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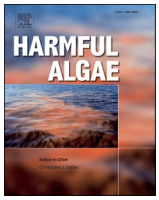

Seasonal dynamics of biotoxins and potentially toxic phytoplankton in three Baltic Sea blue mussel farms

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ABSTRACT

Cyanobacterial blooms are common in the Baltic Sea during summer, and even though several cyanobacteria are toxin producers, many organisms still ingest them as feed. These and other phytoplankton toxins can be detected in blue mussels accumulating over the season, which represents a potential health hazard for shellfish consumers. On a global scale, biotoxins therefore need to be quantified in shellfish before human consumption. We monitored 11 different groups of biotoxins in three blue mussel farms and the composition of 23 potentially toxin producing phytoplankton taxa from March to November 2022. None of the biotoxins were above available health guideline values nor regulated levels. However, the well-known cyanobacterial toxin in the Baltic Sea, nodularin, produced by *Nodularia spumigena*, was detected in net- and rope-farmed mussels throughout the summer, with the highest concentration of 47 μg kg^{-1}. In contrast, the less studied toxin cylindrospermopsin was only present in mussels in early spring and late fall (surface water temperature approx. 2–10 °C), with the maximum concentration of 19.7 μg kg^{-1} in April, where *Aphanizomenon* is a potential producer, but yet not confirmed. Further, *Dinophysis acuminata*, a potential producer of Diarrhetic Shellfish Toxins (DSTs), was observed above warning levels at two sites with up to 2 400 individuals L^{-1}, although the found concentration of 73 μg kg^{-1} is below the regulated level for DST group (160 μg kg^{-1}). Altogether emphasizing that high species abundance does not necessarily result in a high toxin accumulation. On the other hand, mussels can serve effectively as indicator species, detecting the presence of novel toxins when producer abundance is low. As no period of the year was completely toxin-free, quantitative analysis is recommended when mussels are to be harvested.

1. Introduction

Ongoing climate change and anthropogenic perturbation of aquatic environments globally increase the need for sustainable food production. Aquaculture can be an efficient use of space and resources, where mussel farming is especially promising as it also potentially mitigates eutrophication (Kotta et al., 2020). Currently, the only designated production area for farming of blue mussels for human consumption in the Baltic Sea is located in Kiel, despite mussel growth rates being sufficient for production also further north (Karlsson and Reutgard 2019). Establishing sustainable production in the Baltic Proper would reduce excess nutrient supplies and enable several new products and farming activities. However, naturally produced biotoxins, including those from cyanobacteria, accumulate in mussels that later are intended for food, thus need to be closely monitored to ensure consumer health.

Globally, a number of different marine biotoxins and their producers are regularly monitored in edible bivalves, and adjacent to farms, to ensure safe shellfish consumption. Current monitoring of marine biotoxins in the marine North Sea is focusing on the diarrhetic shellfish toxins (DSTs), mainly produced by species of *Dinophysis* and *Prorocentrum*; amnesic shellfish toxins (AST), produced by *Pseudo-nitzschia* species; azaspiracids (AZA), produced by *Azadinium* species; and

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paralytic shellfish toxins (PSTs), which can be produced by *Alexandrium* species (Persson et al., 2020). These species are typically found in marine waters, but occasionally also reach high concentrations in brackish waters such as the Baltic Sea, especially *Dinophysis* spp. (Setälä et al., 2005, 2011). Further, yessotoxins (YTX) are a group of lipophilic polyether compounds that are mainly produced by the dinoflagellates *Gonyaulax spinifera, Protoceratium reticulatum,* and *Lingulodinium polyedra*, and the toxin was detected in Finnish coastal waters in 2006 (Setälä et al., 2011). Warning limits (individuals L^{-1}) of the potential toxin producers are also monitored and act as indicators for toxin production (Persson et al., 2020).

Although marine biotoxins are typically less problematic in estuarine areas, there is an increased risk of fresh- or brackish water cyanobacterial toxins accumulating in mussels and shellfish (Amzil et al., 2023; Camacho-Muñoz et al., 2021). Therefore, in regions such as the Baltic Sea, where the toxin-producing brackish filamentous cyanobacteria bloom annually, these species would need to be added to the list of monitored species (Karlson et al., 2022; Olofsson et al., 2020). Seafood from such locations would therefore require quantitative analysis of cyanobacterial toxins such as nodularin, microcystin, anatoxin, and cylindrospermopsin in addition to the regulated marine biotoxins mentioned above. Cyanotoxins are a chemically diverse group of toxins that enfold a variety of bioactivities and are produced by a number of different cyanobacteria. The hepatotoxin nodularin, for instance, is mainly produced by *Nodularia spumigena* in the Baltic Sea (R et al., 2023; Lopes and Vasconcelos, 2011), whereas the chemically similar toxins microcystins can be produced by e.g., *Dolichospermum, Aphanizomenon, Microcystis*, and *Planktothrix* species (Overlingė et al., 2021; Paerl and Otten, 2013; Thajuddin et al., 2023). Both the microcystins and nodularin may cause serious damage to the human liver if consumed through drinking water or food. Anatoxin, an alkaloid with neurotoxic effects, is produced by the same species groups as microcystins (Otero and Silva, 2022; Sivonen et al., 1989; Thajuddin et al., 2023). Among others, *Dolichospermum, Aphanizomenon*, and *Microcystis* species are also known to potentially produce the cyto- and hepatotoxin cylindrospermopsin (Meriluoto et al., 2016; Preußel et al., 2006, 2009, 2014; Salmaso et al., 2016). Cylindrospermopsin is toxic to the liver and kidney in humans, and potentially also carcinogenic.

Continuous monitoring of cyanobacterial toxins in the Baltic Sea is lacking, but nodularin was recently quantified in several offshore stations (España Amórtegui et al., 2023; Karjalainen et al., 2008). However, coastal accumulation and dense mats of cyanobacteria potentially reach far higher concentrations and might accumulate significantly in filter feeders like mussels. Nodularin has been quantified previously in wild mussels, with up to approx. 82 μg kg^{-1} wet weight in the Southern Baltic Sea (Mazur-Marzec et al., 2007; 2013). Surprisingly, nodularin was recently detected for the first time on the west coast of Sweden (salinity of around 25), with up to 33.1 μg kg^{-1} in mussels (*Mytilus edulis*) (España Amórtegui et al., 2023), potentially due to freshwater intrusion via rivers nursing blooms. However, there is no recommended action limit for nodularin applicable to sea food in Europe, but Australia suggests 51 μg kg^{-1} (wet weight) to be a health guideline limit for human consumption (Testai et al., 2016). Knowledge on cylindrospermopsin in the Baltic Sea is even more scarce, although recently observed in cyanobacterial mats in Finland (Shishido et al., 2023), and it has been quantified in Swedish as well as central European lakes (Dirks et al. 2024; Wilk-Woźniak et al., 2024). Microcystins are similar to nodularins in its structure, but comparatively more common in freshwater systems (Bartram and Chorus, 1999), with future potential of spreading with the freshening of the Baltic Sea (Olofsson et al., 2020). While there are warning levels for species abundance of potentially toxin producing dinoflagellate and diatoms, this is globally lacking for cyanobacteria, although it would be useful for shellfish producers. With climate change there is an increasing need to gain a better understanding of the mix of naturally produced toxins, with accumulation in food potentially harmful to both humans and aquatic organisms. Especially since cyanobacteria are known to like warmer temperatures and therefore may need more attention than the other biotoxins (Paerl and Huisman, 2008).

Conclusively, both dinoflagellate and cyanobacteria derived toxins can be a challenge to mussel farming in the Baltic Sea as they accumulate in filter-feeding bivalves like mussels, especially with the annual cyanobacterial blooms. Therefore, increased knowledge about temporal periods for safe harvest, lag time of toxins in mussels, as well as insight into the quantitative diversity of toxins and their seasonal magnitude, is essential for mussel farming in their future expansion and production of mussels that are intended for human consumption. The aim of this study was to monitor three blue mussel (*Mytilus edulis*) farms along the Swedish south-east coastline during a complete season with high temporal resolution. The study presents seasonal occurrences of 11 different groups of biotoxins with the potential to accumulate in mussels, as well as the abundance of 23 phytoplankton taxa which are potential toxin producers.

2. Methods

2.1. Site description

Three blue mussel (*Mytilus edulis*) farms in the Baltic Sea were monitored biweekly to weekly from March to November 2022. These sites are currently active mussel farms, and represent a gradient along the Baltic Sea coastline, to capture variation in algal blooms and species distribution. Samples were collected to study the composition of potentially toxin-producing phytoplankton in the surrounding water as well as toxins accumulated in the mussels. The farms are located outside Hagby (56°33′56.4″N 16°14′1.8″E), Hasselö outside of Västervik (57°50′44.4″N 16°45′32.5″E), and Dalarö (59°7′36.9″N 18°27′59.9"E), all along the south-east Swedish coastline (Fig. 1). The water depths at the farms are 32–42 m, 20 m, and 6 m at Dalarö, Hasselö and Hagby, respectively, with mussels hanging from 3 m to 10 m at Dalarö and 0.5 m to 5 m at Hasselö and Hagby. The mussels at Hagby and Hasselö were net-farmed and at Dalarö rope-farmed.

2.2. Phytoplankton abundance and composition

Phytoplankton sampling started March 7th in Hagby and Hasselö, and April 4th in Dalarö. It continued until November 21st in Hagby (n = 24), and November 27th in Hasselö (n = 23) and Dalarö (n = 21) (Table S1). Water samples for phytoplankton community composition were collected at 0–10 m at Dalarö and Hasselö, and 0–5 m at Hagby, using integrated tube sampling technique and directly fixed with Lugol's Iodine solution. Samples were analyzed by the Swedish Meteorological and Hydrological institute (SMHI), according to Utermöhl (1958) with additional staining of dinoflagellates using calcofluor on dark filters. The species in focus were pre-selected to comprise known toxin-producing species along the Swedish coastline. The list (Table S2) was compiled based on species within the control program for mussels on the Swedish west coast (Persson et al. 2020) combined with known toxin producers, including potentially toxic cyanobacteria, in the Baltic Sea (Lundholm et al., 2009). The presence of mussel larvae was also estimated from additional net samples.

Additional monitoring data of phytoplankton abundance (individuals L^{-1}) was extracted from the freely available SHARKweb (sharkweb.smhi.se) for four stations as near the mussel farming sites as possible, B1, BY31, and REFM1V1, as well as one more general open sea location, BY5 (see map). These monitoring data were selected to represent the larger area the farms are located in and to identify if our sampling year is a representative year of this region. Cyanobacterial abundance was therefore transformed to average per month per year, station, and genus and thereafter as mean per month over the years 2000–2023. Zeroes were added when the species were not recorded, and included in the mean estimate.

