

The relationship between toxic phytoplankton species occurrence and environmental and meteorological factors along the Eastern Adriatic coast

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ABSTRACT

In this study, the time series of toxic phytoplankton species collected between 2004 and 2018 from the Northern Adriatic, Šibenik Bay, and Mali Ston Bay was analyzed in relation to environmental (temperature, salinity, water column stability, and river flow) and meteorological parameters (precipitation and wind). Because of the mostly non-linear relation between biotic and abiotic parameters, self-organizing maps (SOM) were used to identify these relationships.

SOM analysis distinguished species of the genus *Dinophysis* from *Gonyaulax spinifera* and *Lingulodinium polyedrum* species, which better tolerate wind-induced disturbance. Among the *Dinophysis* species, *Dinophysis fortii*, *Dinophysis tripos*, and *Dinophysis acuta* preferred higher precipitation rate and river flow in addition to optimal temperatures. The abundances of *Alexandrium* species, which occurred more frequently in estuarine areas, were associated with river flow and maximum stable water column. Regardless of the ecological preferences of individual harmful algae, freshwater inflow-caused stratification is present in all clusters of environmental conditions associated with increased abundances of harmful algae in the SOM analysis. It is highly likely that stratification represents an important factor for the development and maintenance of HABs. The non-linear relationship between the NAO index and rainfall was noted, of which the most important for the development of harmful algae is the proportional correlation between the positive phase of the NAO index and higher rainfall, especially in winter and spring. Such conditions are conducive to the development of harmful algae because, with the increase in temperature accompanying the positive phase of the NAO index, increased rainfall further stimulates their growth. This can be achieved either through nutrient yields or through higher freshwater inflow that further stabilizes the water column.

1. Introduction

Harmful phytoplankton species can produce toxins that may impact birds and higher mammals, including humans, as well as damage local fish and invertebrates. They are present in almost all coastal seas; according to their impact on human health, environment, and food chain, they are categorized into five groups (Lassus et al., 2016). In this study, we focused on toxin-producing phytoplankton that cause food poisoning in humans with neurological and gastrointestinal symptoms associated with paralytic shellfish poisoning, diarrhetic shellfish poisoning, and amnesic shellfish poisoning. At the investigated area, phycotoxins have been sporadically found in various shellfish species analysed along the Eastern Adriatic coast. In general, determined concentrations were low, below regulatory limits. Low concentration of

domoic acid (ASP toxins) was reported in northern and central Adriatic (Ujević et al., 2010; Arapov et al., 2016, 2017; Ujević et al., 2019). Lipophilic toxins, including okadaic acid and its derivatives, pectenotoxins, yessotoxins, gymnodimine and spirolide, were found in all major shellfish breeding area (Ninčević-Gladan et al., 2008; Ninčević Gladan et al. 2010a; Ninčević Gladan et al., 2011; Arapov et al., 2015; Ujević et al., 2019). The higher levels that could endanger human health were reported for okadaic acid (Ninčević Gladan et al. 2010a; Ninčević Gladan et al., 2011; Arapov et al., 2015) and saxitoxins (Ujević et al., 2012).

Increased abundances of toxic phytoplankton species whose inoculum already exists can be attributed to natural processes that depend on meteorological conditions and hydrodynamics as well as anthropogenic factors. However, elevated abundance levels vary for

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different species. For example, the presence of several hundred cells per liter is considered high for the species of the genera *Dinophysis* and *Phalacroma*, whereas 10^6 cells per liter is considered high for the species of the genus *Pseudo-nitzschia* (Lassus et al., 2016). Eutrophication has been considered as one of the factors that stimulate the development of phytoplankton blooms, including harmful species (Lassus et al., 2016). In addition, physical factors such as upwelling (Kudela et al., 2005; Lewitus et al., 2012), downwelling (Reguera et al., 2012), and water column stability (Ryan et al., 2014) are related to toxic bloom occurrence and maintenance. The highly productive upwelling regimes in spring and summer favor the development of chain-forming diatoms, including *Pseudo-nitzschia* spp. With the intensification of stratification, dinoflagellate blooms may begin to appear. Blooms of fast-swimming dinoflagellates such as *Gymnodinium catenatum* and *Alexandrium* spp. are concentrated in the convergence zones of the autumn upwelling–downwelling transitions. According to Reguera et al. (2003, 2012), increased abundances of *Dinophysis* species are associated with periods of stratification between moderate upwelling pulses and downwelling events that promote the accumulation of *Dinophysis* cells. Global warming also increases the risk of toxic dinoflagellate blooms (Peperzak, 2003). Moreover, precipitation affects the increase in the number of harmful algal blooms (HABs), as has been recorded in Puget Sound. The abundance of *Alexandrium catenella* increased under stable and warm surface conditions (Determan, 1998). These conditions are achieved when large perception events are followed by calm and warm weather.

Since toxic phytoplankton species belong to diverse taxonomic groups with specific ecological features related to their cell size and shape, motility, nutrition, response to temperature, salinity, and turbulence, they can occur under different environmental conditions. Determination of species-specific environmental conditions and linkage of toxic species abundances with hydrodynamic conditions might provide information necessary for the prediction of HABs by using numerical models. Phytoplankton abundance changes depending on weather conditions at a local scale, but is also affected by larger-scale variabilities, including ocean atmospheric interactions such as the North Atlantic Oscillation (NAO; Belgrano et al., 1999; Ninčević Gladan et al., 2010b).

In this study, the influence of environmental (temperature, salinity, water column stability, and river flow) and meteorological parameters (precipitation and wind) on the appearance of toxic phytoplankton species was analyzed. Because of the complex nonlinear effects and interactions of environmental variables on the occurrence of toxic species and the existence of species-specific conditions, self-organizing map (SOM) analyses (Šolić et al., 2018) were used in this study. SOM analysis allows the creation of non-orthogonal clusters or best matching units (BMUs) for a certain species. Time series of toxic phytoplankton species collected between 2004 and 2018 along the eastern coast of Adriatic Sea at areas characterized by different hydrodynamic conditions was analyzed in order to (1) record the toxic phytoplankton species occurring along the eastern Adriatic coast, (2) describe the seasonal cycle of the most common species in certain areas, (3) define the environmental and meteorological factors that are conducive to the development of these species, and (4) link their occurrence with hemispheric meteorological processes and climate changes by using the NAO index.

2. Materials and methods

2.1. Study area

The investigated area includes ten sampling stations from major shellfish breeding and harvesting areas along the western coast of Istrian Peninsula, Šibenik Bay, and Mali Ston Bay (Fig. 1). In the northern part, along the western coast of Istrian Peninsula, four stations (P1, P2, P3, and P6) are located. Station P1 (45.485 °N, 13.572 °E;

depth, 7 m) is located in Savudrija Bay. This is a shallow bay, with a maximum depth of 19 m. This area is under the influence of the Dragonja River, causing lower salinity in the surface layer during higher river discharge. Sampling station P2 (45.272 °N, 13.576 °E, depth, 10 m) is located 4–5 km south of the mouth of the River Mirna, without significant freshwater or sewage inflow; the maximum depth of the area is 15 m. Station P3 (45.134 °N, 13.724 °E; depth, 13 m) is located in Lim Bay. This is a narrow bay (width, 600 m), deeply embedded in land (length, 11 km); its maximum depth is 33 m at the entrance of the bay. The inner part, where P3 station is located, is the most productive area, which is considerably shallower and under significant influence of ground waters enriched with organic matter. Station P6 (45.221 °N, 13.050 °E; depth, 25 m) is the only sampling site located at the shellfish harvesting area. In the central Adriatic Sea, sampling was conducted at Šibenik Bay at two stations, SB1 (43.761 °N, 15.853 °E; depth, 33 m) and SB2 (43.749 °N, 15.876 °E; depth, 18 m). The estuary is 22 km long and relatively narrow. This is a highly stratified estuary with a halocline persisting throughout the year. The Krka River is characterized by low yearly nutrient input (Gržetić et al., 1991; Cetinić et al., 2006) and represents the main source of nitrates and silicates for the estuary, whereas phosphorus is mainly anthropogenic in origin, from Šibenik harbor. In the southern Adriatic, samples were collected from Mali Ston Bay at six sampling stations: MSB1 (42.873 °N, 17.703 °E; depth, 7 m), MSB2 (42.847 °N, 17.710 °E; depth, 10 m), MSB3 (42.866 °N, 17.691 °E; depth, 10 m), and MSB6 (42.916 °N, 17.472 °E; depth, 7 m). Mali Ston Bay is 28 km long with maximum depth of 29 m; it is situated at the end of the Neretva River Channel, between Pelješac Peninsula and mainland. The shellfish breeding area is located in the inner shallow part of the bay, which is strongly influenced by submarine springs during winter. The bay is characterized by estuarine circulation; the brackish water at the surface forms the output current, and halocline saltier water enters below from the open sea.

2.2. Toxic phytoplankton

Phytoplankton samples were collected from the bottom to the surface by using a plankton net (mesh size, 20 µm) with a vertical drag. Samples were preserved in alkaline Lugol's solution (71 % distilled water/4 % iodine/7 % potassium iodide/18 % sodium acetate), which was prepared by adding 1 ml of Lugol's solution to 250 ml of the seawater sample. Toxic microalgae were taxonomically identified and counted under an inverted microscope by using the Utermöhl (1958) method within 24–48 h after sampling. Among the non-thecate dinoflagellates, only *Karenia* spp. were determined, because of the difficulty in the taxonomical identification of these dinoflagellates in the preserved samples by using light microscopy.

Abundance is expressed as the number of cells per square meter calculated as $N \times P \times F$, where N is the number of cells in the counting chamber, P is the proportion of total volume to subsample volume, and F is a factor of the phytoplankton net calculated as 1 m^2 divided by the net surface (Ninčević-Gladan et al., 2008).

2.3. Data set

Phytoplankton data set consists of a total of 4487 samples collected between 2004 and 2018, of which 1585 were from the Northern Adriatic, 971 from Šibenik Bay, and 1931 from Mali Ston Bay (Fig. 1). The data are equally distributed in time with the exception of 2006 when sampling was only performed in January, February, and April, and 2007 when sampling data for the first three months are missing. Sampling was conducted regularly in the other years, but at different sampling frequencies. In the Northern Adriatic, sampling was conducted twice a month in 2004 and 2005 and once a month between 2006 and 2010, with the exception of 2010 (from October to December, three times a month). Between 2011 and 2018, sampling was conducted two to three times from January to March and in December and four

