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Biochemical composition and biometric parameters of *Mytilus galloprovincialis* from Boka Kotorska Bay in Southern Adriatic Sea

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ABSTRACT: This study reports, for the first time, different physico-chemical analyses, of Mediterranean mussels (*Mytilus galloprovincialis*) from harvesting areas in the Montenegro coast of the Adriatic Sea, in order to evaluate the influence of origin on different parameters and assessed the quality of shellfish grown in this area. The Boka Kotorska Bay is situated in Montenegro, in the south-eastern part of the Adriatic Sea The mussel samples were collected at the same time in the spring of 2019 at six locations in Boka Kotorska Bay, Montenegro: Kotor and Tivat Bays. Biometric parameters, percentage of meat, condition index, proximate composition, sensory evaluation and lipid profiles of mussels were studied. The concentrations of some micro and macro elements and heavy metals in mussels, were also analyzed. Significant differences were found between mussels from different locations. Mean biometric parameters of mussels grown in Sv.Nedjelja, were considerably higher than in mussels grown elsewhere.. Protein, lipid, ash and glycogen content were varied from 7.80 to 10.26%; 1.36 to 2.18%; 1.73 to 3.34% and 12.81 to 15.38%, respectively. Gas chromatographic analysis showed that polyunsaturated fatty acids (PUFAs) were dominant lipids in mussels (37.56 to 41.08%), followed by monounsaturated (MUFAs) (30.52 to 38.31%) and saturated (SFAs) (21.89 to 29.45%) fatty acids. Fatty acid profiles were investigated and revealed high contents of n-3 PUFAs and high n-3/n-6 ratios in all mussels from Montenegro In the mussel samples high concentrations of K, Mg, Ca, and Na, and much lower concentrations of Fe, Mn, Zn, and Cu were found. Some of toxic elements (As, Pb, Hg and Cd) were determined also.. The qualitative sensory assessment showed that all mussels were acceptable. All mussels in the current study achieved scores of 3 or more out of 5 in the qualitative category. Data on biochemical composition and quality indices of the mussels cultured in the Boka Kotorska Bay demonstrated that these products could be accepted well by consumers and could compete with other currently available mussels from other locations in Adriatic Sea.

***Keywords: Mytilus galloprovincialis*, proximate composition, fatty acids, heavy metals, Adriatic Sea**

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INTRODUCTION

The popularity of seafood has increased over recent decades, and this food source is becoming increasingly necessary for the growing population of people on the planet (Grienke et al., 2014). In coastal parts of Montenegro, the harvesting and cultivation of marine organisms dates back centuries. Today, Montenegro has limited mussel production, with relatively low commercial demand and value, despite intensive tourism. A specific national mariculture development plan has yet to be developed.. However, because of favorable hydrographic conditions in this part of Montenegro coast, there is significant potential for development of aquaculture. Currently there are 19 small mussel farms in Montenegro, each producing 5 to 30 tonnes per year, and these are all situated in Boka Kotorska Bay. During the period of 2013–2015, the average annual production of *Mytilus galloprovincialis* was 182 tonnes respectively (Macic, 2005).

Bioactive compounds present in the flesh of seafood, and which have positive effects on human health, also contribute to the popularity of this food (Grienke et al., 2014). Bivalve molluscs are considered to be highly nutritive, as they are a rich source of proteins, carbohydrates, minerals and provide an almost unlimited variety of fatty acids (FAs), primarily highly valuable polyunsaturated fatty acids (PUFAs) (Bongiorno et al., 2015). The contents of Na, Mg, K and Ca were noticeably highter than those reported for Spanish and Italian mussels (Fuentes et al., 2009; Bongiorno et al., 2015). As the Montenegrin coastal area, especially the Boka Kotorska Bay, receives a heavy influx of sewage and industrial effluents, as well as domestic and agricultural wastes (Jovic et al., 2011), the determination of the levels of trace metals, especially those of potentially toxic metals (Hg, Pb, Cd), in mussels is very important. The Boka Kotorska Bay is known as one of the regions in Europe with the highest amount of precipitation, and so the quantity of pollutants reaching the sea from this source cannot be neglected as an adverse factor in aquaculture (Jovic et al., 2011). Quality analysis of mussels from various sites could show the importance of differing sites for shellfish cultivation, because seawater is a complex geomorphological matrix. Several environmental factors can affect the overall quality of mussels in terms of microbiological, nutritional, technological and sensory characteristics (Vernocchi et al., 2007). In this study we compared the biochemical composition of mussels cultured at six most productive harvesting areas, three in Tivat Bay and three in Kotor-Risan Bay, with the aim of investigating if shellfish composition varied with location of the culture units. The study aims to provide new biological information for the potential development of aquaculture species in Montenegro, and to compare the quality of mussels from the Montenegrin aquatorium with mussels that is harvesting on other coast of the Adriatic Sea.

