Pol. J. Environ. Stud. Vol. XX, No. X (XXXX), 1–7
DOI:*10.15244/pjoes/188029* ONLINE PUBLICATION DATE:

Original Research

First Record of Species *Ostreopsis* cf. *ovata* in the Water Column on the Montenegrin Coast of the Adriatic Sea (Boka Kotorska Bay)

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Received: 3 March 2024
Accepted: 27 April 2024

Abstract

Distribution of the phytoplankton community and presence of potentially toxic and toxic species were studied on mussel farms in the mesotrophic area of Boka Kotorska Bay as part of the national monitoring of aquaculture farms.

In this area, the presence of an epiphytic potentially toxic dinoflagellate species, *Ostreopsis* cf. *ovata*, was noticed for the first time. Species of the genus *Ostreopsis* are marine, benthic dinoflagellates. The high abundance of *Ostreopsis* species can result in a brownish mucilaginous biofilm forming on various substrates. Additionally, an abundance of toxic dinoflagellates from the genus *Ostreopsis* is known to cause dangerous respiratory symptoms in humans exposed to aerosols.

We performed national monitoring on four mussel farms in the area of Boka Kotorska Bay. During this monitoring at the aquaculture position Dražin vrt (BK1), the presence of species from the genus *Ostreopsis* was recorded for the first time. The abundance of the species reached a value of 10^3 cells/l.

The presence of the potentially toxic species from the genus *Pseudo-nitzschia* was also observed and reached an abundance of 10^4 cells/l. Additionally, among the potentially toxic dinoflagellates, *Dinophysis acuta* and *Phalacroma rotundatum* were recorded, although their abundance was lower, reaching values up to 10^2 cells/l.

The noticed presence of the toxic species Ostreopsis cf. ovata, along with several other potentially toxic and toxic phytoplankton species, highlights the importance of raising awareness about the necessity of continuous monitoring activities and implementing preventive measures.

Keywords: toxic dinoflagellates, genus Ostreopsis, Montenegrin coast

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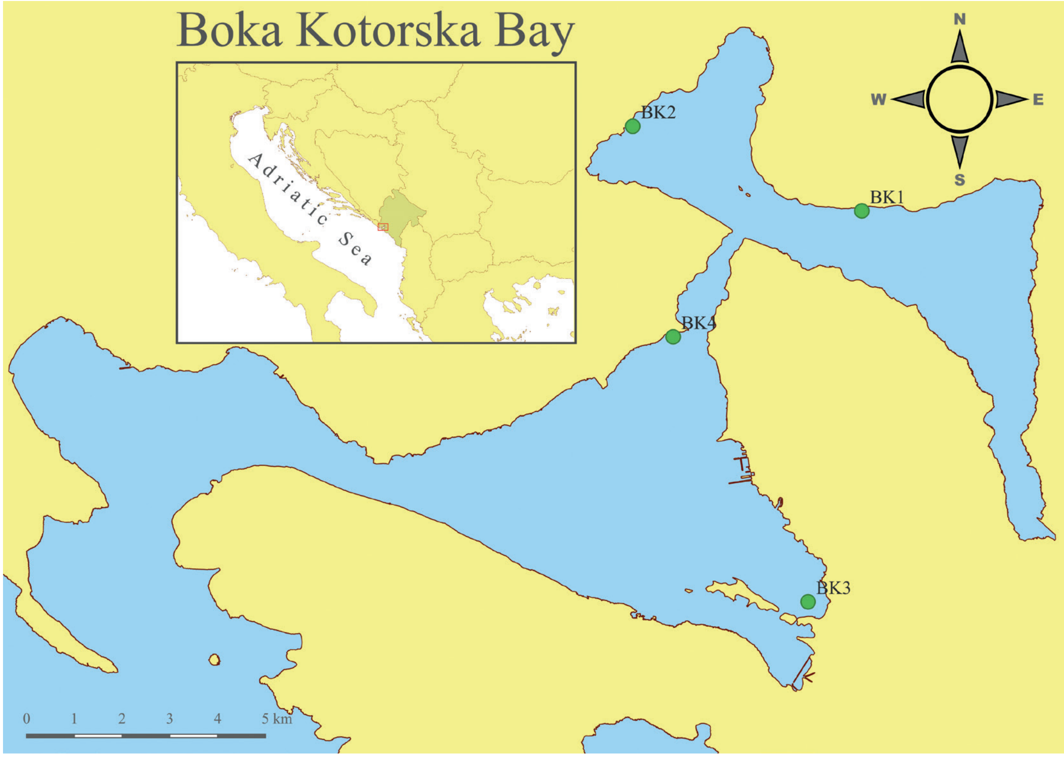

Fig. 1. Area of investigation on which sampling of phytoplankton communities was performed in the Bay area (BK1, BK2, BK3, and BK4) during 2019 (QGIS48) (http://www.qgis.org).

