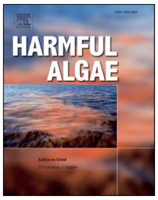
Harmful Algae 151 (2026) 103025

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal



Occurrence of toxic microalgae and associated toxins in the western Black Sea: insights from the PHYCOB cruise in September 2021

Nina Dzhembekova [a], Fuat Dursun [b], Urban Tillmann [c], Ivelina Zlateva [a], Oana Vlas [d], Nataliya Slabakova [a], Kristof Möller [c,1], Snejana Moncheva [a], Florian Koch [c], Laura Boicenco [d], Ertuğrul Aslan [e], Sabri Mutlu [e], Ivan Popov [a], Satoshi Nagai [f], Bernd Krock [c,*]

[a] Institute of Oceanology “Fridtjof Nansen” – Bulgarian Academy of Sciences, Varna 9000, Bulgaria
[b] Istanbul University, Institute of Marine Sciences and Management, Istanbul 34134, Türkiye
[c] Alfred Wegener Institut-Helmholtz Zentrum für Polar- und Meeresforschung, Ökologische Chemie, Bremerhaven 27570, Germany
[d] National Institute for Marine Research and Development "Grigore Antipa", Constanţa 900581, Romania
[e] TÜBİTAK Marmara Research Center, Gebze, Kocaeli 41470, Türkiye
[f] Fisheries Research and Education Agency, Fisheries Technology Institute, Yokohama, Kanagawa 236-8648, Japan

ARTICLE INFO

Keywords:
Plankton
Light microscopy
Scanning electron microscopy
DNA metabarcoding
Phycotoxins

ABSTRACT

Harmful Algal Blooms (HABs) represent a significant global challenge to human health, economies, and ecosystems, including those of the Black Sea. Despite previous reports of potentially toxic microalgae and phycotoxins in the basin, the taxonomy and occurrence of toxigenic species and their associated toxins remain poorly resolved. During the PHYCOB cruise in September 2021, the diversity and distribution of toxigenic microalgae and phycotoxins were investigated across 23 stations in the western Black Sea, covering Bulgarian and Romanian waters. Numerous potentially toxic microalgal taxa were identified using complementary morphological (light microscopy and scanning electron microscopy) and molecular (DNA metabarcoding) methods. The genus *Pseudo-nitzschia* was the only representative of potentially toxic diatoms, but no domoic acid was found. Among toxic dinoflagellates, *Dinophysis* spp., *Protoceratium reticulatum, Lingulaulax polyedra*, and *Gonyaulax* spp. were frequently observed, along with the related pectenotoxins and yessotoxins. Species distribution modelling indicated that the western Black Sea provides favorable conditions for *Dinophysis* spp., *L. polyedra*, and *P. reticulatum*. Additionally, several *Alexandrium* species were identified, including the first record of *A. fragae* in the basin, along with the detection of the associated phycotoxins (GTX-2/3 and GDA). This study provides the first integrated assessment combining light and scanning electron microscopy, DNA metabarcoding, and chemical analyses of toxigenic microalgae in field samples from the western Black Sea, contributing to an improved understanding of their region-specific profiles.

1. Introduction

The Black Sea is a unique marine basin, largely isolated from the global ocean (connected to the Mediterranean Sea only through the narrow Bosphorus Strait), and characterized by extensive freshwater input, strong vertical stratification, low salinity, and permanent euxinic conditions below depths of 150–200 m (Bakan and Büyükgüngör, 2000; Zaitsev, 2008). These factors create challenging physiological conditions for its inhabitants, resulting in an ecosystem with lower species diversity compared to other marine basins and one that is highly vulnerable to anthropogenic pressures (Zaitsev and Mamaev, 1997). The Black Sea ecosystem has been strongly affected by eutrophication in the past, particularly in its north-western and western regions (Aubrey et al., 1996). The anthropogenic nutrient enrichment and the associated increase in microalgal blooms have been identified as key ecological problems for the health of the Black Sea environment (Moncheva et al., 2001; Nesterova et al., 2008).

Harmful algal blooms (HABs), along with associated problems, have

---

[*] Corresponding author.
E-mail address: bernd.krock@awi.de (B. Krock).

[1] Present address: IAEA Marine Environment Laboratories, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, 98000 Monaco, Principality of Monaco.

https://doi.org/10.1016/j.hal.2025.103025
Received 21 May 2025; Received in revised form 5 November 2025; Accepted 11 November 2025

been observed globally (Anderson et al., 2012b; Gobler, 2020; Hallegraeff et al., 2021a). The species that cause HABs are diverse, and some produce potent toxins (phycotoxins). Phycotoxins can either directly kill fish and other marine organisms or bioaccumulate through the food web, ultimately affecting higher trophic consumers, including humans (Landsberg, 2002; Vilariño et al., 2018). There is a growing concern that climate change could impact the geographic distribution, frequency, and severity of HABs (Gobler et al., 2017). HAB occurrences are highly variable at the regional scale and thus necessitate high resolution monitoring at a local level to assess their current status and forecast future trends (Anderson et al., 2012b; Hallegraeff et al., 2021b). Monitoring programs and increased awareness of microalgae, especially HAB species, are urgently needed, since these programs can protect human health from intoxication and fatalities (Trainer et al., 2012).

HAB species in the Black Sea have been documented since the beginning of the 20th century, with new species reported thereafter (Ryabushko, 2003; Dzhembekova et al., 2017, 2018; Krakhmanyi and Terenko, 2019; Moncheva et al., 2019; Krakhmalnyi et al., 2024). Many potentially toxic species are common in the Black Sea plankton community, and some of them form blooms (Nesterova et al., 2008; Moncheva et al., 2019). Additionally, various phycotoxins have been found in the Black Sea, though at levels below EU regulatory limits. Amnesic Shellfish Poisoning (ASP) toxin domoic acid (DA) has been detected in mussel and plankton samples from Bulgarian waters (Peneva et al., 2011; Peteva et al., 2018, 2020a, 2020b), as well as in cultures of *Pseudo-nitzschia calliantha* isolated from the Ukrainian Black Sea (Besiktepe et al., 2008). Diarrhetic Shellfish Poisoning (DSP) toxins (okadaic acid – OA, dinophysistoxins – DTX-1 and DTX-2), pectenotoxins (PTX-2 and PTX-2sa), and yessotoxins (YTXs) have been quantified in mussels and plankton samples from Bulgarian and Russian waters (Vershinin and Kamnev, 2000; Morton et al., 2007, 2009; Peteva et al., 2020a, 2020b, 2023). Paralytic Shellfish toxins (PST) (saxitoxin – STX, B1, and gonyautoxins – GTX-2/3), as well as spirolides (13-desmethyl spirolide C – SPX1) and goniodomin A (GDA), have also been reported in mussels and plankton samples (Vershinin et al., 2006; Kalinova et al., 2015; Peteva et al., 2020a, 2020b). Furthermore, putative phycotoxin-related intoxications have been reported in the past (Moncheva et al., 1993; Vershinin and Kamnev, 2000; Morton et al., 2009). Despite this, knowledge of toxic microalgae in the Black Sea remains fragmentary and insufficient, and expanded, targeted research is required to address issues related to HAB problems in a globally changing environment.

In spring 2019, during an oceanographic cruise in Romanian and Bulgarian waters, the presence of numerous potentially toxic species and phycotoxin variants was reported (Dzhembekova et al., 2022). An integrated morphological and molecular approach identified 20 potentially toxic species, some of which were widely distributed within the investigated area. Among the detected phycotoxins, PTX-1 and PTX-13, as well as several YTX variants, were recorded for the first time in the basin. The PHYCOB project was subsequently developed to further expand knowledge of HAB threats in the Black Sea. As part of this project, detailed data on the distribution and abundance of potentially toxic microalgae in the western Black Sea (Bulgarian and Romanian waters) were collected in September 2021 using morphological and molecular techniques. Additionally, phycotoxin levels in size fractionated plankton samples were determined. In parallel, potentially toxic algal species were isolated and brought into culture for a detailed morphological, phylogenetic, and toxinological characterization (Dursun et al., 2025). Similarities and differences between the recent data and prior findings from May 2019 in the same study region (Dzhembekova et al., 2022) are discussed to explore temporal patterns in species distribution and associated phycotoxins. Data from both campaigns were used in species distribution models to map habitat suitability in the western Black Sea for potentially toxic species associated with the detected phycotoxin variants.

2. Material and methods

2.1. Sampling and sample processing

23 stations (Fig. 1) were sampled in the western part of the Black Sea (Bulgarian and Romanian waters) using the R/V TÜBİTAK MARMARA in September 2021 (Fig. 1). At each station, water samples for plankton community composition analyses and phycotoxins (azaspiracid and karlotoxin determination) were taken from specific depths by utilizing Niskin bottles mounted on a conductivity-temperature-depth (CTD) rosette (Table S1).

In addition, at each station two vertical net tows from 30 m depth to surface were conducted using a plankton net (20 μm mesh size, 40 cm diameter, Hydro-Bios, Kiel, Germany) for analyses of plankton and phycotoxins. The collected net tow concentrates were adjusted to 1 L with filtered seawater before being split into several fractions. One fraction of live plankton net tow samples, together with Niskin bottle samples (1 L sample pooled from three depths: surface, thermocline, and deep chl-a maximum, and gently concentrated using 5 μm pore size polycarbonate filters), was inspected and documented onboard for microplankton community composition and potentially toxic species using an inverted microscope (Axioskop 35, Zeiss) equipped with an HD digital camera (Gryphax Jenoptik, Jena, Germany). Aliquots from the net tow concentrates were preserved for microscopic analyses (20 mL used for qualitative identification and 50 mL used for identification and cell counting, fixed with formaldehyde solution buffered to pH 8.0–8.2 with disodium tetraborate (Marvin Ltd., Plovdiv, Bulgaria) to 2 % final concentration). For DNA metabarcoding analysis, a 150 mL aliquot from the net tow concentrates was filtered under gentle vacuum (<0.2 bar) through 1 μm pore-size polycarbonate filters (Whatman, USA). For phycotoxin analyses, 730 mL from the net tow concentrates was size-fractionated over a sieve array, consisting of 200 μm, 50 μm and 20 μm Nitex sieves. The residue of each mesh was rinsed with filtered seawater and each fraction was transferred to a 50 mL centrifuge tube and adjusted to 30 mL with filtered seawater. The contents of each tube were separated into two aliquots for the analysis of lipophilic toxins (including domoic acid) and hydrophilic PSTs. Prior to aliquoting, homogenization of the samples was ensured, and the rapidly sedimenting material was brought back into suspension by shaking. Subsequently, the aliquots were centrifuged at 3220 x g for 15 min. After centrifugation, the supernatants were decanted and the remaining cell pellets were resuspended with a small volume of filtered seawater and transferred to cryovials containing ceramic beads (lysing matrix D, Thermo BIO 101, Illkirch, France). The samples were centrifuged again, the supernatants were carefully removed with a pipette, and the cell pellets were stored at −20 °C until further processing and analysis.

For azaspiracid and karlotoxin determination, a total of 8 to 9.5 L of pooled water from surface (3 m), 10 m depth, and the thermocline, prescreened with 200 μm gauze, were filtered through 5 μm polycarbonate filters. Filters were stored in 50 mL falcon tubes at −20 °C until further processing and analysis.

2.2. Microscopy of fixed samples

Identification and cell enumeration of the fixed Niskin bottle samples (hereafter referred to as bottle samples) were performed using an IX 73 Olympus inverted microscope in Utermöhl chambers (Moncheva and Parr, 2010). The bottle samples (1 L) were concentrated in the laboratory using the sedimentation method, reducing the volume to approximately 30 mL by settling and removing the supernatant in two stages (Moncheva and Parr, 2010). The sample was then homogenized, and a 1 mL sample fraction was completely analyzed. Considering only the potentially toxic species discussed in this paper, an average of 41 cells per sample was counted. Based on these primary data, the abundance (cells L^{-1}) was calculated for each species, or the lowest taxonomic level, and used as quantitative estimates in this study.

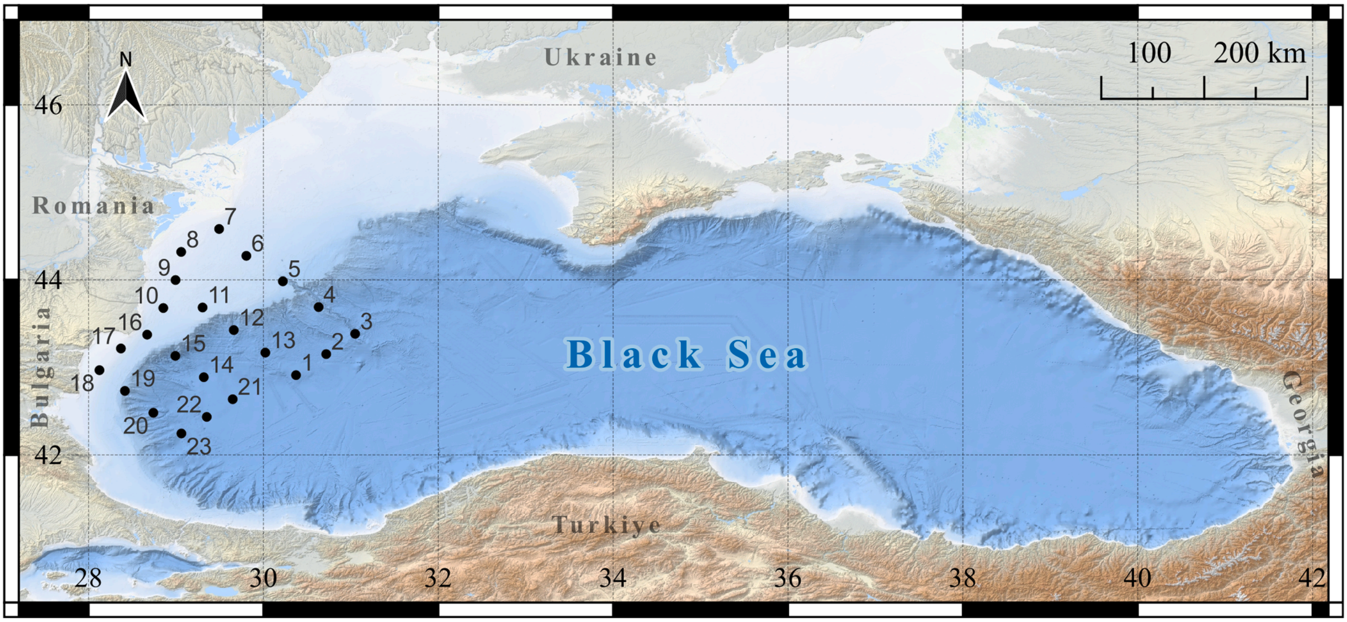


Fig. 1. Map of study region and station locations.

Taxonomic identification and cell counting of the net tow samples were conducted using an inverted microscope (Nikon Eclipse TE2000-U) connected to a video-interactive image analysis system (L.U.C.I.A, Version 4.8, Laboratory Imaging Ltd, Prague, Czech Republic) at 400 × magnification in Sedgewick-Rafter counting chambers. A total of 400 cells were counted from each sample, while all rare and large species were counted (Moncheva and Parr, 2010). Cell abundance, expressed as cells per net tow (cells NT^{-1}), was used both for Spearman rank correlation to compare species and phycotoxin data, and as presence data to indicate species distribution. Quality control (QC) and quality assurance (QA) of the data were performed following the Quality Control Guidelines for Phytoplankton (Moncheva, 2010).

In addition, formalin-fixed net tow samples were inspected using scanning electron microscopy (SEM, FEI Quanta FEG 200) for a qualitative identification of potentially toxic species. Samples for SEM were processed as described by Tillmann et al. (2017).

2.3. DNA metabarcoding analysis

DNA from the filters was extracted immediately after filtration using a 5 % Chelex buffer, following the protocol described in Tanabe et al. (2016), and stored at −20 °C. For detection of eukaryotic species, universal primers targeting the 18S rRNA gene V7–V9 variable region (18S-V7F: TGGAGYGATHTGTCTGGTTDATTCCG and 18S-V9R: TCACCTACGGAWACCTTGTTACG; modified from Tanabe et al. 2016) were used. The construction of paired-end libraries and sequencing on Illumina Miseq 300 PE platform (Illumina, San Diego, CA, USA) were performed by Macrogen Inc. (Seoul, South Korea). The procedures for sequence processing, selection, and taxonomic identification of operational taxonomic units (OTUs) followed the workflow described in Dzhembekova et al. (2017), with the exception that sequences with length over 300 bp were truncated to 300 bp by trimming the 3′ tails. The trimmed sequences shorter than 250 bp were filtered out. Sequences were clustered to OTUs at a similarity level of $\geq$ 99.1 %. Taxonomic assignment was performed using BLAST against a sequence database downloaded from GenBank. For taxonomic identification, a reference similarity threshold $\geq$ 99 % was set for species-level identification. As an additional verification, representative sequences of all OTUs associated with toxic species were manually BLAST-searched in the GenBank online database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

DNA sequences for this study can be found in the DDBJ Sequence Read Archive under accession number DRA014629 (biosamples SAMD00515617– SAMD00515639).

