TÜBİTAK

Research Article

Turk J Zool
2011; 35(6) 837-849
© TÜBİTAK
doi:10.3906/zoo-1002-5

A comparison of the fatty acid composition of the phospholipid and neutral lipid of *Unio elongatulus* (Bourguignat, 1860) (Bivalvia: Unionidae) mussels from 4 different localities in southeastern Anatolia, Turkey

İhsan EKİN*, Mehmet BAŞHAN, Rıdvan ŞEŞEN
Department of Biology, Science Faculty, Dicle University, 21280 Diyarbakır - TURKEY

Received: 02.02.2010

Abstract: The quantitative and qualitative fatty acid compositions of phospholipid and neutral lipid were investigated and compared for specimens of the freshwater mussel *Unio elongatulus*. Samples were collected from 4 different localities in Turkey: Çağçağ Brook in Nusaybin (Mardin), Fabrika Brook in Çınar (Diyarbakır), the Tigris River under the Sadi Bridge (Diyarbakır), and Kırkçeşme Spring in Sultanköy (Mardin). Phospholipids and neutral lipids of the mussels' whole tissues were fractionated by thin layer chromatography (TLC) and fatty acid analyses were done by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Both in the phospholipid and neutral lipid fractions, C16:0 (17.20%-22.17%), C16:1ω7 (9.21%-15.31%), C18:1ω9 (12.21%-23.12%), C18:2ω6 (12.12%-16.10%), and C20:4ω6 (5.86%-13.10%) acids were found to predominate. The percentage of C20:4ω6 acid, a precursor of eicosanoids, was shown to be high in the phospholipid fractions. In the neutral lipids, total monounsaturated fatty acids (ΣMUFA) levels were determined to be higher than total saturated fatty acids (ΣSFA) and total polyunsaturated fatty acids (ΣPUFA) levels. In the phospholipid total, however, ΣPUFA levels were noted to be higher than ΣMUFA and ΣSFA levels. As is characteristic of freshwater mussels, Σω6/Σω3 ratios were found to be high in all lipid fractions.

Key words: Fatty acids, *Unio elongatulus*, freshwater mussel, southeastern Anatolia

Türkiye, Güneydoğu Anadolu Bölgesi'nin dört farklı lokalitesinden toplanan *Unio elongatulus* (Bourguignat, 1860) (Bivalvia: Unionidae) midyesinin fosfolipit ve nötral lipit yağ asiti kompozisyonunun karşılaştırılması

Özet: Çalışmada, Çağçağ Irmağı (Nusaybin - Mardin), Fabrika Çayı (Çınar - Diyarbakır), Dicle Nehri (Sadi Köprüsü - Diyarbakır) ve Kırkçeşme (Sultanköy - Mardin) kaynak su akıntısı gibi 4 farklı lokaliteden toplanan tatlısu midyesi *Unio elongatulus*'un fosfolipit ve nötral lipit yağ asidi içeriği kalitatif ve kantitatif olarak araştırıldı ve karşılaştırıldı. Midyenin, fosfolipit ve nötral lipitleri ince tabaka kromatografi (TLC) ile fraksiyonlandı. Yağ asidi analizleri gaz kromatografi (GC) ve gaz kromatografi - kütle spektrometre cihazları (GC-MS) ile yapıldı. Yüzde dağılımda hem fosfolipitte hem de nötral lipitte C16:0 (% 17,20-% 22,17), C16:1ω7 (% 9,21-% 15,31), C18:1ω9 (% 12,21-% 23,12), C18:2ω6 (% 12,12-% 16,10) ve C20:4ω6 (% 5,86-% 13,10) asitler major yağ asitleri olarak tespit edildi. Fosfolipit fraksiyonlarında eikosanoidlerin öncül maddesi olan C20:4ω6 asidin yüzde oranı daha yüksek saptandı. Nötral lipit fraksiyonlarında ΣTDYA (total tekli doymamış yağ asidi) oranı, ΣÇDYA (total çoklu doymamış yağ asidi) ve ΣDYA (total doymuş yağ asidi) oranlarından

* E-mail: ekinihsan@gmail.com

daha yüksek bulundu. Fosfolipit fraksiyonlarında ise ΣÇDYA oranı, ΣDYA ve ΣTDYA oranlarından daha yüksek saptandı. Tatlısu midyelerine özgü olduğu gibi, Σω6/Σω3 değeri tüm lipit fraksiyonlarında yüksek bulundu.

Anahtar sözcükler: Yağ asitleri, *Unio elongatulus*, tatlısu midyesi, Güneydoğu Anadolu

Introduction

In terms of species number, Mollusca is the second largest phylum, following only Arthropoda (Ekman, 1953). Its members attract the attention of investigators due to their physiology and biochemistry as well as their morphological variety and particular behavior (Vernberg and Vernberg, 1972). Bivalvia is the most investigated class of Mollusca and it is very important for many reasons. Apart from their commercial value as a human foodstuff and in the feeding of several marine crustaceans (Deshimaru et al., 1979; Cotroneo et al., 1980), the biological and pharmacological uses of the polyunsaturated fatty acids (PUFA) contained within these mollusks are of notable interest (Joseph, 1982).

Traits such as the lipid composition and metabolism of many marine bivalves have been well documented; however, only a limited amount of information is available on the fatty acid composition of freshwater forms. Among the known studies on freshwater bivalves, *Carunculina texasensis* (Hagar and Dietz, 1986), *Diplodom patagonicus* (Pollero et al., 1981), *Ligumia subrostrata* (Dietz and Graves, 1981), *Diplodon delodontus* (Pollero et al., 1983), *Dreissena polymorpha*, *Unio* sp. (Dembitsky et al., 1992), *Anadonta piscinalis* (Dembitsky et al., 1993a), and *Dreissena siouffi* (Ekin et al., 2008) have been studied.

Lipids have been reported to function most importantly as energy storage substances and the variability in both the number of carbon atoms and the number of double bonds in fatty acids determines to a large extent the physical properties of biological membranes (Spector and Yorek, 1985). Phospholipids, neutral lipids (monoacylglycerol, diacylglycerol, triacylglycerol, sterol, free fatty acids, and fatty acids esters), and cholesterol are the most important lipid classes that have been studied in bivalves. The accumulation of these lipids can be differentiated by either external factors, such as fluctuations in environmental conditions, temperature, and food availability, or by internal factors, such as metabolic and physiological activities.

Unio elongatulus is a medium sized freshwater mussel that usually lives in rivers, brooks, ponds, springs, and lakes and is generally found buried into the mud or hiding under pebbles. The species is one of the most commonly distributed mussels in the freshwaters of Turkey, and is mostly seen in the Tigris River and a few other Anatolian rivers. Although it is not eaten by Turkish people, this species has a significant role in the local food chain because it is consumed by fish, water birds, mammals, and reptiles living in the river. It is also sometimes used as foodstuff during the production of animals such as fish, chickens, and pigs. For these reasons, every study on freshwater mussels from Turkey gains importance.

The present study had 2 main goals: to investigate and compare the levels of phospholipid and neutral lipid fatty acids in *U. elongatulus* populations collected from 4 different localities and to focus on the effects of regional factors on the distribution of these fatty acids.

Materials and methods

Mussels

U. elongatulus freshwater mussels were collected from 4 different localities: Çağçağ Brook in Nusaybin (Mardin) (altitude: 600 m, coordinates: 37°03.0′N/41°13.2′E); the Tigris River, under the Sadi Bridge (Diyarbakır) (altitude: 583 m, coordinates: 37°55.2′N/40°13.8′E); Fabrika Brook in Çınar (Diyarbakır) (altitude: 545 m, coordinates: 37°43.8′N/40°25.2'E); and Kırkçeşme Spring in Sultanköy (Mardin) (altitude: 920 m, coordinates: 37°25.2′N/41°00.0′E). The water temperatures at the locations were 16 °C in Çağçağ Brook, 18 °C in the Tigris River, 15 °C in Fabrika Brook, and 10 °C in Kırkçeşme Spring. All of the sampling locations are situated in the southeastern part of Turkey and the mussels were collected in May 2009. Individually, 3 healthy adult mussels of similar size (length: 8 ± 2.00 cm, wet weight: 11 ± 1.45 g) were sampled for

each lipid analysis. Adult mussels were divided into 2 groups and their whole bodies were dissected for the neutral and phospholipid analyses. The samples were transferred into a mixture of chloroform and methanol (2:1, v/v) and kept frozen (–80 °C) until use.

