Studia Marina 2019, 32 (2): 52-64 UDC: 543.31:561.23]:597(497.16Boka Kotorska)
Original scientific paper DOI: 10.5281/zenodo.3584222

Assessment of microbial sea water quality and health status of farmed European seabass (*Dicentrarchus labrax*) in Eastern Adriatic Sea (Montenegro and Croatia)

Damir KAPETANOVIĆ[1*], Ana GAVRILOVIĆ[2], Jurica JUG-DUJAKOVIĆ[3], Irena VARDIĆ SMRZLIĆ[1], Snježana KAZAZIĆ[1], Mirjana BOJANIĆ - RAŠOVIĆ[4], Anamarija KOLDA[1], Ana PEŠIĆ[5], Lorena PERIĆ[1], Jakov ŽUNIĆ[1], Milica MANDIĆ[5], Aleksandar JOKSIMOVIĆ[5], Darija VUKIĆ LUŠIĆ[6], Mirko ĐUROVIĆ[5]

[1]Institute Ruđer Bošković Zagreb, Croatia; e-mail: kada@irb.hr
[2]Faculty of Agriculture, University of Zagreb, Croatia;
[3]Sustainable Aquaculture Systems Inc. Frenchtown, USA;
[4]Biotechnical Faculty, University of Montenegro Podgorica, Montenegro;
[5]Institute of Marine Biology, University of Montenegro Kotor, Montenegro;
[6]Faculty of Medicine, University of Rijeka, Croatia.

ABSTRACT

Semi-enclosed bays are generally considered important environmental resources for mariculture. This study was conducted within cage fish farms in two semi-enclosed bays of the Eastern Adriatic Sea, at Boka Kotorska Bay (Montenegro), and at Mali Ston Bay (Croatia). A total of 16 sea water samples, and 46 swabs from the gills and skin of European seabass were collected from two sea bass farms during two samplings in autumn and spring. The aim of this study was to determine the health status of European seabass in Montenegro and Croatia, to evaluate the environmental conditions in both farming areas, and to assess the presence of *Vibrio* and its impact on fish health. Most of the isolated bacteria from the samples were Gram-negative and comprised of *Vibrio*, *Photobacterium* and *Pseudomonas* genera. Assessment of fish health status showed that fish were clinically healthy and results present a bacterial community associated with healthy farmed European seabass. At the same time, some of the isolated bacterial strains are known to be pathogenic (*V. alginolyticus, V. harveyi, V. anguillarum, P. damselae*), and present a potential reservoir of infection. Simultaneous survey confirmed microbial impact in both bays due to an anthropogenic influence. The approach presented in this study is valuable in the assessment of farming conditions in semi-enclosed aquatic environments and is easily applicable to other similar locations worldwide**.**

Keywords: European sea bass, Boka Kotorska Bay, Mali Ston Bay, *Vibrio*, *Photobacterium*

INTRODUCTION

European sea bass (*Dicentrarchus labrax* (Linnaeus, 1758)) is one of the main aquacultured finfish in the Europe with an annual production of around 148.367 tons in 2014 (Vendramin *et al*., 2016). Aquaculture is an important industrial sector for both Montenegro and Croatia, where marine fish culture is dominated by European sea bass.

The incidence of diseases and main bacterial pathogens obtained in farmed European sea bass from Croatian marine farms have been well documented in several publications (Vendramin *et al*., 2016; Zrnčić *et al*., 2015; Oraić & Zrnčić, 2005; Haenen *et al*., 2014; Mladineo *et al*., 2016; Gavrilović *et al*., 2012). In contrast to reports on the microbiology in Croatian European sea bass farming, little is known about cultured sea bass in Montenegro despite its economic importance. There is a shortage of information available for disease management and microbiology in Montenegro mariculture (Joksimović, 2007, 2010, Mandić *et al*., 2017; Pešić *et al*., 2015, 2016).

Fish diseases of bacterial origin are one of the most important factors of economic losses in the European sea bass farming (Vendramin *et al*., 2016). That highlights the need for the insight of the bacterial community naturally associated with farmed European sea bass in Montenegro and possible health problems in their mariculture.

The aim of this study was to simultaneously determine the health status of the European sea bass in Croatia and Montenegro for the first time, to evaluate the environmental conditions affecting farming of this species, and to obtain more knowledge about presence of species of *Vibrio* genus and their impact on the health of farmed fish.

MATERIALS AND METHODS

Sampling

The study areas (Fig. 1) are located in the Eastern part of the Adriatic Sea in Croatia, and in Montenegro. Simultaneous sampling was carried out in two semi-enclosed bays situated on the southern coast of Croatia - Mali Ston Bay, and the coast of Montenegro - Boka Kotorska Bay. The distance between these two locations is approximately 100 km. Mali Ston Bay is characterized by influence of Neretva River in the outer part of the bay, and underwater springs in its inner part (Viličić *et al*., 1998.). Similarly, Boka Kotorska Bay is characterized by influence of the karstic rivers (Škurda, Ljuta, Široka River, Gradiošnica and Sutorina) and underwater springs, which influence temperature, salinity and species distribution (RAC/SPA - UNEP/MAP, 2013). Two samplings were carried out at both locations in autumn 2017 and spring 2018.

Samples of the seawater were collected at four depths (0.5 m, 5 m and 10 m below surface, and 0.5 m above bottom) in sterilized plastic 0.5-l bottles using an 8-l Niskin sampler. Seawater samples were serially diluted prior to the analysis using sterile Phosphate Buffered Saline (PBS) (Sigma–Aldrich). All bacterial analyses were performed in duplicate.

Seawater analysis

Fecal indicators were identified by using a defined substrate technology using Colilert-18 (IDEXX) for total coliforms and *E. coli*, and by using Enterolert-E (IDEXX) for enterococci. The enumeration of total coliforms, *E. coli* and enterococci was obtained using the Quantitray2000 (IDEXX), which utilizes 97 wells-test systems and provides the most probable numbers (CFU/100 ml).

