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Mass mortality of farmed mussels – a phenomenon without explanation?

Milica MANDIĆ[1*], Slađana NIKOLIĆ[1], Ines KOKIĆ[1] & Sandra JOKANOVIĆ[1]

[1] Institute of marine biology, University of Montenegro, Put I Bokeljske brigade 68, 85330, Kotor, Montenegro, [*]e-mail: mamilica@ucg.ac.me

ABSTRACT

First mass mortality event of farmed mussels (*Mytilus galloprovincialis* Linnaeus, 1758), took place in the area of the Boka Kotorska Bay (southern Adriatic Sea) in the period from June to September 2020. The mortality caused a loss of production in the range of 80-90%. Possible ecological and environmental causes of this phenomenon were analyzed by the analyses of mussel cultivation technology, basic physico-chemical parameters of sea water, sanitary water quality analyses, condition index, analysis of the presence of the ectoparasite *Stylochus mediterraneus* Galleni, 1976 (ex *Imogine mediterranea* (Galleni, 1976)) and temperature and salinity values on the farms during the last decade. The occurrence of mass mortality in farmed shellfish is a phenomenon that in most investigated cases has no concrete and clear explanation. It is most likely that this event is the consequence of cumulative impact of several different factors: changes in the marine ecosystem that affect the health of the coastal ecosystem, climate change, the impact of pathogens, genetic mutations or abnormalities, water quality and food availability, characteristics of the location where the farm is located, geographical origin of spat, i.e. procedures for planting and translocation spat from one location to another, the impact of various pollutants originating from land.

Keywords: mass mortality, Bivalve, mussels, *Stylochus mediterraneus*, Mediterranean Sea

INTRODUCTION

Coastal marine ecosystems represent a very sensitive and dynamic network of coexistence of different living beings, organic and inorganic matter, with pronounced mutual interactions and different tolerances to change. Coastal ecosystems are one of the most productive ecosystems on earth, with resources that provide a range of economic benefits for humans, especially for the fisheries, aquaculture and tourism sectors, which is why about 1/3 of the world's population lives in the coastal zones (UNEP/MAP & Plan Blue, 2020). During the last decades, the pressure on the marine ecosystem has been increasing. Climate change, sea levels rising, extreme natural events (droughts, heat waves, algal blooms), marine pollution, invasive species input, intensive coastal construction, irresponsible use of water resources and overfishing, are just some of the risk factors for diversity loss, habitat degradation and overall marine ecosystem health (Walther *et al*., 2002; Danovaro, 2003; Galil, 2007; Gambaiani *et al.*, 2009; Tsikliras *et al*., 2013; Vasilakopoulos *et al.*, 2014).

The consequences that different changes may have on the marine ecosystem and the health of marine organisms are of different intensity and scope and largely depend on the tolerance of organisms to the level of change.

Mediterranean mussels (*Mytilus galloprovincialis* Linnaeus, 1758) is, together with blue mussel (*M. edulis* Linnaeus, 1758) one of the most important species in European marine aquaculture. *M. galloprovincialis* is a warm-temperate species, native of the Mediterranean Sea and highly tolerant to temperature and salinity changes (Skibinski *et al.*, 1983; McDonald *et al*., 1991; Gosling, 1992). Due to its well-known responsiveness to environmental contaminants and changes, *M. galloprovincialis* is widely used as an indicator organism in number of national and international bio-monitoring programmes (Martin-Diaz *et al*., 2009; Nikolić *et al.*, 2019). Intertidal mussels are important ecosystem engineers through their attachment to the substrate in dense mono- and multi-layered beds that create microhabitats that remain moist and thermally benign during low tides (Lathlean *et al*., 2017; Seuront *et al*., 2018; 2019) and offer protection against wave action during high tides (Oswald *et al*., 2014).

