The lipid composition of the abdominal muscle of shrimp *Crangon crangon* from the Gulf of Gdansk in spring and winter periods

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The composition of fatty acids and sterols of Crangon crangon *abdomen muscle was determined during two periods in the year 2010/2011. For determination of lipids classes, especially of fatty acids and sterols, high performance liquid chromatography with a laser light-scattering detector and gas chromatography–mass spectrometry were applied. Diversity and variety of saturated and unsaturated fatty acids was the highest during the spring period. Twenty-seven free fatty acids with from nine to 24 carbon atoms were determined in the spring periods. Among this fraction, 14 saturated and 13 unsaturated fatty acids (eight mono- and five polyunsaturated) were present. Only seven saturated and four unsaturated free fatty acids (14:2, 17:1, 16:1 and 18:1) were identified in December 2010. Arachidonic acid (20:4) and eicosapentaenoic acid (20:5) were detected in these two periods, and during the spring season an additional essential fatty acid—docosahexaenoic acid (22:6)—was present, which was not detected in the winter period. The number of identified sterols was correlated with phytoplankton, which was abundant during April 2011. Also, the sterol fraction in winter periods was very poor—only cholesterol was detected.*

INTRODUCTION

Crangon crangon, the common brown shrimp, settles sedimentary and coastal areas at 0–20 m, but including maximum depth to 130 m. The brown shrimp popularly is found in the north-eastern Atlantic, in the Baltic Sea, Mediterranean Sea and in the Black Sea (Holthuis, 1980). The maximum size is 89 mm, but mainly shrimps are 30–40 mm in length. (Tiews, 1970). Reproduction occurs several times a year, to maximum age 3–5 years (Luttikhuizen *et al.*, 2008). It is an important taxon, ecologically, commercially and economically (Luttikhuizen *et al.*, 2008). *Crangon crangon* is the major predator of shallow marine ecosystem (Van der Veer and Bergman, 1987) and is prey of few larger fish (Amara *et al.*, 2001). *Crangon crangon* is a commercially fished resource, and is food for many marine animals, being the major food for flatfish (*Pleuronectes platessa*), crabs (*Carcinus maenas*), seals (*Phoca vitulina*), various waders (Limicolae), seagulls (Laridae) and auks (Alcidae) (Jung & Zauke, 2008). This benthic organism, with *Carcinus maenas*, represents 90% of macrocrustaceans in the shallow marine ecosystem (Selleslagh & Amara, 2008). Muscles (tails), heads and shells of *Crangon crangon* are rich in proteins, calcium, vitamins, flavour components, natural antioxidants (Heu *et al.*, 2003) and lipids like fatty acids (FAs) and essential fatty acids

(Holub, 2002). Moreover, recent research suggests the possibility of the shrimp shells application to 'transform production of biodiesel fuel into a faster, less expensive and more environmentally friendly' process. Chitin only represents 69% dry weight of shrimp shell, and in appropriate conditions chitin can be transformed into saccharides, which have relatively high catalytic performance for biodiesel production, exhibiting interesting acid catalytic properties (Yang *et al.*, 2009).

Crangon crangon is the main supplier of essential fatty acids (Holub, 2002). The concentration of two main essential fatty acids in *C. crangon* amounted to about 300 mg/100 g, and it is comparable with the concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in cod (Holub, 2002).

Lipids play an important role in the organism: as a source of energy, precursors of steroid hormones, intercellular mediators, compounds of phospholipids and sphingolipids, molecules are responsible for increasing protein barrier integrity and, most importantly, dietary lipids are essential representatives of every living cell (Zhao *et al.*, 2008). Lipids are important in parasites (Mika *et al.*, 2010), insects (Gołębiowski, 2012; Gołębiowski *et al.*, 2012, 2013), marine organisms (Mika *et al.*, 2012) as well as in mammals (Green *et al.*, 2007). It is impossible for humans to synthesize some lipids, which are needed for different life processes. The new function of two main acids (EPA, DHA) is 'immunonutrients'—with a role in beneficial effects on inflammatory bowel diseases and autoimmune disease (Alexander, 1998; Stulnig, 2003), a reduction of cancer effect (Holub, 2002; Logan, 2004) and an increase of intensity of the immune system (Sijben &