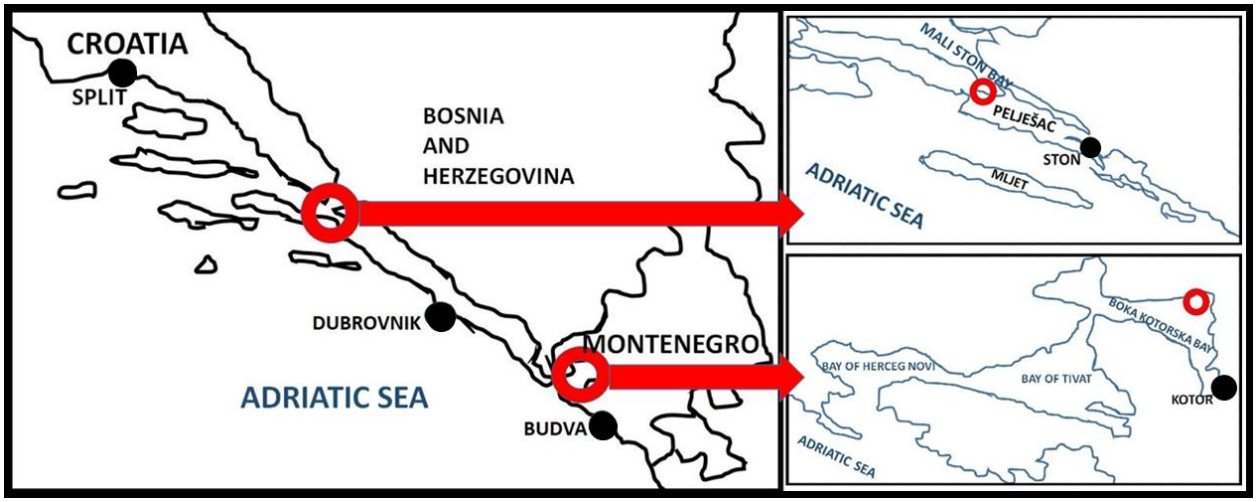


Figure 1. Study area: sampling locations of European seabass in the Mali Ston Bay (Croatia) and Boka Kotorska Bay (Montenegro)

Fecal coliforms were calculated based on determined number of *E. coli* (Surfrider Fundation, 2003) using the formula Fecal coliforms = *E. coli* x 1.25.

For enumeration of marine heterotrophic bacteria – HPC serially diluted seawater samples were spread-plated onto Difco™ Marine Agar 2216 BD (BD DifcoTM), and plates were incubated at 22°C.

Vibrio was isolated using the spread plate method on Thiosulphate Citrate Bile Salt Sucrose (BD Difco^{TM}) – TCBS Agar and Incubation for 24 to 48 h at 22°C and 35°C. Identification of isolated *Vibrio* was done by Bruker Microflex LT MALDI TOF mass spectrometer equipped with the Bruker Biotyper 3.0 software (Bruker Daltonics, Bremen, Germany).

Assessment of fish health status

A total of 23 European sea bass were examined from two marine fish farms in the Eastern Adriatic Sea, in Croatia and in Montenegro, during autumn 2017 and spring 2018. Assessment of the health status of farmed fish included clinical examination and necropsy. Gills and skin below the dorsal fin were swabbed over a 1cm^2 area (Kapetanović *et al*., 2013) prior to clinical examination. Swab samples from the skin and gills were taken by sterile swab sticks with a 1 cm long cotton apex (Deltalab). Swab samples were diluted in 10 ml of sterile PBS (Sigma–Aldrich) in tubes, and then stirred and agitated.

Undiluted and 10-fold diluted swab samples were then plated onto an appropriate medium for bacterial isolation. To isolate *Vibrio*, samples were inoculated onto selective TCBS Agar (BD Difco^{TM}) in duplicate and incubated at 22°C and 35°C. To isolate heterotrophic bacteria, samples were inoculated onto non-selective Difco™ Marine Agar 2216 (BD Difco^{TM}), and plates were incubated at 22°C. Clonies of different morphology that grown on the plates were randomly picked (to pick many different phenotypes) and restreaked onto plates three times to obtain pure culture. Such isolated colonies were characterized and identified using the MALDI TOF mass spectrometry.

Antimicrobial susceptibility

The antimicrobial susceptibility of isolated strains was determined by Kirby-Bauer disk

diffusion method on Difco™ Mueller Hinton II agar (BD DifcoTM) using the discs obtained from the same manufacturer. The following antimicrobial discs with their concentrations given in parentheses were used in antibiogram: ampicillin (10µg), streptomycin (10µg), gentamicin (10µg), imipenem (10µg), chloramphenicol (30µg), florfenicol (5µg), ciprofloxacin (5µg), enrofloxacin (5µg), erythromycin (15µg), oxytetracycline (30µg), sulfamethoxazole/trimethoprim (23.75/1.25µg), vancomycin (30µg) and flumequine (30µg).

The inoculum for antimicrobial susceptibility testing was prepared in 5 ml of sterile 0.85% Suspension medium (bioMérieux), and turbidity was adjusted to 0.5 MacFarland's standard using Vitek Systems ATB 1550 (bioMérieux). Inoculated plates were inverted and incubated at 22°C during 24–48 h. The diameter of the zones of inhibition was read twice at right angles with a ruler graduated to 0.5 mm, and expressed by referring to the manufacturer standard table and reported as susceptible, intermediate or resistant.

Data processing and statistical analyses

For each microbial indicator, the average level was calculated from the data determined at four depths to obtain representative information for the entire seawater column in autumn and spring. Statistical analyses (descriptive statistics, Mann-Whitney test) were performed using SigmaStat statistical package. The obtained differences were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSION

Seawater analysis

The results obtained at four sampling depths (0.5 m, 5 m and 10 m below surface, and 0.5 m above bottom) at both sampling locations didn't show any significant differences between values of analyzed bacterial indicators. Based on that, at each sampling, bacterial indicators counts were averaged over the four depths to obtain a mean value for the water column.