Lipid extraction

In order to extract the total body lipids, 3 samples were homogenized in the chloroform/methanol (2:1, v/v) solution (Bligh and Dyer, 1959). The autoxidation of unsaturated components was minimized by adding 50 µL of 2% butylated hydroxytoluene in chloroform to each sample during the extraction process. The total lipid extracts were dried under a stream of N_2. Then the total lipids were spotted on preparative thin layer chromatography (TLC) plates (made in the lab) using silica gel TLC plates (20 × 20 cm, 0.25 mm thick). After applying the lipid extracts, the TLC plates were developed in a mixture of petroleum ether:diethyl ether:acetic acid (80:20:1 v/v). Lipid fractions were made visible by spraying 2', 7'-dichlorofluorescein (Supelco, Supelco Park, PA, USA) on a small part of the TLC plates, and phospholipid and neutral lipid fractions were identified by corresponding standards. Neutral lipid and phospholipid fractions were scraped into reaction vials and the associated fatty acids were transmethylated by refluxing the fractions in acidified (sulfuric acid) methanol for 90 min at 85 °C. Fatty acid methyl esters (FAMEs) of lipid classes were extracted from the reaction vials 3 times with hexane and concentrated (Stanley-Samuelson and Dadd, 1983).

Gas chromatography and gas chromatography-mass spectrometry conditions

FAMEs were separated and quantified by capillary gas chromatography. The chromatography system consisted of a Hewlett Packard (Wilmington, DE) gas chromatograph (model 6890), a DB-23 capillary column (60 m × 0.25 mm i.d., 0.250 µm film thickness, Bonded 50% cyanopropyl) (J & W Scientific, Folsom, CA), a flame ionization detector, and Hewlett-Packard ChemStation software. The injection port and the detector temperatures were 270 °C and 280 °C, respectively. The split ratio was 1:20. The flow rate of compressed air was 300 mL/min and the corresponding rate for hydrogen was 30 mL/min. Helium was used as a carrier gas (2.8 mL/min). The oven was programmed to increase the temperature at a rate of 6.5 °C/min from 130 °C (with a 1 min hold) to 170 °C; after, it increased at a rate of 2.75 °C/min to 215 °C before increasing again, at a rate of 40 °C/min, to 230 °C, a temperature which was maintained for 12 min. The total fatty acids levels and spectra of FAMEs were obtained using the HP 3365 ChemStation computer program. The chemical structures of the FAMEs were determined by analyses of spectra and by comparing the obtained spectra with those of authentic standards (Sigma-Aldrich Chemicals). Individual FAMEs were identified by comparison with the chromatographic behaviors of authentic standards (Sigma-Aldrich Chemicals).

The chemical structures of the FAMEs were confirmed by capillary gas chromatography-mass spectrometry (GC-MS). GC-MS analyses were performed using GC-MS equipment (HP 5890-E series GC-System, Hewlett-Packard, Palo Alto, CA, USA) with mass-selective detection. An Innowax column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was used, and the temperature was programmed to increase from 150 °C to 230 °C at a rate of 2 °C/min, with an initial hold of 6 min. The carrier gas used was helium (1 mL/min) and the split ratio was 1:50. The injection port and the detector temperatures were 250 °C and 300 °C, respectively. The mass spectrometer was operated in the electron impact ionization mode (70 eV). Chemical structures of the FAMEs were identified by comparison with the Wiley 275 and the Nist 98 databank; they were again determined by the analyses of spectra and by comparing the obtained spectra with those of the authentic standards.

Statistical analyses

The analyses were performed using a commercial statistical program (SPSS 12.0). All analytical determinations were performed in triplicate and the mean values were reported. The statistical analyses of percentages of fatty acid were tested by an analysis of variance (ANOVA) and comparisons between means were performed using Tukey's test. Differences between means were considered to be significant at $P < 0.05$.

Results

The current study has provided deeper insight into the fatty acid composition of *U. elongatulus*

mussels from 4 different locations in Turkey: Çağçağ Brook in Nusaybin (Mardin), the Tigris River under the Sadi Bridge (Diyarbakır), Fabrika Brook in Çınar (Diyarbakır), and Kırkçeşme Spring in Sultanköy (Mardin). Fatty acids detected in the analyses of the phospholipid and neutral lipid fractions from *U. elongatulus* were C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, and C18:0 saturated fatty acids (SFAs); C16:1ω7, C18:1ω9, and C20:1ω9 monounsaturated fatty acids (MUFAs); and C18:2ω6, C18:3ω3, C20:2ω6, C20:3ω6, C20:4ω6, C20:5ω3, C22:2ω6, C22:5ω6, and C22:6ω3 polyunsaturated fatty acids (PUFAs). In the analyses, the proportions of C16:0, C16:1ω7, C18:1ω9, C18:2ω6, and C20:4ω6 acids were found to be higher and the proportions of C10:0, C12:0, C13:0, C15:0, C17:0, C20:2ω6, C20:3ω6, C22:2ω6, C22:5ω6, and C22:6ω3 acids were discovered to be lower (Tables 1 and 2).

Phospholipid fractions

The lowest level of ΣSFA in the phospholipid was 26.80%, found in the mussels from Kırkçeşme Spring, and the highest level, 29.00%, was observed in the mussels from Fabrika Brook (Table 1 and Figure 1). The most abundant SFA were C16:0 and C18:0 acids.

The percentage of ΣMUFA was 29.35% in Kırkçeşme mussels, 32.17% in Fabrika mussels, 34.32% in Tigris mussels, and 34.47% in Çağçağ mussels (Table 1 and Figure 1). In every analysis, the proportion of C18:1ω9 acid was found to be greater than that of C16:1ω7 and C20:1ω9 acids (Table 1). Its percentage (between 15.22% and 16.22%) was almost the same in the mussels from the Çağçağ, Tigris, and Fabrika sampling sites; in the mussels from Kırkçeşme, however, the level of this acid was measured at 12.21%. No important fluctuations in C16:1ω7 acid were found in any of the localities. Its percentage varied from 11.05% to 13.60% (Table 1). The highest level of C20:1ω9 acid was detected in Çağçağ mussels (7.20%) while the lowest level was observed in the Tigris mussels (4.50%) (Table 1).

ΣPUFA levels were recorded at 44.53%, 39.46%, 39.17%, and 37.43% in Kırkçeşme, Fabrika, Çağçağ, and Tigris mussels, respectively (Table 1 and Figure 1). The variation in ΣPUFA levels was mainly associated with the changes in C18:2ω6 and C20:4ω6 acids, which were major components of PUFAs. The quantity of C18:2ω6 acid was 14.71% in Çağçağ mussels, 13.11% in Tigris mussels, 15.70% in Fabrika mussels, and 16.10% in Kırkçeşme mussels (Table 1). The second major fatty acid was C20:4ω6 acid and its percentage was determined at 10.40%, 12.12%, 11.60%, and 13.10% in Çağçağ, Fabrika, Tigris, and Kırkçeşme mussels, respectively (Table 1). The proportion of C20:5ω3 acid varied from 3.20% to 6.12%. In the analyses, the observed percentage of C10:0, C13:0, C15:0, C17:0, C20:3ω6, C22:2ω6, and C22:5ω6 acids did not exceed 1.00% (Table 1).