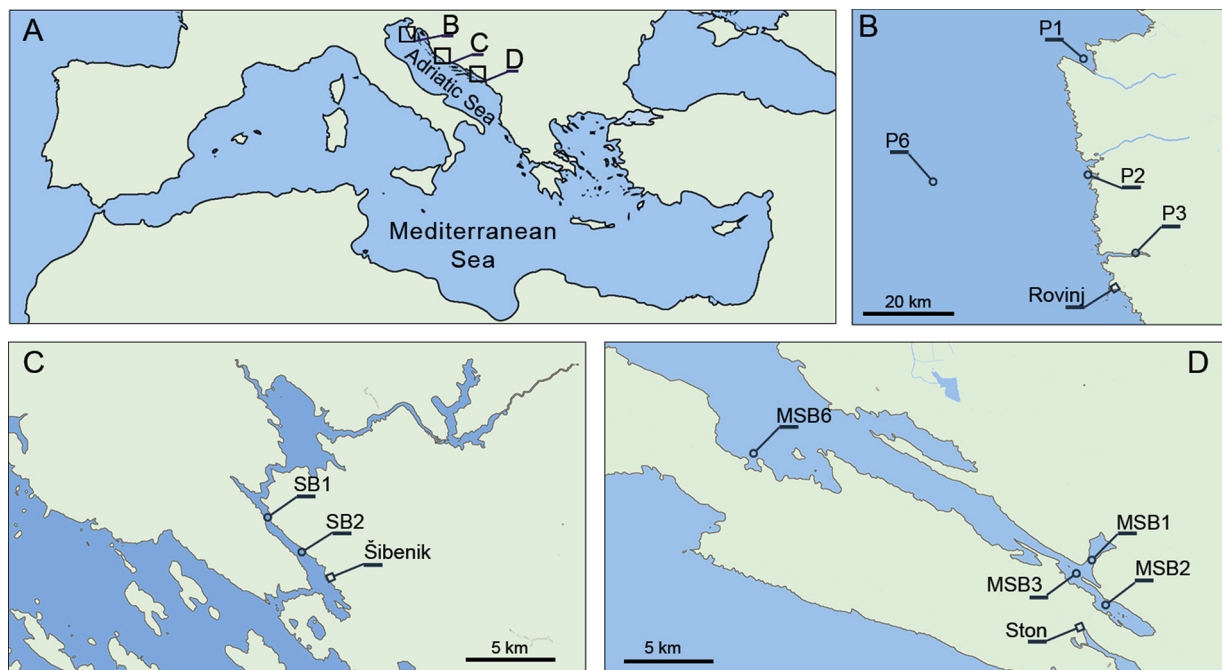


Fig. 1. Investigated area with sampling sites (circles) and the nearest meteorological data collection stations (squares). A) Adriatic Sea with marked study areas, B) stations in the Northern Adriatic placed along the Istrian coast, C) stations in the Šibenik Bay, D) stations in the Mali Ston Bay.

times from April to November. In Šibenik Bay, sampling was conducted once a month in 2004 and 2005 and twice a month between 2007 and 2008. Between 2009 and 2018, sampling was conducted two to three times from January to March and in December and four times from April to November. In Mali Ston Bay, sampling was conducted twice a month in 2004 and 2010, with the exception in 2009 and 2010, when sampling was conducted four times a month from October to December. In 2011, sampling was conducted four times a month; between 2012 and 2018, sampling was conducted two to three times a month from January to March and in December and four times a month from April to November.

2.4. SOM analysis

The SOM method, a neural network technique that combines k-means clustering and data smoothing, was used in this study (Kohonen, 1982). This method reduces the dimensionality of the input data space on a prescribed number of neurons, usually located on a two-dimensional grid. During the SOM learning process, the iteration algorithm attempts to determine the minimum Euclidean distance between neurons and data vectors. At the end of the process, neurons with minimum distance from the data vector are identified as the BMUs of the input vector. SOM provides two measures of mapping quality: average quantization error and topographic error, which describes the average distance between the data vector and BMUs and the percentage of data vectors for which the first and second BMUs are not neighboring units, respectively (Kohonen, 2001).

The model was setup such that the entire data set can be used as the training data set. One data vector was a set of phytoplankton species and environmental variable measurements. Environmental variables were selected from the time of phytoplankton sampling to the previous 12 days and calculated as cumulative precipitation, river runoff, and average wind speed in that period. The data vector was introduced to the SOM as 14-value data vector (6 environmental variables + 8 phytoplankton species). The number of data vectors varied between the SOM models (Table 1). If a selected phytoplankton species was not recorded, a non-existing label was used in the vector. Non-existing values are not considered in the analyses, which is an advantage of SOM

widely used in environmental sciences (Matić et al., 2018; öolić et al., 2018; Matic et al., 2019). However, for distance calculation, each individual vector was masked so that the unknown values were not considered. As distance is used to define the neighborhood of the winning neuron, neighborhood is defined on the collapsed subspace (Vesanto et al., 2000).

The environmental variables were normalized, and phytoplankton time series was logarithmically transformed using natural logarithm and normalized, which is a prerequisite for the equal treatment of different parameters when using SOM analysis (Kohonen, 2001). The SOM was calculated using MATLAB toolbox version 2.0 developed by Vesanto et al. (2000).

For each sampling area, two types of SOM were performed, both including all environmental factors, but different number of phytoplankton species (Table 1). The first SOM type included all phytoplankton species with appearance in time series greater than 10 % for Istra, 8 % for Šibenik Bay, and 7 % for Mali Ston Bay. Therefore, the data vector contained environmental variables and selected numbers of phytoplankton species. The second SOM type, instead of joint modeling of all selected phytoplankton species, models only one species with the environmental variables. Finally, for each of the three areas, 7 SOM analyses (one joint and 6 for selected phytoplankton species), total 21 different SOM analyses, were conducted.

Trend statistics of selected phytoplankton species was determined using a non-parametric Mann–Kendall test (Mann, 1945; Kendall, 1970). This test does not require normal distribution of data and has low sensitivity to outliers in the time series. The test was performed on collected data without any monthly and annual averaging.

2.5. Meteorological data, river flow, teleconnection indices, and satellite Chl *a*

Meteorological data (precipitation and wind speed) collected at the nearest meteorological stations (Fig. 1) were obtained from the Croatian Meteorological and Hydrological Service. The distances of the analyzed marine stations from the nearest meteorological stations were 9–49 km in the northern Adriatic Sea, 2–4 km in Šibenik Bay, and 1.4–20 km in Mali Ston Bay (Fig. 1). The river flow data collected at the

Table 1

Characteristic and errors of SOM models all set as follows: the hexagonal neural lattice of “sheet” shape, the linear SOM initialization, the “ep” neighbourhood function, and the batch training algorithm. Quantization error (QE), Topographic error (TE), Environmental variables: sea surface temperature¹, temperature² and salinity³ vertical gradient, precipitation⁴, wind speed⁵ and river runoff⁶. Phytoplankton spec: *A. concavum*^A, *A. cf. tamarense*^B, *D. acuta*^C, *D. caudata*^D, *D. fortii*^E, *Ph. rotundatum*^F, *D. sacculus*^G, *D. tripos*^H, *G. spinifera*^I, *L. polyedrum*^J, *P. cordatum*^K, *Pseudo-nitzschia* spp.^L

Zone	Environmental variables	Phytoplankton Spec.	Number of samples	SOM size	QE	TE
Istra	1, 2, 3, 4, 5	D, F, G, H, I, J, L	1585	3 × 3	2.413	2.1 %
Šibenik	1, 2, 3, 4, 5, 6	B, C, D, F, G, H, K, L	2557	3 × 3	2.677	0.4 %
Mali Ston	1, 2, 3, 4, 5, 6	A, D, E, F, G, I, J, L	1931	9 × 1	1.939	3.6 %

mouth of the river were obtained from the legal entity for water management “Hrvatske vode.” The NAO index was used from the web page “The Climate Data Guide: Hurrell North Atlantic Oscillation (NAO) Index (PC-based)” retrieved from <https://climatedataguide.ucar.edu/climate-data/hurrell-north-atlantic-oscillation-nao-index-pc-based>. Satellite Chl *a* for Northern Adriatic was obtained from the web page (www.oceancolour.org). Surface Chl *a* maps could not be obtained for the other two areas owing to their very small spatial domains.

The satellite Chl *a* patterns and NAO index were not included in the SOM analysis, but were calculated later as a composite average of each index for each of the BMUs.

3. Results

3.1. Environmental and meteorological data

3.1.1. Northern Adriatic Sea

On the stations in the northern Adriatic, the seawater temperature ranged from 7 to 28.80 °C, with the highest temperature in July and the lowest in January (Fig. 2). Thermocline developed from April to August

except at station P3 where it was noted from March to September. In August and September, vertical heating and mixing of shallow water column was strong enough to push the thermocline to the bottom, making the entire water column vertically homogenous. Temperature inversion was noted from October to December. Salinity in the surface layer differed across the stations. At stations P2 and P6, salinity ranged from 31.3 to 38.8, with the lowest salinity recorded during summer. At station P1, salinity ranged from 25.0 to 38.6, whereas the lowest salinity (5.7 to 38.4) was noted at station P3. The lowest salinity at these stations was recorded during winter and autumn because of the high precipitation and freshwater inflow. Salinity at the bottom layer at all stations ranged from 32.4 to 38.9. The cumulative monthly precipitation ranged from 0 to 356.9 mm, with average of 73.02 mm and standard deviation of 57.10. The daily average wind strength ranged from 0 to 6 bft with maximal strength noted in March; from May to September, the maximal strength was < 4 bft.

3.1.2. Šibenik Bay

At the stations in Šibenik Bay, the seawater temperature ranged from 2.3 to 28.2 °C, with the highest temperature noted in July and the

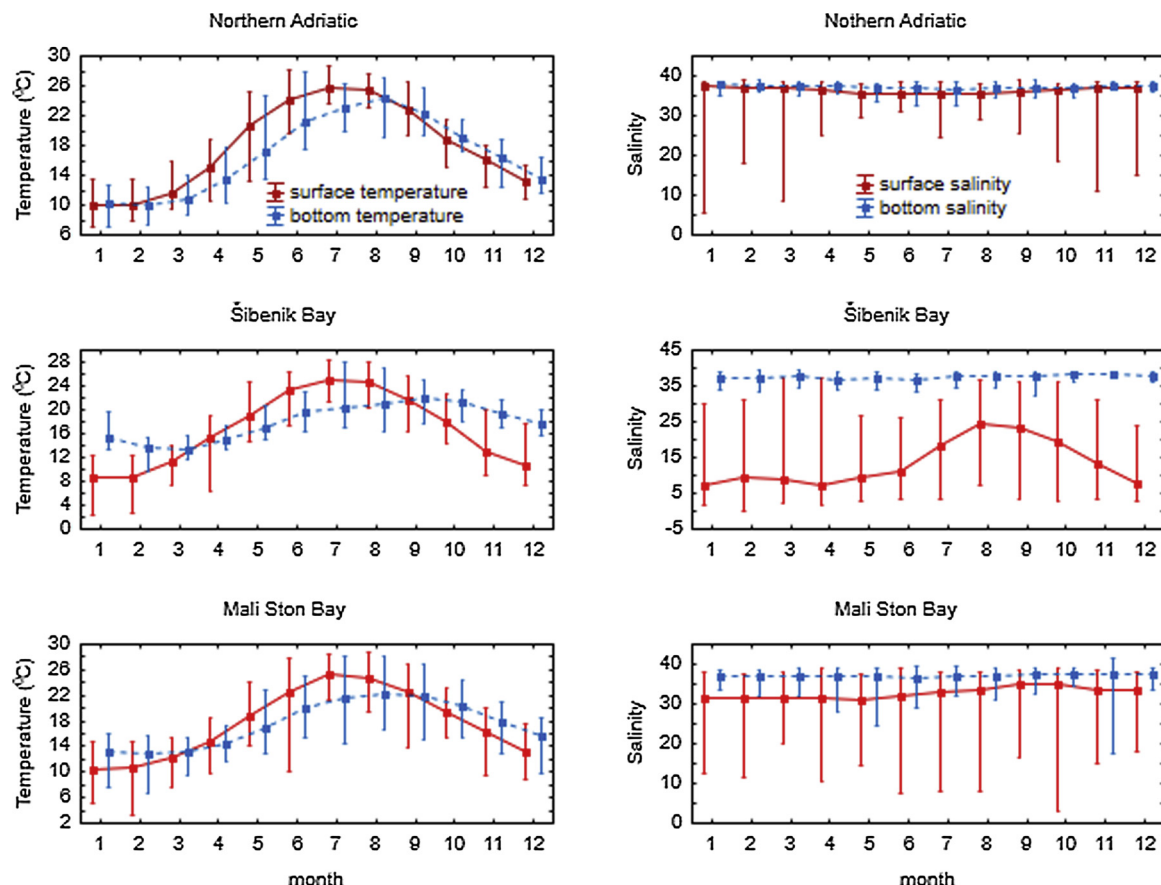


Fig. 2. Temperature and salinity at the surface and the bottom layer at study areas.

lowest in January (Fig. 2). Thermocline developed from May to August, but the depth and gradient were modulated by cold freshwater from the River Krka associated with a strong halocline present throughout the year with the lowest gradient noted in summer and late summer. Temperature inversion was noted from October to February. Salinity ranged from 0.5 to 37 and from 32.4 to 38.8 in the surface and bottom layers, respectively. Cumulative monthly precipitation ranged from 0 mm to 370.1 mm, with average of 70.3 mm and standard deviation of 58.9. The daily average wind strength ranged from 0 to 7.7 bft, with maximal strength in March, November, and December; in summer, the maximal strength was < 6 bft.

