BioRisk 20: 71–81 (2023)
doi: 10.3897/biorisk.20.97557
https://biorisk.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
BioRisk

Occurrence of marine biotoxins on Bulgarian Black Sea coastal waters in 2021

Zlatina V. Peteva[1,2], Stanislava K. Georgieva[1], Bernd Knock[3], Thomas Max[3], Mona D. Stancheva[1], Simona Valkova[1]

1 *Medical University Varna, Tsar Osvoboditel 84, 9002 Varna, Bulgaria* **2** *Dobrudzha Technological College, Dobrotitsa 12, 9302 Dobrich, Bulgaria* **3** *Alfred Wegener Institut, Helmholtz Zentrum für Polar- und Meeresforschung, Chemische Ökologie, am Handelshafen 12, 27570 Bremerhaven, Germany*

Corresponding author: Zlatina V. Peteva (zlatina_peteva@mail.bg)

Academic editor: St. Chankova | Received 14 November 2022 | Accepted 23 December 2022 | Published 15 May 2023

Citation: Peteva ZV, Georgieva SK, Knock B, Max T, Stancheva MD, Valkova S (2023) Occurrence of marine biotoxins on Bulgarian Black Sea coastal waters in 2021. In: Chankova S, Danova K, Beltcheva M, Radeva G, Petrova V, Vassilev K (Eds) Actual problems of Ecology. BioRisk 20: 71–81. https://doi.org/10.3897/biorisk.20.97557

Abstract

Marine biotoxins are produced by certain phytoplankton species and used to accumulate in filter-feeding marine organisms. The occurrence of marine biotoxins in all aquatic environments and latitudes is variable in time and space. Thus, it is an essentially natural phenomenon, but the occurrence of toxigenic phytoplankton cannot be completely avoided or eliminated. A serious concern appears if these substances accumulate at high levels in seafood. If it is consumed by mammals including humans, severe illness of consumers of intoxicated seafood may result. The aim of this study is to assess the presence of marine biotoxins in plankton samples taken in 2021 and to compare the determined levels with a previous period. Plankton samples (n = 21) were collected in 2021 along the whole Bulgarian coastline (Black Sea). The presence of hydrophilic (domoic acid (DA)) and lipophilic toxins (okadaic acid, dinophysis toxin – 1, dinophysis toxin -2, azaspiracid-1, goniodomin A, pectenotoxin-2 (PTX2), yessotoxin, spirolide-1 and gymnodimine A) was investigated via liquid chromatography – tandem mass spectrometry (LC-MS/MS). Results indicated the presence of only DA in three samples and PTX2 in two samples. The positive samples were sporadically distributed throughout the study period. During 2016–2019, LC-MS/MS analysis confirmed the presence of DA, PTX2, YTX, SPX-1 and GDA in plankton net samples collected from the same locations reported here. The matching toxins (DA and PTX2) were at comparable levels in both periods of investigation, thus lower than in other European waters where harmful algal blooms are registered. These results show the persistent appearance of some marine biotoxins in Bulgarian waters. Although levels were low in the monitored periods, a constant monitoring is required in order that toxic events by seafood consumption be avoided.

Keywords
Domoic acid, monitoring, pectenotoxins, the Black Sea

Introduction

Bulgarian Black Sea coastline comprises 432 km (Stanchev et al. 2013). It is important for recreational, touristic (Stoyanova et al. 2019; Ihtimanski et al. 2020; Nikolova et al. 2021) and commercial (Raykov and Nicheva 2018; Stancheva et al. 2022) activities. The Black Sea is a commercial seafood source, including shellfish and fish, as well as providing popular recreational fisheries (General Doctorate for Internal Policies 2011; EAFA 2020).

Black Sea mussel production and catchment has increased in recent years (EAFA 2021). Mussels have been documented to contain beneficial values of polyunsaturated fatty acid, proteins, vitamins etc. and, therefore, are a preferred food worldwide (Hyung et al. 2018; Carboni et al. 2019; Yaghubi et al. 2021).