Neutral lipid fractions

In the neutral lipid of *U. elongatulus*, the highest level of ΣSFA noted was 34.49% and was found in the mussels from the Tigris, while the lowest level of ΣSFA, 28.19%, was seen in the Kırkçeşme mussels (Table 2 and Figure 2). The percentage of C16:0 acid varied from 20.12% to 22.17% (Table 2) but no significant fluctuations in the C16:0 acid proportions were found among the samples from different localities. The second major constituent, C18:0, also demonstrated levels (5.32%-9.24%) that were higher than those of other SFAs.

The level of ΣMUFA was found to be 35.32% in the Çağçağ mussels, 36.10% in the Tigris mussels, 37.69% in the Fabrika mussels, and 43.98% in the Kırkçeşme mussels (Table 2 and Figure 2). The highest level of MUFA, observed in Kırkçeşme mussels, was based on the highest level of C18:1ω9 and C16:1ω7 acids. C18:1ω9 acid was also determined to be the most abundant component in comparison with the other MUFAs. Its value was relatively high, with findings of 23.12% (Kırkçeşme), 21.07% (Tigris), 18.10% (Fabrika), and 17.10% (Çağçağ) (Table 2). The second major MUFA was C16:1ω7 acid and its level ranged between 9.21% (Tigris) and 15.31% (Kırkçeşme) (Table 2).

ΣPUFA level in the neutral lipid was as follows: 28.63% in the Kırkçeşme mussels, 29.17% in the Tigris mussels, 31.63% in the Çağçağ mussels, and 32.23% in the Fabrika mussels (Table 2 and Figure 2). In all of the analyses, C18:2ω6 acid was found to be predominant, with a percentage that varied from 12.12% to 14.05% (Table 2). C18:3ω3 and C20:4ω6 acids were the other major components and their levels were higher than that of C20:5ω3 acid. The proportion of C18:3ω3 acid was 4.72% in Çağçağ, 4.33% in Fabrika, 5.70% in Tigris, and 3.02% in

Table 1. The fatty acid composition (%) of the phospholipids from 4 populations of *Unio elongatulus* (results are expressed as % of the total phospholipid and neutral lipid fatty acids).

Fatty acids	***U. elongatulus*[1]** (mean* ± S.D.)**	***U. elongatulus*[2]** (mean* ± S.D.)**	***U. elongatulus*[3]** (mean* ± S.D.)**	***U. elongatulus*[4]** (mean* ± S.D.)**
C10:0	0.20 ± 0.02a	-	0.15 ± 0.01a	0.50 ± 0.04b
C12:0	1.20 ± 0.14a	0.43 ± 0.03b	1.46 ± 0.18a	1.00 ± 0.12ab
C13:0	-	0.25 ± 0.02a	-	0.15 ± 0.01a
C14:0	2.18 ± 0.24a	1.86 ± 0.16b	1.25 ± 0.12b	2.20 ± 0.20a
C15:0	0.76 ± 0.08a	0.63 ± 0.05a	0.22 ± 0.03b	-
C16:0	17.20 ± 1.05a	20.10 ± 1.13b	18.74 ± 1.10a	18.40 ± 1.06a
C17:0	-	-	-	0.10 ± 0.01
C18:0	5.60 ± 0.60a	5.14 ± 0.52a	7.18 ± 0.80b	4.45 ± 0.48c
ΣSFA	**27.14 ± 1.22a**	**28.41 ± 1.24b**	**29.00 ± 1.23b**	**26.80 ± 1.25a**
C16:1ω7	12.05 ± 0.88a	13.60 ± 0.86b	11.05 ± 0.89a	11.70 ± 0.81a
C18:1ω9	15.22 ± 1.01a	16.22 ± 1.00b	16.02 ± 1.02b	12.21 ± 0.90c
C20:1ω9	7.20 ± 0.71a	4.50 ± 0.39b	5.10 ± 0.64c	5.44 ± 0.78c
ΣMUFA	**34.47 ± 1.32a**	**34.32 ± 1.30a**	**32.17 ± 1.28b**	**29.35 ± 1.21c**
C18:2ω6	14.71 ± 0.95a	13.11 ± 1.00b	15.70 ± 1.08c	16.10 ± 1.10c
C18:3ω3	6.52 ± 0.54a	6.10 ± 0.45a	4.08 ± 0.50b	5.71 ± 0.62ab
C20:2ω6	1.00 ± 0.13a	1.23 ± 0.14a	1.32 ± 0.15a	1.10 ± 0.10a
C20:3ω6	0.65 ± 0.05a	-	0.24 ± 0.02b	0.30 ± 0.04b
C20:4ω6	10.40 ± 0.94a	11.60 ± 1.02b	12.12 ± 1.06b	13.10 ± 1.04c
C20:5ω3	4.32 ± 0.23a	3.20 ± 0.18b	5.09 ± 0.42c	6.12 ± 0.53d
C22:2ω6	-	0.36 ± 0.04a	0.57 ± 0.06a	-
C22:5ω6	0.91 ± 0.03a	0.80 ± 0.03a	-	0.40 ± 0.06b
C22:6ω3	0.66 ± 0.04a	1.03 ± 0.17b	0.34 ± 0.02c	0.70 ± 0.01a
Σω6/ω3	**2.41**	**2.62**	**3.15**	**2.47**
ΣPUFA	**39.17 ± 1.35a**	**37.43 ± 1.33b**	**39.46 ± 1.36a**	**44.53 ± 1.41c**

* Means are the averages of 3 replicates. The values are shown as mean ± standard deviation (SD).
**Means followed by different letters in the same line are significantly different ($P < 0.05$) according to the results of Tukey's test.
SFA: Saturated Fatty Acids, **MUFA:** Monounsaturated Fatty Acids, **PUFA:** Polyunsaturated Fatty Acids
***U. elongatulus*[1]**: collected from Çağçağ Brook in Nusaybin (Mardin)
***U. elongatulus*[2]**: collected from the Tigris River under the Sadi Bridge (Diyarbakır)
***U. elongatulus*[3]**: collected from Fabrika Brook in Çınar (Diyarbakır)
***U. elongatulus*[4]**: collected from Kırkçeşme Spring in Sultanköy (Mardin)

Kırkçeşme mussels; the proportion of C20:4ω6 acid was 7.60% in Çağçağ, 7.58% in Fabrika, 5.86% in Tigris, and 6.02% in Kırkçeşme mussels (Table 2).

The total level of omega 6 acids (Σω6) was higher than the total of omega 3 acids (Σω3) in both of the phospholipid and neutral lipid fractions. Apart from

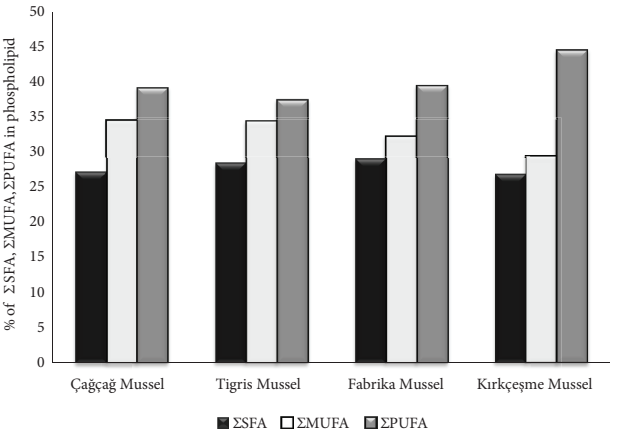

Figure 1. The distribution of ΣSFA, ΣMUFA, and ΣPUFA percentages in the phospholipids of *Unio elongatulus* collected from Çağçağ Brook, the Tigris River, Fabrika Brook, and Kırkçeşme Spring.

the Fabrika mussels, Σω6/Σω3 ratios in the neutral lipids were higher than those of the phospholipids. In the phospholipids, the Σω6/Σω3 ratio was determined at 2.47 in Kırkçeşme mussels, 2.41 in Çağçağ mussels, 2.62 in Tigris mussels, and 3.15 in Fabrika mussels (Table 1). In the neutral lipids, the Σω6/Σω3 ratio was found to be 2.63, 2.72, 2.73, and 2.88 in Çağçağ, Kırkçeşme, Tigris, and Fabrika mussels, respectively (Table 2).