HPC and *Vibrio* counts were higher in autumn 2017 than in spring 2018 at both marine fish farms (Tab. 1; Fig. 2a), although the differences achieved statistical significance only for HPC obtained at 22°C (Mann–Whitney test, $p<0.05$). Generally, marine fish farm located in the Mali Ston Bay had higher HPCs and *Vibrio* counts compared to marine fish farm located in the Boka Kotorska Bay. However, the differences were statistically significant for HPC at 22°C obtained in 2017 and 2018 (Mann–Whitney test, $p<0.05$).

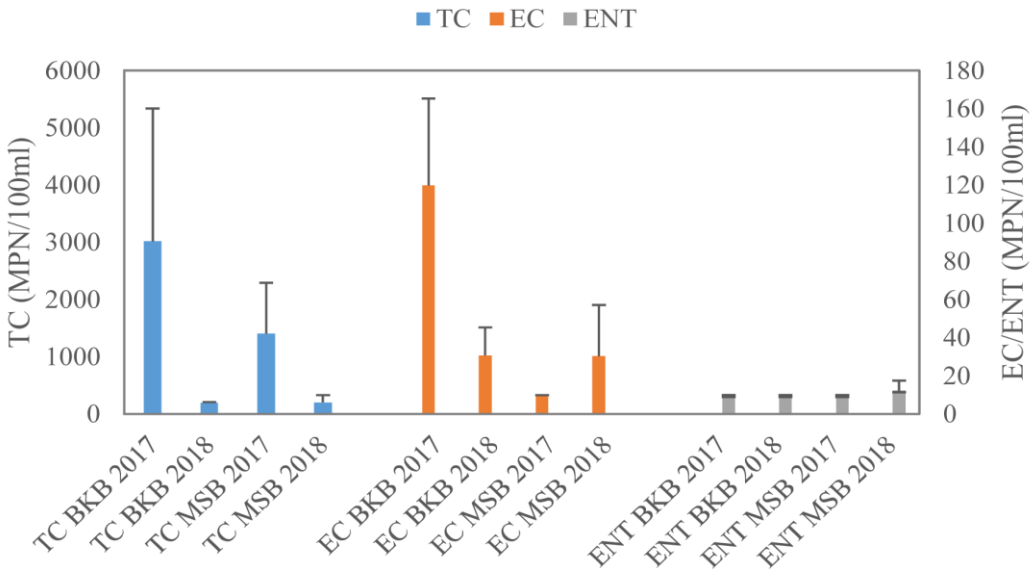
The comparison of the results obtained in autumn 2017 and spring 2018 indicated significantly higher number of total coliforms in autumn samples (Mann–Whitney test, $p<0.05$), at the marine fish farms located in the Mali Ston Bay and in the Boka Kotorska Bay, but the differences in *E. coli* was statistically significant only at the marine fish farm located in the Boka Kotorska Bay ($p<0.05$). In both sampling periods, the number of fecal indicators was higher at the marine fish farm located in the Boka Kotorska Bay than at the marine fish farm located in the Mali Ston Bay. A high standard deviation of most bacterial indicators reported at both sampling locations (Fig. 2) indicated a pronounced temporal variability of bacterial density in the sea water column, especially for the *Vibrio* sp..

Increased concentrations of fecal indicator bacteria are usually associated with influences of wastewaters on the ecosystem (Vezzulli *et al.*, 2008). It was reasonable to presume the increased number of fecal indicators at locations in two semi-enclosed bays, since the study areas has possibly been impacted by

Table 1. Results (mean ± standard deviation) of the heterotrophic bacteria (HPC) at 22°C, *Vibrio* sp. at 22°C and 35°C, total coliforms, *E. coli*, and enterococci in the sea water column at fish farms in Mali Ston Bay and Boka Kotorska Bay in two seasons.

Season	Locations	HPC CFU/ml	*Vibrio* sp. CFU/ml 22°C	*Vibrio* sp. CFU/ml 35°C	Total coliforms MPN/100 ml	*E. coli* MPN/100 ml	Enterococci MPN/100 ml
Autumn 2017	Mali Ston Bay	7025.0±3242.8	35.0±56.9	0	1408.9± 886.0	9.9±0.0	9.9±0.1
	Boka Kotorska Bay	575.0±236.3	25.5±25.8	5±5.8	3018.0± 2318.5	119.8± 45.7	9.9±0.1
Spring 2018	Mali Ston Bay	2200.0±1525.3	50.0±19.6	25±5.8	202.1± 128.2	30.3±26.9	12.4±5.1
	Boka Kotorska Bay	77.5±26.3	16.3±22.8	0	195.3± 14.6	30.8± 14.6	9.9±0.1

a)



b)

