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Variability of fatty acid components of marine and freshwater gastropod species from the littoral zone of the Red Sea, Mediterranean Sea, and Sea of Galilee

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Abstract

Eight marine gastropod species from the littoral zone of the Red and Mediterranean Seas, and four freshwater gastropods from the Sea of Galilee were analysed for their fatty acid composition using TLC (silver nitrate impregnated silica gel) and gas chromatography–mass spectrometry (GC/MS). The major fatty acid components in all twelve samples were polyunsaturated fatty acids (PUFA). The total lipids of the Sea of Galilee species contained considerably more 22:6n-3 (from 10.33% to 12.63%) and 20:5n-3 (up to 2.67%) than those from marine species. The differences among the PUFA in these species appear to be due mainly to different environmental conditions, dietary habits and geographical spreading.

Keywords: Fatty acids; GC-MS; Marine; Freshwater; Gastropods

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1. Introduction

The fatty acid distributions of a large number of marine and/or freshwater molluscs, usually of commercial importance (Ackman, 2000; Karakoltsidis et al., 1995; Soriguer et al., 1997), have been reported upon and reviewed in varying degrees of detail (Joseph, 1989). The phylum Mollusca with four main classes, *Polyplacophora*, *Gastropoda*, *Bivalvia* and *Cephalopoda*, form an interesting group of invertebrates displaying a variety of lipid and fatty acid components (Ackman et al., 1980; Joseph, 1989) and embracing both freshwater and marine members. Lipids from marine molluscs are more extensively studied than those from freshwater or terrestrial representatives. Substantial sets of data on marine species are reported in some publications (Ackman, 2000; Johns et al., 1979; Joseph, 1982, 1989). Nevertheless, freshwater and terrestrial molluscs deserve special attention from the point of view of their evolutional relationship within the sequence: marine molluscs → freshwater molluscs → terrestrial molluscs. A comparative biochemical study on fatty acid components from molluscs belonging to one and the same class but living in different habitats, is expected to provide an insight into the adaptive capabilities of molluscs and the influence of the environment on the mollusc lipid composition. Marine members from the mollusc class *Gastropoda* have been found to contain 160, 18:1n-9, 20:4n-6, 20:5n-3 and 22:5n-3 as the major fatty acids (Joseph, 1982). Freshwater species of the same class contain 160, 18:1n-9, 20:4n-6 and 22:6n-3 (Freid et al. (1993); Dembitsky et al. (1992, 1993a, 1993b, 1994)as the major fatty acid components.

Compared with information on fatty acid composition, correlation with environmental factors and/or evolutionary relationships in the Gastropoda is notably limited in the literature. This paper discusses the fatty acid distributions in a range of marine molluscs common in littoral zones of the Red and Mediterranean Seas and also freshwater gastropod species in the Sea of Galilee.

2. Experimental

2.1. Marine gastropod species and sea environmental conditions

Nassa serata, *Nassarius albescens*, *Nodilittorina subnodosa*, and *Planaxis sulcata* were collected in a rocky littoral zone (0–1 m depth; water temperature 26.7 °C) in October 1995 in the Red Sea, Gulf of Aqaba (Eilat, Israel). Twenty to twenty-five samples of each gastropod species were use for lipid extraction. The Gulf of Aqaba, the northernmost extension of the Red Sea, is a semi-enclosed sea 180 km long, oriented north-to-south. It is home to the world's northernmost coral reef ecosystems, due to the exceptional tropical climate of the area. Summer temperatures in the surrounding desert areas, and the nearly constant brilliant sunshine, heat the water to 29 °C at some shallow waters, and 26 ° on average at the surface. These unusual geographic features are due to the Gulf being situated within the Syro-African Rift Valley, which stretches from East Africa all the way to Turkey. Very little water flows into the Gulf of Aqaba, and its southern mouth at the Strait of Tiran is

extremely narrow. As a result of these factors, the Gulf is a highly saline, still environment, with little hydrological interface with the Red Sea or the larger Indian Ocean (Zwirn, 1998). Chlorine ion concentration in the Gulf of Aqaba is 20,557, pH = 8.87, Salinity 41.51 ‰. The main seaweed characteristic of the Aqaba Gulf are: *Ulva lactuca* (Chlorophyta), *Enteromorpha compressa* (Chlorophyta), *Padina pavonica* (Phaeophyta) and *Laurencia obtusa* (Rhodophyta) (Wahbeh, 1997). Other macrophytic algae are also present (Lundberg and Popper, 1999). *Ulva lactuca* and *Enteromorpha compressa* were found as the dominant algal species in the mollusc habitats.

Monodonta turbinata, *Gibula cineraria*, *Littorina neritoides* were collected in a littoral zone (0–1 m depth; water temperature 27.4 °C) in July 1996 in the Mediterranean Sea (Ashdod, Ashkelon). Eighteen to twenty-three samples of each gastropod species were used for lipid extraction. Some physical features characteristic of the Israeli Mediterranean coast were: pH = 8.17 and salinity 37.61‰. Two red algae species, *Spaerococcus coronopifolius* and *Corallina elongata*, were dominant on rocks where gastropod species collected.

