Seasonal variation in the nutrient composition of mussels (*Mytilus galloprovincialis*) from farms in Boka Kotorska Bay, Southern Adriatic Sea

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ARTICLE INFO

Article History
Received : 14.05.2021
Accepted : 07.06.2022
DOI: 10.33988/auvfd.920577

Keywords
Adriatic Sea
Mytilus galloprovincialis
Nutrient Composition

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How to cite this article: Grkovic N, Bogdanovic IZ, Djuric S, Karabasil N, Suvajdzic B, Čobanović N, Djordjevic V, Vasilev D, Dimitrijevic M (2023): Seasonal variation in the nutrient composition of mussels (*Mytilus galloprovincialis*) from farms in Boka Kotorska Bay, Southern Adriatic Sea. Ankara Univ Vet Fak Derg, 70 (3), 319-326. DOI: 10.33988/auvfd. 920577.

ABSTRACT

The aim of this study was to investigated the seasonal variation in the nutrient composition of Mytilus galloprovincialis, cultivated in Boka Kotorska Bay, Montenegro, during all seasons in one year. Biometric parameters, meat yield, condition index, proximate composition, minerals, and the lipid and fatty acid compositions of mussels were analyzed. The most significant factors determining these parameters were temperature, food amount and gametogenesis The biometric parameters showed no significant differences during the sampling period. In the spring, meat yields and mussel condition index increased at substantial levels. Low values od meat yield and condition index during cold months have resulted from food shortage and reproductive cycle, when mussels use carbohydrates and protein reserves. The highest amount of protein was detected in mussels harvested in August (10.76%), while the highest amount of lipids was recorded in the winter months (2.11%). Docosahexaenoic acid and eicosapentaenoic acid were the most abundant PUFA. The concentration of metals found in mussels from the study area is within the range of mean values reported in the literature. Our results indicate that the best period for mussels harvesting was during the spring and summer (April and August), opposite the winter months when the mussels were not favorable for harvesting.

Introduction

Mussels are a highly nutritious foodstuff, rich sources of digestible proteins, vitamins, and minerals. The mussel lipid contents are rather low, the polyunsaturated fatty acids (n-3 PUFAs), eicosapentaenoic acid (EPA, C20:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3) composition are crucial. Mussels feed on the phytoplankton from which they acquire proteins, carbohydrates, lipids and other components for their biomass (10). Also, different types of metals were detected in their tissue, such as calcium, zinc, copper, iron, iodine, manganese, however they can be contaminated with heavy metals which are dangerous to human health (18).

Factors influencing mussel biochemical composition, meat content and condition index are water temperature, plankton composition and gametogenesis cycle, which change during the season (12). Phytoplankton growth and microalgal blooms rates increase with increasing water temperature in spring and summer and reduce during the cold months (autumn and winter) (19). In addition, the abundance of food resources (e.g., diatoms, dinoflagellates) leads to an increase in mussels biomass (15).

The contribution of the aquaculture in the Montenegrin national economy is insignificant, but there is significant potential for its development because suitable oceanographic, physical, chemical and biological conditions in the Boka Kotorska Bay (8). The natural environment with sheltered bays and estuaries give this area an ideal geoformation.

The objective of this study was to analyze the biometric and biochemical characteristics of mussels during one year. Based on the obtained results, we can propose an ideal time for mussel harvesting. On this location, the study for the first time considers such a large number of analyzes, therefore it constitutes encourages a model for local entrepreneurs and could be helpful for developing the mussel as a functional food.

Materials and Methods

Mussel sampling and environmental conditions of seawater: In the southern Adriatic Sea, at the border between Montenegro and Croatia there is a Boka Kotorska Bay, one of the largest bays of this part of the coast (87.3 km^2 of maritime zone). Bay consists of four smaller sub-bays: the Bay of Kotor, the Bay of Risan, the Bay of Tivat, and the Bay of Herceg Novi. Three large farms located in this Bay: Orahovac, Lipci and Kukuljina, were selected as sampling sites.