Cases of mass mortality of cultured mussels in Europe have become more frequent in recent years and relates mostly to the species of *M. edulis*, while recently, the cases of mass mortality of cultivated *M. galloprovincialis* have become more and more intense (Benabdelmouna & Ledu, 2016; Benabdelmouna *et al.*, 2018; Polsenaere *et al.*, 2017; Bracchetti *et al*., 2024). Mass mortality of living organisms is generally defined by a loss exceeding 30% of stocks (Soletchnik *et al.*, 2007). Some mass mortality events on bivalve molluscs are consequence of a disease caused by parasites and has been widely studied for commercial bivalve molluscs (Peters, 1988; Elston *et al*., 1992; Myrand & Gaudreault 1995; Barber, 2004; Guillotreau *et al*., 2018). Mass mortality of endemic Mediterranean pen shell (*Pinna nobilis* Linnaeus, 1758) in all Mediterranean Sea was caused by a parasitic disease caused by a protozoan of the genus *Haplosporidium* with worrying high mortality rates reaching up to 100% (Vázquez-Luis *et al*., 2017). The extent of mortalities can be highly variable for different mussel stocks, even in the same environments (Dickie *et al*., 1984; Mallet *et al*., 1987; 1990; Mallet & Carver 1989; Myrand 1990; Myrand & Gaudreault, 1995). Several analyses of the causes of high mortality events have shown that the genetic factor is one of the leading causes of mortality, although the cumulative impact of environmental, genetic and other factors is equally important in the analysis of numerous cases of mass mortality events (Myrand, 1990; Myrand & Gaudreault, 1995; Benabdelmouna & Ledu, 2016). Among the environmental factors, the most common causes of mass mortality are high temperatures and reduced oxygen concentration, especially in bays with poor water circulation and slow sea current regime (Guillotreau *et al*., 2017). In addition to environmental factors, an important cause of high mortality is the stress caused by the re-immersion of cultivated mussels after treatment of de-clumping, washing, selecting and de-byssing. High mortality rates could be organism's stress response to the procedure of handling, processing and storage practices before transportation to final marketplace (Theodorou *et al*., 2018).

Marine aquaculture in Montenegro

include farming of two species of bivalves – mussels (*Mytilus galloprovincialis*) and oysters (*Ostrea edulis* Linnaeus, 1758) with annual production of about 200 tonnes of mussels and between 19-32 tons of oysters (MONSTAT, 2022; 2023). One of the major problems of aquaculture sector in Montenegro is absence of a centre for dispatch and depuration of live molluscs (absence of sanitary-hygiene conditions necessary for exports), problem of predation, which is spread throughout the Mediterranean, as well as lack of organized market. The aim of this study is to analyse first mass mortality event of cultured mussel *Mytilus galloprovincialis* during summer period in Montenegrin part of the southern Adriatic Sea through analyses of condition index (CI), sea water quality and series of long-term data of temperature and salinity.

Study area

The research area includes the Boka Kotorska Bay, which is located in the southern part of the Adriatic Sea (Fig. 1). The waters of the Boka Kotorska Bay, with total area 87.3 km^2, are divided into three parts: the internal, the middle and the external one (Fig. 1). The internal and the middle part are under the great influence of the freshwater inputs (underwater springs, rivers, streams and precipitations), whereas the external part is under the influence of the open sea. The relief basis of the Boka Kotorska is one of the best indented parts of the Adriatic coast (Magaš, 2002). The coastline of the bay is 105.7 km long. At the entrance to the bay on the western side is Cape Oštra, while on the eastern is Cape Mirišta, and the passage between them leads to Herceg Novi Bay - the first of the four bays of the Boka Kotorska Bay. The Bay of Herceg Novi continues into the Bay of Tivat via the Kumbor Strait, which continues through the Straits of Verige into the Risan and Kotor Bays. The characteristic of the whole bay is the relatively large depth in the bays and straits. The maximum depth is 64 m (DerMap Project Report, 2011), while the average depth of all four bays is 27.6 m. Given the depths, the entire Bay of Kotor belongs to the coastal or littoral system. Precipitation in the Boka Kotorska reaches the point of the highest precipitation in Europe (4584 mm per year near Crkvice) due to the enormous mass of freshwater flowing into the basin of Risan Bay, which is relatively small and closed (Parenzan & Stjepčević, 1980). Precipitation varies greatly throughout the year. After the rainy season, that takes place in autumn, winter and early spring, there is a period of summer drought, with no rainfall for 3-4 months (July-September).

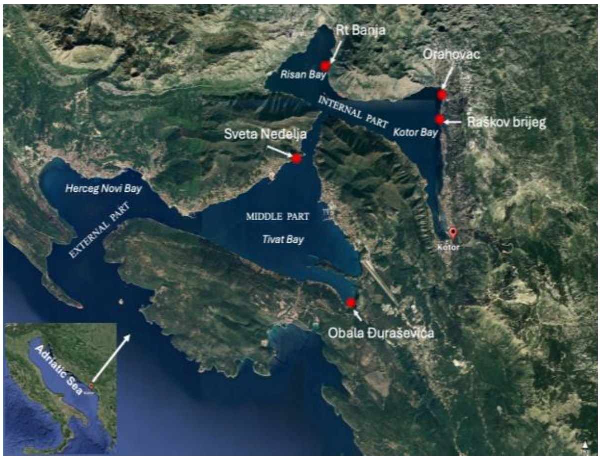


Figure 1. Boka Kotorska Bay with location of sampling (red stars)

