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MORPHOMETRY AND CONDITION INDEX IN MEDITERRANEAN MUSSELS (*MYTILUS GALLOPROVINCIALIS* LAMARCK, 1819) FROM BOKA KOTORSKA BAY (MONTENEGRO, SOUTHEAST ADRIATIC SEA)

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ABSTRACT

Mediterranean mussel is the main farming bivalve species in the area of Boka Kotorska Bay. In this paper results about biometric parameters of cultivated Mediterranean mussel in the area of Boka Kotorska Bay are present. Research was performed on two farms in Orahovac and Kamenari since January 2015 up to January 2016. Biometric parameters indicate morphological plasticity, but further and detailed analysis as morphological ratios are required. Condition index (CI) was calculated according to three different methods. All three methods gave similar results and indicate on spatial and temporal CI variation. The lowest CI was during September, while the highest was during February/March. Very strong and strong correlation between all three methods are obtained. Methods based on wet meat weight are reliable for temporal and spatial CI interpretation in mussels.

Keywords: biometry, condition index, *Mytilus galloprovincialis*, Boka Kotorska Bay

INTRODUCTION

Aquaculture is the fastest growing food production sector in the world. Intense growth of the human population and fact that food availability is decreasing worldwide, make aquaculture sector as important food source in the near future. Marine aquaculture production in the period 2009–2014 was 23.9 million tonnes per year (FAO, 2016a; 2016b), and marine bivalves took 14% of global marine production (Wijsman *et al*., 2019).

Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) is the main farming bivalve species in area of

Boka Kotorska Bay, as well as in the Adriatic Sea. Since 1960s experiments regarding mussel farming started in Boka Kotorska Bay (Stjepčević, 1968), while commercial farming started about thirty years ago. Today, there are about twenty mussel farms in the Boka Kotorska Bay, all using long-lines system (Mandić *et al*., 2016; Gvozdenović *et al*., 2017), with annual production of 179 tonnes (MONSTAT, 2020).

Mussels from genus *Mytilus* are an important source of proteins, essential vitamins, minerals and fatty acids (Simopoulos & Cleland, 2003; Fuentes *et al*., 2009; Grienke *et al*., 2014), known to have benefits for human health. Biochemical composition and condition index (CI) are important indicators of nutritional and commercial quality of bivalves (Orban *et al*., 2002). Župan & Šarić (2014) indicate that besides CI, growth is also important factor in mussel farming.

CI presents the percentage of meat in the shell and regarding Prgić (2019) CI reflects physiological and bio-energetic changes in an organism. Gosling (1992) reported food availability and changes in the reproduction phases as the most important factors affecting CI in bivalves. As Okumuș & Stirling (1998) indicate that harvesting mussels season should be in the period when CI values are the highest, data about annual CI fluctuations are very important and useful for farmers.

Among bivalves, different methods for CI calculation exist, based on wet, dry or cooked meat weight. According to Davenport & Chen (1987) three the most reliable methods are based on cooked and dry meat (equations 1, 2, 3). The same authors also suggest other methods which can be used (equations 4, 5, 6, 7). According to Almeida *et al*. (1999), CI can be calculated as ratio between wet meat weight and total weight, which is actually meat yield index (equation 8). Hickman & Illingworth (1980) also suggest method based on wet meat weight (equation 9). Opposite of all authors, Dabrowska *et al*. (2013) as well as Ruessler *et al*. (2011) suggest methods which represent ratio of wet/dry meat weight and shell length (equations 10, 11).

