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Integrative Investigation on the Ecology of the Black Sea Mussel Mytilus galloprovincialis Lam. and its Habitat

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Abstract. The present work is reporting a preliminary investigation on the ecology of the Black sea mussel (*Mytilus galloprovincialis* Lam.) and its habitat by monitoring of the physicochemical indicators of the water, the microbiology and the radionucleids in the region of Shkorpilovci and Kavarna (NE Bulgaria). The samples were collected in the period of August 2018 to October 2019. The microbiological determination was performed by the use of the system "BIOLOG". *Enterococcus durans* and *E. haere* were isolated from the Shkorpilovci region and *Streptococcus gallolyticus ss gallolyticus*, *E. haere, Lactobacillus brevis* and *L. sakei ss sakei* were found from Kavarna. We isolated the lactic acid bacterium *Streptococcus gallolyticus ss gallolyticus*, a species associated with colon-rectal tumors on the one hand and as a producer of bacteriocins on the other. Additionally, the study includes information on the naturall and tehnogenic radionuclides in the marine environment. No technogenic radionuclides were found in the mussel samples. The calculated indicators for radiation hazard were in accordance with the norms quoted fixed in the local an interntional legislation.

Key words: Bivalvia, *Mytilus galloprovincialis* Lam, radionucleids, Black Sea, monitoring of the water, radiology, marine habitats.

Introduction

Marine mussels of the genus *Mytilus* are grown in aquaculture farms for human consumption worldwide. The economic value of these bivalve molluscs has resulted in considerable efforts to improve production by increasing survival and growth rates. Mussels, as filter feeders, are important for the marine ecology, but they also have an exceptional nutritional value, making them important foof in the human diet. Consumption of bivalve molluscs provide highly valuable proteins, essential vitamins, and minerals, as well as polyunsaturated fatty acids with health beneficial effects (Bongiorno et al., 2015).

The implementation of the Marine Strategy Framework Directive 79/923/EEC (CEC, 1979) requires adaptive management to improve the status of the marine environment. This adaptive management implies monitoring of the ecosystems using a set of appropriate indicators for their functional state (Guerry, 2005). Due to their sessile lifestyle, the filter-feeding apparatus and the lack of excretion mechanisms, the sea mussels have a significant capacity to bioaccumulate different xenobiotics from the environment and bioconcentrate them in their tissues to concentrations, that may significantly exceed the values in the environment. Therefore, a number of researchers share the view, that concentrations of pollutants in the tissues of organisms recognized as bio-indicators reflect most accurately the degree of environmental pollution and their analysis gain more valuable information than the analysis of the sea water (Baumard et al., 1999; Maanan, 2007). Such data can be used to quantify, evaluate, map and track the distribution of bioavailable components of different pollutants in marine and estuarine ecosystems (Phillips, 1994; Farrington et al., 1983).

The Black Sea mussel (*Mytilus galloprovincialis* Lamarck, 1819) is one of the few species that are considered to be a reliable species for the monitoring of the environment conditions in the marine ecosystems (Danellakis et al., 2011). The concept of using mussels as bioindicators for monitoring of coastal marine waters was termed "mussel-watch" (Goldberg, 1975). The mussels *M. galloprovincialis* are globally recognized as biomonitors and serve as control organisms in national and international programs (BIOMAR, BEEP, IOC-IMO, UNEP-GIPME). The Bulgarian Black Sea aquatory is seriously affected by chemical pollutants entering the sea with the waste waters of urban agglomerations, industry, agriculture and river runoffs. Molluscs, especially shellfish, live in the ever-changing environment of marine ecosystems concerning the increase of pollutions, climate change, eutrophication, changes in the water salinity, etc. A number of studies reported, that shellfish can be used as bioindicators to assess the toxic effects of chemical pollutants, heavy metals and other types of pressure. Typically, the effect of such pressure is related to changes in the response of the biological systems, as the effects were mediated at the cellular level. The estimation of the biological response revealing the effects of anthropogenic pressure even at the cell/molecular level may allow indirect integral assessment of the status of the ecosystem (Rodriguez-Ortega et al., 2009; Hering et al., 2003). The Black Sea mussel is in fact the only species of mussels grown as aquaculture in the Bulgarian Black sea aquatory. In Bulgaria its production in 2005 amounted to 170 tons, which accounted for 5.4% of the total aquaculture production in the country. In recent years, there is a steady trend towards the development of marine aquaculture on the Bulgarian Black Sea coast. The Black Sea mussel is used mainly for food in Bulgaria. The mussel tissue and beef are almost equal concerning the protein content, however the mussels contain 11 times more calcium and 2 times more phosphorus. Mussels can effectively purify seawater, so the cultivated production of Black Sea mussel may support the natural process of self-purification of the marine environment.