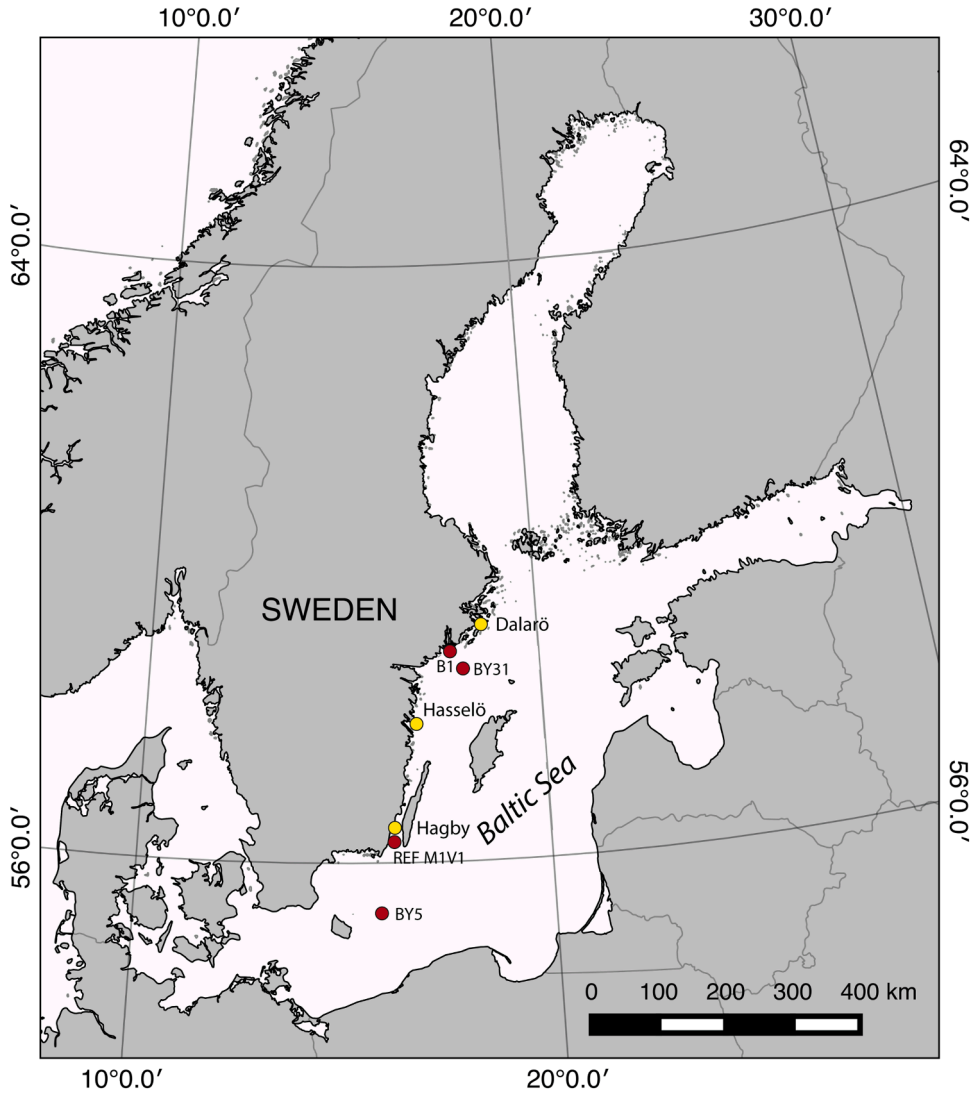

Fig. 1. Map showing the locations of the mussel farms Dalarö, Hasselö, and Hagby as yellow dots, and SMHI monitoring stations B1, BY31, BY5, and Ref M1V1 as red dots, distributed along the Swedish south-east coastline.

2.3. Biotoxins in mussels

Sample collection for analysis of biotoxins accumulated in mussels started March 14th in Hagby, March 21st in Hasselö, and April 4th in Dalarö (Table S1). Sampling for analysis of toxin concentrations in mussels was thereafter performed every second week except during October and November when it was once per month. Sampling continued until November 20th in Hagby ($n = 17$), November 27th in Hasselö ($n = 17$), and November 28th in Dalarö ($n = 17$). Mussels were pooled from the shallow, middle, and deepest parts of each farm, to a total of 500 g each in bags and directly frozen. Mussels were thereafter thawed in aluminum foil covered jars to keep away from light. Mussel meat was removed from the shell, whereafter byssus threads and epiphytes were removed to avoid inclusion of the external algae in the samples. 15 g of mussel meat was sent on ice to Eurofins for analyses of lipophilic toxins and saxitoxins, and 50 g of mussel meat was sent to Wageningen Food Safety Research for analysis of cyanotoxins.

At Eurofins, LC-MS/MS analyses of lipophilic toxins and paralytic shellfish toxins (PSTs) were performed in consistency with the EU-harmonized standard operation procedure (SOP) using acidic chromatography conditions for determination of lipophilic marine biotoxins (European Union Reference Laboratory for Marine Biotoxins, 2015; Villar-González et al., 2011) as well as the EURLMB SOP for the analysis of Paralytic shellfish toxins (PST) from the European Union Reference Laboratory for Marine Biotoxins (2020) for the analysis of PSTs using precolumn-oxidation HPLC-FLD method based on OMA AOAC 2005.06 (European Union Reference Laboratory for Marine Biotoxins, 2020; Gago-Martínez et al., 2005). All samples were analyzed for the presence of 15 lipophilic toxins, 10 of which are regulated lipophilic marine biotoxins, and 13 paralytic shellfish toxins comprised by the SOPs, according to which some toxin isomers are detected in pairs (C1/2, dcGTX2/3, GTX2/3 and GTX1/4). The analysis was performed using certified reference standards that were obtained from the Institute for Marine Biosciences, National Research Council (NRC), Halifax, Nova Scotia, Canada. The two analogues in yessotoxins (YTX) group, 45-OH-YTX and 45-OH-homo-YTX, were analyzed indirectly using YTX and hYTX assuming an equal response factor as there were no certified reference standards commercially available for these two toxins at the time of the study. The limit of quantification (LOQ) in μg kg^{-1} for each of the toxins can be found in Table S3.

Cyanotoxins were analyzed by Wageningen Food Safety Research in The Netherlands. Mussel samples were analyzed for microcystins (MCs: dmMC-RR, MC-RR, MC-YR, dmMC-LR, MC-LR, MC-LY, MC-LA, MC-LW and MC-LF), nodularin (NOD), anatoxins (ATXs: anatoxin-a (ATX) and homoanatoxin-a (hATX)) and cylindrospermopsins (CYNs: CYN, 7-epi-CYN and 7-deoxy-CYN) by LC-MS/MS. One extraction procedure was

applied for analysis of MCs and NOD, and another extraction procedure was used for ATXs and CYNs. For both approaches, 1 g of homogenized mussel tissue was used. For MC/NOD analysis, the tissue was extracted with aqueous methanol (80 methanol: 20 water) at room temperature. After addition of the aqueous methanol, samples were vortexed for 2 min and centrifuged for 5 min. An aliquot of the supernatant was transferred to a filter vial and analyzed by LC-MS/MS. For the analysis of ATX/CYN, samples were extracted twice in 0.1 % formic acid, as the extraction solution. After adding 2 ml of the extraction solution, samples were placed in a water bath at 95 °C for 10 min and centrifuged for 5 min. The supernatant was transferred to a clean tube whereafter the extraction procedure was repeated on the same sample portion using 2 ml of the acidic extraction solution. The supernatants were pooled, the pool was centrifuged from which a portion was transferred and filtered in a filter vial and analyzed with LC-MS/MS.

LC-MS/MS analysis was performed on a Water Xevo TQ(X)S system. MCs and NOD were separated with an Acquity UPLC BEH C18, 1.7 µm 2.1 × 100 mm column, using a 5 min gradient with 0.1 % formic acid in water as eluent A and 0.1 % formic acid in acetonitrile as eluent B. ATXs and CYNs were separated on a Acquity UPLC HSS T3 1.8 µm 2.1 × 100 mm column, using an 8 min gradient using the same mobile phases. Samples within the validated range were quantified against matrix matched calibration curves which were prepared by spiking blank mussel samples with toxin standards before extraction. A first line control was used in each analytical series for quality control.

Both LC-MS/MS methods were validated in-house as quantitative confirmation methods, based on SANTE/12682/2019 (2020) guidelines on analytical quality control, and fulfilled the criteria for accuracy, selectivity, linearity, repeatability and precision. For the analysis of hATX, quantification was only possible with the standard addition method. The validated range (in µg kg^{-1}) for each of the toxins can be found in Table S3.

2.4. Environmental factors

Surface water temperature was recorded each sampling occasion at Hasselö and Dalarö, while not in Hagby. The bottom water temperatures and surface salinities for all farms were acquired from the Copernicus Marine MyOcean Viewer (data.marine.copernicus.eu) for the corresponding sampling dates. The visibility depth at the mussel farms was recorded using a Secchi disc.

2.5. Statistical analyses

We aimed to correlate the occurrence of toxins in mussels with the phytoplankton abundance in the water as well as with the environmental parameters water temperature and Secchi depth. Since the toxin and phytoplankton data contained a large number of zeros and were not normally distributed, we went for the non-parametric Spearman's rank correlation analysis. The data set was reduced to the phytoplankton taxa and toxins which were at least observed once during the study period at any of the three investigated locations. Further, the test was done for each location separately in order to observe location-specific trends in correlation patterns between phytoplankton taxa and toxins. Toxin values at the quantification threshold with unknown concentrations were set to zero.

3. Results

3.1. Phytoplankton species distribution

The abundance of potentially harmful phytoplankton taxa varied greatly between the farms, with generally lower abundance at Hagby as compared to Hasselö and Dalarö (Table 1), except for *Nodularia spumigena, Dolichospermum* spp. and *Dinophysis norvegica.* The phytoplankton abundance generally peaked during summer, with the exception of *Dinophysis acuminata*, whose abundance also increased in October (Figs. 2 and 3). Overall, 12 out of the 23 potentially toxin-producing taxa of phytoplankton were identified at least once in any of the mussel farms (Tables 1 and S2). Species biomass per group could not be directly compared because the number of individuals per liter of cyanobacteria was higher than that of dinoflagellates at all mussel farms (Table 1), while the latter are much larger per cell in terms of volumes.

Table 1
Highest species abundance (individuals L^{-1}) at the three farms, potential toxin produced, and warning levels when available. Species that were observed at least once during the season in at least one farm are shown (all species are listed in Table S2). Missing values indicate that there are no available warning levels for these species or that they were not present at the sampling site.

Species	Toxin	Warning value ind/L	Highest number ind/L Hagby	Highest number ind/L Hasselö	Highest number ind/L Dalarö
Alexandrium ostenfeldii	PST	200*	–	120	40
Alexandrium spp.	PST	200*	17	80	80
Aphanizomenon flos-aquae	Anatoxin, PST	–	19 572	35 454	137 151
Aphanizomenon spp.	CYN, PST, anatoxin	–	34 887	234 476	109 208
Dinophysis acuminata	DST	1500*	748	1 938	2 400
Dinophysis norvegica	DST	4 000*	442	1 040	80
Dinophysis acuta	DST	200*	–	–	120
Dolichospermum spp.	Anatoxin, microcystin	–	101 453	619 916	100 170
Nodularia spumigena	Nodularin	–	7 922	4 480	3 421
Phalacroma rotundatum	DST	1 500*	40	80	170
Prorocentrum cordatum	Unclear	–	–	311	–
Prymnesiales	Unclear	–	7 076	14 150	8 625

* Warning levels in Persson et al. (2020).

