Seasonal and geographical variations in chemical composition and fatty acid profile of Mediterranean mussels

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Summary

This study investigates basic chemical composition and fatty acid profile of Mediterranean mussels (*Mytillus galloprovincialis*) cultivated in aquaculture facilities in six locations on the North Adriatic Istrian peninsula over an annual farming cycle. Mussels were sampled on a monthly basis. Mussels sampled during summer had a lower water content ($p = 0.022$), higher protein content ($p = 0.006$) and higher lipid content ($p < 0.001$), with the highest nutritional quality seen in samples from Limski Bay. Additionally, geographical location significantly influenced the fatty acid profile, in which dominated saturated fatty acids (SFA) followed by monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The shares of SFA, MUFA and PUFA were highest in Savudrija Bay, Budava and Limski Bay, respectively. As compared to SFA and MUFA, the amount of PUFA varied in an opposite fashion, being higher during warmer months ($p < 0.001$). The major PUFA was eicosapentaenoic acid, whose share was significantly higher as compared to docosahexaenoic acid during the entire annual farming cycle at all sampling locations ($p < 0.001$). Based on the basic chemical composition and fatty acid profile, an optimal location and timing for harvesting Mediterranean mussels is Limski Bay during summer.

Keywords

chemical composition; fatty acid profile; Mediterranean mussel; *Mytilus galloprovincialis*; geographical variation; seasonal variation

Mussels are a highly nutritious foodstuff, since they contain appreciable quantities of digestible proteins, essential amino acids, bioactive peptides, long-chain polyunsaturated fatty acids (PUFA), astaxanthin and other carotenoids, vitamin B12 and other vitamins, many minerals, and other nutrients, which offer a variety of health benefits to consumers [1]. Owing to the rise in health consciousness, modern society is interested in taking more seafood due to its nutritional superiority. Proteins are generally the most abundant component of the mussel meat, followed by carbohydrates [2–6]. Although the mussel lipid content is rather low, one of their most important benefits to human health is their fatty acid composition, namely the presence of omega-3 polyunsaturated fatty acids (n-3 PUFA), such as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3). Human body is incapable of synthesising them from their precursor α-linolenic

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fatty acid (18:3 n-3), so they have to be provided by food [6]. PUFA have been recognized as important in the prevention of cardiovascular diseases and alleviation of various inflammations [7]. Moreover, mussels are appreciated by consumers due to their sensory attributes and competitive price as compared to other bivalves [3].

Although the Mediterranean mussel (*Mytilus galloprovincialis)* is a species that spreads from south-west Great Britain, Atlantic French to Spanish, Portuguese and Morocco coasts, it is also an important commercial species reared on the Mediterranean coast, particularly that of the North Adriatic Sea [3, 6, 8]. In Croatia, it represents commercially the most important farmed shellfish species. In the year 2017, 982 tons of shellfish were produced in Croatia, the largest share of it (920 tons) being Mediterranean mussels [9]. Despite its high aquaculture potential and a long tradition of its shellfish industry, Croatian shellfish farming is still a small-scale and very traditional one, and is based exclusively on two shellfish species, i.e. Mediterranean mussels and European flat oyster (*Ostrea edulis*) [9].

Nutritional attributes of bivalves vary both seasonally and geographically. Identification of natural factors influencing the chemical composition and fatty acid profile of mussels has been a point of scientific interest during the last decade. Factors influencing mussel commercial quality and organoleptic characteristics are water temperature, food availability, plankton composition and gametogenesis cycle [2–6].

The objective of this study was to analyse chemical composition and fatty acid profile of Mediterranean mussels cultivated in six different locations on the Istrian peninsula (North Adriatic) at monthly intervals during one-year period. Based on the obtained results, an ideal time for mussel harvesting and further processing could be proposed.

Fig. 1. Sampling points seated along the Istrian peninsula coast.

MATERIALS AND METHODS

Sampling sites

Sampling sites, chosen because they represent the most important aquaculture facilities from the commercial standpoint (Fig. 1), are situated along the Istrian peninsula coast, as follows:

1. Savudrija Bay (N45.484488, E13.57366), situated in the northeast part of the Adriatic coast in the Piran Bay, the maritime border with Slovenia;
2. Čivran (N45.270227, E13.575056), situated on the western coast of the Istrian peninsula, 5 km south of the Mirna River mouth;
3. Limski Bay (N45.133442, E 13.721652) situated on the western coast of the Istrian peninsula, a narrow and long bay penetrating deep into the mainland;
4. Medulin Bay (N44.823094, E13.915248), an outspread bay in the southern part of the Istrian peninsula, southeast of Pula;
5. Budava (N44.897104, E13.986256) situated in the southeastern part of the Istrian peninsula;
6. Raša Bay (N45.011125, E14.055234), a 6.5 km long and 1–2 km wide bay situated in the eastern part of Istria.

At all of the above locations, temperatures vary from 6 °C to 8 °C in winter months up to 26 °C in summer. During the summer months (June to October), rainfalls are rare, while their amount rises in November up to May. The farming starts with the collection of seed from the environment using various types of collectors, which is then put into plastic meshes hung on rafts for approximately 12 to 15 months, depending on the farming, until the commercial size of over 60 mm is reached.