$$CI = \frac{\text{Cooked meat weight}}{\text{Cooked meat weight + shell weight}} \times 100 \quad (1)$$

$$CI = \frac{\text{Dry meat weight}}{\text{Total volume} - \text{shell volume}} \times 100 \quad (2)$$

$$CI = \frac{\text{Dry meat weight}}{\text{Shell weight}} \times 100 \quad (3)$$

$$CI = \frac{\text{Cooked meat weight}}{\text{Total wet weight}} \times 100 \quad (4)$$

$$CI = \frac{\text{Wet meat weight}}{\text{Total volume} - \text{shell volume}} \times 100 \quad (5)$$

$$CI = \frac{\text{Wet meat weight}}{\text{Shell weight}} \times 100 \quad (6)$$

$$CI = \frac{\text{Wet meat volume}}{\text{Total volume} - \text{shell volume}} \times 10\,0 \quad (7)$$

$$CI = \frac{\text{Wet meat weight}}{\text{Total weight}} \times 100 \quad (8)$$

$$CI = \frac{\text{Wet meat weight}}{\text{Total weight} - \text{shell weight}} \times 100 \quad (9)$$

$$CI = \frac{\text{Wet meat weight}}{\text{Shell length}^3} \times 100 \quad (10)$$

$$CI = \frac{\text{Dry meat weight}}{\text{Shell length}} \times 100 \quad (11)$$

MATERIAL AND METHODS

The study was conducted on two farms in Boka Kotorska Bay, Montenegro (Fig. 1). One farm was fish and mussel farm located in Orahovac (42° 29′ 07.79″ N, 18° 44′ 42.47″ E), while the other one was mussel farm located in Kamenari (42° 27′ 30.89″ N, 18° 40′ 21.42″ E).

Mussels of similar size and ages (N=1040 individuals) were sampled from the experimental farm placed near the Institute of Marine Biology (42° 26′ 12.73″ N, 18° 45′ 48.45″ E). All sampled individuals were cleaned of fouling organisms and placed in 26 nylon mesh nets (40 individuals per mesh net). On both farms, Orahovac and Kamenari, 13 mesh nets with mussels were placed at depths between 2 and 3 m. Once per month, since January 2015 up to January 2016, one net from each farm was taken for analysis. From each net, 30 mussel individuals were separated and processed in the laboratory the same day. Every month, temperature and salinity were recorded at depth between 2 and 3 m on both farms, using the Multiline P4 WTW probe.

Following biometric parameters were measured: shell width (SWi), shell height (SH), shell length (SL), total weight (TW), wet meat weight (WMW), shell weight (SWe), as well as condition index (CI). SWi was measured as maximal lateral axis, SH as maximal dorso-ventral axis, and SL as maximal anterior-posterior axis (Prgić, 2019). Shell measurements were taken using vernier caliper to the nearest 0.01 mm, while weighing was done by balance to the nearest 0.01 g. The gender of each individual was determined based on gonads color (Dardignac-Corbel, 1990; Gosling, 2003). CI was calculated according three different methods suggested by Hickman & Illingworth (1980) – CI_1, Almeida *et al.* (1999) – CI_2, and Dabrowska *et al.* (2013) – CI_3. Different CI methods were compared by the linear regression as described by Gavrilović *et al.* (2012).

Descriptive statistic (average and standard deviation) of biometric parameters, as well as linear regression are done in Microsoft Excel Program 2013. Correlation coefficient – R^2 is ranged as: $R^2 > 0.70$ (very strong correlation), $R^2 = 0.40–0.69$ (strong correlation), $R^2 = 0.30–0.39$ (moderate correlation), $R^2 = 0.20–0.29$ (weak correlation), $R^2 = 0.01–0.19$ (no or negligible correlation) (https://www.statisticshowto.com/probability-and-statistics/correlation-coefficient-formula/).

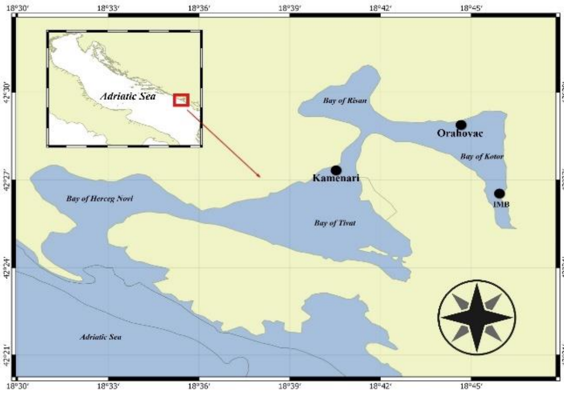

Figure 1. Black circles show sampling locations, farms in Orahovac and Kamenari and farm in front of the Institute of Marine Biology (IMB)