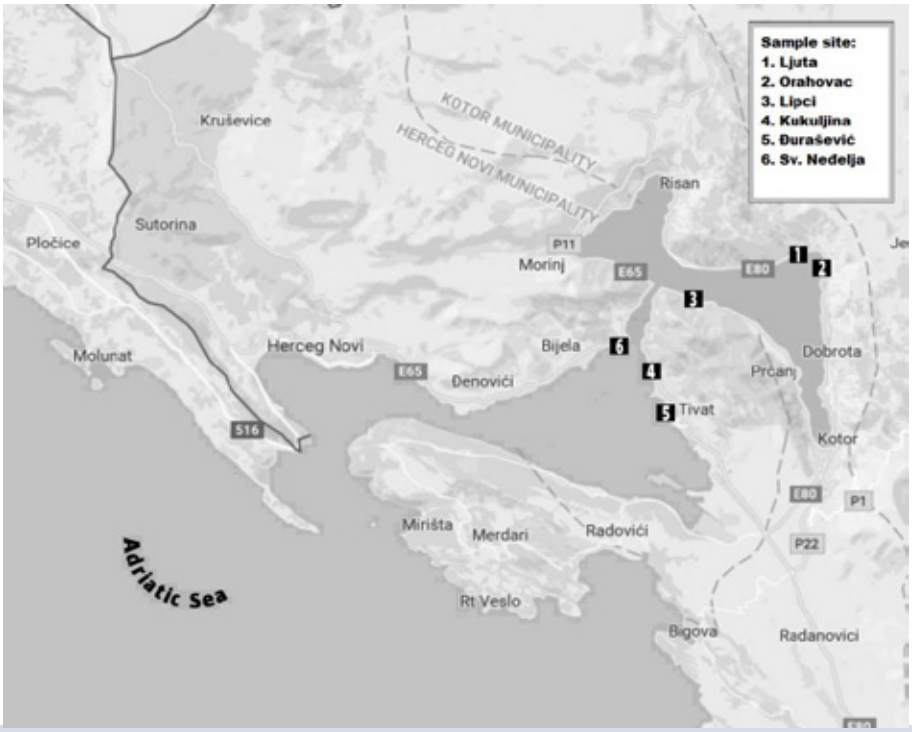

Figure 1. Sampling sites on the Boka Kotorska Bay, Montenegro (Ilic et al., 2017)

MATERIALS AND METHODS

Study site and mussel sampling

The mussels samples were collected in the same time in April 2019, from six commercial sampling sites in Bokokotorska Bay: Ljuta, Orahovac, Lipci, Kukuljina, Obala Djurasevic and Sv. Nedjelja (Fig.1). The sampling sites are influenced by different hydrological characteristics of the Boka Kotorska Bay. Ljuta, Orahovac and Lipci are located in Kotor Bay, and Kukuljina, Obala Djurasevic and Sv. Nedjelja in Tivat Bay, which is under the influence of the open sea. At each sampling site, mussels were manually collected at a depth of approximately 2 m, using standard commercial protocols (Official Gazette of Montenegro, 2009). Samples were washed, placed in nylon bags with seawater and immediately transported to the laboratory. The sampling of seawater was also performed using Niskin bottles, volume of 5 l, at a depth of 0.5 m at same mussel sampling sites. Water temperatures and salinity were measured by automatic probes (Multiline 4, WTW, Germany).

Biometric parameters, meat yield, Condition index

Biometric parameters were assessed only from samples of commercial size (≧50 mm) (Ljuta, n = 39; Orahovac, n = 34; Lipci, n = 40; Kukuljina, n= 38; Obala Djurasevica, n = 34; Sv. Nedjelja, n = 35 mussels). Mussels were measured individually using a 0.05 mm precision calliper for the following size parameters: length (maximum measure along the anterior-posterior axis), width (maximum lateral axis), and height (maximum dorso-ventral axis). For each mussel, the total wet weight (g) was measured to the nearest 0.001 g, after which the meat was carefully dissected away from the shell.

Meat yield (MY), i.e. the percentage ratio between meat content (WT) and total wet weight of mussels (WW) is an important aspect of marketability of mussels and was calculated according to Okumus and Stirling (1998) as follows:

MY (%) = (meat weight (g) /whole mussel weight (g)) x 100

After the determination of individual total weight and wet meat and shell weights, we were registered meat and shell weights after oven-drying (105 °C). Condition index (CI) was calculated according to Orban et al. (2002) as follows:

CI (%) = (meat dry weight (g) /shell dry weight (g)) x 100.