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Calder, 2007; Zhao et al., 2008). Marine organisms—fish, mussels and crustaceans—are rich in polyunsaturated fatty acids (PUFAs), especially the fatty acids omega-3 (Bragagnolo & Rodriguez-Amaya, 2001; Garofalaki et al., 2006). These organisms have important commercial nutritional and economic consequences (Garofalaki et al., 2006). The higher levels of sterols (especially cholesterol) with highly unsaturated fatty acids are important components of biological membranes (Le Nechet et al., 2007), are the intermediaries in the biosynthesis of ecdysteroids (Boutry et al., 1979) and are important in reproductive biochemistry (Allen, 1999). The concentration and amount of fatty acids and sterols, and also the content of unsaturated fats, depend on many factors. The main reasons for change in the composition of the fatty acids (FAs) are: access to food and direct access to essential fatty acids omega-3, as well as to their precursors, linoleic acid 18: 2 n-6 and α-linolenic18: 3 n-3, which create the endogenous fatty acids (Maazouzi et al., 2007). However, the compositions of fatty acids and sterols are also dependent on weather conditions, climate (Limbourn & Nichols, 2009), seawater temperature (Philips et al., 2006), and season (Kasai & Sakai, 2004; Garofalaki et al., 2006). Their composition changes with the availability of plankton (Limbourn & Nichols, 2009) and directly with the type of the analysed tissue (Perez-Velazquez et al., 2003). The lowest-energy calorific and poorly energetic components are present in the winter–spring period (November–March) (Campos et al., 2009).

The primary aim of the current research is the determination of variation in total lipids, lipid classes and fatty acids composition, particularly the fatty acid profiles in the triacylglycerols (TAGs), as well as the sterols profiles. We examined quantitative lipid composition in the abdomen muscle of C. crangon in winter and spring.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals were obtained as follows: dichloromethane (high-performance liquid chromatography (HPLC) grade), ethanol (99.8%), methanol (HPLC grade), aceton (HPLC), n-hexane (HPLC), acetonitrile (HPLC), isooctane (analytically pure), 0.9% NaCl from Chempur (Piekary Śląskie, Poland), 2,5-dihydroxybenzoic acid (DHB) (HPLC grade) from Sigma-Aldrich (Poznań, Poland), and NaCl (0.9%) from Fresenius-Kabi (Kutno, Poland). Ethanol (99.8%), sodium hydrate (pills) were purchased from POCH S.A. (Gliwice, Poland) and BSTFA + TMCS (99:1) (BSTFA (N, O-bis(trimethylsilyl)trifluoroacetamide) and TMCS (trimethylchlorosilane)) was obtained from Supelco (Bellefonte, USA).

Biological material

Baltic shrimps were caught in the coastal area of the Gulf of Gdansk near Sobieszewo Island on 22 December 2010 (seven adults, weight 2236 mg) and 6 April 2011 (6 adults, weight 1953 mg) at $54°35'88''$N $18°83'16''$E. Shrimps were collected from a depth between 80 and 100 m both in the winter and spring. The Crangon crangon were identified, sexed, measured, recorded and sacrificed shortly after collection. Only non-ovigerous females were used for the analysis. Their abdominal muscles were dissected, separated from the cuticles and frozen at $-80°C$ in glass tubes. Before the analytical procedure the abdominal muscles were weighted frozen and then water was removed from the examined tissues by lyophilization. The tissues were homogenized (dissected completely) in physiological saline (0.9% NaCl) and lipids were extracted by using 20 ml dichloromethane for 20 min. The low weight of shrimps during these two periods was determined and the low number of marine organisms.

HPLC analyses

The solvents of all the extracts were evaporated in tared flasks on a roto evaporator and under a stream of nitrogen. Lipid extracts were separated into several classes using high performance liquid chromatography with laser light scattering detector (HPLC–LLSD) (Shimadzu, Japan) in the normal-phase on an analytical column (250 × 4.6 mm) filled with Econosil Silica (particle size = 5 μm). The mobile phase consisted of hexane (Solvent A) and dichloromethane with the addition of 15% acetone (Solvent B). The gradient was programmed linearly from A to B within 20 min.

GC–MS analyses

The free fatty acids (2) and sterols (3) fractions separated by HPLC–LLSD were silylized by treatment with 50 μl of 1% bis(trimethylsilyl)acetamide and 99% chlorotrimethylsilane (Sigma Aldrich), and heated at 100°C for 1 h. Triacylglycerols (1) and polar lipids (PL) (4) (detected in lipids of spring period only) were hydrolysed by heating in 100 μl of a methanolic solution of 0.5 M KOH for 3 h at 70°C in sealed ampoules. Analyses of the lipid derivatives were carried out by gas chromatography mass spectrometry (GC–MS) on a Hewlett Packard GC-MS 5890 (Finnigan Mat) equipped with a fused silica capillary column HP-5, 30 m × 0.25 mm i.d., and with film of thickness of 0.25 μm. The GC–MS was programmed from 60°C to 300°C at the rate of 4°C/min, with helium carrier gas at the column head pressure of 60 kPa.

