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Fatty acid composition of lipid classes in two mussel populations (*Anodonta piscinalis* and *Corbicula fluminalis*) living in Tigris River

[Dicle Nehri'nde yaşayan iki midye popülasyonun (*Anodonta piscinalis* ve *Corbicula fluminalis*) lipid sınıflarının yağ asitleri kompozisyonu]

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ABSTRACT

Purpose: The aim of this work was to investigate quantitative and qualitative fatty acid composition of phospholipid, monoacylglycerol, diacylglycerol, triacylglycerol and total body lipid of two adult freshwater mussels, *Anodonta piscinalis* (Nilsson, 1822) and *Corbicula fluminalis* (Müller, 1774).

Methods: Mussels' body lipids were fractionated by thin layer chromatography. Gas chromatography and gas chromatography-mass spectrometry instruments were used to detect the fatty acids.

Results: C16:0, C18:0, C16:1ω7, C18:1ω9, C18:2ω6 and C20:4ω6 were the dominant fatty acids. Their total percentages are between 70.37% and 82.11% in *A. piscinalis* and between 66.70% and 75.53% in *C. fluminalis*. As characteristic to freshwater mussels, Σω6 / Σω3 ratio was defined high in all lipid fractions. In both of the mussels, the maximum level of total polyunsaturated fatty acids was found in the phospholipids, and the maximum level of the total saturated fatty acids was found in the monoacylglycerol fractions. However, the level total monounsaturated fatty acids showed differentiations among fractions. In comparison to other fractions, the phospholipid contained high amount of C20:4ω6 and C20:5ω3, involved in eicosanoids formation.

Conclusion: The study provided insight into the biochemistry and nutritional value of the widely distributed freshwater mussels from Tigris River.

Key words: fatty acids, *Anodonta piscinalis*, *Corbicula fluminalis*, freshwater mussel, Tigris River

Conflict of interest: Authors have no conflict of interest.

ÖZET

Amaç: Bu çalışmanın amacı, tatlısu midyesi *Anodonta piscinalis* (Nilsson, 1822) ve *Corbicula fluminalis* (Müller, 1774)'in fosfolipit, monoaçilgliserol, diaçilgliserol, triaçilgliserol ve total vücut lipidinin yağ asidi içeriğini kalitatif ve kantitatif olarak araştırmaktır.

Metotlar: Midyelerin total vücut lipitleri, ince tabaka kromatografi ile fraksiyonlandı. Yağ asidi içeriği, gaz kromatografi ve gaz kromatografi-kütle spektrometre cihazları kullanılarak tespit edildi.

Bulgular: C16:0, C18:0, C16:1ω7, C18:1ω9, C18:2ω6 ve C20:4ω6 asitler çoğunlukta bulunan bileşenlerdi. Toplam oranları *A. piscinalis*'te %70.37 ve %82.11 arasında; *C. fluminalis*'te ise %66.70 ve %75.53 arasında değişti. Tatlısu midyelerine özgü olduğu gibi, Σω6 / Σω3 değeri tüm fraksiyonlarda yüksek miktarda bulundu. Her iki midye türünde de en yüksek total çoklu doymamış yağ asidi oranı, fosfolipitte; en yüksek total doymuş yağ asidi oranı ise monoaçilgliserolda saptandı. Total tekli doymamış yağ asidi oranı ise fraksiyonlar arasında farklılık gösterdi. Diğer fraksiyonlar ile kıyaslanınca, fosfolipit fraksiyonunda eikosanoidlerin yapımında görev alan C20:4ω6 ve C20:5ω3 asitler daha yüksek oranda bulundu.

Sonuç: Çalışma, Dicle Nehri'nde geniş dağılış gösteren tatlısu midyelerinin besinsel ve biyokimyasal özelliklerine ait bilgiler sağlamıştır.

Anahtar kelimeler: yağ asitleri, *Anodonta piscinalis*, *Corbicula fluminalis*, tatlısu midyesi, Dicle Nehri

Çıkar çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Marine and freshwater bivalves are valued sea foods of maritime countries and grown in aquaculture farms for consumption worldwide [1]. They are also alternatively consumed by fish, birds, crustacean, aquatic reptiles and mammals as diets in nature. Therefore, most of investigators have extensively studied bivalves. Numerous studies are present on marine bivalves at large, only a few studies have been done on freshwater forms. Especially, fatty acid compositions of marine forms are well established; however only limited information is present on fatty acid composition of freshwater mussels.