The present work is reporting a preliminary investigation on the ecology of the Black sea mussel and its habitat by monitoring of the physicochemical indicators of the water, the microbiology and the radionucleids in the region of Shkorpilovci and Kavarna.

Material and Methods

The study was conducted at the Department of Biology, University of Shumen, Bulgaria. The samples were collected from the regions of Kavarna (43.411059 N; 28.356250 E) and Shkorpilovci (42.957889 N; 27.89899 E) in the period of August 2018 to October 2019 (Fig. 1).

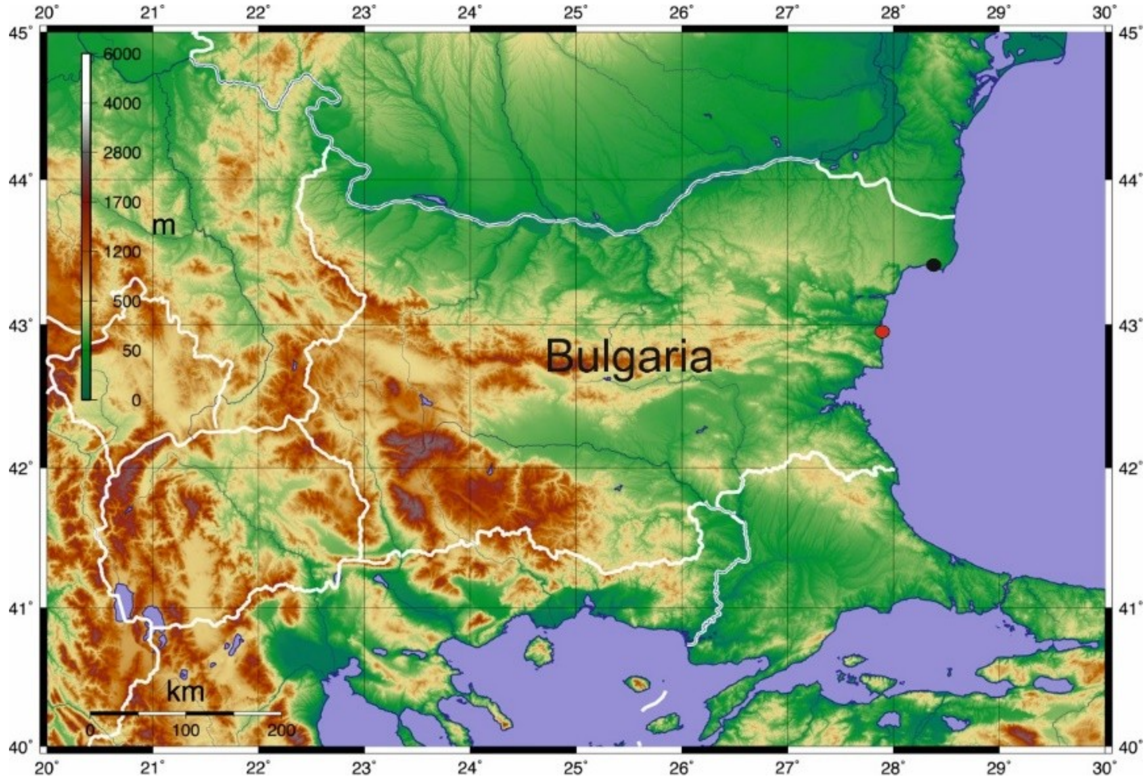

Fig. 1. Sampling locations at the Black sea coast. The red circle represent the sampling site at Shkorpilovci and the black one the sample site at Kavarna.