Preparation of samples

Mediterranean mussels were sampled in the locations detailed above each month throughout the year 2018. Samples of market-size mussels were collected randomly from various parts of the farm

and at various water column depths (from both deeper and shallower waters). In total, 3 kg of mussels were harvested. Samples were delivered to the laboratory in thermal insulation boxes cooled to 8 °C. Upon arrival, individual mussels were mechanically cleaned and opened in order to collect soft tissues. This was continued until 300 g of the latter tissues, required for the analysis, was obtained. The total retrieved amount was further homogenized using a Grindomix GM200 knife mill (Retch, Haan, Germany), so as to obtain a homogeneous sample for determination of basic chemical composition and fatty acid profile. An amount of 5 g of the sample was used for the water content analysis, while 2 g, 3 g and 1 g of the sample were used for the ash, lipid and protein content analysis, respectively. After determining their water content, the samples were stored at 4 °C pending determination of other chemical and fatty acid parameters within 48 h post sampling.

Basic chemical composition

The water content was established gravimetrically [10] at the sample-drying temperature of 103 °C using a Memmert UF75 Plus drier (Memmert, Schwabach, Germany). The total protein content was determined using the Kjeldahl technique [11]. A digestion unit (Unit 8 Basic; Foss, Höganas, Sweden) was used for degradation of the sample by sulfuric acid. Sample distillation via NaOH solution and water, and sample titration via boric acid solution were done in an automated device (Vapodest 50s, Gerhardt, Königswinter, Germany). The total content of lipids was determined gravimetrically using the Soxhlet technique [12] by virtue of hydrolysis by hydrochloric acid and ether-mediated lipid extraction (Soxtherm 416 automated extractor, Gerhardt). The total ash content was established via incineration in a muffle furnace (LV9/11/P320, Nabertherm, Lilienthal, Germany) at 550 °C [13]. The carbohydrate content was calculated by subtracting the sum of water, ash, protein and lipid contents from the total content. All chemicals used for the analyses were of analytical grade. Means obtained from duplicate analyses in the form of weight percentage were considered as typical of a target sample. Quality control of analytical methods was performed using the Certified Reference Material (CRM) T0149 (FAPAS, York, United Kingdom).

Fatty acid profile

Fatty acid methyl esters were prepared from the extracted lipids according to EN ISO 12966-2:2017 [14] and then analysed using gas chromatography (GC) according to EN ISO 12966-4:2015 [15]. For this purpose, a 7890BA gas chromatograph equipped with flame ionization detector (FID) and a 60 m DB-23 capillary column with an internal diameter of 0.25 mm and the stationary phase thickness of 0.25 μm (Agilent Technologies, Santa Clara, California, USA) was used. The components were detected by FID and identified as described by Pleadin et al. [16]. All samples were analysed in duplicate. The results were expressed as the mean value of the share of an individual fatty acid in total fatty acids. Fatty acid methyl ester values were converted to fatty acid values per 100 g of edible fish sample part [17]. Quality control was done using the certified reference material BCR 163 (Institute for Reference Materials and Measurements, Geel, Belgium) containing seven fatty acids in known quantities.

Statistical analysis

Statistical analysis was performed using SPSS Statistics Software 22.0 (IBM, New York, New York, USA) and Two-way ANOVA test with the statistical significance being set at $p < 0.05$.

RESULTS

Influence of seasonality and geographical variations on basic chemical composition

Proximate chemical composition of Mediterranean mussels harvested monthly over a year in various locations on the Istrian peninsula is shown in Tab. 1 and Tab. 2. Water content was found to fluctuate within a very narrow range from 845.62 ± 18.14 g·kg^{-1} (Limski Bay) to 863.04 ± 18.61 g·kg^{-1} (Savudrija Bay). ANOVA confirmed statistically significant differences among mean values of water content during the sampling period ($p < 0.001$, Tab. 3). The minimal water content was recorded in the summer period (May to August), while the highest values were recorded during winter (in January). From August on, water content continuously increased until January, thereafter it decreased again until May.

Although the highest values of proteins, which are the main quality component of Mediterranean mussels, were recorded in summer (September), after which the protein content continuously decreased during winter (from October to April), the relationship between seasonality and protein content was not statistically significant ($p = 0.305$, Tab. 3).

Regarding geographical variations, mussels with the lowest protein value were sampled in Savudrija Bay (87.63 ± 6.63 g·kg^{-1}), while the samples richest in protein originated from Limski

Bay (99.23 ± 7.51 g·kg^{-1}, Tab. 1). Nevertheless, the influence of geographical location on the protein content of our mussel samples was stronger as the two-way ANOVA showed statistically significant differences between mussels obtained from different sampling sites, with the highest share of protein in those obtained from the Limski Bay (p = 0.006, Tab. 1, Tab. 3).

The carbohydrate content was found to be the highest in summer (June to August) and then decreased (p < 0.001, Tab. 2, Tab. 3). The carbohydrate content range seen across the sampling locations was rather narrow (from 22.7 ± 12.76 g·kg^{-1} for Savudrija Bay to 27.91 ± 15.63 g·kg^{-1} for Budava, Tab. 2), the influence of geographic location being proven to be statistically insignificant (p = 0.059, Tab. 3).

The amount of ash was determined with the aim to obtain information on inorganic substance content of the mussel meat (Tab. 1). Although it was fair to assume that seasonality would affect the ash content due to the depletion of biochemical reserves during the peak spawning period, the results showed that the differences were insignificant either across the sampling locations or across the seasons (p = 0.370 for location and p = 0.063 for season, Tab. 3). The mean ash content ranged from 17.41 ± 2.86 g·kg^{-1} (Limski Bay) to 19.62 ± 3.81 g·kg^{-1} (Čivran, Tab. 1), fluctuating from 15.33 ± 1.44 g·kg^{-1} (in May) to 20.61 ± 1.92 g·kg^{-1} (in February, Tab. 2).