The present study aims to define fatty acid composition of the phospholipid, monoacylglycerol, diacylglycerol, triacylglycerol and total body lipid classes of *A. piscinalis* and *C. fluminalis* mussels distributed in most part of Eastern Anatolian Rivers. Meanwhile, the study provides insight into the biochemistry and nutritional value of the widely distributed freshwater mussels from Tigris River.

Materials and methods

Collection and Preparation of Mussels

A. piscinalis and *C. fluminalis* mussels were harvested in April 2009 from Tigris River under Sadi Bridge (Diyarbakır) (Altitude: 583 m, Coordinate: N 37° 55.2' / E 40° 13.8'). The temperature of the river water was 16 °C. Three healthy adult mussels of similar size were sampled for each lipid analysis, totally fifteen individuals. The mussels were divided into five groups and rinsed with tap water to remove dirtiness. Whole bodies of the mussels were removed from shells, and wet weight of their flesh was determined. Average wet weight of *A. piscinalis* meat was 14.5 ±3.4 g, and average wet weight of *C. fluminalis* meat was 6.2 ±2.1 g. Dissected tissues were transferred into chloroform / methanol (1:2 v / v) and stored at -80 °C until required for analyses.

Lipid Extraction and Quantification

Samples were homogenized in chloroform / methanol (1:2, v / v) solution in order to extract whole body lipids [2]. Autoxidation of unsaturated components was minimized by adding 50 μL of 2%butylated hydroxytoluene (BHT) in chloroform to each sample during the extraction process. Total lipid extracts were dried under a stream of N_2. Then, whole lipids were spotted on preparative thin layer chromatography (TLC) plates, using silicagel TLC plates (20 × 20 cm, 0.25 mm thick). After applying the lipid extracts, the TLC plates were developed in 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 the phospholipid, monoacylglycerol, diacylglycerol and triacylglycerol fractions were identified by corresponding standards. The 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. FAMEs (Fatty Acid Methyl Esters) of lipid classes were extracted from the reaction vials three times with hexane and concentrated [3].

GC and GC-MS Conditions

FAMEs were separated and quantified by capillary gas chromatography (GC) system, consisted of a Hewlett Packard (Wilmington, DE) gas chromatograph (model 6890), a DB–23 capillary column (60 m x 0.25 mm i.d x 0.250 μm film thickness and 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 rates of compressed air and hydrogen were 300 ml / min, 30 ml / min, respectively. Helium was used as carrier gas (2.8 ml / min). The oven temperature was programmed at a rate of 6.5 °C / min from 130 °C (1 min hold) to 170 °C, then increased at a rate of 2.75 °C / min to a 215 °C, then again increased at a rate of 40 °C / min to 230 °C, was held for 12 min. Total fatty acids levels and spectra of FAMEs are obtained by HP 3365 ChemStation computer program. Chemical structures of the FAMEs were determined by analyses of spectra and by comparing obtained spectra with the spectra of authentic standards (Sigma–Aldrich Chemicals). Individual FAME was 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 a 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 from 150 °C to 230 °C at a 2 °C / min increase with an initial hold of 6 min. The carrier gas 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. Chemical structures of the FAMEs were determined by the analyses of spectra and by comparing obtained spectra with the spectra of authentic standards.

Statistical Analysis

Kruskal-Wallis non-parametric test was used for measurements to evaluate statistical differences across subjects among the five conditions (phospholipid, monoacylglycerol, diacylglycerol, triacylglycerol and total body lipid). When the Kruskal-Wallis test showed a statistical difference, Mann-Whitney U test was used for multip-

le comparisons to evaluate the statistical significance of the difference between different groups. *$p < 0.05$ was considered statistically significant in all analyses.*