Introduction

The Montenegrin coast comprises the semi-enclosed, fjord-like Boka Kotorska Bay, as well as the open coastal area of the sea. Boka Kotorska Bay is a relatively small transitional system that presents an environmental boundary between land and sea, with specific community assemblages [1]. The area of Boka Kotorska Bay has higher precipitation due to the presence of high karstic mountain massifs [2]. Additionally, the bay area is under greater influence from the land compared to the open coastal region.

The species of the genus *Ostreopsis* are marine, benthic dinoflagellates. They primarily inhabit epiphytically on benthic macroalgae, although they can also be found on sand, rocks, and in the water column. The increased abundance of *Ostreopsis* species can lead to the formation of a brownish mucilaginous biofilm on various substrates.

Toxic species belonging to the marine dinoflagellate genus *Ostreopsis* are known to be present in various environments, from tropical and sub-tropical to temperate areas [3]. Mass appearances of the toxic dinoflagellate genus *Ostreopsis* (≥10000 cells/l) have been noticed to cause dangerous respiratory problems in humans exposed to aerosols [4-6]. Additionally, *Ostreopsis* species produce different toxins that can be transferred to certain fish and invertebrates, posing a potential risk of food poisoning to humans as well [3, 7, 8]. Also *Ostreopsis* blooms have a negative impact, causing mass mortalities of certain invertebrates [3] caused either by oxygen limitation due to the excess of microalgal biomass or specific ecotoxic compounds.

In Croatian waters, the first record of *Ostreopsis* cf. *ovata* was reported from the central Adriatic Kaštela Bay in 1984 [9]. Available literature confirms that, in the Adriatic Sea, the highest abundances of Ostreopsis species were observed along the Marche and Apulia coasts [9, 10]. The highest abundances on macroalgae were noted in 2008 and 2009, while the highest abundances in seawater were reported in 2006, 2010, and 2016 (reaching values up to 10^7 cells/l) [9].

The scope of this paper is to inform about the first records of toxic species on the Montenegrin coast and to emphasize the necessity of monitoring the presence and abundance of these species, knowing the negative effects they have on marine ecosystems and human health.

Material and Methods

Study Area

Sampling for *Ostreopsis* analysis was carried out as part of the monthly monitoring program at four mussel farms in the area of Boka Kotorska Bay. It is important to note that only data from October 2023 are considered for both total phytoplankton and Ostreopsis analysis (see Fig. 1). Water samples for physico-chemical and phytoplankton analysis were taken from one depth, up to 5 meters (maximal

Table 1. Maximal (Max.), minimal (Min.), and average values of temperature (Tem.) and Salinity (Sal.) and maximal, minimal, and average abundances of total microplankton (TMP) and total nanoplankton (TNP) at investigation area during 2023.

	Max	Min	Avg	STD
Tem. (°C)	21.8	20.2	21.25	0.73
Sal. (psu)	37.2	35.2	36.05	0.83
TMP (cells/l)	8.96×10^4	4.74×10^4	6.98×10^4	1.93×10^4
TNP (cells/l)	1.35×10^5	8.72×10^4	1.08×10^5	2.13×10^5

Max. – maximal; Min. – minimal; AVG-average; STD – standard deviation

Tem. (°C).– temperature; Sal.(psu) – salinity; TMP(cells/l) – total microplankton; TNP (cells/l) – total nanoplankton

depth at position Dražin vrt (BK1) where *Ostreopsis* was observed is 23.3 m, while on other investigated positions maximal depths were BK-2-24.5 m, BK3-21.9 m, and BK4-7.6 m). Samples were taken with 5 L Niskin bottles. Two parameters (temperature and salinity) were measured directly in situ using a multi-parameter portable device (Multiline P4; WTW).