Lipid content in mussels spanned from the minimum of 6.00 ± 0.62 g·kg^{-1} (recorded in January) to the maximum of 13.03 ± 2.52 g·kg^{-1} recorded in summer (July, p < 0.001, Tab. 2, Tab. 3). After the summer period, the content of lipids continuously decreased from autumn to winter, until March when it started to increase again. As for geographical variations, a significant influence of harvesting location was shown, with the highest total lipid content recorded in mussels recovered from Limski Bay (p < 0.001, Tab. 1, Tab. 3).

Tab. 1. Chemical indicators descriptive of Mediterranean mussels retrieved from Istrian peninsula.

Location	Water	Ash	Lipids	Proteins	Carbohydrates
	[g·kg^{-1}]				
Raša Bay	858.52 ± 22.81	18.62 ± 1.94	8.84 ± 3.00	87.91 ± 9.61	26.24 ± 12.83
Medulin Bay	855.45 ± 25.52	18.41 ± 4.14	9.64 ± 3.02	89.21 ± 6.84	27.45 ± 15.74
Savudrija Bay	863.04 ± 18.61	18.42 ± 2.04	8.31 ± 2.33	87.63 ± 6.63	22.71 ± 12.76
Limski Bay	845.62 ± 18.14	17.41 ± 2.86	12.14 ± 3.15	99.23 ± 7.51	25.74 ± 9.82
Budava	853.82 ± 20.53	17.44 ± 1.85	10.31 ± 2.90	90.63 ± 5.34	27.91 ± 15.63
Čivran	851.02 ± 15.00	19.62 ± 3.81	9.94 ± 2.82	93.71 ± 11.63	25.84 ± 10.61
All locations	854.62 ± 6.01	18.35 ± 0.82	9.80 ± 1.34	91.43 ± 4.44	26.00 ± 1.82

Values represent mean ± standard deviation (n = 12).

Tab. 2. Seasonal changes in chemical indicators descriptive of Mediterranean mussels reared on the Istrian peninsula during the study period.

	Water	Ash	Lipids	Proteins	Carbohydrates
	[g·kg^{-1}]				
January	882.30 ± 10.11	19.01 ± 3.80	6.00 ± 0.62	90.91 ± 11.32	1.71 ± 1.62
February	865.21 ± 11.12	20.61 ± 1.92	7.51 ± 2.33	87.34 ± 6.32	19.43 ± 6.51
March	867.52 ± 15.14	18.33 ± 1.33	11.50 ± 2.61	88.72 ± 8.61	14.04 ± 3.82
April	865.03 ± 5.70	18.32 ± 1.10	10.32 ± 1.51	86.53 ± 5.82	19.90 ± 3.42
May	847.34 ± 16.90	15.33 ± 1.44	10.72 ± 3.63	90.81 ± 14.23	35.91 ± 4.50
June	838.74 ± 12.31	17.91 ± 5.80	10.84 ± 1.91	88.02 ± 4.53	44.61 ± 7.82
July	833.00 ± 10.83	17.82 ± 1.33	13.03 ± 2.52	94.72 ± 5.62	41.53 ± 7.74
August	831.31 ± 7.522	17.20 ± 1.51	12.72 ± 0.83	96.31 ± 1.94	42.51 ± 7.02
September	834.52 ± 11.01	20.52 ± 4.23	11.71 ± 2.92	97.90 ± 8.42	35.42 ± 9.51
October	856.34 ± 23.33	17.82 ± 1.77	7.81 ± 2.24	91.12 ± 13.13	27.02 ± 6.41
November	866.02 ± 9.12	17.91 ± 2.03	7.82 ± 2.22	93.71 ± 6.65	14.62 ± 3.53
December	867.51 ± 16.10	18.92 ± 2.44	8.00 ± 1.51	90.91 ± 11.32	14.71 ± 4.64
Whole period	854.60 ± 17.02	18.31 ± 1.40	9.84 ± 2.30	91.43 ± 3.61	25.91 ± 13.91

Values represent mean ± standard deviation (n = 24).

Influence of seasonality and geographical variations on fatty acid profile

In total, 15 fatty acids were identified and their seasonal variations were recorded over a year at various locations (Tab. 4, Tab. 5). Data on fatty acid profile, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) showed significant seasonal changes with a persistent and significant predominance of SFA, followed by MUFA and PUFA. As regards sampling locations and specific fatty acids, based on our results, Savudrija Bay offered mussels with the highest share of SFA (76.8 ± 10.2 %), while Budava mussels had the highest share of MUFA (25.4 ± 9.6 %). Mussels with the highest share of PUFA were sampled in Limski Bay (13.5 ± 10.6 %, Tab. 4).

The share of C18:0 was significantly higher in Savudrija Bay ($p < 0.001$) compared to other locations. However, the highest values for the share of other SFA, namely, C14:0 (Limski Bay, $p = 0.052$) and C17:0 (Čivran, $p = 0.078$) did not differ significantly in comparison with the shares of these fatty acids obtained for other locations (Tab. 3).