Results

In the analyses, 22 types of fatty acids were identified. They were C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C14:1ω9, C16:1ω7, C18:1ω9, C20:1ω9, C22:1ω9, C18:2ω6, C18:3ω3, C20:2ω6, C20:3ω6, C20:4ω6, C20:5ω3, C22:2ω6, C22:5ω6 and C22:6ω3. The proportions of C16:0, C18:0, C16:1ω7, C18:1ω9, C18:2ω6 and C20:4ω6 were found higher than others (Table 1 and 2). They totally accounted for 70.37%in the phospholipid, 77.74%in the monoacylglycerol, 82.11%in the diacylglycerol, 81.06%in the triacylglycerol and 76.68%in the total body lipid of *A. piscinalis* and 66.70%in the phospholipid, 72.87%in the monoacylglycerol, 75.53%in the diacylglycerol, 75.43%in the triacylglycerol and 71.28%in the total body lipid of *C. fluminalis.*

Fatty acid analysis of A. piscinalis: The most abundant SFA (Saturated Fatty Acids) was C16:0 and its percentage was 15.43%in the phospholipid, 20.06%in the monoacylglycerol, 20.47%in the diacylglycerol, 24.09%in the triacylglycerol and 21.24%in the total body lipid. The ΣSFA level of the monoacylglycerol (36.93%), triacylglycerol (35.95%) and total body lipid (35.34%) were found at close percentages. The lowest value of ΣSFA was registered in the phospholipid (28.29%). C16:1ω7 and C18:1ω9 were predominant MUFA (Monounsaturated Fatty Acids). The level of C16:1ω7 varied from 9.60%(in the triacylglycerol) to 11.43%(in the phospholipid), and the level of C18:1ω9 ranged from 13.34%(in the phospholipid) to 25.63%(in the diacylglycerol). The level of ΣMUFA was 33.78%in the phospholipid, 35.78%in the monoacylglycerol, 38.48%in the diacylglycerol, 37.63%in the triacylglycerol and 35.02%in the total body lipid. The major PUFA were C18:2ω6, C20:4ω6 and C20:5ω3 and their highest values were registered in the phospholipid. The highest level of ΣPUFA was also registered in the phospholipid (Table 1).

Fatty acid analysis of C. fluminalis: The percentage of C16:0 was 19.63%in the phospholipid, 26.05%in the monoacylglycerol, 22.96%in the diacylglycerol, 24.77%in the triacylglycerol and 20.68%in the total body lipid. The maximum level of ΣSFA was found in the monoacylglycerol. Predominant MUFA were C16:1ω7 and C18:1ω9, and their percentages were 4.48%and 9.71%in the phospholipid, 7.59%and 16.70%in the monoacylglycerol, 8.64%and 20.97%in the diacylglycerol, 9.94%and 19.01 in the triacylglycerol, 8.38%and 15.44 in the total body lipid, respectively. The level of ΣMUFA was 19.43%in the phospholipid, 25.19%in the monoacylglycerol, 30.26%in the diacylglycerol, 32.04%in the triacylglycerol and 27.22%in the total body lipid. C18:2ω6, C20:4ω6 and C20:5ω3 were predominantly found PUFA, and their highest values were registered in the phospholipid. The highest ΣPUFA level was found in the phospholipid, and the lowest one was in the monoacylglycerol (Table 2).

The total of ω6 (omega 6) acids was higher than total of ω3 (omega 3) acids in all of the lipid analyses from *A. piscinalis* and *C. fluminalis*. In *A. piscinalis*, the highest ratio of Σω6 / Σω3 was defined as 2.57 in the phospholipid (Table 1) and in *C. fluminalis*, the highest ratio of Σω6 / Σω3 was 1.95 in the monoacylglycerol (Table 2).

Discussion

In contrast with most of detailed studies on biochemical and physiological composition of marine bivalves [4-14], there are a few studies on freshwater species. *Diplodom patagonicus* [15], *Diplodon delodontus* [16], *Caruncula texasensis* [17], *Dreissena polymorpha* [18], *Dreissena siouffi* [19] and *Unio elongatulus* [20] are some of the studied freshwater mussels. Furthermore, their fatty acid compositions were studied only at restricted respects.