At all three sampling locations, *Dinophysis* species were detected during almost the entire season, where *D. acuminata* was especially prevalent with varying concentrations over the investigated months (Fig. 2). *Alexandrium* spp., and *Phalacroma rotundatum* were also observed in all mussel farms. The dinoflagellate *Alexandrium ostenfeldii* was observed at Hasselö and Dalarö, *Prorocentrum cordatum* in Hasselö and *Dinophysis acuta* in Dalarö. The only exceeded warning limits for dinoflagellates occurred in Dalarö during two occasions with above 1500 individuals L^{-1} for *D. acuminata*, on May 30th and July 4th, and in Hasselö in October (Fig. 2). The dinoflagellate *Phalacroma rotundatum* was only found during the summer and autumn months (June-October) with quite low numbers of individuals ($\leq$ 170 L^{-1}) compared to other dinoflagellates, like *D. acuminata* and *D. norvegica*. Individuals of *P. cordatum* were only found in the middle of October in Hasselö. *A. ostenfeldii* was detected in mid July in Dalarö and mid April in Hasselö, whereas *Alexandrium* spp. was found in Hagby during spring season (March-May) and in August, while in Hasselö during summer (June-July) and beginning of October.

The filamentous cyanobacteria *Aphanizomenon flos-aquae, Aphanizomenon* spp., *Dolichospermum* spp., and *N. spumigena* were observed in all mussel farms (Fig. 3) similarly to haptophytes of the genus *Prymnesiales* (Table 1). The prevalence of the four cyanobacteria taxa *A. flos-aquae, Aphanizomenon* spp., *Dolichospermum* spp., and *N. spumigena* varied among the three sample sites and the season (Fig. 3). Overall, the cyanobacteria were mainly found during June-August, however with slight variations between the four species. *A. flos-aquae* first appeared in April and individuals of this species were only detected until August, whereas *Aphanizomenon* spp. was found from the end of June until the

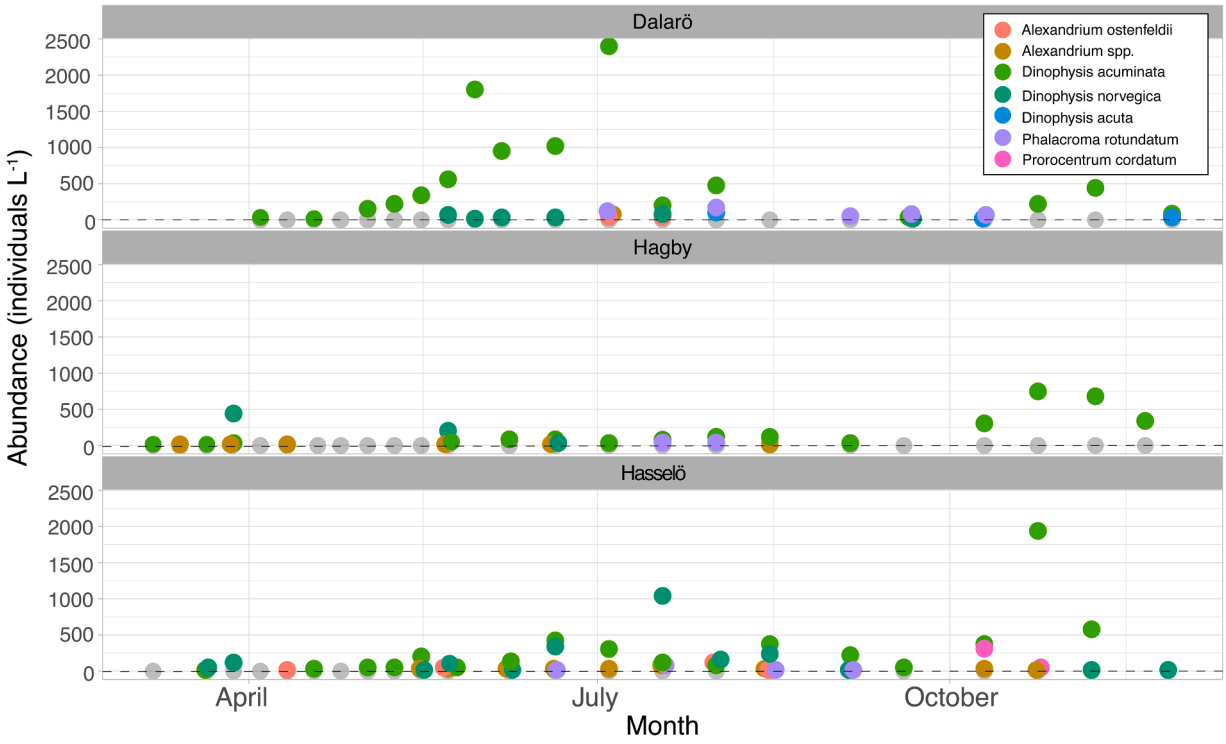

Fig. 2. Dinoflagellate abundance (individuals L^{-1}) at the three farms throughout the season. Gray symbols represent sampling occasions where none of the colored species were observed.

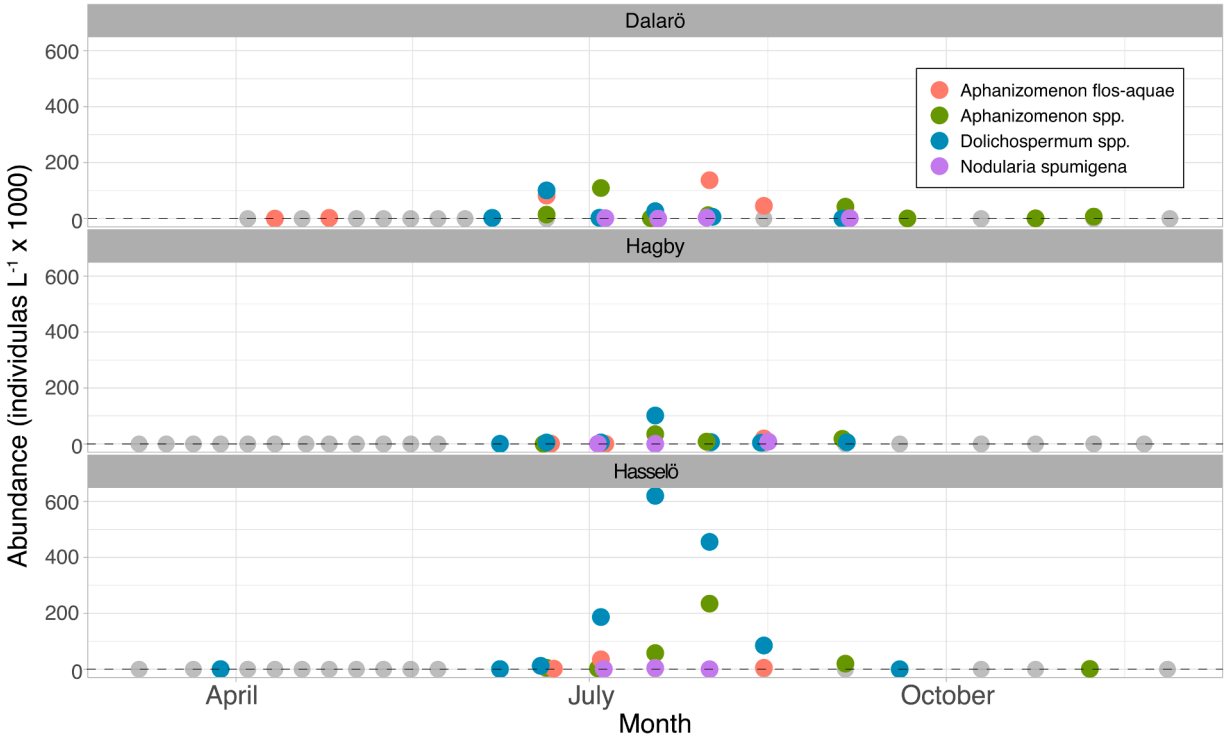

Fig. 3. Cyanobacterial abundance (individuals L^{-1} x 1000) at the three blue mussel farms throughout the season. Gray symbols represent sampling occasions where none of the colored species were observed.

beginning of November. Also, *Dolichospermum* spp. appeared already in April, but only in Hasselö, and was thereafter detected until the end of September. The cyanobacterium *N. spumigena* was only found from July to September at all the locations. To our knowledge there are no health risk guidelines available for cyanobacteria abundances (individuals L^{-1}) associated with mussel farms even on a global scale.

3.2. Biotoxin analyses

One biotoxin belonging to the group of paralytic shellfish toxin

(PST), N-sulfo-carbamoyl Gonyautoxins 2&3 (denoted as C1/2), was detected in one sample in Hagby at a concentration of 40 μg kg^{-1} on August 15th, which is below the regulated limit of 800 μg kg^{-1} (Tables 2 and S5). One toxin belonging to the group of diarrhetic shellfish toxins (DSTs), dinophysistoxin DTX-1, occurred in all mussel farms, although with seasonal differences (Fig. 4). At the farm outside Dalarö, DTX-1 was only present in the first three samples collected in April, but not detectable in samples collected during the rest of the season. In samples from Hasselö, DTX-1 was only detected once, at the end of October, while in Hagby DTX-1 occurred three times, from the end of October until sampling was completed in November. During this study, the regulated limit value of 160 μg kg^{-1} was never reached for DTX-1 (Table 2). The yessotoxin (YTX) was present in mussels from all farms; in Hagby in June and July, and in Hasselö in July (Fig. 4). Outside Dalarö, the toxin was present in the mussels from the beginning of July to the beginning of September, a total of five sampling occasions, but never above the regulated limit for human consumption (Table 2).

For the cyanotoxins, microcystin (only as MC-RR) was detected only in Hasselö, with <5 μg kg^{-1} on April 14 (Tables 2 and S5). Nodularin was the most common cyanotoxin, present in mussels from all farms, peaking in summer (Fig. 5). Nodularin levels were <10 μg kg^{-1} in the mussels from Dalarö when the sampling started in the beginning of April, and were then present in all but two samples during the season. In samples from Hagby and Hasselö, nodularin was detected from June, until the end of October. The maximum concentration of nodularin occurred in July in samples from Hagby which was near the Australian guideline value for mussel consumption of 51 μg kg^{-1} (Testai et al., 2016).

Total concentration of cylindrospermopsin, including dCylindrospermopsin, was only present in the mussel farm near Dalarö (Fig. 5). Further, this toxin group was only present in the mussels at the beginning and end of the sampling season and not during the summer. Total cylindrospermopsin concentration in early April was 19.7 μg kg^{-1} which was above the Australian guideline value of 18 μg kg^{-1} for fish consumption, but below the guideline value for mussels, 39 μg kg^{-1} (Testai et al., 2016).

The presence of other marine biotoxins, including domoic acid (AST), azaspiracids (AZA), anatoxins, pectenotoxins (PTXs) or okadaic acid (OA), could not be confirmed in any samples from the three studied mussel farms (Tables 2 and S5).

Table 2
Regulated limits of marine biotoxins in bivalves aimed for human consumption (provided for those available). Toxins for which there is no regulatory limit are referred to health guideline values in Australia or USA (Testai et al., 2016). [A-C] Highest toxin values detected in blue mussels from the three studied mussel farms in Hagby, Hasselö and Dalarö, respectively. For cyanobacteria toxins microcystin and nodularin, and cylindrospermopsin variants, respectively, the sum of toxins should be below the health guideline values. All values are in μg kg^{-1}.

Toxin	Limit value	Highest value Hagby[A]	Highest value Hasselö[B]	Highest value Dalarö[C]
AST	20 000[+]	–	–	–
PST	800[+]	40	–	–
DST	160[+]	73	30	49
AZA	160[+]	–	–	–
YTX	3 750[+]	170	190	280
Microcystin	51[*]	–	<5	–
Cylindrospermopsin	39[*]	–	–	8.7
dCylindrospermopsin	39[*]	–	–	11
Anatoxin (for fish)	5 000[#]	–	–	–
Nodularin	51[*]	47	26	22

[+] Regulated values for concentrations in mussels (Persson et al., 2020).
[*] Health guideline values for mussels in Australia (Testai et al., 2016).
[#] Health guideline values for fish (when not available for mussels), in the USA (Testai et al., 2016).