As for fluctuation of all fatty acids over a year, it is notable that seasonality significantly affected the fatty acid share. The lowest share of SFA was recorded in January and the highest in December ($p < 0.001$), as opposed by MUFA, whose share was the lowest in December and the highest in January ($p = 0.004$) (Tab. 3). The predominant SFA were palmitic acid (C16:0), whose share ranged from 27.7 ± 12.8 % (January) to 49.4 ± 2.6 % (February, $p < 0.001$), and stearic acid (C18:0), also with the highest share in winter (32.7 ± 5.1 %, December, $p < 0.001$, Tab. 3, Tab. 5). The most abundant MUFA was oleic acid (C18:1 n-9c), whose share, similarly to that of SFA, was highest in the winter period (26.2 ± 16.6 % in January, $p < 0.001$).

Mussels with the significantly highest share of PUFA were sampled in July ($p < 0.001$). Of note, in December PUFA fell below the limit of detection. The contents of total PUFA found in our samples were not statistically different regarding study locations ($p = 0.116$, Tab. 3), but they were lower in comparison with the results of other authors, who reported a significant prevalence of PUFA over MUFA and SFA. The seasonality significantly affected PUFA content ($p < 0.001$), Tab. 5, Tab. 3). The prevailing PUFA were EPA (C20:5 n-3) and DHA (C22:6 n-3), found in the highest quantities in the summer period ($p < 0.001$, Tab. 3). In July, the shares of EPA and DHA in total fatty acids were the highest (3.7 % and 10.3 %, respectively). The mean values of n-6/n-3 ratio of Mediterranean mussels varied from 0.2 ± 0.1 (Čivran, Tab. 4) to 1.2 ± 1.1 (January, Tab. 5).

The ratio of PUFA over SFA established in this study at various sampling locations ranged from value of 0.2 (Savudrija Bay, Raša Bay, Medulin Bay and Čivran) to 0.3 (Budava and Limski Bay), with insignificant differences ($p = 0.514$, Tab. 3). However, across seasons this ratio varied significantly ($p < 0.001$) from undetectable in December to the highest in January (Tab. 3).

Tab. 3. Significance of factors affecting the biochemical composition and fatty acid profile of Mediterranean mussels harvested on the Istrian peninsula.

Parameter	Statistical significance	
	Location	Seasonality
Water	0.022*	<0.001*
Ash	0.370	0.063
Lipids	<0.001*	<0.001*
Proteins	0.006*	0.305
Carbohydrates	0.059	<0.001*
Fatty acid profile		
C8:0	0.479	0.001*
C14:0	0.052	<0.001*
C15:0	0.018*	<0.001*
C16:0	0.313	<0.001*
C17:0	0.078	<0.001*
C18:0	0.001*	<0.001*
C16:1 n-7c	0.094	0.260
C18:1 n-7	0.005*	0.002*
C18:1 n-9c	0.031*	<0.001*
C18:2 n-6c	0.049*	<0.001*
C18:3 n-3	0.296	<0.001*
C18:4 n-3	0.004*	<0.001*
C20:1 n-9	0.059	0.043*
C20:5 n-3 (EPA)	0.059	<0.001*
C22:6 n-3 (DHA)	0.619	<0.001*
SFA	0.010*	<0.001*
MUFA	0.002*	0.004*
PUFA	0.116	<0.001*
n-6	0.041*	<0.001*
n-3	0.196	<0.001*
n-6/n-3	0.295	<0.001*
PUFA/SFA	0.514	<0.001*

Statistical significance set at $p < 0.05$ (* – statistically significant values).
EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, n-6 – omega-6 PUFA, n-3 – omega-3 PUFA.

Tab. 4. Fatty acid composition of Mediterranean mussels harvested on the Istrian peninsula.

