number of the Antarctic specimens was available, we used sub-samples from the animals in order to have pseudo-replicates. We analyzed two sub-samples for *T. challengeriana* and three for the other two Antarctic species, and two sub-samples for each type of tissue analyzed. Each body section was extracted separately

Table II. Content (µg per mg of total lipid extract) of SL, PL and FFA in mantle and viscera of the studied gastropods.
Tabla II. Contenido (µg per mg del extracto lipídico total) de SL, PL y FFA en manto y vísceras de los gasterópodos estudiados.

	SL		PL		FFA	
	Mantle	Viscera	Mantle	Viscera	Mantle	Viscera
Hypselodoris picta	71.0	322.5	14.6	31.0	44.4	75.3
Dendrodoris limbata	16.1	15.1	90.3	119.3	80.6	47.0
Marseniopsis mollis	344.8	58.5	293.1	265.4	193.1	61.2
Bathydoris clavigera	208.3	146.5	8.0	338.4	237.5	77.5
Tritonia challengeriana	242.8	242.0	158.5	228.5	250.0	35.7

by following the method of Bligh and Dyer (HAMILTON, HAMILTON AND SEWELL, 1992). The sample (ca. 8.5 mg) was homogenized by blending with a mixture of $CHCl_3$ (8.5 ml) and MeOH (17 ml) for two minutes. Then 8.5 ml of $CHCl_3$ were added to the solution and blended for 30 sec. more. Distilled water (8.5 ml) was added to the solution and the mixture was blended again for 30 sec. The suspension was filtered through paper on a Buchner funnel and the filtrate was recovered. The residue was transferred into the blender and the extraction was repeated. The $CHCl_3$ layer of the combined filtrates was separated and dried at reduced pressure to give a lipid extract containing glycerides, fatty acids, sterols and phospholipids.

Fractionation of the lipid extracts

The $CHCl_3$ soluble fractions were separated by SiO2 column (typically 70 mg silica *per* 1 mg of extract). Briefly, the lipid extract was solved in petroleum ether (typically 50 µl *per* 1 mg of extract) and loaded onto a column. Then 0.1 ml of 37% aqueous ammonia were added to the column and elution started with petroleum ether/diethyl ether (95:5 v/v) to give triglycerides, wax esters and sterols. Then glacial acetic acid (0.1 ml) was added to the column and the elution was completed by diethyl ether and a mixture of chloroform/methanol/water (60:40:2 v/v), to give free fatty acids and polar glycerides, respectively. The results of the separation by the SiO_2 column were checked by TLC for accuracy. In order to be further analyzed, the samples were transformed into fatty acid methyl esters (FAMEs).

Preparation of FAMEs

Fractions containing free fatty acids were concentrated at reduced pressure to a volume of 0.5 ml and reacted in open vials with a saturated solution of CH_2N_2 in Et_2O (0.5 ml). The reaction was performed for 30 min at room temperature. The excess of CH_2N_2 was removed by bubbling a stream of nitrogen. Solutions were concentrated under nitrogen flow to ca. 0.3 ml and analysed by GC-MS under the analytical conditions reported below.

Phospholipids and storage lipids (triglycerides and wax esters) were converted to FAMEs by a base catalyzed transesterification with Na_2CO_3 in dry methanol. Briefly, lipid samples were transferred to graduate screw-top vials and treated with 1.5 ml of saturated sodium carbonate in dry methanol. The reaction solution was heated at 40°C for 2 h, cooled at room temperature, transferred to a separating funnel and extracted with 5 ml diethyl ether and 8 ml of brine. The upper phase was removed and the extraction was repeated three times. The organic layers were combined, reduced to small volume (ca. 1 ml) and analyzed by GC-MS under the conditions reported below. We did not separate triglycerids

Table III. Relative amounts (mean % w/w ± SD) of the main fatty acids identified in mantle sections of Antarctic molluscs. SL: storage lipids (triglycerids and wax esters). PL: phospholipids. FFA: free fatty acids. nr: below the measurement limit. -: this sample could not be analyzed.
Tabla III. Cantidad relativa (porcentaje medio del peso húmedo ± SD) de los principales ácidos grasos identificados en las secciones del manto de moluscos Antárticos. SL: lípidos de almacenamiento (triglicéridos y ésteres de ceras). PL: fosfolípidos. FFA: ácidos grasos libres. nr: por debajo del límite de resolución. -: muestra no analizada.