RESULTS

Total lipid content and lipid classes

The chemical composition of lipids showed three classes during the winter period and four lipid classes during the spring (Figure 1). The following lipid classes were present in the winter extract: triacylglycerols (TAG); free fatty acids (FFA); and sterols. The polar fraction (PL) was present only in the spring extract. Table 1 summarizes differences not only in the number of classes but also the domination of classes and quantitative composition. Considerable difference was seen in the total lipid content. Level of lipids was three times higher during the spring period. The total lipid content of Crangon crangon abdomen muscle ranged from 1.1% during the winter to 3.2% of total dry mass in the spring. The percentage contents of particular fractions were as follows: TAGs 20.5% (winter) and 10.0% (spring), FFAs 44.5% (winter) and 63.0% (spring), sterols 35.0% (winter—only cholesterol) and 17.0% (spring), and PLs detected only in spring, with the same value as the TAG fraction, 10.0%. However, in both cases, the FFAs were the major lipid class and the next dominated group was sterols (Table 1).
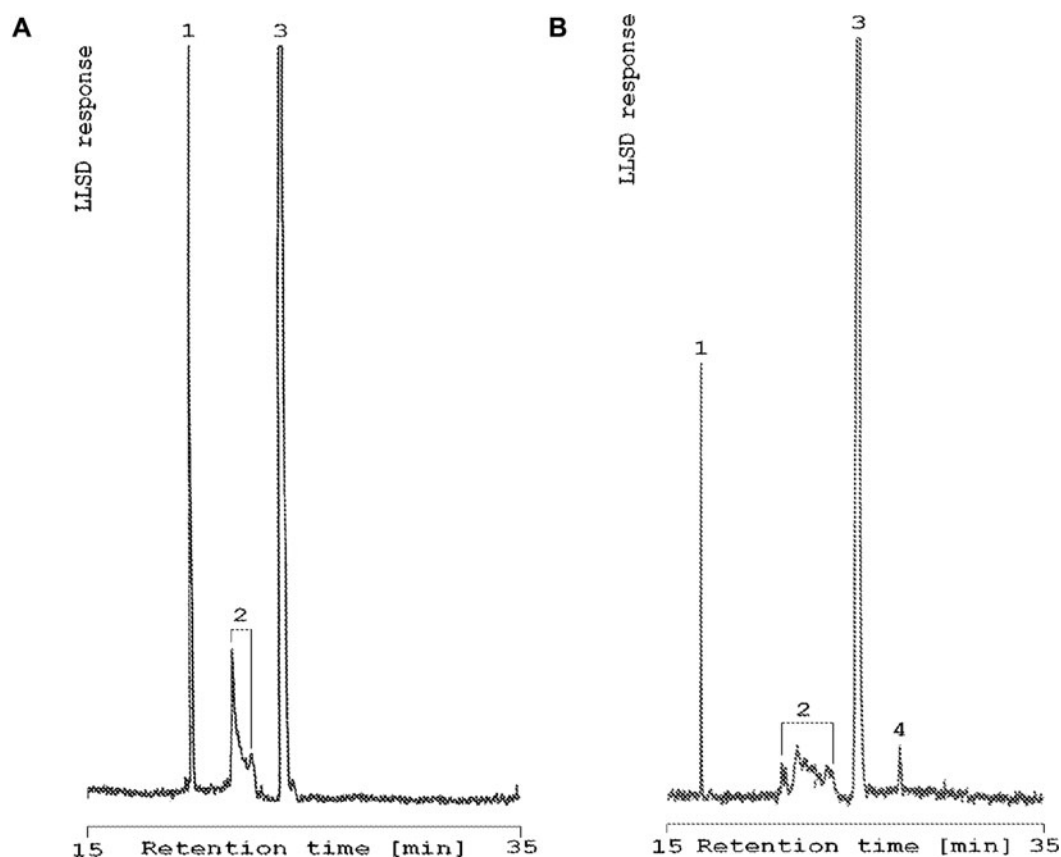
Fig. 1. Separation of lipid classes of abdomen's muscle of *Crangon crangon* during winter (A) and spring period (B) using a normal-phase HPLC method and the LLSD detector. 1, Triacylicerols; 2, free fatty acids; 3, sterols; 4, polar lipids fraction.

The fatty acids composition

Table 2 summarizes the results obtained during the identification and quantification of fatty acids (FAs) in the lipid fractions of *C. crangon* abdomen muscle. Among the FFAs were 13 FAs in winter (seven saturated (SFA), three monounsaturated (MUFA) and three polyunsaturated (PUFA) fatty acids) and 27 FAs in spring (14 SFAs, eight MUFAs and five PUFAs). Among TAGs there was a higher diversity in winter. 12 FAs were identified, including seven SFAs and five MUFAs, with no PUFAs present. A similar profile was identified in spring, where among eight FAs, only two were unsaturated, with one double bond. Seven FAs identified (as fatty acids methyl esters (FAMEs)) in the PL fraction were the same as in the TAG fraction: only SFAs (7) and MUFAs (2). Only one essential fatty acid (EFA)—EPA; 20:5 n-3—was identified during winter time (Figure 2). In the second period DHA; n−3 was identified additionally (Figure 2).