3.1.3. Mali Ston Bay

At the stations in Mali Ston Bay, the seawater temperature ranged from 3.1 to 28.8 °C, with the highest temperature noted in July and the lowest in January and February (Fig. 2). Thermocline developed from May to August, but was modulated with the Neretva River freshwater as well as submarine springs. Temperature inversion was noted from October to February. Salinity ranged from 3.1 to 38.8 and from 24.6 to 38.9 in the surface and bottom layers, respectively. Cumulative monthly precipitation ranged from 0 to 382.9 mm, with average of 115.5 mm and standard deviation of 92.6. The daily average wind strength ranged from 0 to 9 bft with maximal strength in January to March, November, and December; in summer, the maximal strength was < 5.5 bft.

3.2. Toxic phytoplankton species

At the investigated area, 25 toxic dinoflagellate species were

identified, and the diatoms *Pseudo-nitzschia* spp. were not determined to the species level in the frame of the monitoring program (Table 2). Most of the dinoflagellate species belonged to genera *Dinophysis*: *D. acuminata* Claparède & Lachmann, 1859; *D. acuta* Ehrenberg, 1839; *D. caudata* Saville-Kent, 1881; *D. fortii* Pavillard, 1923; *D. hastata* Stein, 1883; *D. odiosa* (Pavillard) Tai & Skogsberg, 1934; *D. ovum* F. Schütt, 1895; *D. rapa* (Stein) T.H. Abé, 1967; *D. sacculus* Stein, 1883; and *D. tripos* Gourret, 1883. This was followed by the genus *Alexandrium*: *A. concavum* (Gaarder) Balech, 1985; *A. insuetum* Balech, 1985; *A. minutum* Halim, 1960; *A. ostenfeldii* (Paulsen) Balech & Tangen, 1985; *A. pseudogonyaulax* (Biecheler) Horiguchi ex Kita & Fukuyo, 1992; *A. cf. tamarense* (Lebour, 1925) Balech, 1995; and *A. taylori* Balech, 1994. Other recorded dinoflagellates were *Phalacroma mitra* F. Schütt, 1895; *Ph. rotundatum* (Claparède & Lachmann) Kofoed & Michener, 1911; *Prorocentrum cordatum* (Ostenfeld) J.D. Dodge, 1975; *P. lima* (Ehrenberg) F. Stein, 1878; *Gonyaulax spinifera* (Claparède & Lachmann) Diesing, 1866; *Karenia* sp. G. Hansen & Moestrup, 2000; *Lingulodinium polyedra* (F. Stein) J.D. Dodge, 1989, and *Protoceratium reticulatum* (Claparède & Lachmann) Bütschli, 1885.

Diatoms *Pseudo-nitzschia* spp. and dinoflagellate *D. caudata* were commonly found in all investigated areas, whereas some species appeared more often in certain areas, and some species were rare and poorly represented. Some species are very rare, and their inclusion in the SOM analysis would have resulted with data vectors having zeros (species not found). Therefore, in each investigated area, the seasonal cycle and the relation with environmental factors were analyzed only for those species that represented at least 8 %–10 % of the analyzed samples.

Table 2

Maximal abundance, mean with standard deviation of abundance of toxic phytoplankton species (cells m⁻²) recorded at investigated area. The genus name is abbreviated. A. *Alexandrium*, D. *Dinophysis*, Ph. *Phalacroma*, G. *Gonyaulax*, L. *Lingulodinium*, P. *Prorocentrum*. N = 1585 in Northern Adriatic, N = 971 in Šibenik Bay, N = 1931 in Mali Ston Bay.

Species	Northern Adriatic		Šibenik Bay		Mali Ston Bay	
	Max	Mean (± SD)	Max	Mean (± SD)	Max	Mean (± SD)
<i>A. concavum</i>	7.7 × 10 ³	19 (± 267)	6.6 × 10 ³	28 (± 332)	2.4 × 10 ⁴	273 (± 1.6 × 10 ³)
<i>A. insuetum</i>	3.3 × 10 ³	4 (± 103)	1.1 × 10 ³	1 (± 35)	3.5 × 10 ⁴	58 (± 996)
<i>A. minutum</i>	1.1 × 10 ⁴	76 (± 525)	9.9 × 10 ³	169 (± 781)	3.0 × 10 ⁴	54 (± 726)
<i>A. ostenfeldii</i>	1.1 × 10 ³	1 (± 28)	7.7 × 10 ³	22 (± 366)	4.4 × 10 ²	0.4 (± 10)
<i>A. pseudogonyaulax</i>	4.4 × 10 ³	10 (± 149)	1.9 × 10 ⁴	135 (± 1.1 × 10 ³)	2.2 × 10 ³	5 (± 79)
<i>A.cf. tamarense</i>	1.1 × 10 ⁴	55 (± 449)	3.3 × 10 ⁵	3.2 × 10 ³ (± 1.7 × 10 ⁴)	1.1 × 10 ³	47 (± 326)
<i>A. taylori</i>	3.3 × 10 ³	2 (± 83)	1.6 × 10 ⁴	24 (± 545)		
<i>D. acuminata</i>	1.1 × 10 ⁴	36 (± 427)	5.0 × 10 ⁴	338 (± 2.2 × 10 ³)	1.9 × 10 ⁴	21 (± 444)
<i>D. acuta</i>	3.3 × 10 ³	10 (± 133)	3.5 × 10 ⁴	431 (± 2.0 × 10 ³)	1.1 × 10 ³	7 (± 8)
<i>D. caudata</i>	3.5 × 10 ⁵	7.1 × 10 ³ (± 2.5 × 10 ⁴)	1.1 × 10 ⁵	3.5 × 10 ³ (± 8.2 × 10 ³)	1.7 × 10 ⁵	2.6 × 10 ³ (± 8.1 × 10 ³)
<i>D. fortii</i>	1.6 × 10 ⁶	4.4 × 10 ³ (± 4.8 × 10 ⁴)	2.1 × 10 ⁵	739 (± 7.5 × 10 ³)	5.8 × 10 ⁴	195 (± 1.8 × 10 ³)
<i>D. hastata</i>	1.3 × 10 ⁴	19 (± 692)	8.8 × 10 ³	82 (± 481)	3.3 × 10 ³	37 (± 235)
<i>D. odiosa</i>	2.8 × 10 ⁴	158 (± 833)			1.1 × 10 ³	1 (± 25)
<i>D. ovum</i>	2.2 × 10 ³	4 (± 74)	1.3 × 10 ³	3 (± 56)	1.1 × 10 ³	1 (± 25)
<i>D. rapa</i>	8.8 × 10 ³	32 (± 323)	3.3 × 10 ³	29 (± 220)	1.3 × 10 ⁴	70 (± 596)
<i>D. sacculus</i>	1.8 × 10 ⁵	1.3 × 10 ³ (± 7.3 × 10 ³)	8.8 × 10 ⁴	1.6 × 10 ³ (± 6.0 × 10 ³)	5.5 × 10 ³	124 (± 458)
<i>D. tripos</i>	1.1 × 10 ⁶	5.6 × 10 ³ (± 4.6 × 10 ⁴)	3.1 × 10 ⁵	2.3 × 10 ³ (± 1.6 × 10 ⁴)	2.0 × 10 ⁴	88 (± 756)
<i>Ph. mitra</i>	7.7 × 10 ³	40 (± 368)	3.3 × 10 ³	14 (± 172)	5.5 × 10 ³	22 (± 231)
<i>Ph. rotundatum</i>	2.2 × 10 ⁴	451 (± 1.4 × 10 ³)	7.7 × 10 ³	227 (± 623)	1.1 × 10 ⁴	193 (± 633)
<i>G. spinifera</i>	3.6 × 10 ⁴	328 (± 1.7 × 10 ³)	6.7 × 10 ³	197 (± 707)	6.4 × 10 ⁴	268 (± 1.9 × 10 ³)
<i>Karenia</i> spp.	2.2 × 10 ⁴	49 (± 607)	3.3 × 10 ³	53 (± 312)	3.3 × 10 ³	25 (± 208)
<i>L. polyedra</i>	1.0 × 10 ⁷	7.9 × 10 ³ (± 2.5 × 10 ⁵)	4.3 × 10 ⁶	8.4 × 10 ³ (± 1.6 × 10 ⁵)	5.1 × 10 ⁵	620 (± 6.2 × 10 ⁴)
<i>P. cordatum</i>	1.0 × 10 ⁶	1.7 × 10 ³ (± 3.4 × 10 ⁴)	9.9 × 10 ⁵	2.5 × 10 ⁴ (± 8.9 × 10 ⁴)	6.1 × 10 ⁴	105 (± 2.0 × 10 ³)
<i>P. lima</i>	3.3 × 10 ³	13 (± 161)	2.2 × 10 ³	7 (± 103)	3.9 × 10 ⁴	64 (± 1.1 × 10 ³)
<i>Protoceratium reticulatum</i>	8.8 × 10 ³	47 (± 438)	9.9 × 10 ³	34 (± 383)	3.3 × 10 ⁴	63 (± 983)
<i>Pseudo-nitzschia</i> spp.	5.0 × 10 ⁹	2.1 × 10 ⁷ (± 1.9 × 10 ⁸)	4.8 × 10 ⁸	1.1 × 10 ⁷ (± 3.6 × 10 ⁷)	9.5 × 10 ⁷	2.6 × 10 ⁶ (± 7.4 × 10 ⁶)