	Marseniopsis mollis			*Bathydoris clavigera*			*Tritonia challengeriana*		
	SL	PL	FFA	SL	PL	FFA	SL	PL	FFA
16:0	28.8±7.2	16.2±5.5	5.0±1.9	-	14.7±3.1	10.7±3.3	1.6±1.1	nr	1.3±0.4
17:0	8.5±3.7	4.8±2.8	1.1±0.7	-	1.6±0.5	1.5±0.7	5.0±2.2	0.9±0.6	0.6±0.2
18:0	22.1±6.7	6.1±2.1	3.3±1.4	-	9.1±3.1	6.4±2.4	8.8±2.4	8.6±3.2	7.6±2.8
16:1 ω7	4.5±2.1	0.6±0.2	1.2±0.5	-	5.0±2.8	3.8±1.0	28.5±7.7	13.9±3.6	14.8±4.4
18:1 ω7/ ω 9	14.5±4.4	7.1±3.0	5.3±1.4	-	6.6±2.8	7.5±2.0	13.0±5.1	5.6±2.1	9.7±3.4
20:1	11.0±3.9	14.1±5.6	12.2±6.5	-	9.1±2.8	10.4±4.1	10.4±5.1	10.2±2.8	9.5±3.6
18:2 ω6	nr	nr	nr	-	3.6±1.6	1.2±0.7	nr	nr	1.1±0.4
20:4 ω6	1.0±0.4	5.6±1.8	26.6±3.1	-	13.8±4.1	22.2±6.3	20.7±7.2	43.8±7.9	40.9±5.4
20:5 ω3	2.8±1.3	29.2±8.6	15.9±4.9	-	11.6±4.2	11.1±4.3	2.7±1.4	4.8±1.2	5.5±1.5
22:5 ω6	nr	4.9±1.5	5.5±2.1	-	10.1±3.4	6.7±3.2	0.7±0.4	6.5±3.3	0.7±0.2
22:6 ω3	1.4±0.8	6.5±2.4	19.7±3.4	-	8.8±2.6	15.3±6.4	1.2±0.3	3.0±1.6	3.1±1.7

from wax esters, since this was not the aim of this study.

GC-MS analysis

Analysis of FAMEs was carried out on a Fisons MD800 Mass Spectrometer coupled to a Fisons GC8000 Chromatograph equipped with a J&W Scientific DB5-MS column (30 m x 0.25 mm x 0.25 μm). Helium was used as carrier gas at a flow rate of 1 ml/min. Each sample (1 μl) was injected in split mode (1:20). The oven temperature was programmed initially at 100°C for 3 min, and then increased to 300°C at 3°C/min; the injector and the transfer line temperatures were 260°C and 240°C, respectively. Mass spectra were recorded in continuous scan mode from 50 to 450 u.m.a. with an ionization current of 70 eV; the source temperature was set at 200°C. FAMEs were identified by both retention time, previously determined on a standard mixture with alkyl chains from C-12 to C-24, and by library-assisted interpretation of mass-spectra. Percentages were measured by analysis of the peak areas in the chromatogram, by using HP G1034C Chemstations software. The results were expressed as relative percentages (% w/w) of the total fatty acid content, and were compared by using t-tests to determine statistically significant differences.

RESULTS

The total lipid content in the viscera was similar in all the studied animals, with means of 28.0±2.4 mg/g of wet tissue in Antarctic samples and 25.2±3.3 mg/g in Mediterranean samples. In the mantle, the total lipid content was consistently higher in the Mediterranean molluscs (15.8±3.1 mg/g of wet tissue) respect to the Antarctic ones (3.2±0.4 mg/g of wet tissue). Three different lipid pools: free fatty acids (FFA), storage lipids (SL) and phospholipids (PL) were considered for each body part (Table II). The fatty acid composition of FFA, SL and PL was determined from mantle and viscera, and it is reported here for the Antarctic molluscs (Tables III and IV). Although fatty acid composition of FFA, SL and PL was not very different, the relative percentages varied

Table IV. Relative amounts (mean % w/w ± SD) of the main fatty acids identified in the viscera of Antarctic molluscs. SL: storage lipids (triglycerids and wax esters). PL: phospholipids. FFA: free fatty acids. nr: below the measurement limit.

Tabla IV. Cantidad relativa (porcentaje medio del peso húmedo ± SD) de los principales ácidos grasos identificados en las vísceras de moluscos Antárticos. SL: lípidos de almacenamiento (triglicéridos y ésteres de ceras). PL: fosfolípidos. FFA: ácidos grasos libres. nr: por debajo del límite de resolución.