In the analyzed lipid classes of *A. piscinalis* and *C. fluminalis*, C16:0, C18:0, C16:1ω7, C18:1ω9, C18:2ω6, C20:4ω6 and C20:5ω3 were determined as major and C10:0, C12:0, C13:0, C15:0, C14:1ω9, C22:1ω9, C20:2ω6, C20:3ω6 and C22:2ω6 were minor (Table 1 and 2). The results obtained in the present work reveal that these major and minor fatty acids coincided with other studies, and the fatty acid profile conformed to the common pattern of bivalves in general [7-11]. The quantity of C16:0, which is key component in biosynthesis of fatty acids, varies over a wide range in marine and freshwater molluscs [12]. In most of the both marine and freshwater mussels, C16:0 was reported as major fatty acid. In *C. texasensis*, *D. delodontus*, *D. patagonicus*, *D. polymorpha*, *U. elongatulus* and *D. siouffi,* C16:0 was found as predominant component. This fatty acid was also found as major fatty acid in 51 marine invertebrates living in Japanese Sea [13]. Presumably, metabolism and physiology of mussels cause to average level of C16:0. On the other hand, specific differences in fatty acid composition of mussels may be, to some extent, affected by external factors such as diet, vegetation, temperature, salinity, dirtiness or sun light. The most important external factor is undoubtedly diet. Phytoplanktons and zooplanktons are known as nutrition of most aquatic organisms. There are a number of studies on lipid and fatty acid compositions of planktons [21-23]. To get some important approaches to current study, we have identified the planktonic composition of Tigris River water filtering by *A. piscinalis* and *C. fluminalis*. The water mostly contained *Gomphonema*, *Synedra*, *Navicula*, *Amphora*, *Cocconeis*, *Cymbella*, *Cyclotella*, *Rhoicosphenia*, *Nitzschia*, *Meridion*, *Bacillaria*, *Spirogyra*, *Oscillatoria*, *Lyngbya*, *Stigeoclonium*, *Peridinium*, *Ceratium*, rotifers, blue-green algae, bacteria and detritus. Notably, most biologically important fatty acids are synthesized *de novo* in phytoplanktons and are transferred to zooplanktons and other

Table 1. Fatty acid composition of phospholipid, monoacylglycerol, diacylglycerol, triacylglycerol and total body lipid of *Anodonta piscinalis* (Results expressed as %of whole lipids fatty acids).