2.4. Selection of toxic microalgal species

The IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Lundholm et al., 2009) was used as a general reference database for the selection of toxic microalgal species for both microscopy and NGS data. However, the dataset was supplemented by information on *Dinophysis hastata*, which is not included on the list of toxic species due to a lack of targeted studies and has nevertheless been monitored within monitoring programs for *Dinophysis* species and diarrhetic shellfish toxins in different regions (Farrell et al., 2018; Fernández et al., 2019; Salas and Clarke, 2019). Additionally, members of the genus *Gonyaulax* and *Sourniaea diacantha* (the accepted taxonomic name of *Gonyaulax verior*), were also considered due to their unresolved taxonomy and toxic potential, which remain insufficiently characterized (Riccardi et al., 2009; Huang et al., 2025b). All cells of the *Gonyaulax spinifera*-type (based on cell size and shape) were included in the counting category *Gonyaulax* cf. *spinifera*. Although, under light microscopy (LM), *Pseudo-nitzschia* and *Alexandrium* were not identified at the species level, they were included in the dataset of toxic species at the genus level (as *Pseudo-nitzschia* spp. and *Alexandrium* spp.) due to the generally high number of toxic representatives within these genera.

On the other hand, *Phalacroma rotundatum* was excluded from the analyses, because this species is likely a vector rather than a direct source of phycotoxins (González-Gil et al., 2011).

2.5. Phycotoxin extraction

For the extraction of paralytic shellfish toxins (PSTs) and lipophilic toxins (including domoic acid (DA)), 500 μL 0.03 M acetic acid (p.a., Merck, Darmstadt, Germany) for PSTs and 500 μL methanol (HPLC-grade, Merck) for lipophilic toxins and DA were added to the respective cell pellets. To each sample, 0.9 g of ceramic beads (Lysing Matrix D, Thermo BIO 101) were added as well. After sealing and vortexing the cryovials, cells were lysed by reciprocal shaking at maximum speed (6.5 m s^{-1}) for 45 s in a shaker (FastPrep, Bio 101). Subsequently, homogenates were centrifuged at 16,100 x g and 4 °C for 15 min (5415R, Eppendorf, Hamburg, Germany). The extracts were transferred to spin filters with a pore size of 0.45 μm (Milipore Ultrafree, Eschborn, Germany) and centrifuged at 16,100 x g and 10 °C for 1 min. The filtrates were finally transferred to high performance liquid chromatography (HPLC) sample vials (2 mL, Agilent, Waldbronn, Germany) and vials were sealed with rubber crimp caps (Agilent). If necessary, the extract was transferred to a cone-shaped HPLC sample vial (Vial, crimp top, micro sampling, Agilent Technologies) to increase the fill level. Samples were stored at −20 °C until mass spectrometric analysis.

For the extraction of azaspiracids (AZAs) and karlotoxins (KmTxs), the filters were rinsed with 500 μL methanol until complete discoloration of the filters. Then the methanolic extracts were transferred to spin filters and filtered at a cut-off of 0.45 μm by centrifugation. Filtrates were transferred into analytical glass vials for subsequent mass spectrometric analysis.

2.6. LC-MS/MS analysis

PST measurements were performed in the selected reaction monitoring (SRM) mode on a Xevo TQ-XS triple quadrupole mass spectrometer equipped with a Z-Spray source (Waters, Eschborn, Germany). Chromatographic separation was achieved on an Acquity UPLC Glycan BEH Amide column (130 Å, 150 mm × 2.1 mm, 1.7 μm, Waters, Eschborn, Germany) equipped with an in-line 0.2 μm Acquity filter and thermostated at 60 °C with an isocratic elution to 5 min with 98 % eluent B followed by a linear gradient of 2.5 min to 50 % B and 1.5 min isocratic elution. The flow rate was 0.4 mL min^{-1}, and the injection volume was 2 μL. Mobile A comprised water with 0.15 % formic acid and 0.6 % ammonia. Mobile B comprised water/acetonitrile (3:7, v/v) with 0.1 % formic acid (Liu et al., 2021). Instrument parameters are given in Table S2 and used mass transitions in Table S3. PSTs were quantified by external calibration with standard mix solutions of four concentration levels consisting of the following PSTs: STX, NEO, GTX2/3, GTX1/4, dcSTX, dcNEO, dcGTX2/3, dcGTX1/4, B1(GTX5), B2(GTX6), C1/2, and C3/4 purchased from the Certified Reference Materials Program (CRMP) of the Institute for Marine Biosciences, National Research Council (Halifax, NS, Canada).

For the measurement of lipophilic toxins, including DA, ultra-performance liquid chromatography (UPLC®) coupled with tandem quadrupole mass spectrometry (LC-MS/MS) was used. The UPLC system included a column oven, an autosampler and a binary pump (AQUITY I UPLC Class, Waters, Eschborn, Germany). The separation was carried out on a RP-18 column (Purospher®STAR endcapped (2 μm) Hibar®HR 50–2.1 UPLC, Merck) equipped with a precolumn (0.5 μm, OPTSSOLV®EXP™, Sigma-Aldrich, Hamburg, Germany). This system was coupled to a triple quadrupole mass spectrometer (Xevo®TQ-XS, Waters). Data were acquired and analyzed with Masslynx (version 4.2, Waters). To uniquely identify the phycotoxins, in addition to the mass transitions defined in the selected reaction monitoring (SRM) mode, the retention times of the phycotoxins of the standards were compared with those in the samples. For quantification, an evaluation method was used which contained the specific transitions and default settings, except for the smoothing function, which was turned off. In some cases, enhanced production spectra were recorded. These were used for the identification of known substances by comparing the recorded characteristic fragmentation patterns with those in the literature.

The various eluents and gradients used for the different phycotoxin analyses are described in supplementary information (Tables S4–S9). Certified standard solutions were used to identify and quantify phycotoxins. These were gymnodimine A (GYM-A), 13-desmethylspirolide C (SPX-1), okadaic acid (OA), dinophysistoxin-1, and -2 (DTX-1, DTX-2), pectenotoxin 2 (PTX-2), yessotoxin (YTX), domoic acid (DA), and azaspiracid-1 (AZA-1). GYM-*A*, SPX-1, OA, and PTX-2, like the PST standards, were obtained from the Institute of Marine Biology from Canada, while DTX-1, DTX-2, YTX, DA, and AZA-1 were obtained from the Laboratorio CIFGA S.A., Lugo, Spain. In addition, a goniodomin A (GDA) standard was used, which was obtained from A. Andersen and a KmTx-2 standard was provided by A. Place. The mass transitions of the screened phycotoxins are given in Table S6 (various phycotoxins), Table S7 (AZAs), Table S8 (KmTxs), and Table S9 (YTXs).

2.7. Statistical analysis: spearman rank correlation and species distribution modelling

Spearman rank correlation was employed to identify statistically significant correlations between species abundances and phycotoxin levels in net tow samples, using a two-tailed p-value threshold of 0.05. The analyses and graphical representations were conducted in the R environment utilizing the statistical and programming software R (R Core Team, 2021), along with the packages ‘tidyverse’ (Wickham et al., 2019), ‘patchwork’ (Pedersen, 2025), ‘viridis’ (Garnier et al., 2024), ‘scales’ (Wickham et al., 2025), ‘corrplot’ (Wei and Simko, 2024) and ‘Hmisc’ (Harrell, 2024), accessible online at the CRAN repository (http://CRAN.R-project.org/).

Habitat distributions of three *Dinophysis* species, as well as *L. polyedra* and *P. reticulatum*, were examined using MaxEnt (Maximum Entropy) species distribution models (SDMs). Species occurrence data were derived from light microscopy analyses of net tow samples collected during the spring 2019 campaign (Dzhembekova et al., 2022) and from net and bottle samples collected in the present study, and were implemented as presence-only records. Environment variables used as model inputs included temperature, salinity, currents, dissolved oxygen, pH, chlorophyll *a*, and concentrations of phosphate and nitrate. The two sampling campaigns were considered as distinct events due to the dynamic nature of the processes involved, and separate MaxEnt models were developed for each. Consequently, two series of species distribution models (SDMs) were developed to assess species distribution influenced by unique seasonal variations. The obtained habitat suitability maps were examined in relation to phycotoxin levels detected in parallel in net samples.

MaxEnt is a machine learning method that has been employed in the modeling of distribution and habitat suitability of HAB species, including in the Black Sea (Townhill et al., 2018; Goncharenko et al., 2021; Rodríguez-Gómez et al., 2021; Borges et al., 2022, 2023; Hu et al., 2024). It estimates habitat suitability using environmental predictors and presence-only data (Phillips and Dudík, 2008; Elith et al., 2011; Phillips et al., 2017). The probability distribution that is most uniform (i.e., nearest to maximum entropy) while still constrained by the environmental characteristics at the observed occurrence locations is determined by employing the principle of maximum entropy. This method is particularly effective when working with presence data and has been demonstrated to perform well across a diverse spectrum of ecological contexts and sample sizes (Phillips and Dudík, 2008; Elith et al., 2011; Merow et al., 2013; Phillips et al., 2017).

SDM models were forced with averaged monthly values of the chosen variables throughout the periods of interest (April−June 2019 and July−September 2021). The latter were obtained using the E.U. Copernicus Marine Service Information (CMEMS) from the Black Sea Physics Reanalysis (Lima et al., 2023) and the Black Sea Biogeochemistry Reanalysis (Grégoire et al., 2020). The Black Sea Physics Reanalysis offers monthly and daily oceanic data for the Black Sea basin commencing from January 1st, 1993. The hydrodynamical core utilizes the NEMO general circulation ocean model, configured in the Black Sea domain with a horizontal resolution of 1/27° x 1/36° and 31 vertical levels. The MAST/ULiege Production Unit generates the biogeochemical reanalysis for the Black Sea utilizing the BAMHBI biogeochemical model.

The species distribution models were obtained by using the r package ‘dismo’ (Hijmans et al., 2023), ‘rJava’ (Urbanek, 2024), ‘rmaxent’ (Baumgartner and Wilson, 2022), ‘raster’ (Hijmans, 2024a), ‘terra’ (Hijmans, 2024b), and ‘maps’ (Becker et al., 2023) employing the Maximum entropy (MaxEnt) machine learning method (Phillips and

Dudík, 2008; Elith et al., 2011; Merow et al., 2013; Phillips et al., 2017). The maps were created using QGIS software (GGI Development Team, 2025).

The input data for the MaxEnt model comprises a compilation of observed species' occurrence locations and a collection of environmental predictors within a user-specified spatial extent divided into grid cells, along with the possibility to modify background or pseudoabsence data. MaxEnt samples background locations, contrasts them with presence locations, and produces an estimate of species probability occurrence (or relative environmental suitability) on a scale from 0 (least likely) to 1 (most likely) (Phillips and Dudík, 2008; Elith et al., 2011; Phillips et al., 2017).

The models were evaluated using both threshold dependent and threshold independent metrics, specifically Area under the Receiver operating characteristic (ROC) curve (AUC) (Bradley, 1997), which represents the overall model performance across all thresholds and strengths of prediction and the True skill statistics (TSS) to assess the model reliability (agreement between predictions and known occurrences, across various binary thresholds) (Pearce and Ferrier, 2000; Allouche et al., 2006; Liu et al., 2009, 2011). TSS was introduced as a metric less affected by the prevalence (Liu et al., 2009): $TSS = \left(\frac{TP}{TP+FN}\right) + \left(\frac{TN}{TN+FP}\right) - 1$, where: TP is the number of true positive instances, TN- the number of true negative instances, FP- the number of the false positive instances and FN is the number of the false negative instances.

The threshold used to calculate TSS was the sum of the maximized true positive rate $TPR = \left(\frac{TP}{TP+FN}\right)$ and the true negative rate: $TNR = \left(\frac{TN}{TN+FP}\right)$ for each model. The latter was considered optimal, ensuring that both the number of incorrectly predicted presences and the number of incorrectly predicted absences are of equal importance.

K-fold cross-validation was used to assess models' performance with the number of folds (k) set to a maximum of five. The choice of k followed sensitivity analyses conducted with values ranging from 3 to 5, considering the number of occurrence records (presence-only) and the spatial extent of the study area.

3. Results

3.1. Diversity and abundance of potentially toxic microalgae

Multiple potentially toxic taxa were identified using morphological and molecular methods (Table 1).

Potentially toxic microalgal taxa were observed at all stations and across all sampled depths, in varying but generally very low abundances (Tables S10–S11). Most species belonged to the class Dinophyceae.

Among the Gonyaulacales, *Gonyaulax* (*Gonyaulax* cf. *spinifera, G. polygramma*, and unidentified *Gonyaulax* spp.), *Lingulaulax polyedra, Protoceratium reticulatum*, and *Sourniaea diacantha* were detected (Fig. 2).

As representatives of *Alexandrium*, both PST-producing species (*A. fragae*), GDA-producers (*A. pseudogonyaulax*), spiroimine producers (*A. ostenfeldii*), as well as species considered non-toxic (*A. insuetum, A. margalefii*, and *A. tamutum*) were identified. However, the low abundance of *Alexandrium* spp. hindered their reliable quantification.

Table 1
Potentially toxic taxa identified during the investigation using different methods: by light microscopy on board using live samples (LM live), in SEM preparations (SEM), in the fixed bottle samples (LM/BS), fixed net tow samples (LM/NS), and DNA metabarcoding (DNA). For completeness, records based on isolated strains from the companion paper (Dursun et al., 2025) are also listed (strain-based). Identification is indicated by "+" (identified), "−" (not identified), and "?" (uncertain identification). Phycotoxins potentially associated with these species are also listed, with those detected in the current study shown in bold. Some non-toxic representatives of the genera are also included for additional context.

Species	Associated toxins	LM Live	SEM	LM/BS	LM/NS	DNA	Strain-based
Pseudo-nitzschia spp.	DA	+	+	+	+	+	−
Pseudo-nitzschia calliantha	DA	−	−	−	−	+	+
Alexandrium spp.*	**PSTs**, SPX, **GDA**, haemolytic products	+	+	+	+	+	+
Alexandrium fragae	PSTs	−	+	−	−	−	−
Alexandrium pseudogonyaulax	GDA	+	−	−	−	?	+
Alexandrium ostenfeldii	PST, spiroimines	−	−	−	−	−	+
Alexandrium insuetum	non-toxic	−	+	−	−	−	−
Alexandrium margalefii	non-toxic	−	−	−	−	+	−
Alexandrium tamutum	non-toxic	−	−	−	−	+	+
Alexandrium andersonii	PSTs	−	−	−	−	−	+
Gonyaulax spp.	**YTXs**	+	+	+	+	+	+
Sourniaea diacantha	non-toxic	+	+	−	+	+	+
Lingulaulax polyedra	**YTXs**	+	+	+	+	+	+
Protoceratium reticulatum	**YTXs**	+	+	+	+	+	+
Dinophysis acuminata	OA, DTXs, **PTXs**	+	−	+	+	+	−
Dinophysis acuta	OA, DTXs, **PTXs**	+	+	+	+	+	−
Dinophysis caudata	OA, DTXs, **PTXs**	+	+	−	+	−	−
Dinophysis sacculus	OA, DTXs, **PTXs**	+	+	+	+	−	−
Dinophysis hastata	not known	+	+	−	−	−	−
Polykrikos hartmannii	unclear	+	−	−	+	+	+
Karlodinium spp.	KmTxs	+	−	−	−	+	+
*Prorocentrum cordatum***	unclear	+	+	+	+	+	+
Amphidomataceae***	AZA	+	+	+	+	+	+
Azadinium spinosum	AZA	−	+	−	−	−	−
Azadinium trinitatum	non-toxic	−	+	−	−	−	−
Azadinium luciferelloides	not known	−	+	−	−	−	−
Amphidoma languida	AZA	−	+	−	−	−	−
Amphidoma pontica	non-toxic	−	+	−	−	−	+

DA – domoic acid; PSTs – Paralytic Shellfish Toxins; SPX – spirolides; GDA – goniodomin A; OA – okadaic acid; DTXs – dinophysistoxins; PTXs – pectenotoxins; YTXs – yessotoxins; KmTxs – karlotoxins; AZAs – azaspiracids.