Discussion

Studies of the fatty acid composition of bivalves provide substantial information for making chemotaxonomic inferences and help in locating the sources of fatty acids, especially PUFA, which possess important physiological and structural benefits for organisms. In contrast with most comparative studies on the biochemical composition of marine mussels, there are not many studies on freshwater mussels and even fewer on their fatty acid compositions. *C. texasensis*, *D. patagonicus*, *L. subrostrata*, *D. delodontus*, *D. polymorpha*, *Unio* sp., *A. piscinalis*, and *D. siouffi* are some of the freshwater mussels that have been examined. Notably, studies on the lipid and fatty acid composition of these species have been quite limited, making the examination of the fatty acid composition of *U. elongatulus* worthy of investigation.

The detailed investigation of 4 different *U. elongatulus* populations considered in this study indicated that the phospholipids and neutral lipids of the mussels contained C16:0 (17.20%-22.17%), C16:1ω7 (9.21%-15.31%), C18:1ω9 (12.21%-23.12%), C18:2ω6 (12.12%-16.10%), and C20:4ω6 (5.86%-13.10%) acids predominantly (Tables 1 and 2). The minor fatty acids were C10:0, C12:0, C13:0, C15:0, C17:0, C20:3ω6, C22:2ω6, C22:5ω6, and C22:6ω3 acids. The results obtained in the present work reveal that the most qualitatively important fatty acids in bivalves are similar to those of *U. elongatulus*; furthermore, the fatty acid profiles of these mussels conform to the common pattern characteristic of both freshwater and marine bivalves in general (Pollero et al., 1981, 1983; Klingensmith and Stillway, 1982; Misra et al., 1985; Hagar and Dietz, 1986; Piretti et al., 1988; Wenne and Polak, 1989; Dembitsky et al., 1992; Pazos et al., 1996, 2003; Alkanani et al., 2007; Ekin et al., 2008; Ekin and Başhan, 2010).

The quantity of C16:0 acid, a key component in the biosynthesis of fatty acids, varies widely among marine and freshwater mollusks (Go et al., 2002). Notably, specific differences in the fatty acid composition of mollusks are, to some extent, affected by external factors such as diet, vegetation, water temperature, salinity, water cleanliness, and sunlight. In this study, C16:0 acid was found in high levels and its percentages ranged from 17.20% to 20.10% in the phospholipids (Table 1) and from 20.12% to 22.17% in the neutral lipids (Table 2). In most of the studies on freshwater mussels, including *C. texasensis*, *D. patagonicus*, *L. subrostrata*, *D. delodontus*, *D. polymorpha*, *Unio* sp., *A. piscinalis*, and *D. siouffi*, C16:0 acid was also determined to be a predominant component (Hagar and Dietz, 1986; Pollero et al., 1981, 1983; Dietz and Graves, 1981; Dembitsky et al., 1992, 1993a; Ekin et al., 2008; Ekin and Başhan, 2010). Moreover, C16:0 acid has also been reported at high percentages in marine bivalves. For instance, it was detected between 19.60% and 26.90% in *Tapes philippinarum* (Caers et al., 1998) and up to 34.25% in *Argopecten pupuratus* (Caers et al., 2003). Isay and Busarova (1984) also reported that C16:0 acid was a major fatty acid in 51 marine invertebrates living in the Sea of Japan.

The ΣMUFA level in the phospholipid was observed to be 29.35% (Kırkçeşme), 32.17% (Fabrika), 34.32% (Tigris), and 34.47% (Çağçağ)

Table 2. The fatty acid composition (%) of the neutral lipids from 4 populations of *Unio elongatulus* (results are expressed as % of the total phospholipid and neutral lipid fatty acids).

Fatty acids	***U. elongatulus*[1]** (mean* ± S.D.)**	***U. elongatulus*[2]** (mean* ± S.D.)**	***U. elongatulus*[3]** (mean* ± S.D.)**	***U. elongatulus*[4]** (mean* ± S.D.)**
C10:0	0.34 ± 0.04a	0.40 ± 0.05a	-	0.25 ± 0.02a
C12:0	1.60 ± 0.18a	0.50 ± 0.06b	0.74 ± 0.06b	0.90 ± 0.08b
C13:0	-	0.12 ± 0.01a	0.18 ± 0.01a	-
C14:0	3.54 ± 0.41a	3.11 ± 0.38a	1.54 ± 0.19b	0.75 ± 0.06c
C15:0	1.10 ± 0.13a	-	0.23 ± 0.01b	0.48 ± 0.03b
C16:0	20.12 ± 1.12a	20.30 ± 1.15a	22.17 ± 1.22b	20.25 ± 1.26a
C17:0	0.24 ± 0.03a	0.82 ± 0.07b	-	0.24 ± 0.02a
C18:0	6.80 ± 0.69a	9.24 ± 0.88b	6.64 ± 0.71a	5.32 ± 0.54c
ΣSFA	**33.74 ± 1.28a**	**34.49 ± 1.26a**	**31.50 ± 1.25b**	**28.19 ± 1.27c**
C16:1ω7	14.72 ± 0.90a	9.21 ± 0.94b	13.72 ± 0.94c	15.31 ± 1.07a
C18:1ω9	17.10 ± 1.02a	21.07 ± 1.23ab	18.10 ± 1.20a	23.12 ± 1.18b
C20:1ω9	3.50 ± 0.28a	5.82 ± 0.38b	5.87 ± 0.46b	5.55 ± 0.47b
ΣMUFA	**35.32 ± 1.34a**	**36.10 ± 1.32a**	**37.69 ± 1.39b**	**43.98 ± 1.40c**
C18:2ω6	13.30 ± 0.94a	14.05 ± 1.04a	13.36 ± 0.91a	12.12 ± 0.84b
C18:3ω3	4.72 ± 0.42a	5.70 ± 0.53b	4.33 ± 0.32a	3.62 ± 0.31c
C20:2ω6	0.71 ± 0.07a	1.02 ± 0.10b	1.62 ± 0.01b	1.45 ± 0.18b
C20:3ω6	0.90 ± 0.08a	0.31 ± 0.03b	-	1.10 ± 0.10c
C20:4ω6	7.60 ± 0.81a	5.86 ± 0.42b	7.58 ± 0.73a	6.02 ± 0.52ab
C20:5ω3	3.99 ± 0.13a	1.98 ± 0.14b	3.97 ± 0.27a	3.02 ± 0.35c
C22:2ω6	0.41 ± 0.05a	-	0.73 ± 0.02b	0.25 ± 0.02a
C22:5ω6	-	0.10 ± 0.01a	0.64 ± 0.01b	-
C22:6ω3	-	0.15 ± 0.01a	-	1.05 ± 0.17b
Σω6/ω3	**2.63**	**2.73**	**2.88**	**2.72**
ΣPUFA	**31.63 ± 1.27a**	**29.17 ± 1.25b**	**32.23 ± 1.19a**	**28.63 ± 1.17b**

* Means are the averages of 3 replicates. The values are shown as mean ± standard deviation (SD).
**Means followed by different letters in the same line are significantly different ($P < 0.05$) according to the results of Tukey's test.
SFA: Saturated Fatty Acids, **MUFA:** Monounsaturated Fatty Acids, **PUFA:** Polyunsaturated Fatty Acids
***U. elongatulus*[1]**: collected from Çağçağ Brook in Nusaybin (Mardin)
***U. elongatulus*[2]**: collected from the Tigris River under the Sadi Bridge (Diyarbakır)
***U. elongatulus*[3]**: collected from Fabrika Brook in Çınar (Diyarbakır)
***U. elongatulus*[4]**: collected from Kırkçeşme Spring in Sultanköy (Mardin)