Water activity and pH measurement

All the measurements were performed using the whole soft body of the mussels. The pH of homogenized mussel meat (around 100g of meat from one sampling site) was determined using a pH meter (Testo 205; Testo AG, Lenzkirch, Germany). The water activity (a_w) was measured with a a_w meter (aw-Wert Messer, Fa-St/1; GBX Scientific Instruments, Tallaght, Ireland).

Chemical composition

Samples of the soft parts obtained from suitable pools of individual mussels were analysed for chemical composition. Twenty individuals from each batch/ origin were dissected and minced in a food processor (IKAR M 20 universal mill, IKA 1603601; IKA, Germany) and subjected to biochemical characterization. Proximate composition was determined in triplicate. Moisture and ash were determined gravimetrically. Briefly, minced tissue pools (3g) were placed in preweighed porcelain trays for drying at 80°C for 24 h and then weighed to the nearest 0.001 g. Subsequently, dried mussel tissue was ashed at 450°C for 4 h in a muffle furnace.

Total protein was determined according to the Kjeldahl method (AOAC, 1997) from the nitrogen concentration of mussel. Because the Kjeldahl method does not measure the protein content directly a conversion factor (F) is needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used to calculate total proteins in mussels.

Glycogen content (mg g^{-1} of wet weight) was measured by colorimetric reaction using 25 µL of prepared minced mussel meat. KOH (30%) was added to 0.5 g of homogenized mussels' tissue (ratio 2:1), then they were heated in a shaking-water bath (100 °C/ 20 min) vortexed for 30 s and subsequently chilled on ice for 5 min. Then, in each sample was added 200 µL of 95% ethanol and 1.2 mL of lukewarm water.Volumes (10 µL) of 80% aqueous phenol and 200 µL of sulphuric acid were added to the mussel meat in 96-well plates. Absorbance was measured using a multi-detection microplate reader (Synergy HT, BIO-TEK) at 490 nm. Glycogen concentration was calculated based on a mussel glycogen standard (Sigma, Saint Louis, MO) following the methodology reported by GALLARDI et al. (2014).The Total lipids (TL) were extracted from the mussel meat (2g) by accelerated solvent ex-

traction (ASE 200, Dionex, Sunnyvale, CA) with a mixture of n-hexane and isopropanol (60:40, v/v) in 33 ml extraction cell at 100°C and nitrogen pressure of 10.3 MPa (Spiric et al., 2010). The solvent was removed under a nitrogen stream at 50°C until dryness in a solvent evaporator (SE 500, Dionex, Sunnyvale, CA). The fat extract was further used for fatty acid determination. Total lipids were further converted to fatty acid methyl esters (FAMEs) by using 0.25 M trimethylsulfonium hydroxide (TMSH) in methanol (EN ISO 5509:2000). FAMEs were determined by capillary gas chromatography using a GC Shimadzu 2010 (Kyoto, Japan) equipped with flame ionization detector and capillary HP-88 column (100 m x 0.25 mm x 0.20 μm, J&W Scientific, USA). Separation and detection were performed under the following temperature program: initial temperature 125 ºC, rate 10 ºC min^{-1} to 175 ºC, hold 10 min, rate 5 ºC min^{-1} to 210 ºC, hold 5 min, rate 2 ºC min^{-1} to final temperature of 230 ºC, hold 12 min. Total analysis time was 50.5 min. The injector and detector temperatures were 250 ºC and 280 ºC, respectively; split ratio 1:50; volume 1 μL; carrier gas, N_2, 1.33 m min^{-1}; make-up gas, N_2, 30 ml min^{-1}; detector gases, H_2, 40 ml min^{-1}; synthetic air, 400 ml min^{-1}. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA).

Concentration of micro and macro elements were determined by inductively coupled plasma-mass spectrometry (ICP-MS) after digestion of the minced mussel meat. The instrumentation employed for pre-treatment of minced mussel meat was the microwave digestion system Start D, Milestone (Sorisole, Italy). Minced mussel meat was prepared as follows: about 0.5 g homogenized minced mussel meat was weighed into a teflon digestion vessel and then 5 ml of nitric acid and 1.5 ml of hydrogen peroxide were added. The digestion vessels were closed and heated in the microwave oven using a preselected program (5 minutes from room temperature to 180°C, 10 minutes at 180°C). After cooling (20 minutes) the digests were quantitatively transferred using deionised water into 100 ml polypropylene volumetric flasks. Digests were then filtered through syringe-nylon filters into polypropylene autosampler cuvettes and used for determination of elements.