[*] Toxin profiles vary between species and strains.

[**] PHYCOB strains of *P. cordatum* are reported in Tillmann et al. (2023).

[***] detailed data on Amphidomataceae are reported in Tillmann et al. (2025).

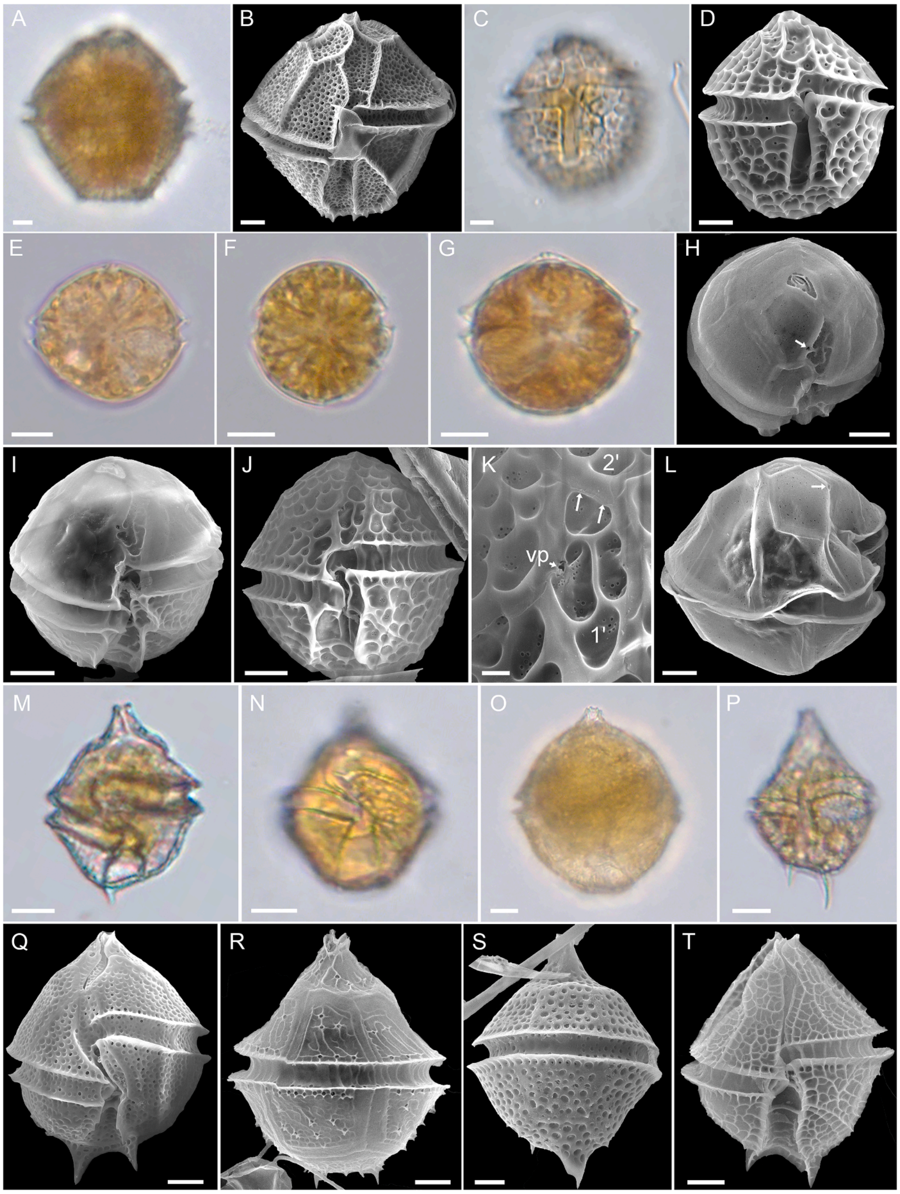


Fig. 2. Light microscopy of living cells (A, C, E–G, M–P) and SEM micrograph documentations (B, D, H–L, Q–T) of potentially toxic Gonyaulacales from the PHYCOB 2021 survey. (A, B) *Lingulaulax polyedra*. (C, D) *Protoceratium reticulatum*. (E) *Alexandrium pseudogonyaulax* (note the compressed cell shape, species determination in this case was supported by a swimming behaviour typical for *A. pseudogonyaulax*). (F, G) Two different cells of *Alexandrium* spp. (H, I) Two different views of the same cell of *Alexandrium fragae*, note the ventral pore (white arrow in H) and the reticulate hypotheca visible in I. (J, K) Two different views of the same cell of *Alexandrium insuetum*, note the ventral pore (vp) and the disconnection of the first apical plate from the pore plate (arrows in K). (L) Right-lateral view of *Alexandrium* sp., note the ventral pore (arrow), but the width of plate 6’’ is not discernable. (M–O) Three different cells of *Gonyaulax* spp. (P) *Sourniaea diacantha*. (Q–S) Three different cells of *Gonyaulax* spp. in ventral (Q) or dorsal (R, S) view. (T) Ventral view of *Sourniaea diacantha*. Scale bars = 5 μm (A–D, H–J, L, Q–T) or 10 μm (E–G, M–P) or 1 μm (K).

YTX-associated species occurred in low abundances (maximum counts ≤270 cells L^{-1} in bottle samples) and were more frequently observed in net samples than in bottle samples. *P. reticulatum* was recorded at all stations, reaching up to 168 cells L^{-1} (station 15). *L. polyedra* was detected at 15 stations with the highest abundance of 220 cells L^{-1} (station 9), whereas *G.* cf. *spinifera* was found at more stations (at 20 stations) with a maximum abundance of 270 cells L^{-1} (station 8).

Among the Dinophysales, species known to produce DTXs and PTXs (*D. acuminata, D. acuta, D. caudata*, and *D. sacculus*) were identified, along with *D. hastata*, a representative not yet tested for phycotoxin production (Fig. 3). *Dinophysis* species occurred widely but at low abundances (<200 cells L^{-1}). *D. acuminata, D. acuta*, and *D. sacculus* were found in both net tow and bottle samples, whereas *D. caudata* was observed only in net tow samples. *D. acuta* was the most frequent *Dinophysis* species, present at 70 % of the stations. *D. acuminata* and *D. sacculus* were detected at 61 % of the stations, whereas *D. caudata* was scarcely found, present in only four samples.

Members of the Gymnodiniales, including *Polykrikos hartmannii* and *Karlodinium* spp., as well as Prorocentrales (*Prorocentrum cordatum*), were also detected (Fig. 4). *P. hartmannii* was detected only in the net tow samples, whereas *Karlodinium* was recorded in both types of samples. In bottle samples, *Karlodinium* was included within the larger group of small unarmored dinoflagellates and not counted separately. In net tow samples, *P. hartmannii* was rare, whereas *Karlodinium* was most frequently observed (at 20 stations). Among a few other planktonic species of *Prorocentrum, P. cordatum* was common in both sample types, occurring in higher abundances than other dinoflagellate species, up to

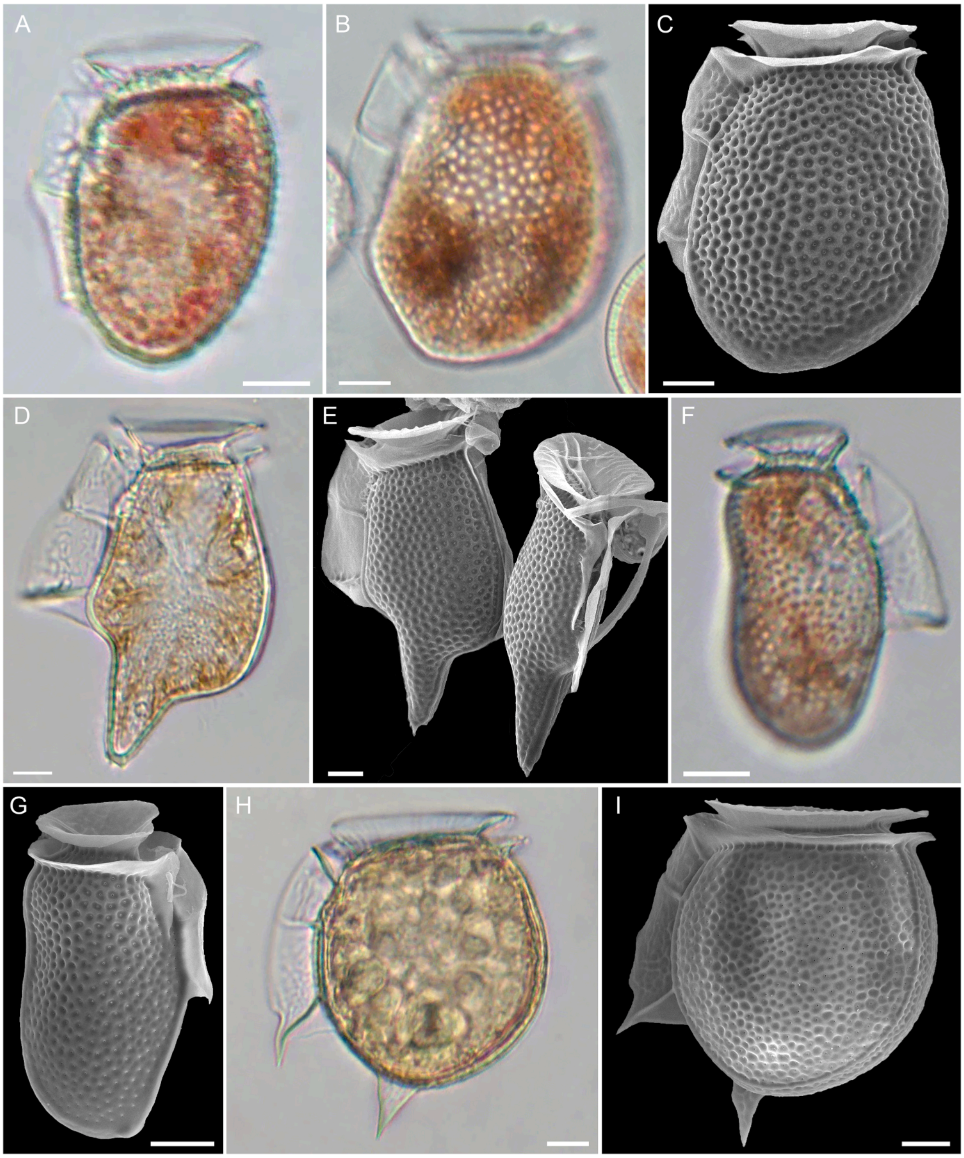


Fig. 3. Light microscopy of living cells (A, B, D, F, H) and SEM micrograph documentations (C, E, G, I) of potentially toxic Dinophysales from the PHYCOB 2021 survey. (A) *Dinophysis acuminata*. (B, C) *Dinophysis acuta*. (D. E) *Dinophysis caudata*. (F, G) *Dinophysis sacculus*. (H, I) *Dinophysis hastata*. Scale bars = 10 μm.

3520 cells L^{-1} (station 7).

Detailed data on the distribution, abundance, and taxonomy of the Amphidomataceae from the PHYCOB cruise are presented in Tillmann et al. (2025). Briefly, two of the known AZA producers, *Azadinium spinosum* and *Amphidoma languida*, were detected, along with other representatives not associated with toxin production (*Az. luciferelloides, Az. trinitatum*, and the newly described *Amphidoma pontica*). Amphidomataceae species occurred at all stations, with the highest total abundance of 13.6 × 10^3 cells L^{-1} (Tillmann et al., 2025).

DNA metabarcoding confirmed many of these dinoflagellate species, generally with low read counts, except for *Karlodinium veneficum*, which was recorded with higher read numbers in some samples (Table S12). Species-level identification was hampered for one OTU showing equal similarity to two different toxic species of *Alexandrium* (*A. pseudogonyaulax* and *A. hiranoi*), two OTUs related to *Gonyaulax* that did not meet the reference similarity threshold, and one OTU assigned only to the genus level (best match to a reference sequence deposited as *Karlodinium* sp.).

Potentially toxic diatoms *Pseudo-nitzschia* were identified to the genus level only under LM (Fig. 4F), while metabarcoding data and strain isolation (Dursun et al., 2025) revealed the presence of *P. calliantha. Pseudo-nitzschia* occurred at all stations reaching up to 8404 cells L^{-1} in bottle samples (station 9).

3.2. Phycotoxin distribution

Among the screened toxins, gonyautoxins (GTX-2/3), goniodomin A (GDA), pectenotoxins (PTXs), and yessotoxins (YTXs) were found in the study region (Tables 2, 3, Supplementary material Table S14). On the other hand, azaspiracids (AZAs), karlotoxins (KmTxs), domoic acid (DA), cyclic imines (gymnodimines and spirolides), okadaic acid (OA), and dinophysistoxins were not detected in the field samples. The respective detection limits are provided in the Supplementary material (Table S13).

GTX-2/3 was detected at a total of 13 stations, with levels ranging from 0.2 to 1.8 ng NT^{-1} in the 20–50 μm size fraction, the highest value being measured at station 8. GDA was detected at only three stations (stations 7, 13, and 23), with levels of 1.9, 1.7, and 3.0 ng NT^{-1} in the 20−50 μm size fraction, respectively (Table 2). Among the PTXs, PTX-2 was observed at all stations. The highest levels detected during the

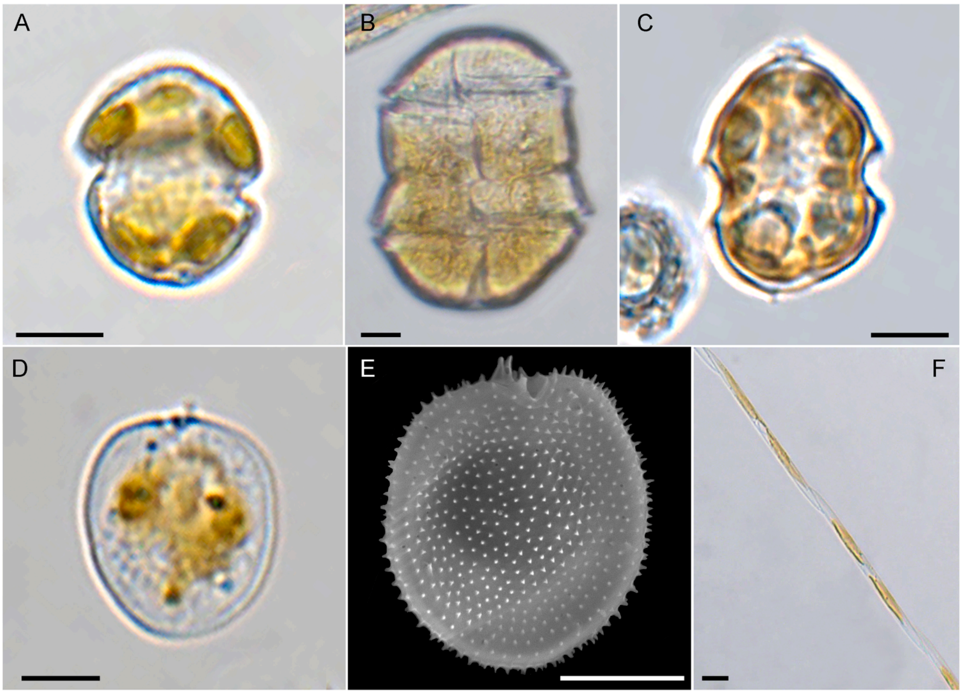


Fig. 4. Light microscopy of living cells (A–D, F) and SEM micrograph documentation (E) of potentially toxic microalgae from the PHYCOB 2021 survey. (A) *Karlodinium* sp. (B) *Polykrikos hartmannii.* (C) *Azadinium* sp., (D, E) *Prorocentrum cordatum.* (F) *Pseudo-nitzschia* sp. Scale bars = 5 μm (A, C–E) or 10 μm (B, F).

Table 2
Phycotoxin contents [ng NT^{-1}] of the 20–50 μm size fractions of 30 m vertical net tows. "–" indicates toxin levels below the respective limit of detection (Table S13). PTX-11 and PTX-12 are expressed as PTX-2 equivalents. All YTX variants are expressed as YTX equivalents. [a] YTX numbers refer to Miles et al. (2005).