Mussel samples for this study were collected from three harvesting areas during four seasons (April, August, October 2018 and February 2019). Specific properties of this bay are caused by variable weather conditions, like abundant, rainfall during the autumn and winter and a lot of arid days during the summer. More than 2 kg of mussels were collected at each sampling site and were transported to the laboratory under refrigeration (+4°C) immediately. The biggest 25-30 mussels were separated for condition index, meat yield determination and biometric measurements. The rest of the mussels were washed, opened, and the flesh scraped out of the shells. At the same time, seawater environmental parameters were measured (below 1 m surface). Basic physical-chemical water parameters (temperature, salinity) were measured every season in situ using the multiparameter instrument (MultiLine 4, WTW, Germany). This research was conducted under commercial conditions.

Biometric parameters, condition index and meat yield: *Mytilus galloprovincialis* collected were measured for their biometrical parameters - length (maximum measure along the anterior-posterior axis), height (maximum dorsoventral axis) and width (maximum lateral axis). Mussels were measured using a 0.05 mm precision caliper (21). After opening the adductor muscle with a scalpel, the total weight of mussels, wet meat and shell weight were measured.

Using the following we calculate the condition index and meat yield of mussels. Condition Index (%): (dry meat weight (g) / dry shell weight (g)) × 100 (23).

Meat Yield (%): (wet meat weight (g)/total weight (g)) × 100 (22).

For condition index determination, to obtain dry weight, mussels (soft tissues and shells) were dried at 60°C for 48 h.

Proximate composition and mineral analysis: For proximate chemical composition studies, three pooled samples of mussels from each sampling site were minced in a processor for food (IKAR M 20 universal mill, IKA 1603601; IKA, Germany) and, until further analyzed, kept in dry conditions. Biochemical analyses on homogenized samples of mussels tissues were carried out in triplicate.

For chemical composition (moisture, ash, proteins and lipids) mussels samples were analyzed according to AOAC (3). In pre-measured porcelain trays, the moisture and ash content were established gravimetrically at the 80°C for 24 h. Then the dried mussel was ashed at 450°C for four h in a muffle furnace and weighed to the nearest 0.001 g. The total protein content was determined using the Kjeldahl technique (N × 6.25). In a digestor (BÜCHI B-435, Labortechnik AG, Flawil, Switzerland) mussel tissue was digested and then distilled using a Rotavapor R-210 (BÜCHI Labortechnik AG, Flawil, Switzerland) and automatic volumetric titration (T-50 automatic titrator, Mettler Toledo, United States).

The total fat content was determined gravimetrically, using the Soxhlet technique, by virtue of ether-mediated lipid extraction. In a muffle furnace (550°C/16h) the amount of inorganic material was measured by incinerating the samples to ash. Fatty acid profiles were determined by capillary gas chromatography using GC Shimadzu 2010 (Kyoto, Japan) gas chromatograph with flame ionization detector and capillary HP-88 column (100 m x 0.25 mm x 0.20 µm, J&W Scientific, USA). Working conditions were as previously described (23). Comparison of retention times to authentic standards were used for fatty acids identification. After digestion of the samples, the concentration of micro and macro elements were determined by inductively coupled plasma-mass spectrometry (ICP-MS). During ICP-MS, simultaneously with the samples, a working solution of internal standard was introduced into the system. The internal standard included low, middle and high mass elements. Based on the recorded values (i.e. according to the percentage of intensity reduction or increase) of the internal standard, the software performed an automatic correction of the obtained concentration of elements in the minced mussel tissue digests. Quality control was carried out by analyzing the certified reference material NIST 1577c.

DOI: 10.33988/auvfd.920577

Statistical analysis: Results were presented by descriptive statistical parameters mean±standard deviation. Significance was fixed at level P<0.05 in all cases. At all sampling times, analyses were carried out in duplicate. For all parameters conducted to test significant seasonal fluctuations, the analysis of variance (One-Way ANOVA) was using (14). All obtained results are presented in tables and figures.

