



## Toxic and Potentially Toxic Phytoplankton in the Mussel and Fish Farms in the Transitional Area of Montenegrin Coast (South-Eastern Adriatic Sea)

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### Abstract

The distribution of toxic and potentially toxic phytoplankton species in relation to environmental factors was investigated from November 2014 to April 2015 in Boka Kotorska Bay, in the south-eastern part of the Adriatic Sea. The RDA analysis of environmental parameters showed that the system is mostly temperature-driven. The phytoplankton community was mainly composed of diatoms and less with dinoflagellates. *Pseudo-nitzschia* was the dominant diatom, present in 88.40 % of samples, with a maximum (of  $1.85 \times 10^5$  cells/l). Among toxic dinoflagellates, frequent were *Dinophysis acuminata* (with frequency of 33.33%), *D. fortii* (with frequency of 23.19%) and *Prorocentrum cordatum* (with frequency of 21.74%). The RDA analysis showed that diatoms correlated slightly positively with nutrients and negative correlation was recorded with temperature and salinity. Dinoflagellates showed strong positive association, indicating a positive correlation with oxygen concentration, chl *a*, SiO<sub>4</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> concentrations and negative correlation with temperature and salinity.

**Keywords:** Harmful phytoplankton, spatial distribution, Boka Kotorska Bay.

### Introduction

The eastern coast of the Adriatic Sea consists mainly of numerous islands, bays, coastal embayments, and also some transitional areas can be found. As of recently, increasing attention has been paid on sub-basins, such as coastal embayments, gulfs and lagoons as important part in coastal hydrodynamic studies in the Adriatic Sea (Bellafiore *et al.*, 2011). A lot of information exists about these systems in the northern Adriatic, i.e. the Venice Lagoon (Bellafiore & Umgiesser, 2010; Ferrarin, Cucco, Umgiesser, Bellafiore, & Amos, 2010a) and the Grado and Marano lagoons (Ferrarin *et al.*, 2010b) but less for other parts of Adriatic. The southern part of the Adriatic Sea, especially the part along Montenegro and Albania plays the crucial role in the general Adriatic Sea circulation (Bellafiore *et al.*, 2011).

The Boka Kotorska is located in the south-eastern part of the Adriatic Sea along Montenegrin coast. The Bay is a transitional system as it is a boundary environment between land and sea, characterized with specific biological communities which differ from other adjacent marine and

continental biomes (Sarno, Zingone, Saggiomo, & Carrada, 1993). Area of the Boka Kotorska Bay can be classified as a ROFI (Region of Freshwater Influence) due to presence of rivers and high precipitation caused by the high karstic mountain massifs (Bellafiore *et al.*, 2011, Campanelli *et al.*, 2009). Phytoplankton organisms are very sensitive to changes in its environment. The enrichment of water with nutrients (primarily nitrogen, silicon and phosphorus compounds) may result in the growth of algal biomass. As for that, they are useful indicator of water quality before these organisms grow up and become visible in higher trophic levels and result in intensive eutrophication (Brettum & Andersen, 2005). In recent years, human influence in the Bay has increased due to accelerate urbanization of the coastal zone and increasing tourist activities. Nevertheless, based on physico-chemical parameters and phytoplankton biomass expressed as chlorophyll *a* concentration the Bay is considered as a system where natural eutrophication still prevails over anthropogenic (Krivokapić, Pestorić, Bosak, Kušpilić, & Riser, 2011). For getting a better biological quality assessment of the ecosystem, a trophic estimation based on physico-chemical parameters and