RESULTS AND DISSCUSION

Temperature and salinity fluctuations during investigated period on both farms are given in Table 1. Minimum temperature was recorded on farm in Kamenari 10.5 °C during January 2016, while maximum temperature was recorded on farm in Orahovac 27.9 °C during July 2015. Minimum salinity was on farm in Orahovac 13.8 ‰ during February 2015, while maximum salinity was on farm in Kamenari 37.1 ‰ during December 2015.

Temperature and salinity showed typical monthly variations depending on different climate conditions. The lowest values were during winter months and the highest during summer months, what is also obtain by other authors for Boka Kotorska Bay (Drakulović *et al*., 2014; Pestorić *et al*., 2014). Quite low salinity, e.g. during February 2015 especially on Orahovac farm, can be explained by intensive influx of fresh water. According to Bellafiore *et al*. (2011) inner part of the Bay (where Orahovac farm is located) is under a significant influence of underwater freshwater sources and freshwater influx form the land which have great impact on salinity, especially on the surface layer. Drakulović *et al*. (2014) also obtained very low salinity on the surface water layer in Orahovac during winter months. Interesting fact is that the maximum salinity on both farms was during December 2015, what is not usual. This has been happened most likely because quite warm and dry period during autumn with absence of rain.

In total, 780 mussel individuals were processed (390 individuals from each farm). Descriptive statistics (average ± standard deviation) of biometric parameters (TW, WMW, SWe, SWi, SH, SL), as well as number of females and males, among each investigated month and farm is given in Table 2. Spatial and temporal differences among all parameters are present. Those differences indicate on morphological plasticity in mussels. Morphological plasticity is a strategy to mitigate the effects of intra-specific competition at the individual level (Cubillo *et al*., 2012). Mussels are characterized by high morphological plasticity as a result of variations in local environmental conditions what is showen by Cubillo *et al*. (2012) and Prgić (2019).

Compared to wild population, mussels growth and shell morphology in suspended culture are under high impact of cultivation density. Cultivation density is recognized as very important factor affecting shell shape (Lauzon-Guay *et al*., 2005; Cubillo *et al*., 2012) and growth (Gascoigne *et al*., 2005; Filgueira *et al*., 2008) in mussels. For better interpretation of morphological plasticity, morphological ratios as SWi/SL, SH/SL, SH/SWi, SL/TW should be considered in future investigation.

Table 1. Temperature and salinity values on investigated farms (T–Temperature; S–Salinity)

	Orahovac		**Kamenari**	
	T (°C)	**S (‰)**	**T (°C)**	**S (‰)**
Jan´15	12.4	21.3	11.3	23.8
Feb´15	11.6	13.8	12.0	19.8
Mar´15	13.0	18.6	12.7	26.4
Apr´15	16.3	31.3	18.0	30.0
May´15	20.2	30.9	19.0	31.0
Jun´15	22.8	31.0	23.5	33.5
Jul´15	27.9	34.8	24.0	35.0
Aug´15	26.7	35.1	26.5	36.4
Sep´15	24.3	31.0	24.1	36.0
Oct´15	15.7	21.4	19.4	31.1
Nov´15	15.6	20.8	17.7	36.1
Dec´15	16.0	35.2	17.2	37.1
Jan´16	12.2	28.9	10.5	21.0

There was slightly more females than males (214 females, 175 males) on Orahovac farm, while one individual was undetermined. On the other hand, in Kamenari farm ratio between females and males was the same (194 females,195 males) and also one individual was undetermined. Similar results are obtained for mussel populations in other areas (Sunila, 1981; Da Ros *et al*., 1985; Toro *et al*., 2002; Suárez *et al*., 2005; Bhaby *et al*., 2014; Bhaby, 2015). Regarding Gosling (2003) mussels are dioecious species i.e. the sexes are separate, and there are usually equal numbers of males and females, what our results confirmed.