Results

Environmental parameters (temperature, salinity) in the water samples from three different stations in the Boka Kotorska Bay were monitored in April, August, October 2018, and February 2019. During the seasons, the lowest seawater temperature and water salinity recorded in February (11.6°C; 14.9‰) and the highest values recorded in August, when the seawater temperature was 27.6°C and salinity was 33.9‰. The seasonal distribution of mean temperature and salinity were shown in Figure 1.

The biometric of mussel results in all seasons are shown in Table 1. Width shell showed significant variation (P<0.05) reaching minimum values in April (20.72±1.02 mm) and maximum values in October (24.31±0.83 mm). In spring, both meat yield (MY) and conditions index (CI) values began to grow significantly, and this high values continued all summer. CI values varying between 7.73+1.04% in February, and 18.83+0.55% in August and MY was maximum (31.07+0.76%) in April and minimum (22.63+0.45%) in October (Figure 2).

Table 1. Biometric measurement (length, width, height) of *Mytilus galloprovincialis* collected from Boka Kotorska Bay.

	n	April	August	October	February
Length (mm)	25	51.99±1.51^{a}	60.66±6.85^{a}	56.42±2.84^{a}	53.32±1.80^{a}
Width (mm)	25	20.75±1.02^{a}	23.07±1.35^{ab}	24.31±0.83^{b}	21.01±0.95^{a}
Height (mm)	25	11.25±0.49^{a}	13.88±3.14^{a}	11.23±0.31^{a}	12.15±0.60^{a}

^{a,b,c} Values within a row with different superscripts differ significantly at P<0.05.
n= number od samples

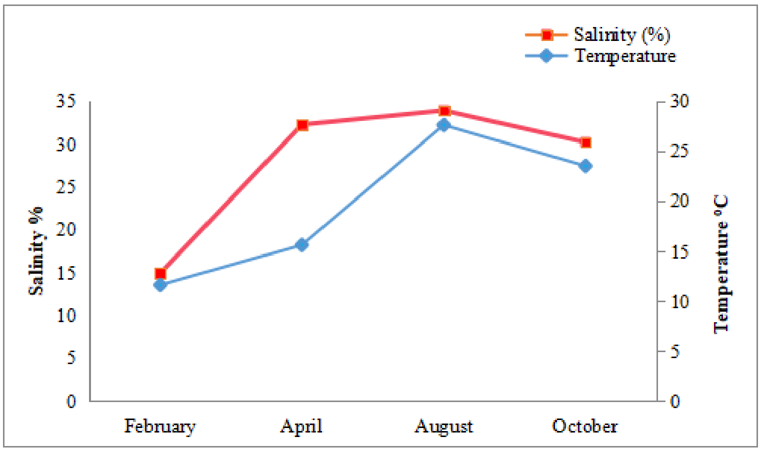

Figure 1. Seasonal distribution of mean temperature and salinity.

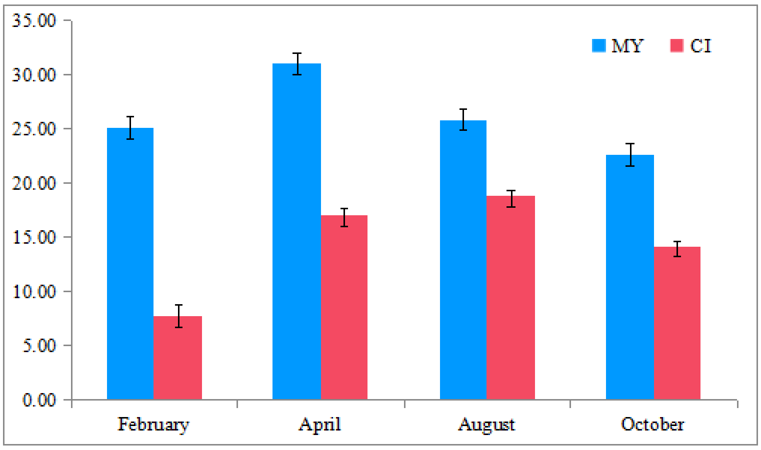

Figure 2. Seasonal variations in percentage condition index and meat yield of mussels. CI, condition index; MY, meat yield.