	Pectenotoxins (PTX)				Yessotoxins (YTX)							PST/ GDA	
Station	PTX-2	PTX-2 sa	PTX-11	PTX-12	YTX	41-a-homo-YTX	Carboxy-YTX	e# 21/22 [a]	#20 [a]	undesc. [a]	#21[a]	GTX-2/3	GDA
1	12.6	–	–	–	122.9	1.8	–	–	–	–	–	–	–
2	5.4	–	–	–	27.6	1.3	–	–	–	–	–	–	–
3	5.0	–	–	–	14.8	–	–	–	0.6	–	–	–	–
4	21.1	–	–	–	106.2	1.7	–	0.6	0.6	4.4	1.2	–	–
5	23.4	–	–	–	120.3	4.3	–	–	0.6	–	–	–	–
6	45.6	3.0	–	–	36.1	3.1	–	–	–	–	–	0.7	–
7	155.0	11.8	1.4	–	28.7	5.4	–	–	–	–	–	0.7	1.9
8	152.4	10.1	–	–	22.2	5.4	–	–	–	–	–	1.8	–
9	84.2	3.1	–	–	20.6	4.8	–	–	–	–	–	0.4	–
10	154.5	4.1	–	–	49.6	7.2	–	–	–	–	–	0.9	–
11	9.4	–	–	–	31.7	1.1	–	–	–	–	0.6	–	–
12	14.4	–	–	–	81.6	0.8	11.4	–	–	1.9	–	–	–
13	5.6	–	–	–	19.4	1.2	–	–	–	–	–	–	1.7
14	4.5	–	–	–	60.7	1.8	–	–	–	–	–	–	–
15	3.5	–	–	–	51.9	2.9	–	–	–	–	–	0.3	–
16	59.9	3.8	–	–	13.4	3.8	–	–	–	–	–	0.8	–
17	61.5	6.4	2.1	–	31.4	8.2	–	0.6	0.6	–	–	0.6	–
18	54.3	6.4	–	–	24.4	4.7	–	–	0.6	–	–	0.4	–
19	2.7	–	–	–	–	–	–	–	–	–	–	0.4	–
20	18.7	–	–	–	60.1	3.9	–	–	–	–	–	0.3	–
21	4.7	–	–	–	35.5	2.7	–	–	–	–	–	0.2	–
22	27.0	1.7	–	–	43.7	1.5	–	–	–	–	–	–	–
23	12.4	–	–	–	319.7	10.3	–	–	1.6	–	–	0.3	3.0

expedition were 155.0 ng NT^{-1} in the 20–50 μm size fraction at station 7 (Table 2), up to 70.1 ng NT^{-1} in the 50–200 μm size fraction at station 10 (Table 3), and 44.3 ng NT^{-1} in the >200 μm size fraction at the same station (Table S14). Moreover, PTX-2 seco acids were detected in all size fractions, with levels up to 11.8 ng NT^{-1} in the 20–50 μm size fraction at station 7, 11.3 ng NT^{-1} in the 50–200 μm size fraction at station 10, and 11.5 ng NT^{-1} in the >200 μm size fraction at station 10. In addition to PTX-2 and PTX-2 seco acids, PTX-12 was also detected at low levels, with maxima of 2.2 ng NT^{-1} in the 50–200 μm size fraction at station 20 (Table 3), and 2.2 ng NT^{-1} in the >200 μm size fraction at station 1. The third and least abundant PTX was PTX-11, which reached maxima of 2.1 ng NT^{-1} in the 20–50 μm size fraction at station 17 (Table 2) and 2.5 ng NT^{-1} in the 50–200 μm size fraction at station 10 (Table 3).

The other lipophilic toxin group present in the region was the yessotoxin group, of which seven variants were detected in total. In addition to the base compound yessotoxin (YTX), six other variants were detected during the expedition. These variants were: 41a-homo-YTX (compound #5), carboxy-YTX (#3), entries #21, 22 (m/z 1061 > 981), undescribed (m/z 1131 > 1051), #20 (m/z 1273 > 1193), and #21 (m/z 1405 > 1325) (designations of all compounds according to

Table 3

Toxin contents [ng NT^{-1}] of the 50–200 µm size fractions of 30 m vertical net tows. “–“ indicates toxin levels below the respective limit of detection (Table S13). PTX-11 and PTX-12 are expressed as PTX-2 equivalents.

Station	Pectenotoxins (PTX)			
	PTX-2	PTX-2 sa	PTX-11	PTX-12
1	2.9	–	–	–
2	2.0	–	–	1.4
3	8.3	–	–	1.3
4	8.8	–	–	–
5	7.0	–	–	1.6
6	28.6	2.1	–	–
7	30.5	4.8	–	–
8	24.1	3.6	–	2.0
9	23.8	2.5	–	1.6
10	70.1	11.3	2.5	–
11	20.4	–	–	–
12	16.7	–	–	–
13	–	–	–	–
14	7.1	–	–	–
15	3.7	–	–	–
16	15.0	2.8	–	–
17	9.2	–	–	–
18	11.9	1.7	–	1.7
19	23.0	2.0	–	1.5
20	18.0	–	–	2.2
21	4.6	–	–	1.5
22	26.1	–	–	–
23	41.8	1.4	–	1.5

Miles et al. (2005)). YTX was found at 22 of the 23 stations, and with levels up to 319.7 ng NT^{-1} in the 20–50 µm size fraction. The highest levels were detected at stations 23, 1, and 5 (319.7, 122.9, and 120.3 ng NT^{-1}, respectively) (Table 2). The YTX variant with the highest level was 41a-homo-YTX, recorded at 10.3 ng NT^{-1} in the 20–50 µm size fraction at station 23 (Table 2). Compound #20 was the second most abundant YTX variant, with a relatively high level of 1.6 ng NT^{-1} in the same size fraction at station 23. All other YTX variants were detected at a few stations and at lower levels (Table 3). All YTX compounds were found exclusively in the 20–50 µm size fraction, and their levels were higher at deep stations (stations 1, 4, 5, and 23) than at the coastal stations along the cruise transects.

3.3. *Correspondence between detected toxigenic species and phycotoxins*

Across the stations, potentially toxic dinoflagellates and their associated toxins co-occurred (Fig. 5A, B). Spearman rank correlation analyses between the abundances of potential toxin producers and the related phycotoxin levels in the net tow samples revealed significant correlations for part of the dataset. Pectenotoxins showed positive correlations with the cell abundances of *D. acuminata* and *D. sacculus* (Fig. 6A, B). PTX-2 and PTX-2sa levels were strongly correlated with *D. sacculus* cell abundances in both size fractions and moderately correlated with *D. acuminata* in the smaller fraction (20–50 µm). For yessotoxins, the 41-a-homo-YTX variant levels were moderately correlated with *L. polyedra* and *Gonyaulax* cf. *spinifera* cell abundances (Fig. 6C).

3.4. *Relationship between selected dinoflagellates, phycotoxins, and environmental predictors: spatial distribution modeling*

All models showed AUC values ranging from 0.8 to >0.9 and TSS values >0.5 (Tables S15, S16), indicating good to excellent predictive performance. The resulting distribution maps, together with data gathered during the sampling campaigns, suggest that the species of concern co-occur and exhibit substantial overlap in their environmental niche suitability (Figs. 7A-D, 8A–E).

In spring 2019, nitrates were the most influential predictor, while temperature and phosphate contributed considerably less to the probability of occurrence (Table S15). In contrast, during summer 2021, phosphate concentration was the primary variable influencing the predicted species occurrences, whereas currents and dissolved oxygen had considerably weaker effects (Table S16).

The species distribution maps were overlaid with phycotoxin levels to assess whether the highest probabilities align with the highest measured phycotoxin values. In both seasons, the highest toxin abundances were recorded in regions with the highest predicted probabilities of species occurrence; however, due to niche overlap, it remains challenging to identify specific phycotoxin producers.

4. Discussion

The presence of numerous harmful species and phycotoxins was documented in Bulgarian and Romanian Black Sea waters in September 2021, complementing a case study conducted in spring 2019 (Dzhembekova et al., 2022).

During both sampling campaigns, the overall hydrographic conditions were comparable. The greatest difference was observed in water temperature, which was notably lower in May 2019 compared to September 2021, when significantly warmer surface temperatures reflected the late summer maximum (Mohamed et al., 2022). Corresponding to the colder water temperatures, oxygen concentrations were higher in May.

Seasonal variations in plankton composition and abundance, including potentially toxic species, have been documented in different regions of the Black Sea, with some taxa exhibiting a consistent year-round presence (Türkoğlu and Koray, 2002; Vershinin et al., 2005; Yasakova, 2013; Dzhembekova and Moncheva, 2014; Moncheva et al., 2019). The potentially toxic species identified in September 2021 (this study) largely overlapped with those detected in May 2019 (Dzhembekova et al., 2022), but occurred at lower abundances. The Romanian coastal stations (7–10) showed the highest numbers.

The major phycotoxin groups also corresponded well between the two periods, although some toxin variants differed by season, and new phycotoxins appeared only in samples from the summer campaign, as detailed in the sections below.

Pseudo-nitzschia was the most widespread potentially toxic taxon, observed at all stations, similar to the previous study (Dzhembekova et al., 2022). Cell abundances, however, were low, likely reflecting the seasonal dynamics of the genus in the Black Sea (Dzhembekova et al., 2021; Lifanchuk et al., 2023). Only one *Pseudo-nitzschia* species (*P. calliantha*) was identified through DNA metabarcoding, whereas the same approach detected three species (*P. calliantha, P. delicatissima*, and *P. pungens*) in the previous investigation (Dzhembekova et al., 2022). Although *Pseudo-nitzschia* was the most dominant species in the net tow samples during both campaigns, no domoic acid was detected. These findings corroborate the reported lack of DA production in cultures of *P. calliantha* strains isolated during the PHYCOB sampling campaign (Dursun et al., 2025). In contrast, earlier studies reported DA in spring plankton samples from Bulgarian coastal waters (Peteva et al., 2018, 2020b) and in cultures of *P. calliantha* strains isolated from the Ukrainian Black Sea (Besiktepe et al., 2008). *Pseudo-nitzschia calliantha* strains from the Mediterranean Sea have also been reported as toxigenic, both in field studies and under laboratory conditions (Marić et al., 2011; Tanković et al., 2022). The lack of consensus on the key abiotic and biotic factors controlling DA production, the strain-specific response, and the occurrence of both toxic and non-toxic strains within most *Pseudo-nitzschia* species (Bates et al., 2018; Lelong et al., 2012; Trainer et al., 2012) hampers the interpretation of amnesic shellfish toxin patterns, which require further studies to better understand *Pseudo-nitzschia*-related toxicity in the Black Sea.

Alexandrium spp. were detected at almost all sampling stations during this study, albeit at low abundances. The combination of different methodological approaches resulted in the identification of multiple

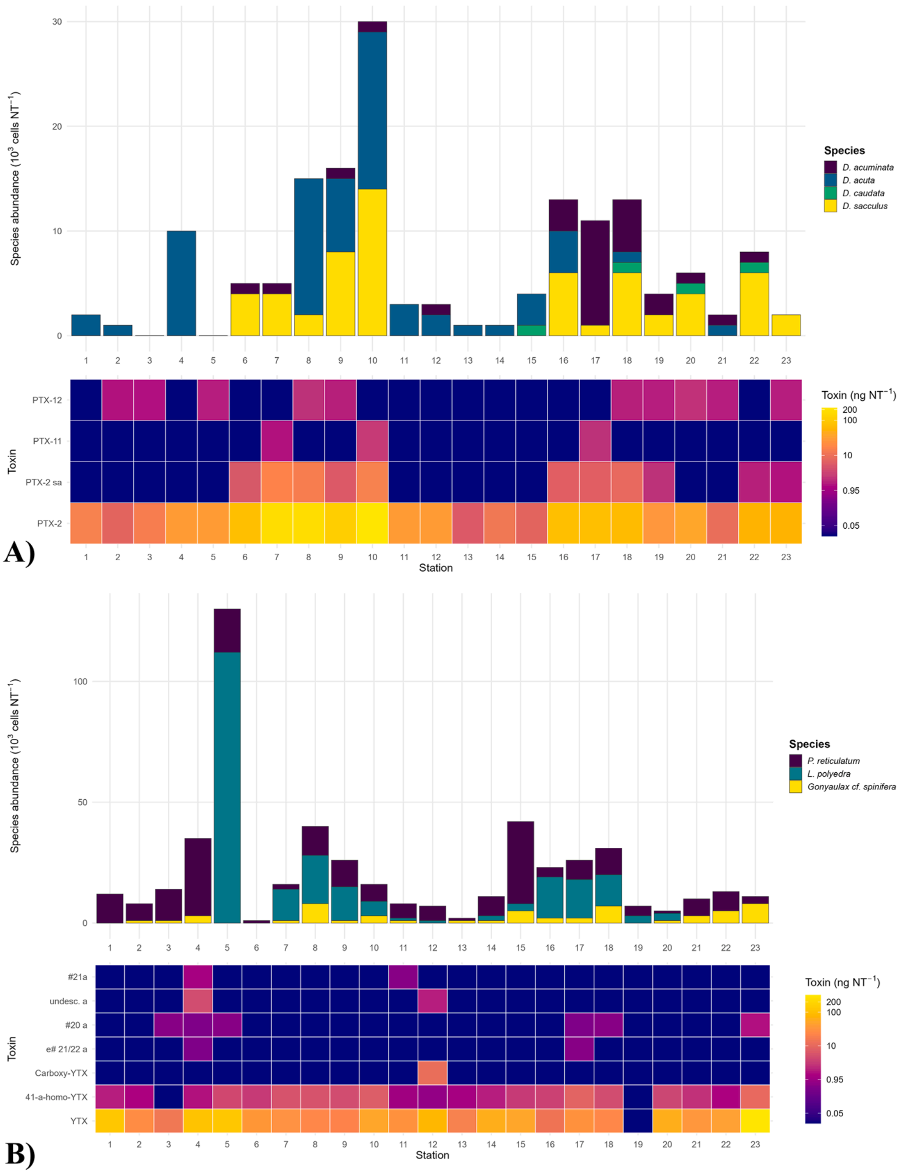


Fig. 5. Distribution of potentially toxic dinoflagellate species and associated phycotoxin levels in net tow samples across sampling stations. A) PTX variants and PTX-producing species; B) YTX variants and YTX-producing species. Stacked bars show the abundances of dinoflagellate species, expressed as 10^3 cells NT^{-1}. The heatmap below displays phycotoxin concentrations (ng NT^{-1} in the combined 20–200 μm fraction) on a log₁₀ scale.

Alexandrium species (this study and Dursun et al., 2025), including both toxigenic (*A. andersonii, A. fragae, A. pseudogonyaulax*, and *A. ostenfeldii*) and non-toxigenic (*A. tamutum, A. insuetum*, and *A. margalefii*) representatives co-occurring in the Black Sea. Similar observations have been reported in the Mediterranean Sea (Anderson et al., 2012a). Additionally, DNA metabarcoding recorded *A. minutum* in May 2019 (Dzhembekova et al., 2022). The high similarity among *A. fragae, A. minutum*, and *A. tamutum* (Branco et al., 2020) is reflected in the performance of the metabarcoding marker used. For instance, only a single base pair difference is observed in this region between *A. minutum* and *A. tamutum* strains originally used for their phylogenetic delineation (Montresor et al., 2004). Thus, although the *A. minutum* and *A. tamutum* OTUs differed by ~1.7 % (over 475 bp) and the BLAST top-hit approach identified them as separate species, species-level delineation with this marker is unreliable and requires further support. Nevertheless, the presence of *A. tamutum* in the Black Sea in 2021 is supported by a strain-based investigation (Dursun et al., 2025), whereas the identity of *A. minutum* detected during the previous cruise remains unconfirmed by corroborating data. On the other hand, OTU sequences identified as *A. minutum* and *A. tamutum* differed by > 2 % (over 410 bp) from a reference sequence of *A. fragae* (KX097019), providing no molecular evidence for the presence of *A. fragae* in the samples, despite its identification by SEM analyses. These findings highlight the importance of using a multifaceted approach that combines field samples and cultures to clarify the identity and toxicity of species and strains in the Black Sea and other ecosystems. This is particularly crucial for species that are challenging to identify under LM or through DNA metabarcoding, such as those with identical (*A. pseudogonyaulax* and *A. hiranoi*) or highly similar (*A. minutum* and *A. tamutum*) marker gene sequences.