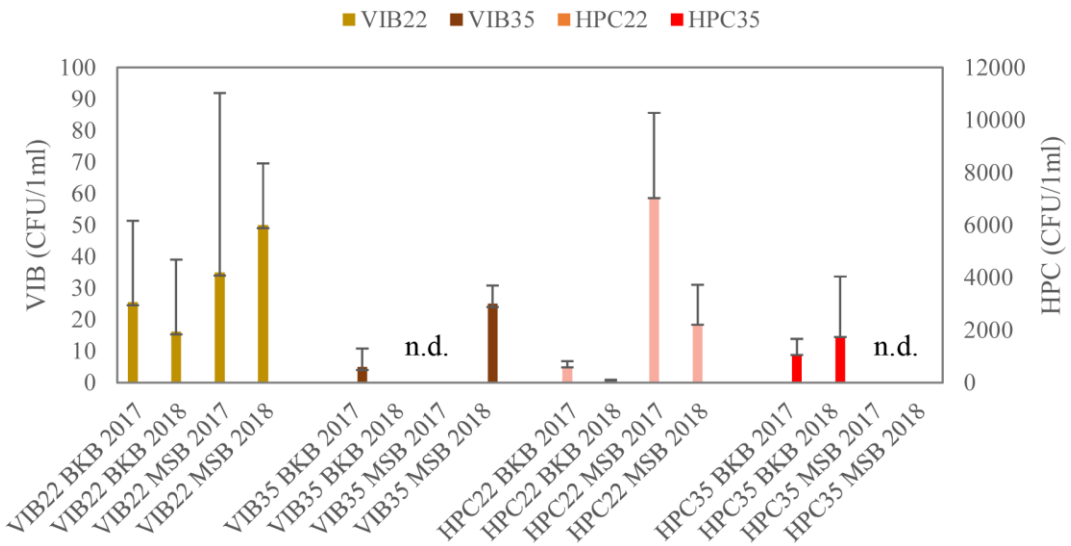


Figure 2. Bacterial concentrations of a) total coliforms (TC; ■), *E. coli* (EC; ■), enterococci (ENT; ■) and b) *Vibrio* at 22°C (VIB22; ■) and at 35°C (VIB35; ■), heterotrophic bacteria at 22°C (HPC22; ■) and at 35°C (HPC35; ■) in the sea water column at fish farms in Mali Ston Bay (MSB) and Boka Kotorska Bay (BKB) in two seasons (autumn 2017 and spring 2018). (n.d. = not determined)

anthropogenic activities. Moreover, the higher HPCs and *Vibrio* counts could be linked to the activities on the marine fish farm and impact on water quality. One of the reasons for a more pronounced anthropogenic influences at the sampling location in the Boka Kotorska Bay than in the Mali Ston Bay could be the possible assessment of the origin of fecal water contamination. Therefore, the ratio of fecal coliforms to enterococci in the sea water column was calculated (Tab. 2), as it is known as the best descriptor of the nature of fecal enrichment in the water (Ashbolt *et al*., 2001).

The maximal ratio between fecal coliforms and enterococci of 2.8 and below in the Mali Ston Bay, indicating according to Ashbolt *et al.* (2001) the animal origin of increased fecal indicators, whereas in the Boka Kotorska Bay the ratio was 4.5 and more, indicating the human pollution, respectively.

Assessment of fish health status

The size of European sea bass ranged from 21.6 to 34.2 cm, and weight from 92.02 to 397.49 g at Montenegro marine fish farm. At Croatian marine fish farm the size ranged from 22.8 to 35.1 cm, while the weight ranged from 142.7 to 512.7 g.

Assessment of fish health status showed that the European sea bass in our study were clinically healthy during these two samplings in Mali Ston Bay and Boka Kotorska Bay, without signs of diseases in clinical examination and necropsy. Therefore, these results present the bacterial community associated with clinically healthy European sea bass farmed in two fish farms.

HPC and *Vibrio* counts were determined from the skin and gills swabs of European sea bass. As in the sea water, both HPC and *Vibrio* counts differed between autumn and spring sampling (Tab. 3).

Within each tissue type (skin and gills), the corresponding seasonal differences were determined for HPC and *Vibrio* count on skin and gills, but they were not significant (Mann–Whitney test, $p>0.05$).

There were no significant differences in the number of HPC and *Vibrio* between Mali Ston Bay and Boka Kotorska Bay in the spring and in autumn periods. These higher HPC and *Vibrio* counts on the skin and gills of European sea bass in the Mali Ston Bay were in agreement with obtained higher HPC and *Vibrio* count in the sea water of Mali Ston Bay than in the Boka Kotorska Bay. These results suggest that HPC and *Vibrio* in the sea water, together with sea water temperature, were primary determinant of HPC and *Vibrio* from skin and gill swabs in this study, like in previous similar studies in Adriatic Sea (Kapetanović *et al*., 2017).

MALDI TOF analysis of isolates from the sea water and skin and gills swabs of European seabass

A total of 24 bacterial isolates were identified from sea water samples in Boka Kotorska Bay (Tab. 4.). Of the 24 isolates from sea water samples, 20.8% were identified as *V. harveyi*. The same frequencies (12.5%) were recorded for *V. chagasii*, *V. fluvialis*, *V. fortis*, *V. parahaemolyticus* and *V. scophtalmy*. Lower frequencies were obtained for *V. anguillarum* (8.3%) and *Halomonas aquamarina* (8.3%).

At the same time, 36 bacterial isolates were identified from the skin and gills swabs of European seabass in Boka Kotorska Bay (Tab. 4.). Most of the isolates were identified as *V. alginolyticus* (25.0%), and then *V. pomeroyi* (16.7%) and *Staphylococcus* sp. (16.7%), respectively. Lower frequencies were displayed by *Shewanella baltica* (13.95%) and *V. parahaemolyticus* (11.1%), as well as

Table 2. Ratio of fecal coliforms (MPN/100 ml) and enterococci (MPN/100 ml) in the sea water column at fish farms in Mali Ston Bay and Boka Kotorska Bay in two seasons.

Season	Locations	Fecal coliforms (FC)	Enterococci (ENT)	Ratio FC:ENT
Autumn 2017	Mali Ston Bay	12.4	9.9	1.3
	Boka Kotorska Bay	155.6	9.9	15,7
Spring 2018	Mali Ston Bay	28.1	9.9	2.8
	Boka Kotorska Bay	45.0	9.9	4.5

Table 3. Heterotroph plate count (HPC) and *Vibrio* count on skin and gills of European sea bass (log CFU/cm^2) in two seasons at Mali Ston Bay and Boka Kotorska Bay.