phytoplankton biomass expressed as chlorophyll *a* concentration need to be showed together with information on the phytoplankton taxonomic composition (Toming & Jaanus, 2007; Jaanus, Toming, Hallfors, Kaljurand, & Lips, 2009). Eutrophication causes numerous physical and chemical changes in the marine environment which lead to pressure on algal populations, and all those changes can result in intensive growth of harmful-toxin producing species. High growth of harmful algae (algal bloom) may cause serious problems related to the ecosystem and public health (mostly by the consumption of contaminated marine products such as shellfish). These blooms are known as Harmful Algal Blooms (HABs). Mainly dinoflagellates are classified as toxic, but confirmation of toxicity exists also for some species of other taxa (diatoms, flagellates, cyanobacteria, prymnesiophytes, raphidophytes) (Codd, 1999; Vershinin & Orlova, 2008; Moestrup et al., 2010). There are three types of harmful blooms: caused by toxic algae (e.g. *Karenia*, *Alexandrium*, *Dinophysis* and *Pseudo-nitzschia*) that can result in poisoning even at low algal abundance; caused by potentially toxic algae (e.g. *Pseudo-nitzschia*); and caused by high-biomass blooms (e.g., *Phaeocystis*, *Lepidodinium*, *Noctiluca*) that induce problems mainly because of the high biomass. This high biomass blooms are known as "red tides" but there can also be brown, green or white discolorations of the sea. Some organisms (e.g. *Alexandrium*) can be toxic and in high biomass (Ferreira et al., 2011). Some non-toxic species caused high biomass (HB) blooms are also classified as harmful, because their occurrence caused water discoloration and anoxic harmful conditions to the ecosystem, which lead to economic problems due to loss to fisheries and tourism (Smayda, 1997).

Potentially toxic diatom species from genus *Pseudo-nitzschia* are permanently a part of phytoplankton community in the Mediterranean and in the Adriatic Sea (Socal et al., 1999; Orsini et al., 2002; Quiroga, 2006; Bosak, Burić, Đakovac, & Viličić, 2009). Reference papers refer to 32 species of this genus and for eleven of them data of toxicity exist (Quiroga, 2006); they produce domoic acid which is responsible for Amnesic Shellfish Poisoning (ASP) (Bates, Garrison, & Horner, 1998). Understanding the seriousness of treating amnesic shellfish poisoning increased after a case of poisoning in Canada in 1987 (Wright et al., 1989) when a toxic episode caused the death of at least three people (Todd, 1993; Ujević et al., 2010). Increased interest in *Pseudo-nitzschia* species has begun also in the Adriatic Sea. The composition of domoic acid, distribution and relation of domoic acid to physico-chemical parameters still needs to be clarified, even some domoic acid records have been confirmed in the northern Adriatic (Marić et al., 2011).

There are over 4000 different species of marine

algae and 2% are known to produce biotoxins (Sournia, 1995). In marine ecosystems, dinoflagellates, along with diatoms, are important components of the phytoplankton. Dinoflagellates of the genus *Dinophysis* occur in various coastal waters (Hallegraff, 2003) including those of the Adriatic Sea (France & Mozetič, 2006). The main dinoflagellates causing DSP belong to genera *Prorocentrum* and *Dinophysis*. In contrast to *Prorocentrum*, *Dinophysis* blooms are rare, but they can induce poisoning even at low cell densities (Bruno et al., 1998). In the Adriatic sea, eighteen dinoflagellate species producing biotoxins have been recorded: *Alexandrium minutum*, *Alexandrium tamarense*, *Alexandrium fundyense*, *Alexandrium lusitanicum*, *Dinophysis acuminata*, *Dinophysis acuta*, *Dinophysis caudata*, *Dinophysis fortii*, *D. norvegica*, *D. mitra*, *D. sacculus*, *D. tripos*, *Phalacroma rotundatum*, *Prorocentrum micans*, *P. cordatum*, *P. lima*, *Gymnodinium catenatum* and *Lingulodinium polyedrum* (Honsell, 1993). Biotoxins are also produced by diatoms as: *Nitzschia navis-varingica*, *Pseudo-nitzschia australis*, *Pseudo-nitzschia multiseriata*, *Pseudo-nitzschia seriata* (Kotaki, 2008).

Since the research was done on shellfish farms operating in monoculture, as well as on fish farms which applying system of integrated multitrophic aquaculture, the impact of the presence of potentially toxic phytoplankton organisms can range from benign to catastrophic. The mussels accumulate toxins from phytoplankton and in some cases algal bloom of potential toxic phytoplankton can cause mild problems if consumed by humans, such as headaches and upset stomachs, while others can cause serious neurological and hepatic symptoms. Even during the occurrence of algal blooms of non-toxic phytoplankton, dense blooms can cause limitation of photosynthesis and dissolved oxygen, which can lead to a buildup of potentially toxic compounds and the consequent contamination of cultured organisms.

Regarding mariculture, there are 17 shellfish farms cultivating mostly mussels, and two fish farms rearing seabass/seabream registered in the Boka Kotorska Bay (MONSTAT, 2014). Of that number, 8 shellfish farms and 2 fish farms are located in the Kotor Bay.