Interestingly, in contrast to the previous study (Dzhembekova et al., 2022), phycotoxins associated with *Alexandrium* species (GTX-2/3 and GDA) were detected. GDA was measured at levels comparable to those reported in plankton samples from the Bulgarian Black Sea (Peteva et al., 2020a), but significantly lower than values in other brackish waters (Kremp et al., 2019). As a possible source, DNA metabarcoding detected an OTU with equal similarity to *A. pseudogonyaulax* and *A. hiranoi*, both known GDA producers (Lassus et al., 2016). Strain-based data confirmed the presence of *A. pseudogonyaulax* in the PHYCOB samples and further

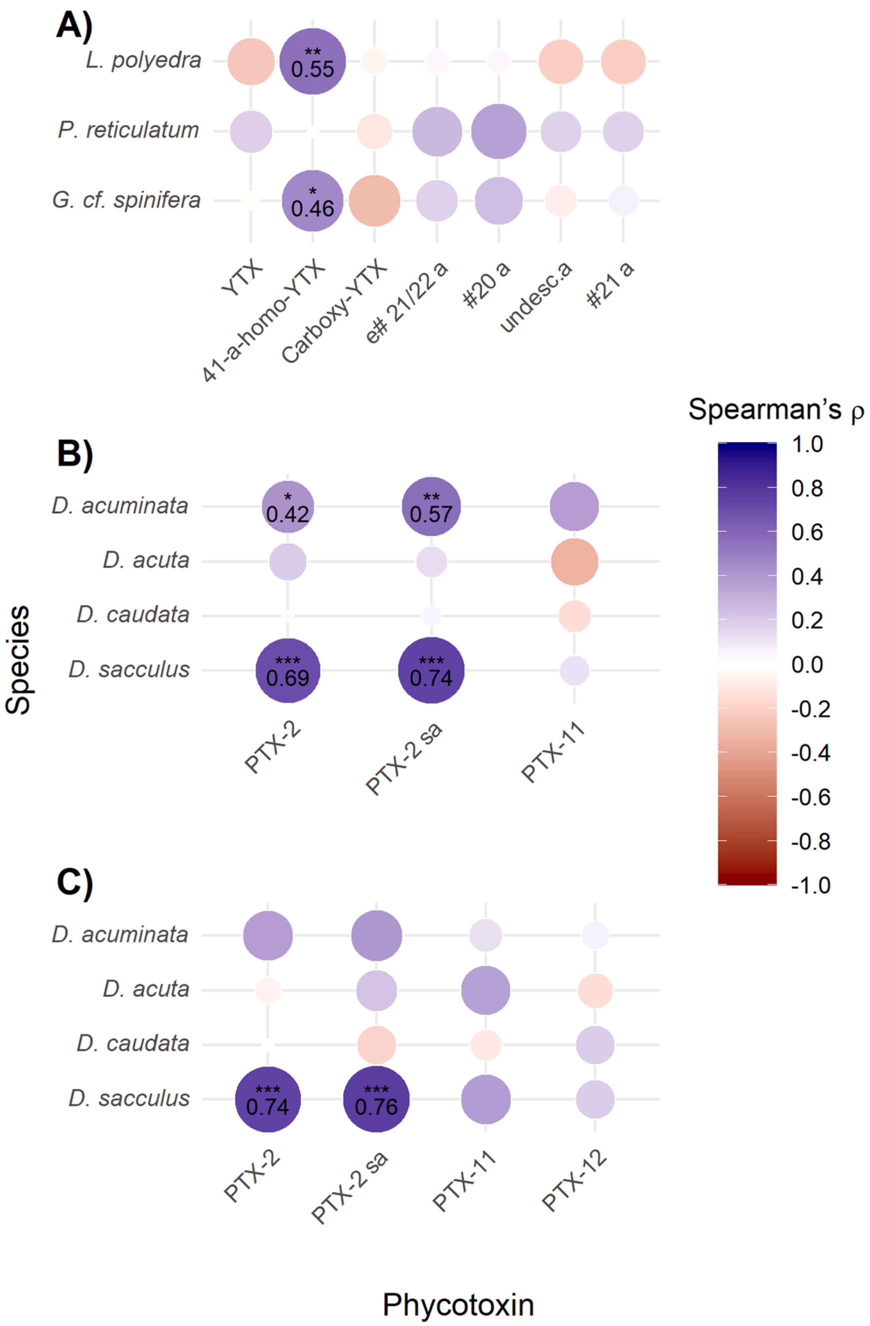


Fig. 6. Correlation analysis between the potentially toxic dinoflagellate species and the relevant phycotoxin variants by fractions: (A, B) 20–50 µm fraction; (C) 50–200 µm fraction. Circle size and color intensity are proportional to the Spearman's rho correlation coefficients. Only significant results are labeled, with the statistical significance annotated as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

revealed its ability to produce GDA (Dursun et al., 2025), clearly identifying this species as a source of GDA in the Black Sea. Compared with GDA, GTX-2/3 were detected more frequently in the samples. Although low levels of GTX-2/3 have previously been reported in wild and cultivated mussels from the Bulgarian Black Sea (Kalinova et al., 2015; Krumova-Valcheva and Kalinova, 2017; Peteva et al., 2019, 2020b) and in Russian waters (Vershinin et al., 2006), this is the first report of these toxins in plankton samples. GTX-2/3 were detected at levels much lower than those reported from other regions (Fabro et al., 2017; D'Agostino et al., 2019), including brackish waters (Kremp et al., 2019). This is most likely due to the low *Alexandrium* abundances, however differences in phycotoxin cell quotas among *Alexandrium* species (Branco et al., 2020), variability in toxin production within strains (Kremp et al., 2019), and environmental effects influencing phycotoxin production (Lim and Ogata, 2005) may also play roles. GTX-2/3 are produced by several *Alexandrium* species, including *A. minutum* (Lewis et al., 2018) and the recently described *A. fragae* (Branco et al., 2020). While no evidence of *A. minutum* was found in this study, SEM analyses confirmed the presence of *A. fragae* in the Black Sea for the first time, suggesting its potential contribution to PST production in the region. This issue is discussed further in the context of previous reports on *Alexandrium* and GTX-2/3 in the Black Sea by Dursun et al. (2025).

Five species of the genus *Dinophysis* were recorded in the investigated area: *D. acuminata, D. acuta, D. caudata, D. sacculus*, and *D. hastata*. All of them, except for *D. hastata*, which was identified only in the current study based on live samples and SEM observations, were also previously reported in the May 2019 net tow samples (Dzhembekova et al., 2022). In contrast to microscopy-based analyses, DNA metabarcoding detected

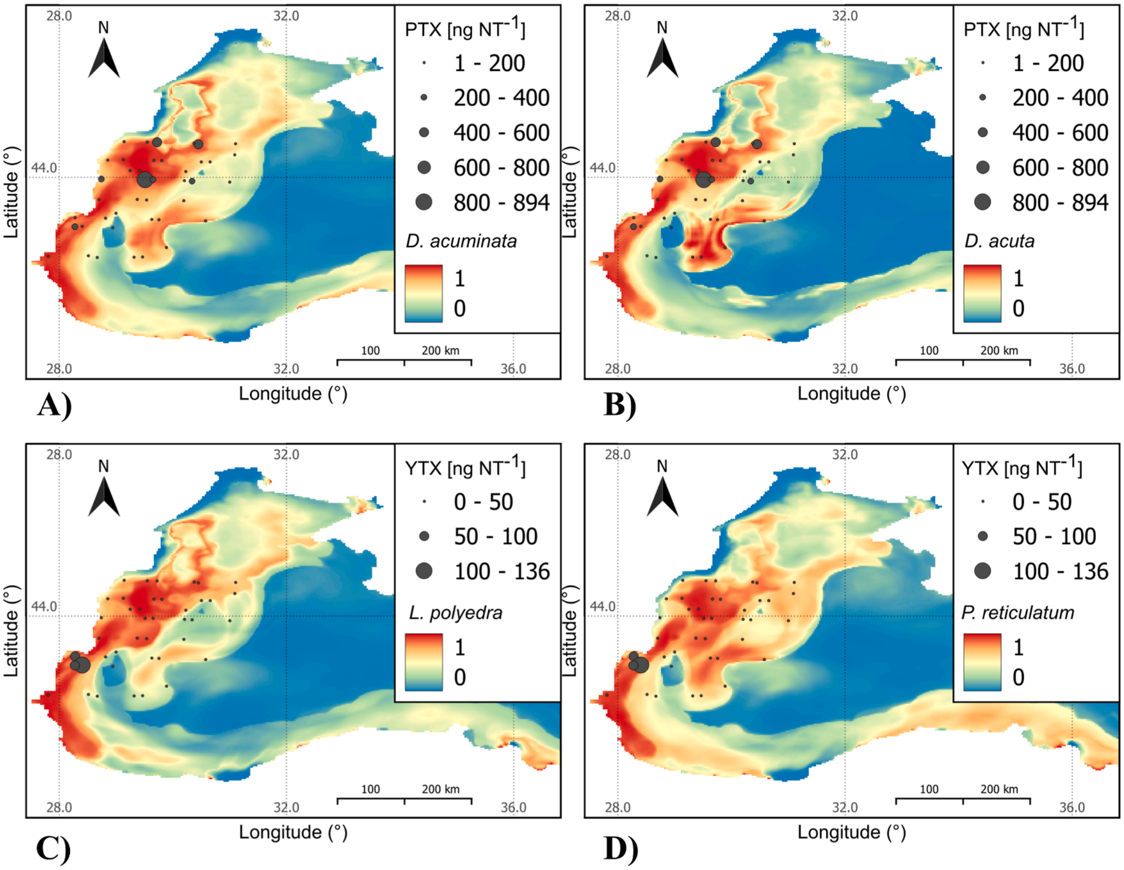


Fig. 7. Species distribution models representing MaxEnt (Maximum entropy) predicted probability of occurrence [0; 1] and the most favorable environment based on the data collected during the spring expedition of 2019 (Dzhembekova et al., 2022) for: A. *D. acuminata*; B. *D. acuta*; C. *L. polyedra*, and D. *P. reticulatum* in the western Black Sea coastal and shelf waters, overlayed with phycotoxin levels. The probability is represented with a color scheme, with light blue indicating the least likelihood of suitable conditions, light yellow indicating conditions matching those where species were found, and red corresponding to the highest predicted probability of a suitable environment.

only two *Dinophysis* species. This highlights methodological limitations of the metabarcoding approach. For instance, the limited resolution of commonly used 18S rDNA markers (V4 and V7-V9) within the *Dinophysis* genus has been documented, and further validation of molecular data has been recommended (Dzhembekova et al., 2022; Gaonkar and Campbell, 2023). In this study, both *D. acuminata* and *D. acuta* were identified using a top BLAST hit approach, although sequence similarity to other *Dinophysis* species was relatively high. The presence of these species in the samples was confirmed by the parallel morphological observations.

The high adaptability of some *Dinophysis* representatives to changing environmental conditions (Rial et al., 2023) likely contributes to the consistent presence of the genus in Black Sea plankton community (Türkoğlu and Koray, 2002; Petrova and Velikova, 2003; Terenko, 2011). Moreover, the western Black Sea appears to provide suitable habitats for *Dinophysis* species, largely overlapping with regions where higher toxin levels were detected.

Among *Dinophysis*-associated toxins, only PTXs were detected in this and previous studies (Peteva et al., 2020b; Dzhembekova et al., 2022). This can be attributed to: (1) the presence of strains that produce only PTXs (Reguera et al., 2014), (2) the release of other phycotoxins (e.g., okadaic acid and dinophysistoxins) into the water column (Nagai et al., 2011), or (3) analytical sensitivity, as PTXs can be detected at much lower levels than OA/DTXs. The detection of only PTXs in mussel tissue from samples collected in Bulgarian waters (Peteva et al., 2018, 2020b) supports the first hypothesis, although uncertainty regarding *Dinophysis* phycotoxins in the western Black Sea still remains, especially considering the more diverse toxin composition in Russian waters (Morton et al., 2009). In this regard, the establishment of cultures is crucial to fill the gap in knowledge about the phycotoxin profiles of local *Dinophysis* strains.

The major PTX, namely PTX-2, was recorded in both seasons, whereas the other PTX variants showed seasonal variability: PTX-2 sa, PTX-11, and PTX-12 were detected in summer samples, while PTX-1 and PTX-13 were found in spring samples. These differences might reflect variability in toxin profiles and cell toxin quotas among species and strains (reviewed in Reguera et al., 2014; Lassus et al., 2016), as well as between seasons and years (Pizarro et al., 2013). In the current investigation, PTXs were positively correlated with *D. sacculus* and *D. acuminata*, whereas in spring, they were associated only with *D. acuta* cell abundance (Dzhembekova et al., 2022). Despite their distinct ecological preferences, distinguishing phycotoxin production by species remains challenging due to the co-occurrence of multiple *Dinophysis* species (Petrova and Velikova, 2003; Escalera et al., 2006; Lindahl et al., 2007; Ajani et al., 2016; Díaz et al., 2019; Baldrich et al., 2021).

Potential YTX-producers (*P. reticulatum, L. polyedra*, and *Gonyaulax* spp.) were present in the plankton community along the western Black Sea during both sampling campaigns. *Gonyaulax* cf. *spinifera* and *L. polyedra* were more frequently observed in the net tow samples during the spring campaign (Dzhembekova et al., 2022), whereas *P. reticulatum* was consistently present in this study. It is worth noting that with the description of *Pentaplacodinium* (Mertens et al., 2018, 2023; Luo et al., 2020), there is general uncertainty about previous identifications of *P. reticulatum*, as both genera are almost undistinguishable in their light microscopy appearance. However, in this study, together with the data presented in Dursun et al. (2025), the identification of *P. reticulatum* is

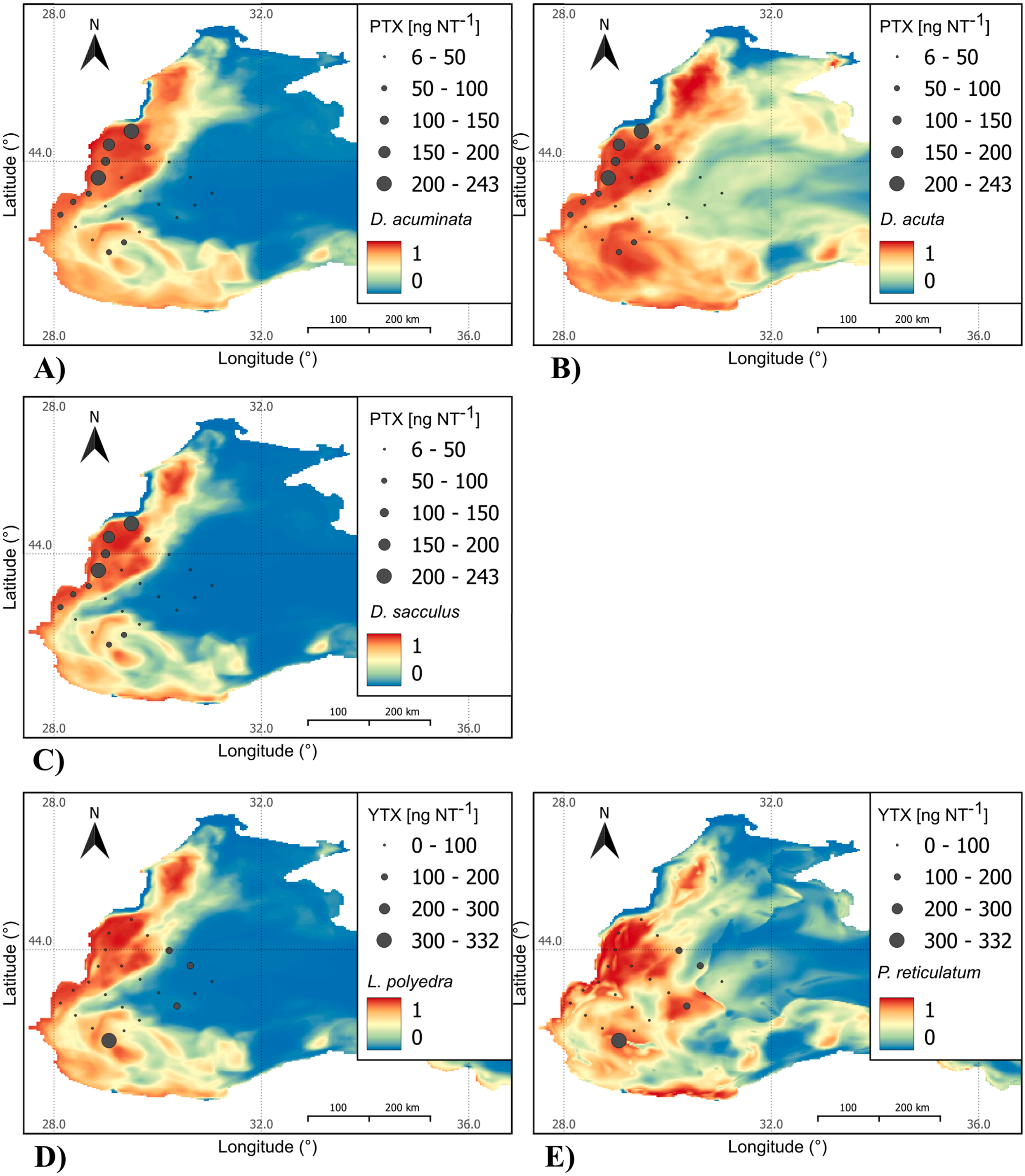


Fig. 8. Species distribution models representing MaxEnt (Maximum entropy) predicted probability of occurrence [0; 1] and the most favorable environment based on the data collected during the summer expedition of 2021 for: A. *D. acuminata*; B. *D. acuta*; C. *D. sacculus*; D. *L. polyedra* and E. *P. reticulatum* in the western Black Sea coastal and shelf waters, overlayed with phycotoxin levels. The probability is represented with a color scheme, with light blue indicating the least likelihood of suitable conditions, light yellow indicating conditions matching those where species were found, and red corresponding to the highest predicted probability of a suitable environment.

unequivocally confirmed by both molecular evidence (metabarcoding data and phylogeny of strains) and morphological analyses (SEM observations of field samples and cultures).