Currently, there are about 23 active shell farms, among which are two fish farms using the integrated multi-trophic aquaculture system (IMTA). All farms are situated in Boka Kotorska Bay, while about 80% belongs to the area of Kotor-Risan bays. Bivalve farming is done using the method of floating long-line system. The analyzes were carried out at one breeding site per each of five production zones for the cultivation of shellfish (Tab. 1)

Table 1. Geographical coordinates (in WGS84 coordinate system) of breeding sites where analyses were performed

Location	Latitude (N)	Longitude (E)
Rt Banja	42° 29’ 57.78”	18° 41’ 29.96”
Sveta Neđelja	42° 27’ 31.63”	18° 40’ 18.60”
Raškov brijeg	42° 28’ 32.13”	18° 45’ 51.16”
Orahovac	42° 29’ 19.97”	18° 45’ 49.82”
Obala Đuraševića	42° 24’ 02.19”	18° 41’ 49.30”

MATERIAL AND METHODS

After reporting by mussel growers about the high mortality of cultivated mussels, field work was carried out in 5 production areas for mussel cultivation. The field work was carried out on September 16th 2020, although the first signs of mortality on the breeding grounds were observed in early June.

Mussel cultivation analyses

Control of cultivation installations implied checking the length and weight of "pergolas" or "rests" on which the growed mussels are knotted (estimation of the amount of marketable shells obtained by processing one pergola). Distance between the buoys and planting density was controlled in accordance with the scientific recommendations, that is, the Projects on the technological characteristics of shellfish farming, prepared for each farm separately by the Institute of marine biology. The project provides detailed information about each location with a proposal of farming technology, optimal planting density, collection of spat, cultivated species and an estimate of the annual mussel production, all based on the specificities of each site. Based on the Project, breeding permits were obtained by responsible Ministry.

Sanitary water quality analyses

Detection of coliforms and *Escherichia coli* was performed using membrane filtration technique according to MEST EN ISO 9308-1:2015/A1:2018. Water samples were filtered through 0.45 µm cellulose nitrate filters and placed on CCA (Chromogenic Coliform agar). It includes a mixture of chromogens: Salmon–Gal, which is hydrolyzed by *β*-D-galactosidase to produce a reddish color in coliform colonies, and X-gluc, hydrolyzed by *β*-D-glucuronidase, giving a blue color to the colonies. Both enzymes are synthesized by *E. coli*, so its colonies exhibit a dark blue color. The plates for total coliforms and *E. coli* were incubated for 21-24 h at 36°C ± 2°C, while fecal coliforms were incubated for 21-24 h at 44°C ± 0.5°C. Total coliform bacteria counted as the sum of oxidase-negative colonies with pink to red color and all dark-blue colonies grown at 36°C ± 2°C, while fecal coliforms counted as the sum of oxidase-negative colonies with pink and dark blue color grown at 44°C ± 0.5°C. Detection of fecal enterococci was performed using membrane filtration technique according to MEST EN ISO 7899-2:2016. Water samples were filtered through 0.45 µm cellulose nitrate filters and placed with forceps on the surface of Slanetz and Bartley agar. The plates were incubated at 36°C ± 2°C for 44 h ± 4 h. The typical colonies were convex red. For the confirmation test, the filter was removed on the Bile esculin agar and incubated at 44°C ± 0.5° C for two hours. All black colonies counted as fecal enterococci. One-time sampling and analyzes were carried out on September 16th, 2020.

Analyses of the basic physico-chemical parameters of sea water

The analysis of the basic physico-chemical parameters of the sea was analysed by monthly dynamics in the period from June to August 2020 as part of the regular monitoring programme of phytoplankton communities in production areas for shellfish cultivation. In order to assess the possible influence of temperature on the mortality rate, the available data collected during water quality monitoring at shellfish farms in the period from 2010 to 2020 were analysed. The values of the temperature were determined by automatic probes MultiLine 4, WTW.

Condition index

For analysing the condition index (CI), 30 adult individuals from each farm were sampled and transported to the laboratory in chiller ice box. One-time sampling and analyzes were carried out on September 16th, 2020. All individuals had a shell length greater than 50 mm, which is the minimum legal size (Official Gazette of Montenegro, 65/15). In the Institute, the individuals were cleaned of fouling organisms, their total weight (TW), wet meat weight (WMW) and shell weight (SWe) were measured with the help of a scale with a precision of 0.1 g. The individuals were also processed morphometrically, using a vernier caliper to the nearest of 0.1 mm, the shell width (SWi) shell height (SH) and shell length (SL) were measured. The average value and standard deviation (ST) were calculated for all parameters. Differences in CI values among locations were tested with the Kruskal-Wallis test, as Levene's test indicated that the variance was not homogeneous.