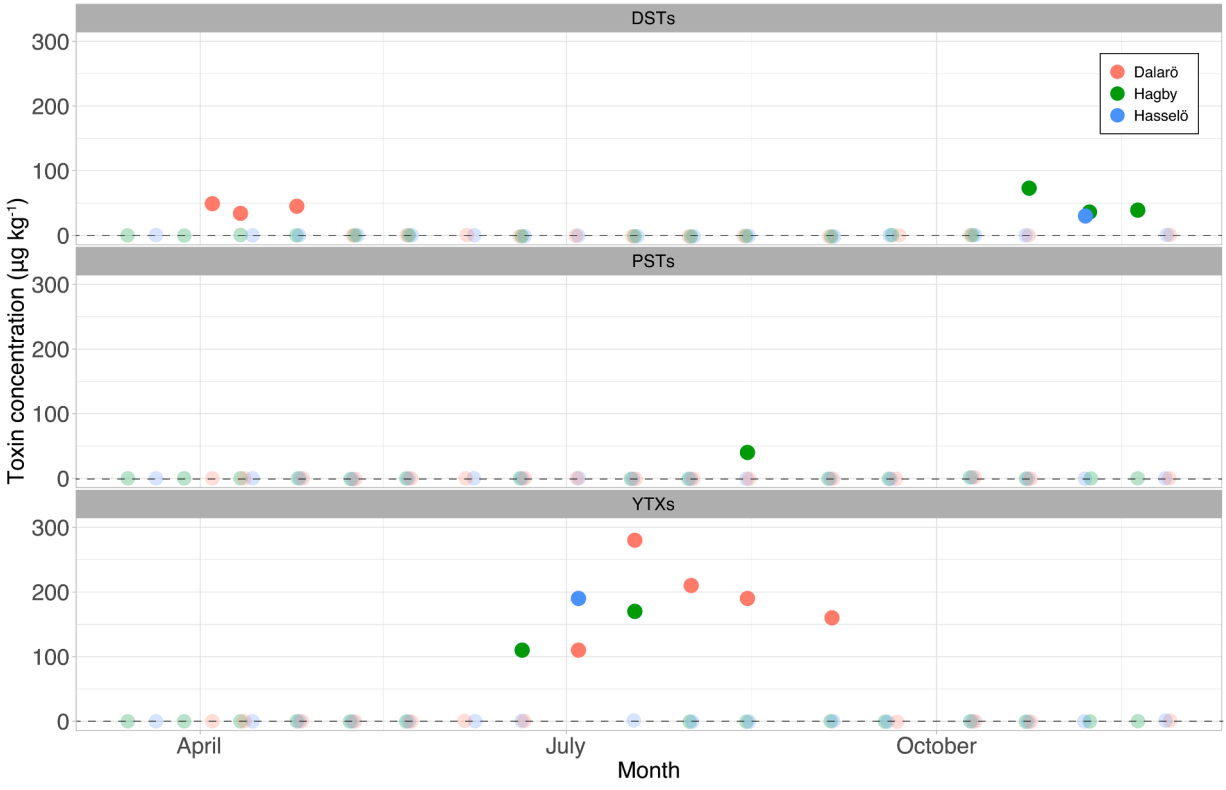

Fig. 4. Concentration of diarrhetic shellfish toxins (DSTs), paralytic shellfish toxins (PSTs) and yessotoxins (YTXs) in mussels (μg kg^{-1}) harvested from the three farms across the season. Regulated limits for food consumption for DSTs is 160 μg kg^{-1}, for PSTs 800 μg kg^{-1}, and for YTXs 3 750 μg kg^{-1}. Dashed lines mark zero values and semi-transparent dots when sampling was conducted but no toxins were detected.

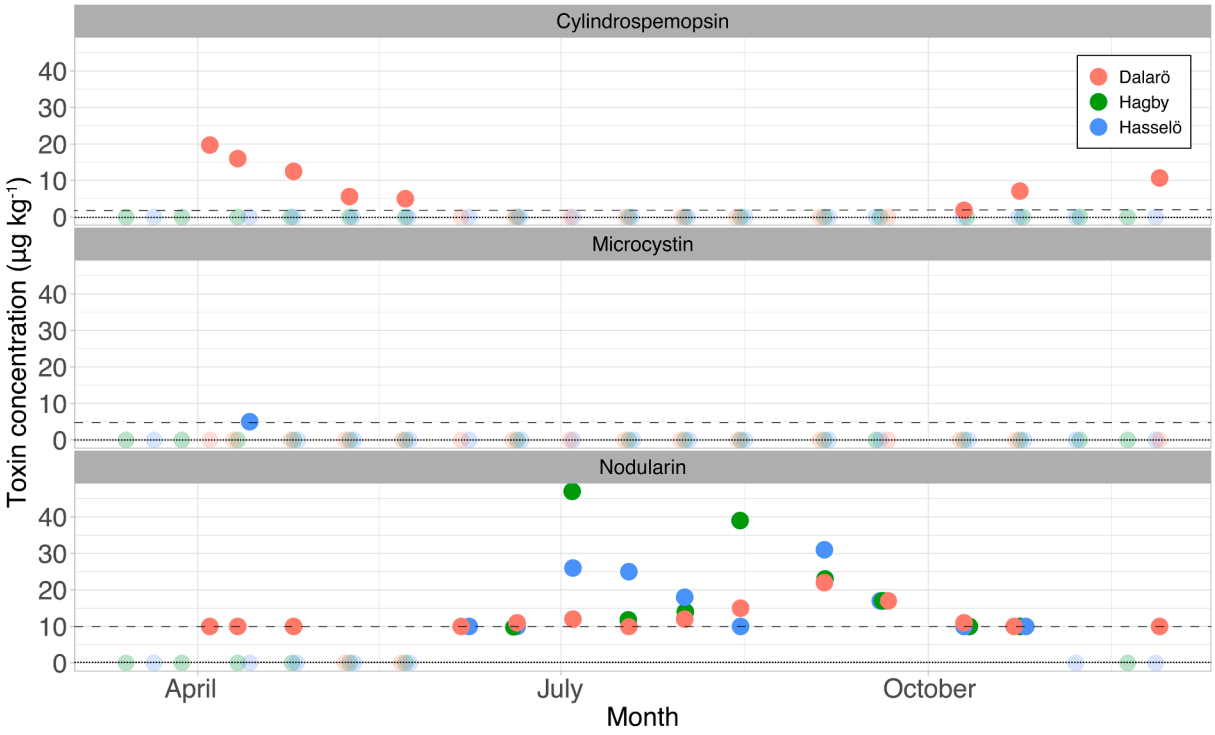

Fig. 5. Cyanobacterial toxins, as the total of each group, at the three studied blue mussel farms. Pale color dots indicate the concentrations that were below the limit of quantification (LOQ) for the method; the LOQs for cylindrospermopsin, nodularin and microcystins are 2.5 µg kg-1, 10 µg kg-1 and 5 µg kg^{-1}, respectively (Table S3). Recommended limits for food consumption are presented in Table 2.

3.3. Environmental variables and mussel larvae

Water temperature at Dalarö reached 18 °C in the surface and 17 °C in the bottom as the highest, Hasselö 20 °C and 16 °C, respectively, and the highest bottom water temperature in Hagby was 20 °C (Supplementary Fig. 1). The temperature when first detecting DTX-1 and cylindrospermopsin in spring was only approx. 2 °C in Dalarö, and in fall when DTX-1 was observed in Hagby and Hasselö around 10 °C. Salinity in Dalarö was 6.1, Hasselö 7.2, and Hagby 7.4 in April 2022 (Copernicus OceanViewer).

The visibility depth in Hagby varied over the sampling season, with around 6 m from the beginning of May to the beginning of September and with generally lower values before and after (Table S6). In contrast, the visibility at Dalarö was around 8–9 m until mid-May when it slowly dropped to reach the lowest visibility depth of 4 m in mid-July before increasing again. Similarly, outside Hasselö, visibility was above 8 m until the end of June and reached its lowest of 4 m in the beginning of August and then started increasing again. Suggested visibility thresholds by HELCOM for the Northern Baltic Proper is 7.1 and Western Gotland Basin 8.4 for good ecological status (HELCOM, 2023). However, these limits are exceeded (lower visibility) in most basins except towards the North Sea.

In Hagby and Dalarö farms, mussel larvae were observed from the beginning of May until the beginning of August (Table S7). In the farm outside Hasselö, mussel larvae were found for the first time on April 4 and occurred at every sampling occasion until August 1. As the method was semi-quantitative based on net sampling, we could not include it in any statistical evaluation.

3.4. Correlations between phytoplankton taxa, toxins and environmental parameters

The Spearman's correlation analysis (Fig. 6) showed slightly different patterns among the investigated sites. In Hagby, four toxins, C1/2, DTX-1, YTX, and nodularin were detected at least once during the study period, thus included in the site-analysis. The correlation matrix for this site revealed a strong positive correlation between the toxin C1/2 and *A. flos-aquae* as well as *N. spumigena*, and somewhat lower with Prymnesiales followed by the dinoflagellate *Alexandrium* spp. and the cyanobacterium *Dolichospermum* spp. (Fig. 6A). DTX-1 only positively correlated with *D. acuminata*. The toxin YTX was positively correlated with *Phalacroma rotundatum* and *Aphanizomenon flos-aquae*, and to a stronger extent with *Aphanizomenon* spp., and *Dolichospermum* spp. The cyanotoxin nodularin was mainly positively correlated to *A. flos-aquae* and *N. spumigena*. Moreover, both YTX and nodularin, revealed a positive correlation with temperature and Secchi depth. On the other hand, the dinoflagellate *Alexandrium* spp. was negatively correlated with both temperature and Secchi depth, and *D. norvegica* partly as well.

In Dalarö (Fig. 6B), five toxins were detected at least once and included in the correlation matrix. Here, similar patterns between DTX-1 and both cylindrospermopsins (7-epi-Cylindrospermopsin and 7-deoxy-Cylindrospermopsin) and phytoplankton taxa were observed where all were negatively correlated with most of the investigated taxa, but positively to Secchi depth. The toxin nodularin was positively related to all investigated cyanobacteria species, as well as to *Phalacroma rotundatum*. The toxin group YTXs was positively correlated with all phytoplankton taxa but strongest to *A. ostenfeldii* and *Dolichospermum* spp. While the two CYN toxins and DTX-1 were negatively correlated to temperature and positively to Secchi depth, YTX and nodularin showed an opposite pattern. All the phytoplankton taxa showed negative correlation with Secchi depth while at least weakly positive with temperature.