Season	Locations	Mali Ston Bay		Boka Kotorska Bay	
		Skin	Gill	Skin	Gill
Autumn 2017	HPC CFU/cm^2	1075±411.3	750.3±525.5	360±28.3	120±31.1
	Vibrio sp. CFU/cm^2	15.8±4.3	9.5±4.2	14±8.5	10±7.1
Spring 2018	HPC CFU/cm^2	1200±432.1	1075±377.5	700±113.1	200±70.7
	Vibrio sp. CFU/cm^2	23.0±4.8	12.5±5.0	31±12.7	1±1.4

Photobacterium damselae (8.3%) and *Pseudomonas brenneri* (8.3%).

A total of 29 bacterial isolates were identified from sea water samples in Mali Ston Bay (Tab. 4). Of the 29 isolates from sea water samples, more than 50% were identified as *V. harveyi* (31.0%) and *Photobacterium damselae* (20.7%). The same frequencies (10.3%) were displayed by *V. chagasii*, *V. fluvialis*, *V. pomeroyi* and *Pantoea aglomerans*, and the lowest by *Pseudomonas segetis* (6.9%).

A total of 33 bacterial isolates were identified from the skin and gills swabs of European seabass in Mali Ston Bay (Tab. 4). *Vibrio* strains again displayed the highest frequency: *V. alginolyticus* (36.4%) and *V. pomeroyi* (21.2%). Two members of the genus *Pseudomonas* were present at lower frequencies: *P. brenneri* (18.2%) and *P. putida* (9.1%). The rest of isolates were identified as *V. fortis* (9.1%), *V. parahaemolyticus* (3.0%) and *Psychrobacter* sp. (3.0%).

Two major bacterial diseases Vibriosis and Photobacteriosis/Pasteurellosis are highlighted in the Central Mediterranean region which includes Adriatic Sea (Vendramin *et al*., 2016). *Vibrio* (*Listonella*) *anguillarum* is most commonly known as causative agent of vibriosis (Hickey & Lee, 2017), but recently *V. harveyi* has been recognized as an emerging problem for seabass (Vendramin *et al*., 2016). Lethal levels of *V. anguillarum* correspond to quantities ranging from Log 4 CFU ml^{-1} to Log 3 CFU ml^{-1} in sea water (Hickey and Lee, 2017). The occurrence of *V. anguillarum* in the sea water of Boka Kotorska Bay was below the lethal dose (< Log 1 CFU ml^{-1}), and that is probably the reason why the outbreak of vibriosis didn't occur. At the same time, *V. harveyi* was a part of *Vibrio* population of the sea water in Boka

Table 4. Bacteria identified in the sea water and on skin and gill swabs of farmed European seabass in Boka Kotorska Bay and Mali Ston Bay.

	Boka Kotorska Bay				Mali Ston Bay			
	Sea water		European seabass		Sea water		European seabass	
Species	No	%	No.	%	No.	%	No.	%
Vibrio alginolyticus			9	25.0			12	36.4
Vibrio anguillarum	2	8.3						
Vibrio chagasii	3	12.5			3	10.3		
Vibrio fluvialis	3	12.5			3	10.3		
Vibrio fortis	3	12.5					3	9.1
Vibrio harveyi	5	20.8			9	31.0		
Vibrio parahaemolyticus	3	12.5	4	11.1			1	3.0
Vibrio pomeroyi			6	16.7	3	10.3	7	21.2
Vibrio scophtalmy	3	12.5						
Photobacterium damselae			3	8.3	6	20.7		
Halomonas aquamarina	2	8.3						
Pantoea aglomerans					3	10.3		
Pseudomonas brenneri			3	8.3			6	18.2
Pseudomonas putida							3	9.1
Pseudomonas segetis					2	6.9		
Psychrobacter sp.							1	3.0
Shewanella baltica			5	13.9				
Staphylococcus sp.			6	16.7				

Kotorska Bay and Mali Ston Bay which represent a potential threat to health of farmed fish European seabass in these two bays.

Prevalence of Vibrio in the sea water and at skin and gills of European seabass

During our study, *Vibrio* counts and the predominant *Vibrio* strains did not vary significantly at the skin and gill swabs, as well as in the sea water across these two samplings. Therefore, culturable *Vibrio* were presented as *Vibrio* populations in the sea water and at the skin and gills swabs of European sea bass in the Boka Kotorska Bay and Mali Ston Bay (Fig. 3).

Vibrio population of sea water in the Boka Kotorska Bay (Fig. 3A) was consisted of seven *Vibrio* strains (*V. anguillarum*, *V. chagasii*, *V. fluvialis*, *V. fortis*, *V. harveyi*, *V. parahaemolyticus*, *V. scophtalmy*), while four *Vibrio* strains (*V. pomeroyi*, *V. harveyi*, *V. chagasii*, *V. fluvialis*) were found in the sea water of the Mali Ston Bay (Fig. 3C).

Comparison of data from both bays revealed that the common *Vibrio* bacteria in the sea water were identified as *V. harveyi*, *V. chagasii*, *V. fluvialis*, accounting for more than 50% of the total *Vibrio* population of the sea water in Boka Kotorska Bay and more than 70% of the *Vibrio* population of the sea water in Mali Ston Bay, respectively.

As in the sea water, there was similarity between *Vibrio* population of European seabass found in Boka Kotorska Bay (Fig. 3B) and Mali Ston Bay (Fig. 3D). *Vibrio* population found in European seabass in Boka