The major FAs in the TAG fraction (winter period) were: 16:0 (35.8%), 18:0 (31.6%), 17:1 (11.0%), 14:0 (5.8%) and 16:1 (4.5%). The similar composition dominated the FAs recorded in the spring period, but their percentage composition differed significantly. The amount of acid 16:0 was almost 62%, the amount of 18:0 was much lower (only 5.8%) and acids 14:0 and 16:1 had almost the same percentage composition (5.7% and 4.7%, respectively) (Table 2). TAG fraction was more abundant in unsaturated FAs in winter than in spring. Additionally, the acids 14:1, 15:1, 17:1 and two saturated with 17 and 19 carbon atoms were present. In the spring sample, in the FFA fraction was noticed diversity.

Nonetheless the percentage content of fatty acids was very different. Lipids contained 19 FAs present in concentrations from 0.1% to 1.4%. In this range, the dominant fatty acids were 20:2 (1.4%) and 22:5 (1.1%). In this fatty acids profile were detected acids: 22:6 n-3 (7%) and 20:5 n-3 (27.8%). 20:5 acid was the major compound also in the second period (9%). Additionally, arachidonic acid 20:4 n-6 was detected at 3% in winter and 0.9% during the spring. In this fraction, similar to the TAG spring fraction, acids 16:0 (42% and 27.8%) and 18:1 (24% and 13%) dominated in winter and spring, respectively. Acid 14:0 was abundant in other acid profiles, but in the FFA fraction was present only in smaller percentages.

Among polar fraction in spring time, dominant saturated acids are mainly 16:0 (59%) and 18:0 (more than 31%), as well as acid 14:0 (5.7%—percentage content very similar to the remaining profiles of FAs). The common feature of all fatty acid profiles was dominance of SFAs as the result of organic material which originated from fresh phytoplankton food (spring) and phytodetritus (Maazouzi et al., 2007). The following typical SFAs were present: 12:0, 14:0, 15:0, 16:0 and 18:0 and even 17:0 (absent in the TAG spring sample) (Table 2). These acids almost always were characteristic in all lipid profiles for Baltic marine shrimp during the two periods. The next feature was the presence of MUFAs, mostly 16:1 and 18:1 in all fractions and 17:1 during the winter period (in TAG fraction acid comprising 11% of total FAs). In the polar lipids and TAG fractions EPA and DHA

Table 1. Lipid content and class composition of adult *Crangon crangon* determined during winter and spring period. Number of measurements $N = 3$.

	Total weight of abdomen's muscle (mg dry mass)	Total lipid (mg/g dry mass)	Total lipid (% dry mass)	Triacylglycerols		Free fatty acids		Sterols		Phospholipids	
				mg/g dry mass	% of total lipid	mg/g dry mass	% of total lipid	mg/g dry mass	% of total lipid	mg/g dry mass	% of total lipid
Winter	2236	10.73 ± 0.54	1.1	2.19 ± 0.11	20.5	4.78 ± 0.24	44.5	3.76 ± 0.19	35.0	0	0.0
Spring	1953	32.26 ± 1.61	3.2	3.23 ± 0.16	10.0	20.32 ± 1.10	63.0	5.48 ± 0.27	17.0	3.23 ± 0.16	10.0

acids were not detected. While the FFA fraction was dominant in the spring sample, this class was two times more abundant in PUFAs than the other fractions in this sample and in winter time.

Sterols

The bigger diversity was in sterol fractions. The sterols composition of *C. crangon* abdomen muscle is shown in Table 3. The spring sample was very rich. The following sterols were identified: 24-norcholestadienol (retention time (RT) 67.5 min); cholesterol (RT 71.9 min); campesterol (RT 74.0 min); campestanol (RT 74.4 min); brassicasterol (RT 74.8 min); β-sitosterol (RT 76.0 min); and ergosta-7-en-3β-ol (RT 78.9 min) (Figure 3). Table 3 shows the characteristic ions of sterol trimethylsilyl ethers such as: 24-norcholestadienol—m/z 442, 313, 337, 352, cholesterol—m/z 458, 443, 368, 329, campesterol—m/z 472, 457, 343, 382, campestanol—m/z 474, 459, 384, 369, brassicasterol—m/z 470, 455, 380, 341, β-sitosterol—m/z 486, 396, 357, 381 and ergosta-7-en-3β-ol—m/z 472, 457, 367, 382. Characteristic ion for all sterols, except of campestanol (m/z 215) and ergosta-7-en-3β-ol (m/z 255) was m/z 129 peak. In the winter time only cholesterol was identified. Among eight sterols identified in this study, seven representatives appeared in traces. Only campesterol and campestanol had percentage content close to 1% (campesterol 1.0% and campestanol 0.9%). The other sterols ranged from 0.4% to 0.2%. Cholesterol was predominant among the group of sterols and amounted to 97% (Table 3).