	Marseniopsis mollis			*Bathydoris clavigera*			*Tritonia challengeriana*		
	SL	PL	FFA	SL	PL	FFA	SL	PL	FFA
16:0	5.2±1.4	4.5±1.8	2.2±1.2	2.9±2.0	4.3±0.7	2.5±0.6	16.7±3.7	9.5±2.1	6.9±1.9
17:0	5.3±1.4	8.7±0.6	0.4±0.1	0.7±0.2	1.8±0.6	0.5±0.1	7.3±1.5	5.5±1.0	3.1±0.9
18:0	12.4±0.3	29.6±0.6	3.6±1.6	9.1±0.4	14.2±1.8	4.3±0.9	5.9±1.1	9.1±2.2	3.3±2.2
16:1 ω7	4.7±0.2	4.5±1.3	1.1±0.7	3.3±0.4	2.6±1.0	6.9±1.4	30.3±5.5	12.5±3.6	28.4±4.0
18:1 ω7 /ω 9	13.5±1.0	17.9±0.9	18.7±1.0	18.3±0.8	12.0±1.1	15.3±1.4	23.4±2.7	11.8±1.4	15.1±1.6
20:1	24.2±2.2	17.2±3.2	13.3±0.8	15.5±1.6	18.7±1.4	16.2±0.6	2.3±0.9	4.0±1.9	0.7±0.4
18:2 ω6	4.0±0.9	5.4±1.1	3.0±0.8	5.3±0.5	3.8±0.3	1.1±0.5	8.9±2.3	11.8±3.0	18.2±3.7
20:4 ω6	6.0±0.9	1.8±0.9	7.9±1.3	18.1±1.2	15.9±0.9	19.3±1.0	nr	16.9±1.8	10.3±1.5
20:5 ω3	9.6±1.4	4.6±0.6	20.2±2.1	10.8±0.9	8.7± 0.9	11.4±0.8	nr	6.9±2.1	6.6±1.1
22:5 ω6	4.0±1.5	nr	7.2±0.4	3.5±0.9	5.0±0.4	5.1±1.1	nr	1.6±0.3	1.5±0.6
22:6 ω3	5.3±0.2	0.9±0.3	18.1±0.3	8.2±1.0	7.5±0.3	12.5±1.4	nr	5.0±0.7	1.8±0.2

from mantle to viscera in both Antarctic and Mediterranean molluscs. Lipids from mantle of Antarctic animals contained the highest levels of polyunsaturated fatty acids (PUFA) counting for ca. 50%. Also, the fatty acid composition varied considerably in SL and PL of the same individual (Table III).

In general, the SL of the mantle of Antarctic molluscs consisted largely of saturated (SFA) and monounsaturated (MUFA) components, with high SFA/PUFA and MUFA/PUFA ratios (Table V). Particularly, the SFA/PUFA ratio in SL of Mediterranean species was significantly lower than for Antarctic species. The PL of the mantle of Antarctic molluscs were featured by a high content of PUFA, with similar ratios for the SFA/PUFA and MUFA/PUFA ratios (Table V). Accordingly, the analysis of the SL composition of the mantle of Antarctic molluscs revealed that palmitic acid (16:0) and stearic acid (18:0) were the main acyl residues, whereas arachidonic acid (20:4 ω6) and eicosapentaenoic acid (20:5 ω3) were the dominant species in PL (Fig. 1). Compared to Antarctic gastropods, the mantle PL of Mediterranean molluscs contained higher levels of MUFA (Table V), with a particularly large content of octadecaenoic acid (15.98±12.4; 18:1 ω7/ω9) (Fig. 1). The MUFA/PUFA ratio was significantly higher than the Antarctic value (Table V).

Mantle sections of *T. challengeriana* contained levels of palmitoleic acid (16:1 ω7) considerably higher than those of palmitic acid and stearic acid, but the other two Antarctic species revealed an opposite composition (Table III). A similar trend was found in the viscera (Table IV). The overall percentage of PUFA, mainly arachidonic and eicosapentaenoic acids, was very similar in mantle PL from *M. mollis*, *B. clavigera* and *T. challengeriana* (49.2%, 44.3% and 58.0%, respectively) although the specific composition varied according to the species (Table III).