Microalgae are the primary food source for mussels (Brown 2002; Pleissner et al. 2012), but some microalgal species are reported as toxic or harmful. These phytoplankton species tend to produce potent toxins that accumulate in filter feeders. Yearly, potential producers of marine toxins (Pseudo-nitzschia, Alexandrium, Dinophysis) are registered in Bulgarian coastal waters (Dzhembekova et al. 2021; Dzhembekova et al. 2022). Marine toxins are transferred through the food chain to the higher trophic levels and may cause severe illness in them. A wide range of symptoms, from dizziness, digestive (nausea and vomiting) to nervous complaints, are associated with human intoxication by biotoxins, characterising different and specific syndromes, called shellfish poisonings. The risk assessment of the occurrence of toxigenic phytoplankton is complicated by the fact that toxin levels of plankton samples do not always correlate with biomass and abundance of potentially toxigenic species.

The aim of this study is to evaluate the levels of marine biotoxins in plankton samples of the year 2021 collected from areas of different function and economic importance. Furthermore, the determined levels will be compared to marine toxins levels from previous periods of investigation.

Materials and methods

Sampling plan

Phytoplankton samples (n = 21) (Table 1) were hauled vertically from depths between one and five metres from the surface with a conical plankton net (20 µm mesh size, 40 cm outer diameter) along the Bulgarian coast in the period March-December 2021.

Table 1. Collected plankton samples.

№	Sample №	Sampling site (Coordinates)	Type of the region	Sampling date
1	**ME6**	North 43°32'348"N, 029°18'224"E	Intensive fishing activities	31.03.2021
2	**ME8**	South 42°26'296"N,027°41'360"E	Mussel farming site	25.05.2021
3	**ME9**	North 43°21'276"N, 028°27'053"E	Intensive fishing activities	31.03.2021
4	**ME15**	North 43°39'961"N, 029°39'793"E	Intensive fishing activities	13.04.2021
5	**ME17**	North 43°21'885"N, 028°20'528"E	Mussel farming site	13.04.2021
6	**ME38**	North 43°01'23.5"N, 27°53'22.0"E	Protected area	18.07.2021
7	**ME47**	North 43°24'14.3"N, 28°21'11.8"E	Mussel farming site	26.07.2021
8	**ME48**	North 43°23'58.9"N, 28°09'34.5"E	Intensive fishing activities	27.07.2021
9	**ME49**	South 42°38'14.3"N, 27°40'26.5"E	Area with anthropogenic activities	11.08.2021
10	**ME57**	North 43°07'09.8"N, 28°02'52.1"E	Protected area	08.10.2021
11	**ME58**	Varna 43°13'31.9"N, 28°02'12.3"E	Areas with anthropogenic activities	08.10.2021
12	**ME59**	Varna 43°16'52.5"N, 28°07'03.9"E	Areas with anthropogenic activities	08.10.2021
13	**ME66**	South 42°33'19.4"N, 27°38'19.4"E	Mussel farming site	1.11.2021
14	**ME68**	South 42°39'58.6"N, 27°43'06.5"E	Protected area	1.11.2021
15	**ME74**	North 43°21'01.7"N, 28°28'49.8"E	Protected area	4.11.2021
16	**ME75**	North 43°23'49.9"N, 28°19'36.1"E	Mussel farming site	4.11.2021
17	**ME76**	South 42°43'15.0"N, 27°55'26.1"E	Protected area	4.11.2021
18	**ME83**	South 43°01'23.5"N, 27°53'22.0"E	Protected area	29.11.2021
19	**ME86**	Varna 43°10'28.3"N, 27°54'60.0"E	Areas with anthropogenic activities	5.12.2021
20	**ME89**	Varna 43°12'42.2"N, 27°57'30.1"E	Areas with anthropogenic activities	5.12.2021
21	**ME92**	Varna 43°11'36.8"N, 27°51'46.5"E	Areas with anthropogenic activities	5.12.2021

Sampling sites close to mussel farming areas, areas used for harvesting of wild mussels (including areas of intensive fisheries and areas with anthropogenic activities), as well as protected areas, were included in the sampling plan.

Experimental plan

Immediately after sampling, net haul concentrates were adjusted to a defined volume of 500–1000 ml (depending on the net tow volume) using 20 µm filtered seawater. After centrifugation (4000 × g, 10 min at 10 °C), the supernatant was discarded. The cell pellets were stored in at -20 °C until further processing.

Plankton pallets were suspended washed with 1000 µl 100% methanol for domoic acid and lipophilic toxins extraction. The methanolic acid suspensions were than sonicated (40 Hz, 15 min) and centrifuged by 4000 x g for 10 min at 10 °C. The supernatant was filtered through syringe filters (0.45 µm pore size, ø25 mm, Minisart, Sartorius, Germany). Filtrates (1000 µl) were transferred into chromatographic vials and kept at -20 °C until further analysis.