(Figure 1 and Table 1); in the neutral lipid, however, it was 43.98% (Kırkçeşme), 37.69% (Fabrika), 36.10% (Tigris), and 35.32% (Çağçağ) (Figure 2 and Table 2). It can thus be seen that the neutral lipid of the mussels consistently contained a higher quantity of ΣMUFA than the phospholipid. The highest level of ΣMUFA was based on a high level of C18:1ω9 acid. This fatty acid was the second most abundant component. Its proportion was relatively high, comprising 23.12% in the Kırkçeşme mussels, 21.07% in the Tigris mussels, 18.10% in the Fabrika mussels, and 17.10% in the Çağçağ mussels. Another important finding

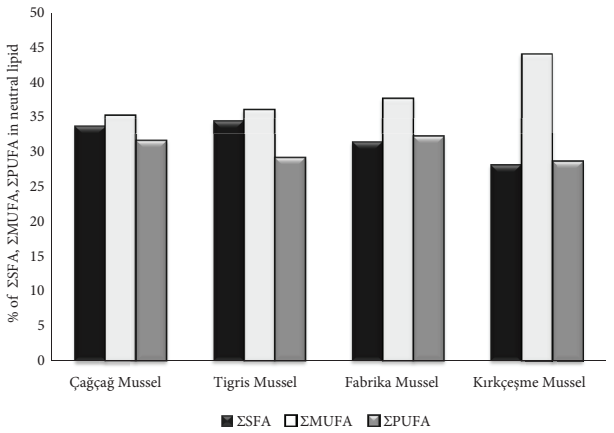

Figure 2. The distribution of ΣSFA, ΣMUFA, and ΣPUFA percentages in the neutral lipids of *Unio elongatulus* collected from Çağçağ Brook, the Tigris River, Fabrika Brook, and Kırkçeşme Spring.

centered on C16:1ω7 acid, which ranged between 9.21% and 15.31%. The maximum levels of C16:1ω7 and C18:1ω9 acids were found in the neutral lipid of Kırkçeşme mussels and measured 15.31% and 23.12%, respectively. In most of the studies on vertebrates and invertebrates, C16:1ω7 acid has usually been reported in low percentages. However, in Diptera (Thompson, 1973), some Heteroptera (Spike et al., 1991; Başhan et al., 2002) and Diatoms (Kharlamenko et al., 1995), the level of C16:1ω7 acid was reported to be high.

After determining the zooplankton and phytoplankton composition of the waters of Kırkçeşme Spring, Fabrika Brook, the Tigris River, and Çağçağ Brook, the dietary content of *U. elongatulus* was revealed to consist mostly of diatoms (*Amphora*, *Cocconeis*, *Cymbella*, *Cyclotella*, *Gomphonema*, *Synedra*, *Navicula*, *Rhoicosphenia*, *Nitzschia*, *Meridion*, and *Bacillaria*). It is similar to the diet of other filter feeding mollusks which generally comprise diatoms, dinoflagellates, and bacteria as well as dissolved and particulate organic material. Diatoms are the most important food component of mollusks. According to Kaitaranta et al. (1986), the main fatty acids of the lipids (mainly triacylglycerol: neutral lipid) in diatoms from the Gulf of Finland were C16:1ω7 (up to 51.9%), C16:0 (up to 26.2%), and C20:5ω3 acids (up to 7.8%). It has also been reported that the fatty acid composition of an organism can be dictated by metabolic activities (Pollero et al., 1983). It therefore seems likely that the MUFAs found to be predominant in *U. elongatulus* (C16:1ω7 and C18:1ω9 acids) have 2 possible origins: exogenous, in the form of dietary intake of diatoms; or endogenous, through the desaturation of C16:0 and C18:0 acids.

Dembitsky et al. (1992) found C16:1ω7, C16:1ω9, and C18:1ω9 acids to be major MUFAs and they reported that bivalve species showed limited diversity in terms of monoenoic fatty acids. The species studied (*D. polymorpha* and *Unio* sp.) contained only 4 types of monoenoic fatty acids while gastropod species such as *Coretus corneus* contained 24 types of monoenoic fatty acids. Additional reports revealed 6 types in *C. texasensis* (Hagar and Dietz, 1986) and 4 types in *D. delodontus* (Pollero et al., 1983). In *U. elongatulus* analyses, only 3 types (C16:1ω7, C18:1ω9, and C20:1ω9) could be detected.

The quantity of C18:2ω6 acid was 14.71% (Çağçağ), 13.11% (Tigris), 15.70% (Fabrika), and 16.10% (Kırkçeşme) in the phospholipid of the populations studied. In the neutral lipid, the level of this acid was 13.30% (Çağçağ), 14.05% (Tigris), 13.36% (Fabrika), and 12.12% (Kırkçeşme). The overall ΣPUFA level was mainly associated with C18:2ω6 acid, a major component of PUFAs. The percentages of C18:3ω3 acid varied from 4.08% to 6.52% in the phospholipids and from 3.02% to 5.70% in the neutral lipids. With the exception of the Tigris River mussels, all the populations contained more C18:2ω6 acid in the phospholipid than in the neutral lipid. C18:2ω6 and C18:3ω3 acids are highly likely to have an exogenous origin since they are not synthesized by most animals (Abad et al., 1995). It is generally known that in animals PUFAs are mainly incorporated in the phospholipid and C18:2ω6 acid is preferentially converted into fatty acids of longer chain high unsaturation. Therefore, the high levels of C18:2ω6 acid in both the phospholipid and neutral lipid of *U. elongatulus* may be related to the conversions required by the mussels for structural and metabolic processes.

C20:4ω6 and C20:5ω3 acids were observed to be the other major PUFAs found in the phospholipids and neutral lipids. The high levels of these acids were not surprising since the percentages of the components in the majority of previously examined bivalve mollusks were reportedly higher than those

of other highly unsaturated fatty acids (Gardner and Riley, 1972; Pollero et al., 1983; Dembitsky et al., 1992; Ekin et al., 2008). The proportion of C20:4ω6 acid in the phospholipid was 10.40% (Çağçağ), 11.60% (Tigris), 12.12% (Fabrika), and 13.10% (Kırkçeşme) and for C20:5ω3 the figures were revealed to be 3.20% (Tigris), 4.32% (Çağçağ), 5.09% (Fabrika), and 6.12% (Kırkçeşme) (Table 1). In the neutral lipid, C20:4ω6 acid was found to be 7.60% (Çağçağ), 7.58% (Fabrika), 6.02% (Kırkçeşme), and 5.86% (Tigris) while the levels for C20:5ω3 were 3.99% (Çağçağ), 3.97% (Fabrika), 3.62% (Kırkçeşme), and 1.98% (Tigris) (Table 2). Always, the percentage of C20:4ω6 acid was recorded to be higher than that of C20:5ω3 acid. Their percentages were also generally noted in significant quantities in other freshwater mussel studies. For example, the level of C20:4ω6 acid was reported to be between 0.92% and 15.13% in *C. texasensis* (Hagar and Dietz, 1986), between 3.04% and 6.75% in *A. piscinalis* (Dembitsky et al.,1993a), 9.03% in *D. delodontus* (Pollero et al., 1983), 10.42% in *D. polymorpha*, and 3.69% in *Unio* sp. (Dembitsky et al., 1992). On the other hand, the percentages of C20:5ω3 acid were discovered to be between 1.60% and 10.85% in *C. texasensis*, between 0.02% and 0.65% in *A. piscinalis*, 3.70% in *D. delodontus*, 10.71% in *D. polymorpha*, and 20.06% in *Unio* sp.

Marine bivalves have been shown to contain less C20:4ω6 acid in comparison to freshwater mollusks, with this acid accounting for only 0%-5% of the total fatty acids in marine bivalves (Gardner and Riley, 1972; Watanabe and Ackman, 1974; Paradis and Ackman, 1977; Joseph, 1982) and 5%-10% in marine gastropods (Paradis and Ackman, 1977; Johns and Nichols, 1980; Joseph, 1982). In previous studies of marine bivalves, the percentage of C20:4ω6 acid was reported to be between 3.11% and 6.60% in *O. edulis* (Napolitano et al., 1988), between 2.5% and 1.0% in *Mytilus platensis* (De Moreno et al., 1980), between 2.07% and 10.8% in *M. balthica* (Wenne and Polak, 1989), and between 0.9% and 4.3% in *C. gigas* (Pazos et al., 1996); the percentage of C20:5ω3 acid characteristic to marine mollusks was noted to be between 5.54% and 14.20% in *O. edulis* (Napolitano et al., 1988), between 17.9% and 20.7% in *M. platensis* (De Moreno et al., 1980), between 7.70% and 19.9% in *M. balthica* (Wenne and Polak, 1989), and between 13.4% and 27.8% in *C. gigas* (Pazos et al., 1996).