DISCUSSION

Winter and spring periods differed considerably in total lipids content, lipid classes, fatty acids and sterols composition. It was caused particularly by adaptation to various conditions of weather, temperature of water (in lower temperature shrimp are going to in lower level of sea and settling in the sand or muddy bottom (Holthuis, 1980)). The second main case is the lack of food, especially of zooplankton and phytoplankton or other sources of nutrition (Donaldson, 1976). The muscle lipid of the abdomen of *Crangon crangon* in winter and spring time amounted to 24.0 ± 1.29 mg/g dry weight (1.1% dry mass) and 63.0 ± 4.08 mg/g dry weight (3.2% dry mass), respectively. This lipid content of the abdomen's muscle was similar to another crustacean *Palinurus vulgaris* (1.0–2.6%) (Garofalaki *et al.*, 2006). The lower values of total lipid content are dependent upon the degree of development of shrimp—the larval stage contained much more lipid than the adult stage (Allen, 1999), which the shrimp used for growth and moulting (Tocher, 2003), survival and pigmentation success (Coutteau & Mourente, 1997).

During spring four lipid classes (TAGs, FFAs, sterols and PLs) were detected. The PLs are the major part of the lipid composition (Garofalaki *et al.*, 2006; Limbourn & Nichols, 2009). In our study, the content of PLs was the same as the TAG content (10% dry mass) and this group was diverse and abundant (Table 2). Small amounts or the lack of PL detection may be dependent upon the detection limit of liquid chromatography to this type of compound (Allen, 1999) and method used for separation of the abundant lipid group. In our research, HPLC–LLSD was used. Triacylglycerols occur as an energy source in storage lipids in adult shrimp (Allen,

Table 2. Main fatty acids in polar and neutral lipid fractions of abdomen muscle's of adult *Crangon crangon* detected by GC–MS methods during winter and spring period (μg/g dry mass). Number of measurements $N = 3$.

Fatty acid	Methyl derivatives		Trimethylsilyl derivatives		Methyl derivatives	
	F1—triacylglycerols		F2—free fatty acids		F4—phospholipids	
	Winter	Spring	Winter	Spring	Winter	Spring
9:0	–		–	1.03 ± 0.06	–	–
10:0	–	52.95 ± 3.18	–	1.03 ± 0.06	–	–
11:0	–		–	1.03 ± 0.06	–	–
12:0	3.02 ± 0.18	32.01 ± 1.92	3.12 ± 0.18	6.21 ± 0.36	–	–
13:0	–	–		1.03 ± 0.06	–	–
14:0	57.91 ± 3.48	57.99 ± 3.48	39.66 ± 2.41	25.85 ± 1.56	–	56.85 ± 3.42
15:0	13.12 ± 0.78	32.01 ± 1.92	13.00 ± 0.78	7.23 ± 0.42	–	6.34 ± 0.36
16:0	357.78 ± 21.48	613.86 ± 36.84	418.52 ± 25.19	277.33 ± 16.68	–	588.68 ± 35.41
17:0	20.99 ± 1.26	–	14.23 ± 0.84	7.92 ± 0.48	–	6.53 ± 0.36
18:0	315.75 ± 18.96	57.99 ± 3.48	62.96 ± 3.72	51.26 ± 3.06	–	312.52 ± 18.78
19:0	21.44 ± 1.26	–	1.01 ± 0.06	1.03 ± 0.06	–	–
20:0	–	–	–	4.29 ± 0.24	–	–
22:0	–	–	–	3.08 ± 0.18	–	–
24:0	–	–	–	3.08 ± 0.18	–	–
ΣSFA	790.01 ± 47.41	846.81 ± 50.81	552.50 ± 50.81	391.4 ± 23.48	-	970.92 ± 58.53
14:1	7.98 ± 0.48	–	–	–	–	–
15:1	16.01 ± 0.96	–	–	1.03 ± 0.06	–	–
16:1	45.00 ± 2.70	47.10 ± 2.82	34.27 ± 2.04	58.79 ± 3.54	–	6.34 ± 0.36
17:1	110.05 ± 6.60	–	48.43 ± 2.88	17.32 ± 1.02	–	–
18:1	30.95 ± 1.86	106.09 ± 6.36	238.46 ± 14.25	128.85 ± 7.81	–	22.74 ± 1.33
19:1	–	–	–	3.08 ± 0.18	–	–
20:1	–	–	–	12.19 ± 0.72	–	–
22:1	–	–	–	3.08 ± 0.18	–	–
24:1	–	–	–	2.49 ± 0.12	–	–
ΣMUFA	209.99 ± 12.6	153.19 ± 9.19	321.16 ± 19.27	226.83 ± 13.61	-	29.08 ± 1.75
14:2	–	–	5.05 ± 0.30	–	–	–
20:5	–	–	91.17 ± 5.45	277.69 ± 16.68	–	–
20:4	–	–	30.12 ± 1.81	9.10 ± 0.54	–	–
20:2	–	–	–	13.89 ± 0.84	–	–
22:6	–	–	–	69.88 ± 4.20	–	–
22:5	–	–	–	11.21 ± 0.66	–	–
ΣPUFA	-	-	126.34 ± 7.58	381.77 ± 22.91	-	-