Fatty **Acids**	Phospholipid (Mean* ±S.D.) **	Monoacylglycerol (Mean* ±S.D.) **	Diacylglycerol (Mean* ±S.D.) **	Triacylglycerol (Mean* ±S.D.) **	Total body lipid (Mean* ±S.D.) **
Saturated					
C10:0	0.05 ±0.01a	0.09 ±0.02b	-	0.04 ±0.01a	0.12 ±0.02b
C12:0	0.03 ±0.01a	-	0.04 ±0.01a	0.06 ±0.02b	0.05 ±0.02ab
C13:0	-	0.04 ±0.01a	0.03 ±0.01a	-	0.04 ±0.01a
C14:0	3.93 ±0.24a	4.58 ±0.31a	2.46 ±0.18b	2.86 ±0.21b	2.12 ±0.16b
C15:0	0.88 ±0.05a	0.94 ±0.08a	0.91 ±0.09a	0.68 ±0.06b	0.50 ±0.04b
C16:0	15.43 ±1.13a	20.06 ±1.18b	20.47 ±1.17b	24.09 ±1.18c	21.24 ±1.21b
C17:0	1.63 ±0.12a	1.28 ±0.15b	1.66 ±0.12a	1.01 ±0.06c	1.53 ±0.12a
C18:0	6.34 ±0.54a	9.94 ±0.54b	7.40 ±0.54ab	7.21 ±0.54ab	9.74 ±0.56b
ΣSFA	28.29 ±1.26	36.93 ±1.34	32.97 ±1.30	35.95 ±1.32	35.34 ±1.31
Monoenoic					
C14:1ω9	0.50 ±0.05a	0.32 ±0.08b	-	0.40 ±0.02ab	0.24 ±0.02c
C16:1ω7	11.43 ±1.21a	9.80 ±0.56b	10.91 ±1.01ab	9.60 ±0.92b	10.28 ±1.01ab
C18:1ω9	13.34 ±1.02a	20.68 ±1.14b	25.63 ±1.21c	23.87 ±1.20bc	17.04 ±1.11ab
C20:1ω9	7.49 ±0.56a	4.98 ±0.38b	1.89 ±0.11c	3.68 ±0.24d	7.32 ±0.68a
C22:1ω9	1.02 ±0.10a	-	0.05 ±0.01b	0.08 ±0.01c	0.14 ±0.02d
ΣMUFA	33.78 ±1.32	35.78 ±1.33	38.48 ±1.35	37.63 ±1.35	35.02 ±1.32
Polyenoic					
C18:2ω6	12.66 ±0.85a	9.28 ±0.85b	10.95 ±0.89ab	10.27 ±0.98ab	8.60 ±0.56b
C18:3ω3	1.09 ±0.14a	1.00 ±0.10a	1.63 ±0.09b	1.74 ±0.09b	0.95 ±0.11a
C20:2ω6	0.64 ±0.05a	0.55 ±0.06a	0.28 ±0.03b	0.52 ±0.06a	0.46 ±0.04ab
C20:3ω6	0.45 ±0.03a	0.12 ±0.01b	0.39 ±0.02a	0.23 ±0.06ab	0.42 ±0.03a
C20:4ω6	11.17 ±0.74a	7.98 ±0.95b	6.75 ±0.74b	6.02 ±0.52b	9.78 ±0.89a
C20:5ω3	5.98 ±0.41a	5.63 ±0.42a	3.10 ±0.24b	3.53 ±0.35b	4.27 ±0.42c
C22:2ω6	0.75 ±0.05a	-	0.03 ±0.01b	0.02 ±0.01b	-
C22:5ω6	1.82 ±0.14a	1.10 ±0.12b	1.09 ±0.10b	1.21 ±0.13b	1.68 ±0.11a
C22:6ω3	3.62 ±0.02a	1.49 ±0.12b	4.40 ±0.21c	3.36 ±0.02a	4.02 ±0.35c
Σω6 / Σω3	2.57	2.34	2.13	2.12	2.26
ΣPUFA	38.18 ±1.35	27.15 ±1.28	28.62 ±1.29	26.90 ±1.25	30.18 ±1.31

* Values are means ±S.D (Standard Deviation) for three replicates.
Results expressed as percentage of total fatty acids methyl esters
** Different letters (a, b, c, d) in the same row represent significant statistical differences ($p < 0.05$).
SFA: Saturated Fatty Acids, **MUFA:** Monounsaturated Fatty Acids, **PUFA:** Polyunsaturated Fatty Acids

Table 2. Fatty acid composition of phospholipid, monoacylglycerol, diacylglycerol, triacylglycerol and total body lipid of *Corbicula fluminalis* (Results expressed as %of whole lipids fatty acids).