Table 2. Total fatty acid composition (saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids) and ratio ω-3/ω-6 fatty acids for *Mytilus galloprovincialis* samples from different locations in Bokakotorska Bay during four season.

	April	August	October	February
C14:0	2.39 ± 0.43	3.76 ± 0.62	2.53 ± 0.09	1.66 ± 0.55
C15:0	0.50 ± 0.03	0.88 ± 0.05	0.79 ± 0.04	0.62 ± 0.07
C16:0	23.69 ± 0.82	25.86 ± 0.82	23.26 ± 0.02	22.12 ± 1.22
C17:0	0.68 ± 0.08	2.43 ± 0.08	1.91 ± 2.07	1.17 ± 0.21
C18:0	3.42 ± 0.20	3.94 ± 0.11	4.11 ± 0.12	3.11 ± 0.07
ΣSFA	**30.68 ± 0.75**	**36.87 ± 1.02**	**32.6 ± 2.07**	**28.68 ± 0.84**
C16:1	4.33 ± 1.02	2.72 ± 0.26	3.51 ± 0.12	5.11 ± 1.29
C18:1 cis-9	3.21 ± 0.80	3.42 ± 0.12	3.44 ± 0.16	2.53 ± 0.39
C20:1	3.55 ± 0.04	5.39 ± 0.29	5.98 ± 0.04	3.16 ± 1.00
ΣMUFA	**11.09 ± 1.05**	**11.53 ± 0.64**	**12.93 ± 1.01**	**10.8 ± 1.23**
C18:2 n-6	2.98 ± 0.22	2.12 ± 0.18	3.47 ± 1.45	1.26 ± 0.28
C18:3 n-3	1.41 ± 0.32	1.13 ± 0.12	1.41 ± 0.12	1.33 ± 0.26
C20:2 n-6	4.41 ± 0.27	5.22 ± 0.08	4.81 ± 0.21	3.98 ± 0.31
C20:3 n-3	1.42 ± 0.18	1.84 ± 0.21	1.98 ± 0.30	1.32 ± 0.10
C20:3 n-6	1.14 ± 0.02	0.87 ± 0.03	0.98 ± 0.12	0.68 ± 0.12
C20:5 n-3 (EPA)	11.31 ± 0.71	9.23 ± 0.31	7.99 ± 0.34	13.05 ± 3.21
C22:5 n-3	0.55 ± 0.04	1.46 ± 0.11	1.08 ± 0.21	0.97 ± 0.55
C22:6 n-3 (DHA)	12.26 ± 0.65	16.58 ± 0.21	12.41 ± 1.11	12.23 ± 1.44
ΣPUFA	**38.48 ± 1.29**	**38.45 ± 0.74**	**34.13 ± 0.59**	**34.92 ± 1.66**
Total n-3 PUFA	26.95 ± 0.58	30.13 ± 0.42	24.87 ± 0.61	28.9 ± 0.95
Total n-6 PUFA	8.53 ± 0.72	8.21 ± 0.87	9.26 ± 0.75	5.92 ± 0.60
n-3/n-6	3.16 ± 0.03	3.67 ± 0.02	2.68 ± 0.03	4.88 ± 0.19

All samples were analyzed in duplicate (n = 2) from a pooled sample (20 numbers) and expressed as mean ± standard deviation.

Table 3. The mean concentrations of metals (mg/kg dry weight) in the sampled mussels.