1999), but PLs contained much more PUFAs than TAGs. Moreover, neutral lipids are transformed to PLs, transported to ovaries and exploited during development, reproduction and maturation (Mourente & Rodriquez, 1991). Polar lipids, as easily emulsified, are better sources of PUFAs than neutral lipids (TAGs or wax esters) (Limbourn & Nichols, 2009). In our work, among all determined lipid fractions during the spring period, the highest diversity characterized FFAs and sterols. The sterols fractions were determined as follows: 4-norcholestadienol, cholesterol, campesterol, campestanol, brassicasterol, β-sitosterol, and ergosta-7-en-3β-ol, which are the characteristic sterols of crustaceans. They lack the capacity to synthesize sterols, and among 16 known sterols, none is synthesized (Kanazawa, 2001), so all sterols come from dietary sources (Virtue *et al.*, 1993; Kanazawa, 2001). Sterols are converted by crustaceans into the requisite cholesterol (Virtue *et al.*, 1993). In turn, amongst the FFA samples, 27 acids with from nine to 24 carbon atoms were determined. There were saturated acids and also monounsaturated, polyunsaturated and highly unsaturated fatty acids. Approximate composition was noted in other studies (Jeffs *et al.*, 2002; Limbourn & Nichols, 2009). As reported by some authors (Jeffs *et al.*, 2002), SUFA dominated in spring period, which agrees with our results. Monounsaturated fatty acids were the second most

popular subclass of FAs, which was a surprise because PUFAs occurred in the lowest numbers. Additionally, in our study, short-chain fatty acids (>9:0) and 23:0 were detected. The rest of the fatty acids composition was the same (Table 2 and Jeffs *et al.*, 2002).

In winter three groups of lipids (TAGs, FFAs and sterols) were identified and the least abundant was the sterols fraction, where only cholesterol occurred at levels above the detection limit. The great variety of marine sterols is dependent on human production. Some drugs accumulate in the marine environment, and a drug used in human and veterinary medicine blocks sterol synthesis by inhibiting cytochrome P450-dependent 14α-demethylases (Stenersen, 2004). Cholesterol is synthesized in animals via the 14α-demethylase substrate lanosterol (Porsbring *et al.*, 2009), clotrimazole exposure decreases the level of cholesterol and its derivatives: 22-dehydrocholesterol and desmosterol (Porsbring *et al.*, 2009). It is probably the cause of the low level or absence of the other sterols during the winter period. On the other hand, high variability of crustaceans sterols is related to nature of the environment and adaptation to difficult environmental conditions (Boutry *et al.*, 1979). Moreover, crustaceans cannot synthesize sterol *de novo*, and the basic level of their sterols is lower and they must have dietary source of sterols.
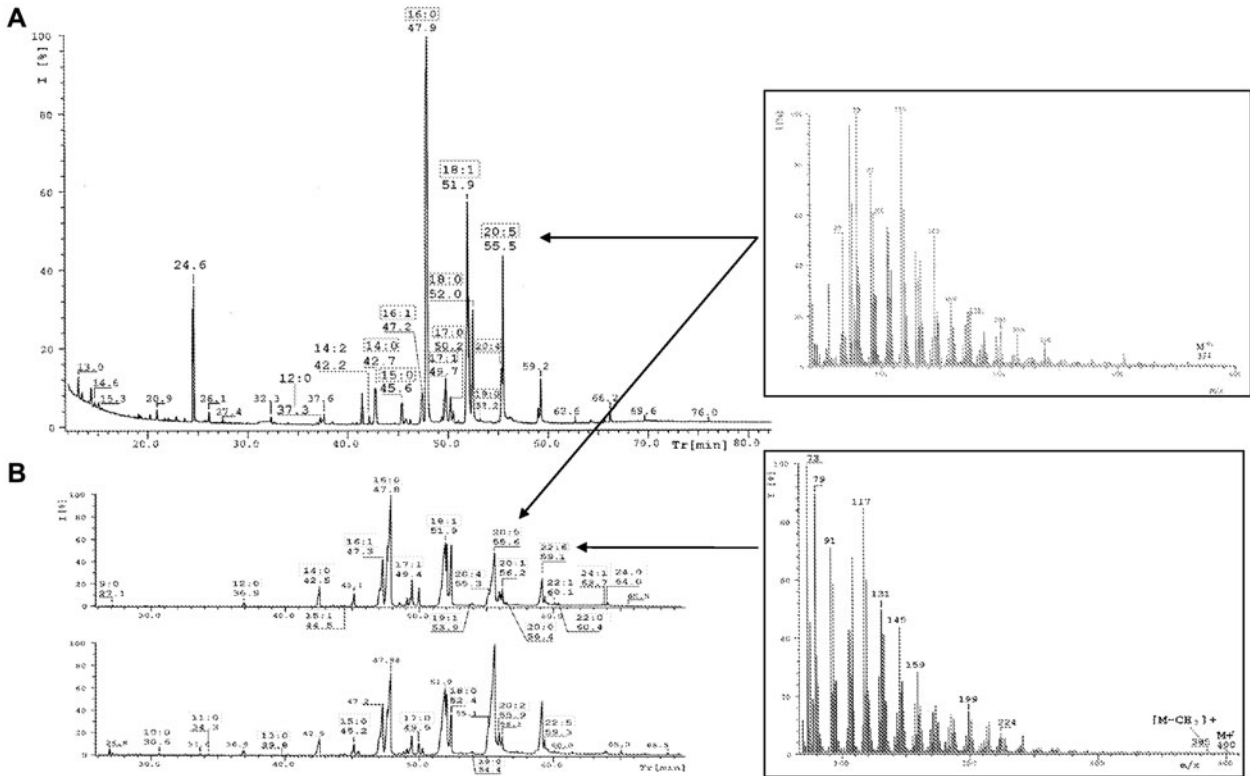
Fig. 2. The total ion current chromatogram of fatty acid trimethylsilyl ethers and mass spectrum of the TMS of EPA and DHA during winter (A) and spring period (B) detected by GC–MS.