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. Calculation of the content of elements in the minced mussel tissue digests was done by the software, taking into account the equation of the calibration formula for each element, the mass of the minced tissue and the total dilution, as well as the correction based on the internal standards. In accordance with the instructions for handling the ICP-MS device, before each readout of the concentration of elements in the minced mussel tissues, and using the basic calibration solution (Tune B), the instrument parameters were automatically adjusted to meet the specified criteria, after which the instrument was ready for operation. Quality control was carried out by analyzing the certified reference material NIST 1577c. Values determined were in agreement with the certified values (data not shown).

Sensory analyses

Sensory analysis sessions were performed in a sensory analysis room compliant with ISO 8587:2006. They were conducted using six panellists who had experience with seafood. In sensory evaluations, appearance, odor, texture and elasticity of raw mussels were assessed. The sensory analysis conducted on fresh mussel samples. Eighteen individual mussels were randomly selected from each batch of different origin. Prior to the evaluation, mussels were washed with cold water and scrubbed in order to remove debris and any remaining byssal thread, and then they were stored on ice. Three raw mussels of each batch were manually shucked, immediately before testing and presented to each panellist in a white, plastic plate labelled with a 3-digit random number. Sensory evaluations were conducted using a 5-point scale (0-5), where a score of 5 was defined as excellent (Caglak et al., 2008). All the panellists were allowed into the room together and had unlimited time to complete the testing.

Statistical analysis

Statistical analysis of the results was conducted using software GraphPad Prism version 7.00 for Windows, GraphPad Software, San Diego CA, USA, www.graphpad.com. All parameters were described by descriptive statistics (mean ± standard deviation).

Data were subjected to one-way ANOVA to test significant differences for various traits. Assumptions of normality and homogeneity of variances were tested prior to ANOVA with Shapiro – Wilk and D`Agostino-Pearson test, respectively.

RESULTS

Biometric parameters, MY, CI, pH and aW values

The mussels from Lipci were statistically smaller in ($p < 0.05$) length (51.05 ± 3.10 mm), height (28.9 ± 2.06 mm) and width (19.83 ± 1.68 mm) compared with the mussels from the other sites. Conversely, mussels from Sv. Nedjelja displayed significantly higher morphometric values than others, as their average length was 56.19 ± 4.79 mm, height 32.40 ± 2.45 mm and width 22.37 ± 2.22 mm (Table 1). Meat yield ranged from 20.89 ± 6.41% in Ljuta to 26.39 ± 7.93% in Obala Djurasevic. The differences between the meat yield and condition index (CI) of mussels from Obala Djurasevic were statistically significant ($p < 0.05$) compared to mussels from the other locations. No significant differences ($p > 0.05$) were found in CI between Lipci and Sv. Nedjelja mussels in spite of the differences found in shell morphology (Table 1). There were no significant differences ($p > 0.05$) in the pH and aW values of mussels from the six sites, ranging from 7.0 to 7.2 and from 0.96 to 0.97,. respectively.

Proximate composition of mussels

Significantly higher ash and protein levels in mussels were detected in Orahovac and Sv. Nedjelja areas compared to the other ones. Mussel tissue protein content ranged from 7.80 in mussels from Ljuta to 10.26% in mussels from Orahovac. Mussels from Kotor Bay (Ljuta and Lipci) showed higher moisture values than mussels from Tivat Bay. Lipid contents did not show any significant differences between mussel from the different areas. Finally, glycogen levels (15.11 $mg \cdot g^{-1}$ and 15.38 $mg \cdot g^{-1}$ in mussels from Orahovac and Sv. Nedjelja, respectively) were slightly higher, although differences were not statistically significant ($p<0.05$) (Table 2.). The polyunsaturated fatty acids (PUFA) predominated over the monounsaturated fatty acid (MUFA) and saturated fatty acids (SFA). Significant differences in the PUFA profile were found between mussel from Sv. Nedjelja and mussels from other location. Palmitic acid (16:0), the major SFA in mussels, was clearly predominant in all samples. With regard to PUFA, a high proportion of n-3 long-chain PUFAs was found, and eicosapentaenoic (20:5 n-3) and docosahexaenoic (22:6 n-3) acids were the most important FAs in this fraction. High levels of n-3 PUFA (18.62 ± 2.32% to 25.75 ± 2.2%), low levels of n-6 PUFA (2.86 ± 0.17% to 3.38 ± 0.14%) and high n-3/n-6 ratios (5.70 ± 1.41 to 8.99 ± 1.33) characterized all sample of mussels from Bokokotoska Bay (Table 3.). Metal concentrations, of the investigated micro and macro elements and heavy metals in mussels taken at six locations, expressed in mg/kg are listed in Table 4. All mussel samples are a rich source of the macrominerals Ca, K, Na, Mg and essential elements Fe and Mn.