The condition index was calculated based on the method proposed by Almeida *et al*. (1999), as the ratio of wet meat weight to total weight (equation 1).

$$CI = (WMW / TW) * 100 \qquad (1)$$

RESULTS AND DISCUSSION

Analyses of mussels stocking density

By the control of five breeding sites it was found that the distance between the buoys was done in accordance with the recommendations, that is, the Projects on the technological characteristics of shellfish farming. Between each buoy on one line rope (at a distance of about 10 meters) there were an average of 20-22 pergolas with an average length of about 1.5-2 m. The inspection determined that the density of stocking was optimal, i.e. that it could not cause higher mortality of shellfish due to the lack of nutrients and/or limited space for growth and development.

A visual inspection revealed the poor condition of the mussels, with the byssus threads weakly connected between the individuals, while the empty shells of the larger individuals were easily broken by hand. Given that the mussel planting on all farms was carried out in the late autumn of the previous year and that the growers reported the onset of mortality by early June 2020, this fact indicates the possibility that mortality occurred at least 2 months ago in relation to the time of the analysis.

The total weight of one pergola with consume size of mussels under normal conditions is on average about 20 kg (Fig. 2). On the spot, one pergola was processed on each farm and all consume individuals were separated. In "normal" conditions, one 1.5-2 meter long pergola yields 10-15 kg of marketable mussels, while on-site processing has determined a quantity of 2-3 kg of marketable mussels at each farm. This data points to extremely high mortality, in the range of 80-90%, or to the occurrence of mass mortality of cultivated mussels.

Figure 2. The pergola impacted by mass mortality (left) and healthy pergola (right)

Analysis of sanitary water quality

Total and faecal coliform bacteria, *E. coli* (according to the MEST EN ISO: 9308-1:2015 standard) and intestinal enterococci (according to the MEST EN ISO: 7899-2:2011 standard) were analysed using the membrane filtration method (Tab. 2). These analyses, although one-time, were performed in order to include as many seawater parameters as possible during the period of the most intense mussel mortality.

In accordance with the national legislation, i.e. Table 2 of the "Regulations on the method and deadlines for the implementation of measures to ensure the preservation, protection and conservation of bathing water" (Official Gazette of the Republic of Montenegro, 028/19), the water quality at all tested locations belongs to the class of "excellent quality", with the exception of the location Orahovac when it comes to intestinal enterococci, which belongs to the class of "good quality" water.

Table 2. Results of the analysis of sanitary water quality

Location	**Total coliform/100ml**	**Faecal coliforms/100ml**	***E. coli***	**Intestinal enterococci /100ml**
Rt Banja	3	1	1	17
Sveta Neđelja	2	1	1	1
Raškov brijeg	1	0	0	0
Orahovac	23	21	20	70
Obala Đuraševića	58	4	3	15

Analysis of the basic physico-chemical parameters of the sea

The analysis of the basic physico-chemical parameters of the sea was analyzed from a series of existing data collected as part of the national monitoring programme of shellfish farms in the period from 2010 to 2020 (Fig. 1-3, Tab. 3-5). The data are presented for eight farms where monitoring was carried out, in order to show the values of temperature and salinity in the entire researched area. Measuring was done from the surface (0.5 m depth).

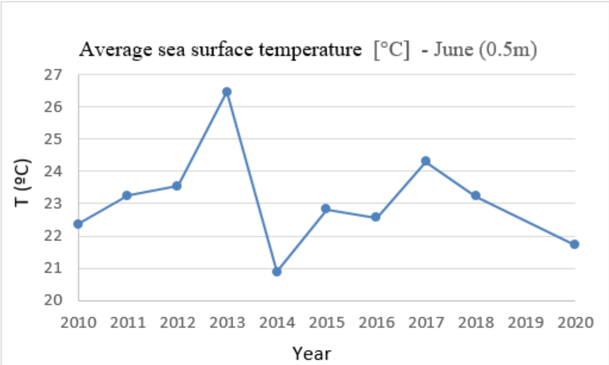
Analysis of temperature and salinity for the period June-August from 2010 to 2020 (except for June 2019 when monitoring activities were not performed) were tested for significance by Kruskal Wallis test. Analyses showed a statistically significant difference in temperature values for the period from 2010-2020 for June (H = 24.74, $p < 0.05$), July (H = 51.02, $p < 0.05$) and August (H = 28.71, $p < 0.05$). A statistically significant difference in salinity values was obtained for all analyzed months for the period 2010-2020: June (H = 32.72, $p < 0.05$), July (H = 32.77, $p < 0.05$) and August (H = 44.73, $p < 0.05$).

The lowest temperature in June was recorded in 2016 (17.7°C), and the highest in June 2013 (29.1°C). For July, the minimum temperature was recorded in 2013 (20.3°C) and the maximum in 2015 (27.05°C). In August, the minimum temperature was observed in 2012 (22.7°C) and the maximum in 2015 (27.85°C).