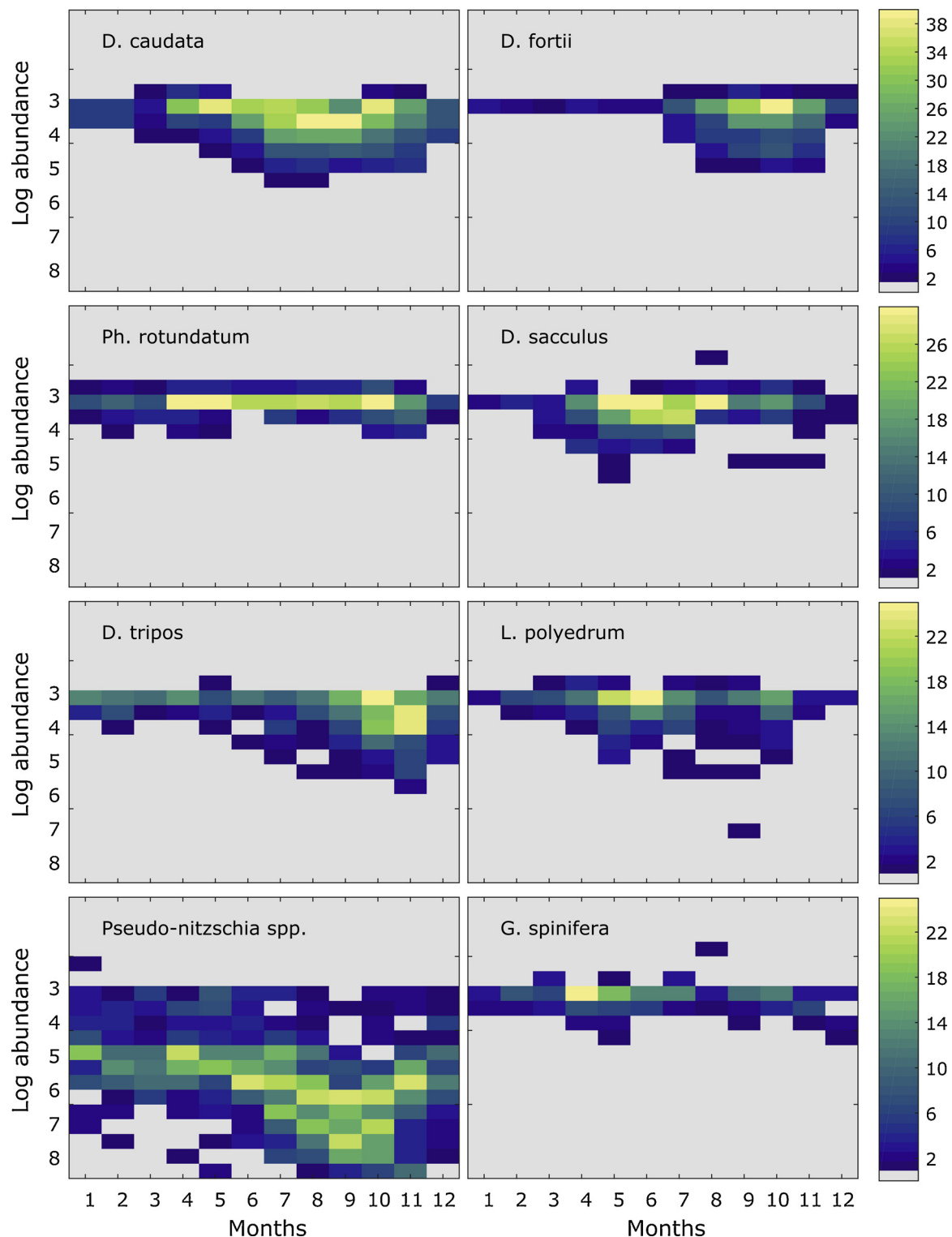


Fig. 3. Seasonal cycle of the abundance of the most common species in the Northern Adriatic. The colours shown in the legend on the right-side represent the normalized numbers of samples with recorded log abundances on the y-axis.

3.3. Seasonal distribution

3.3.1. Northern Adriatic

All the most common species in Northern Adriatic could be found in the water column throughout the year, but their abundance and frequency of occurrence differed (Fig. 3). The incidence of *D. caudata* became higher in April and lasted until October with larger abundances in the summer and autumn periods. *D. fortii* was the most common in

late summer and autumn, whereas *D. sacculus* occurred in spring and summer periods, and *D. tripos* was the most common in the late autumn. *Phalacroma rotundatum* was not noted abundantly and most often occurred between April and October, whereas *L. polyedra* was common during spring. The dinoflagellate *G. spinifera* occurred in low abundances in April. Diatoms *Pseudo-nitzschia* spp. are the most abundant and were present across the year.

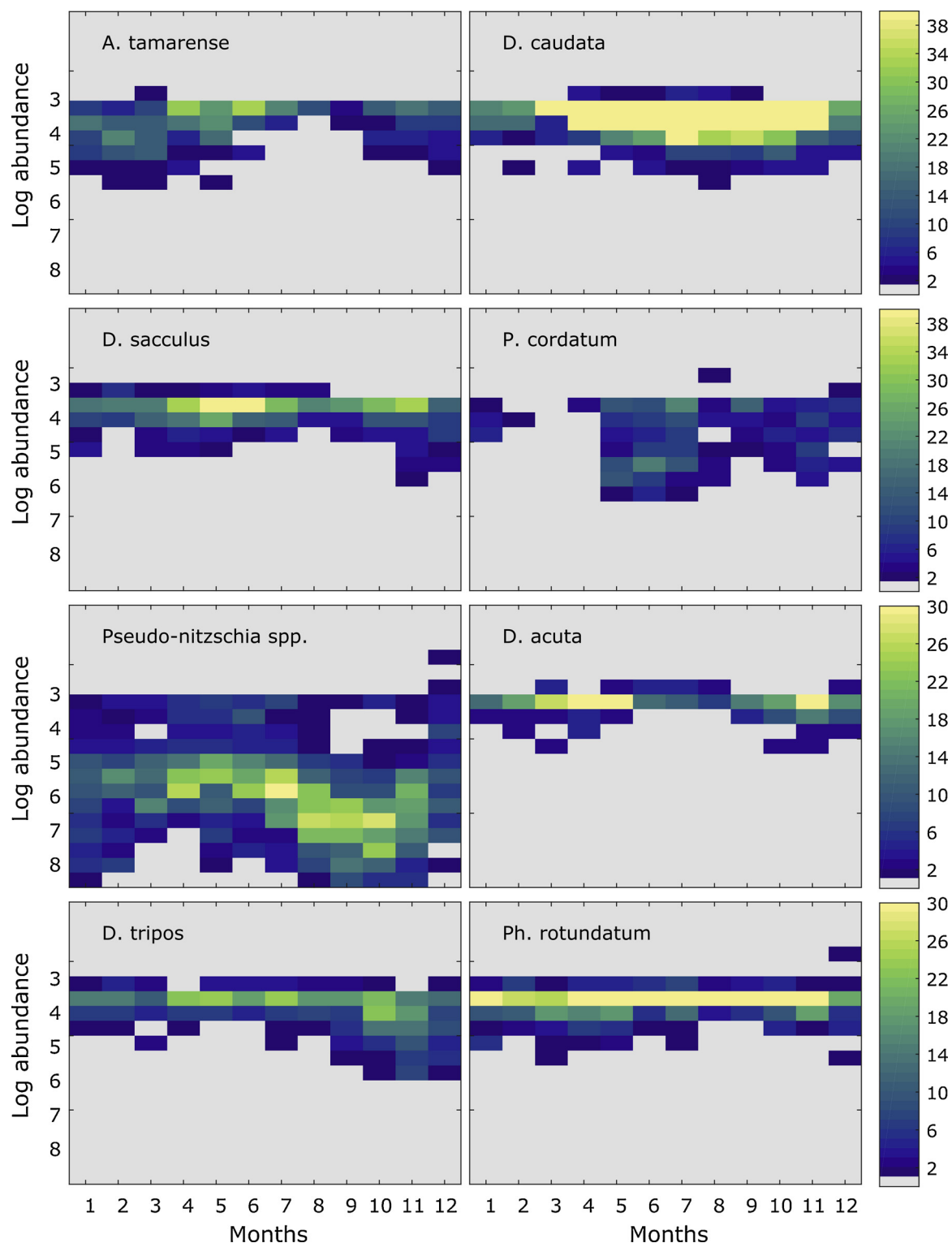


Fig. 4. Seasonal cycle of the abundance of most common species in Šibenik Bay. The colours shown in the legend on the right-side represent the normalized numbers of samples with recorded log abundances on the y-axis.

3.3.2. Šibenik Bay

The species *D. caudata*, *D. sacculus*, *D. tripos*, *Ph. rotundatum*, and *Pseudo-nitzschia* spp. had the same or very similar seasonal cycle as that in Northern Adriatic (Fig. 4). The only difference was noted for *D. caudata*, which begins to appear more frequently a month earlier (March) with abundances uniformly distributed throughout the year without clearly expressed peak abundances. Species of *A. cf. tamarensis*

occurred in higher abundances in the winter or spring and autumn periods with the most often occurrences noted during the spring. *P. cordatum* occurred from May until the end of the year. *D. acuta* was the most common in the spring and autumn periods.

3.3.3. Mali Ston Bay

Species that occurred more frequently in Mali Ston Bay were almost

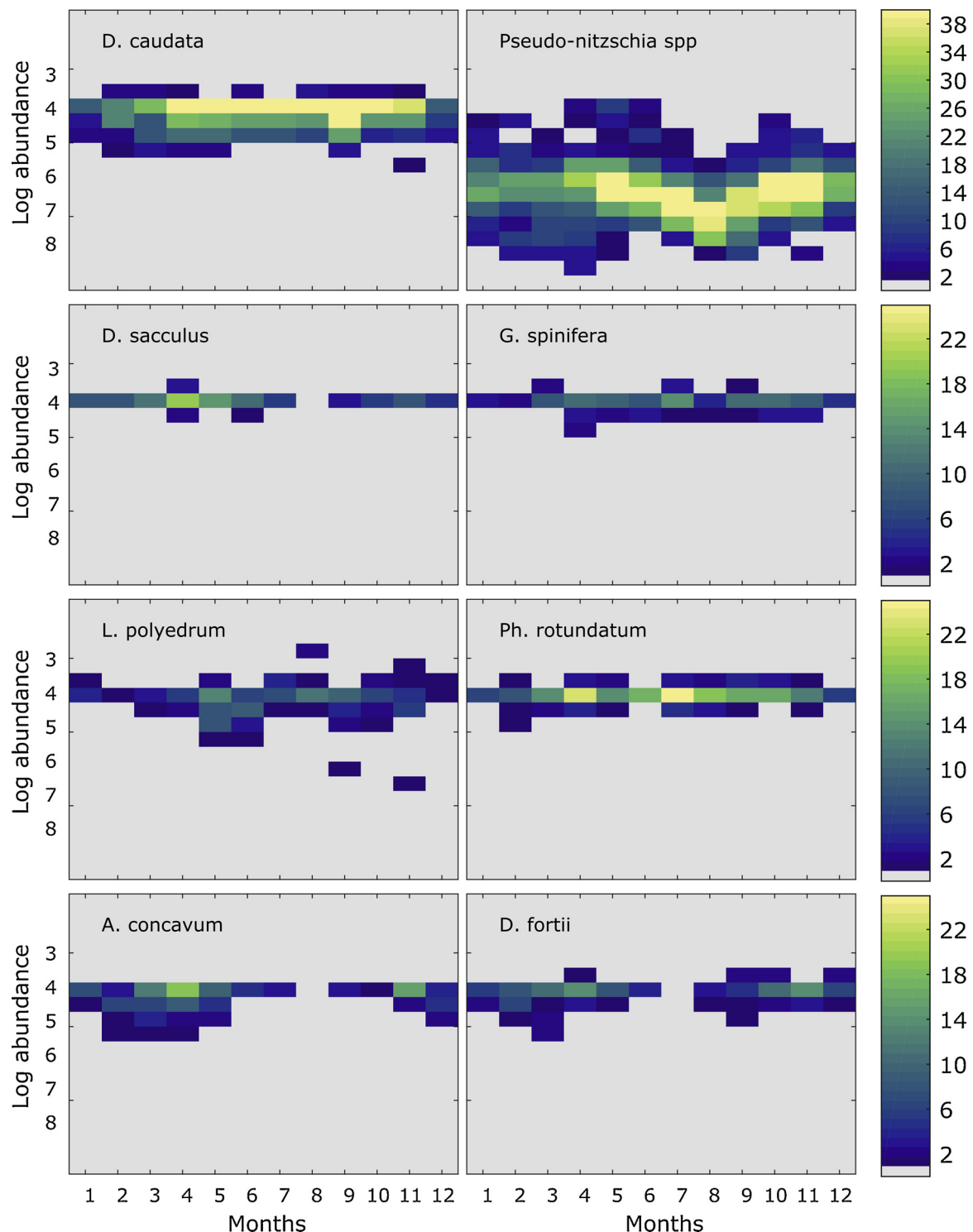


Fig. 5. Seasonal cycle of the abundance of most common species in Mali Ston Bay. The colours shown in the legend on the right-side represent the normalized numbers of samples with recorded log abundances on the y-axis.

the same as those most commonly occurring in the Northern Adriatic (Fig. 5). All species had a similar or the same seasonal cycle, as already recorded in the Northern Adriatic, with slight differences for *D. sacculus* and *D. fortii*. Species *D. sacculus* appeared in lower abundances in Mali Ston Bay with low frequency of occurrence in summer, and *D. fortii* showed pronounced abundance in winter or spring and showed maximum abundance even in autumn. The distribution of *D. caudata* was more similar to that recorded in Šibenik Bay than in the Northern Adriatic because it is uniformly distributed throughout the year. Species

A. concavum, which more frequently occurred in Mali Ston Bay only, showed a spring and slightly weaker autumn maximum, whereas the species was not recorded or was rarely recorded in small abundances during summer.