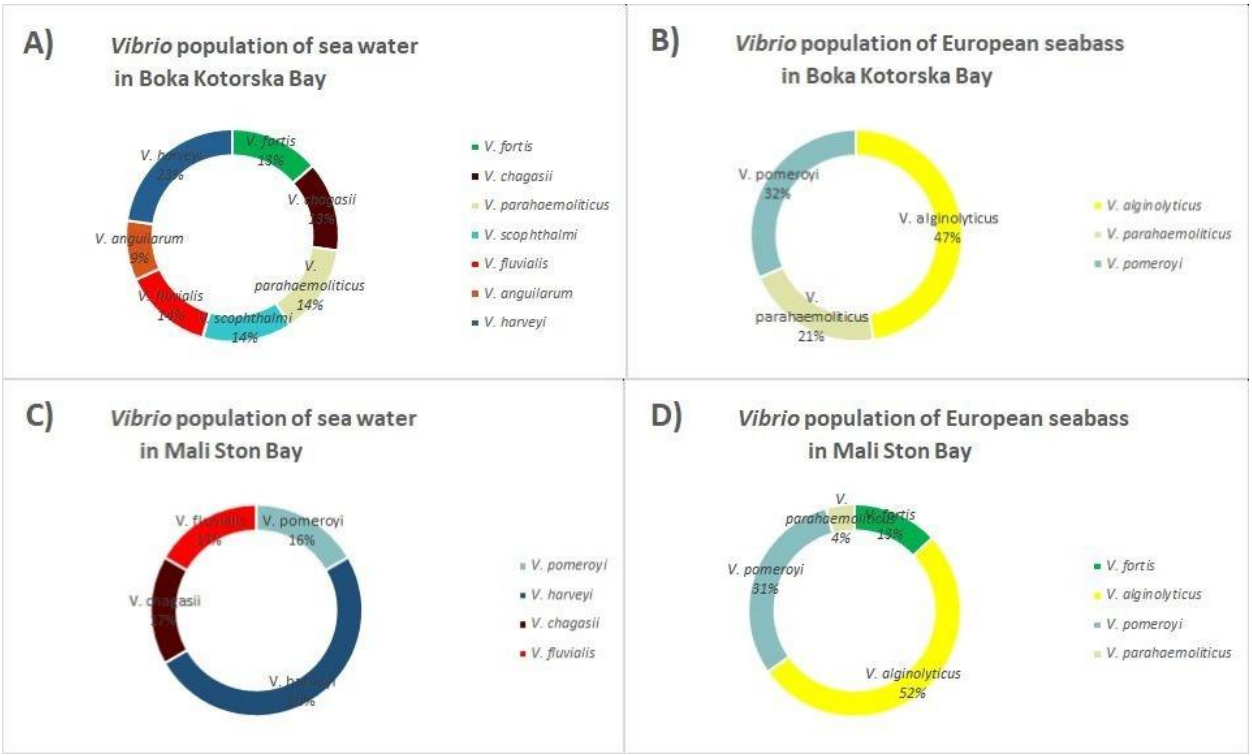


Figure 3. *Vibrio* population found in the sea water and European seabass in Boka Kotorska Bay (A and B) and Mali Ston Bay (C and D)

Kotorska Bay was consisted of *V. alginolyticus*, *V. parahaemolyticus* and *V. pomeroyi*. These three *Vibrio* species accounted for 87% of the total *Vibrio* population found in the European seabass in Mali Ston Bay, whereas the rest of 13% belong to *V. fortis*.

Bacterial growth and no occurrence of infections or diseases in examined European seabass suggest that most of the bacteria isolated are transient or commensal organisms, which are associated with a bacterial community of surrounding sea water (Austin, 2002; Kapetanović *et al*., 2017). Our results of bacterial occurrence suggests that sea water influences the bacterial community on the fish (*V. parahaemolyticus* and *V. pomeroyi*).

Antimicrobial susceptibility

The most effective antibiotics for treatment of *Vibrio* infections (Lee *et al*.,2018) were tested for antimicrobial susceptibility of *Vibrio* isolates from sea water and fish samples in the Boka Kotorska Bay and Mali Ston Bay. The percentages of antibiotic resistant profiles of isolated *Vibrio* strains from these two bays were summarized in the Tab. 5.

The isolated *Vibrio* strains in Boka Kotorska Bay were resistant to florfenicol (57%), ampicillin (100%), erythromycin (71%), vancomycin (100%) and chloramphenicol (57%). In contrast, high susceptibility rate was determined for gentamicin (100%) and flumequine (85%).

At the same time, high resistance rate of the *Vibrio* strains isolated in Mali Ston Bay was recorded for ampicillin (100%), erythromycin (70%) and vancomycin (100%) like in Boka Kotorska Bay. *Vibrio* strains isolated in Mali Ston Bay were susceptible to florfenicol (80%), gentamicin (70%), sulfamethoxazole / trimethoprim (80%), flumequine (80%) and imipenem (100%).

Table 5. Percentage of antibiotic susceptible, intermediate, and resistant *Vibrio* isolated from sea water and fish samples.

Antibiotics	**Boka Kotorska Bay** (Total no. of isolates n=7)						**Mali Ston Bay** (Total no. of isolates n=10)					
	Susceptible (S)		**Intermediate (I)**		**Resistant (R)**		**Susceptible (S)**		**Intermediate (I)**		**Resistant (R)**	
	No. of isolates	%[a]	No. of isolates	%[a]	No. of isolates	%[a]	No. of isolates	%[a]	No. of isolates	%[a]	No. of isolates	%[a]
Enrofloxacin	4	57.1	1	14.3	2	28.6	1	10	6	60	3	30.0
Florfenicol	3	42.9			4	57.1	8	80.0	1	10.0	1	10.0
Gentamicin	7	100					7	70.0	1	10.0	2	20.0
Ampicillin					7	100					10	100.0
Erythromycin			2	28.6	5	71.4			3	30.0	7	70.0
Oxytetracycline			6	85.7	1	14.2	2	20.0	4	40.0	4	40.0
Sulfamethoxazole/ Trimethoprim	3	42.9	4	57.1			8	80.0			2	20.0
Vancomycin					7	100					10	100.0
Flumequine	6	85.7			1	14.2	8	80.0	2	20.0		
Imipenem	4	57.1	1	14.2	2	28.6	10	100				
Ciprofloxacin	4	57.1	1	14.2	2	28.6	4	40.0	5	50.0	1	10.0
Streptomycin			6	85.7	1	14.2	3	30.0	3	30.0	4	40.0
Chloramphenicol	3	42.9			4	57.1	4	40.0	2	20.0	4	40.