The lowest salinity values for June, July and August were found in 2010 (6.51 ‰, 24.45 ‰ and 20.16 ‰, respectively), while the maximum values were found in 2020 (36.64 ‰, 38.31 ‰ and 38.32 ‰ for June, July and August, respectively).

However, it is important to note that the measurements were carried out only once a month, which significantly affects the reliability of the data. During the summer months of 2020, in numerous interviews with local fishermen and mussel growers, extremely high sea surface temperatures were reported in the area of Boka Kotorska Bay (up to 32°C, personal comm.), i.e. several heat waves, which could certainly cause the occurrence of mass mortality of mussels.

Although thermal tolerance range for adult *M. galloprovincialis* is from 10°C to 32°C (Braby & Somero, 2006; Denny *et al.*, 2011; Somero, 2011). Previous research shows that *M. galloprovincialis* cannot survive sea water temperatures of and beyond 26°C over extended periods of time; temperatures higher than 24°C might be attributed to reduced ability to assimilate food and associated energy (Anestis *et al.*, 2007).



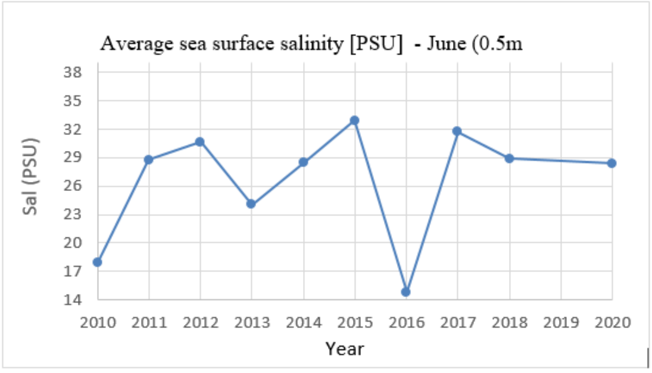


Figure 1. Average SST (°C) - left and SSS (‰) - right for June (2010-2020)

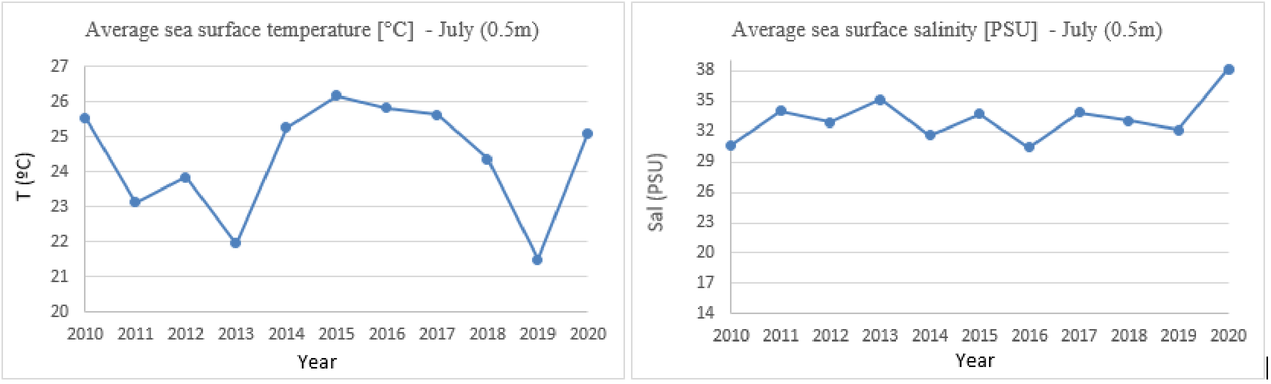


Figure 2. Average SST (°C) - left and SSS (‰) - right for July (2010-2020)

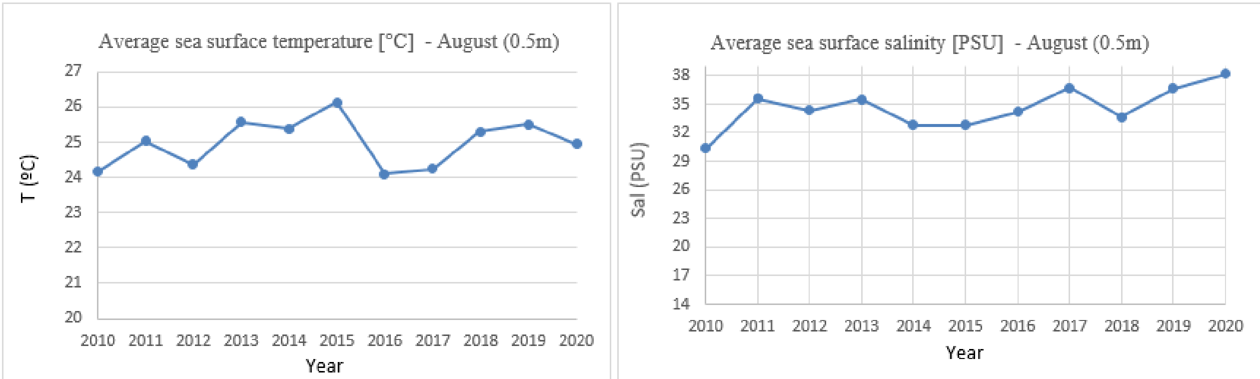


Figure 3. Average SST (°C) - left and SSS (‰) - right for August (2010-2020)