For the sampling of phytoplankton, 5-L Niskin bottles were used, and the collected samples were transferred into 250 ml plastic bottles. Preservation of samples was performed using a 4% neutralized formalin solution. Samples were transported in a refrigerator under dark conditions at a temperature of approximately 8 ± 3°C. The sampling was performed according to the guidelines in MEST ISO 5667-9:2020-Water quality – Sampling Part 9: Guidance on sampling from marine waters.

Phytoplankton cells were counted using a Zeiss Axio inverted microscope (Axio Observer A1) in accordance with the method of Utermöhl (1958) [11] (MEST EN 15204: 2014-Water quality – Guidance standard on the enumeration of phytoplankton using inverted microscopy). In the laboratory, samples were settled in sedimentation chambers of 25 ml, and after a period of sedimentation of 24 h, we started with the processing of the determination. Enumeration was performed at the following magnifications: 200 ×, 400 ×, and 630 ×. The whole bottom chamber (taxa larger than 30 µm) was crossed at a magnification of 200 × (enumeration of *Ostreopsis* cells was done this way), while at the same magnification, two transects were used for counting abundant microplankton (> 20 µm). Small phytoplankton (nanophytoplankton 2–20 µm) were counted at magnifications of 400 × and 630 × using 10 randomly selected fields. Determination of phytoplankton species was performed using an appropriate key [12–15]. Non-identified microalgae were classified into taxonomic categories: nanoflagellates, small dinoflagellates, small coccolithophores, and chrysophytes.

Results and Discussions

Environmental Data

During the research period (October 2023), maximal temperature was recorded at position BK1-Dražin vrt and it was 21.8°C, while minimal temperature values were observed at position BK2-Lipci (20.2°C). During the summer period, temperature ranged from 24.1–25.0°C (Table 1). In the north Adriatic Sea, it was observed that the highest abundances of *Ostreopsis* do not match with the highest water temperature values in all areas.

Accoroni and Totti [16] suggested that environmental factors play a crucial role in *Ostreopsis* blooms, as the species tends to produce more toxins under suboptimal conditions. It is interesting that *Ostreopsis* cf. *ovata* can produce both temporary and resting cysts. Specifically, resting cysts have been noticed to germinate for up to 5 months after formation at 25°C, but not at 21°C. This temperature-dependent cyst germination suggests that temperature may be a key factor influencing the *Ostreopsis* cf. *ovata* blooms. This observation could explain the presence of *Ostreopsis* in our study during October, when temperatures were lower compared to the summer period.

In the northern Adriatic Sea, *Ostreopsis* blooms often occur approximately 30 days after temperatures reach 25°C, indicating that other factors besides temperature may also impact the growth of *Ostreopsis* cf. *ovata* [16]. Several authors have similarly emphasized the role of temperature in favoring *Ostreopsis* cf. *ovata* blooms [17].

Regarding values of salinity concentration, the highest salinity was measured (37.2 psu) in position BK3-Kalardovo, while the lowest value of salinity was in position BK1-Dražin vrt (35.2 psu) (Table 1).

Accoroni and Totti 2016; Pezzolesi et al. [16, 18] observed that benthic dinoflagellates growth is favored by lower salinity waters, and mostly occurs in periods of maximal rainfall, which was noticed in our study. During the study of the relationships between algal growth and salinity, other factors, such as levels of nutrients (which are typically associated with low salinity waters) have to be considered [16]. On the other side, some studies showed that *Ostreopsis* cf. *ovata* grew better under higher salinity conditions, but others suggested that it depends on strains [17]. A lot of studies have emphasized the influence of environmental factors on *Ostreopsis* blooms, yet the full complexity of conditions leading to these blooms remains incompletely understood [16]. Both abiotic and biotic factors play significant roles, including interactions with other organisms and the use of organic forms of nutrients. It is interesting that several harmful algal bloom (HAB) genera have been shown to need organic forms of nutrients to meet their nutritional requirements. Furthermore, the effects of biotic interactions on *Ostreopsis* should be considered. However, only a few studies have investigated these interactions, focusing on bacteria, diatoms, and macroalgae [16, 19].

In the south-eastern Mediterranean Sea [20], it was observed that between salinity of 25 psu and 35 psu, *Ostreopsis* cf. *ovata* cells were almost absent in the water column. Its growth is recorded, in the Gulf of Gabès at a salinity of 35 psu and reaching its peak at a salinity of 45 psu.