According to Hickman & Illingworth (1980) method (CI_1) the highest values on both farms were during February 2015 (Orahovac 52.74; Kamenari 48.73). The lowest CI values on both farms were during September 2015 (Orahovac 31.23; Kamenari 31.42) (Fig. 2).

Table 2. Biometric parameters of mussels among investigated months and farms (average ± standard deviation) (TW – total weight; WMW – wet meat weight; SWe – shell weight; SWi – shell width; SH – shell height; SL – shell length; F – female; M – male)

	Orahovac						
	TW (g)	**WMW (g)**	**SWe (g)**	**SWi (cm)**	**SH (cm)**	**SL (cm)**	**Gender**
Jan´15	11.65±2.87	3.52±1.12	4.25±0.95	2.75±0.24	1.87±0.19	5.12±0.45	17F, 13M
Feb´15	9.10±3.09	2.99±1.12	3.20±0.98	2.56±0.29	1.69±0.24	4.62±0.57	15F, 15M
Mar´15	11.03±3.45	2.96±0.90	3.63±1.11	2.70±0.24	1.74±0.19	4.89±0.47	13F, 17M
Apr´15	13.18±4.06	3.24±1.12	4.40±1.33	2.83±0.32	1.90±0.22	5.22±0.53	20F, 10M
May´15	15.74±3.93	4.63±1.29	5.75±1.32	3.08±0.30	2.06±0.20	5.56±0.48	15F, 15M
Jun´15	18.26±3.77	5.95±2.05	6.27±1.39	3.17±0.22	2.15±0.22	5.96±0.52	19F, 11M
Jul´15	11.75±3.48	2.58±0.82	4.32±1.29	2.72±0.25	1.80±0.20	4.98±0.45	12F, 18M
Aug´15	15.69±4.28	3.88±1.30	5.84±1.39	2.97±0.28	1.97±0.22	5.47±0.56	16F, 14M
Sep´15	21.59±6.03	4.04±1.43	7.04±1.71	3.22±0.30	2.24±0.28	6.23±0.61	19F, 11M
Oct´15	21.29±7.69	6.50±2.42	7.37±2.75	3.09±0.30	2.22±0.30	6.05±0.75	16F, 13M, 1?
Nov´15	21.29±6.39	6.41±3.00	6.54±2.09	3.21±0.26	2.21±0.25	6.15±0.53	17F, 13M
Dec´15	23.72±4.83	7.42±1.93	7.98±2.40	3.30±0.21	2.29±0.21	6.42±0.37	17F, 13M
Jan´16	20.50±4.41	5.97±1.49	6.71±1.54	3.13±0.25	2.15±0.22	6.08±0.45	18F, 12M

	Kamenari						
	TW (g)	**WMW (g)**	**SWe (g)**	**SWi (cm)**	**SH (cm)**	**SL (cm)**	**Gender**
Jan´15	9.31±2.58	2.54±0.67	3.44±0.82	2.64±0.19	1.75±0.18	4.92±0.46	14F, 16M
Feb´15	9.04±2.65	2.88±1.00	3.07±0.75	2.55±0.26	1.67±0.22	4.64±0.53	15F, 15M
Mar´15	11.20±2.91	3.45±1.11	3.87±0.99	2.73±0.24	1.77±0.20	4.87±0.47	17F, 13M
Apr´15	12.41±2.21	3.37±0.85	4.16±0.80	2.89±0.18	1.88±0.14	5.11±0.32	10F, 20M
May´15	17.53±4.95	4.74±1.68	6.40±1.80	3.14±0.25	2.07±0.26	5.75±0.53	11F, 19M
Jun´15	18.60±4.39	5.53±1.94	6.63±1.39	3.24±0.25	2.09±0.24	5.88±0.59	17F, 13M
Jul´15	21.80±4.52	5.14±1.83	7.25±1.46	3.37±0.26	2.17±0.19	5.94±0.45	13F, 17M
Aug´15	17.69±4.50	4.34±1.49	6.84±1.58	3.11±0.28	2.05±0.22	5.62±0.53	9F, 21M
Sep´15	18.51±3.63	3.58±0.86	7.05±1.35	3.19±0.24	2.06±0.15	5.71±0.37	15F, 14M, 1?
Oct´15	20.48±4.38	5.18±2.07	7.72±1.56	3.35±0.24	2.13±0.19	5.92±0.51	17F, 13M
Nov´15	22.67±4.20	5.51±1.65	8.07±1.55	3.33±0.19	2.27±0.23	6.09±0.36	17F, 13M
Dec´15	24.89±5.80	6.14±1.53	8.86±2.27	3.48±0.63	2.35±0.23	6.30±0.56	16F, 14M
Jan´16	30.25±4.41	8.56±2.01	11.02±2.09	3.66±0.15	2.54±0.20	6.78±0.45	23F, 7M