Table 3. Sea surface temperature (°C at 0.5 m depth) in June at shellfish farming locations in the period from 2010 to 2020

Bay	Location	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Kotor	IBMK	21.3	25.05	26	22.1	19.8	21.4	19.15	22.9	22.25	/	
Kotor	Raškov brijeg	22.4	22.6	22.6	29.1	20.8	21.55	17.85	23.35	22.35	/	
Kotor	Orahovac	21.6	20.05	22.4	28.6	20.3	22.25	17.7	22.35	23.15	/	21.3
Kotor	Brbat	22.15	24.35	24.4	26.4	21.3	22.4	25.5	25.2	23.2	/	
Risan	Lipci	21.05	20.25	22	/	/	/	/	/	/	/	21.5
Tivat	Sveta Neđelja	21.75	20.3	21.9	26.5	20.6	23.35	24.65	25.5	22.7	/	21.3
Tivat	Kalardovo	24.7	26.55	22.8	26.6	21.8	24.45	26.95	26.55	24.6	/	22.72
Tivat	Obala Đuraševića	24	26.85	26.2	26	21.6	24.35	26.15	24.3	24.4	/	
	AVERAGE	22.37	23.25	23.54	26.47	20.89	22.82	22.56	24.31	23.24	/	21.71
	ST.DEV	1.31	2.85	1.76	2.27	0.72	1.25	4.14	1.53	0.94	/	0.68

Table 4. Sea surface temperature (°C at 0.5 m depth) in July at shellfish farming locations in the period from 2010 to 2020

Bay	Location	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Kotor	IBMK	24.75	22.9	23.7	21	23.8	25.7	24.4	24.7	21.55	22.5	25.2
Kotor	Raškov brijeg	26.65	23.6	23.8	20.6	25.1	25.75	25.85	24.2	24.25		
Kotor	Orahovac	26.4	21.9	23.4	20.3	25.6	25.4	25.55	23	21.65	22	
Kotor	Brbat	26.55	23.4	23.7	21.9	25.2	26.55	26.05	26.5	25.75		26
Risan	Lipci	24.6	22.2	23.7	22.4	24.2	26.2	25.9	26.7	24.95	21	24.3
Tivat	Sveta Neđelja	23.9	23	23.9	21.4	25.3	25.65	25.55	26.3	24.75	20.5	25.3
Tivat	Kalardovo	25.8	24.5	24.7	24.6	26.5	27.05	26.95	27.1	26.25		24.7
Tivat	Obala Đurasevića	25.65	23.7	23.9	23.4	26.3	27.05	26.35	26.7	25.75		
	AVERAGE	25.54	23.13	23.85	21.74	25.25	26.17	25.83	25.63	24.36	21.50	25.10
	ST.DEV	1.02	0.86	0.38	1.45	0.93	0.65	0.73	1.47	1.82	0.91	0.64

Table 5. Sea surface temperature (°C at 0.5 m depth) in August at shellfish farming locations in the period from 2010 to 2020

Bay	Location	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Kotor	IBMK	23.45	25.8	24.2	24.4	24.9	24.35	24.55	23.75	24.3	25.5	23.3
Kotor	Raškov brijeg	24.1	25.2	25.3	25.1	24.5	26.35	23.6	24.05	24.9	/	24.7
Kotor	Orahovac	23.25	23.45	24.3	26.2	24.7	25.9	23.4	22.95	24.2	25	
Kotor	Brbat	25.4	25.55	25.5	26.7	25.5	25.5	24.1	24.7	26.3		
Risan	Lipci	22.75	23.9	24	/	/	/	/	/	/		24.7
Tivat	Sveta Neđelja	23.85	25.3	22.7	25.7	24.8	26.25	24.45	24.9	25.1	25	25.5
Tivat	Kalardovo	25.8	24.4	25.1	25.4	26.1	26.7	23.4	24.1	25.7	26	25.9
Tivat	Obala Đurasevića	24.65	26.55	23.8	25.5	27.1	27.85	25.1	25.2	26.55	26	25.5
	AVERAGE	24.16	25.02	24.36	25.57	25.37	26.13	24.09	24.24	25.29	25.50	24.93
	ST.DEV	1.06	1.03	0.92	0.74	0.94	1.08	0.65	0.77	0.92	0.50	0.93

Results of the condition index analysis

Results related to morphometry and condition index of in total 150 individuals of *Mytilus galloprovincialis* are presented below.