	April	August	October	February
Ca	1401 ± 506^{a}	3714 ± 206^{b}	2370 ± 710^{c}	7085 ± 445^{d}
K	1876 ± 109.8^{a}	2216 ± 140.9^{b}	2037 ± 83.91^{c}	1824 ± 207.8^{d}
Ni	4.1 ± 0.03^{a}	7.9 ± 0.35^{b}	5.1 ± 0.15^{c}	7.2 ± 0.11^{d}
Cr	2.6 ± 0.06^{a}	1.3 ± 0.04^{b}	2.4 ± 0.06^{a}	5.3 ± 0.12^{c}
Fe	120.7 ± 15.27^{a}	267.2 ± 1.28^{b}	381.8 ± 7.96^{c}	341.7 ± 8.52^{d}
Zn	148.4 ± 2.11^{a}	189.1 ± 1.84^{b}	224.8 ± 2.09^{c}	246.8 ± 2.88^{d}
Mn	57.4 ± 2.43^{a}	15.8 ± 0.29^{b}	62.6 ± 1.60^{c}	77.5 ± 2.78^{d}
Pb	0.22 ± 0.02^{a}	0.27 ± 0.06^{b}	0.28 ± 0.06^{bcd}	0.30 ± 0.03^{d}
Cd	0.13 ± 0.04^{a}	0.14 ± 0.02^{a}	0.12 ± 0.02^{a}	0.19 ± 0.03^{b}
Hg	0.2 ± 0.001^{a}	0.3 ± 0.004^{a}	0.3 ± 0.01^{a}	0.4 ± 0.001^{a}

All samples were analyzed in duplicate (n = 2) and expressed as mean ± standard deviation.

The fluctuations of total lipid (0.72–2.12%), ash (2.56-3.59%), and protein (8.42–10.76%) contents of mussels on a dry basis during the experimental period are shown in Figure 3. Quantitative analyses of proteins, responsible, for energy storage in animals, revealed elevated levels in spring and summer and lower levels during autumn and winter. The percentage of lipids ranged from 0.72 ± 0.18% in October to 2.11 ± 0.69% in the winter months, when it reached its highest value. The fatty acid profile of the mussels in Boka Kotorska Bay is shown in Table 2, where a total of 16 FAs were identified. Polyunsaturated fatty acids (34.13-38.48%) predominated oversaturated (28.68-36.87%) and monounsaturated fatty acids (10.8-12.93%) throughout the year. Amongst all PUFAs, a high proportion of ω-3 long-chain PUFAs, especially EPA and DHA, is apparent. Within the group of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), palmitic (C16:0) and palmitoleic (C16:1n-7) acids were the most abundant.

The mean concentrations of the investigated metals (Ca, K, Ni, Cr, Fe, Zn, Mn, Pb, Cd and Hg) in mussel *M. galloprovincialis* samples are given in Table 3. The concentrations of the these metals in the mussel along the Montenegrin coastline decreased in the following order: Ca > K > Fe > Zn > Mn > Ni > Cr > Pb > Cd > Hg for all investigated samples. As can be seen from Table 3, the greatest mean metal concentrations were observed in samples from the autumn and winter seasons.