Collection of samples

The mussels were harvested from the northern part of the Bulgarian Black Sea aquatory. After collection, the samples (about 10 kg) were immediately refrigerated (4 °C) and transported to the laboratory for the further analyses.

In this study, we examined *Mytilus* mussels of similar size, weight, and shape to ensure maximal uniformity in the applied methods (Duquesne et al., 2004). The average length of mussels used in the study was 4.676 ± 0.47 cm.

Physico-chemical analysis of water

During mussel sampling we measured in situ the temperature, total salinity (by using YSI Model 33 salinity meter), and pH (by using ATC Piccolo HI 1280 pH-meter).

ɣ-*Spectrometric measurements*

The study included both determination of the concentration of natural radionuclides such as ^{40}K, ^{232}Th, ^{226}Ra, ^{214}Pb and others, as well as monitoring of the accumulation of the technogenic radionuclides.

Microbiological analysis

Three subsamples (each of about 1 kg of mussels) were used for the microbiological analyses. The mussels were scrubbed free of dirt, washed in hypochlorite solution (20 mg l^{-1}), rinsed with sterile distilled water, and shucked with a sterile knife. Tissue liquor samples (about 100 g) were homogenized.

Microbial Identification Databases for the "Biolog" Systems

The microbial identification was performed by a manual microbial identification system Biologist VIO45101AM (Diahim LTD, Sofia, Bulgaria). The isolated strains were screened on BL4021502 Tryptic Soy Agar (TCA), cultured for 24 hours at 37°C and then subjected to Gen III plaque identification to identify Gram positive and Gram negative aerobic bacteria. The microscopic pictures were performed using stereomicroscope OPTIKA (Italy) with a DinoEye, Eyepiece camera with 5 megapixels. Photographs were performed by using a with a Canon EOS 60D camera.

Results

The microorganisms were isolated from *M. galloprovincialis* Lam. collected from both sample sites at north Bulgarian Black Sea aquatory.

After 24 h of cultivation on different media, various microbial colonies were obtained. Data are represented on Table 1, Fig. 2 and 3.

Parallel to the performance of the microbiological experiments we conducted a physicochemical analysis of the sea waters. The results are summarized in Table 2.

Gamma spectrometry analysis of the samples was performed with Ge(Li) detector for a period of 24 h. Gamma spectra were analyzed with the ANGES program (Mishev & Vidolov, 2020, Program ANGES, Research Contact 9493/RO, Vienna, Austria, IAEA). The analysis of the gamma spectrums include: detecting of the peaks and determination of their energy and area; identification of the nuclides; calculation of the specific activities of the nuclides. Specific activities of radionuclides identified in the samples are represented on Fig. 4 and 5.

In the sand samples, collected from Kavarna and Shkorpilovci were found natural radionuclides ^{212}Pb, ^{214}Pb, ^{214}Bi, ^{208}Tl, ^{228}Ac, ^{226}Ra and ^{40}K. The values obtained for the specific activities of radionuclides in the samples are close. The values of the specific activities of ^{40}K in both samples were about 500 $Bq.kg^{-1}$.

The calculated indicators for radiation hazard (I_r), external hazard (H_{ex}) (Beretka & Mathew, 1985), absorbed dose (D) and annual effective dose (E) (UNSCEAR 2000; Veiga et al., 2006) were in accordance with the norms termed in the local and international legislation. The obtained values are presented in Table 3.