2.2. Freshwater gastropod species and Lake environmental conditions

Melanoides tuberculata, *Thedoxus jorduni*, *Pyrgula barroisi* and *Melanopsis praemorsum* were collected from 0–1.2 m depth, in September 1996 (water temperature was 28.1 °C) in the Sea of Galilee (Lake Tiberias = Lake Kinneret). Twenty-six to thirty-two samples of each gastropod species were used for lipid extraction. Lake Kinneret (The Biblical Sea of Galilee) is a warm, monomictic lake, located at the northern end of the Afro Syrian Rift Valley in Northern Israel with a surface area of 170 km^2, a maximum depth of 43 m and a mean depth of 24 m (Serruya, 1978). Chlorine ion concentration in Lake Kinneret has declined from 400 mg/l to 210–240 mg/l since 1967 and water pH (0–12 m depth) varies from 8.9 in June to 8.0 in January/December (Serruya and Pollingher, 1983). Lake salinity is 10.23 ‰. Lake flora that are characteristic of the emerged macrophytes: *Phragmites australis*, *Arundo donax*, *Juncus actus*, and also of the submerged macrophytes: *Myriophyllum spicatum*, *Najas marina* (Serruya, 1978).

2.3. Extraction of fatty acids and GC–MS analysis

Fresh material of each sample was put in methanol and kept at –10°C, the solvent concentrated under nitrogen and lipids extracted by means of dichloromethane-methanol (1:2, v/v). Fatty acid methyl esters (FAME) were prepared by the basic transesterification of the total lipids that had been extracted from the molluscs using a mixture of CH_2Cl_2–MeOH (Řezanka and Dembitsky, 1999). The oxazolines were prepared by a modification of the literature methods (Fay and Richli, 1991). Oxygen was removed from the TLC mobile phase by bubbling with helium. Butylated hydroxytoluene was added to the other solvents (70 mg/L). All the vessels and the rotary evaporator were flushed with nitrogen before use. For qualitative study, preparative TLC was employed (10 mg of esters was separated on a 20 × 20 cm plate coated

with 0.5 mm silica gel containing 10% silver nitrate) using the solvent system *n*-hexane-diethyl ether (2:3, v/v). After visualising under UV (254 nm), the components with 4–6 double bonds (fraction IV) were eluted from the adsorbent with CH_2Cl_2–MeOH (9:1, v/v). The fraction contaminated with silver ions was eliminated from the extract by washing with dilute ammonia (pH ~ 9). However, only the original fatty acid samples were used for quantification. The total FAMEs, and total oxazolines after separation on silver nitrate-impregnated silica layers were identified using a Finnigan MAT 1020 B spectrometer (Finnigan MAT, San Jose, CA). A 60 m × 0.25 mm SUPELCOWAX 10 column with a film thickness of 0.25 μm (Supelco, Gland, Switzerland) was used. Injection temperature was 100 °C. Temperature program was as follows: 100 °C for 1 min, then 100 °C to 230 °C over 7 min, 230°C to 280 °C over 25 min and 280 °C for 10 min. The carrier gas was helium at a linear flow rate of 70 cm/s. Mass spectra were scanned within *m/z* 50–700.

2.3.1. Algal species collected at the same sites and time from gastropod habitats

Analysis of FAME from marine algae and lake macrophyte were carried out with a gas chromatograph Hewlett-Packard 5890 (series II) (Palo Alto, CA), equipped with a 5971B MSD mass selective detector. FAME were analyzed by GC–MS using two coupled capillary columns with different stationary phases: a RTX-l column; 30 m, ID = 0.32 mm, film thickness 0.25 μm, (Restek, PA) coupled with a second capillary column; RTX-1701 30 m, ID = 0.32 mm × 0.25 μm film thickness (Restek). The GC oven program had an initial temperature of 40 °C for 2 min, 2 °C/min run to 300 °C and final hold at 300 °C (20 min). Injector temperature was kept at 180 °C (splitless), and carrier gas (helium) flow rate was 25 cm/sec. The MS Detector was operated at 194 °C, scan range was from 30 to 650 m/z at 0.9 scan/set scan rate. Solvent delay was 10 min. FAME were identified by mass spectral library search (Wiley, 7th Edition) as described by Dembitsky et al. (1999).

3. Results and discussion

Taxonomic and food chain studies are important in understanding interrelationships in marine and freshwater environments, but surprisingly for the littoral zone, no data were available in the literature on the gastropods which inhabit this environment. This is particularly true for the Jordan Rift Valley species. The gastropods reported in this paper are primarily from three temperate and saline water sites which differ from one another quite markedly in the variety of species of algae which they offer to the animals.

The Jordan Rift Valley is one of the sections of the great rift system extending from the southern edges of the Anatolian highland to the Red Sea and crossing the African continent (Por, 1963). The small number of mollusc species living in the Sea of Galilee can be explained only by the drastic geological events that occurred in the Jordan Rift Valley during the Pleistocene (Tchernov, 1975). Most of the prosobranchs presently inhabiting the Sea of Galilee are Tethyan relicts.

Fatty acids isolated from twelve gastropod species were identified in both forms

as methyl esters and oxazolines by GC/MS as described recently by Řezanka and Dembitsky (1999).