Fatty acid [%]	Raša Bay	Medulin Bay	Savudrija Bay	Limski Bay	Budava	Čivran
C8:0	< *LOD*	0.1 ± 0.4	0.2 ± 0.6	0.1 ± 0.3	0.1 ± 0.3	< *LOD*
C14:0	3.9 ± 2.0	4.3 ± 1.5	4.7 ± 2.9	5.6 ± 1.8	4.3 ± 1.9	5.3 ± 1.4
C15:0	0.2 ± 0.5	0.5 ± 0.7	0.2 ± 0.5	0.7 ± 0.6	0.8 ± 0.7	0.7 ± 0.8
C16:0	42.2 ± 10.1	42.0 ± 5.9	43.8 ± 5.3	40.8 ± 6.9	39.2 ± 9.2	41.9 ± 6.2
C17:0	1.1 ± 1.7	1.1 ± 1.3	1.8 ± 2.6	1.7 ± 1.7	1.7 ± 1.7	2.2 ± 2.0
C18:0	23.5 ± 10.0	19.6 ± 6.9	26.2 ± 9.0	18.0 ± 7.2	16.4 ± 8.6	21.1 ± 8.9
C16:1 n-7c	4.5 ± 2.7	5.0 ± 1.4	3.8 ± 2.1	5.9 ± 2.6	6.2 ± 3.2	5.6 ± 1.3
C18:1 n-7	1.0 ± 1.3	1.7 ± 1.5	0.9 ± 1.3	1.5 ± 1.4	2.7 ± 1.5	1.9 ± 1.3
C18:1 n-9c	10.9 ± 10.9	10.9 ± 5.1	8.5 ± 2.1	8.4 ± 4.3	13.6 ± 11.5	6.8 ± 1.6
C18:2 n-6c	3.7 ± 3.9	4.3 ± 4.1	2.2 ± 2.1	4.2 ± 5.2	4.8 ± 4.1	2.7 ± 1.9
C18:3 n-3	1.3 ± 2.2	0.8 ± 1.4	1.0 ± 2.5	1.7 ± 1.9	1.9 ± 2.1	1.4 ± 2.6
C18:4 n-3	0.4 ± 0.8	0.7 ± 1.4	< *LOD*	1.4 ± 1.7	0.3 ± 0.9	1.1 ± 1.5
C20:1 n-9	1.9 ± 1.9	3.9 ± 1.1	2.6 ± 2.1	3.0 ± 1.3	2.9 ± 1.8	2.5 ± 1.4
C20:5 n-3 (EPA)	1.2 ± 1.5	1.0 ± 1.6	0.5 ± 1.3	1.7 ± 1.5	1.6 ± 1.4	2.0 ± 2.0
C22:6 n-3 (DHA)	3.9 ± 4.9	3.0 ± 4.0	3.2 ± 4.3	4.4 ± 4.1	2.8 ± 3.2	4.1 ± 4.6
SFA	70.9 ± 17.9	67.7 ± 10.6	76.8 ± 10.2	67.0 ± 11.9	62.5 ± 15.5	71.2 ± 11.6
MUFA	18.3 ± 11.1	21.5 ± 3.7	15.7 ± 3.9	18.8 ± 4.3	25.4 ± 9.6	16.9 ± 2.9
PUFA	10.4 ± 9.3	10.1 ± 7.9	6.9 ± 7.5	13.5 ± 10.6	11.4 ± 7.7	11.2 ± 10.0
n-6	3.7 ± 3.9	4.3 ± 4.0	2.2 ± 2.1	4.3 ± 5.2	4.8 ± 4.1	2.7 ± 1.9
n-3	6.7 ± 7.4	5.8 ± 7.3	4.7 ± 5.8	9.2 ± 8.2	6.6 ± 5.3	8.5 ± 9.1
n-6/n-3	0.4 ± 0.4	0.3 ± 0.2	0.3 ± 0.3	0.4 ± 0.7	0.5 ± 0.6	0.2 ± 0.1
PUFA/SFA	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.3	0.2 ± 0.2

Values given as mean ± standard deviation represent the percentage of total fatty acids (n = 12).
EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, *LOD* – limit of detection (*LOD* = 0.05 %).

DISCUSSION

Influence of seasonality and geographical location on basic chemical composition

Within the frame of this study, it was revealed that mussels sampled in the summer were richer in proteins, carbohydrates and lipids as compared to those reared during the rest of the year. Across the six study locations, Limski Bay was identified as the location in which the highest nutritional quality mussels were reared. Basic chemical composition of Mediterranean mussels fluctuates as a result of synergistic interaction between the quality and quantity of mussel diet, geographical location and reproductive cycle [2].

Regarding the water content, the higher values obtained during winter and the lower ones obtained during summer were related to the mussel reproductive cycle. These results are similar to the results of other authors, who also confirmed minimum water contents during spring and summer [4, 6]. In our previous work, we also showed that the inverse relationship between mussel water and lipid content is much stronger than that between water and protein or ash content [5].

The determined protein contents and their seasonal variation, with the highest value established in spring and summer, are similar to those descriptive of Mediterranean mussels inhabiting other locations in the Mediterranean Sea [4, 6, 18, 19]. Protein content is mostly influenced by the mussel reproductive cycle, since proteins are major components of bivalve eggs and are utilized as energy source during egg development [20]. Due to their important role in gonadal maturation, low protein content can be expected after the spawning season.

Carbohydrates have two major biological functions, namely, they pose as long-term energy storage and they are structural elements [21]. Under unfavourable environmental conditions (low temperature, lack of food or oxygen), they represent the basic energy stock of shellfish for reeling gametogenesis and can be considered

Tab. 5. Fatty acid composition of Mediterranean mussels reared on the Istrian peninsula during the study period.