Fatty **Acids**	Phospholipid (Mean* ±S.D.) **	Monoacylglycerol (Mean* ±S.D.) **	Diacylglycerol (Mean* ±S.D.) **	Triacylglycerol (Mean* ±S.D.) **	Total body lipid (Mean* ±S.D.) **
Saturated					
C10:0	-	0.04 ±0.01a	0.02 ±0.01b	0.03 ±0.01ab	-
C12:0	0.01 ±0.01a	-	0.03 ±0.01b	0.07 ±0.02c	0.07 ±0.01c
C13:0	-	0.04 ±0.01a	0.02 ±0.01b	0.04 ±0.01a	0.06 ±0.01c
C14:0	1.83 ±0.09a	4.66 ±0.29b	3.17 ±0.12c	4.33 ±0.28b	3.53 ±0.21c
C15:0	0.76 ±0.07a	2.08 ±0.18b	1.94 ±0.17b	0.93 ±0.09a	0.77 ±0.03a
C16:0	19.63 ±1.45a	26.05 ±1.07b	22.96 ±1.12ab	24.77 ±1.02b	20.68 ±2.01a
C17:0	2.84 ±0.09a	4.33 ±0.28b	2.57 ±0.12a	2.33 ±0.11a	2.75 ±0.20a
C18:0	11.66 ±0.74a	10.49 ±0.87a	8.05 ±0.72b	7.56 ±0.56b	11.54 ±0.85a
ΣSFA	36.73 ±1.30	47.69 ±1.70	38.76 ±1.32	40.06 ±1.36	39.40 ±1.34
Monoenoic					
C14:1ω9	0.10 ±0.02a	-	0.07 ±0.02ab	0.06 ±0.01b	0.08 ±0.02a
C16:1ω7	4.48 ±0.28a	7.59 ±0.54b	8.64 ±0.69b	9.94 ±0.92b	8.38 ±0.67b
C18:1ω9	9.71 ±0.65a	16.70 ±1.05b	20.97 ±1.05c	19.01 ±1.07c	15.44 ±1.08b
C20:1ω9	5.06 ±0.42a	0.86 ±0.62b	0.53 ±0.32b	2.98 ±0.19c	3.22 ±0.18c
C22:1ω9	0.08 ±0.02a	0.04 ±0.01b	0.05 ±0.01b	0.05 ±0.01a	0.10 ±0.02a
ΣMUFA	19.43 ±1.21	25.19 ±1.24	30.26 ±1.31	32.04 ±1.30	27.22 ±1.25
Polyenoic					
C18:2ω6	10.83 ±1.02a	7.01 ±0.54b	8.44 ±0.95b	9.00 ±0.87ab	7.97 ±0.56b
C18:3ω3	3.10 ±0.21a	2.18 ±0.15b	3.93 ±0.15a	3.83 ±0.29a	3.14 ±0.25a
C20:2ω6	0.60 ±0.05a	0.59 ±0.02a	0.47 ±0.06a	0.77 ±0.05b	0.72 ±0.02b
C20:3ω6	0.38 ±0.05a	-	0.36 ±0.06a	0.12 ±0.03a	0.30 ±0.02a
C20:4ω6	10.39 ±0.74a	5.03 ±0.54b	6.47 ±0.50b	5.15 ±0.50b	7.27 ±0.68ab
C20:5ω3	7.10 ±0.58a	3.65 ±0.15b	3.69 ±0.21b	2.56 ±0.16b	4.61 ±0.38c
C22:2ω6	0.15 ±0.05a	-	0.12 ±0.02a	0.18 ±0.02a	-
C22:5ω6	5.30 ±0.38a	5.35 ±0.48a	2.64 ±0.12b	1.65 ±0.16b	4.21 ±0.31c
C22:6ω3	6.27 ±0.52a	3.38 ±0.28b	5.11 ±0.51a	4.82 ±0.31ab	5.59 ±0.14a
Σω6 / Σω3	1.68	1.95	1.45	1.50	1.53
ΣPUFA	44.12 ±1.56	27.19 ±1.25	31.23 ±1.33	28.08 ±1.26	33.81 ±1.34

* Values are means ±S.D (Standard Deviation) for three replicates.
Results expressed as percentage of total fatty acids methyl esters
** Different letters (a, b, c) in the same row represent significant statistical differences ($p < 0.05$).
SFA: Saturated Fatty Acids, **MUFA:** Monounsaturated Fatty Acids, **PUFA:** Polyunsaturated Fatty Acids

primary consumers [22]. Small phytoplankton species including diatoms and flagellates contain significant amount of long chain PUFA (especially C20:5ω3 and C22:6ω3) as well as C16:0, C16:1ω7 and C14:0 [24] while larger phytoplanktons such as dinoflagellates, contain C20:5ω3, C22:6ω3, C16:0 and C18:4ω3 [25]. C16:1ω7 is believed to be a diatom marker, whereas C18:1ω9 is not restricted to a single phytoplankton group. C18:4ω3 and C16:4ω3 (not detected in the current study) were determined as minor components in phytoplankton samples [11]. It is wise to express that the diet of *A. piscinalis* and *C. fluminalis* mostly contained diatoms and some flagellates. C22:6ω3 and predominant fatty acids such as C16:0, C16:1ω7 and C20:5ω3 of the mussels may have two origins, exogenous from the diets or endogenous by desaturation and elongation process. However, it remains difficult to correlate fatty acid composition of the planktons with their food value since it is impossible to take all the interspecific differences between different planktonic diets into account. Furthermore, certain minor components such as vitamin and mineral may also play an important role on bivalve fatty acid composition [26].