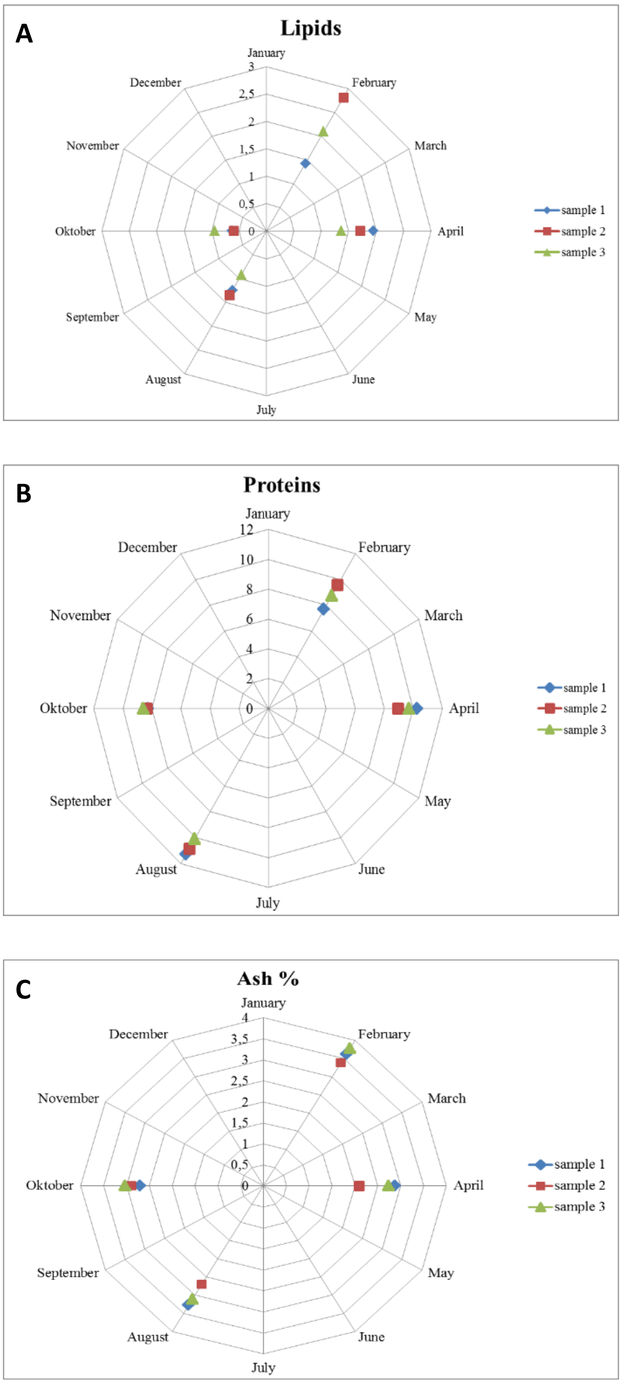

Figure 3. Polar diagram - seasonal variation a) lipids; b) proteins; c) ash

Discussion and Conclusion

The results suggest an influence of environmental parameters such as water temperature, food availability, and the reproductive cycle of the mussels on their biochemical composition. Boka Kotorska Bay has very pronounced seasonal water temperature fluctuations. Perennial research shows minimum temperatures in January and February and peak temperatures in July and August (28). Our study reported the mean minimum temperature in February (11.6°C), and the maximum temperature was recorded in August (27.6°C). The salinity of the Adriatic Sea (38.30‰) is higher than the western Mediterranean (37‰) and but slightly lower than the eastern Mediterranean (39‰) (28). In our examination the salinity was constant throughout the year (30-33‰), except in February when it was 14.9‰, resulting from higher amounts of precipitation.

In 2004, Ojea et al. found some variations in the weight, length, width, height and proximal composition of mussels that occur in response to changes in environmental conditions, especially in food availability (20). While parameters such as the decrease of sunlight or the cooling of the sea surface reduce phytoplankton biomass production during autumn and winter, they have the opposite effect in spring and summer, resulting in microalgal blooms (19). When there is enough food, mussels use the energy to grow their tissues and develop the gonads. It takes about 18 months to reach a commercial size of at least 50 mm, because during the winter months, they generally stagnate and lose weight, and after that they grow again. Shell growth can be continuous during the year as it is formed mainly from dissolved calcium present in seawater, still meat weight appears to be seasonal, with gross growth concentrated in specific seasons (2). In our study, the biometric parameters showed no significant differences during the sampling period.

The biochemical compositions of mussels are characterized by phases of accumulation and depletion of food reserves in the spawning season. Shortage of food typically occurs between late September and early December. Then low values of biochemical resources may be expected (17).

The commercial quality and physiological state of bivalve molluscs are adequately described by the condition index (CI), and also the meat yield (MY), parameters of economic relevance reflecting the ecophysiological conditions and the health of *Mytilus galloprovincialis* (7). The fluctuations in condition index and meat weight have important implications for the cultivation and harvesting strategy. For optimum exploitation the harvesting season should be timed according to the peak period for the condition index. In our study, in spring, both meat yield (MY) and conditions index (CI) began to grow significantly. A variety of extrinsic and intrinsic factors, like salinity and temperature of the water, the gametogenic cycle of animals, and food availability, affect these environmental parameters (22). A positive correlation between CI and biochemical constituents of bivalves has been reported in different mollusc species (23). Low meat yield values and condition index values during winter have been attributed to the utilisation of carbohydrates (i.e., glycogen) reserves and the depletion of protein contents due to of food shortage and gametogenesis.