Fatty acids [%]	January	February	March	April	May	June	July	August	September	October	November	December
C8:0	0.6 ± 0.7	< *LOD*	0.3 ± 0.4	0.3 ± 0.6	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
C14:0	1.8 ± 0.7	2.9 ± 2.7	4.5 ± 2.2	5.5 ± 1.7	5.7 ± 0.7	6.9 ± 0.8	4.8 ± 0.7	5.1 ± 1.2	3.8 ± 0.6	6.2 ± 3.0	4.2 ± 1.3	4.6 ± 1.4
C15:0	< *LOD*	< *LOD*	0.3 ± 0.	0.5 ± 0.5	0.6 ± 0.7	1.1 ± 0.9	1.3 ± 0.1	1.2 ± 0.6	0.6 ± 0.7	0.6 ± 0.9	0.2 ± 0.5	< *LOD*
C16:0	27.7 ± 12.8	49.4 ± 2.6	34.2 ± 2.6	40.7 ± 2.2	39.8 ± 5.5	47.0 ± 4.3	37.8 ± 3.2	43.6 ± 2.6	44.4 ± 3.1	43.1 ± 2.1	45.6 ± 3.4	46.2 ± 1.5
C17:0	< *LOD*	< *LOD*	4.9 ± 2.5	0.8 ± 0.9	1.6 ± 1.3	1.7 ± 1.9	2.7 ± 0.3	3.3 ± 0.4	3.1 ± 0.3	< *LOD*	0.7 ± 1.1	0.4 ± 0.9
C18:0	20.1 ± 11.0	27.5 ± 7.5	17.4 ± 3.7	20.8 ± 5.3	13.0 ± 4.8	16.6 ± 6.6	11.2 ± 2.8	14.3 ± 3.2	21.0 ± 5.2	24.5 ± 6.7	30.6 ± 9.0	32.7 ± 5.1
C16:1 n-7c	3.0 ± 0.8	4.6 ± 4.0	4.8 ± 0.8	5.4 ± 1.9	5.1 ± 1.5	5.9 ± 1.1	5.7 ± 1.1	7.1 ± 2.9	4.1 ± 0.5	6.2 ± 2.9	5.5 ± 4.5	4.9 ± 1.6
C18:1 n-7	< *LOD*	1.5 ± 2.3	1.7 ± 1.5	1.7 ± 1.4	1.2 ± 1.3	1.3 ± 1.4	2.4 ± 0.2	2.6 ± 1.3	2.6 ± 0.2	2.6 ± 1.4	1.3 ± 1.4	0.4 ± 1.1
C18:1 n-9c	26.2 ± 16.6	8.3 ± 1.8	8.0 ± 3.5	10.1 ± 6.0	9.9 ± 3.2	11.0 ± 2.3	8.3 ± 1.7	6.6 ± 2.1	6.9 ± 2.1	8.2 ± 1.2	6.7 ± 1.8	7.9 ± 0.9
C18:2 n-6c	12.6 ± 6.6	2.1 ± 2.5	3.2 ± 1.0	4.0 ± 1.3	4.9 ± 1.0	3.1 ± 2.4	4.3 ± 0.3	3.3 ± 0.6	3.2 ± 1.0	2.3 ± 1.2	0.8 ± 1.4	< *LOD*
C18:3 n-3	1.7 ± 2.7	0.6 ± 1.4	6.7 ± 1.8	1.9 ± 1.3	1.6 ± 1.8	0.4 ± 1.0	1.7 ± 0.2	0.7 ± 0.8	0.5 ± 0.7	0.4 ± 0.6	< *LOD*	< *LOD*
C18:4 n-3	< *LOD*	< *LOD*	2.9 ± 1.6	1.5 ± 1.4	1.3 ± 2.1	0.4 ± 0.9	1.2 ± 0.9	< *LOD*	0.2 ± 0.5	0.2 ± 0.5	< *LOD*	< *LOD*
C20:1 n-9	1.6 ± 0.9	1.9 ± 2.9	1.91 ± 0.2	2.1 ± 1.1	3.5 ± 1.9	2.9 ± 2.3	3.6 ± 0.3	3.9 ± 0.5	4.3 ± 0.5	3.2 ± 1.9	2.5 ± 2.0	2.3 ± 1.8
C20:5 n-3 (EPA)	1.3 ± 1.4	< *LOD*	1.8 ± 1.4	1.2 ± 1.0	2.3 ± 2.6	0.5 ± 1.2	3.7 ± 0.5	2.5 ± 0.5	1.2 ± 1.3	1.0 ± 1.6	0.9 ± 1.6	< *LOD*
C22:6 n-3 (DHA)	2.2 ± 2.3	0.8 ± 1.9	3.7 ± 2.7	3.1 ± 2.9	9.5 ± 5.2	1.3 ± 3.1	10.3 ± 2.4	5.7 ± 1.9	3.8 ± 3.0	1.1 ± 1.7	1.0 ± 2.5	< *LOD*
SFA	51.4 ± 20.7	80.4 ± 7.9	61.9 ± 6.1	68.7 ± 7.2	60.7 ± 8.3	73.3 ± 8.5	58.1 ± 4.7	67.5 ± 3.9	73.1 ± 7.2	74.7 ± 7.0	81.3 ± 10.3	84.4 ± 3.0
MUFA	30.8 ± 16.7	16.1 ± 4.9	16.3 ± 5.3	19.3 ± 7.0	19.6 ± 3.5	21.1 ± 2.5	20.0 ± 1.6	20.3 ± 5.0	18.0 ± 2.5	20.3 ± 3.7	16.0 ± 8.3	15.6 ± 3.0
PUFA	17.9 ± 9.5	3.5 ± 3.9	21.7 ± 4.9	12.0 ± 5.2	19.6 ± 9.7	5.6 ± 7.4	21.9 ± 4.2	12.3 ± 3.2	9.0 ± 5.4	4.9 ± 4.4	2.5 ± 3.8	< *LOD*
n-6	12.6 ± 6.6	2.1 ± 2.5	6.5 ± 2.0	4.0 ± 1.3	5.1 ± 1.0	3.1 ± 2.4	4.9 ± 1.4	3.3 ± 0.6	3.3 ± 1.1	2.3 ± 1.2	0.8 ± 1.4	< *LOD*
n-3	5.3 ± 4.3	1.3 ± 2.1	15.3 ± 4.3	8.1 ± 5.3	14.6 ± 10.1	2.5 ± 6.2	17.0 ± 3.1	8.9 ± 2.7	5.7 ± 4.7	2.7 ± 4.2	1.9 ± 3.9	< *LOD*
n-6/n-3	1.2 ± 1.1	0.3 ± 0.5	0.5 ± 0.2	0.8 ± 0.7	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.2	0.1 ± 0.2	0.1 ± 0.4	N/A
PUFA/SFA	0.5 ± 0.5	0.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	N/A

Values given as mean ± standard deviation represent the percentage of total fatty acids (n = 24).
EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, *LOD* – limit of detection (*LOD* = 0.05%). N/A – not applicable.

as a bioindicator of the availability of food in a given environment. During the development of gonads, carbohydrates are converted to lipids, so the highest carbohydrate content can be found at commencement of gametogenesis, while the lipid content at that time is minimal [22].