Therefore crustaceans collect these lipids' elements from chemoautotrophic and phytodetrital source of nutrition (Allen, 1999). Membrane permeability, cell plasticity and the exchange of solutes depends on the variations of the side chain structures (Boutry *et al.*, 1979), hence there is such a large number of all sorts of steroidal compounds at living organisms. It is difficult to say much about the dominant lipid fraction from the analysed percentage content of
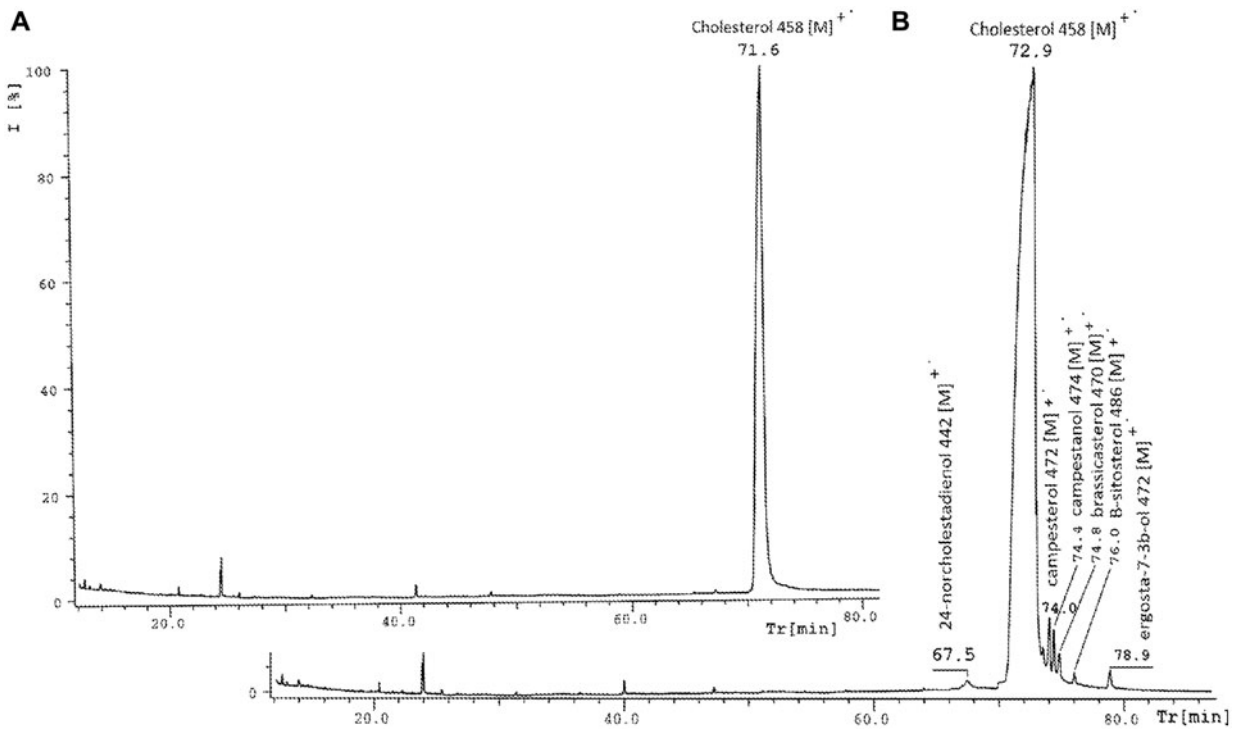

Fig. 3. Mass spectra of the TMS derivatives of sterols in abdomen's muscle of *Crangon crangon* during winter (A) and spring period (B) detected by GC–MS.

Table 3. Molecular masses and major fragmentation ion of TMS ether derivatives of each peak of *Crangon crangon* collected in GC–MS during spring period. Number of measurements $N = 3$.