The variations in protein content appeared to be due to the seasonal differential availability of food (microalgae). The protein content varied from 8.42-10.76%; a maximum of 10.76% was recorded in samples collected in August. In general, a rapid tissue restoration occurred in spring and mussels remained in good condition during summer when protein was maximal and decreased through winter. During winter the lipid reserves in the developing eggs leads to higher lipid content in mussels (5).

Considering the economic value of biochemical components from marine mussels, fatty acids (FAs), especially PUFAs, are regarged as the single most crucial nutritional indicator dictating the quality of *Mytilus galloprovincialis*. The prevalence of PUFAs, amounting to about 34 - 38% of total fatty acids and predominated over the SFAs and MUFAs. Similar profiles have been reported previously for *M. galloprovincialis* grown in the northwest of Spain (9), the Adriatic Sea (29) and two different Italian sites (23). The main factor explaining these results might be the availability of phytoplankton, the bivalve's major source for n-3 PUFAs, particularly linoleic (C18:2n-6) and linolenic (C18:n-3) acids, known to be synthesized by diatoms and dinoflagellates (2). Therefore, the quantitative and qualitative availability of food influences the composition of fatty acids (11). The proper balance of dietary n-3/n-6 PUFAs is integral to prevent chronic and cardiovascular diseases. It is potentially significant because the ratios of essential fatty acids in the tissues are determined mainly by their proportions in the diet (6). In present study n-3/n-6 ratios were 2.7–4.9 which is paralleled previous studies.

Mussel fatty acid profiles usually contain about ~30–40% SFA (1), a level found in the present study. The predominant SFA was recorded to be 16:0 (22–25% of total fatty acids), with a maximum recorded in August. It is as expected because SFAs are used for energy storage, and therefore, their concentration increases during periods of enhanced feeding activity (13).

The metal level in mussel tissues represents integrated response to bioavailable metal in seawater and the food available to them. Because they are filter feeders, metals in their interior can also be found in higher concentrations in seawater (24). In the mussel samples K, Mg, and Ca concentrations were higher than the remaining tested elements. That is understandable since these macronutrients have many vital roles in the organisms (27). The high content of Ca and Mg in mussels is their requirement for the shell formation while the mussel growth (4).

Based on the measured concentrations of metals in mussel sampled in four different seasons, it can be observed that generally the highest average concentrations were measured in the winter and autumn months. This result agrees with that of other authors (26, 16) and is explained by the higher solubility of heavy metals and minerals due to increased rainfall and fresh water inflow. It can also be explained by the decrease in the weight of mussels during this time of year, less food available, while the metal concentration remains the same. A significant seasonal variability was observed for the tissue concentrations of thesome trace metals. Chromium, iron, calcium and zinc exhibited higher levels in winter and autumn, and lower in spring or summer months (Table 3).

The present study provided a detailed biochemical profile of the *Mytilus galloprovincialis* collected from Boka Kotorska Bay for the first time from this part of the Southern Adriatic Sea. The CI, MY and biochemical parameters proved suitable conditions for mussel cultivation in Boka Kotorska Bay. The most significant factors determining these parameters were temperature, food amount, and gametogenesis. In addition the metal concentrations found in the mussels are within the range of the mean values reported in the literature. Therefore, we can conclude that ideal periods for mussels harvesting were April and August. Spawning periods following these months and winter (between December and February) were not suitable for harvesting.

Acknowledgements

The authors are grateful to the Diagnostic Veterinary Laboratory of Montenegro, Podgorica for assistance in sample collection.

Financial Support

The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract number 451-03-9/2021-14/200143).

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

NG, VDj and MD conceived and planned the experiments. NG, IZB, NČ and SB carried out the experiments. NG, MD planned and carried out the simulations. NG, VD and

IZB contributed to sample preparation. NG, NK and SDj contributed to the interpretation of the results. NG took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

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DOI: 10.33988/auvfd.920577