Tab. 6 shows the mean values of total weight, wet meat weight, shell weight, shell width, shell height and shell length. It can be seen that the individuals differ the most in the mean values of the total weight, while the mean values of the other parameters are mostly similar in the individuals from all analysed farms.

Tab. 7 shows the mean values of the condition index of the tested individuals at five farms. Based on the obtained mussels CI, spatial differences are evident and statisticaly significant (H = 51.416, p < 0.05). CI values were below 20 at all breeding grounds except for the Rt Banja, where CI is 23.15 ± 9.06. The obtained results indicate low values of CI in

Table 6. Morphometry of the analyzed individuals (TW – total weight; WMW – wet meat weight, SWe – shell weight, SWi – shell width, SH – shell height, SL – shell length, SD – standard deviation

Locality	**TW (g) ± SD**	**WMW (g) ± SD**	**SWe (g) ± SD**	**SWi (cm) ± SD**	**SH (cm) ± SD**	**SL (cm) ± SD**
Obala Đuraševića	31.28 ± 6.8	4.61 ± 1.13	12.57 ± 2.97	3.55 ± 0.28	2.57 ± 0.21	7 ± 0.7
Sveta Neđelja	28.57 ± 7.37	4.15 ± 0.98	11.44 ± 2.49	3.53 ± 0.27	2.48 ± 0.22	6.65 ± 0.9
Rt Banja	19.78 ± 5.07	4.54 ± 2.25	9.01 ± 1.9	3.35 ± 0.21	2.27 ± 0.24	6.37 ± 0.53
Orahovac	23.1 ± 5.6	3.4 ± 0.96	8.33 ± 2.19	3.29 ± 0.29	2.28 ± 0.24	6.27 ± 0.65
Raškov brijeg	22.26 ± 6.46	3.72 ± 1.01	9.07 ± 2.5	3.33 ± 0.3	2.34 ± 0.23	6.54 ± 0.52

the tested individuals at all farms. These results coincide with the results given by Gvozdenović *et al*. (2017) and Nikolić *et al*. (2023) for the area of the Boka Kotorska Bay during September.

Table 7. Mean values of the condition index (CI) on the farms (SD – standard deviation)

Locality	**CI ± SD**
Obala Đuraševića	15.02 ± 3.01
Sveta Neđelja	14.85 ± 2.94
Rt Banja	23.15 ± 9.06
Orahovac	14.83 ± 3.12
Raškov brijeg	17.09 ± 3.16

It is known that changes in the reproductive cycle of mussels significantly influence changes in the condition index, and that depending on the environmental conditions in the same locality, mussels can change their reproductive strategy (Gosling, 1992). For this reason, the lower values of the CI during the summer period are most likely the result of empty gonads and the resting stage that mussels enter after spring spawning what is also confirmed by Gvozdenović *et al*. (2017) and Nikolić *et al*. (2023). The resting stage of the gonads is correlated with the seawater temperature and the individuals remain in a state of rest until the seawater temperature drops below 20°C (Da Ros *et al*., 1985; Gvozdenović *et al*., 2017; Nikolić *et al*., 2023).

Stylochus mediterraneus

The presence of the ectoparasitic flatworm *Stylochus mediterraneus* Galleni, 1976, which belongs to the order Polycladida, family Stylochidae, was observed in all samples (Fig. 3). It is generally known that species of this order feed on sessile invertebrates, primarily mussels and scallops (Gammoudi *et al.*, 2017). The mechanism of predation of this worm on mussels was described by Gammoudi *et al.* (2017). The worm goes to the edges of the mussel shells, and when the mussels open the shells, it first digests the adductor muscles, after which it eats the rest of the tissue. Several authors suggest the use of different "preparations" to control this parasite, such as: the use of calcium hypochlorite solution (Yang, 1974) as well as increasing salinity (Espinosa, 1981).

Given that the control determined the highest mortality in specimens over 6 cm in size, we believe that this predator could not have caused such a high mortality rate. The earlier findings of this worm at breeding sites in the Boka Kotorska Bay area indicated a certain mortality rate, but not higher than the rate caused by predation by other species (e.g. starfish).