Table 1. Biometric characteristic, meat yields and Condition index of mussels (Mytilus galloprovincialis) from six different location in Boka Kotorska Bay.

	Ljuta	Orahovac	Lipci	Kukuljina	Obala Djurasevic	Sv.Nedjelja
L (mm)	53.29 ± 2.07^{A}	54.10 ± 3.27^{B}	51.05 ± 3.10^{C}	53.92 ± 3.91^{BD}	53.89 ± 4.32^{BE}	56.19 ± 4.79^{F}
H (mm)	30.10 ± 2.23^{A}	30.89 ± 2.32^{B}	28.9 ± 2.06^{C}	30.51 ± 3.47^{BD}	30.62 ± 3.76^{BE}	32.40 ± 2.45^{F}
W (mm)	21.21 ± 1.53^{A}	21.75 ± 1.62^{B}	19.83 ± 1.68^{C}	21.29 ± 2.65^{AF}	21.51 ± 2.42^{BDF}	22.37 ± 2.22^{E}
MY (%)	20.89 ± 6.41^{A}	21.95 ± 5.11^{B}	23.01 ± 6.76^{C}	22.80 ± 5.74^{CD}	26.39 ± 7.93^{E}	21.68 ± 6.46^{BF}
CI (%)	17.32 ± 15.09^{A}	17.24 ± 13.41^{A}	17.6 ± 18.09^{A}	16.91 ± 11.35^{A}	18.9 ± 14.99^{B}	16.5 ± 20.69^{A}

Values are given as Mean ± S.D. from all samples from each sampling site. . Different letters in the same row denote statistically significant difference ($P <0.05$) between groups

Table 2. Proximate composition of mussels from different sampling sites

	Ljuta	Orahovac	Lipci	Kukuljina	Obala Djurasevic	Sv.Nedjelja
Ash (%)	2.40 ± 0.21^{A}	3.34 ± 0.24^{B}	1.73 ± 0.32^{A}	2.46 ± 0.63^{A}	2.16 ± 0.21^{A}	3.30 ± 0.27^{C}
Moisture (%)	81.39 ± 0.52^{A}	77.87 ± 1.16^{B}	81.42 ± 1.21^{A}	78.9 ± 0.52^{B}	78.06 ± 0.82^{B}	78.12 ± 0.87^{B}
Total protein (%)	7.80 ± 0.24^{A}	10.26 ± 0.33^{B}	8.95 ± 0.48^{A}	8.55 ± 0.45^{A}	8.66 ± 0.45^{A}	9.70 ± 0.40^{B}
Total lipid (%)	2.18 ± 0.25^{A}	1.71 ± 0.22^{A}	1.36 ± 0.32^{A}	1.96 ± 0.61^{A}	2.13 ± 0.28^{A}	2.13 ± 0.35^{A}
Glycogen (mg/g)	12.81 ± 0.09^{A}	15.11 ± 0.06^{A}	14.34 ± 2.16^{A}	13.19 ± 0.15^{A}	13.2 ± 0.03^{A}	15.38 ± 0.49^{A}

Values are given as Mean ± S.D. from triplicate ; Different letters in the same row denote statistically significant difference ($P <0.05$) between groups.

Table 3. Fatty acid profile (% of total fatty acids) of mussels from different sampling sites