The hydrophilic domoic acid (DA) and lipophilic toxins – goniodomin A (GDA), okadaic acid (OA), dinophysistoxins -1 and 2 (DTX1,2), pectenotoxins (PTX2, PTX2-sa, epi-PTX-sa), yessotoxins (YTX, OH-YTX), azaspiracid-1 (AZA1), spirolides (SPX1) and gymnodimine A (GYMA) were analysed according Krock et al. (2008) on a LC-MS/MS system. It consists of liquid chromatograph (model 1100 LC, Agilent, Waldbronn, Germany) coupled to a triple quadrupole mass spectrometer (API 4000 QTrap, Sciex, Darmstadt, Germany), equipped with a Turbo Spray interface.

The quality control was performed by regular analysis of procedural blanks and certified reference material (National Research Council, Canada). Limits of detection (LOD) for lipophilic toxins and DA were determined based on 3:1 signal-to-noise ratio.

Calculations

Contents of the toxin are expressed as nanograms per net tow (ng/NT) in order to be compared with previous results and other literature data.

Results

In total, 21 plankton samples were collected in the studied period February-December 2021 along the Bulgarian coastline in accordance with sampling plan (Table 1).

With the aim to analyse for the presence of selected marine biotoxins, appropriate retention times and LODs were achieved (Table 2).

The huge efforts for the toxin profile revealed a scarce presence of marine biotoxins in the plankton samples. Amongst the investigated toxins – DA, GDA, OA, DTX1, DTX2, PTX2, PTX2-sa, epi-PTX-sa, YTX, OH-YTX, AZA1, SPX1) and GYMA, only DA and PTX2 were detected (Table 3).

Table 2. Investigated lipophilic toxins and domoic acid including associated standard solution concentrations, LODs, quantification transitions and retention times.

Marine toxins investigated	Concentration of standard solution pg/µl	LOD ng/NT	Quantification transition (*m/z*)	Retention time (min)
DA	100	4.93	312→266	7.17
OA	500	25.71	822→223	11.57
DTX2	500	36.59	822→223	11.87
DTX1	500	60.00	836→237	12.57
PTX2	100	3.73	876→213	12.14
PTX2-sa	–	–	894→213	11.70
Epi-PTX2-sa	–	–	894→213	11.90
GONA	412.5	30.56	786→607	12.67
YTX	1000	100.00	1176→981	13.00
OH-YTX	–	–	1176→981	11.70
AZA1	100	0.92	842→824	12.62
GYMA	500	1.50	508→490	10.33
SPX1	100	2.58	692→164	11.22

Table 3. Levels of detected toxins in plankton samples.

Sample №	DA, ng/NT	PTX2, ng/NT
ME6	170,94	< LOD
ME9	< LOD	9.23
ME15	138.48	< LOD
ME58	13.38	< LOD
ME76	< LOD	6.51

Results obtained in this study showed that only 14% of the samples were positive for DA and only 9% for PTX2. DA was present in spring and autumn samples from areas with intensive fishing and anthropogenic activities. Pectenotoxin-2 was detected in a spring and autumn sample from an area with anthropogenic activities and a protected region, respectively.

Comparison of the results with results obtained from previous studies in the same regions (Peteva et al. 2018; Peteva et al. 2020) showed that, in 2016, DA was detected in 57% of the samples, in 2017 in 41%, in 2018 – 17% of samples and in 2019, in none of the samples. In 2017 and 2018, plankton samples investigated were many more than in 2016 and in 2021. Comparison of the concentration range of the positive samples showed a great variability between the different periods of investigation (Fig. 1).