Yessotoxin was detected in both May 2019 and September 2021, whereas the other YTX variants differed among campaigns, except for compound e# 21/22, which was detected during both seasons. Moderate correlations were found between 41-a-homo-YTX levels and the cell abundances of *L. polyedra* and *Gonyaulax* spp. in the net tow samples from this study. In May 2019, YTX was associated with both *L. polyedra* and *P. reticulatum*, whereas YTX variants were linked only to *L. polyedra* (Dzhembekova et al., 2022). Toxin screening of Black Sea strains under culture conditions confirmed YTX production by *P. reticulatum* and YTX-variants production by *L. polyedra*, while no toxins were detected in one strain of *Gonyaulax* sp. (Dursun et al., 2025). Similar inconsistent relationships between the presence of YTXs and their putative producers in the field have also been reported in the Mediterranean Sea (Busch et al., 2016). These findings can be attributed to the highly variable toxin profiles and cell toxin quotas of YTX-producing species and strains (Paz et al., 2008), as demonstrated for the tested Black Sea strains of *P. reticulatum* and *L. polyedra* (Dursun et al., 2025). Similar variations have also been reported in other geographic locations (Peter et al., 2018; Sala-Pérez et al., 2016). In addition, YTX profiles and toxin cell quotas may be influenced by environmental factors (Peter et al., 2018; Röder et al., 2012), as observed during the spring campaign in the Black Sea (Dzhembekova et al., 2022).

Despite the low abundances of YTX-producing species, the low levels of YTXs in the samples, and the relatively low oral toxicity of YTX analogues (Tubaro et al., 2003), both *P. reticulatum* and *L. polyedra*, as well as their associated phycotoxins, should be closely monitored. This is particularly important considering the occurrence of massive *L. polyedra* blooms in the region (Velikova et al., 1999; Moncheva et al., 2001; Terenko and Krakhmalnyi, 2021) and the higher YTX cell quotas reported for the Black Sea *P. reticulatum* strains compared to *L. polyedra* (Dursun et al., 2025). In addition, *L. polyedra* resting cysts are widely distributed and abundant in Black Sea sediments, providing a potential inoculum for bloom initiation (Dzhembekova et al., 2024). Furthermore, model outputs indicate that the western coastal area is suitable for *P. reticulatum* and *L. polyedra*, along with other bloom-forming species (Goncharenko et al., 2021).

In the present study, several *Gonyaulax* species were reported. Given the challenging taxonomy of *Gonyaulax*, and particularly the cryptic diversity described for the *Gonyaulax spinifera* species complex (Huang et al., 2025a, 2025b), the identity of the cells reported here as *Gonyaulax* cf. *spinifera* requires further examination and clarification, as thoroughly discussed in Dursun et al. (2025). Unfortunately, both DNA metabarcoding data and strain-based data failed to resolve species-level identification due to low similarity with available reference sequences. No YTX production was found in one strain each of *Gonyaulax* sp. and *Sourniaea diacantha* (Dursun et al., 2025), but further studies are necessary to better elucidate the diversity of *Gonyaulax* species and their associated phycotoxins in the Black Sea.

Among other potentially toxic dinoflagellates, the bloom-forming *P. cordatum* was also observed. Although no toxigenic Black Sea strains have been described under laboratory conditions (Moncheva, 1991), the frequent bloom events documented in the region (Moncheva et al., 2019) and the high risk of mass outbreaks predicted for certain Black Sea areas (Goncharenko et al., 2021) highlights the importance of monitoring this species for potential harmful effects. However, it is important to note the risk of overestimating *P. cordatum* abundance in fixed samples, considering the number of morphologically similar small *Prorocentrum* species, such as *P. thermophilum, P. pervagatum*, and in particular *P. ponticum*, having a comparable shape and size. For example, while live microscopy and SEM analyses confirmed the presence of *P. ponticum* in the present study, this species was not counted in the fixed samples used for quantitative analysis.

Two OTUs in the DNA samples were attributed to *Karlodinium*. One of them was identified as *K. veneficum*, whereas the other could not be resolved to the species level. Examination of *Karlodinium* strains obtained from the PHYCOB samples revealed significant sequence differences from other known *Karlodinium* species, with no karlotoxin production observed (Dursun et al., 2025). In line with these findings, no KmTx was detected in the field samples. Whether the absence of KmTx is due to strain-specific variability in toxin cell quotas of *K. veneficum* (Bachvaroff et al., 2009), low sensitivity of KmTx detection (Krock et al., 2017), or attributed to misidentification remains unclear, especially in light of the low resolution of the 18S rRNA region within some genera and the limited reference sequence data for different *Karlodinium* species available in GenBank.

While this study provides valuable data on the distribution of potentially toxic plankton species and phycotoxins in the western Black Sea, some limitations must be acknowledged. Sampling was limited to a single cruise conducted over a week in mid-September 2021 and restricted to 23 stations within Bulgarian and Romanian waters. Thus, the results represent a temporal snapshot and may not fully capture seasonal or interannual variability in plankton communities and toxin production across the Black Sea. Furthermore, while plankton net tows are useful for detecting the presence and diversity of potentially toxic species, they may be biased for accurate quantitative analyses due to the mesh size selectivity, clogging, and damage of fragile cells (Sournia, 1978), affecting the actual water volume filtered (Mack et al., 2012). Therefore, net-based observations should be complemented by data from discrete water samples collected using Niskin bottles, as implemented in this study, which provide a more standardized approach for quantitative analyses of plankton (Karlson et al., 2010). In addition, the low abundances recorded in the samples represent a limitation for species distribution modelling, as they may introduce potential bias in occurrence data. The sensitivity and specificity of the molecular assay can also influence detection outcomes. Although DNA metabarcoding is a sensitive and powerful instrument for biodiversity monitoring, the method also has limitations related to the resolution of target regions, the completeness and reliability of reference nucleotide databases, and the standardization of workflow. The problem with limited resolution can be mitigated by incorporating multiple target regions to improve identification efficiency (Sildever et al., 2019; Fu et al., 2021). Additionally, the detection of microalgal species via DNA metabarcoding was significantly limited due to the dominance of metazoan sequences, particularly from zooplankton taxa, in the raw data. Future studies might consider pre-filtration of the plankton net tow samples to reduce DNA overshadowing by abundant non-target organisms.

5. Concluding remarks

Comprehensive knowledge of the occurrence of toxic microalgae and phycotoxins in the Black Sea is essential for assessing ecosystem and public health risks. Although this study covers only a limited period and area, the data help to fill existing information gaps regarding their identity and distribution in the western Black Sea. Numerous potentially toxic species and phycotoxins were observed to co-occur in September 2021 in Bulgarian and Romanian waters, largely overlapping with those reported in May 2019. The most prevalent toxins were PTX-2 and YTXs, with GTX-2/3 and GDA also present. The microalgal species identified as the most probable toxin producers were *P. reticulatum* and *L. polyedra* for YTXs, *D. acuminata* and *D. sacculus* for PTXs, *A. pseudogonyaulax* for GDA, and *A. fragae* for GTX-2/3. The latter species represents the first record for the Black Sea, most likely previously overlooked due to limitations associated with the application of a single method for species identification. This finding underscores the value of integrated, high-resolution analyses of microalgal composition and phycotoxin distribution. A notable contribution to improving the understanding of Black Sea species taxonomy and toxicology was provided by the strain-based component of the study (Dursun et al., 2025). The results further emphasize the importance of experimental work using cultured local isolates for comprehensive morphological, phylogenetic, and toxinological characterization.

To date, no cases of human poisoning have been officially reported in the western Black Sea. Similarly, despite the occurrence of numerous potentially toxic species and phycotoxins in the Mediterranean Sea, only extremely rare cases of intoxication and limited effects on aquaculture have been documented (Zingone et al., 2021). Nevertheless, the detected toxins, including potent neurotoxins (GTXs) along with others (GDA, PTXs, YTXs), may pose risks to ecosystems and public health (reviewed in Landsberg, 2002 and Lassus et al., 2016). The co-occurrence of these compounds further raises the possibility of combined impacts on biota (Alarcan et al., 2018), especially under environmental stress (Krishna et al., 2025). Despite generally low toxin levels in plankton, repeated exposure and bioaccumulation may present cumulative ecological and human health risks, warranting further investigation, including regular monitoring of toxic microalgae and toxins in water, as well as toxin measurement in seafood. As this study provides only a snapshot of potentially toxic microalgae and phycotoxins in the area, a temporal analysis covering a wider spatial range and including more sampling stations is recommended to evaluate seasonal variability within a broader regional or basin-scale context.

CRediT authorship contribution statement

Nina Dzhembekova: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Fuat Dursun:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Urban Tillmann:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Ivelina Zlateva:** Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis, Conceptualization. **Oana Vlas:** Writing – review & editing, Formal analysis. **Nataliya Slabakova:** Writing – review & editing, Formal analysis. **Kristof Möller:** Writing – review & editing, Visualization, Software. **Snejana Moncheva:** Writing – review & editing. **Florian Koch:** Writing – review & editing. **Laura Boicenco:** Writing – review & editing. **Ertuğrul Aslan:** Writing – review & editing. **Sabri Mutlu:** Writing – review & editing. **Ivan Popov:** Writing – review & editing, Visualization. **Satoshi Nagai:** Writing – review & editing, Formal analysis. **Bernd Krock:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Captain Tayfun Denizmen and R/V TÜBİTAK MARMARA crew for their technical support and collaboration throughout the entire cruise. The study was financed by EUROFLEETS+ under SEA-Call “REGIONAL” funded from the EU H2020 Research and Innovation programme (GA 824077) and the BRIDGE-BS Project funded from the EU H2020 Research and Innovation Action (RIA) funding scheme (GA 101000240).

The authors acknowledge support by the Open Access Publication Funds of AWI.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2025.103025.

Data availability

Data will be made available on request.

References

Ajani, P., Larsson, M.E., Rubio, A., Bush, S., Brett, S., Farrell, H., 2016. Modelling bloom formation of the toxic dinoflagellates *Dinophysis acuminata* and *Dinophysis caudata* in a highly modified estuary, south eastern Australia. Estuar. Coast. Shelf. Sci. 183, 95–106. https://doi.org/10.1016/j.ecss.2016.10.020.

Alarcan, J., Biré, R., Le Hégarat, L., Fessard, V., 2018. Mixtures of lipophilic phycotoxins: exposure data and toxicological assessment. Mar. Drugs 16 (2), 46. https://doi.org/10.3390/md16020046.

Allouche, O., Tsoar, A., Kadmon, R., 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). J. Appl. Ecol. 43, 1223–1232. https://doi.org/10.1111/j.1365-2664.2006.01214.x.

Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012a. The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. Harmful. Algae 14, 10–35. https://doi.org/10.1016/j.hal.2011.10.012.

Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., 2012b. Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. Annu. Rev. Mar. Sci. 4, 143–176. https://doi.org/10.1146/annurev-marine-120308-081121.

Aubrey, D., Moncheva, S., Demirov, E., Diaconu, V., Dimitrov, A., 1996. Environmental changes in the western Black Sea related to anthropogenic and natural conditions. J. Mar. Syst. 7, 411–425. https://doi.org/10.1016/0924-7963(95)00031-3.

Bachvaroff, T.R., Adolf, J.E., Place, A.R., 2009. Strain variation in *Karlodinium veneficum* (DINOPHYCEAE): toxin profiles, pigments, and growth characteristics[1]. J. Phycol. 45, 137–153. https://doi.org/10.1111/j.1529-8817.2008.00629.x.

Bakan, G., Büyükgüngör, H., 2000. The Black Sea. Mar. Pollut. Bull. 41, 24–43. https://doi.org/10.1016/S0025-326X(00)00100-4.

Baldrich, Á.M., Pérez-Santos, I., Álvarez, G., Reguera, B., Fernández-Pena, C., Rodríguez-Villegas, C., Araya, M., Álvarez, F., Barrera, F., Karasiewicz, S., Díaz, P.A., 2021. Niche differentiation of *Dinophysis acuta* and *D. acuminata* in a stratified fjord. Harmful. Algae 103, 102010. https://doi.org/10.1016/j.hal.2021.102010.

Bates, S.S., Hubbard, K.A., Lundholm, N., Montresor, M., Leaw, C.P., 2018. *Pseudo-nitzschia, Nitzschia*, and domoic acid: new research since 2011. Harmful. Algae 79, 3–43. https://doi.org/10.1016/j.hal.2018.06.001.

Baumgartner, J., Wilson, P., 2022. rmaxent: tools for working with Maxent in R. R package version 0.8.5.9000.

Becker, R., Minka, T.P., Wilks, A.R., Brownrigg, R., Deckmyn, A., 2023. maps: draw geographical maps.

Besiktepe, S., Ryabushko, L., Ediger, D., Yilmaz, D., Zenginer, A., Ryabushko, V., Lee, R., 2008. Domoic acid production by *Pseudo-nitzschia calliantha* Lundholm, Moestrup et Hasle (bacillariophyta) isolated from the Black Sea. Harmful. Algae 7, 438–442. https://doi.org/10.1016/j.hal.2007.09.004.

Borges, F.O., Lopes, V.M., Amorim, A., Santos, C.F., Costa, P.R., Rosa, R., 2022. Projecting future climate change-mediated impacts in three paralytic shellfish toxins-producing dinoflagellate species. Biology 11 (10), 1424. https://doi.org/10.3390/biology11101424. . (Basel).

Borges, F.O., Lopes, V.M., Santos, C.F., Costa, P.R. and Rosa, R., 2023. Impacts of climate change impacts on the biogeography of three amnesic shellfish toxin producing diatom species. Toxins 15 (1), 9. 10.3390/toxins15010009.

Bradley, A.P., 1997. The use of the area under the ROC curve in the evaluation of machine learning algorithms. Pattern. Recognit. 30, 1145–1159. https://doi.org/10.1016/S0031-3203(96)00142-2.

Branco, S., Oliveira, M.M.M., Salgueiro, F., Vilar, M.C.P., Azevedo, S.M.F.O., Menezes, M., 2020. Morphology and molecular phylogeny of a new PST-producing dinoflagellate species: *Alexandrium fragae* sp. nov. (Gonyaulacales, dinophyceae). Harmful. Algae 95, 101793. https://doi.org/10.1016/j.hal.2020.101793.

Busch, J.A., Andree, K.B., Diogène, J., Fernández-Tejedor, M., Toebe, K., John, U., Krock, B., Tillmann, U., Cembella, A.D., 2016. Toxigenic algae and associated phycotoxins in two coastal embayments in the Ebro Delta (NW Mediterranean). Harmful. Algae 55, 191–201. https://doi.org/10.1016/j.hal.2016.02.012.

D’Agostino, V.C., Krock, B., Degrati, M., Sastre, V., Santinelli, N., Krohn, T., Hoffmeyer, M.S., 2019. Occurrence of toxigenic microalgal species and phycotoxin accumulation in mesozooplankton in Northern Patagonian gulfs. Argentina. Environ. Toxic Chem. 38, 2209–2223. https://doi.org/10.1002/etc.4538.

Díaz, P.A., Reguera, B., Moita, T., Bravo, I., Ruiz-Villarreal, M., Fraga, S., 2019. Mesoscale dynamics and niche segregation of two *Dinophysis* species in Galician-Portuguese coastal waters. Toxins 11, 37. https://doi.org/10.3390/toxins11010037.

Dursun, F., Dzhembekova, N., Krock, B., Tebben, J., Tillmann, U., 2025. Morphological, molecular and toxinological characterization of potentially toxigenic microalgal strains from the western Black Sea. Harmful Algae 151, 103026. https://doi.org/10.1016/j.hal.2025.103026.

Dzhembekova, N., Moncheva, S., 2014. Recent trends of potentially toxic phytoplankton species along the Bulgarian Black Sea area. In: Proceedings of the Twelfth International Conference on Marine Sciences and Technologies, pp. 321–329.