The proportion of saturated and branched fatty acids in the examined species varied from 11.85% to 21.60% of total lipids (Table 1). The major saturated acids were 14:0 and 16:0. Most of the acids identified in the course of the present study were monoenoic, ranging from 14:1n-7 to 24:1n-9. *cis*-C_{20} and C_{22} acids were represented by five isomers of each; 16:1 acids were also represented by four isomers, n-9, n-7, n-5 and *trans*-3-16:1. A wide range (28 acids, from C_{14} to C_{24}) of monoenoic acids was detected in ten species, in amounts generally less than 1%. Several acids, 16:1n-9, 18: 1n-9, 20:1n-9 and 22:1n-9, were present in amounts exceeding 1%, and were characteristic of the species studied (Table 2). Fourteen fatty acids were identified as dienoic acids, among which were non-methylene-interrupted (NMI) acids: 5,11-20:2, 5,13-20:2, 7,13-22:2 and 7,15-22:2, in amounts less than 0.4% (Table 3). Paradis and Ackman (1977) found varying amounts of NMI in some molluscs, from 0.5% to 2.6% (for 20:2), and 0.1% to 5.8% (for 22:2). The most interesting are NMI fatty acids, which frequently occur in marine (Ackman, 1989; Zhukova, 1991) and freshwater (Dembitsky et al., 1992, 1993a, 1994) molluscs, including gastropod species. Tri-, tetra-, penta- and hexaenoic compounds are the most abundant fatty acids in the species examined (43.59% to 57.88%). The major acids among total lipids in marine species were 18:3n-3 (7.46% to 13.75%, 18:3n-6 (10.21% to 13.87%) and 20:4n-6 (7.05% to 9.36%) (Table 4). Small amounts of 24:5n-6 and 24:6n-3 acids were found in all species, and high percentages of 22:6n-3 (up to 12.63%) in freshwater species. Small amounts of 18:4n-3 and 16:4n-1 acids were found in both gastropod species, mostly less than 1%. Earlier, we found a high percentage of 18:4n-6 in the glycolipids of two Baikalian gastropod species, in *Benedictia baicalensis* (13.7%) and in *Baicalia oviformus* (15.92%) and 18:4n-3 in phospholipids of *Benedictia baicalensis* (19.35%) (Dembitsky et al., 1993a).

Figure 1 illustrates the chromatogram obtained for the polyunsaturated fatty acids isolated from marine as well as freshwater samples. When the relative proportions of the classes of polyenoic fatty acids are examined in detail, interesting contrasts emerge.

Comparative studies of fatty acids in marine, brackish and freshwater molluscs were reported in many publications (Ackman, 2000; Dembitsky and Řezanka, 1996; Dembitsky et al., 1993b; Joseph, 1989, 1982; Paradis and Ackman, 1977). The relations between fatty acids and diet or environmental conditions have often been complicated by occasional reports of high proportions of polyunsaturated acids such as 20:4n-6, 20:5n-3 and 22:6n-3, and/or saturated acids.

In a comparative study of composition fatty acids, in particular gastropod species, and other invertebrates and fish, it is often difficult to determine which of various factors influences the content: diet, water temperature or salinity, wave action and/or physiological status (Logue et al., 2000; Nichols et al., 2000; Coull, 1999; Ringo and Gatesoupe, 1998; Katter et al., 1998; Wells et al., 1998; Conaway et al., 1996; Cheung and Lam, 1995; Zhu et al., 1994; Williams and Mellor, 1991). In a series of articles it was reported that the algal diet essentially could influence composition

Table 1
Comparison of the proportion of saturated and branched fatty acids of marine and freshwater gastropod species.

FA Type	Marine species												Freshwater species				
	Red Sea						Mediterranean Sea						Sea of Galilee				
	1	2	3	4	A1	A2	5	6	7	8	A3	A4	9	10	11	12	A5
i-13:0		0.02	0.09					0.01		0.02			0.01	0.11			
ai-13:0	0.01	0.03	0.13	0.02			0.01	0.03		0.04			0.03	0.13	0.13		
13:0	0.02	0.06	0.20	0.03	0.31		0.02	0.09	0.01	0.06	1.43		0.08	0.19	0.02	0.01	
i-14:0	0.02	0.07	0.16	0.04			0.03	0.05		0.07			0.05	0.13	0.02		
14:0	7.78	6.49	5.94	7.74	1.24	1.48	8.94	2.44	4.14	2.40	1.74	0.14	2.30	3.59	2.42	2.92	2.35
4,8,12-TM-13:0		0.12	0.20		0.30			0.26		0.14			0.24	0.14	0.08	0.19	
i-15:0	0.03	0.20	0.07	0.05			0.03	0.24	0.02	0.22		0.54	0.22	0.98	0.08	0.02	
ai-15:0	0.06	0.32	0.28	0.11			0.05	0.31	0.24	0.35			0.30	0.60	0.04	0.04	
15:0	0.07	0.37	0.41	0.10	0.70		0.07	0.43	0.06	0.63		0.59	0.41	1.13	0.11	0.05	
i-16:0	0.12	0.38	0.22	0.12			0.12	0.25	0.02	0.42			0.26	0.72	0.04	0.02	
Pristanic	0.02	0.19	0.12	0.03			0.03	0.33	0.01	0.21			0.31	0.94	0.01	0.01	
16:0	5.30	5.93	5.64	5.55	16.70	19.52	5.51	7.82	9.86	8.71	39.45	36.28	7.36	6.78	4.34	6.24	33.12
i-17:0	0.07	0.16	0.11	0.05			0.07	0.15	0.02	0.17			0.14	0.43	0.04	0.02	
ai-17:0	0.05	0.31	0.37	0.04			0.05	0.34	0.09	0.34			0.32	0.65	0.06	0.08	
17:0	0.16	0.30	0.41	0.08	0.60		0.17	0.52	0.05	0.66		0.41	0.49	1.14	0.14	0.04	
i-18:0	0.01	0.12	0.12	0.02			0.01	0.12	0.01	0.21			0.11	0.21	0.02	0.01	
18:0	0.53	0.58	0.60	0.38	0.93	1.67	0.56	3.61	3.11	1.73	1.01	2.35	3.40	2.11	3.12	1.92	2.24
i-19:0	0.02	0.12	0.25	0.02			0.03	0.15	0.01	0.12	0.45		0.14	0.25	0.02	0.01	
ai-19:0		0.07	0.19	0.01				0.13		0.07			0.12	0.22	0.01		
19:0	0.01	0.29	0.10	0.04	0.70		0.01	0.31	0.02	0.32			0.30	0.69	0.04	0.02	