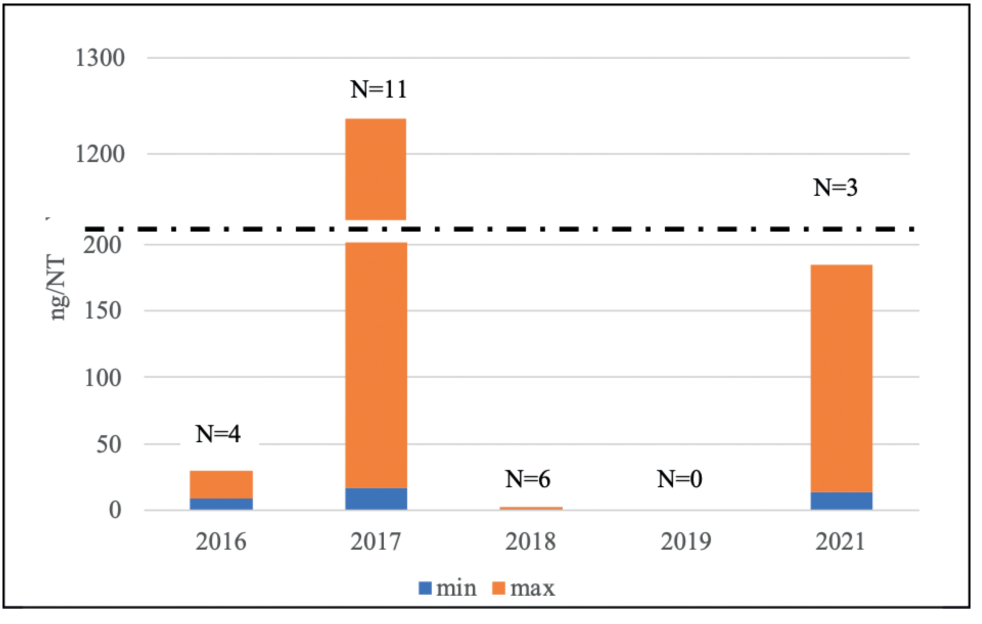

Figure 1. Comparison of DA levels with previous studies (n- indicates the number of positive samples).

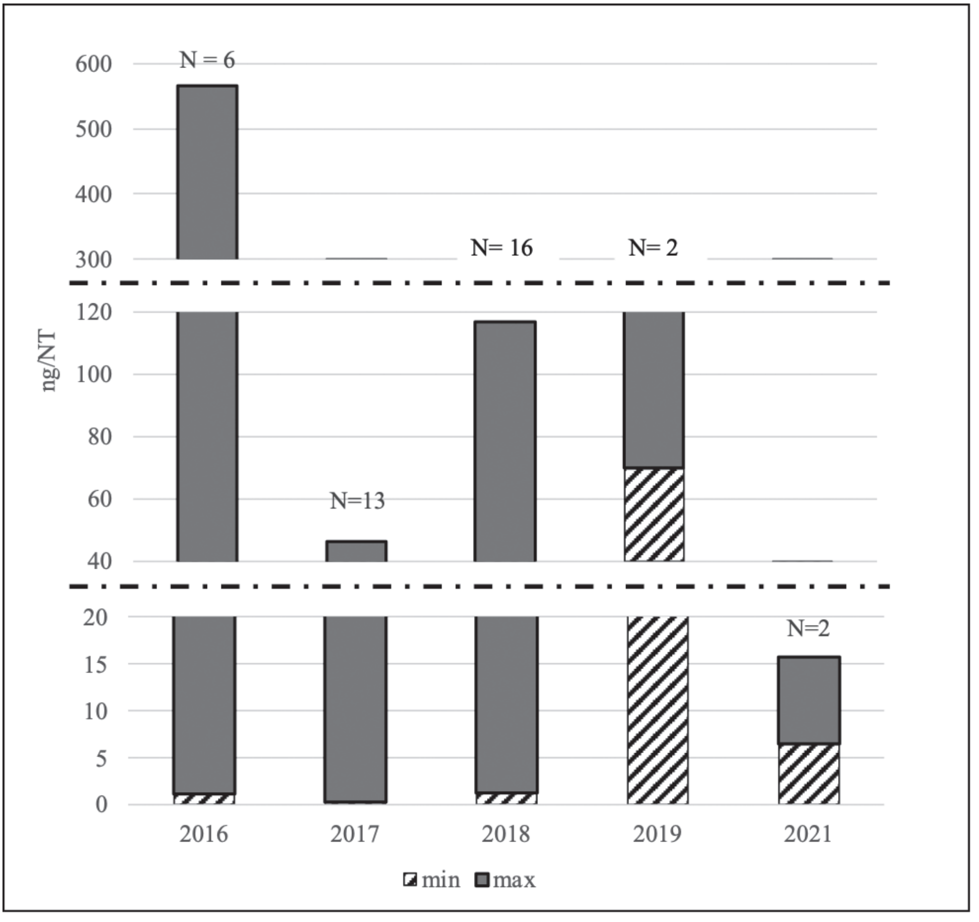

Figure 2. Comparison of PTX2 levels with previous studies.