The aim of this study is to investigate the distribution and taxonomic composition of toxic and potentially toxic phytoplankton assemblages on mussel and fish farms in the Boka Kotorska Bay. With the assumption that in the study area there is no increased abundance of toxic and potentially toxic phytoplankton organisms that could lead to bioaccumulation of toxins in shellfish and consequently endangering (threatening) of the health of consumers, this paper may provide valuable information for now and for future investigations of toxic phytoplankton on mussel and fish farms, for prediction and prevention of its harmful effects and potential toxicity in one of the most sensitive areas of

the Adriatic Sea.

## Materials and Methods

### Study Area

The Boka Kotorska Bay is located in the south-eastern part of the Adriatic Sea and covers an area of 87.3 km<sup>2</sup>. The Bay is considered one of the most important transitional areas of the region, in terms of environmental and socio-economic aspects. The traits distinguishing it among the bays of the Mediterranean are its deep insertion into the land, as well as its historical and geographical role and significance. The entrance to Boka Kotorska is closed by the cape Oštra from the west and by the cape Mirište from the east at the very mouth (Magaš, 2002). It consists of three indented branches. The innermost branch consists of two embayments (Kotor and Morinj-Risan Bays) and is connected by the narrow Verige Strait to the central Tivat Bay. The Kumbor Strait connects the Tivat Bay to the Herceg Novi Bay, which flows into the Adriatic Sea to the South. In the Morinj-Risan and Kotor Bays, there are a lot of subaerial and submarine springs, among them Sopot and Ljuta which can reach higher peak of discharges for a short period (Figure 1.) (Bellafiore *et al.*, 2011). Due to its characteristics, the Bay is considered ROFI (Region of Freshwater Influence) (Bellafiore *et al.*, 2011). Kotor Bay, one of the areas covered by this research, is located in the innermost part of Boka Kotorska Bay, encompassing approximately 30% of the Bay area. The freshwater influx from small rivers, numerous karstic streams and karstic submarine springs greatly affects the hydrological and chemical properties of the water column (Milanović, 2007). Krivokapić, Vuksanović

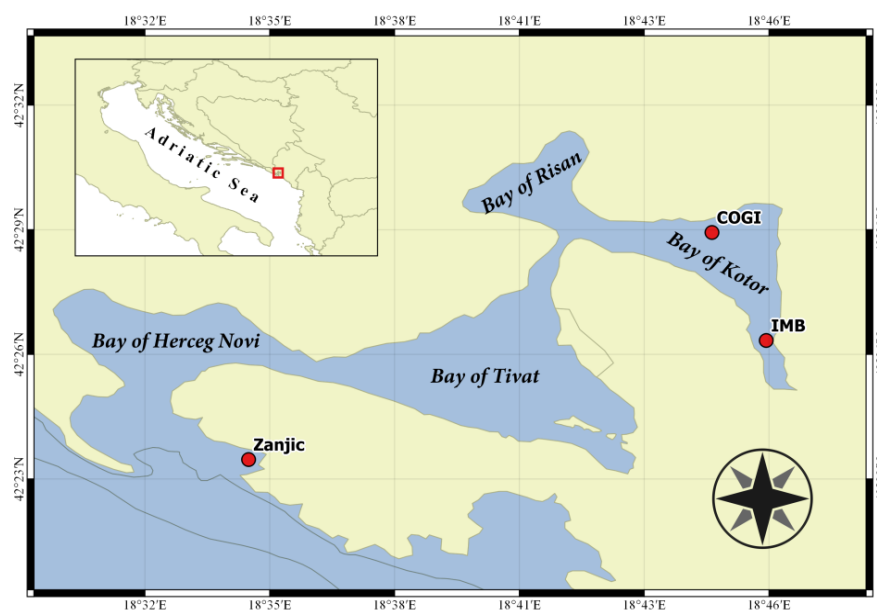
and Stanković (2009) noticed that the annual rainfall pattern has a significant influence on nutrient-loading seasonality in the area.

### Sampling Methods and Analysis

Sampling was carried out from November 2014 to April 2015, at three stations IMB – mussel farm, COGI – mussel and fish farm and Žanjic – from where mussels were taken from the wild and this station is used as reference station for comparison. In November 2014, sampling was performed at three depths: surface, 4m and bottom (9m, 31m, 10m, respectively for IBM, COGI and Žanjic), while from December 2014 to April 2015 at fourth depths: 0m, 2m, 4m, and bottom (Figure 1). Samples were taken with 5l Niskin bottle, with monthly frequency.