% = percentage (number of isolates/total number of isolates tested).

Based on the results of antimicrobial susceptibility testing, the antibiotic resistance profiles of the *Photobacterium damselae* isolated in Boka Kotorska Bay and Mali ston Bay were more different. The isolated *P. damselae* strains in Boka Kotorska Bay were resistant to ampicillin (100%), vancomycin (100%), erythromycin (50%), oxytetracycline (50%) and sulfamethoxazole/trimethoprim (50%). These isolates were susceptible to enrofloxacin (100%), florfenicol (100%), gentamicin (100%), flumequine (100%), imipenem (100%) and chloramphenicol (100%).

On the other side, isolated *P. damselae* strains from Mali Ston Bay were resistant to enrofloxacin (100%), florfenicol (100%), ampicillin (100%), vancomycin (100%), erythromycin (50%), oxytetracycline (50%) and sulfamethoxazole/trimethoprim (50%). High susceptibility rate was also determined for these *P. damselae* isolates for gentamicin (100%), flumequine (100%), imipenem (100%) and chloramphenicol (100%).

CONCLUSION

Etiological agents of vibriosis and photobacteriosis/pasteurellosis outbreaks were identified in seabass cage farms located in two semi-enclosed bays of the Eastern Adriatic Sea, at Mali Ston Bay and at Boka Kotorska Bay. Sea water contained potentially pathogenic species of *Vibrio*, including *Vibrio* (*Listonella*) *anguillarum*, and of *Photobacterium*, presenting a potential reservoir of infection. Bacteria (*V. alginolyticus*, *V. parahaemolyticus* and *V. pomeroyi*) were identified as naturally associated with healthy farmed European seabass in both areas. Antimicrobial sensitivity test showed differences in resistance between isolated and analyzed bacterial strains from these two marine fish farms. These results provide a valuable baseline reference for future studies of how farming conditions in semi-enclosed aquatic environments could influence the microbial community in sea water and in farmed fish. This is particularly important for the Boka Kotorska Bay, because there is no available data with similar interdisciplinary research. Thus, periodical monitoring of microbiological water quality in the farming areas, and seasonal analysis of the bacterial abundance and diversity in farmed European seabass in Boka Kotorska Bay is recommended.

ACKNOWLEDGMENS

This work has been fully supported by the Croatian Science Foundation's funding of the under the project AQUAHEALTH - Grant No IP-2014-09-3494. The, Croatian Ministry of Science and Education of Republic Croatia and Ministry of Science of the Republic of Montenegro are also acknowledged for their funding of Bilateral project Croatia Montenegro: The influence of environmental factors on the health status of European sea bass (*Dicentrarchus labrax*), with emphasis on the occurrence of vibriosis in Croatia and Montenegro.

REFERENCES

Ashbolt, N. J., W. O. K. Grabow & M. Snozzi (2001): Indicators of microbial water quality in Water quality: Guidelines, Standards and Health. In: Fewtrell, L., Bartram, J. (Eds), World Health Organization (WHO), Published by IWA Publishing, London, pp. 290-316.

Austin, B. (2002): The bacterial microflora of fish. Sci World J 2: 558–572.

Gavrilović, A., J. Jug-Dujaković, K. Bonačić, I. Vardić Smrzlić, D. Kapetanović, D. Valić & E. Teskeredžić (2012): Effectiveness of different antibacterial drugs on sea bass vibriosis caused by Vibrio alginolyticus. Proceedings of the 47th Croatian and 7th International Symposium on Agriculture. Zagreb, 591-594.

Haenen, O., B. Fouz, C. Amaro, M. M. Isern, H. Mikkelsen, S. Zrnčić, A. Travers, T. Renault, R. Wardle, A. Hellstrom & I. Dalsgaard (2014): Vibriosis in aquaculture. 16th EAFP Conference, Tampere, Finland, 4th September 2013: workshop report. B Eur Assoc Fish Pat. (0108-0288) 34 (2014), 4; 138-148.

Hickey, M. E. & J. L. Lee (2017): A comprehensive review of *Vibrio* (*Listonella*) *anguillarum*: ecology, pathology and prevention. Rev Aquacult. 0, 1–26.

Joksimović, A., 2007. The Most Common Fish Species of the Montenegrin Coast. Montenegrin Academy of Sciences and Arts, Special Editions (Monographies and Studies, Vol. 58, Section of Natural Scinces, Vol. 30., p. 140. COBISS.CG-ID 11938832

Joksimović A. 2010. „Biološka istraživanja Jadranskog mora, stanje, perspektive i pravci razvoja" Crna Gora u XXI vijeku – u eri kompetitivnosti. Nauka i tehnologija, urednik Jovan Mirković; Crnogorska akademija nauka i umjetnosti, posebna izdanja (monografije i studije), knjiga 73, sveska 11, Podgorica: Crnogorska akademija nauka i umjetnosti 2010, str. 549-558. (ISBN 978-86-7215-249-4).

Kapetanović, D., I. Vardić Smrzlić, D. Valić & E. Teskeredžić (2013): Occurrence, characterization and antimicrobial susceptibility of *Vibrio alginolyticus* in the Eastern Adriatic Sea. Mar Pollut Bull 75: 46–52.

Kapetanović, D., I. Vardić Smrzlić, D. Valić, Z. Teskeredžić & E. Teskeredžić (2017): Culturable microbiota associated with farmed Atlantic bluefin tuna (*Thunnus thynnus*). Aquat. Living Resour. 30: 30.

Lee, L. H., N. S. Ab Mutalib, J. W. F. Law, S. H. Wong & V. Letchumanan (2018): Discovery on Antibiotic Resistance Patterns of *Vibrio parahaemolyticus* in Selangor Reveals Carbapenemase Producing *Vibrio parahaemolyticus* in Marine and Freshwater Fish. Front Microbiol. 9: 2513. Published online 2018 Oct 25. doi: 10.3389/fmicb.2018.02513

Mandić, M., S. Gvozdenović, A. Joksimović & A. Pešić (2017): Condition of cultured sea bass and sea bream and occurrence of their spawning in the cages in Boka Kotorska Bay. 22nd International Scientific-Professional Conference Information Technology 2017.