(*continued on next page*)

Table 1 (*continued*)

FA Type	Marine species												Freshwater species				
	Red Sea						Mediterranean Sea						Sea of Galilee				
	1	2	3	4	A1	A2	5	6	7	8	A3	A4	9	10	11	12	A5
0.20	0.12	0.23	0.25	0.17	0.25		0.12	0.71	0.21	0.80			0.67	0.31	0.22	0.19	
22.0	0.07	0.40	0.16	0.08	0.18		0.07	0.20	0.06	0.44		0.49	0.19	0.13	0.08	0.05	
24:0	0.02	0.23	0.24	0.02			0.02	0.08	0.01	0.25		0.32	0.07	0.02	0.03	0.01	
Total Saturated	14.49	16.99	16.26	14.70	21.91	22.67	15.93	18.58	17.95	18.29	44.08	41.12	17.52	21.60	10.99	11.85	37.71
Correlation r	Pearson 0.2292	–0.1350	– 0.1488	– 0.2408			– 0.2360	0.0372	– 0.1567	0.0195			0.0369	– 0.0663	–0.0347	– 0.1382	
P value (two tailed)	0.3310	0.5392	0.4981	0.2931			0.3166	0.8664	0.5345	0.9297			0.8671	0.7636	0.8781	0.5727	
R squared	0.0525	0.0182	0.0221	0.0579			0.0556	0.0014	0.0246	0.0004			0.0014	0.0044	0.0012	0.0190	

Abbreviations: Marine species. Red Sea: 1, Nassa serata ; 2, Nassarius albescens ; 3, Nodilittorina subnodosa ; 4, Littorina scarba ; 5, PIanaxis sulcata. Marine algae species: A1, Ulva lactuca ; A2, Enteromorpha compressa. Mediterranean Sea: 6, Monodonta turbinata ; 7, Gibula cineraria ; 8, Littorina neritoides. Marine Algae: A3, Spaerococcus coronopifolius; A4, Corallina elongata. Freshwater species: 9, Melanoides tuberculata; 10, Thedoxus neritoides (endemic) ; 11, Pyrgulia barroisi ; 12, Melanopsis praemorsum. Lake macrophyte: A5, Myriophyilum spicatum. i, iso-; ai-, anteiso-.

Table 2
Comparison of the proportion of monoenoic fatty acids of marine and freshwater gastropod species

Acid type	Marine species												Freshwater species				
	Red Sea							Mediterranean Sea					Sea of Galilee				
	1*	2	3	4	A1	A2	5	6	7	8	A3	A4	9	10	11	12	A5
14:1n-7	0.07	0.19	0.34	0.11			0.07	0.17	0.17	0.21			0.16	0.22	0.08	0.15	
14:1n-5	0.02	0.07	0.10	0.05			0.03	0.08	0.04	0.07			0.07	0.08	0.03	0.04	
16:1n-9	0.72	1.51	1.03	1.47	1.21	2.31	0.84	2.44	1.25	2.38	4.22		2.30	3.39	2.16	1.14	2.36
16:1n-7	0.22	0.26	0.85	0.32	0.34	0.47	0.23	0.89	0.19	0.29	6.08	5.93	0.83	1.86	0.24	0.17	1.13
3t-16:1[a]		0.06	0.20					0.04		0.06		0.31	0.04	0.03			
16:1n-5	0.25	0.60	0.34	0.12			0.26	0.35	0.08	0.55			0.33	0.37	0.13	0.07	
17:1n-8	0.04	0.05	0.05	0.03			0.04	0.07	0.01	0.05			0.06	0.08	0.03	0.07	
18:1n-13	0.05	0.21	0.10	0.31			0.05	0.20	0.37	0.24			0.19	0.27	0.23	0.34	
18:1n-11	0.22	0.70	0.55	0.72			0.23	0.68	0.66	0.77			0.64	0.72	0.58	0.60	
18:1n-9	7.57	8.28	8.11	8.83	11.44	9.65	7.80	13.09	10.62	13.16	2.42	9.15	20.02	15.68	19.30	18.74	9.24
18:1n-7	0.45	0.64	0.44	0.20	0.55	0.39	0.47	1.12	1.05	1.07			1.06	0.99	1.68	2.48	
18:1n-5	0.25	0.42	0.64	0.23			0.26	0.17	0.46	0.46			0.16	0.13	0.08	0.42	
19:1[b]	0.01	0.02					0.01	0.02		0.02			0.02		0.01		
19:1n-8	0.13	0.20	0.35	0.14			0.14	0.12	0.03	0.22			0.11	0.13	0.04	0.03	
20:1n-15	0.12	0.16	0.47	0.06			0.13	0.08	0.02	0.14			0.07	0.21	0.14	0.02	
20:1n-13	0.27	0.31	0.65	0.11		0.30	0.28	0.24	0.14	0.34			0.22	0.50	0.29	0.13	
20:1n-11	0.42	0.70	0.54	0.26		0.60	0.44	0.93	0.23	0.77		0.78	0.88	0.84	0.58	0.21	
20:1n-9	0.71	1.36	1.44	0.90		0.10	0.74	1.37	1.69	1.50			1.29	0.95	1.16	1.53	
20:1n-7	0.06	0.12	0.05	0.08			0.06	0.13	0.10	0.14			0.12	0.13	0.22	0.09	
21:1n-9		0.03	0.03					0.03		0.04			0.03	0.02			
22:1n-15	0.01	0.03	0.07	0.02			0.01	0.02		0.04			0.02	0.05	0.01		
22:1n-13	0.02	0.06	0.13	0.02			0.02	0.04	0.02	0.06		2.35	0.04	0.08	0.02	0.02	
22:1n-11	0.25	0.50	0.80	0.30			0.26	0.90	0.38	0.54			0.85	0.50	0.42	0.35	
22:1n-9	0.30	0.88	0.86	0.41			0.31	2.06	1.65	0.97			1.94	1.12	1.01	0.69	
22:1n-7	0.21	0.50	0.34	0.26			0.21	0.33	0.42	0.54			0.31	0.37	0.33	0.39	
23:1n-9	0.01	0.04	0.12	0.02			0.01	0.08	0.02	0.05			0.07	0.08	0.02	0.02	
24:1n-11	0.23	0.12	0.16	0.09			0.24	0.12	0.02	0.14			0.11	0.14	0.05	0.02	
24:1n-9	0.27	0.36	0.89	0.49			0.28	0.55	0.45	0.40			0.52	0.34	0.27	0.41	