The two PTX2 positive samples from 2021 represent 10% of all samples. Thus, in previous studies, the portion of positive samples was much higher – 86% in 2016, 48% in 2017, 47% in 2018 and in 2019 – 67% of the samples. Moreover, in this former period of time, the PTX2 concentration ranges are much wider (Fig. 2).

Discussion

The Bulgarian coast is important for the development of the economy and tourism in the country (Dimitrov and Rangelov 2018; Mooser et al. 2022).

Bulgaria is considered as a minor producer of seafood, responsible for 0.01 percent of world production and 0.4 percent of EU fishery and aquaculture products in terms of volume (EUMOFA 2020). Recent investigations showed a persistent value of the catch (for 2015–2020 – 8476 tonnes), as well as a visible peak in 2019

(Shivarov 2021). Fishing activities are performed almost throughout the whole year and along the whole coastline. Thus, it is known that fishing activities change the environmental parameters in the regions where they are undertaken (Stier et al. 2020; Gissi et al. 2021).

In Bulgarian mariculture farms, Mediterranean mussel (*Mytilus galloprovincialis* Lamarck) is dominant farmed species. The total marine aquaculture production of 2,531 t in 2018 consists mainly of this mollusc (Klisarova et al. 2020). Recent state reports showed that, since 2008, Bulgaria is one of the important suppliers of Mediterranean mussels in the Black Sea region. Nowadays, Bulgarian mussel farms produced over than 1.5% of the cultivated mussels in the world (Ministry of Agriculture and Food Bulgaria 2019).

A number of factors of natural and predominantly anthropogenic nature have a negative impact on the state of the environment of this region of the country (Kotsev and Prodanov 2020, Kotsev et al. 2021). Natural factors, superimposed in a number of cases by anthropogenic activity, are mainly abrasion, landslides, floods of the coast from the sea and climate change (Penchev 2019).

Anthropogenic activities and technological advances are commonly pointed out to justify the increasing occurrence, frequency and intensity of harmful algal blooms and the detection of new toxins or emergence of toxins in regions where they were previously not known (Costa 2019; Otero and Silva 2022). In this regard, investigation and comparison of the toxin profiles of plankton samples from locations of fishing and anthropogenic activities, as well as mussel farming sites, seem meaningful and informative.

Coastal protected areas in Bulgaria are established by national policy instruments/laws and EU Directives to protect a wide range of natural and cultural resources (Stancheva et al. 2016). In these areas, any catch and industrial activities are banned by the law (Ministry of Regional Development and Public Works 1998). Accordingly, protected areas are considered control sites in this study.

Quantitative and qualitative analysis of marine biotoxins was performed by applying liquid chromatography coupled with mass spectrometry which is acknowledged by the scientific community as one of the most powerful analytical tools able to identify multiple toxins (Visciano et al. 2016; Estevez et al. 2019). Results indicate that, in 33% of samples from the areas with anthropogenic activities and in 50% of the samples from the areas of intensive fishing, marine biotoxins were detected. No toxins were detected in the samples from the mussel farms and in one sample from a protected area. Further interpretation of the results would be possible if the investigation is repeated in a future period.

Interestingly, two other marine toxins were detected in previous periods – YTX and SPX1. Yesotoxins were registered in the samples from 2016–2018. The concentration range is very large – 0.001 – 1.959 ng/NT. In the present study, no YTXs were detected. The small number of positive samples in the previous period, as well as the absence hereby, is most likely due to the fact that yesotoxins are exotoxins. Once synthesised, they are rapidly released into the environment and, therefore, difficult to determine in plankton samples (Hess and Aasen 2007). For example, Krock et al. (2013) also investigated the levels of lipophilic toxins along the German and Danish coasts, but yesotoxins were not determined.

Our previous investigation showed that SPX1 was registered in the samples from summer-autumn 2018 in a concentration range from 0.054–0.245 ng/NT. No spirolides were registered in this study. This result might be associated with low abundance or even absence of *A. ostenfeldii*, as SPX 1 production is associated with this species (Van Wagoner et al. 2011; Guinder et al. 2018).