3.4. Relation with environmental and meteorological factors

Relation between the occurrence of toxic phytoplankton species and environmental and meteorological parameters was analyzed using SOM

Table 3

The most frequent toxic phytoplankton species in Northern Adriatic Sea, environmental and meteorological data organized in nine best matching units (BMU) obtained by SOM analysis. Higher values are bolded while lower values are underlined. (Preci. = precipitation, SST = Sea surface temperature, dT/dz = thermocline gradient, sS/dz = halocline gradient).

BMU	Precipitation	SST	Wind	dT/dz	dS/dz	<i>D. caudata</i>	<i>D. fortii</i>	<i>Ph. rotu</i>	<i>D. sacculus</i>	<i>D. tripos</i>	<i>L. polyedra</i>	<i>Pseudo. spp.</i>	<i>G. spinifera</i>
1	28.61	<u>14.83</u>	0.03	<u>−0.65</u>	2.94	<u>0.64</u>	<u>0.27</u>	<u>0.32</u>	<u>0.48</u>	0.50	<u>0.23</u>	8.71	<u>0.21</u>
2	19.11	15.70	−0.02	−1.11	2.56	<u>1.33</u>	0.39	<u>0.46</u>	0.64	0.47	0.38	4.43	0.26
3	16.46	15.53	0.01	−1.44	2.84	<u>1.16</u>	<u>0.25</u>	0.51	0.79	<u>0.28</u>	0.51	<u>1.16</u>	0.30
4	26.20	16.45	−0.02	<u>−0.76</u>	2.15	2.04	0.75	0.50	<u>0.52</u>	0.80	<u>0.30</u>	9.88	<u>0.23</u>
5	12.73	17.34	−0.14	−1.22	1.91	3.22	0.89	0.69	0.67	0.81	0.44	6.93	0.28
6	<u>8.77</u>	16.39	−0.14	−1.56	2.23	2.13	0.48	0.61	0.79	0.44	0.53	<u>2.50</u>	0.30
7	22.52	18.51	−0.11	<u>−0.88</u>	<u>1.64</u>	4.68	1.58	0.84	0.59	1.35	0.38	11.52	0.27
8	<u>9.70</u>	18.68	<u>−0.24</u>	−1.30	<u>1.78</u>	5.07	1.33	0.95	0.71	1.16	0.46	9.54	0.31
9	<u>4.54</u>	17.62	<u>−0.29</u>	−1.61	<u>1.80</u>	3.70	0.79	0.75	0.74	0.70	0.52	5.76	0.29

analysis separately for each investigated area.

3.4.1. Northern Adriatic

SOM analysis yielded 9 clusters for different combinations of environmental factors as well as analyzed species abundance under these environmental conditions (Table 3); these clusters were noted throughout the year (Fig. 6). The first cluster was characterized by lower temperatures, heavy precipitation, strong halocline, and wind intensity above the average; *Pseudo-nitzschia* species were more abundant than all dinoflagellate species under these conditions. This cluster was present throughout the year, but mostly occurred in May and November with minimum abundance in July and August. The SOM method classified the two characteristic populations of *Pseudo-nitzschia* species, one of which appeared in the colder and the other which appeared in the warmer part of the year (Fig. 6, Table 3). Both populations favored more precipitation, whereas their abundances were the lowest during stratification.

Among dinoflagellates, two groups were the most abundant across different seasons. One group described by clusters BMU 7 and BMU 8 included co-occurring species *D. caudata*, *D. fortii*, *D. tripos*, and *Ph. rotundatum*, whereas the second group described by clusters BMU 3 and BMU 6 included *L. polyedra* and *G. spinifera*. Species *D. caudata*, *D. fortii*, *D. tripos*, and *Ph. rotundatum* mostly occurred from July to November during the warm weather; however, within this group, two subgroups could be differentiated. *D. fortii* and *D. tripos* were more abundant when precipitation level and temperature were higher (cluster BMU 7),

whereas *D. caudata* and *Ph. rotundatum* were more abundant when higher temperature was associated with calm weather (cluster BMU 8). *D. sacculus* was abundant during the prevalence of both dinoflagellate groups. *L. polyedra* and *G. spinifera* better tolerate turbulence than *Dinophysis* species and occurred with high abundances when the wind intensity was above average, as described by cluster BMU 3. *Dinophysis* species were the most abundant during warm and calm weather, as described by clusters BMU 7 and BMU 8. *D. sacculus* was the most related to water column stability, as described by clusters BMU 3, BMU 6, BMU 8, and BMU 9. *D. sacculus* and *Lingulodinium polyedrum* co-occurred during moderate temperature, and the amount of precipitation differed within that optimum temperature range. The highest occurrence of these species was noted when precipitation was higher but temperature was lower, and vice versa; these conditions could be related with the stability of the water column.

Individual analyses of each species with environmental parameters are shown in Appendix I. The development of *D. caudata* confirmed its dependence on temperature, whereas the development of *D. fortii*, *Ph. rotundatum*, and *D. tripos* depended on temperature as well as precipitation, which plays an important role in enabling them to develop a more dense population even at lower temperatures and higher wind intensity, as described by the clusters in the tables for the corresponding species (Appendix I).

The lowest sea surface temperature at which dinoflagellate species appeared in seawater column was above 8 °C except *D. tripos*, which occurred below 8 °C. *D. sacculus* and *L. polyedra* appeared at temperatures above 9 °C. The abundance of *D. tripos*, *D. fortii*, and *D. sacculus* was higher at temperatures above 15 °C, whereas that of *D. caudata* was higher above 19 °C.

Considering the stations, the abundance of *D. fortii*, *D. sacculus*, *D. tripos*, and *L. polyedra* was the highest in Lim Bay (P3), whereas that of *D. caudata*, *Ph. rotundatum*, and *G. spinifera* was the highest at station P6.

Satellite Chl *a* patterns (Fig. 7) on the surface layer showed distribution according to the seasonal phytoplankton cycle in the Adriatic Sea, which is characterized by spring (BMU 1, BMU 3) and autumn (BMU 1, BMU 7) maximum, resulting in a mixed column of water and sufficient amount of nutrients in addition to sufficient light. The summer period (BMU 9) was characterized by a small concentration of chlorophyll *a* owing to the consumption of nutrients during spring bloom and stratification that prevented the dispersion of nutrients from the sediment layer. *Pseudo-nitzschia* spp. as autotrophic species were more abundant in spring and autumn during the mixed water column (BMU 1, BMU 7). Mixotrophic species of the genus *Dinophysis* and *Ph. rotundatum* were the most common and abundant when the chlorophyll concentration was high (BMU 7; Fig. 7).

3.4.2. Šibenik Bay

The SOM analysis created 9 clusters within which, the rate of precipitation, river flow, wind, thermocline, and halocline gradually

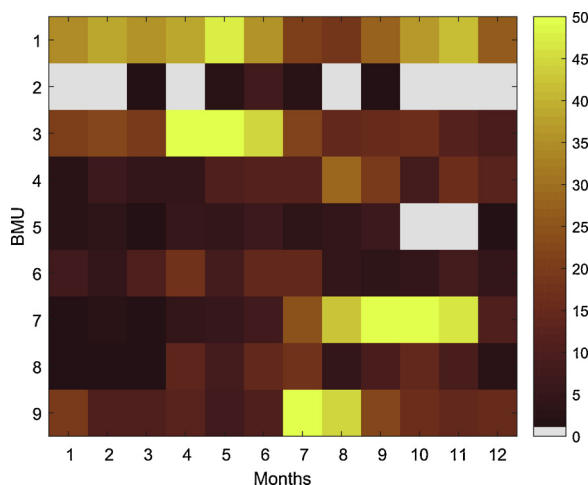


Fig. 6. The appearance of the Best Matching Units (BMU) solution over 12 months for the SOM model in Northern Adriatic. The colours shown in the legend on the right-side represent the normalised numbers of occurrence of each BMU in different months during the study period. Environmental and meteorological conditions and abundances of the most frequent species associated with each BMU are described in Table 3.

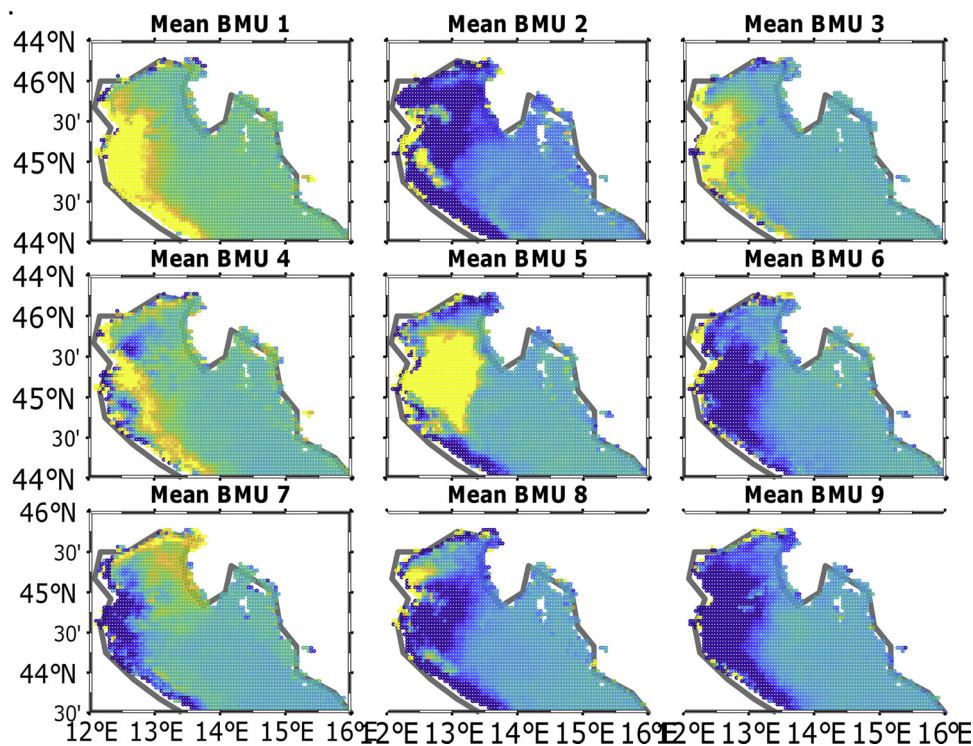


Fig. 7. The satellite Chl a patterns during the each of Best Matching Units (BMUs) in the Northern Adriatic obtained by SOM model. A description of each BMU, containing the environmental conditions and abundances of the most common toxic species, is provided in Table 3.

Table 4

The most frequent toxic phytoplankton species in Šibenik Bay, environmental and meteorological data organized in nine best matching units (BMU) obtained by SOM analysis. (Preci. = precipitation, SST = Sea surface temperature, dT/dz = thermocline gradient, sS/dz = halocline gradient, *Pseudo. spp.* = *Pseudo-nitzschia* spp., *Ph. rotu.* = *Ph. rotundatum*).