Marine bivalve are generally rich in fatty acids of ω3 (especially C18:3ω3, C20:5ω3, and C22:6ω3 acids). However, freshwater bivalves are rich in fatty acids of ω6 (most notably C18:2ω6 and C20:4ω6 acids). While freshwater mussels have Σω6/Σω3 ratios of 2-4, marine mollusks have ratios of 0.1-1.0 (Hagar and Dietz, 1986). The Σω6/Σω3 ratios in *U. elongatulus* ranged from 2.41 to 3.15 in the phospholipids and from 2.63 to 2.88 in the neutral lipids. Both in the phospholipid and in the neutral lipid, the highest Σω6/Σω3 ratio was detected in the Fabrika mussels. The findings were similar to the results yielded by other freshwater mussels. The differences in the fatty acid profiles of marine and freshwater mollusks may be due to diet since marine plankton are rich in ω3 acids, while ω6 acids predominate in terrestrial and freshwater plants (Sargent, 1976).

Prostaglandins appear to be considerably important mediators in basic physiological functions of the mollusk, including tasks such as ion regulation and possibly even renal function and reproductive biology (Stanley-Samuelson, 1987). In a study on the freshwater snail *Helisoma durgi*, it was also proposed that prostaglandins may stimulate egg production (Kunigelis and Saleuddin, 1986). C20:4ω6 and C20.5ω3 acids are necessary for the synthesis of series 2 and 3 prostaglandins, respectively. In the body lipids of freshwater bivalves, prostaglandins are involved in regulating sodium uptake and for this reason its content is relatively high (Hagar and Dietz, 1986). The increased proportion of the C20:4ω6 acid in the gills and, to some extent, in the mantle of *M. balthica* (Wenne and Polak, 1989) suggests an adaptation to the brackish-water conditions of the Gulf of Gdansk. This is confirmed by the low content of C20:4ω6 acid reported in *M. edulis* gills from typical sea-water (Morris et al., 1983). While marine mollusks possess little C20:4ω6 acid, the freshwater bivalves investigated contain a relatively large amount. C20:4ω6 acid was also found to be the most abundant fatty acid in a total lipid extract of *L. subrostrata* gills (Saintsing et al., 1983) and was reported to be a major component of a whole animal extract of the South American freshwater mussel *D. patagonicus* (Pollero et al., 1981). The high level of C20:4ω6 acid in *U. elongatulus* populations may be related to prostaglandin synthesizing in order to regulate sodium uptake.

As a result of water analyses of Çağçağ Brook, the Tigris River, Fabrika Brook, and Kırkçeşme Spring, we were able to determine that the mussels' waters mostly contained the genera *Amphora*, *Cocconeis*, *Cymbella*, *Cyclotella*, *Gomphonema*, *Synedra*, *Navicula*, *Rhoicosphenia*, *Nitzschia*, *Meridion*, and *Bacillaria* from the phylum Bacillariophyta; the genus *Spirogyra* from the phylum Chlorophyta; the genera *Oscillatoria* and *Lyngbya* from the phylum Cyanoprokaryota; the genus *Stigeoclonium* from the phylum Chlorophyta; and the genera *Peridinium* and *Ceratium* from the phylum Dinoflagellata, as well as rotifers, blue-green algae, bacteria, and detritus. Although all of these components could be seen in the 4 sampling locations, Kırkçeşme Spring was denser than the other 3 localities in terms of vegetation and organism density. Çağçağ Brook, the Tigris River, and Fabrika Brook all have a higher current velocity than Kırkçeşme Spring and their depth, at an average of 2-3 m, is greater than that of Kırkçeşme Spring, which averages only 70 cm; the water temperature of Kırkçeşme was also observed to be lower than that at the other localities. We have observed that the fauna and flora of Kırkçeşme Spring contained a lot of organisms, both plant and animal. Feeding activity is probably more intense for Kırkçeşme mussels because of the increased vegetation and low water velocity. Some previous works have emphasized that most lipids and a considerable amount of C20:5ω3 and C22:6ω3 acids are provided by diatoms and dinoflagellates, respectively, while small amounts of lipids and saturated and monounsaturated acid of 14 and 18 carbons are provided by detritus (Williams, 1965; Ackman et al., 1968). In addition, according to Kaitaranta et al. (1986), the lipids of diatoms also contained high levels of C16:1ω7, C16:0, and C20:5ω3 acids. The fact that lipids in the mussels are rich in PUFAs of 20 and 22 carbons seems to indicate that their food consists mainly of phytoplanktonic elements. In another study, it was also stressed that polyenoic fatty acids of 20 and 22 carbons are provided by the diet and can be synthesized from corresponding dietary precursors (De Moreno et al., 1977).

One of the most interesting fatty acid groups is undoubtedly non-methylene interrupted dienoic (NMID) fatty acids (C20:2$\Delta^{5\text{-}11}$, C20:2$\Delta^{5\text{-}13}$, C22:2$\Delta^{7\text{-}13}$, and C22:2$\Delta^{7\text{-}15}$), which have been described in several marine phyla. The origin of NMID fatty acids has been discussed. It was suggested that these fatty acids are derived almost exclusively from food sources and are biochemically inert (Paradis and Ackman, 1977; Johns et al., 1979). On the other hand, some authors have claimed that these fatty acids in aquatic invertebrates have an endogenous origin (Joseph, 1982; Irazu et al., 1984; Zhukova, 1986, 1991). As has been reported in most of the other examinations of freshwater mollusks, in this study on *U. elongatulus* none of the mussel lipids contained the NMID main constituents of the polar lipids of marine mollusks (Pollero et al., 1983; Dembitsky et al., 1992, 1993a, 1993b; Fried et al., 1993), freshwater prosobranch gastropods (Misra et al., 2002), and freshwater fishes (Ackman et al., 2002). Ackman and Hooper (1973) have suggested that NMID fatty acids may be acceptable as mimics for the C20:2ω6 acid more commonly found in mollusks. In this study, C20:2ω6 acid appeared both in the phospholipid and neutral lipid and its percentage varied from 0.71% to 1.62%.

In comparison with ΣSFA in the phospholipid of Çağçağ, Tigris, Fabrika, and Kırkçeşme mussels, it can be seen that the neutral lipid fraction always contained a higher amount of ΣSFA than the phospholipid fractions (Figure 1). For instance, in the phospholipids, the level of ΣSFA was observed to be 27.14%, 28.41%, 29.00%, and 26.80% (Figure 1) while in the neutral lipid, it was 33.74%, 34.49%, 31.50%, and 28.19% (Figure 2) in the mussels of Çağçağ, the Tigris, Fabrika, and Kırkçeşme, respectively. These findings resulted from the investigation of C16:0 and C18:0 acids and were unsurprising due to the fact that sterol, sterol esters, free fatty acids, fatty acid esters, monoacylglycerol, diacylglycerol, and triacylglycerol make up neutral lipids, which contain much more SFA than the phospholipids membrane components of cell and cellular organelles. The accumulation of ΣSFA in the neutral lipids is probably related to energy requirement and the aim of this accumulation may be storing energy-rich material for subsequent consumption during metabolic activity and hard conditions. In the phospholipid, the ΣPUFA level was 44.53%, 39.46%, 39.17%, and 37.43% in Kırkçeşme, Fabrika, Çağçağ, and Tigris mussels, respectively (Table 1 and Figure 1). In the neutral lipid fractions, the ΣPUFA level was 28.63% (Kırkçeşme), 29.17% (Tigris), 31.63% (Çağçağ), and 32.23% (Fabrika)