This further reinforces the belief that toxin production by plankton is an unpredictable phenomenon (Kremp et al. 2019) and studies on it should be continued.

Conclusions

Results obtained in this paper including the values below LOD indicate that abundance of marine biotoxins is not alarming. This suggests that good quality of mussel meat might be expected. Monitoring of harmful phytoplankton composition and biotoxins should be continued in future, so it can provide the opportunity to react in good time in order to prevent negative consequences which can be caused by HABs and biotoxins.

Acknowledgements

This work was supported by the Maritime Affairs and Fisheries Program 2014–2020 co-financed by the European Union through the European Maritime Affairs and Fisheries Fund. Project No BG14MFOP001-6.004-0006-C01, contract No МДР-ИП-01-13/25.01.2021 "Investigation of priority chemical pollutants and biotoxins and assessment of the state of the marine environment".

References

Brown MR (2002) Nutritional value of microalgae for aquculture. In: Cruz-Suárez LE, Ricque-Marie D, Tapia-Salazar M, Gaxiola-Cortés MG, Simoes N (Eds) Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola. 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

Carboni S, Kaur G, Pryce A, McKee K, Desbois AP, Dick JR, Galloway SDR, Hamilton DL (2019) Mussel Consumption as a "Food First" Approach to Improve Omega-3 Status. Nutrients 19:11(6): e1381. https://doi.org/10.3390/nu11061381

Costa PR (2019) Advances and Current Challenges in Marine Biotoxins Monitoring. Journal of Marine Science and Engineering 7(9): e302. https://doi.org/10.3390/jmse7090302

Dimitrov O, Rangelov B (2018) Natural hazards and natural resources of the bulgarian black sea coastal area. Ses 2018. Fourteenth international scientific conference space, ecology. Safety (Basel, Switzerland) 2018: 323–327.

Dzhembekova N, Slabakova N, Slabakova V, Zlateva I, Moncheva S (2021) Long-term Trends in Pseudo-nitzschia Complex Blooms in the Black Sea-is there a Potential Risk for Ecological and Human Hazards. Ecologia Balkanica 13(1): 55–75. http://web.uni-plovdiv.bg/mollov/EB/2021_vol13_iss1/055-075_eb20148.pdf

Dzhembekova N, Moncheva S, Slabakova N, Zlateva I, Nagai S, Wietkamp S, Wellkamp M, Tillmann U, Krock B (2022) New Knowledge on Distribution and Abundance of Toxic Microalgal Species and Related Toxins in the Northwestern Black Sea. Toxins (Basel) 6:14(10): e685. https://doi.org/10.3390/toxins14100685

EAFA [Fisheries and Aquaculture Executive Agency] (2020) Fishing and aquaculture. https://iara.government.bg/wps/portal/iara-web/fishing.and.aquaculture [In Bulgarian]

EAFA (2021) Register of produced fish from fish farms in 2020. https://iara.government.bg/wps/portal/iara-web/home [In Bulgarian]

Estevez P, Castro D, Pequeño-Valtierra A, Giraldez J, Gago-Martinez A (2019) Emerging marine biotoxins in seafood from European coasts: Incidence and analytical challenges. Foods 8(5): e149. https://doi.org/10.3390/foods8050149

EUMOFA (2020) Country profile: Bulgaria. https://eumofa.eu/en/bulgaria

General Doctorate for Internal Policies (2011) Fisheries in Bulgaria. https://www.europarl.europa.eu/RegData/etudes/note/join/2011/460049/IPOL-PECH_NT(2011)460049_BG.pdf [In Bulgarian]

Gissi E, Manea E, Mazaris AD, Fraschetti S, Almpanidou V, Bevilacqua S, Coll M, Guarnieri G, Lloret-Lloret E, Pascual M, Petza D, Rilov G, Schonwald M, Stelzenmüller V, Katsanevakis S (2021) A review of the combined effects of climate change and other local human stressors on the marine environment. Science of The Total Environment 10: 755(Pt 1): e142564. https://doi.org/10.1016/j.scitotenv.2020.142564

Guinder VA, Tillmann U, Krock B, Delgado AL, Krohn T, Garzon Cardona JE, Metfies K, López Abbate C, Silva R, Lara R (2018) Plankton multiproxy analyses in the Northern Patagonian Shelf, Argentina: Community structure, phycotoxins, and characterization of toxic Alexandrium strains. Frontiers in Marine Science 5: e394. https://doi.org/10.3389/fmars.2018.00394