The obtained ash content values were slightly lower as compared to those determined in Mediterranean mussels retrieved from the Gulf of Trieste (22–33 $g \cdot kg^{-1}$) [6], but the pattern of seasonal ash content fluctuation was similar to that reported in other works [2, 6].

In many temperate bivalves, lipid content steadily increases during the summer months until spawning occurs [2, 18]. The trend of lipid fluctuation observed in our study, with the highest values in summer and the lowest values in winter, was also previously recorded in Mediterranean mussels reared in the Mediterranean area [4, 6]. The studies reported lipid values higher than those established in our study (10–26 $g \cdot kg^{-1}$, on average). Only in a single study, conducted also in the Adriatic Sea region, an opposite seasonal trend in total lipid content was recorded [23]. In general, the lipid content increase is linked to the mussel reproductive cycle, contributing to both neutral reserves in gonads and structural lipids, whereas the lipid content decrease is related to spawning. The reproductive cycle starts with gametogenesis taking place during August when water temperatures are 25–26 °C. The production of gametocytes is triggered by the temperature drop down to 20 °C and, by beginning of October, one can find individual mussels in the spawning stage. Spawning continues during winter till late spring and is most vivid during February and March [24].

As for the influence of geographical location on the mussel basic chemical composition, it can be seen that the highest nutritional quality have mussels from Limski Bay. The amount of organic substance, temperature, light and salinity are affecting the primary production in the marine environment providing the abundance of plankton and, consequently, optimal food source for mussels [25]. Except for Limski Bay, all sampling locations were oligotrophic. Limski Bay, on the other hand, is a long, narrow aquatorium in which Istrian underground waters flow all year long. Therefore, the bay is mesotrophic during most of the year. However, in the summer period, environmental temperatures rise, farm activities (feeding) intensifies leading to the rise in nutrients, and the currents cease, allowing for nutrients to remain in place. All of the aforementioned, together with higher insolation, a lot of light as a source of energy required for photosynthesis, contributes to the increase in trophic index and the consequent improvement of mussel meat nutritional composition.

Influence of seasonality and geographical location on fatty acid profile

This study confirmed a strong influence of seasonality and geographical location on fatty acid profile of mussels reared in Istrian peninsula. Fatty acid profiles revealed the predominance of SFA over MUFA and PUFA. Mussels with the highest share of SFA originated from Savudrija Bay, while those with the highest shares of MUFA and PUFA came from Budava and Limski Bay, respectively.

Several patterns of temporal variability of fatty acid profile in bivalve molluscs, reported in previous studies, come as a result of several simultaneously acting geographical and environmental factors, such as food availability, plankton composition, and physiological factors [2, 18]. Studies of seasonal changes in fatty acid composition of shellfish [2, 3, 22] showed that accumulation of lipid reserves mainly depends on the impact of environmental factors on metabolic activity. Considerable contribution of SFA to the mussel fatty acid content, seen at all harvesting locations during all seasons, and the lower representation of PUFA in the latter content, are in line with data published by Prato et al. [18]. However, these results are also in contrast to the results of other investigations carried out on Mediterranean mussels, which claimed these mussels to be a valuable source of PUFA and pointed towards phytoplankton as their main feed [6, 8].

Palmitic acid has been reported by numerous studies as the major fatty acid present in mussels [3, 18, 23]. Prato et al. [18] explained that the predominance of SFA, namely C16:0 and C14:0, indicates omnivorous feeding habits. Fatty acids also become saturated when organic matter is oxidized in the water column, especially in environments with low nutrient availability, high levels of detritus and limited phytoplankton growth [26]. Moreover, in our recent paper in which we analysed fatty acid profile of three different mussels - European flat oyster (*Ostrea edulis*), variegated scallop (*Chlamys varia*) and smooth scallop (*Flexopecten glaber*) - reared in the Adriatic Sea, the predominance of SFA was determined in all three, followed by MUFA and PUFA [5].

Higher PUFA values during the summer period were previously reported by other authors [6, 27]. The amount of PUFA is closely related to the availability of phytoplankton as the major source of linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids, as well as C20:5 n-3 and C22:6 n-3. While

reduced sunlight and water surface cooling reduce the production of phytoplankton during autumn and winter, the opposite effect in spring and summer results in micro-algal blooms [28]. The fact that locations investigated in this study might generally be phytoplankton-poor, together with limited abilities of Mediterranean mussels to accumulate PUFA [27], resulted in lower level of PUFA in comparison with other studies. In the springtime, PUFA are not as abundant in mussel tissues as in summer, which is probably due to post-spawning effects, since female mussels are depleted from PUFA by generating egg biomass.

The relative proportion and seasonal trends of two major PUFA, EPA and DHA, established in this study were similar to previously reported data [4]. During the entire year and irrespective of sampling location, the share of DHA was significantly higher than the share of EPA. The determined predominant shares of DHA and EPA can be explained by the presence of phytoplankton in these production areas. The presence of diatom population in seawater is generally linked to high EPA levels in mussels, while the increase in DHA coincides with the proliferation of dinoflagellate species [2, 27]. When discussing fatty acid profile variations, it should also be taken into account that fatty acid profile of bivalves can be affected by hydrographical parameters [29] and availability of food sources, as well [27].