(Table 2 and Figure 2). Hagar and Dietz (1986), in their study on the phospholipid content of the gill tissue from freshwater mussel *C. texasensis*, found ΣPUFA levels (35.27%-44.03%) to be higher than ΣMUFA (25.10%-35.69%) and ΣSFA levels (14.75%-23.01%). In another study on the freshwater mussel *A. piscinalis*, the levels of ΣSFA, ΣMUFA, and ΣPUFA in phospholipid were reported as 7.29%, 21.64%, and 71.07%, respectively. In the same study the percentages of ΣSFA, ΣMUFA, and ΣPUFA in the neutral lipid were 53.59%, 40.79%, and 5.32%, respectively (Dembitsky et al., 1993a). Abad et al. (1995) also found ΣPUFA level in high percentages in the phospholipid of marine bivalve *Ostrea edulis* (between 52.7% and 73.7%), and the ΣSFA level was reported to be between 16.2% and 27.2%. The level of ΣPUFA in the phospholipid in all *U. elongatulus* populations was found to be higher than that of the neutral lipid. As usual, the phospholipid contains more PUFAs than the neutral lipid. Generally, cell membranes have a higher composition of polyunsaturated fatty acids and a lower composition of saturated fatty acid. In aquatic organisms, increasingly cold environments lead to a uniquely high cell membrane content of both MUFAs and PUFAs, presumably to maintain greater membrane fluidity at the lower temperatures. Neutral lipids are considered as depot or reserve lipids, however, since cellular membranes contain very small amounts. It is generally known that neutral lipids have relatively less polyenoic fatty acids than phospholipids. In *U. elongatulus*, the SFAs and MUFAs levels were found in higher quantities in the neutral lipid than in the phospholipid. It seems likely that these fatty acids were accumulated by the mussels in order to meet future energy requirements, which could be readily supplied after being catabolized. It is also thought that the neutral lipids may be temporary reservoirs of PUFAs that can be transferred to the structural lipids or directed to specific metabolic pathways.

Nevertheless, the investigation on the fatty acid composition of phospholipids and neutral lipids from freshwater mussels living in 4 different localities has demonstrated that the animals in questions contain C16:0, C16:1ω7, C18:1ω9, C18:2ω6, and C20:4ω6 as the predominant fatty acids. The percentages of C20:4ω6 acids, precursors of eicosanoids, were observed to be higher in the phospholipids than in the neutral lipids. In all localities, the phospholipid of the mussel contained more ΣPUFA and the neutral lipid contained more ΣMUFA. There were some differences in the fatty acid percentages among localities; these differences presumably result from the specific vegetal flora found in each location.

Acknowledgements

We are grateful to Prof. Dietz T.H. and Can Buluş for their constructive criticism on the language and content of this study. Thanks are also due to Memet Varol and Aysel Bekleyen for helping us to identify the plankton samples.

References

Abad, M., Ruiz, C., Martinez, D., Mosquera, G. and Sanchez, J.L. 1995. Seasonal variation of lipid classes and fatty acids in flat oyster, Ostrea *edulis*, from San Cibrian (Galicia, Spain). Comp. Biochem. Physiol. 110C (2): 109-118.

Ackman, R.G., Tocher, C.S. and McLachlan, J. 1968. Marine phytoplankter fatty acids. J. Fish. Res. Board Can. 25: 1603-1620.

Ackman, R.G. and Hooper, S.N. 1973. Non-methylene-interrupted fatty acids in lipids of shallow water marine invertebrates: a comparison of two molluscs with the sand shrimp. Comp. Biochem. Physiol. 46B: 153-165.

Ackman, R.G., McLeod, C., Rakshit, S. and Misra, K.K. 2002. Lipids and fatty acids of five freshwater food fishes of India. J. Food Lipids. 9: 127-145.

Alkanani, T., Parrish, C.C., Thompson, R.J. and McKenzie, C.H. 2007. Role of fatty acids in cultured mussels, *Mytilus edulis*, grown in Notre Dame Bay, Newfoundland. J. Exp. Mar. Biol. Ecol. 48: 33-45.

Bashan, M., Akbas, H. and Yurdakoc, K. 2002. Phospholipid and triacylglycerol fatty acid composition of major life stages of sunn pest, *Eurygaster integriceps* (Heteroptera: Scutelleridae). Comp. Biochem. Physiol. 132B: 375.

Bligh, E.G. and Dyer, W.J.A. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Caers, M., Coutteau, P., Lombeida, P. and Sorgeloos, P. 1998. The effect of lipid supplementation on growth and fatty acid composition of *Tapes philippinarum* spat. Aquaculture. 162: 287-299.

Caers, M., Coutteau, P., Sorgeloos, P. and Gajardo, G. 2003. Impact of algal diets and emulsions on the fatty acid composition and content of selected tissues of adult broodstock of Chilean scallop *Argopecten pupuratus* (Lamarck, 1819). Aquaculture. 217: 437-452.

Cotroneo, A., Ziino, M., Alfa M., Salvo, F. and Dogo, G. 1980. Scarti di pesca nell'alimentazione di *Penaeus kerathurus* - Nota IV. Atti Soc. Peloritana di Scienze Fisiche. Matem. e Naturali. 26: 151-160.

De Moreno, J.E., Moreno, V.J. and Brenner, R.R. 1977. Lipids metabolism of the yellow clam, *Mesodesma mactroides*: 3-saturated fatty acids and acetate metabolism. Lipids. 12: 804-808.

De Moreno, J.E.A., Pollero, R.J., Moreno, V.J. and Brenner, R.R. 1980. Lipids and fatty acids of the mussel (*Mytilus platensis* d'Orbigny) from South Atlantic waters. J. Exp. Mar. Biol. Ecol. 48: 263-276.

Dembitsky, V.M, Kashin, A.G. and Stefanow, K. 1992. Comparative investigation of phospholipids and fatty acids of freshwater molluscs from Volga River Basin. Comp. Biochem. Physiol. 102B: 193-198.

Dembitsky, V.M., Rezanka, T. and Kashin, A.G. 1993a. Fatty acid and phospholipids composition of freshwater molluscs *Anadonta piscinalis* and *Limnaea fragilis* from the River Volga. Comp. Biochem. Physiol. 105B, 3(4): 597-601.

Dembitsky, V.M., Rezanka, T. and Kashin, A.G. 1993b. Comparative study of the endemic freshwater fauna of Lake Baikal-I. Phospholipids and fatty acid composition of two mollusc species, *Baicalia oviformis* and *Benedictia baicalensis*. Comp. Biochem. Physiol. 106B (4): 819-823.

Deshimaru, O., Kuroki, K. and Yone, Y. 1979. The compositions and level of dietary lipid appropriate for growth of Prawn. Bull. Japanese Soc. Sci. Fish. 45: 591-594.

Dietz, T. H. and Graves, S. Y. 1981. Sodium influx in isolated gills of the freshwater mussel, *Ligumia subrostrata*. J. Comp. Physiol. 143: 185-190.

Ekin, İ., Başhan, M. and Şeşen, R. 2008. Fatty acid composition of *Dreissena siouffi* (Bivalvia: Dreissenidae) collected from the Fırat River. Science and Eng. J. of Fırat Univ. 20(2): 243-250.

Ekin, İ. and Başhan, M. 2010. Fatty acid composition of selected tissues of *Unio elongatulus* (Bourguignat, 1860) (Mollusca: Bivalvia) collected from Tigris River, Turkey. Turk. J. Fish. Aquat. Sci. 10: 445-451.

Ekman, S. 1953. Zoogeography of the Sea, Plenum Press, London.

Fried, B., Rao, K.S., Sherma, J. and Huffman, J.E. 1993. Fatty acid composition of *Goniobasis virginica, Physa sp*. and *Viviparus malleatus* (Mollusca: Gastropoda) from Lake Musconetcong, New Jersey. Biochem. Syst. Ecol. 21(8): 809-812.