The extracts of the viscera contained similar amounts of FFA in Antarctic and Mediterranean animals (58.1±17.2 μg and 61.1±14.1 μg *per* mg of lipid extract, respectively). However, the content of FFA in the mantle was consistently higher in the Antarctic organisms (226.9±24.4 μg and 62.5±18.1 μg *per* mg of lipid extract, respectively in Antarctic and Mediterranean molluscs). The GC-

Table V. Fatty acid ratios in mantle SL (storage lipids: triglycerids and wax esters) and PL (phospholipids) in Antarctic and Mediterranean gastropods. SFA: Saturated fatty acids; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids. *: significantly different than the Antarctic species value ($p \leq 0.01$; t-test).
*Tabla V. Relación de ácidos grasos en SL del manto (lípidos de almacenamiento: triglicéridos y ésteres de ceras) y PL (fosfolípidos) en gasterópodos Antárticos y Mediterráneos. SFA: Acidos grasos saturados; PUFA: ácidos grasos poliinsaturados; MUFA: ácidos grasos monoinsaturados. *: significativamente diferente del valor obtenido en especies Antárticas ($p \leq 0.01$; t-test).*

	SL		PL	
	Antarctic	Mediterranean	Antarctic	Mediterranean
SFA/PUFA	2.45	0.39*	0.40	0.52
MUFA/PUFA	2.68	1.24	0.47	1.78*

MS analysis of mantle FFA showed a trend in fatty acid distribution similar to that found in mantle PL, with SFA and MUFA predominant in Mediterranean animals and PUFA more abundant in Antarctic organisms (Fig. 2). However, the fatty acid composition of mantle FFA in Antarctic molluscs proved to be rather different from that found in mantle PL and SL of the same species (Table III). Also the FFA profile in the mantle of Mediterranean molluscs showed very few similarities to the fatty acid distribution in FFA, PL and SL from the viscera of the same species (Tables III and IV). Analysis of FFA composition revealed that arachidonic acid (20:4 ω6), eicosapentaenoic acid (20:5 ω3) and docosahexaenoic acid (22:6 ω3) predominated significantly in Antarctic molluscs ($p<0.05$; t-test) whereas Mediterranean animals were mainly featured by a higher percentage of octadecadienoic acid (13.75±0.98; 18:2 ω6) which was almost absent in Antarctic animals (0.76±0.66) (Fig. 2).

DISCUSSION

In this study we analyzed the lipid composition of the opisthobranchs *B. clavigera*, *T. challengeriana*, *H. picta* and *D. limbata*, and the prosobranch *M. mollis*. Although the data are of limited value due to the few number of individuals analyzed from Antarctica, we believe that the results provide useful information on their fatty acid composition, distribution and comparison with Mediterranean species.

The total lipid content was very similar in the viscera of Antarctic and Mediterranean molluscs, but it proved to be higher in mantle sections of Mediterranean animals respect to the Antarctic ones. May be this difference could be related to the abundance in the Mediterranean species of non-polar components, such as terpenoids (ÁVILA, CIMINO, FONTANA, GAVAGNIN, ORTEA AND TRIVELLONE, 1991; ÁVILA, CIMINO, CRISPINO AND SPINELLa, 1991) that were less abundant in the extracts of the Antarctic species studied here.

T. challengeriana, contrary to the other Antarctic species, contained levels of palmitoleic acid (16:1 ω7) in mantle considerably higher than those of palmitic acid and stearic acid. As a similar trend was found in the viscera, we believe this may reflect dietary preferences. Lipid biomarkers have been recently used to clarify Antarctic trophodynamics in krill (see PHLEGER, NELSON, MOONEY AND NICHOLS, 2002). Perhaps further studies in Antarctic opisthobranchs will also help to understand their poorly known trophic relationships with other benthic organisms.