Table 1. Number of obtained colonies on the different media.

Media/ region	MRS agar	Cetrimid agar	Hromokult agar	MacConkey agar	MPA agar	M17	Strain BIOLOG
Kavarna (08.2018)	200.10^{-2}	76.10^{-2}	58.10^{-3}		246.10^{-3}		*Lactobacillus brevis; L. sakei sakei; Streptococcus gallolyticus gallolyticus; Enterobacter aerogenes*
Shkorpilovci region (08.2018)			78.10^{-3}	10.10^{-3}	90.10^{-3}		*Enterococcus hirae; Enterococus durans*
Kavarna (10.2018)	58.10^{-2}		68.10^{-3}	84.10^{-5}	128.10^{-3}		*Streptococcus gallolyticus gallolyticus*
Shkorpilovci region (10.2018)				56.10^{-3}	12.10^{-5}	101.10^{-3}	*Enterococcus hirae*
Shkorpilovci region (02.2019)			71.10^{-3}	94.10^{-1}	130.10^{-3}		*Enterococus durans*
Kavarna (02.2019)	28.10^{-2}		3.10^{-3}	2.10^{-3}	1.10^{-1}		*Streptococcus gallolyticus gallolyticus*
Shkorpilovci region			28.10^{-2}		150.10^{-3}	200.10^{-3}	*Enterococcus hirae;*

(06.2019)							*Enterococcus durans*
Kavarna (06.2019)	67.10^{-3}	46.10^{-3}			132.10^{-3}		*Streptococcus gallolyticus gallolyticus*
Kavarna (10.2019)	70.10^{-2}		55.10^{-5}		14.10^{-5}	79.10^{-3}	*Streptococcus gallolyticus gallolyticus*
Shkorpilovci region (10.2019)				46.10^{-3}	2.10^{-5}	88.10^{-3}	*Enterococcus hirae*

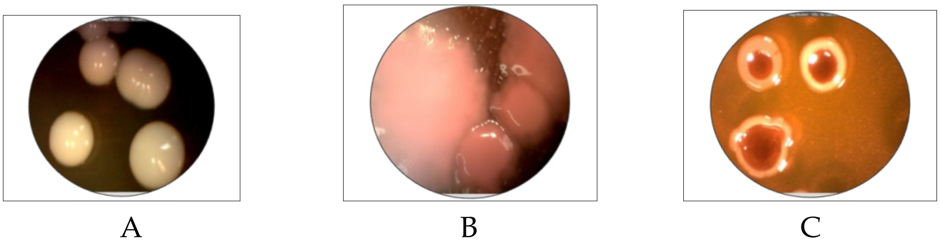

Fig. 2. Photographs of the colonies of the isolated species: A) colonies of Lactic acid bacteria on media MRS agar B) colonies of *Enterococuus durans* on media MacConkey agar; C) colonies of *E. hirae* on media Hromokult.