(*continued on next page*)

Table 2 (*continued*)

Acid type	Marine species												Freshwater species				
	Red Sea							Mediterranean Sea					Sea of Galilee				
	1*	2	3	4	A1	A2	5	6	7	8	A3	A4	9	10	11	12	A5
Monoenoic	12.88	18.28	19.65	15.55	13.54	13.82	13.42	26.32	20.07	25.25	12.42	18.52	32.46	29.28	29.11	28.07	12.73
Correlation	–	-0.1212	–	–			–0.1230	–	–	–			–	–	–	–	
Pearson r	0.1190		0.0998	0.1486				0.1166	0.0966	0.1341			0.1137	0.1748	0.1343	0.1219	
P value (two tailed)	0.5627	0.5391	0.6205	0.4784			0.5493	0.5547	0.6528	0.4962			0.5644	0.3833	0.5131	0.5706	
R squared	0.0142	0.0147	0.0099	0.0220			0.0151	0.0136	0.0094	0.0179			0.0129	0.0305	0.0180	0.0148	

* See abbreviations in Table 1. a, identified only on the basis retention time and mass spectrum of methyl esters.

[a] Unkn/LINKown position of double bond(s), identified by retention time and value of molecular ion of methyl ester.

[b] Prob/LINKably 18:4n-6, but this position of bouble bonds was not confirmed by GC/MS.

Table 3
Comparison of the proportion of dienoic fatty acids of marine and freshwater gastropod species

Acid type	Marine species												Freshwater species				
	Red Sea						Mediterranean Sea						Sea of Galilee				
	1*	2	3	4	A1	A2	5	6	7	8	A3	A4	9	10	11	12	A5
16:2n-4	0.34	0.12	0.03	0.25			0.35	0.35	0.41	0.14	4.33		0.33	0.02	0.04	0.38	
16:2n-9	1.76	1.29	1.54	1.60			1.83	1.10	1.14	1.41			1.04	0.22	0.88	1.04	
18:2n-6	7.22	7.18	7.44	7.94	10.31	4.47	5.80	6.73	8.42	5.68	11.69	5.36	4.15	2.51	6.17	5.99	15.21
18:2n-3	0.08	0.05	0.04	0.04	0.36	0.59	0.09	0.02	0.09	0.05	1.93		0.02	0.05	0.10	0.08	
5,11-20:2	0.35	0.24	0.16	0.32			0.37	0.41	0.23	0.26			0.39	0.24	0.36	0.21	
20:2n-9	0.59	0.70	0.13	0.29		0.90	0.62	0.33	0.31	0.77			0.31	0.70	0.29	0.28	
20:2n-6	0.71	1.87	2.02	2.05			0.74	0.07	1.21	2.05	0.58	0.35	0.66	0.21	0.14	0.19	
20.2n-3	0.62	0.26	0.18	0.41			0.64	0.05	0.19	0.05			0.05	0.26	0.12	0.17	
7,13-22:2	0.27	0.12	0.24	0.27			0.28	0.22	0.27	0.14			0.20	0.13	0.20	0.24	
7,15-22:2	0.12	0.07	0.02	0.07			0.12	0.11	0.21	0.07			0.10	0.06	0.07	0.19	
22:2n-9	0.88	0.81	0.45	0.68			0.91	0.10	0.05	0.89			0.09	0.80	0.13	0.04	
22:2n-6	1.38	0.69	0.52	1.13			1.44	0.08	0.06	0.75			0.07	0.06	0.08	0.05	
24:2b	0.16	0.12	0.05	0.17			0.16	0.07		0.14			0.06	0.13	0.17	0.08	
Dienoic	14.75	13.73	12.81	15.48	10.67	5.96	13.63	9.84	12.73	12.87	23.59	6.71	7.66	5.60	9.42	9.07	15.21
Correlation	–	–	–	–			–	–	–	–			–	–	–	–0.3977	
Pearson r	0.3124	0.3114	0.3319	0.3101			0.3097	0.3955	0.3667	0.3073			0.4379	0.2339	0.3933		
P value (two-tailed)	0.2769	0.2785	0.2464	0.2807			0.2813	0.1616	0.2178	0.2852			0.1173	0.4209	0.1642	0.1590	
R squared	0.0975	0.0969	0.1101	0.0961			0.0959	0.1564	0.1345	0.0944			0.1918	0.0547	0.1547	0.1582	

* See footnote in Table 2.