In Hasselö (Fig. 6C), only three toxins were found at least once and included in the correlation matrix. Here, the toxin DTX-1 was only positively correlated with *D. acuminata* and Secchi depth. YTX was strongly positively correlated with *A. flos-aquae* and weakly with the other cyanobacteria, and negatively with Secchi depth. Nodularin had a positive correlation with all cyanobacteria, strongest with

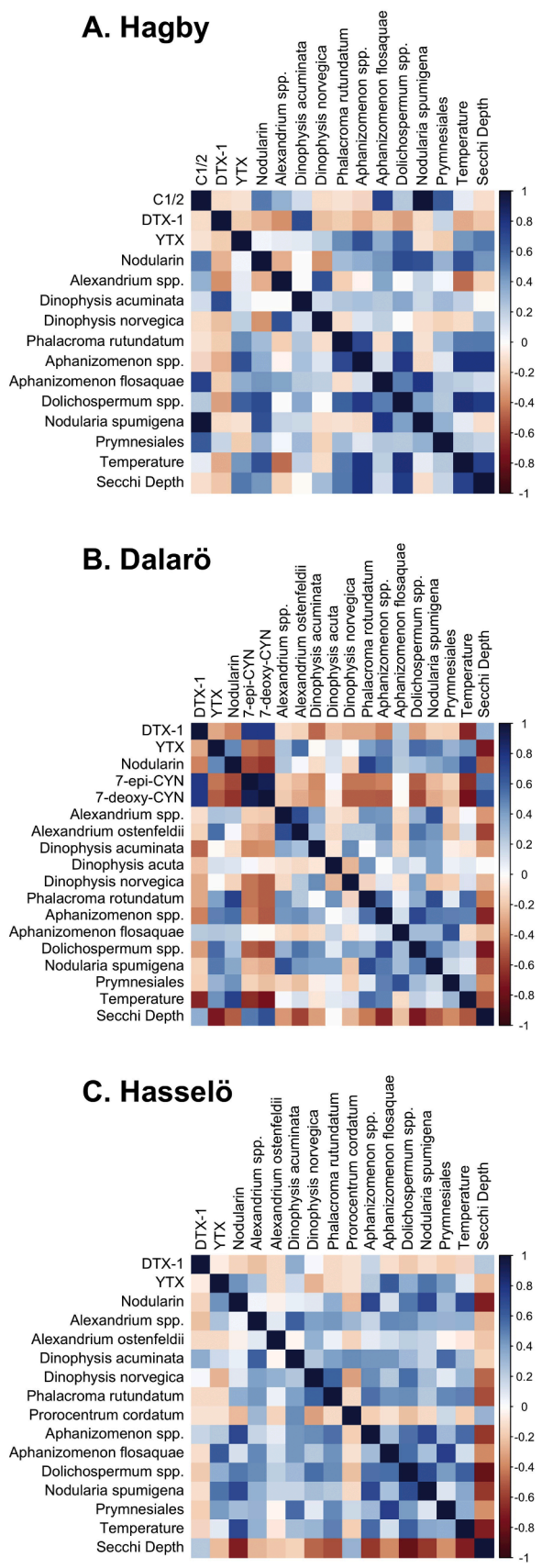

Fig. 6. Correlation matrix between taxa, toxins and environmental parameters based on Spearman's Rank Correlation Coefficients calculated separately for the three locations A. Hagby, B. Dalarö, and C. Västervik.

Aphanizomenon spp. and *N. spumigena*, as well as temperature but was negatively correlated to Secchi depth. Overall, Secchi depth was negatively and temperature positively correlated with all species, except *Prorocentrum cordatum*.

3.5. Comparison to 20 years of monitoring data

Herein, *Aphanizomenon* had the highest abundance in Hasselö with about 270 000 individuals L^{-1}, *Dolichospermum* in Hasselö with 620 000 individuals L^{-1}, and *Nodularia* with 7 922 individuals L^{-1} in Hagby (Table 1). The seasonal average peaks for *Aphanizomenon* over the last 20 years reached about 113 000 as the highest in BY31 and around 50 000 to 70 000 individuals L^{-1} in the other monitoring stations (Fig. 7). For *Dolichospermum* the average was not as high, with about 10 000 - 20 000 individuals L^{-1} in three monitoring stations and up to 50 000 in RefM1V1, which is lower than the values obtained in the current study (Table 1). For *Nodularia* the numbers in 2022 were similar to average for B1 and BY31 but lower than BY5 average peak and highest values similar to Ref M1V1. This means that 2022 was not an extreme year in any sense but a bit lower than it can be for *Nodularia* and slightly higher than average for *Aphanizomenon*. The higher abundances of *Dolichospermum* at Ref M1V1 and of *Nodularia* at BY5 indicates the risk of toxin accumulation in farms further south along the coast, which was also the pattern observed in toxin quantified in the mussels from 2022 (Fig. 5).

4. Discussion

To ensure safe mussel consumption, a number of different biotoxins are globally monitored, including those produced by dinoflagellates, diatoms, and cyanobacteria. In this study, biotoxin content was monitored in mussels farmed along the western coastline of the Baltic Proper in combination with phytoplankton abundances over an entire growth season. We focused on 23 potentially harmful phytoplankton taxa (Table S2), of which 8 genera were encountered at least once in any of the farms (Table 1). Among the detected toxins in this study, representatives from at least one of the groups DSTs, PSTs, yessotoxin, nodularin, microcystin, and cylindrospermopsin were present in any given mussel sample in at least one of the farms on each sampling occasion. Toxin levels were however always below regulatory or recommended health guideline values. Given that toxins were detected throughout the season and can vary significantly both temporally and spatially, it is recommended to conduct toxin analyses at the time of harvest.

The annually occurring dense blooms of filamentous cyanobacteria in the Baltic Sea are often toxin producing (Karlson et al., 2022; Olofsson et al., 2020), which may cause concern for mussel farmers in the region. Filter feeders like mussels ingest phytoplankton through their filtration activity, and are thus susceptible to toxin bioaccumulation and might act as vectors through the food chain (Morais et al., 2008). Therefore, food from freshwater and estuarine environments would need testing for cyanobacterial toxins in addition to those toxins already regulated (Table 2). Here, nodularin, microcystin, anatoxins, and cylindrospermopsins are the most prominent cyanotoxins, and similarly to other biotoxins, they are of concern because of their negative impact on animal and human health (Karjalainen et al., 2007; Thajuddin et al., 2023). Among the monitored cyanotoxins in this study, anatoxin was the only toxin not detected. Despite their well-known risk, there are no suggested health guideline values developed for cyanotoxins for food in Europe, including Sweden. The limit values that exist are for drinking water, with 1 μg L^{-1} for microcystins (Livsmedelsverket, 2018), and in some countries this value also applies for nodularins, anatoxins, and cylindrospermopsins (Testai et al., 2016). As limit values for drinking water are set at levels that are not harmful at 2 liters daily consumption over a lifetime, these cannot be directly translated to foods that are consumed to a lesser extent. In other countries where cyanobacterial toxins occur in food, different limit values have been developed (Chorus and Welker, 2021; Testai et al., 2016) with cylindrospermopsins of

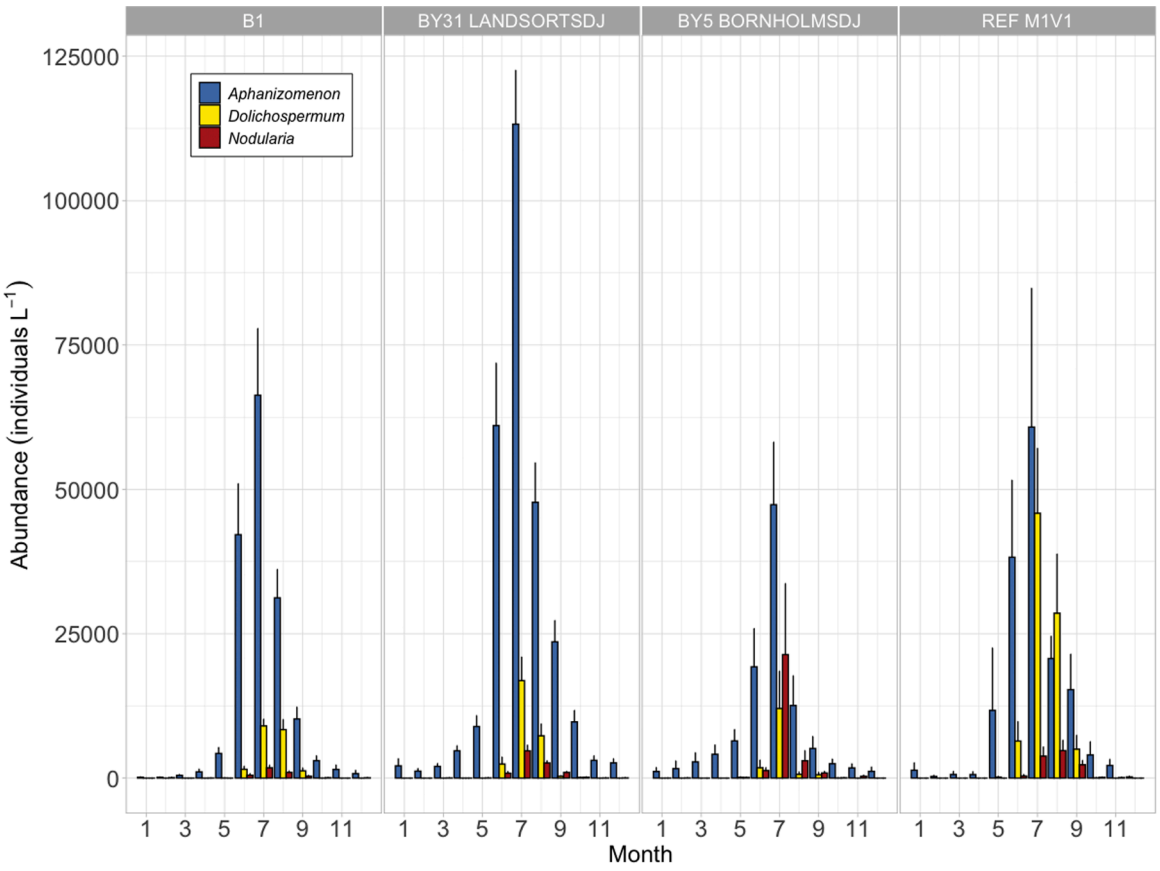

Fig. 7. Cyanobacterial abundance (individuals per L) as average per genera per month for 2000–2023 in four monitoring stations (shown on the map in Fig. 1). Data was downloaded from sharkweb.se, hosted by SMHI.

18–70 μg kg^{-1}, microcystins and nodularins of 51 μg kg^{-1}, and anatoxins of 5 000 μg kg^{-1}.

Among the cyanotoxins, the hepatotoxin nodularin was by far the most prominent detected one in this study: found in mussels in all farms during almost the entire season, showing a clear peak during the summer months. In the Baltic Sea, nodularin is mainly produced by *N. spumigena* (Mazur-Marzec et al., 2013), a species that was also investigated in our study and found to be present during July and August in the three sites. Previous studies also showed accumulation in mussels, with concentrations up to approx. 86 μg kg^{-1} (Mazur-Marzec et al. 2013). As expected, the correlation matrix confirms a positive correlation between nodularin and its well-known producer *N. spumigena* in all three farms, however often together with other cyanobacteria (Fig. 6). The fact that nodularin can still be detected without the presence of *N. spumigena,* especially in April which is long before its growth season, makes it challenging to understand the link between species abundance and toxin concentration. However, cyanotoxins are known to be sustained in the mussels for a long time. A study by Camacho-Muñoz et al. (2021) shows that after three days of exposure to nodularin and microcystin, a depuration period of 27 days was not enough to completely remove accumulated toxins from mussel tissues. One reason may be due to slow release of covalently bound compared to free microcystins (Camacho-Muñoz et al., 2021; Pham et al., 2017; Williams et al., 1997), but also temperature can have an effect, where higher temperature has been demonstrated to result in faster elimination rates of PST (Tang et al., 2021), thus sustaining toxins longer during winter periods as compared to during summer.