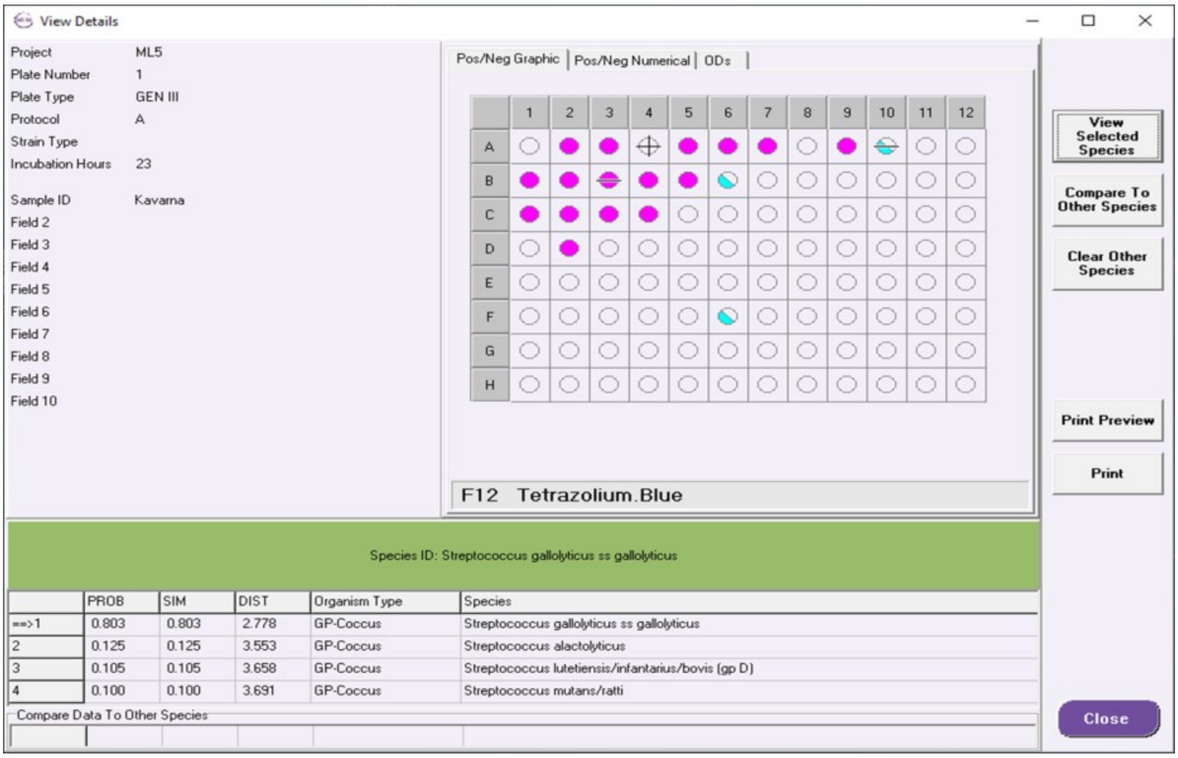

Fig. 3. Diagram from a test with the Microbial identification system Biologist VIO45101AM, demonstrating the presence of *Streptococcus gallolyticus ss gallolyticus.*

Table 2. The physicochemical parameters of the waters.

Region	Date	Depth m	temperature [° C]	pH [pH]	salinity [ppt]	dissolved O2 [mg/l]
Kavarna	08.2018	2 to 4	27.9	8.30	13.7	7.3
Shkorpilovci	08.2018	2 to 4	27.2	8.27	13.6	7.1
Kavarna	10.2018	2 to 4	26.7	7.26	11.5	8.5
Shkorpilovci	10.2018	2 to 4	24.6	7.35	10.5	8.2
Kavarna	02.2019	2 to 4	5.1	7.61	11.1	8.8
Shkorpilovci	02.2019	2 to 4	7.4	7.78	9.2	8.5
Shkorpilovci	06.2019	2 to 4	27.7	8.26	13.5	7.5
Kavarna	06.2019	2 to 4	26.2	8.25	13.5	7.2
Shkorpilovci	10.2019	2 to 4	25.7	7.36	11.2	8.8
Kavarna	10.2019	2 to 4	23.2	7.25	11.2	8.4

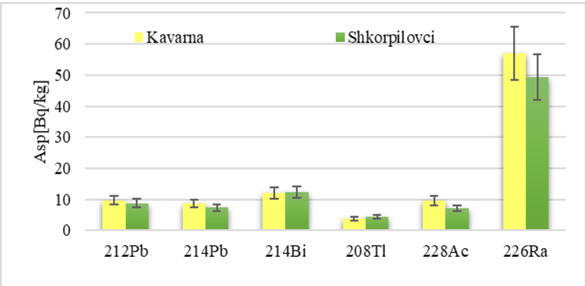

Fig. 4. Activity concentration of samples of sand collected from Kavarna and Shkorpilovci.