Phytoplankton Parameters

The abundance of total phytoplankton during October 2023, at position Dražin vrt in the area of the Boka Kotorska Bay reached values up to 10^5 cells/l. The highest recorded value of microplankton- higher phytoplankton fraction was at position Kalardovo BK3 (8.96×10^4 cells/l), while the lowest abundance was at position Lipci-BK3 and it was 4.74×10^4 cell/s. Nanoplankton – smaller fraction was the highest at position BK3-Kalardovo (1.35×10^5 cells/l) and the lowest abundance was recorded at position Lipci-BK2 (8.72×10^4 cells/l) (Table 1).

The distributions of total microplankton (TMP) and phytoplankton groups-diatoms and dinoflagellates along the research area were presented (Fig. 2). Diatoms were the dominant group of phytoplankton with maximum abundance at position BK3 (8.62×10^4 cells/l).

Dinoflagellates were the second most frequent and abundant phytoplankton group during the research period. Previously, the dominance of diatoms was observed in both Boka Kotorska Bay [21, 22] and the northern Adriatic Sea [23, 24] on both sides.

Dinoflagellates values ranged up to 10^3 cells/l. The highest abundance of dinoflagellates was recorded at the position Dražin vrt (BK1) reaching 6.96×10^3 cells/l, while the lowest abundance of dinoflagellates was recorded at the position Sveta Nedjelja (BK4), with 320 cells/l.

In the area of Boka Kotorska Bay and in the Montenegrin coast the presence of the species from the genus *Ostreopsis* was recorded for the first time. The species was recorded in the water column, up to a depth of 5 m (Fig. 3A, Fig. 3B). The abundance of the species was 5.92×10^3 cells/l while the total abundance of dinoflagellates at position Dražin vrt was 6.96×10^3 cells/l. This confirms that at position Dražin vrt (BK1) in the water mass species, *Ostreopsis* cf. *ovata* contributed mostly to dinoflagellates abundances.

The presence of *Ostreopsis* cf. *ovata* (with an abundance of 5.92×10^3 cells/l in water) was recorded during the fall period in October 2023 as part of our regular monitoring. Additionally, during the research (October 2023) at BK1 position several other dinoflagellate species were recorded: *Gonyaulax* spp., *Gyrodinium fusiforme*, *Prorocentrum compressum*, *P. triestinum*, .*Protoperidinium crassipes*, *Tripos kofoidii,* and *T. lineatus.* On position BK2 it was noticed presence of dinoflagellate *Dinophysis acuta* and at BK3 position presence of *Phalacroma rotundatum*.

Kužat et al. [25] observed higher abundances of *Ostreopsis* in the north-eastern Adriatic coast (Istrian peninsula and Kvarner Bay) compared to the south-eastern Adriatic Sea (the area around the city of Dubrovnik) at the end of the summer period. This discrepancy with our findings may be due to unfavorable ecological conditions, which can change the timing of *Ostreopsis* presence or bloom events. In the study by Kužat et al. in 2021 [25], lower temperatures and salinity resulting from inputs of submersed coastal freshwater springs were found to influence the presence of *Ostreopsis* at some stations. These findings underscore the necessity for more detailed monitoring along the entire Adriatic coast (both north-eastern and south-eastern parts).

Furthermore, in the north-western Adriatic [6, 16], it was documented that the peak of *Ostreopsis* at some stations could occur earlier and with higher abundances due to changes in ecological conditions.

In their study, Kužat et al. [25] observed differences in ecological conditions, including anthropogenic influence and eutrophication, at all sampling stations where the presence and blooms of *Ostreopsis* cells were recorded. This confirms the notion that anthropogenic pressure increases the risk of harmful *Ostreopsis* cf. *ovata* blooms along the generally oligotrophic eastern Adriatic coast.

However, while significantly higher cell abundances corresponded to densely populated areas around the cities of Poreč and Rovinj, the direct influence of anthropogenic factors on the occurrence of *Ostreopsis* blooms is present. Although the correlation between higher abundances of *Ostreopsis* cf. *ovata* and the trophic load discharged into marine waters from anthropogenic sources has been noticed, a clear relationship between anthropogenic impact and *Ostreopsis* cf. *ovata* abundances has yet to be confirmed [16]. Nonetheless, in the northern Adriatic Sea, it was observed that during *Ostreopsis* blooms, concentrations of certain substances were higher than during the rest of the study period [17].