	Ljuta	Orahovac	Lipci	Kukuljina	Obala Djurasevic	Sv.Nedjelja
SFA	28.61 ± 2.12^{A}	29.45 ± 2.21^{A}	25.99±2.25^{AB}	21.89± 3.51^{B}	23.37±3.56^{AB}	28.65 ± 2.22^{A}
MUFA	31.68±1.98^{AC}	30.52± 1.97^{A}	34.28±2.04^{AC}	38.31± 2.11^{B}	35.55±1.97^{BC}	33.79± 2.01^{AC}
PUFA	39.71^{a} ± 0.82	40.03^{a} ± 0.60	39.73^{a} ± 0.83	39.80^{a} ± 0.82	41.08^{a} ± 1.15	37.56^{b} ± 1.12
n-3	25.75 ± 2.2^{A}	25.23± 2.19^{A}	21.61± 2.2^{AC}	18.62±2.32^{BC}	20.19±2.19^{BC}	25.54 ± 2.22^{A}
n-6	2.86 ± 0.17^{A}	3.04± 0.13^{AB}	3.38 ± 0.14^{B}	3.27 ± 0.13^{B}	3.18 ± 0.12^{B}	3.12± 0.13^{AB}
n-3/n-6	8.99 ± 1.33^{A}	8.29± 1.70^{AC}	6.39± 1.35^{BC}	5.70 ± 1.41^{B}	6.34±1.32^{ABC}	8.19± 1.45^{AC}
n-6/n-3	0.11 ± 0.03^{A}	0.12 ± 0.03^{A}	0.16 ± 0.01^{A}	0.18 ± 0.02^{A}	0.16 ± 0.02^{A}	0.12± 0.01^{A}
C20 :5n3 (EPA)	16.10 ± 1.73^{A}	14.09±1.70^{AC}	14.65± 1.82^{AC}	14.31±1.86^{AC}	16.70±2.02^{BC}	15.17± 1.93^{A}
C22 :6n3 (DHA)	16.04±1.07^{AB}	17.25 ± 0.98^{A}	16.09± 1.27^{AB}	16.60 ± 1.14^{B}	15.63±1.09^{AB}	17.41 ± 0.97^{A}
C18:2 n-6	2.98 ± 0.22^{A}	3.45± 0.23^{B}	3.66± 0.18^{B}	3.80± 0.06^{B}	4.1± 0.33^{C}	2.9± 0.21^{A}
C20:2 n-6	4.60 ± 0.60^{A}	5.22 ± 0.08^{B}	5.33± 0.02^{B}	5.09± 0.03^{B}	5.4± 0.21^{B}	4.1± 0.02^{C}
C14:0	11.38± 0.93^{A}	10.22±0.91^{AC}	10.66± 0.94^{AC}	9.36± 0.88^{C}	9.25 ± 0.88^{C}	8.17 ± 0.93^{B}
C16:0	17.23± 0.87^{A}	19.23 ± 0.89^{A}	15.33 ± 0.81^{B}	12.53±0.77^{B}	14.12± 0.76^{B}	20.48± 0.54^{A}
C16:1	4.33 ± 1.02^{A}	4.25 ± 1.10^{A}	4.11 ± 0.12^{B}	4.41 ± 1.03^{AC}	4.35 ± 1.12^{AC}	4.51 ± 1.02^{AC}
C18:1 cis-9	3.21 ± 0.80^{AB}	3.48 ± 0.40^{A}	3.25 ± 0.89^{B}	3.33 ± 0.84^{B}	2.92 ± 0.11^{C}	3.17 ± 0.80^{B}
C20:1	3.55 ± 0.04^{A}	3.63 ± 0.41^{A}	3.41 ± 0.03^{B}	3.41 ± 0.12^{B}	3.53 ± 0.01^{A}	3.49 ± 0.11^{A}

Values are given as Mean ± S.D. from triplicate ; Different letters in the same row denote statistically significant difference (P <0.05) between groups.
PUFA: Polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SAFA: Saturated fatty acids; n-3: omega-3; n-6: omega-6; EPA: C20:5n-3; DHA: C22:6n-3;

Table 4. Metal concentrations, expressed in mg/kg, in the whole soft tissue of mussels.

	Ljuta	Orahovac	Lipci	Kukuljina	Obala Đurašević	Sv.Nedjelja
Ca	936.3	1009.1	1220.0	857.5	738.2	694.8
K	2018.3	1930.6	1749.5	2066.0	2149.8	2136.8
Mg	509.0	773.0	665.9	911.5	888.2	855.6
Na	401.03	461.73	407.15	564.90	541.72	532.03
Cu	1.3	1.1	1.3	1.6	1.4	1.5
Zn	13.1	16.8	12.6	14.2	17.0	17.5
Fe	23.2	27.3	27.6	39.0	29.9	27.9
Mn	1.5	1.4	1.9	3.4	1.6	2.2
As	2.7	2.9	2.3	2.7	3.4	3.3
Cd	0.2	0.2	0.2	0.3	0.3	0.1
Pb	0.3	0.2	0.2	0.2	0.2	0.2
Hg	0.05	0.04	0.05	0.05	0.05	0.05

Table 5. The main sensory descriptors of mussels from Boka Kotorska Bay, Montenegro

	Ljuta	Orahovac	Lipci	Kukuljina	Obala Đurašević	Sv.nedjelja
Odor	4.0 ± 0.63^{A}	5.0 ± 0.0^{B}	4.0 ± 0.0^{A}	4.0± 0.63^{A}	4.0± 0.63^{A}	3.93 ± 0.41^{A}
Appearance	4.17± 0.41^{A}	5.0 ± 0.0^{B}	3.0 ± 2.83^{C}	4.0± 3.83^{AC}	4.0± 3.33^{AC}	4.0± 3.81^{AC}
Texture	4.0 ± 0.00^{A}	5.0 ± 0.0^{B}	3.83± 0.41^{A}	3.33± 0.52^{A}	3.33± 0.82^{A}	4.00 ± 0.89^{A}
Elasticity	3.5 ± 0.55^{A}	5.0 ± 0.00^{B}	3.03± 0.41^{C}	4.0 ± 0.00^{A}	3.83± 0.41^{A}	4.83± 0.41^{BD}

Values are given as Mean ± S.D. from eighteen samples from each sampling sitE. Different letters in the same row denote statistically significant difference (P <0.05) between groups.