Hess P, Aasen J (2007) Chemistry, origins and distribution of Yessotoxin and its analogues. In: Botana LM (Ed.) Phycotoxins, Chemistry and Biochemistry. Blackwell Publishing, 187–202. https://doi.org/10.1002/9780470277874.ch10

Hyung JH, Ahn CB, Je JY (2018) Blue mussel (*Mytilus edulis*) protein hydrolysate promotes mouse mesenchymal stem cell differentiation into osteoblasts through up-regulation of bone morphogenetic protein. Food Chemistry 242: 156–161. https://doi.org/10.1016/j.foodchem.2017.09.043

Ihtimanski I, Nedkov S, Semerdzhieva L (2020) Mapping the natural heritage as a source of recreation services at national scale in Bulgaria. One Ecosystem 5: e54621. https://doi.org/10.3897/oneeco.5.e54621

Klisarova D, Gerdzhikov D, Kostadinova G, Petkov G, Cao X, Song C, Zhou Y (2020) Bulgarian marine aquaculture: Development and prospects–A review. Bulgarian Journal of Agricultural Science 26(1): 163–174. http://www.agrojournal.org/26/01s-20.pdf

Kotsev I, Prodanov B (2020) Linking pattern and process at a spatio-temporal scale: Present-day landscape structure and dynamics of the north Bulgarian Black Sea coast. International Multidisciplinary Scientific GeoConference SGEM 20(2.2): 405–412. https://doi.org/10.5593/sgem2020/2.2/s11.048

Kotsev I, Prodanov B, Bekova R (2021) Long-Term Impacts of Land Use Change Upon the Natural Flood Storage Reservoirs Along the North Bulgarian Black Sea Coast, 233–261. https://doi.org/10.1007/698_2021_765

Kremp A, Hansen PJ, Tillmann U, Savela H, Suikkanen S, Voß D, Barrera F, Jakobsen HH, Krock B (2019) Distributions of three Alexandrium species and their toxins across a salinity gradient suggest an increasing impact of GDA producing A. pseudogonyaulax in shallow brackish waters of Northern Europe. Harmful Algae 87: e101622. https://doi.org/10.1016/j.hal.2019.101622

Krock B, Tillmann U, John U, Cembella A (2008) LC-MS-MS aboard ship: Tandem mass spectrometry in the search for phycotoxins and novel toxigenic plankton from the North Sea. Analytical and Bioanalytical Chemistry 392(5): 797–803. https://doi.org/10.1007/s00216-008-2221-7

Krock B, Tillmann U, Alpermann TJ, Voss D, Zielinski O, Cembella AD (2013) Phycotoxin composition and distribution in plankton fractions from the German Bight and western Danish coast. Journal of Plankton Research 35(5): 1093–1108. https://doi.org/10.1093/plankt/fbt054

Ministry of Agriculture and Food Bulgaria (2019) Annual report on the state and development of agriculture (Agricultural report '2019). https://www.mzh.government.bg/media/filer_public/2020/02/11/agrarian_report_2019.pdf [In Bulgarian]

Ministry of Regional Development and Public Works (1998) Protected areas act. rom. SG. 133/11 Nov 1998. https://www.mrrb.bg/en/protected-areas-act/

Mooser A, Anfuso G, Stanchev H, Stancheva M, Williams AT, Aucelli PP (2022) Most Attractive Scenic Sites of the Bulgarian Black Sea Coast: Characterization and Sensitivity to Natural and Human Factors. Land (Basel) 11(1): e70. https://doi.org/10.3390/land11010070

Nikolova M, Stoyanova V, Varadzhakova D, Ravnachka A (2021) Cultural ecosystem services for development of nature-based tourism in Bulgaria. Journal of the Bulgarian Geographical Society 45: 81–87. https://doi.org/10.3897/jbgs.e78719

Otero P, Silva M (2022) Emerging Marine Biotoxins in European Waters: Potential Risks and Analytical Challenges. Marine Drugs 20(3): e199. https://doi.org/10.3390/md20030199