Dzhembekova, N., Moncheva, S., Ivanova, P., Slabakova, N., Nagai, S., 2018. Biodiversity of phytoplankton cyst assemblages in surface sediments of the Black Sea based on metabarcoding. Biotechnol. Biotechnol. Equip. 32, 1507–1513. https://doi.org/10.1080/13102818.2018.1532816.

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 14, 685.. https://doi.org/10.3390/toxins14100685.

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.

Dzhembekova, N., Urusizaki, S., Moncheva, S., Ivanova, P., Nagai, S., 2017. Applicability of massively parallel sequencing on monitoring harmful algae at Varna Bay in the Black Sea. Harmful. Algae. https://doi.org/10.1016/j.hal.2017.07.004.

Dzhembekova, N., Zlateva, I., Rubino, F., Belmonte, M., Doncheva, V., Popov, I., Moncheva, S., 2024. Spatial distribution models and biodiversity of phytoplankton cysts in the Black Sea. NC 55, 269–296. https://doi.org/10.3897/natureconservation.55.121181.

Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E., Yates, C.J., 2011. A statistical explanation of MaxEnt for ecologists: statistical explanation of MaxEnt. Divers. Distrib. 17, 43–57. https://doi.org/10.1111/j.1472-4642.2010.00725.x.

Escalera, L., Reguera, B., Pazos, Y., Moroño, A., Cabanas, J., 2006. Are different species of *Dinophysis* selected by climatological conditions? Afr. J. Mar. Sci. 28, 283–288. https://doi.org/10.2989/18142320609504163.

Fabro, E., Almandoz, G.O., Ferrario, M., John, U., Tillmann, U., Toebe, K., Krock, B., Cembella, A., 2017. Morphological, molecular, and toxin analysis of field populations of *Alexandrium* genus from the Argentine Sea. J. Phycol. 53, 1206–1222. https://doi.org/10.1111/jpy.12574.

Farrell, H., Ajani, P., Murray, S., Baker, P., Webster, G., Brett, S., Zammit, A., 2018. Diarrhetic shellfish toxin monitoring in commercial wild harvest bivalve shellfish in New South Wales, Australia. Toxins 10 (11), 446. https://doi.org/10.3390/toxins10110446.

Fernández, R., Mamán, L., Jaén, D., Fuentes, L.F., Ocaña, M.A., Gordillo, M.M., 2019. *Dinophysis* species and diarrhetic shellfish toxins: 20 years of monitoring program in Andalusia, South of Spain. Toxins 11 (4), 189. https://doi.org/10.3390/toxins11040189. .

Fu, Z., Piumsomboon, A., Punnarak, P., Uttayarnmanee, P., Leaw, C.P., Lim, P.T., Wang, A., Gu, H., 2021. Diversity and distribution of harmful microalgae in the Gulf of Thailand assessed by DNA metabarcoding. Harmful. Algae 106, 102063. https://doi.org/10.1016/j.hal.2021.102063.

Gaonkar, C.C., Campbell, L., 2023. Metabarcoding reveals high genetic diversity of harmful algae in the coastal waters of Texas, Gulf of Mexico. Harmful. Algae 121, 102368. https://doi.org/10.1016/j.hal.2022.102368.

Garnier, S., Ross, N., Rudis, R., Camargo, A.P., Sciaini, M., Scherer, C., 2024. viridis(Lite) - Colorblind-friendly Color Maps for R. viridis package version 0.6.5.

GGI Development Team, 2025. QGIS geographic information system.

Gobler, C.J., 2020. Climate change and harmful algal blooms: insights and perspective. Harmful. Algae 91, 101731. https://doi.org/10.1016/j.hal.2019.101731.

Gobler, C.J., Doherty, O.M., Hattenrath-Lehmann, T.K., Griffith, A.W., Kang, Y., Litaker, R.W., 2017. Ocean warming since 1982 has expanded the niche of toxic algal blooms in the North Atlantic and North Pacific oceans. Proc. Natl. Acad. Sci. U.S.A. 114, 4975–4980. https://doi.org/10.1073/pnas.1619575114.

Goncharenko, I., Krakhmalnyi, M., Velikova, V., Ascencio, E., Krakhmalnyi, A., 2021. Ecological niche modeling of toxic dinoflagellate *Prorocentrum cordatum* in the Black Sea. Ecohydrol. Hydrobiol. 21, 747–759. https://doi.org/10.1016/j.ecohyd.2021.05.002.

González-Gil, S., Pizarro, G., Paz, B., Velo-Suárez, L., Reguera, B., 2011. Considerations on the toxigenic nature and prey sources of *Phalacroma rotundatum*. Aquat. Microb. Ecol. 64, 197–203. https://doi.org/10.3354/ame01523.

Grégoire, M., Vandenbulcke, L., Capet, A., 2020. Black Sea Biogeochemical reanalysis (CMEMS BS-Biogeochemistry): BLKSEA_REANALYSIS_BIO_007_005. 10.25423/CMCC/BLKSEA_REANALYSIS_BIO_007_005_BAMHBI.

Hallegraeff, G., Enevoldsen, H., Zingone, A., 2021a. Global harmful algal bloom status reporting. Harmful. Algae 102, 101992. https://doi.org/10.1016/j.hal.2021.101992.

Hallegraeff, G.M., Anderson, D.M., Belin, C., Bottein, M.-Y.D., Bresnan, E., Chinain, M., Enevoldsen, H., Iwataki, M., Karlson, B., McKenzie, C.H., Sunesen, I., Pitcher, G.C., Provoost, P., Richardson, A., Schweibold, L., Tester, P.A., Trainer, V.L., Yñiguez, A. T., Zingone, A., 2021b. Perceived global increase in algal blooms is attributable to intensified monitoring and emerging bloom impacts. Commun. Earth. Environ. 2, 117. https://doi.org/10.1038/s43247-021-00178-8.

Harrell Jr, F.E., 2024. Hmisc: harrell miscellaneous. (p. 5.2-0)[Dataset] [online].

Hijmans, R.J., 2024a. raster: geographic data analysis and modeling. 10.32614/CRAN.package.raster.

Hijmans, R.J., 2024b terra: spatial data analysis. 10.32614/CRAN.package.terra.

Hijmans, R.J., Phillips, S., Leathwick, J., Elith, J., 2023. dismo: species distribution modeling. 10.32614/CRAN.package.dismo.

Hu, W., Su, S., Mohamed, H.F., Xiao, J., Kang, J., Krock, B., Xie, B., Luo, Z., Chen, B., 2024. Assessing the global distribution and risk of harmful microalgae: a focus on three toxic *Alexandrium* dinoflagellates. Sci. Total Environ. 948, 174767. https://doi.org/10.1016/j.scitotenv.2024.174767.

Huang, S., Mertens, K.N., Derrien, A., David, O., Shin, H.H., Li, Z., Cao, X., Cabrini, M., Klisarova, D., Gu, H., 2025a. Gonyaulax montresoriae sp. nov. (Dinophyceae) from the Adriatic Sea produces predominantly yessotoxin. Harmful. Algae 141, 102761. https://doi.org/10.1016/j.hal.2024.102761.

Huang, S., Mertens, K.N., Nguyen-Ngoc, L., Doan-Nhu, H., Krock, B., Li, Z., Luong, D.Q., Bilien, G., Pospelova, V., Shin, H.H., Plewe, S., Gu, H., 2025b. Cryptic diversity within the *Gonyaulax spinifera* species complex, its relation to the cyst-defined species *spiniferites bentorii, S. mirabilis* and *S. membranaceus*, with the description of *Gonyaulax carbonell-mooreae* sp. nov. (Gonyaulacales, Dinophyceae). Journal of Phycology jpy, 70005. https://doi.org/10.1111/jpy.70005.

Kalinova, G., Mechkarova, P., Marinova, M., 2015. A study of paralytic toxins in cultured mussels from Bulgarian Black Sea. Rakia J. Sci. 13, 303–308.

Karlson, B., Godhe, A., Cusack, C., Bresnan, E., 2010. Introduction to methods for quantitative phytoplankton analysis. In: Karlson, B., Cusack, C., Bresnan, E. (Eds.), Microscopic and Molecular Methods For Quantitative Phytoplankton Analysis. UNESCO.

Krakhmalnyi, A., Terenko, G., Krakhmalnyi, M., Sokolovska, O., 2024. Toxic and harmful dinoflagellata in Odessa Bay (Black Sea). 10.5281/ZENODO.14363976.

Krakhmanyi, O.F., Terenko, G.V., 2019. *Chimonodinium lomnickii* (Wolosz.) Craveiro et al. (Dinoflagellata) - agent of winter water "bloom" in the Odesa Bay (the Black Sea). Hydrobiol. J. 55, 55–62.

Kremp, A., Hansen, P.J., Tillmann, U., Savela, H., Suikkanen, S., Voß, D., Barrera, F., Jakobsen, H.H., 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, 101622. https://doi.org/10.1016/j.hal.2019.101622.

Krishna, S., Lemmen, C., Örey, S., Rehren, J., Pane, J.D., Mathis, M., Püts, M., Hokamp, S., Pradhan, H.K., Hasenbein, M., Scheffran, J., 2025. Interactive effects of multiple stressors in coastal ecosystems. Front. Mar. Sci. 11, 1481734. https://doi.org/10.3389/fmars.2024.1481734.

Krock, B., Busch, J., Tillmann, U., García-Camacho, F., Sánchez-Mirón, A., Gallardo-Rodríguez, J., López-Rosales, L., Andree, K., Fernández-Tejedor, M., Witt, M., Cembella, A., Place, A., 2017. LC-MS/MS detection of karlotoxins reveals new variants in strains of the marine dinoflagellate *Karlodinium veneficum* from the Ebro Delta (NW Mediterranean). Mar. Drugs 15, 391. https://doi.org/10.3390/md15120391.

Krumova-Valcheva, G., Kalinova, G., 2017. *Escherichia coli* and paralytic shellfish poisoning toxins contamination of mussels farmed in Bulgarian Black Sea coast. Acta Microbiol. Bulg. 33, 30–35.

Landsberg, J.H., 2002. The effects of harmful algal blooms on aquatic organisms. Rev. Fisher. Sci. 10, 113–390. https://doi.org/10.1080/20026491051695.

Lassus, P., Chomérat, N., Hess, P., Nézan, E., 2016. Toxic and harmful microalgae of the world ocean: = micro-algues toxiques et nuisibles de l'océan mondial. Denmark, International Society for the Study of Harmful Algae / Intergovernmental Oceanographic Commission of UNESCO. IOC Manuals and Guides, 68. (Bilingual English/French). In: .

Lelong, A., Hégaret, H., Soudant, P., Bates, S.S., 2012. *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. Phycologia 51, 168–216. https://doi.org/10.2216/11-37.1.

Lewis, A.M., Coates, L.N., Turner, A.D., Percy, L., Lewis, J., 2018. A review of the global distribution of *Alexandrium minutum* (Dinophyceae) and comments on ecology and associated paralytic shellfish toxin profiles, with a focus on Northern Europe. J. Phycol. 54, 581–598. https://doi.org/10.1111/jpy.12768.

Lifanchuk, A.V., Mikaelyan, A.S., Sergeeva, A.V., Silkin, V.A., 2023. Seasonal dynamics and ecology of the *Pseudo-nitzschia delicatissima* group in the Black Sea. Reg. Stud. Mar. Sci. 68, 103249. https://doi.org/10.1016/j.rsma.2023.103249.

Lim, P.-T., Ogata, T., 2005. Salinity effect on growth and toxin production of four tropical *Alexandrium* species (Dinophyceae). Toxicon 45, 699–710. https://doi.org/10.1016/j.toxicon.2005.01.007.

Lima, L., Jansen, E., Azevedo, D., Ilicak, M., Causio, S., Sözer, A., Maslo, A., Cretì, S., Lecci, R., Ciliberti, S.A., Peneva, E.L., Federico, I., Barletta, I., Coppini, G., Masina, S., Pinardi, N., Valchev, N., 2023. Black Sea Physical Reanalysis (Copernicus Marine Service BLK-PHY). 10.25423/CMCC/BLKSEA_MULTIYEAR_PHY_007_004.

Lindahl, O., Lundve, B., Johansen, M., 2007. Toxicity of *Dinophysis* spp. in relation to population density and environmental conditions on the Swedish west coast. Harmful. Algae 6, 218–231. https://doi.org/10.1016/j.hal.2006.08.007.

Liu, C., White, M., Newell, G., 2011. Measuring and comparing the accuracy of species distribution models with presence-absence data. Ecography 34, 232–243. https://doi.org/10.1111/j.1600-0587.2010.06354.x.

Liu, C., White, M., Newell, G., 2009. Measuring the accuracy of species distribution models: a review. Presented at. In: Proceedings of the 18th World IMACS /MODSIM Congress. Cairns, Australia.

Liu, M., Zheng, J., Krock, B., Ding, G., MacKenzie, L., Smith, K.F., Gu, H., 2021. Dynamics of the toxic dinoflagellate *Alexandrium pacificum* in the Taiwan Strait and its linkages to surrounding populations. Water 13, 2681. https://doi.org/10.3390/w13192681. .

Lundholm, N., Churro, C., Escalera, L., Fraga, S., Hoppenrath, M., Iwataki, M., Larsen, J., Mertens, K., Moestrup, Ø., Murray, S., Tillmann, U., Zingone, A., 2009 onwards. IOC-UNESCO taxonomic reference list of harmful micro algae. Accessed at https://www.marinespecies.org/hab on 2024-12-17. 10.14284/362.

Luo, Z., Lim, Z.F., Mertens, K.N., Krock, B., Teng, S.T., Tan, T.H., Leaw, C.P., Lim, P.T., Gu, H., 2020. Attributing *Ceratocorys, Pentaplacodinium* and *Protoceratium* to Protoceratiaceae (Dinophyceae), with descriptions of *Ceratocorys malayensis* sp. nov. And *Pentaplacodinium usupianum* sp. nov. Phycologia 59 (1), 6–23. https://doi.org/10.1080/00318884.2019.1663693.

Mack, H.R., Conroy, J.D., Blocksom, K.A., Stein, R.A., Ludsin, S.A., 2012. A comparative analysis of zooplankton field collection and sample enumeration methods. Limnol. Oceanogr. Methods 10 (1), 41–53. https://doi.org/10.4319/lom.2012.10.41.

Marić, D., Ljubešić, Z., Godrijan, J., Viličić, D., Ujević, I., Precali, R., 2011. Blooms of the potentially toxic diatom *Pseudo-nitzschia calliantha* Lundholm, Moestrup & Hasle in coastal waters of the northern Adriatic Sea (Croatia). Estuar. Coast. Shelf. Sci. 92, 323–331. https://doi.org/10.1016/j.ecss.2011.01.002.

Merow, C., Smith, M.J., Silander, J.A., 2013. A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. Ecography 36, 1058–1069. https://doi.org/10.1111/j.1600-0587.2013.07872.x.

Mertens, K.N., Carbonell-Moore, M.C., Pospelova, V., Head, M.J., Highfield, A., Schroeder, D., Gu, H., Andree, K.B., Fernandez, M., Yamaguchi, A., Takano, Y., 2018. *Pentaplacodinium saltonense* gen. Et sp. nov.(Dinophyceae) And its relationship to the cyst-defined genus *Operculodinium* and yessotoxin-producing *Protoceratium reticulatum*. Harmful. Algae 71, 57–77. https://doi.org/10.1016/j.hal.2017.12.003.

Mertens, K.N., Morquecho, L., Carbonell-Moore, C., Meyvisch, P., Gu, H., Bilien, G., Duval, A., Derrien, A., Pospelova, V., Śliwińska, K.K., Garate-Lizarraga, I., 2023. *Pentaplacodinium lapazense* sp. nov. From Central and Southern Gulf of California, a new non-toxic gonyaulacalean resembling *Protoceratium reticulatum*. Mar. Micropaleontol. 178, 102187. https://doi.org/10.1016/j.marmicro.2022.102187.

Miles, C.O., Samdal, I.A., Aasen, J.A.G., Jensen, D.J., Quilliam, M.A., Petersen, D., Briggs, L.M., Wilkins, A.L., Rise, F., Cooney, J.M., Lincoln MacKenzie, A., 2005. Evidence for numerous analogs of yessotoxin in *Protoceratium reticulatum*. Harmful. Algae 4, 1075–1091. https://doi.org/10.1016/j.hal.2005.03.005.

Mohamed, B., Ibrahim, O., Nagy, H., 2022. Sea surface temperature variability and marine heatwaves in the Black Sea. Remote Sens. 14 (10), 2383.. https://doi.org/10.3390/rs14102383.