In addition, cyanobacterial toxins can be an issue where freshwater and marine waters meet (Amzil et al., 2023; Camacho-Muñoz et al., 2021), which may be the case for the nodularin recently detected in mussels on the west coast of Sweden, with up to 33.1 μg kg^{-1} mussels (*Mytilus edulis*) in August 2020 and 10.9 μg kg^{-1} in July 2021 (España Amórtegui et al., 2023). This observation is not expected due to the higher salinity of about 25. Here adjacent freshwater systems might have nursed the blooms and then filaments were transported to the mussels with water movement. This highlights the need for cyanotoxin determination in all mussels exposed to or adjacent to brackish environments.

Another hepatotoxin with a similar chemical structure to nodularin, is microcystin, which was only found in mussels on one occasion in April outside Hasselö when none of the monitored cyanobacterial species known to produce microcystin were identified in the study. Again, these observations indicate that toxin production does not necessarily correlate with species abundance. Further, microcystin is more known to be found in freshwater environments, so farms located near freshwater runoff might be at higher risk for this toxin accumulating in bivalves.

Surprisingly, the cyanobacterial toxin cylindrospermopsin was observed at its highest concentrations already in April (19.7 μg kg^{-1}), which exceeds the above recommended concentrations for fish of 18 μg kg^{-1} but not mussels of 39 μg kg^{-1} in Australia (Testai et al., 2016). The toxin has only been detected a few times in the Baltic Sea before; in microbial mats in Finland (Shishido et al., 2023) and in the *Kamptonema* sp. strain PCC 7926 (Mazmouz et al., 2010) isolated from the surface water bloom in the southern harbor of Helsinki July 1978 (Vaara et al., 1979). The toxin has been reported to also be produced by *Dolichospermum* (Salmaso et al., 2016) and *Aphanizomenon* (Preußel et al., 2006, 2009, 2014), but from other regions. If *Aphanizomenon* produced the toxin accumulated in the sampled mussels, it would be the first time to detect this in the Baltic Sea, but this would need to be validated by isolating the taxa and determining its toxin production.

Cylindrospermopsin was only observed in the Dalarö mussels in early spring and increasing during autumn, when water temperatures

decreased to 2 °C. *Aphanizomenon* species which were present over almost the entire season, is a possible producer (Bácsi et al., 2006; Banker et al., 1997). However, due to the negative correlation between cylindrospermopsins and the investigated taxa (Fig. 6), it is difficult to identify the producer of the toxins and indicates a time lag between the abundance of potential toxin producers and detectable toxin concentrations in mussels. During spring, *A. flos-aquae* was found in the water at the same time as the toxin was detected in the mussels, but as this taxon was also found in even higher numbers in the summer, the correlation analysis did not capture their co-occurrence. In autumn, it is less clear, as the cyanobacteria have stopped appearing in the water mass when the toxin was found in the mussels, but the species had been present for a number of weeks before. *A. flos-aquae* has been shown to produce cylindrospermopsin in two German lakes (Preußel et al., 2006, 2009, 2014), concentrations related to nitrogen availability, temperature, and light conditions (Preußel et al., 2009). However, sampling at Dalarö did not start until April, so it is not possible to say whether there was cylindrospermopsin in the mussels already during March. Its occurrence can further be linked to slower metabolism in the mussels when the temperature is low which results in an accumulation of cylindrospermopsin as was observed for PST by Tang et al. (2021). In a combination it can also be that *A. flos-aquae* mainly produces toxins when temperature is low, as in Preußel et al. (2006, 2009, 2014).

Other well-known producers of toxins are the diverse group of dinoflagellates. Prominent dinoflagellates found in the Baltic Sea belong to the genus *Alexandrium* and *Dinophysis* (Hakanen et al., 2012; Setälä et al., 2005), where both showed occasional elevated abundances during the investigated period. Dinoflagellates are known for their potential to produce variable toxins (Verma et al., 2019; Visciano et al., 2016). The analyses were conducted to detect the presence of toxins from ASTs, PSTs, DSTs, AZAs, and YTXs groups in the blue mussels, where the only toxin from the PST group, specifically C1/2, as well as from the DST group, specifically DTX-1, and YTX were detected. DSTs can lead to diarrhetic shellfish poisoning and are produced mainly by *Dinophysis* species. According to our correlation analysis, DTX-1 was indeed positively correlated to *D. acuminata* in Hagby and Hasselö, which indicates location-specific traits in taxa-toxin relationships. In Dalarö, DTX-1 was only detected at the beginning of the season when there was low abundance of *D. acuminata* in the water, but as they were observed in much higher abundances during summer, the early spring co-occurrence was not captured in the correlation analysis. With warmer temperatures during spring compared to winter months, the metabolic activity of the mussels might increase, leading to faster metabolization of the toxins; a detoxification process enhanced by temperature previously observed in *Mytilus edulis* (Tang et al., 2021). Accordingly, lower temperatures slow down toxin depuration whereas warmer temperatures promote it. This process could explain the sudden disappearance of the DTX-1 after April outside Dalarö. However, it needs to be considered that besides temperature, also salinity, the species, and the affected mussel tissue considerably influence the detoxification rate. Later in the season, when *Dinophysis* became more common and the warning value of 1 500 individuals L^{-1} was also exceeded outside Dalarö, no DTX-1 could be detected in the mussels. Thus, our results highlight that a higher species abundance does not necessarily correlate with the increased toxin production. Conversely, in Hagby and Hasselö, DTX-1 was present in the mussels when *Dinophysis*, particularly *D. acuminata* had recently been abundant in the water. In addition, at Hasselö, *Prorocentrum cordatum* was present in two samples in October and DTX-1 in the mussels was detected in early November.

Paralytic shellfish toxins (PSTs) refer to a group of toxins which lead to paralytic shellfish poisoning after consumption (Livsmedelsverket, 2022). Saxitoxins are the most prominent and toxic representatives of PSTs and are well-known for the bioaccumulation in shellfish (Thangaraj et al., 2023). They can be produced by marine dinoflagellates like *Alexandrium* species which are mainly found in fully marine waters, but occasionally also reach high concentrations in brackish waters such as the Baltic Sea (Kremp et al., 2019; Sopanen et al., 2011). PST (as C1/2) was only found on August 15 in Hagby even though *Alexandrium* species were detected a few more times over the season and also in Dalarö and Hasselö where PST could not be found. This indicates that the toxin production does not necessarily correlate to the abundance of the species and suggests a strict regulation of the PST biosynthesis by *Alexandrium* species which might be coupled to a number of environmental factors as well as grazing pressure (Selander et al., 2012). Also species of the cyanobacteria *Aphanizomenon* and *Dolichospermum* which were present over July and August in Hagby could be responsible for saxitoxins in the mussels, as this has been observed in freshwaters (Thangaraj et al., 2023). This hypothesis can be underlined by the fact that C1/2 was strongly positively correlated to *A. flos-aquae* and weakly also to *Alexandrium* spp. (Fig. 6). In addition, the levels of *Dolichospermum* build up the weeks before observed in the mussels, with a peak of >100 000 individuals L^{-1}, potentially indicating a delayed accumulation if the cyanobacteria was the producer.

The polyether phycotoxin yessotoxin (Barbosa et al., 2024) was found in mussels from all three farms, but exclusively detected during July and August when water temperatures reached up to 17–18 °C. Previous investigations on this lipophilic toxin emphasize its potential for bioaccumulation in seafood, and YTX contaminated shellfish has been reported worldwide (Tubaro et al., 2010), but here concentrations never exceeded regulated levels of 3 750 μg kg^{-1} in mussels (Persson et al., 2020). To date, no known poisonings in humans have been documented (Barbosa et al., 2024). Previous studies highlight that yessotoxins are primarily produced by the dinoflagellates *Protoceratium reticulatum, Lingulodinium polyedra*, and *Gonyaulax spinifera* (Draisci et al., 1999; Paz et al., 2004; Satake et al., 1997), however, none of the above mentioned species were investigated which makes it challenging to assume from where the toxin originated. In the monitoring program from station B1 near the Dalarö mussel farm, *Protoceratium reticulatum* has been observed at least once per year the most recent decade, between June to October, in 81 - 570 individuals L^{-1} (sharkweb.smhi.se). Further, neither *Lingulodinium polyedra* nor *Gonyaulax spinifera* have been observed, but only *Gonyaulax verior*, although not since 2015. Thus, *Protoceratium reticulatum* was the only known potential producer in these waters, with a warning value of 1 000 individuals L^{-1} in Swedish bivalve monitoring for food consumption (Persson et al., 2020). According to the correlation matrix (Fig. 6), YTX were mainly positively correlated to cyanobacteria species, especially *Aphanizomenon* species but not exclusively. It needs, however, to be considered, that a positive or negative correlation does not necessarily confirm that the toxin is produced by the correlated taxa. Instead, it provides an indication of which taxa and toxins co-occur. While correlation is a valuable tool for identifying potential associations, it does not establish causality.

Many environmental factors may govern or direct toxin production. Since phytoplankton are living in an ever-changing environment, including seasonal changes, nutrient limitation will occur at some point, leading to physiological changes and potentially triggering toxin production (Pimentel and Giani, 2014). Temperature is an important driver for phytoplankton composition and growth and thus influences species abundances and toxin production (Barbosa et al., 2024; Calderini et al., 2023; Edullantes et al., 2023). While blooms of cyanobacteria are generally thought to prefer high temperatures (Paerl and Huisman 2008), their toxin production is not easy to generalize. This we exemplify herein for cylindrospermopsin, being higher in the mussels in the colder season of spring and fall, expanding the season for cyanotoxins.

5. Conclusions

We aimed to disentangle the seasonal variation of phytoplankton abundances and biotoxin production in the Baltic Sea. However, we were not able to identify any time period when mussels were completely toxin free, although they were never above regulatory levels. While dinoflagellate derived toxins were observed in fall and spring,

cyanobacterial toxins were mainly found in summer, except cylindrospermopsin. This underlines the importance of toxin determination in seafood derived from freshwater and estuarine areas, regardless of season. The abundance of the investigated dinoflagellates and cyanobacteria varied strongly during the season, but showed a general tendency of higher abundances during summer months. The correlation analysis between species abundance and toxin levels were not always informative, indicating that species abundance and toxin production underlie complex and dynamic mechanisms. Elevated toxin levels during the absence of potential toxin producers implies that toxins are accumulated in mussels over time, and that detoxification rates might differ, depending on compound and season. Further studies are required to reveal the linkage between species abundance and toxin production and also to elucidate the mechanisms that govern toxin retention and detoxification processes in mussel tissues.