Table 4
Comparison of the proportion of polyenoic fatty acids of marine and freshwater gastropod species

Acid type	Marine species												Freshwater species				
	Red Sea						Mediterranean Sea						Sea of Galilee				
	1*	2	3	4	A1	A2	5	6	7	8	A3	A4	9	10	11	12	A5
16:3n-4	0.85	0.24	0.03	0.49			0.89	0.90	0.91	0.26			0.84	0.48	0.66	0.82	
16:3n-6	2.09	1.16	1.36	1.43	1.11	3.19	2.17	1.35	1.55	1.28		0.47	7.79	8.08	8.33	9.67	4.19
16:4n-1	1.77	1.60	1.47	1.00		5.78	1.84	0.98	1.05	1.76			6.29	4.65	6.55	9.12	3.56
18:3n-6	13.87	10.21	10.90	10.59	12.78	15.11	12.82	11.37	12.22	11.76	9.66	10.58	0.52	1.37	0.43	0.94	23.32
18.4c	0.12	0.05		0.26			0.13			0.05				0.01			
18:3n-3	13.91	13.38	12.57	11.98	12.24	14.12	13.75	9.02	12.19	7.46	0.63	6.24	0.07	0.27	0.23		3.28
18:4n-3	0.65	0.79	0.76	1.42	6.98	7.14	0.68	0.55	0.84	0.87			0.52	0.25	0.48	0.18	
19:4a	0.20	0.05		0.09			0.21			0.05			0.02	0.05	0.06	0.05	
18:5n-3	0.58	0.17	0.40	0.28	3.46		0.52	0.08	0.30	0.19			0.08	0.30	0.35	0.14	
20:3n-9	2.31	3.19	2.98	4.15			3.32	3.15	2.82	3.21			2.73	3.10	2.44	2.39	
5,11,14-20:3	0.05	0.02	0.07	0.06			0.05	0.02	0.06	0.02			0.41	0.25	0.39	0.22	
20:3n-6	0.23	0.26	0.48	0.32			0.24	0.09	0.16	0.29			0.08	0.29	0.42	0.10	
20:4n-6	9.36	8.21	8.20	8.16	13.28	5.99	8.74	8.90	7.05	6.40	7.23	9.96	4.29	5.72	7.99	8.82	
20:3n-3	0.20	0.20	0.19	0.26			0.21	0.43	0.25	0.22		1.14	0.60	1.21	1.27	0.40	
5,11,14,17-20:4	0.25	0.26	0.26	0.30			0.26	0.09	0.11	0.29			0.02	0.05	0.04	0.01	
20:4n-3	0.46	0.33	0.35	0.38	1.29	1.44	0.51	1.12	1.23	0.36			0.14	0.24	0.17	0.13	
20:5n-3	0.27	0.31	0.37	0.41	1.16	3.36	0.28	0.64	0.44	0.34	2.39	6.26	2.35	2.20	2.67	0.42	
21:5n-3	0.02	0.07	0.05	0.05			0.03	0.02	0.01	0.07			0.43	0.45	0.61	0.47	
22:3n-9	0.20	0.18	0.24	0.25			0.21	0.15	0.14	0.20			0.20	0.13	0.27	0.22	
22:3n-6	1.21	0.83	1.05	1.36			1.43	0.37	0.41	0.92			0.49	0.61	0.68	0.78	
22:4n-6	0.69	1.10	1.39	1.33			0.72	0.45	0.52	1.21			0.74	0.43	0.90	0.65	
22:4n-3	1.42	1.58	1.62	2.34	0.36	0.44	1.39	0.52	0.86	1.73			0.23	0.54	0.43	0.65	
22:5n-6	0.23	0.20	0.19	0.44			0.24	0.79	0.71	0.22			0.34	0.15	0.32	0.19	

(*continued on next page*)

Table 4 (*continued*)

Acid type	Marine species												Freshwater species				
	Red Sea						Mediterranean Sea						Sea of Galilee				
	1*	2	3	4	A1	A2	5	6	7	8	A3	A4	9	10	11	12	A5
22:5n-3	1.40	1.11	1.39	1.34			1.62	1.04	1.82	1.21			0.98	1.23	1.49	1.66	
22:6n-3	3.80	3.62	2.90	3.16	1.22	0.98	2.95	2.02	1.71	1.26			11.57	10.33	12.63	11.77	
24:4n-6	0.67	0.74	0.77	0.96			0.70	0.51	0.31	0.82			0.48	0.82	0.49	0.28	
24:5n-6	0.52	0.59	0.69	0.64			0.54	0.45	0.87	0.65			0.07	0.07	0.05	0.03	
24:6n-3	0.55	0.45	0.60	0.57			0.57	0.25	0.71	0.49			0.08	0.14	0.12	0.11	
Polyenoic	57.88	51.00	51.28	54.02	53.88	57.55	57.02	45.26	49.25	43.59	19.91	34.65	42.36	43.52	50.48	51.90	34.35
Correlation	−0.3039	−0.2650	–	–			–	–	–	–			–	–	–	–	
Pearson r			0.3259	0.2638			0.3711	0.3685	0.3750	0.3040			0.1150	0.0929	0.0950	0.2043	
P value (two tailed)	0.1159	0.1729	0.1043	0.1750			0.0994	0.0640	0.0591	0.1158			0.5679	0.6386	0.6373	0.3169	
R squared	0.0923	0.0702	0.1062	0.0696			0.1009	0.1358	0.1406	0.0924			0.0132	0.0086	0.0090	0.0417	

* See footnote in Table 2.