Two sampling locations, Limski Bay and Budava, are somewhat specific, since they represent integrated multi-trophic aquaculture sites where marine aquaculture is integrated with lower trophic level organisms during farming. The depth of Limski Bay open sea border is up to 33 m, while the inner side bordering the mainland is shallower. The inner side is strongly influenced by underground waters rich in organic matters that increase biological productivity. So the shellfish farms are situated in this part of the bay, while its open sea border hosts a marine fish farm. The salinity varies depending on the inflow of fresh underground waters, while the water temperature ranges from 9 °C to 25 °C. Budava is a 2.5 km long and 400–600 m wide bay having the water depth of 2 m close to the shore and up to 40 meters in its central part. There are several freshwater wells in the central part of the bay and, similarly to Limski Bay, a shellfish farm is situated in its inner part, while a marine fish farm occupies its outer part. On these two locations, Mediterranean mussels are cultivated in close vicinity with fish (sea bass and sea bream). Therefore, it is fair to assume that organic matter from fish farm deposition caused changes in chemical environment and, consequently, also in fatty acid profile. When we compared the share of certain fatty acids between Limski Bay and Budava (for the sake of that comparison considered as a single location) and other study locations, it turned out that at Limski Bay/Budava a significantly lower share of SFA ($p = 0.047$), especially that of C18:0 ($p = 0.011$), was found as compared to other locations. Additionally, the shares of MUFA found in these two locations were higher as compared to other sampling sites ($p = 0.038$, data not shown). Both et al. [30] also demonstrated significant changes in fatty acid composition of *Mytilus edulis* feed effluent from Atlantic cod. They recorded an increase in the proportion of 18:1 n-9, 18:2 n-6, 20:4 n-6, 21:5 n-3 and a significant decrease in the proportions of 16:0, 18:4 n-3 and 20:5 n-3.

In Savudrija Bay, water temperature is influenced by the Dragonja River and ranges from 9 °C to 25 °C. The maximal depth is 19 m, which makes these shallow waters quite productive from the aquaculture standpoint. In Čivran location, there is no direct inflow of freshwater on site, the depth of which is 4 m to 15 m, but it is free from any wastewater influx whatsoever. Medulin Bay consists of two sections, the southeaster outer open sea section and the smaller northwestern section, connected by a 400 m wide channel. In the middle of the outer section, the water depth is up to 21 m, while towards the open sea it gets shallower. At the entrance into Raša Bay, the water depth is roughly 46 m, while closer to the dry land it falls down to 5 m. The Bay is characterized by very sharp slants with poor vegetation. On its bottom, the Raša River mouth rich in nutrients is located.

Knowledge on bivalve food sources is important in aquaculture areas where cultured populations are usually denser than natural. Except for phytoplankton, which is considered to be their primary food source, bivalves can utilize other food sources such as detritus, bacteria and zooplankton [27]. Traditionally, bivalves are considered to be herbivores but several studies showed that they can use other food sources, such as detritus, bacteria, micro-zooplankton and meso-zooplankton [31]. Ezgeta-Balić et al. [27] showed that during the periods of plankton abundance, i.e. in summer and spring, bivalves mainly ingest phytoplankton, followed by zooplankton and detritus. During the periods of low plankton presence, i.e. in autumn and winter, zooplankton and detritus become more important in the bivalve diet. However, their research confirmed that, as opposed to other bivalves, Mediterranean mussels do not accumulate significant amounts of PUFA during the

spring and summer periods, which could explain the unexpectedly low shares of PUFA found in our samples.

Our results suggest that mussels utilized various food sources over the study course and that relative contribution of individual dietary components varied over the year. However, since fatty acid markers for dinoflagellates and zooplankton are common when it comes to mussels (16:1/16:0 < 1, DHA/EPA > 1) [32], it can be assumed that dinoflagellates and zooplankton were the main feeding sources of our samples, which could also explain the difference in fatty acid profile in comparison with other studies. The share of n-3 was higher in comparison with that of n-6. Phytoplankton availability and composition could be the main reason for seasonal and geographical differences in n-3/n-6 ratios. Of note, n-6/n-3 ratios in range of 0.2 to 1.5 are recommended in human nutrition, while values higher than 1.5 can result in a higher risk of cardiovascular diseases [1, 7]. The values of the ratio of PUFA over SFA should not be considered favourable, since, according to recommendations, the PUFA/SFA ratio should be higher than 0.4, so as to reduce the risk of cardiovascular, autoimmune and other chronic diseases [7, 33].

CONCLUSIONS

The population of Mediterranean mussels reared on the Istrian peninsula showed significant variations in basic chemical composition throughout a year, as well as at different sampling locations. Higher protein, lipid and carbohydrate contents and lower levels of water and ash were recorded in the summer period, while lower nutritional values were recorded in the winter period. As for the fatty acid profile, the predominance of SFA over MUFA and PUFA was established. The predominant PUFA was eicosapentaenoic acid, whose share was significantly higher as compared to that of docosahexaenoic acid during the entire annual farming cycle at all sampling locations. The shares of SFA, MUFA and PUFA were the highest in Savudrija Bay, Budava and Limski Bay, respectively. Based on the obtained maximum values of total lipids, PUFA, carbohydrates and proteins, it can be concluded that the highest-quality Mediterranean mussels are those reared in summer (July/August) in Limski Bay. Although in this study composition-based markers of Mediterranean mussels inhabiting the Istrian peninsula were identified for the first time ever, future research is needed to complete an in-depth characterization of habitats and food sources of Mediterranean mussels populating the Istrian peninsula so as to amend labelling with the Appellation of Origin.

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