BMU	Preci.	Wind	River flow	SST	dT/dz	sS/dz	<i>A. cf. tamarens</i>	<i>D. caudata</i>	<i>D. sacculus</i>	<i>P. cordatum</i>	<i>Pseudo. spp.</i>	<i>D. acuta</i>	<i>D. tripos</i>	<i>Ph. rotu.</i>
1	24,78	0,06	239,75	15,00	-2,18	21,08	0,76	1,93	0,50	0,15	0,40	0,33	0,31	1,04
2	12,40	-0,02	157,31	16,01	-1,26	18,48	0,56	2,22	0,55	0,24	1,15	0,29	0,36	0,92
3	5,04	-0,08	103,64	16,99	-0,28	17,31	0,48	2,47	0,46	0,20	0,46	0,28	0,34	1,12
4	26,46	0,04	231,80	15,12	-2,18	19,22	0,61	1,99	0,61	0,30	2,80	0,30	0,45	0,72
5	14,80	-0,02	155,93	16,27	-1,21	16,15	0,43	2,62	0,77	0,47	6,21	0,27	0,48	0,60
6	5,95	-0,08	106,36	17,16	-0,24	15,81	0,39	2,77	0,66	0,38	2,96	0,25	0,38	0,81
7	28,98	0,03	216,22	15,40	-2,11	16,73	0,48	2,33	0,77	0,50	9,64	0,30	0,61	0,48
8	17,19	-0,02	151,06	16,59	-1,11	14,46	0,37	3,23	0,95	0,65	12,05	0,28	0,56	0,47
9	7,81	-0,07	111,97	17,48	-0,21	13,98	0,32	3,49	0,94	0,62	9,65	0,24	0,43	0,56

decreased while the temperature increased (Table 4). These gradual changes in environmental factors were observed between clusters BMU 1 to BMU 3, BMU 4 to BMU 6, and BMU 7 to BMU 9. The frequency of occurrence of these clusters throughout the year is shown in Fig. 8. SOM analysis revealed strong relation between precipitation and abundance of *A. cf. tamarens* (BMU 1, BMU 4, and BMU 7) and a somewhat weaker relation of precipitation rate with *D. acuta*, which also has the highest abundance in these clusters. During the greater precipitation, the abundances of the above species were higher during the greater flow of the River Krka and stronger stratification. They better tolerated wind than the other species, and their abundance was more related to an increase in precipitation than in temperature. In contrast, *D. caudata* was associated with rising temperatures within these cluster groups. In addition, the abundance of *D. caudata*, *D. sacculus*, *D. tripos*, and *P. cordatum* increased with the reduction of the halocline, which is strong and constantly present in Šibenik Bay, and the highest abundances were noted for clusters BMU 8 and BMU 9. Within this group, *D. tripos* differs because, along with the weaker halocline, it prefers a higher precipitation rate and thus is the most numerous in the environmental conditions described by the BMU 7 and

BMU 8 clusters. Unlike *D. caudata*, *D. sacculus*, *D. tripos*, *P. cordatum*, and *Pseudo-nitzschia* spp., *Ph. rotundatum* was the most abundant under the conditions described by clusters BMU 1 to BMU 3, which are characterized by a strong halocline. *Pseudo-nitzschia* spp. are the most abundant during the weaker halocline.

Individual analyses of each species with environmental parameters revealed the dependence of *A. cf. tamarens* and *D. acuta* on river flow rather than on precipitation amount (Appendix II). The relation of *Pseudo-nitzschia* spp., *D. caudata*, and *D. tripos* was confirmed using SOM analyses of all investigated species (Table 4). These analyses showed strong relation of *D. sacculus* with stratification condition, although this was not apparent by the joint analysis with other species (Table 4). Similarly, *Ph. rotundatum* was associated with a strong halocline in the joint SOM analysis, whereas it was related with a weaker halocline in individual analyses (Appendix II). *P. cordatum* showed relation with river flow and lower and moderate wind. This disagreement between SOM analyses of individual species (Appendix II) and a joint analysis of all species (Table 4) may be attributed to the difference in signal strength when analyzing optimal environmental conditions of one species versus those of more species in an equal number of clusters. For

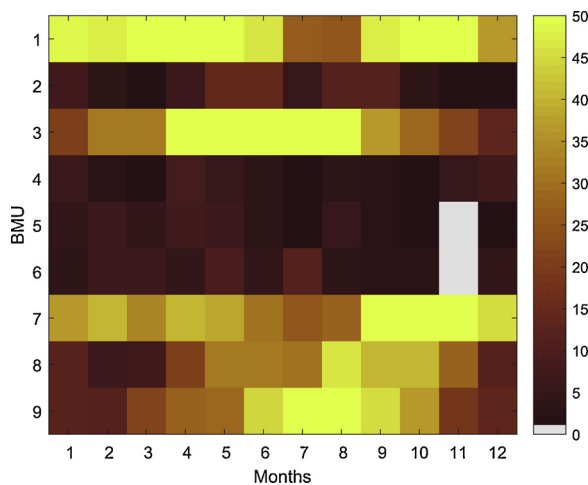


Fig. 8. The appearance of the Best Matching Units (BMU) solution over 12 months for the SOM model in Šibenik Bay. The colours shown in the legend on the right-side represent the normalized numbers of occurrence of each BMU in different months during the study period. Environmental and meteorological conditions and abundances of the most frequent species associated with each BMU are described in Table 4.

example, the abundance of *D. sacculus* has been shown to be dependent on stratification condition; however, during the analysis with other species in Šibenik Bay where permanent halocline is present and stratification is not a limiting factor in the development of the population of this species, this signal was weaker than those of other species, which indicated the association of these species with their limiting factors.

All species in the samples were recorded at extremely low sea surface temperatures (5 °C) because of freshwater inflow into the surface layer.

3.4.3. Mali Ston Bay

The SOM analysis revealed 9 clusters with a gradual increase in precipitation rate, river flow, and halocline and a decrease in temperature and thermocline. Wind speed in relation to average gradually increased in clusters BMU 1 to BMU 6 and then slightly decreased up to BMU 9. *D. caudata* was associated with lower temperatures (BMU 9), in contrast to the results obtained in the Northern Adriatic and Šibenik Bay. The analysis revealed the existence of two populations of *Pseudo-nitzschia* spp., one characteristic of summer (BMU 1, BMU 2, and BMU 3) and another characteristic of lower temperatures and occurring in winter, spring, and autumn (BMU 8 and BMU 9). The association of *D. sacculus* was also confirmed with the stratification condition (BMU 8 and BMU 9), with the lowest abundance noted during the weak thermocline and halocline (BMU 4 and BMU 5). Two groups of dinoflagellates occurred under different environmental conditions. One group includes *G. spinifera*, *L. polyedra*, and *Ph. rotundatum*. These species are more abundant in summer, with their abundance increasing with temperatures above 20 °C and below the average wind speed (BMU 2 and BMU 3). The second group includes *D. caudata*, *D. sacculus*, *D. fortii*, and *A. concavum*, which are more numerous during higher precipitation and river flows and a well-developed halocline. Within the latter group, *A. concavum* and *D. fortii* are more commonly associated with each other, clearly related with precipitation and river flow (BMU 7–BMU 9). The SOM analysis also showed that *Ph. rotundatum* has two populations: summer (BMU 1–BMU 3) and winter or spring population (BMU 9), which occur together with the dinoflagellates from the second group mentioned above, whereas the summer population occurs in diametrically opposite environmental conditions.

Individual analyses of each species with environmental parameters (Appendix III) confirmed the above-mentioned results obtained by the joint analysis of all species (Table 5).

The lowest sea surface temperature at which *D. caudata*, *D. sacculus*, *L. polyedra*, and *A. concavum* appeared in seawater column was above 6 °C, whereas *G. spinifera* appeared at a sea surface temperature above 9 °C. *Pseudo-nitzschia* spp., *D. fortii*, and *Ph. rotundatum* appeared in the water column during sea surface temperature below 6 °C. *A. concavum* was not recorded in the water column at a surface temperature above 24 °C, and *D. fortii* was not recorded above 26 °C, whereas other species were recorded up to the maximum recorded temperatures in the surface layer.

3.5. Trends in the occurrence of toxic phytoplankton species

The trend statistics of the selected phytoplankton species in the analyzed period were calculated separately for each investigated area. Mann–Kendall test revealed a statistically significant ($p < 0.005$) trend of increasing abundance of 1.8×10^5 cells L^{-1} between 2004 and 2018 for *Pseudo-nitzschia* spp. in the Northern Adriatic and Šibenik bay. In addition, statistically significant ($p < 0.005$), but small decreasing trend, was noted for *D. caudata* abundance (approximately 200 cells L^{-1}) in all the three study areas.

3.6. Link with the NAO index

3.6.1. Northern Adriatic

Spearman rank correlation showed statistically significant correlation between NAO index and precipitation ($r = -0.26$; $p < 0.05$), although the correlation changed during the season. Significant negative correlations were recorded in spring and autumn, whereas positive correlation was observed during summer. Because of the strong non-linear effect, the influence of NAO was detected using SOM analysis (Fig. 10A). Cluster BMU 1 was linked to the positive phase of the NAO index, whereas the mutually opposite cluster BMU 9 was related to the negative phase. Unexpectedly, a higher amount of precipitation was associated with the positive phase of the NAO index and vice versa. Similar situations were noted for the other clusters such as clusters BMU 7 and BMU 8, which describe the environmental conditions for *Dinophysis* species where cluster BMU 7 with higher precipitation amounts occurred during the positive NAO index.

3.6.2. Šibenik Bay

Spearman rank correlation showed negative, but non-significant correlation between NAO index and precipitation. Significant correlation was recorded for the winter period (January–March; $r = -0.33$; $p < 0.05$), whereas the correlation was positive, but non-significant in summer. In Šibenik Bay, larger precipitation levels were observed during neutral (BMU 1), negative (BMU 4), and positive (BMU 7) NAO index (Fig. 10B), indicating non-linear NAO effect.

3.6.3. Mali Ston Bay

Spearman rank correlation showed negative, but non-significant correlation between NAO index and precipitation. Significant correlation was recorded for winter (January–March; $r = -0.30$; $p < 0.05$) and autumn periods (October–December; $r = -0.44$; $p < 0.05$), whereas the correlation was positive, but non-significant in summer. In the clusters characterized by higher precipitation rate (BMU 7, BMU 8, and BMU 9), the first seven days were characterized by a positive to neutral NAO index, followed by a negative to neutral NAO index by the twelfth day (Fig. 10C).

4. Discussion

This study analyzed the occurrence of toxic phytoplankton species in relation to environmental and meteorological factors in three areas with different hydromorphological characteristics located along the Croatian coast.

Table 5

The most frequent toxic phytoplankton species in Mali Ston Bay, environmental and meteorological data organized in nine best matching units (BMU) obtained by SOM analysis. Higher values are bolded while lower values are underlined. (Preci. = precipitation, SST = Sea surface temperature, dT/dz = thermocline gradient, sS/dz = halocline gradient, *Pseudo. spp.* = *Pseudo-nitzschia* spp., *Ph. rotu.* = *Ph. rotundatum*).