According to Almeida *et al.* (1999) method (CI_2) the highest values on both farms were during February 2015 (Orahovac 33.45; Kamenari 31.54). The lowest CI values were during September 2015 on both farms (Orahovac 19.28; Kamenari 19.35) (Fig. 3). According to Dabrowska *et al.* (2013) method (CI_3) the highest value on farm in Orahovac was during February 2015 – 2.96, while the highest value on farm in Kamenari was during March 2015 – 2.90. The lowest CI values were during September 2015 on both farms (Orahovac 1.68; Kamenari 1.90) (Fig. 4).

Spatial and temporal variations in mussels CI are evident, and quite similar results are obtained by all three CI methods. Mitrić *et al.* (2016) also suggest on spatial and temporal variations of mussels CI in Boka Kotorska Bay. Annual variation in bivalve CI are natural and are the results of various factors such as: temperature, salinity, oxygen concentrations, food availability, changes in the reproductive cycles (Hrs-Brenko, 1973; Marguš & Teskeredžić, 1984; Gosling, 1992; Çelik *et al.*, 2012). Considering the fact that mussels are good examples among bivalves with a flexible reproductive strategy, adjusting their cycle according to prevailing environmental conditions (Gosling, 2003), it is not unusual that CI in mussels from the same locality varies one year to another. Regarding Gvozdenović (2020) in area of Boka Kotorska Bay ripe gonads in mussels appear during late autumn and winter, spawning happened in winter and spring, while during summer period (especially during August) the most individuals are in resting/inactive stadium. Those results are in accordance to the CI results obtained in this study, indicating that higher CI appear when individuals are in ripe stadium, and CI declines appear after spawning and during resting stadium.

According all three methods, greater CI throughout the most of the investigated period

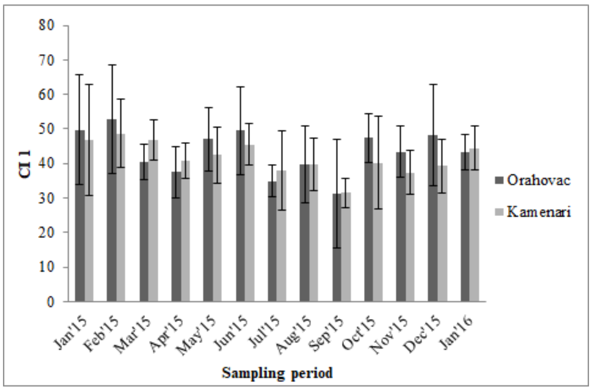

Figure 2. Average monthly CI and standard deviation during investigated period according to method suggested by Hickman & Illingworth (1980)