C20:4ω6 (characteristic to freshwater mollusc) and C20:5ω3 (characteristic to marine mollusc) are the most important precursors of prostaglandins which are considerably important mediators in basic physiological functions, ion regulation, renal function and reproductive process in molluscs [27]. In some studies, C20:4ω6 accounted for only 0%–6.6%of the total fatty acids (8, 11, 22, 26), whereas the percentage of C20:5ω3 was reported at high level in marine bivalves (Table 3). For example it was reported between 5.54%and 14.20%in *Ostrea edulis* [28], between 17.9%and 20.7%in *Mytilus platensis* [29], between 7.70%and 19.9%in *Macoma balthica* [8], between 13.4%and 27.8%in *Crassostrea gigas* [9] and between 12.01%and 17.02%in *Mytilus edulis* (Table 3) [11]. In the present report, the quantity of C20:4ω6 was obtained at high percentages in all lipid classes, especially in the phospholipid (11.17%in *A. piscinalis* and 10.39%in *C. fluminalis*), however the level of C20:5ω3 was not as high as C20:4ω6 (Table 1 and 2). It was suggested that C20:4ω6 is mostly associated with reproductive process and not with growth [11]. Maybe, the high proportion of C20:4ω6 in *A. piscinalis* and *C. fluminalis* was attributed to reproduction process since the mussels were harvested from the river in the spring season which is reproductive period of the mussels. C20:4ω6 is also a substrate for production of prostaglandins involved in regulating Na uptake, and its content is relatively high in lipids of freshwater mussels, especially in gill lipids [17]. The reason of C20:4ω6 high level in the fractions may also be due to the synthesizing process of prostaglandins to regulate Na uptake metabolism.

Generally marine bivalves are rich in fatty acids of ω3 (especially C18:3ω3, C20:5ω3 and C22:6ω3) and freshwater bivalves are rich in fatty acids of ω6 (especially C18:2ω6 and C20:4ω6). Hagar and Dietz [17] reported that freshwater mussels have Σω6 / Σω3 ratios of 2–4, marine forms have ratios of 0.1-1. In the present study, the Σω6 ratios were always obtained higher than Σω3 (Table 1 and 2). The major ω6 and ω3 acids which caused to proportional differences among the lipid classes were C18:2ω6, C20:4ω6, C20:5ω3 and C22:6ω3. The differences in the fatty acid profiles of marine and freshwater molluscs is generally due to dietary differences since marine planktons are rich in ω3 acids, while ω6 acids are predominate in terrestrial and freshwater plants [30].

C18:2ω4 and C22:5ω6 are important fatty acid for mussels. Some of the studies have reported that these fatty acids have a significant role in determining the weight of the mussel despite their low proportions [11]. In addition, it was suggested that C22:5ω6 was essential for scallop growth [31]. In the current study, C18:2ω4 was detected neither in *A. piscinalis* nor in *C. fluminalis.* However, C22:5ω6 was found in both of the mussels. Interestingly, its percentage was noteworthy (between 1.09%and 1.82%in *A. piscinalis* and between 1.65%and 5.35%in *C. fluminalis*). The level of C22:5ω6 may be related to growth, reproduction or other physiological processes, but is not exactly known.

The level of ΣSFA was registered 28.29%and 36.73%in the phospholipid, 36.93%and 47.69%in the monoacylglycerol, 32.97%and 38.76%in the diacylglycerol, 35.95%and 40.06%in the triacylglycerol, 35.34%and 39.40%in the total body lipid, and the level of ΣMUFA was 33.78%and 19.43%in the phospholipid, 35.78%and 25.19%in the monoacylglycerol, 38.48%and 30.26%in the diacylglycerol, 37.63%and 32.04%in the triacylglycerol, 35.02%and 27.22%in the total body lipid of *A. piscinalis* and *C. fluminalis*, respectively (Table 1 and 2). The ΣPUFA level in the phospholipid, monoacylglycerol, diacylglycerol, triacylglycerol and total body lipid was as follows; 38.18%, 27.15%, 28.62%, 26.90%, 30.18%in *A. piscinalis* (Table 1) and 44.12%, 27.19%, 31.23%, 28.06%, 33.81%in *C. fluminalis* (Table 2), respectively. Hagar and Dietz [17] were found ΣPUFA level (35.27%–44.03%) higher than ΣMUFA (25.10%–35.69%) and ΣSFA levels (14.75%–23.01%) in the phospholipid of gill tissue from freshwater mussel *C. texasensis* (Table 4). In addition, the level of ΣPUFA in the phospholipid fraction of freshwater mussel *D. siouffi* was reported as 34.07%(Table 4) [19]. In marine bivalve, the percentage of ΣPUFA was also determined as high quantity (Table 4). For example, in the phospholipid of *O. edulis*, ΣPUFA level was ranged from 52.7%to 73.7%; however ΣSFA level was ranged from 16.2%to 27.2%(Table 4) [32]. In the present paper, the level of ΣPUFA in the phospholipid fraction was also higher than the other four lipid fractions and the level of ΣSFA and ΣMUFA in the monoacylglycerol, diacylglycerol, triacylglycerol and total body lipid were higher than the phospholipid (Table 1 and 2). As usual, phospholipid contains more