BMU	Preci.	Wind	River flow	SST	dT/dz	sS/dz	<i>D. caudata</i>	<i>Pseudo. spp.</i>	<i>D. sacculus</i>	<i>G. spinifera</i>	<i>L. polyedra</i>	<i>Ph. rotu.</i>	<i>A. concavum</i>	<i>D. fortii</i>
1	<u>0,46</u>	<u>-0,21</u>	<u>103,81</u>	21,01	1,39	-3,03	2,12	11,29	0,16	0,26	0,26	0,41	<u>0,09</u>	<u>0,07</u>
2	<u>1,61</u>	-0,14	<u>103,72</u>	20,99	1,32	-2,92	2,93	11,55	0,17	0,31	0,32	0,43	<u>0,11</u>	<u>0,06</u>
3	5,80	-0,05	<u>101,24</u>	20,88	1,13	<u>-2,72</u>	2,65	11,57	0,15	0,35	0,33	0,42	<u>0,09</u>	<u>0,06</u>
4	13,06	-0,01	110,27	19,44	0,57	-2,74	085	10,35	<u>0,13</u>	0,29	0,21	0,33	0,06	0,09
5	23,67	0,00	148,92	16,84	<u>-0,22</u>	-3,18	<u>0,14</u>	8,83	<u>0,15</u>	0,21	0,11	0,22	0,08	0,13
6	43,51	0,03	220,04	15,01	-0,85	-4,13	<u>0,05</u>	8,61	0,17	<u>0,17</u>	<u>0,08</u>	<u>0,15</u>	0,13	0,17
7	67,79	0,05	286,55	<u>14,32</u>	-1,16	-5,07	0,42	9,54	0,19	<u>0,17</u>	<u>0,09</u>	<u>0,15</u>	0,23	0,23
8	68,36	-0,01	266,11	<u>14,99</u>	-0,87	-5,29	2,95	10,55	0,27	0,21	0,14	0,26	0,33	0,27
9	59,14	-0,09	229,16	15,85	-0,49	-5,06	6,72	10,84	0,36	0,24	0,18	0,35	0,36	0,28

4.1. Relationship between environmental variables in SOM analysis

The SOM analysis, used for its ability to detect nonlinear relationships, revealed different ecological preferences between the studied species. Its advantages over other multivariate techniques is that the algorithm is robust in handling missing and spike data, which is a common feature of the time series of harmful algae. In addition, the analysis does not require any a priori assumption on the distribution of data (Richardson et al., 2003). According to Richardson et al. (2003), the SOM array is similar to a combination of cluster analysis and multidimensional scaling (MDS) where patterns in the SOM analysis match the clusters. However, SOM analysis captures relationships among all clusters by placing them in a two-dimensional space while the relative positions of the groups in the dendrograms are arbitrary. Both MDS and principal component analysis efficiently capture the relationships among samples, but do not explicitly define groups. Furthermore, SOM analysis allows the clear visualization of data and monitoring of particular events by using the trajectory through time. It also provides insights into the frequency through time of a particular node. Studied environmental characteristics are the inversion links between temperature and precipitation: increase in sea surface temperature is followed by a decrease of precipitation, and vice versa. Only exception was noted for BMU 7 in the Istria SOM model, in which very high temperature was associated with high precipitation. Another environmental characteristic is the synchronized connection between precipitation and river flow in Šibenik Bay where the maximum of precipitation was followed by maximum river flow. In Mali Ston Bay, no such synchronized connection was noted between precipitation and river flow connected with the same BMU, because of the relatively large distance between Neretva delta and Mali Ston Bay sampling stations (Fig. 1).

The analyzed areas are under the influence of freshwater inflow so the halocline is present in all the BMUs. The thermocline is formed during the warm part of the year (Istra), but it can be strongly modified (Mali Ston Bay) or completely destroyed (Šibenik Bay) by the presence of colder river water.

The wind speed is the most variable parameter; in general, low wind speed is connected with low precipitation, river flow, and high temperature, but many nonlinear effects occur in all three SOM models, isolating wind speed from the rest of the environmental variables.

4.2. Relationship between toxic phytoplankton species with environmental variables in SOM analysis

In this study, the relationship of 11 dinoflagellate species and diatoms of *Pseudo-nitzschia* spp. with environmental and meteorological conditions was analyzed. Among the species analyzed, the most common species such as *D. caudata*, *D. sacculus*, *Ph. rotundatum*, and *Pseudo-nitzschia* spp. were present in all the three study areas, followed by *D. fortii*, *L. polyedra*, and *G. spinifera*, which were more common in

the Northern Adriatic and Mali Ston Bay. *D. tripos* occurred more frequently in Northern Adriatic and Šibenik Bay, and *A. cf. tamarense*, *D. acuta*, and *P. cordatum* species occurred more frequently only in Šibenik Bay; *A. concavum* occurred more frequently in Mali Ston Bay.

Diatoms of the genus *Pseudo-nitzschia* were clearly separated from dinoflagellates; in accordance with Margalef's mandala (Margalef, 1978; Margalef et al., 1979; Glibert, 2016), they showed better tolerance to the turbulence required to keep them in a suspended state, preferred a mixed column of water, and occurred most commonly in the spring and autumn. In Northern Adriatic and Mali Ston Bay, they were associated with higher precipitation and river flow because of their need for nutrient-rich conditions, particularly for the silicon needed to build the cell wall. The same results were obtained in previous studies, which showed that the bloom of *Pseudo-nitzschia* followed a period of sustained precipitation, elevated river flow, and persistent southeasterly winds (Trainer et al., 2007, 1998). These conditions cause an increased concentration of nutrients that favor the development of diatoms of *Pseudo-nitzschia* spp. The results are also confirmed by a study performed along the west coast of South Africa, where upwelling-favorable winds favor the development of *Pseudo-nitzschia* diatoms, whereas periods of weak winds were associated with warmer surface waters, strengthening stratification and dinoflagellate, including *Dinophysis* species, dominance (Lucas et al., 2014). These results indicate that the stability of the water column is an important indicator of the dominance of a particular taxonomic group of phytoplankton. These differences result from not only the available nutrient concentration but also the origin of nutrients. That is, during the mixing period, the regenerated nutrients are responsible for the so-called new production, whereas the stratification is dominated by recycled nutrients preferred by dinoflagellates (Glibert, 2016). In Šibenik Bay, which is characterized by permanent halocline, because of the strong influence of the River Krka, this connection was not observed. The highest abundances were noted during the weakest halocline in summer and late summer. This is probably because of the higher amount of nitrogen and silicon nutrients in Šibenik Bay than in Mali Ston Bay (Bužančić, 2013; Ninčević et al., 2015), which remains high throughout the year to support the growth of *Pseudo-nitzschia* spp. The SOM analysis revealed the existence of two populations of *Pseudo-nitzschia* spp. in Northern Adriatic and Mali Ston Bay that differed in their temperature preferences. These findings can be explained by the existence of different species within *Pseudo-nitzschia* spp. The same results were obtained in Northern Adriatic by Ljubešić et al. (2011), who found that *P. calliantha* and *P. manii* dominated the summer bloom, whereas *P. fraudulenta* and *P. pungens* species dominated the winter bloom. In Šibenik Bay, *P. calliantha* and *P. pseudodelicatissima* were the most abundant in the winter bloom (Arapov et al., 2016). Blooming of *P. calliantha* species has been documented in both warmer and colder parts of the year (Caroppo et al., 2005; Burić et al., 2008), indicating their tolerance over a wide temperature range.

Among the harmful dinoflagellates, diverse habitat preferences

were observed, which led to the formation of a group of species according to the environmental preference. Almost the same species of dinoflagellates occurred more frequently in Northern Adriatic and Mali Ston Bay, whereas the assemblage of harmful dinoflagellates in Šibenik Bay differed owing to the specific environmental conditions, confirming the first assembly rule that specific habitat conditions select for specific life forms (Smayda and Reynolds, 2003).

The SOM model distinguished groups of species belonging to different life-forms and having different adaptive strategies for survival, which was developed by Smayda and Reynolds (2003). These authors introduce three types of strategists: fast-growing, high-surface-to-volume colonist species (C); the acquisitive, large, slow-growing nutrient stress-tolerant species (S); and tolerant of shear/stress in physically disturbed water masses (R). Within the abovementioned basic strategies, classical r- and K-strategies can be distinguished.

In the Northern Adriatic and Mali Ston Bay, the SOM model separated (S) strategist *Dinophysis* species from (R) strategist *G. spinifera* and *L. polyedra*. These species occupied different niches in different areas. In Northern Adriatic, *L. polyedra* and *G. spinifera* occurred in slightly increased abundances during elevated turbulence (wind above average, BMU 3), but also during stratification together with *Dinophysis* (Table 3), confirming that these species are (R) strategists that endure diminished but pronounced vertical mixing. According to their growth and nutritional requirements, these forms of taxa are between diatoms and other dinoflagellates, which prefer strong stratification conditions and tolerate smaller amounts of nutrients (e.g., *Dinophysis*). In Mali Ston Bay, elevated abundances of these species have been recorded at higher temperatures and below-average wind intensities. These species were possibly located within actively mixed or circulating water layers owing to estuarine circulation and continued to grow with increasing temperature. Despite the stated differences and ecological preferences of each species, all dinoflagellates were more abundant during stratification, which was mainly caused by the freshwater inflow in the study area. Previous studies have shown the dominance of dinoflagellates during stratification and have explained it as accumulation of cells (*Dinophysis*) on the pycnocline (Maestrini, 1998; Reguera et al., 2003; Ninčević-Gladan et al., 2008) or as an advantage for swimmers (Peacock and Kudela, 2014). More recent studies have shown that stratification also promotes the intensification of the pycnocline, which potentially leads to the formation of thin layers. In these layers that diversify and expand the available ecological niche space, planktonic organisms accumulate (Sullivan, 2010 and references therein). Thin phytoplankton layers are often dominated by high concentrations of a single species, including numerous harmful algal species, indicating that the understanding of these thin layers could be important for HAB research (Sullivan, 2010 and references therein). The occurrence of *Dinophysis* species in sub-surface layers in Irish waters was explained by gyrotactic trapping at various densities during the vertical migration of these species with the subsequent increases in densities (Raine, 2014). In the Bay of Biscay, retentive eddies were associated with *D. acuminata* accumulation (Lazure et al., 2009). The maximal abundance of *Dinophysis* species in the pycnocline layer was reported in Galician Rias (Reguera et al., 2012); Thermaikos Bay, Greece (Koukaras and Nikolaidis, 2004); Chilean (Díaz et al., 2011); and Swedish fjords (Lindahl et al., 2007). Even the diatoms of *Pseudo-nitzschia* spp. are commonly found in layers (Ryan et al., 2010). The bloom of *L. polyedrum* in Baja California Bay was thought to be formed and maintained by a combination of temperature stratifications and sea breeze (Ruiz-de la Torre et al., 2013). Increased abundances of this species during thermal stratification were also observed during this study in Mali Ston Bay. A review by Berdalet et al. (2014) indicated that stratification acts as a barrier against the mixing of adjacent layers, allowing the vertical distribution of different phytoplankton. In addition, retentive environments such as investigated semi-closed bays increase the water residence time, allowing the creation of distinct physical conditions, which can strongly influence the ecology of HABs. These layers also

provide high prey concentration for mixotrophic species such as *Dinophysis* spp. (Velo-Suárez et al., 2008). The elevated abundances of *D. fortii* and *D. acuta* in the estuary area were associated with higher river flows and precipitation, which could be related to the mixotrophic nutrition of these species. They feed on the ciliate *Mesodinium rubrum*, which feeds on cryptophytes (Park et al., 2006). That is, nutrients brought in by the river could support cryptophyte blooms recorded in the Krka River estuary (Öupraha et al., 2014), which further supports the growth of *M. rubrum* (Peterson et al., 2013). Most of the dinoflagellate species are mixotrophic, but have different feeding rates. In the Northern Adriatic, high abundance of *D. fortii* was associated with higher temperatures and higher precipitation, which are specific conditions in the area (BMU 7; Table 3). These results suggest that higher precipitation in summer and late summer in the Northern Adriatic could cause an increase in *D. fortii* abundance.

Increased abundances of *Alexandrium* species characterized the highly stratified Šibenik Bay, which is under strong influence of the River Krka. In addition, high abundances of the cysts of *Alexandrium* species were also identified in Šibenik Bay (Di Poi et al., 2019). The strong association of these species with freshwater has been well documented (Anderson et al., 2005; Fauchot et al., 2005, 2008); furthermore, the importance of the existence of cyst seabeds for their bloom dynamics has been reported (Anderson et al., 2005; McGillicuddy et al., 2005). The estuarine circulation could inoculate surface waters with newly germinated cells. In addition, nutrient-rich freshwater stimulates cell growth and generates stratification conditions that favor the proliferation and accumulation of *Alexandrium* cells, which are linked to particular water masses. The bloom dynamics of *Alexandrium* species in estuarine areas have not yet been completely elucidated, but their ability to swim, vertically migrate, or physiologically adapt are the most important features for understanding these aspects (Anderson et al., 2012). Many studies indicate the importance of the proper combination of meteorological, hydrological, and biological behaviors as the main factors that lead to the development of *Alexandrium* bloom. For example, in St Lawrence Bay (Canada), *Alexandrium* bloom is associated with maximum water stability, low nutrients and winds, and high surface temperature (Therriault et al., 1985), which is below 15 °C in that area. The review by Anderson et al. (2012) found the fastest growth of *Alexandrium* species at 15–20 °C under laboratory condition. Larger blooms (> 800 cells L⁻¹) observed in Brisbane Water occurred between 16 and 20 °C (Farrell et al., 2013). In our study (Šibenik Bay), high abundances occurred at surface temperatures between 15 and –17.48 °C, with maximum abundances noted at about 15 °C along with the strongest rainfall, river inflow, and stratification. Spring appearance of *Alexandrium* bloom with increased rainfall, freshwater inflow, and water column stabilization was reported for the Mediterranean lagoon by Giacobbe et al. (1996). The largest bloom (22,000 cells L⁻¹) of *A. catenella* reported at the Georges River occurred after a period of intense rainfall (Farrell et al., 2013). The importance of stratification and vertical migration for bloom maintenance is reported for Salt Pond by Anderson and Stolzenbach (1985).