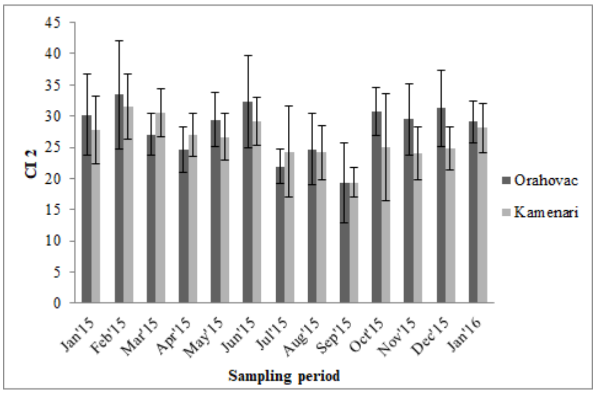

Figure 3. Average monthly CI and standard deviation during investigated period according to method suggested by Almeida *et al.* (1999)

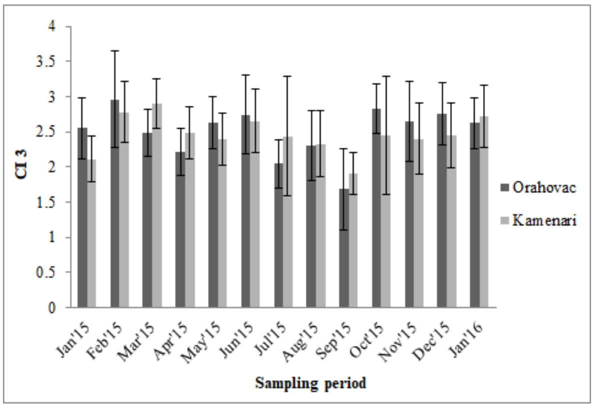

Figure 4. Average monthly CI and standard deviation during investigated period according to method suggested by Dabrowska *et al.* (2013)

was on farm in Orahovac. Comparison between farms according to all three methods showed the same pattern expect during August 2015 and January 2016 (Tab. 3).

Table 3. Comparison between farms according to all three CI methods (Orah. – Orahovac; Kam. – Kamenari)

	CI_1	CI_2	CI_3
Jan´15	Orah.>Kam.	Orah.>Kam.	Orah.>Kam.
Feb´15	Orah.>Kam.	Orah.>Kam.	Orah.>Kam.
Mar´15	Orah.<Kam.	Orah.<Kam.	Orah.<Kam.
Apr´15	Orah.<Kam.	Orah.<Kam.	Orah.<Kam.
May´15	Orah.>Kam.	Orah.>Kam.	Orah.>Kam.
Jun´15	Orah.>Kam.	Orah.>Kam.	Orah.>Kam.
Jul´15	Orah.<Kam.	Orah.<Kam.	Orah.<Kam.
Aug´15	Orah.>Kam.	Orah.>Kam.	Orah.<Kam.
Sep´15	Orah.<Kam.	Orah.<Kam.	Orah.<Kam.
Oct´15	Orah.>Kam.	Orah.>Kam.	Orah.>Kam.
Nov´15	Orah.>Kam.	Orah.>Kam.	Orah.>Kam.
Dec´15	Orah.>Kam.	Orah.>Kam.	Orah.>Kam.
Jan´16	Orah.<Kam.	Orah.>Kam.	Orah.<Kam.

Greater CI on farm in Orahovac during the most of the investigated period can be attributed to fact that this is IMTA farm (Integrated multi-trophic aquaculture) where mussels are farmed together with two fish species (*Dicentrarchus labrax* (Linnaeus, 1758) and *Sparus aurata* Linnaeus, 1758) and most probably are additionally feed on the nutrients originating from fish farm. Higher CI in mussels from integrated farming compare to monoculture or referent locations is also obtained by other authors (Peharda *et al*., 2007; Sará *et al*., 2009; Lander *et al*., 2012; Bajnoci, 2014; Irisarri *et al*., 2014; Župan *et al*., 2014).