In temperate areas, as noted by Accorini and Totti [16], the growth of *Ostreopsis* cells typically occurs as summer events. This trend is consistent with the research in the northern Adriatic Sea, where peaks generally occur in September-October. In the Ligurian Sea, peaks are observed in mid-summer, toward the end of July. Studying the growth trend of *Ostreopsis* cells in Mediterranean areas reveals that the highest abundance can occur from spring to fall [6, 25].

In the northern Adriatic Sea in the Conero Riviera (Ancona, northern Adriatic Sea) blooms of O. cf. ovata have been constantly reported between the end of the summer and the beginning of the fall since 2006 [6, 26]. This temporal pattern suggests that specific environmental conditions during this period may favor the development of the *Ostreopsis* cells. Several studies have shown the importance of hydrodynamics on the development of *Ostreopsis* cf. *ovata* blooms [3, 6]. Moderate waves can cause cells to settle and rough seas and strong wind would rather favor cell dispersion, which reduces the cells' growth [3, 16]. On the contrary, relatively low wind intensities were sources of matters necessary for *Ostreopsis* growth [27–30].

The abundance of *Ostreopsis* cf. *ovata* in our study reached a value of 6.96 × 10^3 cells/l. This finding was similar to research in the south-eastern Mediterranean Sea (Gulf of Gabès), where the maximum concentration was in fall during October (5x 10^3 and 7 × 10^3 cells/l) and November (8 × 10^3 cells/l). In the Gulf of Gabès the highest abundance of *Ostreopsis* (7 × 10^3 cells/l) was reached at temperatures between 22.5 and 30 °C, which is similar to our study [20]. The abundance of *Ostreopsis* cf. *ovata* in the coastal areas along the Gulf of Gabès showed a seasonal trend, as previously noticed in other temperate areas in the Mediterranean Sea [17, 20].

Along the coasts of the Ligurian and Catalan Seas, the first peaks of *Ostreopsis* cells are typically recorded in mid-summer (July), with a second bloom often occurring in fall [27]. In the Northern Adriatic Sea, blooms tend to appear later in fall, between September and October [9]. These findings suggest that temperature has an important role in the occurrence of *Ostreopsis* cf. *ovata* blooms, out of simple growth patterns correlated with increasing temperatures.

Sea depth and substratum also have a specific role in studying the occurrence of blooms, as *Ostreopsis* spp. cells predominantly grow in shallow waters, and high abundances have been observed on macroalgae [3, 31].

Also, among the potentially toxic dinoflagellates, *Dinophysis acuta* and *Phalacroma rotundatum* were recorded still with lower abundances (the abundance reached values up to 10^2 cells/l).

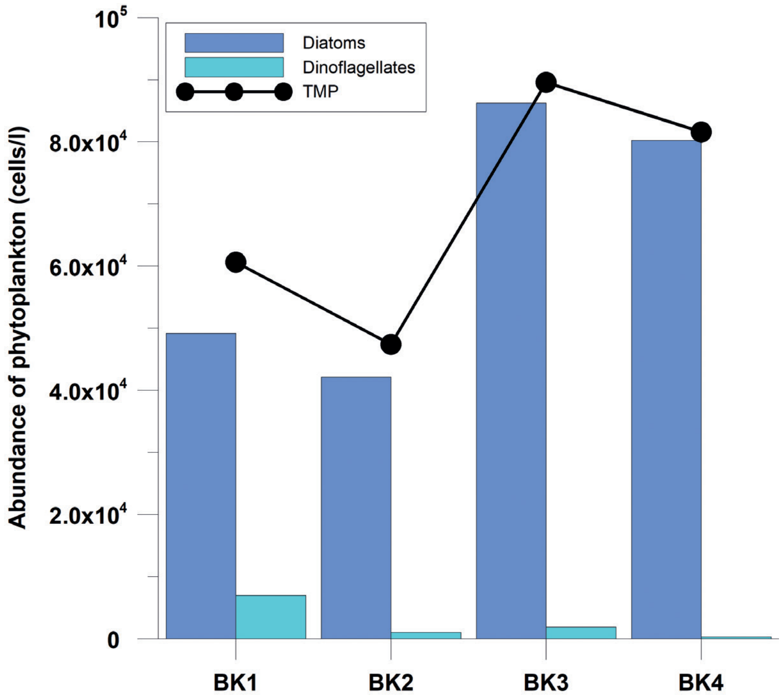

Fig. 2. Distribution of abundances (cells/l) of total microplankton (TMP) and phytoplankton groups (diatoms and dinoflagellates) during the research period on mussel farms (BK1, BK2, BK3, BK4).