In theory, lipids from the viscera should be more dependent on factors

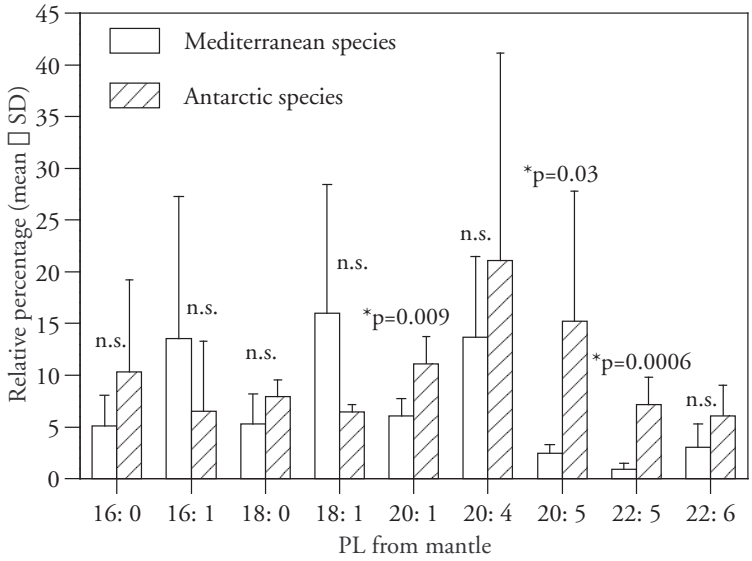

Figure 1. Relative percentage (mean ± SD) of phospholipid fatty acids (PL) in the mantle of the studied gastropod molluscs from the Antarctic and the Mediterranean. Statistical differences were determined by t-tests. *: p<0.05. n.s.: not significant. Acids: 16:0 palmitic acid, 16:1 palmitoleic acid, 18:0 stearic acid, 18:1 octadecaenoic acid, 20:1 eicosanoic acid, 20:4 arachidonic acid, 20:5 eicosapentaenoic acid, 22:5 docosapentaenoic acid, 22:6 docosahexaenoic acid.

*Figura 1. Porcentaje relativo (media ± SD) de ácidos grasos asociados a fosfolípidos (PL) en el manto de los moluscos gasterópodos estudiados de la Antártida y del Mediterráneo. Las diferencias estadísticas se determinaron mediante t-tests. *: p<0,05. n.s.: no significativo. Nombres de los ácidos: 16:0 ácido palmítico, 16:1 ácido palmitoleico, 18:0 ácido esteárico, 18:1 ácido octadecaenoico, 20:1 ácido eicosanoico, 20:4 ácido araquidónico, 20:5 ácido eicosapentaenoico, 22:5 ácido docosapentaenoico, 22:6 ácido docosahexaenoico.*

such as diet and reproductive cycles, whereas fatty acid composition of mantle should be far more responding to environmental conditions, such as temperature or depth. The fatty acid composition in SL and PL of mantle extracts were approximately similar in all animals studied, although the mantle PL of Antarctic species showed a higher content of unsaturated fatty acids (Fig. 1). In fact, the overall levels of PUFA in PL were similar in the three species of Antarctic molluscs, but were consistently higher than those of molluscs from the Mediterranean. MUFA/PUFA ratios in mantle PL were significantly divergent in Antarctic and Mediterranean molluscs, and suggested a different composition of membrane phospholipids from the two groups. In fact, fatty acids in PL have positional specificity, being the *sn*-1 and *sn*-2 position of glycerol usually occupied by saturated (or trans-unsaturated) and polyunsaturated groups, respectively. At a molecular basis, the fatty acid composition of the mantle of Antarctic specimens supported the presence of a large fraction of PL with polyunsaturated/polyunsaturated or monounsaturated/polyunsaturated chains, whereas the analysis of the Mediterranean specimens indicated an opposite trend in PL with saturated or monounsaturated species at both the 1-position and the 2-position. In a study on fish (DEY *ET AL.*, 1993), it was suggested that some phospholipids, such as those containing oleic/docosahexaenoic and oleic/eicosapentaenoic acids, play an