Gardner, D. and Riley, J.P. 1972. The components fatty acids of the lipids of some species of marine and freshwater molluscs. J. Mar. Biol. Assoc. U.K. 52: 827-838.

Go, J.V., Rezanka, T., Srebnik, M. and Dembitsky, V.M. 2002. Variability of fatty acid component of marine and freshwater gastropod species from the littoral zone of the Red Sea, Mediterranean Sea and Sea of Galilee. Biochem. Syst. Ecol. 30: 819-835.

Hagar, A.F. and Dietz, T.H. 1986. Seasonal changes in the lipid composition of gill tissue from the freshwater mussel *Carunculina texasensis*. Physiol. Zool. 59(4): 419-428.

Isay, V.S. and Busarova, N.G. 1984. Study on fatty acids composition of marine organisms–I. Unsaturated fatty acids of Japan Sea invertebrates. Comp. Biochem. Physiol. 77B (4): 803-810.

Irazu, C.E., Pollero, R.J. and Brenner, R.R. 1984. Occurrence of a 22:2 nonmethylene interrupted dienoic fatty acids and its seasonal distribution among lipids and tissues of the freshwater bivalve *Diplodon delodontus* from an isolated environment. Lipids. 19: 649-655.

Johns, R.B., Nichols, P.D. and Perry, G.J. 1979. Fatty acid composition of ten marine algae from Australian waters. Phytochemistry. 18: 799-802.

Johns, R.B., Nichols, P.D. and Perry, G.J. 1980. Fatty acid components of nine species of molluscs of the littoral zone from Australian waters. Comp. Biochem. Physiol. 65B: 207-214.

Joseph, J.D. 1982. Lipid composition of marine and estuarine invertebrates. Part II: Mollusca. Progr. Lipid Res. 21: 109-153.

Kaitaranta, J.K., Linko, R.R. and Voulera, R. 1986. Lipids and fatty acids in plankton from the Finnish coastal waters of Baltic Sea. Comp. Biochem. Physiol. 85B: 427-433.

Kharlamenko, V.I., Zhukova, N.V., Khotimchenko, S.V., Svetashev, V.I. and Kamenev, G. M. 1995. Fatty acids as markers of food sources in a shallow water hydrothermal ecosystem (Kraternaya Bight,Yankich Island, Kurile Islands). Mar. Ecol. Prog. Ser. 120: 231-241.

Klingensmith, S.J. and Stillway, W.L. 1982. Lipid composition of selected tissues of hardshell clam *Mercenaria mercenaria*. Comp. Biochem. Physiol. 71B: 111-112.

Kunigelis, S.C. and Saleuddin, A.S.M. 1986. Reproduction in the freshwater gastropod, *Helisoma*: involvement of prostaglandin in egg production. Int. J. Invert. Reprod. Dev. 10: 159-167.

Misra, K.K., Shkrob, I., Rakshit, S. and Dembitsky, V.M. 2002. Variability in fatty acids and fatty aldehydes in different organs of two prosobranch gastropod molluscs. Biochem. Syst. Ecol. 30: 749-761.

Misra, S., Ghosh, K.M., Choudhury, A., Dutta, K.A., Pal, K.P. and Ghosh, A. 1985. Fatty acids from *Macoma sp.* of bivalve mollusc. J. Sci. Food Agric. 36: 1193-1196.

Morris, R.J., Culkin, F., Lockwood, A.P.M. and Jensen, A.C. 1983. Effect of chlorination on the gill lipids of the mussel *Mytilus edulis*. Chem. Ecol. 1(3): 173-189.

Napolitano, G.E, Ratnayake, W.M.N. and Ackman, R.G. 1988. Fatty acid components of larval *Ostrea edulis* (L.): importance of triacylglycerols as fatty acid reserve. Comp. Biochem. Physiol. 90B: 875-883.

Paradis, M. and Ackman, R.G. 1977. Potential for employing the distribution of anomalous non-methylene-interrupted dienoic fatty acids in several marine invertebrates as part of food web studies. Lipids. 12: 170-176.

Pazos, J.A., Ruiz, C., Martin, G.O., Abad, M. and Sanchez, L.J. 1996. Seasonal variation of the lipid content and fatty acid composition of *Crassostrea gigas* cultured in E1 Grove, Galicia, N.W. Spain. Comp. Biochem. Physiol. 114B (2): 171-179.

Pazos, J.A., Sanchez, L.J., Roman, G., Perez-Parelle, M.L. and Abad, M. 2003. Seasonal changes in lipid classes and fatty acids composition in digestive gland of *Pecten maximus*. Comp. Biochem. Physiol. 134B: 367-380.

Piretti, M.V., Zuppa, F., Pagliuca, G. and Taioli, F. 1988. Variations of fatty acid constituents in selected tissues of the bivalve mollusc *Scapharia inaequivaleis*. Comp. Biochem. Physiol. 89B (1): 183-187.

Pollero, R.J., Brenner, R.R. and Gros, G.E. 1981. Seasonal changes in lipid and fatty acid composition of the freshwater mollusc *Diplodom patagonicus*. Lipids. 16(2): 109-113.

Pollero, R.J., Irazu, C.E. and Brenner, R.R. 1983. Effect of sexual stage on lipids and fatty acids of *Diplodon delodontus*. Comp. Biochem. Physiol. 76B: 927-931.

Saintsing, D.G., Hwang, D.H. and Dietz, T.H. 1983. Production of prostaglandin E_2 and $F_{2\alpha}$ in the freshwater mussel *Ligumia subrostrata*: relation to sodium transport. J. Pharmacol. Exp. Ther. 226: 455-461.

Sargent, J.R. 1976. The structure, metabolism and function of lipids in marine organisms. In: Biochemical and Biophysical Perspectives in Marine Biology (eds. D.C. Malin and J.R. Sargent), Academic Press, London, pp.149-212.

Spector, A.A. and Yorek, M.A. 1985. Membrane lipid composition and cellular function. J. Lipid Res. 26: 1015-1035.

Spike, B.P., Wright, R.J., Danielson, S.D. and Stanley-Samuelson, D.W. 1991. The fatty acid compositions of phospholipids and triacylglycerols, from two chinch bug species *Blissus leucopterus leucopterus* and *B. iowensis* (Insecta; Hemiptera; Lygaeidae) are similar to the characteristic dipteran pattern. Comp. Biochem. Physiol. 99B: 799-802.

Stanley-Samuelson, D.W. and Dadd, R.H. 1983. Long chain polyunsaturated fatty acids: Patterns of occurrence in insects. Biochemistry. 13: 549-558.

Stanley-Samuelson, D.W. 1987. Physiological roles of prostaglandins and other eicosanoids in invertebrates. Biol. Bull. 173: 92-109.

Thompson, S.N. 1973. A review and comparative characterization of the fatty acid compositions of seven insect orders. Comp. Biochem. Physiol. 45B: 467-482.

Vernberg, W.B. and Vernberg, F.J. 1972. Environmental Physiology of Marine Animals, Springer, Berlin.

Watanabe, T. and Ackman, R.G. 1974. Lipids and fatty acids of the American (*Crassostrea virginica*) and European flat (*Ostrea edulis*) oysters from a common habitat and after one feeding with *Dicrateria inornata* or *Isochrysis galbana*. J. Fish. Res. Board Can. 31: 403-409.

Wenne, R. and Polak, L. 1989. Lipid composition and storage in the tissues of the *Macoma balthica*. Biochem. Syst. Ecol. 17: 583-587.

Williams, P.M. 1965. Fatty acids derived from marine origin. J. Fish. Res. Board Can. 22: 1107-1122.

Zhukova, N.V. 1986. Biosynthesis of non-methylene interrupted dienoic fatty acids from [C^{14}] acetate in molluscs. Biochim. et Biophys. Acta. 878: 131-133.

Zhukova, N.V. 1991. The pathway of the biosynthesis of non-methylene-interrupted dienoic fatty acids in molluscs. Comp. Biochem. Physiol. 110B: 801-804.