Moncheva, S., 2010. Guidelines For Quality Control of Biological Data-Phytoplankton. Black Sea Commission, Istanbul, Türkiye.

Moncheva, S., 1991. Ecology of Marine Phytoplankton from the Black Sea in Conditions of Anthropogenic Eutrophication (Doctoral Dissertation). Institute of Oceanology-Bulgarian Academy of Sciences, Varna, Bulgaria.

Moncheva, S., Boicenco, L., Mikaelyan, A.S., Zotov, A., Dereziuk, N., Gvarishvili, C., Slabakova, N., Mavrodieva, R., Vlas, O., Pautova, L.A., Silkin, V.A., Medinets, V., Sahin, F., Feyzioglu, A.M., 2019. Phytoplankton, in: state of the environment of the black sea (2009-2014/5). Publications of. In: the Commission on the Protection of the Black Sea Against Pollution (BSC). Istanbul, Turkey, pp. 225–285.

Moncheva, S., Gotsis-Skretas, O., Pagou, K., Krastev, A., 2001. Phytoplankton blooms in Black Sea and Mediterranean Coastal ecosystems subjected to anthropogenic eutrophication: similarities and differences. Estuar. Coast. Shelf. Sci. 53, 281–295. https://doi.org/10.1006/ecss.2001.0767.

Moncheva, S., Parr, B., 2010. Phytoplankton Manual – Manual For Phytoplankton Sampling and Analysis in the Black Sea. Black Sea Commission, Istanbul, Türkiye.

Moncheva, S., Petrova-Karadjova, V., Palasov, A., 1993. Harmful algal blooms along the Bulgarian Black Sea coast and possible patterns of fish and zoobenthic mortalities. Harmful Marine Algal Blooms. In: Proceedings of the Sixth International Conference on Toxic Marine Phytoplankton. Nantes, France. Lavoisier Publishing, pp. 193–198.

Montresor, M., John, U., Beran, A., Medlin, L.K., 2004. *Alexandrium tamutum* sp. nov. (Dinophyceae): a new nontoxic species in the Genus *Alexandrium* 1. J. Phycol. 40 (2), 398–411. https://10.1111/j.1529-8817.2004.03060.x.

Morton, S.L., Vershinin, A., Leighfield, T., Smith, L., Quilliam, M., 2007. Identification of yessotoxin in mussels from the Caucasian Black Sea Coast of the Russian Federation. Toxicon 50, 581–584. https://doi.org/10.1016/j.toxicon.2007.05.004.

Morton, S.L., Vershinin, A., Smith, L.L., Leighfield, T.A., Pankov, S., Quilliam, M.A., 2009. Seasonality of *Dinophysis* spp. And *Prorocentrum lima* in Black Sea phytoplankton and associated shellfish toxicity. Harmful. Algae 8, 629–636. https://doi.org/10.1016/j.hal.2008.10.011.

Nagai, S., Suzuki, T., Nishikawa, T., Kamiyama, T., 2011. Differences in the production and excretion kinetics of okadaic acid, dinophysistoxin-1, and pectenotoxin-2 between cultures of *Dinophysis acuminata* and *Dinophysis fortii* isolated from Western Japan^1. J. Phycol. 47, 1326–1337. https://doi.org/10.1111/j.1529-8817.2011.01076.x.

Nesterova, D., Moncheva, S., Mikaelyan, A., Vershinin, A., Akatov, V., Boicenco, L., Aktan, Y., Sahin, F., Gvarishvili, T., 2008. Chapter 5. The state of phytoplankton. State of the Environment of the Black Sea (2001–2006/7), in: State of the Environment of the Black Sea (2001–2006/7). Black Sea Commission Publications, Istanbul, Turkey, pp. 133–167. Commission on the Protection of the Black Sea Against Pollution.

Paz, B., Daranas, A.H., Norte, M., Riobó, P., Franco, J.M., Fernández, J.J., 2008. Yessotoxins, a group of marine polyether toxins: an overview. Mar. Drugs 6, 73–102. https://doi.org/10.3390/md6020073.

Pearce, J., Ferrier, S., 2000. Evaluating the predictive performance of habitat models developed using logistic regression. Ecol. Modell. 133, 225–245. https://doi.org/10.1016/S0304-3800(00)00322-7.

Pedersen, T., 2025. Patchwork: The Composer of Plots. R package version 1.3.2.9000. https://patchwork.data-imaginist.com.

Peneva, G., Gogov, Y., Kalinova, G., Slavova, A., 2011. Application of HPLC method for determination of ASP toxins in bivalve mulluscs. In: Proceedings of the Jubilee Scientific Session—110 Years. National Diagnostic Science-and-Research Veterinary Medical Institute, pp. 210–213.

Peter, C., Krock, B., Cembella, A., 2018. Effects of salinity variation on growth and yessotoxin composition in the marine dinoflagellate lingulodinium polyedra from a Skagerrak fjord system (western Sweden). Harmful. Algae 78, 9–17. https://doi.org/10.1016/j.hal.2018.07.001.

Peteva, Z., Kalinova, G., Krock, B., Stancheva, M., Georgieva, S., 2019. Evaluation of paralytic shellfish poisoning toxin profile of mussels from Bulgarian North Black Sea coast by HPLC-FlD with post and pre-column derivatization. Bulg. Chem. Commun. 51, 233–240.

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. IJAES 5, 1–11. https://doi.org/10.14445/23942568/IJAES-V5I4P101.

Peteva, Z., Krock, B., Georgieva, S., Stancheva, M., Max, T., 2023. Food safety status of mussels from Bulgarian coast in regard of marine biotoxins. Bulg. J. Agric. Sci. 29, 536–543.

Peteva, Z., Krock, B., Max, T., Stancheva, M., Georgieva, S., 2020a. Detection of marine biotoxin in plankton net samples from the Bulgarian coast of Black Sea. Bulg. Chem. Commun. 52, 22–27.

Peteva, Z., Krock, B., Stancheva, M., Georgieva, S., 2020b. Comparison of seasonal and spatial phycotoxin profiles of mussels from South Bulgarian coast. Bulg. Chem. Commun. 52, 16–21.

Petrova, D., Velikova, V., 2003. A report of potentially toxic species *Dinophysis* in Bulgarian Black Sea waters. In: Proceedings of the 4th IWA Specialized Conference on Assessment and Control of Hazardous Substances in Water Ecohazard 2003. Presented at the Ecohazard 2003. Aachen, Germany.

Phillips, S.J., Anderson, R.P., Dudík, M., Schapire, R.E., Blair, M.E., 2017. Opening the black box: an open-source release of Maxent. Ecography 40, 887–893. https://doi.org/10.1111/ecog.03049.

Phillips, S.J., Dudík, M., 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31, 161–175. https://doi.org/10.1111/j.0906-7590.2008.5203.x.

Pizarro, G., Moroño, Á., Paz, B., Franco, J., Pazos, Y., Reguera, B., 2013. Evaluation of passive samplers as a monitoring tool for early warning of *Dinophysis* toxins in shellfish. Mar. Drugs 11, 3823–3845. https://doi.org/10.3390/md11103823.

R Core Team, 2021. R: a language and environment for statistical computing.

Reguera, B., Riobó, P., Rodríguez, F., Díaz, P., Pizarro, G., Paz, B., Franco, J., Blanco, J., 2014. *Dinophysis* toxins: causative organisms, distribution and fate in shellfish. Mar. Drugs 12, 394–461. https://doi.org/10.3390/md12010394.

Rial, P., Sixto, M., Vázquez, J.A., Reguera, B., Figueroa, R.I., Riobó, P., Rodríguez, F., 2023. Interaction between temperature and salinity stress on the physiology of *Dinophysis* spp. and *Alexandrium minutum*: implications for niche range and blooming patterns. Aquat. Microb. Ecol. 89, 1–22. https://doi.org/10.3354/ame01994.

Riccardi, M., Guerrini, F., Roncarati, F., Milandri, A., Cangini, M., Pigozzi, S., Riccardi, E., Ceredi, A., Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Forino, M., Tartaglione, L., Pistocchi, R., 2009. *Gonyaulax spinifera* from the Adriatic sea: toxin production and phylogenetic analysis. Harmful. Algae 8, 279–290. https://doi.org/10.1016/j.hal.2008.06.008.

Rodríguez-Gómez, C.F., Vázquez, G., Maya-Lastra, C.A., Aké-Castillo, J.A., Band-Schmidt, C.J., Moreno-Casasola, P., Rojas-Soto, O., 2021. Potential distribution of the dinoflagellate *Peridinium quadridentatum* and its blooms in continental shelves globally: an environmental and geographic approach. Mar. Biol. 168 (3), 29. https://doi.org/10.2112/SI92-004.1.

Röder, K., Hantzsche, F.M., Gebühr, C., Miene, C., Helbig, T., Krock, B., Hoppenrath, M., Luckas, B., Gerdts, G., 2012. Effects of salinity, temperature and nutrients on growth, cellular characteristics and yessotoxin production of *Protoceratium reticulatum*. Harmful. Algae 15, 59–70. https://doi.org/10.1016/j.hal.2011.11.006.

Ryabushko, L.I., 2003. Atlas of Toxic Microalgae of the Black Sea and the Sea of Azov. EKOSI-Gidrofizica, Sevastopol, Ukraine.

Sala-Pérez, M., Alpermann, T.J., Krock, B., Tillmann, U., 2016. Growth and bioactive secondary metabolites of arctic *Protoceratium reticulatum* (Dinophyceae). Harmful. Algae 55, 85–96. https://doi.org/10.1016/j.hal.2016.02.004.

Salas, R., Clarke, D., 2019. Review of DSP toxicity in Ireland: long-term trend impacts, biodiversity and toxin profiles from a monitoring perspective. Toxins 11 (2), 61.. https://doi.org/10.3390/toxins11020061.

Sildever, S., Kawakami, Y., Kanno, N., Kasai, H., Shiomoto, A., Katakura, S., Nagai, S., 2019. Toxic HAB species from the Sea of Okhotsk detected by a metagenetic approach, seasonality and environmental drivers. Harmful. Algae 87, 101631. https://doi.org/10.1016/j.hal.2019.101631.

Sournia, A., 1978. Phytoplankton Manual. UNESCO, Paris.

Tanabe, A.S., Nagai, S., Hida, K., Yasuike, M., Fujiwara, A., Nakamura, Y., Takano, Y., Katakura, S., 2016. Comparative study of the validity of three regions of the 18S-rRNA gene for massively parallel sequencing-based monitoring of the planktonic eukaryote community. Mol. Ecol. Resour. 16, 402–414. https://doi.org/10.1111/1755-0998.12459.

Tanković, M.S., Baričević, A., Gerić, M., Domijan, A.-M., Pfannkuchen, D.M., Kužat, N., Ujević, I., Kuralić, M., Rožman, M., Matković, K., Novak, M., Žegura, B., Pfannkuchen, M., Gajski, G., 2022. Characterisation and toxicological activity of three different *Pseudo-nitzschia* species from the northern Adriatic Sea (Croatia). Environ. Res. 214, 114108. https://doi.org/10.1016/j.envres.2022.114108.

Terenko, G., Krakhmalnyi, A., 2021. Red tide of the *Lingulodinium polyedrum* (Dinophyceae) in Odessa Bay (Black Sea). Turk. J. Fisher. Aquat. Sci. 22. https://doi.org/10.4194/TRJFAS20312.

Terenko, L.M., 2011. The genus *Dinophysis* Ehrenb.(Dinophyta) in the Ukrainian Black Sea coastal waters: species composition, distribution, dynamics. Int. J. Algae 13 (4). https://doi.org/10.1615/InterJAlgae.v13.i4.30.

Tillmann, U., Jaén, D., Fernández, L., Gottschling, M., Witt, M., Blanco, J., Krock, B., 2017. *Amphidoma languida* (Amphidomatacea, Dinophyceae) with a novel azaspiracid toxin profile identified as the cause of molluscan contamination at the Atlantic coast of southern Spain. Harmful. Algae 62, 113–126. https://doi.org/10.1016/j.hal.2016.12.001.

Tillmann, U., Dzhembekova, N., Vlas, O., Krock, B., Boicenco, L., Dursun, F., 2025. Diversity of Amphidomataceae (Dinophyceae) in the Black Sea, including description of *Amphidoma pontica* sp. nov. Phycol. Res. https://doi.org/10.1111/pre.70001.

Tillmann, U., Mitra, A., Flynn, K.J., Larsson, M.E., 2023. Mucus-trap-assisted feeding is a common strategy of the small mixoplanktonic *Prorocentrum pervagatum* and *P. cordatum* (Prorocentrales, Dinophyceae). Microorganisms 11, 1730. https://doi.org/10.3390/microorganisms11071730.

Townhill, B.L., Tinker, J., Jones, M., Pitois, S., Creach, V., Simpson, S.D., Dye, S., Bear, E., Pinnegar, J.K., 2018. Harmful algal blooms and climate change: exploring future distribution changes. ICES J. Mar. Sci. 75 (6), 1882–1993. https://doi.org/10.1093/icesjms/fsy113.

Trainer, V.L., Bates, S.S., Lundholm, N., Thessen, A.E., Cochlan, W.P., Adams, N.G., Trick, C.G., 2012. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful. Algae 14, 271–300. https://doi.org/10.1016/j.hal.2011.10.025.

Tubaro, A., Sosa, S., Carbonatto, M., Altinier, G., Vita, F., Melato, M., Satake, M., Yasumoto, T., 2003. Oral and intraperitoneal acute toxicity studies of yessotoxin and homoyessotoxins in mice. Toxicon 41, 783–792. https://doi.org/10.1016/S0041-0101(03)00032-1.

Türkoğlu, M., Koray, T., 2002. Phytoplankton species' succession and nutrients in the southern Black Sea (Bay of Sinop). Turk. J. Botany 26 (4), 235–252.

Urbanek, S., 2024. rJava: low-level R to Java interface. 10.32614/CRAN.package.rJava.

Velikova, V., Moncheva, S., Petrova, D., 1999. Phytoplankton dynamics and red tides (1987–1997) in the Bulgarian black sea. Water Sci. Technol. 39, 27–36. https://doi.org/10.1016/S0273-1223(99)00184-5.

Vershinin, A., Kamnev, A., 2000. Harmful algae in Russian European coastal waters. Harmful Algal Blooms 2000.. In: Proceedings of the Ninth International Conference on Harmful Algal Blooms, United Nations Educational, Scientific and Cultural organization. Hobart, Australia, pp. 112–115.

Vershinin, A., Morton, S., Leighfield, T., Pankov, S., Smith, L., Quilliam, M., Ramsdell, J., 2006. *Alexandrium* in the Black Sea — Identity, ecology and PSP toxicity. Afr. J. Mar. Sci. 28, 209–213. https://doi.org/10.2989/18142320609504149.

Vershinin, A.O., Moruchkov, A.A., Leighfield, T., Sukanova, I., Kamnev, A.N., Pankov, S., Morton, S.L., Ramsdell, J.S., 2005. Potential toxic algae in northeast Black Sea coastal phytoplankton in 2000-2002. Okeanologiya 45, 240–248.

Vilariño, N., Louzao, M., Abal, P., Cagide, E., Carrera, C., Vieytes, M., Botana, L., 2018. Human poisoning from marine toxins: unknowns for optimal consumer protection. Toxins 10, 324. https://doi.org/10.3390/toxins10080324. .

Wei, T., Simko, V., 2024. R package "corrplot": visualization of a correlation matrix.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. J. Open. Source Softw. 4 (43), 1686. https://doi.org/10.21105/joss.01686.

Wickham, H., Pedersen, T., Seidel, D., 2025. Scales: Scale Functions For Visualization. R package version 1.4.0. https://scales.r-lib.org.

Yasakova, O.N., 2013. The seasonal dynamics of potentially toxic and harmful phytoplankton species in Novorossiysk Bay (Black Sea). Russ. J. Mar. Biol. 39 (2), 107–115. https://doi.org/10.1134/S1063074013020090.

Zaitsev, Y., 2008. Introduction to the Black Sea Ecology. Smil Edition and Publishing Agency ltd.

Zaitsev, Y., Mamaev, V., 1997. Biological Diversity in the Black Sea: A Study of Change and Decline. Black Sea Environmental Series. United Nations Publications, New York.

Zingone, A., Escalera, L., Aligizaki, K., Fernández-Tejedor, M., Ismael, A., Montresor, M., Mozetič, P., Taş, S., Totti, C., 2021. Toxic marine microalgae and noxious blooms in the Mediterranean Sea: a contribution to the Global HAB Status Report. Harmful. Algae 102, 101843. https://doi.org/10.1016/j.hal.2020.101843.