Mass mortality of farmed mussels was determined, in the range of 80-90% of adult individuals. The appearance of the ectoparasite *S. mediterranea* determines a certain mortality rate, but so far it has not been recorded to cause a mass mortality events. The values of temperature and salinity showed significant differences during the summer months of the last decade (2010-2020), but considering the

Figure 3. *Stylochus mediterraneus* Galleni, 1976 in shellfish samples from mussel farms in the Boka Kotorska Bay

fact that the mussels are organisms with a wide range of temperature and salinity tolerance, we assume that these parameters are not the cause of mass mortality of mussels. It is absolutely necessary to carry out regular measurements of basic parameters (temperature and salinity) at all shellfish farms by using data loggers or similar devices. However, in personal communication with fishermen and mussel

CONCLUSION

Analyses of sanitary water quality and basic physico-chemical parameters of seawater indicated that the water quality at the farms was within class A (excellent quality), except for the farm in Orahovac (in relation to the amount of intestinal enterococci). The control of stocking density and cultivation technology showed that the cultivation was optimal, and that the stocking density could not cause any disturbances in the growth and development of the cultivated individuals.

breeders who monitor the sea temperature with fish finders (sonars with temperature sensors) temperatures above 30 degrees were reported, that is, several heat waves during the summer of 2020, which could potentially cause mass mortality event. Given that the first signs of mortality were observed at the beginning of June 2020, it is assumed that the temperature was not a decisive factor for this phenomenon.

Although the analysis and control of the farms did not provide an answer about the exact cause of mass mortality, the occurrence of mass mortality of farmed mussels was determined, as a phenomenon that occurs in many countries of the world for no apparent reason, but with enormous consequences, both for the continuation of the breeding process and for socio-economic circumstances of growers.

During the last decade, regular cases of mass mortality events have been recorded in different species of marine bivalves along the French coast: *Magallana gigas* (Miossec *et al.*, 2009; Garcia *et al.*, 2014), *Donax trunculus* (Garcia *et al.*, 2019), *Cerastoderma edule*(Garcia *et al.*, 2019) and mussels *Mytilus* sp. (Garcia *et al.*, 2015; Lupo & Prou, 2016).

The etiology of these events is often unknown, and the current consensus within the scientific community is that their origin is multifactorial (Lupo *et al.*, 2021). It is most likely that it is the cumulative impact of changes in the marine ecosystem that affect the health of the coastal ecosystem, the impact of pathogens, the quality of water in the breeding grounds, the characteristics of the locality where the breeding ground is located, the geographical origin of the spat, i.e. the procedures for stocking and translocation spat from one location to another, the impact of various pollutants originating from land and climate change.

Namely, all the mentioned impacts are individually risk factors for increasing the mortality rate, but for the area of the Boka Kotorska Bay, based on all the analyses carried out by the Institute of Marine Biology, it is not possible to conclude what is the exact cause of mass mortality events of farmed mussels. In order to monitor further changes and determine the causes of mass mortality events, it is necessary to develop regional (or wider international) projects that, through data sharing, standardization of methods and with the involvement of mussel farmers, will enable significant progress in understanding the changes that lead to mass mortality events.

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Masovni mortalitet uzgojenih mušulja – fenomen bez objašnjenja?

Milica MANDIĆ, Slađana NIKOLIĆ, Ines KOKIĆ i Sandra MARKOVIĆ

SAŽETAK

Prvi slučaj masovnog mortaliteta mušulja (*Mytilus galloprovincialis* Linnaeus, 1758), zabilježen je na području Bokokotorskog zaliva (južni Jadran) u periodu od juna do septembra 2020. godine. Mortalitet je uzrokovao gubitak proizvodnje u opsegu od 80-90%. Mogući ekološki i sredinski uzroci ove pojave analizirani su provjerom tehnologije uzgoja dagnji, analizom osnovnih fizičko-hemijskih parametara morske vode, analizama sanitarnog kvaliteta mora, kondicionog indeksa, analizom prisutnosti ektoparazita *Stylochus mediterraneus* Galleni, 1976 (ex *Imogine mediterranea* (Galleni, 1976)) i vrijednostima temperature i saliniteta na uzgajalištima tokom posljednje decenije. Pojava masovnog mortaliteta školjkaša iz uzgoja fenomen je koji u većini istraženih slučajeva nema konkretno i jasno objašnjenje. Najvjerovatnije je to posljedica kumulativnog uticaja više različitih faktora: promjena u morskom ekosistemu koje utiču na zdravlje obalnog ekosistema, klimatskih promjena, uticaja patogena, genetskih mutacija ili abnormalnosti, kvaliteta vode i dostupnosti hrane, karakteristike lokacije na kojoj se uzgajalište nalazi, geografsko porijeklo mlađi, odnosno postupci nasada i premještanja mlađi sa jednog mjesta na drugo, uticaj raznih zagađivača sa kopna.

Ključne riječi: masovni mortalitet, školjke, mušulje, *Stylochus mediterraneus*, Mediteran