Penchev G (2019) Legal protection of the environment in the region of the Black sea costal Zone of the Republic of Bulgaria: current problems. Revista Crítica de la Historia de las Relaciones Laborales y de la Política Social (12): 81–87. https://www.semanticscholar.org/paper/Legal-protection-of-the-environment-in-the-region-Penchev/8005f7ed7d1b6edce4911719787dbdafa83610d6

Peteva Z, Krock B, Georgieva S, Stancheva M (2018) Occurrence and Variability of Marine Biotoxins in Mussel (*Mytillus galloprovincialis*) and in Plankton Samples from Bulgarian

Coast in Spring 2017. International Journal of Agriculture & Environmental Science 5(4): 1–11. https://doi.org/10.14445/23942568/IJAES-V5I4P101

Peteva Z, Krock B, Max T, Stancheva M, Georgieva S (2020) Detection of marine biotoxin in plankton net samples from the Bulgarian coast of Black Sea. Bulgarian Chemical Communications 52(B): 22–27.

Pleissner D, Eriksen NT, Lundgreen K, Riisgård HU (2012) Biomass composition of blue mussels, Mytilus edulis, is affected by living site and species of ingested microalgae. ISRN Zoology 2012: 1–12. https://doi.org/10.5402/2012/902152

Raykov VS, Nicheva S (2018) Governance and Socio-Economic Implications of the Black Sea Small Scale Fisheries (Bulgaria). In: Finkl C, Makowski C (Eds) Diversity in Coastal Marine Sciences. Springer, Cham, 413–442. https://doi.org/10.1007/978-3-319-57577-3_25

Shivarov A (2021) Global interdependencies in seafood trade: the case of Bulgaria. Izvestia Journal of the Union of Scientists-Varna. Economic Sciences Series 10(3): 110–121. https://journals.mu-varna.bg/index.php/isuvsin/article/view/8346

Stanchev H, Young R, Stancheva M (2013) Integrating GIS and high resolution orthophoto images for the development of a geomorphic shoreline classifica- tion and risk assessmentda case study of cliff/bluff erosion along the Bulgarian coast. Journal of Coastal Conservation 17(4): 719–728. https://doi.org/10.1007/s11852-013-0271-2

Stancheva M, Stanchev H, Peev P, Anfuso G, Willliams AT (2016) Coastal protected areas and historical sites in North Bulgaria–Challenges, mismanagement and future perspectives. Ocean and Coastal Management 130: 340–354. https://doi.org/10.1016/j.ocecoaman.2016.07.006

Stancheva M, Stanchev H, Zaucha J, Ramieri E, Roberts T (2022) Supporting multi-use of the sea with maritime spatial planning. The case of a multi-use opportunity development-Bulgaria, Black Sea. Marine Policy 136: e104927. https://doi.org/10.1016/j.marpol.2021.104927

Stier AC, Olaf Shelton A, Samhouri JF, Feist BE, Levin PS (2020) Fishing, environment, and the erosion of a population portfolio. Ecosphere 11(11): e03283. https://doi.org/10.1002/ecs2.3283

Stoyanova M, Hristova S, Stankova S (2019) Analysis and evaluation of resource potential of the Black Sea SPA Riviera in Bulgaria. In: Dimitrov N (Ed.) Proceeding 2nd International Scientific Conference Challenges of Tourism and Business Logistics in the 21st century. Stip (North Macedonia), September 2019. Faculty of Tourism and Business logistics Publisher, Stip, 225–234.

Van Wagoner RM, Misner I, Tomas CR, Wright JL (2011) Occurrence of 12-methylgymnodimine in a spirolide-producing dinoflagellate *Alexandrium peruvianum* and the biogenetic implications. Tetrahedron Letters 52(33): 4243–4246. https://doi.org/10.1016/j.tetlet.2011.05.137

Visciano P, Schirone M, Berti M, Milandri A, Tofalo R, Suzzi G (2016) Marine biotoxins: Occurrence, toxicity, regulatory limits and reference methods. Frontiers in Microbiology 7: e1051. https://doi.org/10.3389/fmicb.2016.01051

Yaghubi E, Carboni S, Snipe RMJ, Shaw CS, Fyfe JJ, Smith CM, Kaur G, Tan SY, Hamilton DL (2021) Farmed Mussels: A Nutritive Protein Source, Rich in Omega-3 Fatty Acids, with a Low Environmental Footprint. Nutrients 13(4): e1124. https://doi.org/10.3390/nu13041124