Sterols		µg/g dry mass	$[M]^{+\cdot}$	B.P.	Fragment ions		
Trivial name	Scientific name						
β-sitosterol	24-ethylcholest-5-en-3β-ol	2.53 ± 0.12	486	129	396	357	381
campestanol	24-methyl-5α-cholestan-3β-ol	9.15 ± 0.54	474	215	459	384	369
campesterol	24-methylcholest-5-en-3β-ol	9.84 ± 0.60	472	129	457	343	382
ergosta-7-en-3β-ol	24-methyl-cholesta-7-en-3 -ol	2.51 ± 0.12	472	255	457	367	382
brassicasterol	24-methylenecholest-5-en-3β-ol	4.22 ± 0.24	470	129	455	380	341
cholesterol	cholest-5-en-3β-ol	968.39 ± 58.17	458	129	443	368	329
24-norcholestadienol	22-trans-24-norcholesta-5,22E-lien-3β-ol	3.36 ± 0.18	442	129	313	337	352

individual factions from the winter sample, because percentage value of each fraction was very similar. During spring the situation was similar, percentage contents of TAGs, sterols and PLs were similar (10%, 17%, 10%, respectively), and only FFAs occurred in higher amounts (63%). Donaldson also described smaller values of lipids in winter and spring, and indicated this period as richer than during other seasons of the year (Donaldson, 1976).

In each lipid sub-sample the same composition of typical acids is repeated: 14:0, 15:0, 16:1, 16:0, 18:1 and 18:0. Similarly as described by Clarke, the major fatty acids in all neutral and polar lipid fraction were 14:0, 16:1, 16:0, 18:0, 18:1, 20:5, 22:6, characteristic compounds for benthic crustaceans (Clarke, 1979; Virtue et al., 1993). In our study, only the spring sample of FFAs contained 14:0 and 18:0. Polyunsaturated fatty acids descended from another source of food—phytoplankton. Main acids: EPA and DHA are biomarkers for phytoplankton, of which a significant amount was recorded during spring (Allen, 1999; Lee et al., 2006). Additionally, PUFAs are needed for working membranes in a deep-sea environment. For example, long-chain unsaturated fatty acids of shrimps (e.g. acid 18:1) can derive from bacteria, which are characteristic of deeper areas (Allen, 1999). The major differences were observed between the TAG fraction during winter and spring. The winter sample of TAGs was much more varied; however, not in the number of PUFAs, but only in number of SFAs and MUFAs. Monounsaturated fatty acids are used by crustaceans as a primary source of energy (Limbourn & Nichols, 2009). Thus, the main food source in winter is phytodetritus, which is rich in these components (Maazouzi et al., 2007).

The following factors are decisive in the amounts and diversity of FAs: the weather; the temperature of water; and the habitat. The PUFA content is correlating with the life cycle of *C. crangon*—during spring and summer the development and the growth of young larval stages occurs (Abad et al., 1995). Simultaneously, the lowest contents of energy elements and the lowest values of the energy are noted in the period November–March (Campos et al., 2009), which explains the low level or the lack of PUFA in autumn and winter (Mika et al., 2013 and Table 2). The next factor—habitat—is directly connected with the type of food taken up. The bigger quantity of omega-3 FA comes from fresh organic matter, whose source is the phytoplankton inhabiting the water depths. Algae and a waterside flora are rich in acids omega-6, as well as detritus, which provides SFAs additionally (Abad et al., 1995; Maazouzi et al.,

2007). The next factor is the duration of solar exposure of water, causing an increase of saturated acids (Kasai & Sakai, 2004). Concurrently type of the analysed tissue is also deciding on the kind and amounts of PUFA. Muscles are the store of essential fatty acids and sterols. Muscular tissue has considerable affinity for concentrating these lipid associations in the form of phospholipids. Phospholipids are a better store for PUFA than neutral lipids (Perez-Velazquez et al., 2003; Limbourn and Nichols, 2009) and they play a role also as a main component of cell membranes. High concentration of PUFA in organisms influences their survival and their tolerance to changes of salinity. The presence of these acids modifies the permeability of the cell membrane and modulates the activity of the sodium–potassium pump, as an essential osmoregulatory mechanism (Maazouzi et al., 2007).

CONCLUSIONS

For the first time the lipid content of the abdomen muscle of benthic taxon of adult stage *Crangon crangon* is determined. As the result of these studies the composition of lipid classes, and the fatty acid profiles in all fractions have been determined, and also the composition of marine sterols in *C. crangon*. The composition of lipids did not differ widely in comparison to the other crustaceans (Allen, 1999). Minor differences are noticeable in fatty acids composition, and depended on the season, weather conditions, availability of nutrients, age (Çelik et al., 2004) and sex of the organisms— rapid gonad maturation and maximum concentration of lipid during the spring period (March–April). Dietary composition of nutrients much more affected the PUFA content than temperature and weather condition (Abad et al., 1995). Consumption of shrimps increases not only because of taste, but also due to the usefulness of shrimps as a supply of healthy essential fatty acids (Holub, 2002). Despite the small size of *C. crangon* it stands out in accessibility of biological material. It is worthwhile to continue study of these organisms and consider them as PUFAs source.

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