Table 3. C20:4ω6 and C20:5ω3 acid compositions of some marine bivalves.

Fatty acids	*Ostrea edulis*	*Mytilus platensis*	*Macoma balthica*	*Crassostrea gigas*	*Mytilus edulis*
C20:4ω6	3.11%–6.6%	1.8%–2.5%	3.0%	0.9%–4.3%	2.76%–2.85%
C20:5ω3	5.54%–14.2%	17.9%–20.7%	7.7%–19.9%	13.4%–27.8%	12.%–17.02%

Table 4. ΣSFA, ΣMUFA and ΣPUFA percentages of some marine and a freshwater (*D. siouffi*) bivalve

	Ostrea edulis	*Carunculina texasensis*	*Dreissena siouffi*	*Crassostrea gigas*	*Mytilus edulis*
ΣSFA	16.2%-27.2%	14.75%–23.01%	39.42%-48.11%	219%-35.9%	25.38%-23.64%
ΣMUFA	9%–17.5%	25.10%- 35.69%	21.43%–35%	13.8%-20.2%	14.53%-17.70%
ΣPUFA	52.7%-73.7%	35.27%- 44.03%	22.3%- 34.07%	45.1%-61.2%	60.80%-61.88%

of the long chain highly unsaturated fatty acids than neutral lipid (monoacylglycerol, diacylglycerol and triacylglycerol). Generally, cellular membranes have a higher composition of PUFA and lower composition of SFA. In aquatic organisms, cold environments lead to increasingly high cell membrane content of both monounsaturated and polyunsaturated fatty acids to maintain greater membrane fluidity at the lower temperatures. However, neutral lipids are considered as depot or reserve lipids since cellular membranes contain very small amounts. It is generally known that neutral lipids have relatively less PUFA than phospholipids. Probably, SFA and MUFA were accumulated in the monoacylglycerol, diacylglycerol, triacylglycerol and total body lipid by *A. piscinalis* and *C. fluminalis* to meet future energy requirements, and PUFA was accumulated in the phospholipid to maintain and form cellular membrane structures.

Conclusively, data in the study has demonstrated that *A. piscinalis* and *C. fluminalis* contained C16:0, C18:0, C16:1ω7, C18:1ω9, C18:2ω6, C20:4ω6 and C20:5ω3 as predominant fatty acids. The percentage of C20:4ω6 and C20:5ω3, precursors of eicosanoids playing important role in reproductive processes and regulating Na uptake from the gills, were high in the phospholipid fractions than monoacylglycerol, diacylglycerol, triacylglycerol and total body lipid. The highest value of ΣPUFA was found in the phospholipid, the highest value of ΣMUFA was found in the diacylglycerol, and the highest value of ΣSFA was registered in the monoacylglycerol both in *A. piscinalis* and *C. fluminalis*. As in freshwater mussels, the Σω6 ratio was always higher than Σω3. On the basis of these results, the mussels are good source for some important fatty acids mentioned above and the study may be significant guide for further biochemical investigation on freshwater mussels, especially chemosystematics study of the Mollusca. Although not eaten by native people, they have got important roles in food chain since they are consumed by fish, water birds, mammals and reptiles in the river. Even, in the future, they may be eaten as edible freshwater food after studying pathologically.

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