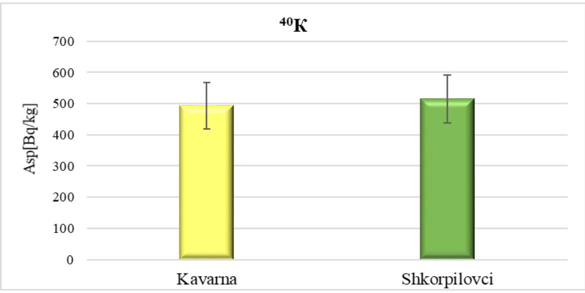

Fig. 5. Specific activity of 40K in sand samples collected from Kavarna and Shkorpilovtsi.

Table 3. Value for the indicators of radiation hazard, external hazard, absorbed dose and annual effective dose obtained for the sand samples collected from Kavarna and Shkorpilovci, and their referent norms.

	I_r [$Bq.kg^{-1}$]	H_{ex} [$Bq.kg^{-1}$]	D [$nGy.h^{-1}$]	E [$mSv.y^{-1}$]
Kavarna	0.60	0.21	38.96	0.048
Shkorpilovci	0.59	0.20	38.08	0.047
Norms	1	1	55	1

Discussion

The values observed for Fecal coliforms (FC) were conformed also for *E. durans* and *E. hirae* counts (Table 1 and Figures 3 A). In particular, *Enterococcus* was responsible for the FC peak in August (the two counts coincided), suggesting the presence of particular environmental conditions, which influenced the quality of the mussels harvested in that month. These bacteria were found in the region of Shkorpilovci. The lactic acid bacteria, which were isolated, belonged to *Streptococcus gallolyticus gallolyticus*, *Lactobacillus brevis* and *L. sakei sakei* from the region of Kavarna, as the peak in the concentration was detected in August (Table 1 and Figures 3 A, 4). The high concentrations of FC, detected in the warmer months may be related to the increased metabolic activity of the moluscs. The high metabolic activity of the mussels is directly related to the increase of the temperature of the sea waters from one side (Table 2) and their biological cycle from the other. In previous investigations of our working group we demonstrated experimentally, that from the black mussels can be isolated two stems of lactic acid bacteria belonging to *L. plantarum*, which show antifungal activities (Ibryamova et al., 2020). For the north section of the Bulgarian Black sea aquatory, for June 2018 were reported high levels of *E. coli* and *Pseudomonas aeroginosa* (Ignatova-

Ivanova et al., 2018). The temperature of the sea waters may affect the number of the microbs by concentration of the nutients. Simmilar results were reported previously (Grimes et al., 1986; Ramon & Richardson, 1992). The peak of *Enterobacter* observed in August can be related to some particular environmental conditions. In fact, the characteristics of the mussels can be affected by a variety of extrinsic and intrinsic factors, such as water temperature and salinity, food availability and gametogenic cycle of the animals (Okumus & Stirling, 1998). According to Ordinance No. 4 from 20.10.2000 for the quality of fisheries water and the breeding of shellfish, the amount of fecal coliforms in the inter-shell content should be less than 300 NVB - in our samples from October 2018, the values were exceeded by 40 times. The probable cause for the increased quantities of fecal coliforms is the pollution of the seawater at the end of the summer season, which remains relatively high until the beginning of October. No pollution was reported at the same site in June.

The gamma-spectrometric analysis revealed, that technogenic radionuclides can be detected in the sand samples, collected from the Kavarna and Shkorpilovci region. Tehnogenic radionuclides were not detected in the shells and the soft tissues of the mussels colected from theses regions. The indexes for radiation hazard, external hazard, absorbed dose and annual effective dose obtained from the sand samples are lower than the referent values set in the UNSCEAR.

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