Sensory analysis

The main sensory descriptors of mussels from Boka Kotorska Bay are listed in Table 4. In our study, we confirmed significant differences ($p < 0.05$) in sensory profiles between the mussels from Orahovac and those from the other sites. Orahovac mussels had the best ratings for odor, appearance and texture (5.0 ± 0.0).

DISCUSSION

The Boka Kotorska Bay is composed of several smaller bays and is the only part of the Southern Adriatic with jagged coastline which are influenced by specific hydrological and climatic conditions that are unique in Europe (Vukanic et al., 2016). The results of environmental monitoring indicated that the Boka Kotorska Bay seawater had a variable salinity, but similar temperature in harvesting areas that were investigated. It is important to know that this area is under the influence of freshwater inflows from numerous springs and submarine sources, which is the main factor contributing to a decline in salinity. During the sampling, the superficial waters (0–5 m) of the sampling sites showed similar temperature (15–18 °C). Since the optimum thermal range for this mussel species physiology is generally reported as 10–20 °C (Bayne, 1976), all sampling site had biological conditions for a suitable growth. Salinity was lower at Tivat Bay than at Kotor Bay (19.3 ppm vs 30.56 ppm, respectively), reflecting the lesser mixing processes in the water column reasonably attributable to its greater distance from the open sea. Several authors have highlighted the influence of extrinsic and intrinsic factors, such as water temperature and salinity, food availability and gametogenic cycle of animals on these parameters in mussels (Fernández et al. 2015; Henderikx, 2017; Cherifi et al., 2018). In areas where the eutrophication process is more pronounced microplankton population density was higher (Kotor Bay). Conversely, in Tivat Bay the microplankton populations was decreased due to the impact of the open sea (Vukanic et al., 2016). The mussel's shell is formed largely through deposition of ions, mostly calcium from the seawater and its changes in biometric characteristics is less susceptible to food availability and variety of extrinsic and intrinsic factors than mussel tissue (Alluno-Bruscia et al., 2001).In our study the mussels from Sv. Nedjelja displayed significantly higher morphometric values than others, and the mussels from Obala Đurašević had the maximum values of meat yield (26.39 ± 7.93) and condition index (18.9 ± 14.99) in spite of lower values of microplankton population in that part of Bay. The condition index and meat content in mussels are parameters of biological and technological interest, and they have a direct influence on the quality of the shellfish. The results of meat yield were comparable with previously reported data for cultivated mussels in Adriatic sea (25%) (Vernocchi et al., 2007), but they had lower values than mussels reported in the same period from different Spanish areas (> 30%) (Fuentes et al., 2009) and from the Gulf of Trieste (32.7%) (Bongiorno et al., 2015). The physical, chemical and microbiological stability of mussels strongly depends on the water activity of the meat. Mussels can be considered to be food products with high a_w (recorded a_w values were ≥ 0.95) and high pH (6.7–7.1) (Jay, 1996). In our study we have obtained results that are in accordance with those.

In this research, we obtained different results on biochemical composition between mussels from all sampling sites. Biochemical composition of mussels is affected by water temperature, nutrient availability, and the reproductive cycle of individuals (Fernández-Reiriz et al. 2007). Depending on the time of the year, levels of nutrients are different. In spring, mussels had a higher glycogen and protein content because they reflect the interaction between food supply, temperature, growth and reproductive cycle (Gallardi et al., 2014). In that time of the year, energy is stored for gametogenesis. Glycogen and proteins are considered the main energy substrate and they have a dominant role in bivalve metabolism for nutrient storage and to supply energy for gametogenesis (Baek et al., 2014). Our research was conducted in the spring and we also observed high levels of glycogen and protein concentrations. On the other side, changes in mussels composition are closely related to the food available. The highest chlorophyll levels have been recorded during spring, due to higher seawater temperatures (Irisarri et al., 2014).