The correlation between different CI methods, obtained by linear regression, are present in Table 4. Results showed very strong correlation (R^2 = 0.936) among CI_1 and CI_2 method, as well as among CI_2 and CI_3 (R^2 = 0.821), while strong correlation is observed among CI_1 and CI_3 methods (R^2 = 0.693). Gavrilović *et al*. (2012) in *Chamelea gallina* found very strong correlation among all five CI methods, all obtained R^2 values were above 0.990, even 1, suggesting that based on the knowledge of CI obtained by one method, it is possible to determine values of other CI methods with great precision. Authors also pointed that methods based on dry meat weight are more precise compare to wet meat weight methods for CI calculation in *C. gallina*. Phernambucq & Vroonland (1983) compared four different CI methods in *Ostrea edulis*, all based on dry meat weight, and results also showed very strong correlation among used methods. The same authors suggest that correlations between methods may vary during the year, influenced by seasonal cycle of oysters CI. In contrast to above mentioned authors, who conducted their research on seasonal scale or just during one season, our results are more detailed and implied CI during 13 months among two localities in area of Boka Kotorska Bay.

Table 4. Correlation among three different CI methods interpreted as equation obtained by linear regression ($y = a + bx$; $y = bx$, when $a = 0$); R^2 – correlation coefficient

Method	**Linear regression equation**	**R^2**
$CI_1 \times CI_2$	$y = 1.5651x$	0.936
$CI_2 \times CI_3$	$y = 10.961x$	0.821
$CI_1 \times CI_3$	$y = 17.148x$	0.693

We consider that methods based on wet meat weight are reliable for temporal and spatial CI interpretation in bivalve, especially meat yeald index suggested by Almeida *et al*. (1999). In contribution to this fact are results obtained by Chelyadina *et al*. (2018) who suggest that this method is recommended to farmers, as it is easily applied and the most representative to determine the harvest time of mussels (mussels have been shown to acquire optimal commercial quality if the meat yield index is more than 20%). The same authors proven that it is sufficient to use total weight of only ten mussels for convenient determination of the meat yield index.

CONCLUSIONS

Results of this study indicate on morphological plasticity in mussels. For better interpretation, morphological ratios (SWi/SL; SH/SL; SH/SWi; SL/TW) should be included in future investigation, as well as comparison with wild populations. Obtained results also showed spatial and temporal differences in mussels CI in area of Boka Kotorska Bay. All three CI methods showed quite similar spatial and temporal results, and correlation between methods were strong. Although, the most used and suggested CI methods in the literature are those based on dry meat, we consider that methods based on wet meat are also reliable for temporal and spatial CI interpretation, especially method suggested by Almeida *et al*. (1999). As this method is used as an indicator of the market value in *Ostrea edulis* (Fleury *et al*., 2003), we consider that it should be further used as market value indicator among commercial important bivalves, including mussels, because of easy application and representativeness.

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MORFOMETRIJA I KONDICIONI INDEKS KOD MEDITERANSKE MUŠULJE (*MYTILUS GALLOPROVINCIALIS* LAMARCK, 1819) U BOKOKOTORSKOM ZALIVU (CRNA GORA, JUGOISTOČNI JADRAN)

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SAŽETAK

Mediteranska mušulja je glavna uzgojna školjka u oblasti Bokokotorskog zaliva. U ovom radu su predstavljeni rezultati biometrijskih karakteristika mediteranske mušulje uzgajane u oblasti Bokokotorskog zaliva. Istraživanje je sprovedeno na dva uzgajališta u Orahovcu i Kamenarima od januara 2015. do januara 2016. godine. Vrijednosti biometrijskih karakteristika ukazuju na morfološku plastičnost ove vrste, ali su neophodne sistematičnije i detaljnije analize koje uključuju odnose morfoloških karaktera. Kondicioni indeks je izračunat na osnovu tri različite metode. Rezultati sve tri metode su dali slične rezultate i ukazuju kako na prostorne tako i na vremenske razlike. Najniže vrijednosti kondicionog indeksa su bile tokom septembra, dok su najvisočije vrijednosti bile tokom februara/marta. Vrlo jaka i jaka korelacija je uočena između sve tri korišćene metode. Metode bazirane na masi mokrog mesa su pouzdane za prostornu i vremensku interpretaciju kondicionog indeksa kod mušulja.

Ključne riječi: biometrija, kondicioni indeks, *Mytilus galloprovincialis*, Bokokotorski zaliv