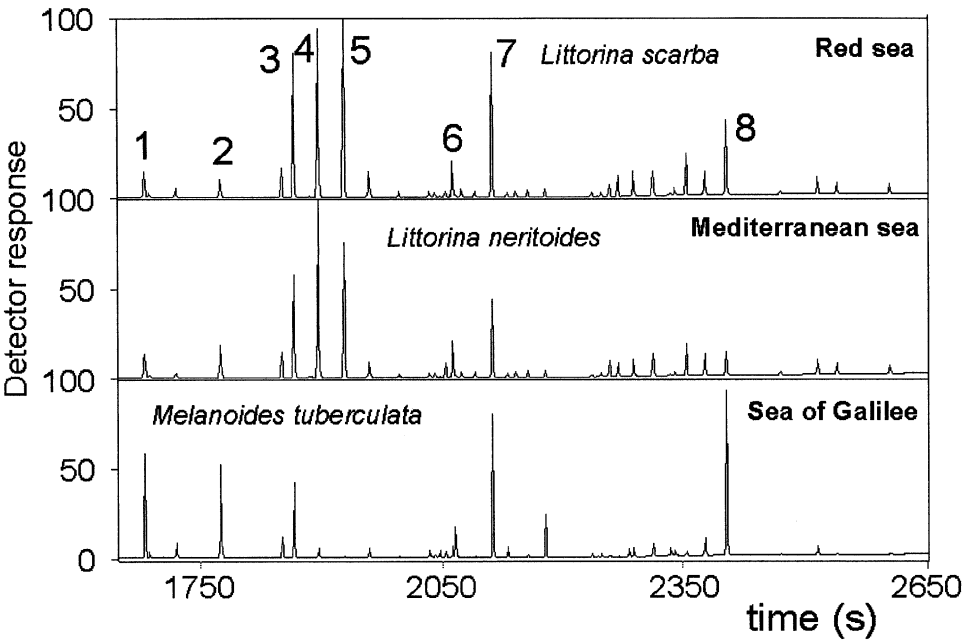

Fig. 1. The regions of the gas chromatogram (full run is 3000 set) for the separation of polyunsaturated fatty acid methyl esters isolated from marine and freshwater gastropods. Main peaks identified: **1**, 16:3n-6; **2**, 16:4n-1; **3**, 18:2n-6; **4**, 18:3n-6; **5**, 18:3n-3; **6**, 20:3n-9; **7**, 20:4n-6, and **8**, 22:6n-3. GC-MS conditions, see in Experimental section.

of fatty acids of invertebrates and fish (Ackman, 2000; Lundberg and Golani, 1995; Mustafa and Nakagawa, 1995).

The scallops *Argopecten purpuratus* were fed a mixed algal diet either alone or supplemented with an emulsion rich in ethyl esters of docosahexaenoic acid (22:6n-3) or eicosapentaenoic acid (20:5n-3) at a concentration of 50% lipids per algal dry weight. Lipid supplementation resulted in a significant increase of the total lipid content of the eggs. The fatty acid quality of the diet had no significant effect on the fatty acid profile of the polar lipids. However, supplementation of the 22:6n-3-rich emulsion resulted in a significant increase of the 22:6n-3 level in the total and neutral lipids of the eggs compared to eggs from scallops fed solely on algae. The absolute 22:6n-3 content (mg/g) increased by 47% (Caers et al., 1999).

The nutritional role of PUFA in dietary macroalgae *Alaria esculenta*, *Laminaria digitata*, *Laminaria saccharina*, *Palmaria palmata* and *Ulva lactuca* for the abalone, *Haliotis tuberculata* and *Haliotis discus hannai* were reported (Mai et al., 1996). Fatty acid analysis revealed that C18 and C20 families of unsaturated fatty acids, such as 18:4n-3, 18:3n-3, 20:4n-6 and 20:5n-3, were dominant in the brown algae, *A.esculenta*, *L. digitata* and *L. saccharina.* The red alga, *P. palmata*, was characterised by the highest proportion of 20:5n-3 among the selected algae. In the green alga, *Li. lactuca*, however, the dominant PUFA were C16 and C18 fatty acids, while C20 fatty acids were minimal. All the selected algae consistently contained very low

levels of C22 fatty acids. The authors indicated that the PUFA of both n-3 and n-6 families seem to be essential for growth of *H. discus hannai*; for *H. tuberculata.* However, growth enhancement appeared to depend largely on n-3 PUFA.

Taking into consideration that fatty acids of algae are the main component of a gastropoda diet, it is interesting to compare the contents of polyunsaturated acids of algae and studied molluscs. Fatty acids of studied algal species are presented in Tables 1–4. Thus, marine gastropod species from the Red Sea: *Nassa serata*, *Nassarius albescens*, *Nodilittorina subnodosa*, *Planaxis sulcata*, and marine algae *Ulva lactuca* and *Enteromorpha compressa* contain the same high level of 18:3n-6, 18:3n-3 and 20:4n-6. (Table 4). We found that gastropods from the Mediterranean Sea: *Monodonta turbinata*, *Gibula cineraria*, *Littorina neritoides*, and algae: *Spaerococcus coronopifolius* and *Corallina elongata*, also contain high levels of 18:3n-3 and 20:4n-6. Another pattern was observed for freshwater molluscs from Lake Kinneret. We found that the major trienoic acid was 18:3n-6 (23%) in *Myriophyllum spicatum* and that the main fatty acids in gastropod species *Melanoides tuberculata*, *Thedoxus jordani*, *Pyrgula barroisi* and *Melanopsis praemorsum* were 16:3n-6, 18:3n-6, 20:4n-6 and 22:6n-3. We cannot at this time provide an explanation for these differences, but some relationships between content of fatty acids in molluscs and macrophyte can be found among di- and monoenoic acids (Tables 2 and 3).