4.3. Link with NAO index

Many studies showed the influence of the North Atlantic Oscillation on Mediterranean and Adriatic Sea climate, which is mostly related to air temperature and precipitation rate in the Northern hemisphere (Hurrell, 1995, 1996; Hurrell and Loon, 1997; Trigo et al., 2002, 2004; Grbec et al., 2009, 2018; Matić et al., 2019). These studies showed that the positive phase of the NAO index in the winter months was related to higher temperatures and lower precipitation, whereas the negative phase was associated with lower temperatures and greater precipitation in the Mediterranean. The nonlinear influence of NAO on environmental variables over the Adriatic Sea was analyzed by Matić et al. (2019), and all combinations between positive or negative anomalies were found. In the Northern Adriatic, out of four of the most commonly

reported clusters (BMU 1, BMU 3, BMU 7, and BMU 9; Fig. 6), two (BMU 1 and BMU 7) were found during the positive phase of the NAO index (Fig. 10A) and were associated with higher amount of precipitation (Table 3). Higher temperatures related to the positive phase of the NAO index were favored for more intensive development of the species in the spring period, such as *Pseudo-nitzschia* (BMU 1), *D. sacculus*, *L. polyedrum*, and *G. spinifera* (BMU 3; Fig. 6). In addition to temperature, the development of the abovementioned species as well as those of the genus *Dinophysis* and *Ph. rotundatum* (BMU 7) were favored by the higher precipitation rate, which was not, as expected, associated with the negative phase of the NAO index. These results could be explained by the findings of a nonlinear connection between the hemispheric index NAO and precipitation anomalies (Matić et al., 2019). In particular, the NAO index and precipitation amount are inversely proportional, especially for extreme NAO values, but a proportional relation was also noted between the NAO index and precipitation rate. According to the authors, wet conditions associated with the positive NAO index usually occur in spring and autumn, as was noted in our study. Higher precipitation during the positive and negative NAO index was also recorded in Šibenik Bay and Mali Ston Bay. In addition, Spearman rank correlation showed a positive and negative relationship between NAO index and precipitation depending on season. These findings suggest the importance of season on the influence of the NAO index on the development of phytoplankton species. Interestingly, clusters that occur more frequently in summer were associated with a negative NAO index, whereas clusters more frequently found in the winter or spring and autumn were associated with a positive to neutral NAO index (Figs. 6, 8–10). This can be clearly noted in the Northern Adriatic by comparing the diametrically opposed clusters (BMU 1 and BMU 9). BMU 9 was dominant in summer and was associated with low precipitation rate, low wind intensity, and a negative NAO index. BMU 1 was dominant in the rest of the year and was associated with positive NAO, higher precipitation rate and wind intensity. These findings indicate a nonlinear effect of NAO that needs to be investigated in the future.

4.4. Relation between HAB and climate change

Recently, because of the increased number of reports on climate change, there are concerns about their impact on HABs. Among the analyzed species, Mann–Kendall test showed a trend of increasing

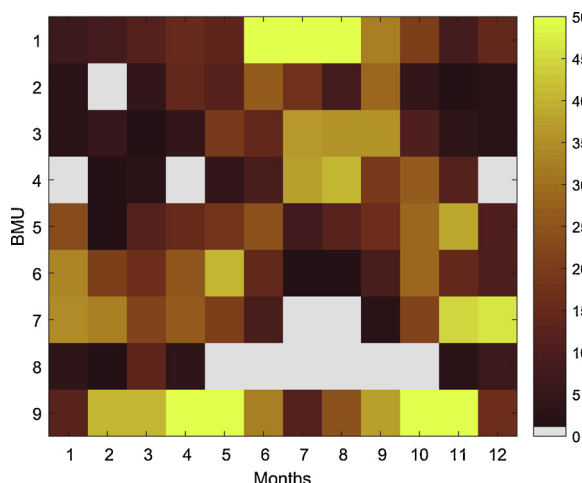


Fig. 9. The appearance of the Best Matching Units (BMU) solution over 12 months for the SOM model in Mali Ston Bay. The colours shown in the legend on the right-side represent the normalized numbers of occurrence of each BMU in different months during the study period. Environmental and meteorological conditions and abundances of the most frequent species associated with each BMU are described in Table 5.

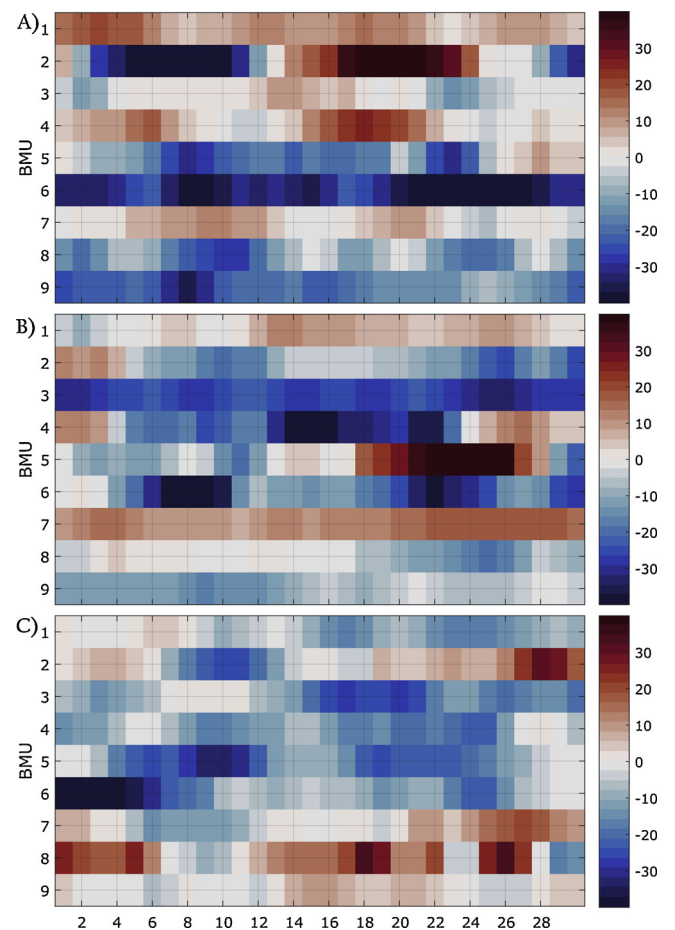


Fig. 10. NAO index 30 days before sampling the phytoplankton species, calculated as composite average state for each of the BMUs obtained in: a) Northern Adriatic, b) Šibenik Bay, c) Mali Ston Bay. The vertical column indicates negative (blue) and positive (red) NAO index.

abundance of *Pseudo-nitzschia* species. This trend, according to the results of SOM analyses, could be associated with an increase in precipitation, which is recorded in the colder part of the year, according to the Croatian Meteorological and Hydrological Service. Furthermore, the decrease of precipitation in summer was recorded. The precipitation simulations obtained by different climate model projections for Mediterranean Sea differ from each other (Raible et al., 2010; IPCC, 2013), but the strong inter-annual variability seems to be one of the main feature of the precipitation in the Mediterranean basin (Lionello, 2012). Under these conditions, spatial heterogeneity and local climatic conditions are of remarkable importance (Jacob et al., 2014). Our data indicate an increasing trend in the abundance of *Pseudo-nitzschia* spp. with increased precipitation. According to our results, an increase in winter population was mainly caused by higher runoff and nutrient input, what is commonly noted during the development of high-biomass HABs (Paerl et al., 2014). In contrast, the *Pseudo-nitzschia* spp. population would decrease in summer, because of the increasing warming and prolongation of the stratification period, which are unfavorable for the development of these species (Wells et al., 2015). The warming of the surface layer in the Mediterranean Sea is clearly predicted by global and regional models (Ducrocq, 2016) as well as by *in situ* measurements (Grbec et al., 2018). Since the SOM analysis detected increased abundances of the species of *D. caudata*, *Ph. rotundatum*, *G. spinifera*, and *L. polyedra* with an increase in sea surface temperature, the abundance of these species could be assumed to increase in the future. However, for such predictions, defining a temperature window for the studied species is necessary. The consequence of warming is an

extended stratification period; however, in this study, stratification was mainly due to halocline rather than thermocline because of the influence of freshwater at the investigated area. In line with previous studies, stratification favors the development of dinoflagellates (Jester et al., 2009). This is supported by research findings in the United States, the west coast of Canada, and coastal South Africa, where a link was noted between increased stratification and emergence of *Dinophysis* spp. (Hattenrath-Lehmann et al., 2013; Hubbart et al., 2012; Taylor et al., 2013; Trainer et al., 2013). The results of SOM analysis for *Alexandrium* species, which are the most abundant in spring during high river inflow and heavy rainfall, suggested that increased precipitation in spring might be thought to increase the bloom occurrence, especially since a trend of increasing temperature was noted in the spring period from 2007 to 2018, according to the Croatian Meteorological and Hydrological Service. In support of these findings is the non-linear relationship between the positive NAO index and higher rainfall, which, with increasing temperature, further stimulates the growth of harmful algae.

5. Conclusion

SOM analysis proved to be a useful tool for grouping phytoplankton species with optimal environmental conditions as well as in identifying groups of species that coexist. The analysis of long-term datasets of toxic phytoplankton species confirmed the dinoflagellate assembly rules set by Smayda and Reynolds (2003). Thus, in areas with different hydromorphological characteristics, the species composition was different. Šibenik Bay differed in the more frequent occurrence of *A. cf. tamarensis*, *D. acuta*, and *P. cordatum*. In addition, different life forms of dinoflagellates that have different survival strategies were clearly separated. Thus, the *Dinophysis* species from *Gonyaulax* and *Lingulodinium* were clearly separated. Among the *Dinophysis* species, those that prefer higher temperatures (*D. caudata*) over those preferring higher precipitation rate (*D. fortii*, *D. acuta*, and *D. tripos*) were also clearly separated. Specific conditions have been identified in the Northern Adriatic Sea, leading to an increased abundance of *D. fortii*. In particular, increased precipitation rate with elevated temperature favors the intense development of this species in the summer and late summer.

The species of the genus *Alexandrium* were strongly associated with freshwater inflow and maximum water stability. The amount of precipitation that causes stronger river flows, bring nutrients and has a positive effect on the development of toxic phytoplankton populations showed a non-linear relationship with the NAO index depending on season. Recorded wet conditions associated with positive phase of NAO index could favor the development of *Alexandrium* bloom as well as *D. fortii*. Identified trend of increasing abundance of *Pseudo-nitzschia* spp. associated with high precipitation is caused by increasing precipitation in a colder part of the year recorded by Croatian Meteorological and Hydrological Service and was associated by positive NAO index phase.

Since water column stability due to freshwater inflow was recorded during HAB occurrence, it could play an important role in the formation of HABs because of formation of layers with specific environmental conditions.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.hal.2020.101745>.

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