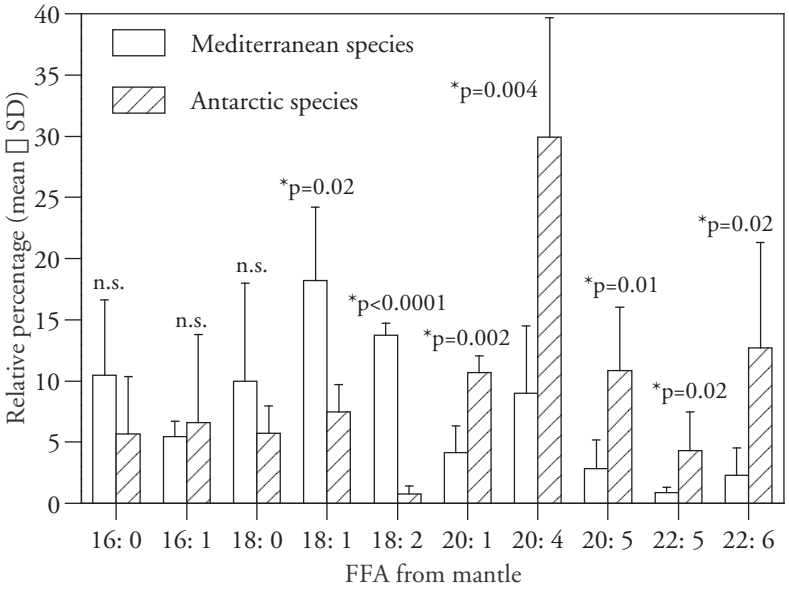

Figure 2. Relative percentage (mean ± SD) of free fatty acids (FFA) in the mantle of the studied gastropod molluscs from the Antarctic and the Mediterranean. Statistical differences were determined by t-tests. *: p<0.05. n.s.: not significant. Acids are as in Figure 1, and 18:2 is octadecadienoic acid.
*Figura 2. Porcentaje relativo (media ± SD) de ácidos grasos libres (FFA) en el manto de los moluscos gasterópodos estudiados de la Antártida y del Mediterráneo. Las diferencias estadísticas se determinaron mediante t-tests. *: p<0,05. n.s.: no significativo. Nombres de los ácidos como en la Figura 1, y 18:2 es el ácido octadecadienoico.*

important role in the membrane homeostasis. In particular, an increase of the unsaturated fatty acid percentage in the *sn*-1 position of phospholipids, such as that due to the replacement of palmitic acid by oleic acid, may affect the membrane structure in order to maintain its functional integrity at cold temperatures (DEY *ET AL.*, 1993). Our data, therefore, are in agreement with this, having found high levels of polyunsaturated or monounsaturated fatty acids in position *sn*-1 of mantle phospholipids in Antarctic animals.

Our results also showed a very high content of FFA in the Antarctic samples. Free fatty acids may be produced due to degradation of SL and PL during handling and storage. Consequences of slow frozen storage autolysis are well know in fish research (HARDY, MCGILL AND GUNSTONE, 1979) and these may have affected our FFA results, since our procedure consisted in a very fast storage of samples at –30°C until extraction, while only a storage a –80°C completely blocks enzymatic activity. However, there are some evidences suggesting a physiological meaning for these FFA levels. First, degradation did not occur at a similar rate in mantle and viscera of the animals, since we detected higher quantities of FFA in the mantle. Moreover, no apparent correlation was observed between the FFA composition of the mantle and that of the other lipid pools from both mantle and viscera. We believe that the production and occurrence of high levels of FFA may be a distinct characteristic of these Antarctic species, reflecting the chemical-physical properties of the cold-adapted metabolism of these organisms (e.g. lipases). Further research should investigate this possibility with larger numbers of specimens.

Besides the reported high amounts, the specific composition of FFA in the mantle of Antarctic and Mediterranean molluscs was also remarkably different (Fig. 2). While Antarctic species were characterized by higher levels of PUFA, Mediterranean animals showed a dominance of saturated and monounsaturated species. It seems probable that the different distribution of FFA may indicate an environmental adaptation. Whether and how the accumulation of FFA is related in any way to the membrane homeostasis remains to be thoroughly investigated. It is interesting to note, however, that the high concentration of FFA can be one of the ways to transcend the positional specificity of fatty acids in phospholipids (MEAD, ALFIN-SLATER, HOWTON AND POPJÀK, 1986). In fact, the formation of phospholipids involves the transfer of an acyl group from CoA to either *sn*-1 or *sn*-2 positions of the corresponding lysophosphoglycerides. Such transacylation is catalysed by acyltransferases, which are enzymes sensitive to the chemical features of the fatty acid chains. As discussed above, the final result of this preference is the positioning of saturated fatty acids at the 1-position and of *cis*-unsaturated fatty acids at the 2-position. It has been demonstrated that the position-dependent specificity of acyltransferases can be overridden by the fatty acid concentration (MEAD *ET AL*., 1986). In the Antarctic molluscs, the presence of high levels of PUFA, therefore, may be needed for the synthesis of phospholipids with polyunsaturated chains at both the 1-position and the 2-position of glycerol. Further studies are, however, necessary to clarify this point.

Our results show that gastropod molluscs, and opisthobranchs in particular, possess biochemical mechanisms of adapting the fatty acid composition of phospholipids to temperature. Similarly, Viarengo and coworkers (VIARENGO, ACCOMANDO, ROMA, BENATTI, DAMONTE AND ORUNESU, 1994) demonstrated that fatty acid differences between Antarctic and Mediterranean scallops were related to cell membrane adaptation to low temperatures. Although the number of species investigated in our study is very limited, we suggest that these data are representative of the lipid adaptation in gastropod molluscs from cold and temperate environments.

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