Recognising that the algal diet for molluscs could be one of the major factors determining the composition of fatty acids, we have carried out a comparative analysis of the relationship of fatty acid composition between algal and molluscs species (see Fig. 2). Such a comparison, certainly, is conditional; however, it evolves definite

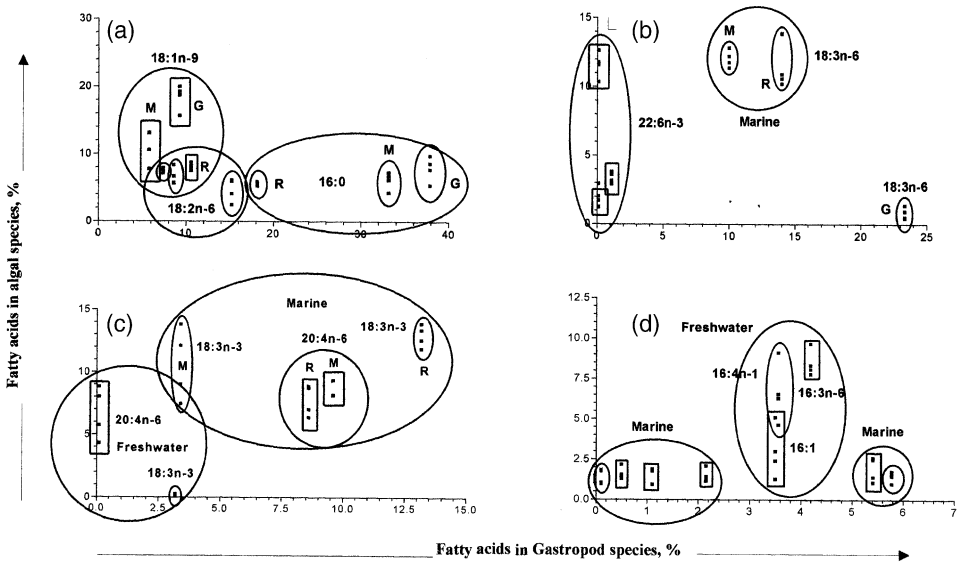

Fig. 2. Comparative allocation of the contents of some fatty acids retrieved in algae and gastropod species; both marine and freshwater representatives. See text for explanation. Abbreviation: M - Mediterranean Sea; R — Red Sea; G — Sea of Galilee.

cluster areas of allocation fatty acids, and also allows us to make some comparative conclusions. Figure 2A shows the ratio of three acids — 16:0, 18:1n-9 and 18:2n-6. Though the content of palmitic acid (16:0), which is key in a biosynthesis of fatty acids, varies over a wide range in marine and freshwater molluscs, nevertheless the distribution of acids 18:1n-9 and 18:2n-6 is compact, and large differences are not found. Figure 2B shows the variability of docosahexaenoic (22:6n-3) and 18:3n-6 acids. It is possible to explain the different contents of 22:6n-6 in marine and freshwater species and is apparently due to the difference in the biosynthesis of this acid in these species. It is known that docosahexaenoic acid is the end product in the n-3 fatty acid synthetic pathway and is an important membrane fatty acid of fish and mammals — it is not found, for example, in terrestrial slugs and snails (Zhu et al., 1994). However, high contents of 22:6n-3 (10–27 %) were found in marine oysters, clams and scallops (Ackman, 2000; Zhu et al., 1994). According to Katter et al. (1998), this acid is predominantly contained in polar lipids (up to 31%) of *Clione limacina* dwelling in both Arctic and Antarctic waters. Figure 2C demonstrates the allocation of linolenic (18:3n-3) and arachidonic (20:4n-6) acids in both marine and freshwater species. Here, it is possible to define only two cluster areas, one of which is characteristic only for the marine and freshwater species. The high level of 18:3n-3 is marked for both algae and mollusc species. Figure 2D show the ratio of the family of the C16 fatty acids, 16:1 (sum of isomers), 16:3n-6 and 16:4n-1. The contents of these acids in freshwater species of algae and molluscs differ from their marine representatives, though a strong correlation between the contents of these acids in marine algae and molluscs is not found.

The present results show that marine gastropods, in their fatty acid compositions, differ substantially from freshwater gastropod species belonging to the same class, though the diversity of fatty acids present in the freshwater group is more modest than in marine species. This statement suggests that representatives of the freshwater fauna, endemic to the Middle Eastern section of the Rift Valley, when changing their habitat and experiencing adaptation to specific freshwater conditions, preserve the basic principles of marine fatty acid biosynthesis, at least with regard to their fatty acids. We do not know the origin of some unsaturated fatty acids identified in high proportions from freshwater gastropod species. However it is possible to assume that they could accumulate from microalgae or synthesis from exogenous fatty acids. Corroborating this hypothesis requires additional studies.

The statistical analysis was done using the statistical software package GraphPad Prism ver. 3.01 (GraphPad Software, Inc., San Diego, CA, USA), and results are indicated in Tables 1–4. Correlation between saturated, monounsaturated, dienoic and polyenoic fatty acids in each group of gastropod species was also done.

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