There are no health guideline values of cyanobacterial toxins in mussels available in Europe, but occasionally the concentrations were close to levels suggested by Australia, which demonstrates the need to also have such regulations in Sweden and Europe. Further, as we present data from only one year, our study does not capture annual variation in species abundance and toxin concentration, and other years might have less toxins accumulating as well as vice versa. For example, Mazur-Marzec (2007) found higher nodularin content in mussels as compared to our observations, collected in Southern Baltic Sea during summer. We also leave some open questions from our results, including identifying the producer of cylindrospermopsin in the Dalarö region. Here species of *Aphanizomenon* spp. is a probable producer but needs to be clarified to know which taxa to monitor. Combining toxin analyses, microscopy, and molecular tools will be needed to understand this production completely. Until today, knowledge on these toxins and food products from the Baltic Sea is rather low, as mussels and other molluscs from the area are currently only farmed for human consumption in one location, but is expected to be expanded. With nodularin and cylindrospermopsin close to the limits advised in Australia we suggest regulation limits should be developed also for European waters in order to expand food production in areas exposed to cyanobacteria. This is especially relevant in the light of climate change as filamentous cyanobacteria in the Baltic Sea are changing in community composition and abundance, with freshening and elevated temperatures (Olofsson et al., 2020). This is of increasing concern also on a global scale, where cyanobacterial blooms may cause disruption to aquaculture and drinking water production.

CRediT authorship contribution statement

Malin Olofsson: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Martin Karlsson:** Writing – review & editing, Resources, Methodology. **Kimberly Melkersson:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Susanna Minnhagen:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Malin Persson:** Writing – review & editing, Validation, Methodology. **Martin Reutgard:** Writing – review & editing, Resources, Methodology. **Manuela Seehauser:** Writing – review & editing, Writing – original draft, Visualization, Data curation. **Aida Zuberovic Muratovic:** Writing – review & editing, Validation, Methodology.

Declaration of competing interest

The authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2025.102885.

Data availability

Data will be made available on request.

References

Amzil, Z., Derrien, A., Terre Terrillon, A., Savar, V., Bertin, T., Peyrat, M., Duval, A., Lhaute, K., Arnich, N., Hort, V., Nicolas, M., 2023. Five years monitoring the emergence of unregulated toxins in shellfish in France (EMERGTOX 2018–2022). Mar. Drugs 21 (8), 435. https://doi.org/10.3390/md21080435.

Bácsi, I., Vasas, G., Surányi, G., M-Hamvas, M., Máthé, C., Tóth, E., Grigorszky, I., Gáspár, A., Tóth, S., Borbely, G., 2006. Alteration of cylindrospermopsin production in sulfate- or phosphate-starved cyanobacterium *aphanizomenon ovalisporum*. FEMS Microbiol. Lett. 259 (2), 303–310. https://doi.org/10.1111/j.1574-6968.2006.00282.x.

Banker, R., Carmeli, S., Hadas, O., Teltsch, B., Porat, R., Sukenik, A., 1997. Identification of cylindrospermopsin in *aphanizomenon ovalisporum* (cyanophyceae) isolated from Lake Kinneret, ISRAEL†. J. Phycol. 33, 613–616. https://doi.org/10.1111/j.0022-3646.1997.00613.x.

Barbosa, M., Costa, P.R., David, H., Lage, S., Amorim, A., 2024. Effect of temperature on growth and yessotoxin production of *protoceratium reticulatum* and *lingulodinium polyedra* (Dinophyceae) isolates from the Portuguese coast (NE Atlantic). Mar. Environ. Res. 194, 106321. https://doi.org/10.1016/j.marenvres.2023.106321.

Bartram, J., Chorus, I. (Eds.), 1999. Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management. CRC Press. https://doi.org/10.1201/9781482295061, 0 ed.

Calderini, M.L., Pääkkönen, S., Salmi, P., Peltomaa, E., Taipale, S.J., 2023. Temperature, phosphorus and species composition will all influence phytoplankton production and content of polyunsaturated fatty acids. J. Plankton Res. 45 (4), 625–635. https://doi.org/10.1093/plankt/fbad026.

Camacho-Muñoz, D., Waack, J., Turner, A.D., Lewis, A.M., Lawton, L.A., Edwards, C., 2021. Rapid uptake and slow depuration: health risks following cyanotoxin accumulation in mussels? Environ. Pollut. (Barking, Essex: 1987) 271, 116400. https://doi.org/10.1016/j.envpol.2020.116400.

Chorus, I., Welker, M., 2021. Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management, 2nd ed. CRC Press. https://doi.org/10.1201/9781003081449.

Dirks, C., Cappelli, P., Blomqvist, M., Ekroth, S., Johansson, M., Persson, M., Drakare, S., Pekar, H., Zuberovic Muratovic, A., 2024. Cyanotoxin occurrence and diversity in 98 cyanobacterial blooms from Swedish lakes and the Baltic Sea. Mar. Drugs 22 (5), 199. https://doi.org/10.3390/md22050199.

Draisci, R., Ferretti, E., Palleschi, L., Marchiafava, C., Poletti, R., Milandri, A., Ceredi, A., Pompei, M., 1999. High levels of yessotoxin in mussels and presence of yessotoxin and homoyessotoxin in dinoflagellates of the Adriatic Sea. Toxicon: Offic. J. Int. Society Toxinol. 37 (8), 1187–1193. https://doi.org/10.1016/s0041-0101(98)00254-2.

Edullantes, B., Low-Decarie, E., Steinke, M., Cameron, T., 2023. Comparison of thermal traits between non-toxic and potentially toxic marine phytoplankton: implications to their responses to ocean warming. J. Exp. Mar. Biol. Ecol. 562, 151883. https://doi.org/10.1016/j.jembe.2023.151883.

España Amórtegui, J.C., Pekar, H., Retrato, M.D.C., Persson, M., Karlson, B., Bergquist, J., Zuberovic-Muratovic, A., 2023. LC-MS/MS analysis of cyanotoxins in bivalve mollusks—Method development, validation and first evidence of occurrence of nodularin in mussels (*Mytilus edulis*) and oysters (*Magallana gigas*) from the west coast of Sweden. Toxins (Basel) 15 (5), 329. https://doi.org/10.3390/toxins15050329.

European Union Reference Laboratory for Marine Biotoxins. (2015). *EU-harmonised Standard Operating Procedure for determination of lipophilic marine biotoxins in molluscs by LC-MS/MS*. (Version 5). https://www.aesan.gob.es/en/CRLMB/docs/docs/metodos_analiticos_de_desarrollo/EU-Harmonised-SOP-LIPO-LCMSMS_Version5.pdf.

European Union Reference Laboratory for Marine Biotoxins. (2020). *EURLMB SOP for the analysis of paralytic shellfish toxins (PST) by precolumn HPLC-FLD according to OMA AOAC 2005.06* (Version 1). https://www.aesan.gob.es/en/CRLMB/web/public_documents/seccion/crlmb_standard_operating_procedures.htm.

Gago-Martínez, A., Lawrence, J.F., Leão, J.M., & Vilariño, O. (2005). *AOAC 2005.06 Standard Operating Procedure simplified version for the screening and semi-quantitation of PSP toxins*.

Hakanen, P., Suikkanen, S., Franzén, J., Franzén, H., Kankaanpää, H., Kremp, A., 2012. Bloom and toxin dynamics of *alexandrium ostenfeldii* in a shallow embayment at the SW coast of Finland, northern Baltic Sea. Harmful Algae 15, 91–99. https://doi.org/10.1016/j.hal.2011.12.002.

HELCOM. (2023). *Water clarity. HELCOM core indicator report.* https://indicators.helcom.fi/wp-content/uploads/2023/04/Water-clarity_Final_September_2023.pdf.

Karjalainen, M., Engström-Öst, J., Korpinen, S., Peltonen, H., Pääkkönen, J.-P., Rönkkönen, S., Suikkanen, S., Viitasalo, M., 2007. Ecosystem consequences of cyanobacteria in the Northern Baltic Sea. AMBIO: A J. Human Environ. 36 (2), 195–202. https://doi.org/10.1579/0044-7447(2007)36[195:ECOCIT]2.0.CO;2.

Karjalainen, M., Pääkkönen, J.-P., Peltonen, H., Sipiä, V., Valtonen, T., Viitasalo, M., 2008. Nodularin concentrations in Baltic Sea zooplankton and fish during a cyanobacterial bloom. Mar. Biol. 155 (5), 483–491. https://doi.org/10.1007/s00227-008-1046-4.

Karlsson, M., Reutgard, M., 2019. Musselodling som närsaltsreducerande åtgärd i Norra Östersjöns vattendistrikt. Report Life IP Rich Waters. https://www.richwaters.se/wp-content/uploads/Musselodling-som-n %C3 %A4rsaltsreducerande- %C3 %A5tg %C3 %A4rd-i-Norra- %C3 %96stersj %C3 %B6ns-vattendistrikt.pdf. Online March 13, 2025.

Karlson, B., Arneborg, L., Johansson, J., Linders, J., Liu, Y., Olofsson, M., 2022. A suggested climate service for cyanobacteria blooms in the Baltic Sea – Comparing three monitoring methods. Harmful Algae 118, 102291. https://doi.org/10.1016/j.hal.2022.102291.

Kotta, J., Futter, M., Kaasik, A., Liversage, K., Rätsep, M., Barboza, F.R., Bergström, L., Bergström, P., Bobsien, I., Díaz, E., Herkül, K., Jonsson, P.R., Korpinen, S., Kraufvelin, P., Krost, P., Lindahl, O., Lindegarth, M., Lyngsgaard, M.M., Mühl, M., Virtanen, E., 2020. Cleaning up seas using blue growth initiatives: mussel farming for eutrophication control in the Baltic Sea. Sci. Total Environ. 709, 136144. https://doi.org/10.1016/j.scitotenv.2019.136144.

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.

Livsmedelsverket, 2018. Handbok Dricksvattenrisker—Cyanotoxiner i dricksvatten. Livsmedelsverket. https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2018/2018-handbok-dricksvattenrisker-cyanotoxiner-i-dricksvatten.pdf.

Livsmedelsverket, 2022. Livsmedelsverkets författningssamling—Livsmedelsverkets föreskrifter om dricksvatten. Livsmedelsverket. https://www.livsmedelsverket.se/globalassets/om-oss/lagstiftning/dricksvatten—naturl-mineralv—kallv/livsfs-2022-12_web_t.pdf.

Lopes, V.R., Vasconcelos, V.M., 2011. Planktonic and benthic cyanobacteria of European brackish waters: a perspective on estuaries and brackish seas. Eur. J. Phycol. 46 (3), 292–304. https://doi.org/10.1080/09670262.2011.602429.

Lundholm, N., Bernard, C., Churro, C., Escalera, L., Hoppenrath, M., Iwataki, M., Larsen, J., Mertens, K., Moestrup, Ø., Murray, S., Salas, R., Tillmann, U., Zingone, A. (Eds) (2009 onwards). IOC-UNESCO Taxonomic reference List of harmful micro algae. Accessed at https://www.marinespecies.org/habon2025-03-24. doi:10.14284/362.

Mazmouz, R., Chapuis-Hugon, F., Mann, S., Pichon, V., Méjean, A., Ploux, O., 2010. Biosynthesis of cylindrospermopsin and 7-epicylindrospermopsin in *Oscillatoria* sp. Strain PCC 6506: identification of the *cyr* gene cluster and toxin analysis. Appl. Environ. Microbiol. 76 (15), 4943–4949. https://doi.org/10.1128/AEM.00717-10.

Mazur-Marzec, H., Tymińska, A., Szafranek, J., Pliński, M., 2007. Accumulation of nodularin in sediments, mussels, and fish from the Gulf of Gdańsk, southern Baltic Sea. Environ. Toxicol. 22 (1), 101–111. https://doi.org/10.1002/tox.20239.