Fatty acid composition is associated with seasonal periods and geographical origin. The results showed that of all fatty acids identified in all samples, the polyunsaturated fatty acids (PUFA) is the group with the highest percentage. This group of fatty acid, PUFAs (37.56 ± 1.12% to 41.08 ± 1.15%) were dominated followed by the monounsaturated FA (MUFA) (30.52 ± 1.97% to 38.31 ± 2.11%), while SFA levels were lower (21.89±3.51% to 29.45±2.21%). These results were similar to those reported by other authors (Bongiorno et al., 2015; Fernández et al., 2015). Moreover, these findings concur with studies of numerous bivalve species distributed in other regions of Europe and America. Within PUFAs, the C20:5n-3 and C22:6n-3 fatty acids showed the highest levels. The low levels of PUFA in mussels from Sv. Nedjelja probably is due to the reduced access to phytoplanktonic food. Phytoplankton represents the largest food source for bivalve molluscs and contains a high pro-

portion of polyunsaturated fatty acids of more than 20 carbons (Fernández-Reiriz et al., 1989). As discussed earlier, shellfish are rich in n-3 PUFA, with a ratio of n-3 to n-6 PUFA above 1.0 (Sriket et al., 2007). High levels of total n-3 and low levels of total n-6 PUFA were detected also in our samples and consequently high n-3/n-6 ratio values characterised the mussels cultivated in the Boka Kotorska Bay.

Mytilus galloprovincialis, like other bivalve molluscs, may also be considered a good source of nutritionally important minerals. Since these mussels are edible and marketed commercially, the presence of metals in high conentrations could limit the quantity of mussels that humans can consume, as excessive consumption of metal-contaminated mussels could be toxic to humans (Stankovic et al., 2012). In the mussel samples K, Mg, Ca, and Na concentrations were significantly higher than the remaining tested elements. Several studies have shown that K is usually found to have the highest concentration among the macro minerals in shellfish (Bilandzic et al.,2015, Manthey-Karl et al., 2015. Also, the reason for the high content of Ca and Mg in mussels is their requirement for the shell formation during the mussel growth (Bokori et al., 1995). Since mussels are seawater filter-feeders, their feeding depends on plankton. Phytoplankton are relatively rich in Ca, K and Mg content (Fujita 1971). Seafood provides a major source of Zn and Fe to humans, which are important elements in their diet, but in excess may be harmful for human health (Plum et al., 2010). Comparing data from the literature with the present results showed that concentrations of Zn were lower to those reported for Albanian coast (Cullaj et al., 2007), Croatia (Kljakovic-Gaspic et al., 2006), and Slovenia (Ščančar et al., 2007).

The amount of As, Pb, Hg and Cd in marine organisms reflects the degree of metal pollution in the aquatic environment (Stankovic et al., 2011). Contaminants in mussels have been examined from the standpoint of food safety, and in some places, including the Adriatic Sea, the levels of contaminants exceeded health standards, (Giusti and Zhang, 2002, Kljakovic-Gaspic et al., 2006). In comparison with the permissible limits set by the Montenegrin Food Regulation (2002) for total Hg (1.0 mg kg-1), Cd (1.0 mg kg-1), Pb (1.0 mg kg-1), all concentrations of these metals from all locations were lower than the legislated limits.

The main sensory descriptors of mussels were those related to appearance, odor, texture and, to lesser extent, elasticity. The differences in the assessment of "orange color", "firmness" or "consistency" in fresh mussels have a biological explanation since these are the result of different natural stimuli (Costell and Durán, 2005). Our evaluation coincides with Caglak et al. (2008), who suggested that a numeric acceptability scale from 0 to 5 points is suitable to evaluate fresh mussels. The qualitative sensory assessment showed that all mussels were acceptable. All mussels in the current study achieved scores of 3 or more out of 5 in the qualitative category. From a marketing perspective, the mussels cultured in the Boka Kotorska Bay could be accepted well by consumers, and these shellfish fulfill the minimum requirements to fit and compete in the existing local market.

CONCLUSION

This is the first comprehensive report of biometric, sensory and nutritional assessment of mussels harvested in this part of Southern Adriatic Sea (Montenegro). In general, mussels from the Bay of Kotor have shown higher nutritional quality and better sensory and biometric characteristics than mussels from the Bay of Tivat. Consequently, recommendation for mussel growers from Montenegro is to concentrate their production on this area. In addition, the study determined that mussels cultured in the Boka Kotorska Bay could compete with other currently available mussels from other Adriatic Sea locations. The data obtained from the present study and further microbiological studies on the mussels from these locations can confirm the quality of these products and can be valuable for estimating the cost-effectiveness of mussel production and to support marketing of mussels to consumers.

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CONFLICT OF INTEREST

None declared by the authors.

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