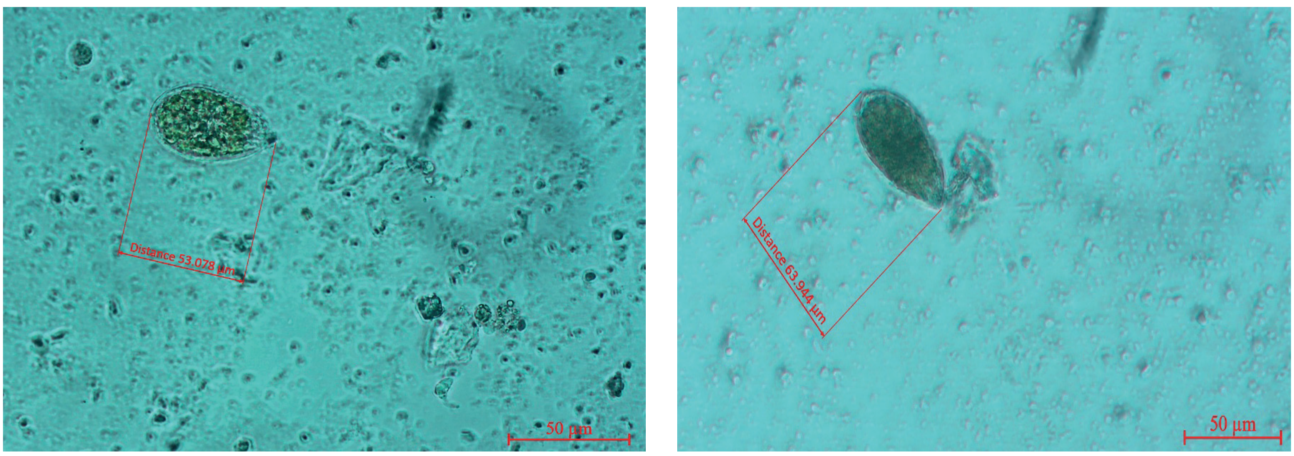

Fig. 3. Species from the genus *Ostreopsis* recorded on a Zeiss microscope (Axio Observer A1) in the area of Boka Kotorska Bay- Montenegro (A. magnification is 40X, distance 53.078 **µm, scale bar 50 µm**; B. magnification is 40X, distance 63.944 **µm**, scale bar 50 µm).

Our finding is important, knowing that if *Ostreopsis* species appeared in a frequent "blooming", it could cause harmful effects on marine benthic communities and human health through skin irritations after direct contact with the seawater, toxic aerosols which cause respiratory problems from inhalation of aerosol [4–6, 27] and contaminated seafood [3, 7, 8, 32, 33].

Conclusion

The reported findings show that the area of Boka Kotorska Bay can be a suitable habitat for *Ostreopsis* cf. *ovata.* The occurrence of *Ostreopsis* cf. *ovata* in the Boka Kotorska Bay-Montenegrin coast is noticed for the first time, and further investigation will show the occurrence and spread of this species along the entire Montenegrin coast. This record highlights the potential for *Ostreopsis* to form blooms even under small-scale and localized favorable conditions, emphasizing the need for careful planning of activities in these areas.

Our data should be considered as preliminary, and further detailed studies and analyses of *Ostreopsis*, particularly on benthic substrates such as macroalgae and rocks, as this species is a benthic dinoflagellate, will be conducted in the future.

Monitoring the presence and abundance of these microalgae is crucial, considering their potential impacts on human health, the ecology of marine organisms, and the negative economic effects on fisheries, aquaculture, and tourism. Such monitoring efforts are essential for preventing risks to both human and environmental health.

Acknowledgments

This study was funded by the Ministry of Agriculture, Forestry, and Water Management, Directorate for Food Safety, Veterinary and Phytosanitary Affairs in the frame of National monitoring of marine ecosystem on mussel farms.

Conflict of Interest

The authors declare no conflict of interest.

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