Mladineo, I., I. Bušelić, J. Hrabar, I. Radonić, A. Vrbatović, S. Jozić & Ž. Trumbić (2016): Autochthonous Bacterial Isolates Successfully Stimulate *In vitro* Peripheral Blood Leukocytes of the European Sea Bass (*Dicentrarchus labrax*). Front Microbiol. 2016; 7: 1244 https://doi.org/10.3389/fmicb.2016.01244

Oraić, D. & S. Zrnčić (2005): An Overview of Health Control in Croatian Aquaculture. Vet Res Commun. (0165-7380) 29 (2005), S2; 139-142.

Pešić, A., M. Đurović, A. Joksimović, O. Marković & Z. Ikica (2015): Condition of fish, sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) from fish farm in Bokakotorska Bay (South-East Adriatic). VII international conference "Water & Fish" - zbornik predavanja Faculty of Agriculture, Belgrade Zemun, Serbia. 419-424.

Pešić A., Joksimović A., Ikica Z., Nikolić M., Gvozdenvić S. 2016. Condition factor of Sea bass (Dicentrachus labrax) from fish farm in Boka Kotorska Bay. 45. Konferencija o aktuelnim temama korišćenja i zaštite voda „VODA 2016". Zlatibor, 15-17. jun 2016. Zbornik radova: 445-448. (ISBN 978-86-916753-3-2)

RAC/SPA - UNEP/MAP (2013): Ecological quantitative description of Boka Kotorska Bay marine area (Montenegro). By Golder Associates. Ed. RAC/SPA - MedMPAnet Project, Tunis: 82 pp + Appendices.

Surfrider Fundation (2003): Blue water task force – Coastal water quality monitoring manual. Version 1.4., pp. 42. http://www.surfrider.org/files/BWTF_manual_June2003.pdf

Vendramin, N., S. Zrncic, F. Padrós, D. Oraic, A. Le Breton, C. Zarza & N. J. Olesen (2016): Fish health in Mediterranean Aquaculture, past mistakes and future challenges. Bull. Eur. Ass. Fish Pathol. 36(1), 38-45.

Vezzulli, L., M. Moreno, V. Marin, E. Pezzati, M. Bartoli, & M. Fabiano (2008): Organic waste impact of caputre-based Atlantic bluefin tuna aquaculture at an exposed site in the Mediterranean Sea. Estuar. Coast. Shelf S. 78, 369-384.

Viličić, D., Jasprica, N., Carić, M., Burić, Z., 1998. Taxonomic composition and seasonal distribution of microphytoplankton in Mali Ston Bay (eastern Adriatic). Acta Bot. Croat. 57, 29-48.

Zrnčić, S., N. Vendramin, T. S. Boutrup, M. Boye, M. S. Bruun, D. Brnić & D. Oraić (2015): *Piscirickettsia salmonis* infection in European sea bass – An emerging disease in Mediterranean mariculture. 17th International Conference on Diseases of Fish and Shellfish, Book of Abstracts / EAFP - Las Palmas de Gran Canaria: EAFP, 2015, 153-153.

Received: 18. 11. 2019.
Accepted: 19. 12. 2019.

Procjena kvaliteta morske vode i zdravstvenog stanja brancina (*Dicentrarchus labrax*) u istočnom Jadranskom moru (Crna Gora i Hrvatska)

Damir KAPETANOVIĆ, Ana GAVRILOVIĆ, Jurica JUG-DUJAKOVIĆ, Irena VARDIĆ SMRZLIĆ, Snježana KAZAZIĆ, Mirjana BOJANIĆ - RAŠOVIĆ, Anamarija KOLDA, Ana PEŠIĆ, Lorena PERIĆ, Jakov ŽUNIĆ, Milica MANDIĆ, Aleksandar JOKSIMOVIĆ, Darija VUKIĆ LUŠIĆ, Mirko ĐUROVIĆ

SAŽETAK

Poluzatovoreni zalivi se smatraju važnim potencijalima životne sredine za potrebe marikulture. Ovaj rad je sproveden u okviru kaveznog uzgoja riba u dva poluzatvorena zaliva u istočnom Jadranskom moru, u Bokokotorskom zalivu (Crna Gora) i Malostonskom zalivu (Hrvatska). Ukupno 16 uzoraka morske vode i 46 briseva škrga i kože brancina je sakupljeno sa dva uzgajališta, tokom dva uzorkovanja, u jesen i proljeće. Cilj ovog istraživanja je bio da utvrdi zdravstveni status brancina u Crnoj Gori i Hrvatskoj, da procijeni stanje abiotičkih faktora u obije oblasti marikulture i da procijeni prisustvo *Vibrio* i njegovog uticaja na zdravlje riba. Većina izolovanih bakterija su Gram-negativne i čine ih rodovi *Vibrio, Photobacterium* i *Pseudomonas.* Procjena statusa zdravlja riba pokazuje da su ribe bile klinički zdrave i nađena bakterijska zajednica je prisutna kod zdravih gajenih brancina. U isto vrijeme, neki od izolovanih sojeva bakterija su poznati kao patogeni (*V. alginolyticus, V. harveyi, V. anguillarum, P. damselae*) te predstavljaju potencijalni rezervoar infekcije. Paralelna istraživanja potvrđuju mikrobiološki uticaj zbog antropogenog uticaja u oba zaliva. Pristup prikazan u ovom radu je značajan u procjeni stanja gajilišta u poluzatvorenim zalivima i lako je primjenljiv u drugim, sličnim lokacijama širom svijeta.

Keywords: brancin, Bokokotorski zaliv, Malostonski zaliv, *Vibrio*, *Photobacterium*