Mazur-Marzec, H., Sutryk, K., Kobos, J., Hebel, A., Hohlfeld, N., Błaszczyk, A., Toruńska, A., Kaczkowska, M.J., Łysiak-Pastuszak, E., Kraśniewski, W., Jasser, I., 2013. Occurrence of cyanobacteria and cyanotoxin in the Southern Baltic Proper. Filamentous cyanobacteria versus single-celled picocyanobacteria. Hydrobiologia 701 (1), 235–252. https://doi.org/10.1007/s10750-012-1278-7.

Meriluoto, J., Spoof, L., Codd, G.A, 2016. Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis, 1st ed. Wiley. https://doi.org/10.1002/9781119068761.

Morais, J., Augusto, M., Carvalho, A.P., Vale, M., Vasconcelos, V.M., 2008. Cyanobacteria hepatotoxins, microcystins: bioavailability in contaminated mussels exposed to different environmental conditions. Eur. Food Res. Technol. 227 (3), 949–952. https://doi.org/10.1007/s00217-007-0779-5.

Olofsson, M., Suikkanen, S., Kobos, J., Wasmund, N., Karlson, B., 2020. Basin-specific changes in filamentous cyanobacteria community composition across four decades in the Baltic Sea. Harmful Algae 91, 101685. https://doi.org/10.1016/j.hal.2019.101685.

Otero, P., Silva, M., 2022. The role of toxins: impact on human health and aquatic environments. The Pharmacological Potential of Cyanobacteria. Elsevier, pp. 173–199. https://doi.org/10.1016/B978-0-12-821491-6.00007-7.

Overlingė, D., Toruńska-Sitarz, A., Kataržytė, M., Pilkaitytė, R., Gyraitė, G., Mazur-Marzec, H., 2021. Characterization and diversity of microcystins produced by cyanobacteria from the Curonian Lagoon (SE Baltic Sea). Toxins (Basel) 13 (12), 838. https://doi.org/10.3390/toxins13120838.

Paerl, H.W., Huisman, J., 2008. Blooms like it hot. Science (1979) 320 (5872), 57–58. https://doi.org/10.1126/science.1155398.

Paerl, H.W., Otten, T.G., 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microb. Ecol. 65 (4), 995–1010. https://doi.org/10.1007/s00248-012-0159-y.

Paz, B., Riobó, P., Fernández, M.L., Fraga, S., Franco, J.M., 2004. Production and release of yessotoxins by the dinoflagellates protoceratium reticulatum and lingulodinium polyedrum in culture. Toxicon: Offic. J. Int. Society Toxinol. 44 (3), 251–258. https://doi.org/10.1016/j.toxicon.2004.05.021.

Persson, M., Karlson, B., Zuberovic Muratovic, A., Simonsson, M., Bergkvist, P., Renborg, E., 2020. Kontrollprogrammet För Tvåskaliga Blötdjur Årsrapport 2014-2019. Livsmedelsverkets Rapportserie. Livsmedelsverket.

Pham, T., Shimizu, K., Dao, T., Motoo, U., 2017. First report on free and covalently bound microcystins in fish and bivalves from Vietnam: assessment of risks to humans. Environ. Toxicol. Chem. 36 (11), 2953–2957. https://doi.org/10.1002/etc.3858.

Pimentel, J.S.M., Giani, A., 2014. Microcystin production and regulation under nutrient stress conditions in toxic microcystis strains. Appl. Environ. Microbiol. 80 (18), 5836–5843. https://doi.org/10.1128/AEM.01009-14.

Preußel, K., Chorus, I., Fastner, J., 2014. Nitrogen limitation promotes accumulation and suppresses release of cylindrospermopsins in cells of *aphanizomenon* sp. Toxins (Basel) 6 (10). https://doi.org/10.3390/toxins6102932. Article 10.

Preußel, K., Stüken, A., Wiedner, C., Chorus, I., Fastner, J., 2006. First report on cylindrospermopsin producing *aphanizomenon flos-aquae* (Cyanobacteria) isolated from two German lakes. Toxicon 47 (2), 156–162. https://doi.org/10.1016/j.toxicon.2005.10.013.

Preußel, K., Wessel, G., Fastner, J., Chorus, I., 2009. Response of cylindrospermopsin production and release in *Aphanizomenon flos-aquae* (Cyanobacteria) to varying light and temperature conditions. Harmful Algae 8 (5), 645–650. https://doi.org/10.1016/j.hal.2008.10.009.

R, S.K.G., Sundar, R.D.V., K, A.K., Ravi, L., 2023. Identification of toxic blooms of cyanobacteria in estuarine habitat. In: Thajuddin, N., Sankara Narayanan, A., Dhanasekaran, & D. (Eds.), Protocols For Cyanobacteria Sampling and Detection of Cyanotoxin. Springer, Nat. Singapore, pp. 35–45. https://doi.org/10.1007/978-981-99-4514-6_5.

Salmaso, N., Bernard, C., Humbert, J., Akçaalan, R., Albay, M., Ballot, A., Catherine, A., Fastner, J., Häggqvist, K., Horecká, M., Izydorczyk, K., Köker, L., Komárek, J., Maloufi, S., Mankiewicz-Boczek, J., Metcalf, J.S., Quesada, A., Quiblier, C., Yéprémian, C., 2016. Basic guide to detection and monitoring of potentially toxic cyanobacteria. In: Meriluoto, J., Spoof, L., Codd, G.A. (Eds.), Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis, 1st ed. Wiley, pp. 46–69. https://doi.org/10.1002/9781119068761.ch6.

SANTE/12682/2019 of 1st January 2020, 2019. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed. European Commission, Brussels, Belgium, pp. 1–53.

Satake, M., MacKenzie, L., Yasumoto, T., 1997. Identification of protoceratium reticulatum as the biogenetic origin of yessotoxin. Nat. Toxins 5 (4), 164–167. https://doi.org/10.1002/nt.7.

Selander, E., Fagerberg, T., Wohlrab, S., Pavia, H., 2012. Fight and flight in dinoflagellates? Kinetics of simultaneous grazer-induced responses in *Alexandrium tamarense*. Limnol. Oceanogr. 57 (1), 58–64. https://doi.org/10.4319/lo.2012.57.1.0058.

Setälä, O., Autio, R., Kuosa, H., Rintala, J., Ylöstalo, P., 2005. Survival and photosynthetic activity of different *dinophysis acuminata* populations in the northern Baltic Sea. Harmful Algae 4 (2), 337–350. https://doi.org/10.1016/j.hal.2004.06.017.

Setälä, O., Sopanen, S., Riitta, A., Kankaanpää, H., Erler, K., 2011. Dinoflagellate toxins in northern Baltic Sea phytoplankton and zooplankton assemblages. Boreal Environ. Res. 16, 509–520.

Shishido, T.K., Delbaje, E., Wahlsten, M., Vuori, I., Jokela, J., Gugger, M., Fiore, M.F., Fewer, D.P., 2023. A cylindrospermopsin-producing cyanobacterium isolated from a microbial mat in the Baltic Sea. Toxicon 232, 107205. https://doi.org/10.1016/j.toxicon.2023.107205.

Sivonen, K., Himberg, K., Luukkainen, R., Niemelä, S.I., Poon, G.K., Codd, G.A., 1989. Preliminary characterization of neurotoxic cyanobacteria blooms and strains from Finland. Toxicity Assess. 4 (3), 339–352. https://doi.org/10.1002/tox.2540040310.

Sopanen, S., Setala, O., Piiparinen, J., Erler, K., Kremp, A., 2011. The toxic dinoflagellate *alexandrium ostenfeldii* promotes incapacitation of the calanoid copepods *eurytemora affinis* and *acartia bifilosa* from the northern Baltic Sea. J. Plankton Res. 33 (10), 1564–1573. https://doi.org/10.1093/plankt/fbr052.

Tang, Y., Zhang, H., Wang, Y., Fan, C., Shen, X., 2021. Combined effects of temperature and toxic algal abundance on paralytic shellfish toxic accumulation, tissue distribution and elimination dynamics in mussels *mytilus coruscus*. Toxins (Basel) 13 (6), 425. https://doi.org/10.3390/toxins13060425.

Testai, E., Buratti, F.M., Funari, E., Manganelli, M., Vichi, S., Arnich, N., Biré, R., Fessard, V., Sialehaamoa, A., 2016. Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food. EFSA Supp. Pub. 13 (2). https://doi.org/10.2903/sp.efsa.2016.EN-998.

Thajuddin, N., Sankara narayanan, A., Dhanasekaran, D. (Eds.), 2023. Protocols For Cyanobacteria Sampling and Detection of Cyanotoxin, 1st ed. Springer, Nat. Sing.. https://doi.org/10.1007/978-981-99-4514-6 2023.

Thangaraj, R., Ramnath, S., Thajuddin, N., 2023. Isolation of toxin-producing cyanobacteria from aquatic samples with *Anabaena*. In: Thajuddin, N., Sankara Narayanan, A., Dhanasekaran, D. (Eds.), Protocols For Cyanobacteria Sampling and Detection of Cyanotoxin. Springer, Nature Singapore, pp. 71–76. https://doi.org/10.1007/978-981-99-4514-6_9.

Tubaro, A., Dell'Ovo, V., Sosa, S., Florio, C., 2010. Yessotoxins: a toxicological overview. Toxicon 56 (2), 163–172. https://doi.org/10.1016/j.toxicon.2009.07.038.

Utermöhl, H., 1958. Zur Vervollkommnung der Quantitativen Phytoplankton-Methodik. Schweizerbart.

Vaara, T., Vaara, M., Niemelä, S., 1979. Two improved methods for obtaining axenic cultures of cyanobacteria. Appl. Environ. Microbiol. 38 (5), 1011–1014. https://doi.org/10.1128/aem.38.5.1011-1014.1979.

Verma, A., Barua, A., Ruvindy, R., Savela, H., Ajani, P.A., Murray, S.A., 2019. The genetic basis of toxin biosynthesis in dinoflagellates. Microorganisms 7 (8), 222. https://doi.org/10.3390/microorganisms7080222.

Villar-González, A., Rodríguez-Velasco, M.L., Gago-Martínez, A., 2011. Determination of lipophilic toxins by LC/MS/MS: single-laboratory validation. J. AOAC Int. 94 (3), 909–922.

Visciano, P., Schirone, M., Berti, M., Milandri, A., Tofalo, R., Suzzi, G., 2016. Marine biotoxins: occurrence, toxicity, regulatory limits and reference methods. Front. Microbiol. 7, 1051. https://doi.org/10.3389/fmicb.2016.01051.

Wilk-Woźniak, E., Krztoń, W., Budziak, M., Walusiak, E., Žutinič, P., Udovič, M.G., Koreivienė, J., Karosienė, J., Kasperovičienė, J., Kobos, J., Toporowska, M., Bańkowska-Sobczak, A., Budzyńska, A., Domek, P., Dunalska, J., Frąk, M., Gołdyn, R., Grabowska, M., Jakubowska-Krepska, N., Pfeiffer, T.Ż., 2024. Harmful blooms across a longitudinal gradient in central Europe during heatwave: cyanobacteria biomass, cyanotoxins, and nutrients. Ecol. Indic. 160, 111929. https://doi.org/10.1016/j.ecolind.2024.111929.

Williams, D.E., Dawe, S.C., Kent, M.L., Andersen, R.J., Craig, M., Holmes, C.F.B., 1997. Bioaccumulation and clearance of microcystins from salt water mussels, *Mytilus edulis*, and in vivo evidence for covalently bound microcystins in mussel tissues. Toxicon 35 (11), 1617–1625. https://doi.org/10.1016/S0041-0101(97)00039-1.