Temperature and salinity were measured in situ with a universal meter (Multiline P4; WTW). Subsamples for the determination of dissolved nutrients – total nitrogen (NTOT), nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), total phosphorus (PTOT), phosphate (PO<sub>4</sub><sup>3-</sup>) and silicate (SiO<sub>4</sub><sup>-</sup>) – were analyzed on Analytic Jena UV/VIS spectrophotometer at a different wavelength for each nutrient using standard methods Strickland and Parsons (1972), while oxygen concentration was measured according Winkler (1888) that was modified by Strickland and Parsons (1968) by fixation with a reagent that lead to oxidize iodide ion (I<sup>-</sup>) to iodine (I<sub>2</sub>) quantitatively. The iodine amount was determined by titration with a standard thiosulfate solution.

Subsamples (1 l) for the determination of chlorophyll *a* were filtered onto Whatman GF/F (47 mm and 0.7 μm pore size) filters and then the pigment was extracted in 90% acetone. Finally, chlorophyll *a*



**Figure 1.** Map of the Boka Kotorska Bay showing its four small bays. The sampling locations are located in the inner part of the Kotor Bay and in the outside part of the Bay (Žanjic).

concentrations were determined on a Perkin Elmer spectrophotometer by measuring the absorbance at four wavelengths, and calculated according to Jeffrey, Mantoura and Wright (1997).

Phytoplankton samples were taken with 5 L Niskin bottles and preserved in 250 ml bottles using a 4% neutralized formaldehyde solution. Cells were identified and counted using Leica DMI4000 B inverted microscope (Heerbrugg, Switzerland) in subsamples of 25 ml after 24 h of sedimentation, following Utermöhl (1958). Enumeration was carried out using phase contrast and bright field illumination at magnifications of 100, 200, and 630 $\times$ . At a 100 $\times$  magnification, total chamber bottom was scanned for taxa larger than 30  $\mu\text{m}$ , while abundant microphytoplankton (>20  $\mu\text{m}$ ) were counted at two transects at magnification of 200 $\times$ . Nanophytoplankton cells (2–20  $\mu\text{m}$ ) were counted in 10 randomly selected fields with magnifications of 630 $\times$ . For determination, appropriate key for the specified field of investigation was used (Cupp, 1933; Hustedt, 1930; H. Peragallo & M. Peragallo, 1965; Dodge, 1985; Schiller, 1933, 1937; Sournia, 1989). Microalgae that could not be identified to species level were assigned to generic groups. They were classified into recognizable taxonomic category: green nanoflagellates, small dinoflagellates and small coccolithophores.

### Data Analysis

For sorting of the data, numeric and graphic presentation as well as for basic statistical analysis, several computer packages were used: Microsoft Excel (Microsoft Corporation 2007), Grafer 7 and Statistica 7.0. RDA analysis was performed using the R package "vegan" for community ecology and the R software.

### Results

From November to December 2014, a slight variation of temperature in terms of maximum and minimum values was noticed. In January 2015, temperature decreased and maximal temperature in this month reached the value of 14.8 $^{\circ}\text{C}$ . In February 2015, maximal temperature was similar to that in January. Temperatures in March and April 2015 slowly increased. Maximal temperature during research was recorded in November 2014 (19.9  $^{\circ}\text{C}$ ), while the minimal was in January 2015 (9.8  $^{\circ}\text{C}$ ) (Table 1). Salinity concentration showed lower temporal variations related to maximal values, while pronounced variations occurred with relation to minimum ones. The highest salinity concentration was in December 2014 (38.1 psu) and the lowest was in January 2015 (2 psu). (Table 1).

Concentration of total nitrogen showed an increase from November 2014 to February 2015, when the concentration decreased and in March 2015

started to increase to the maximal concentration of 1077.24  $\mu\text{mol/l}$  and then in April 2015 sharply decreased to the maximal concentration of 53.51  $\mu\text{mol/l}$ . Nitrate concentration varied slightly with the highest concentration in February 2015 (15.70  $\mu\text{mol/l}$ ) and the lowest in April 2015 (0.23  $\mu\text{mol/l}$ ). Total phosphorus reached the highest concentration in November 2014, after that its value was slowly decreasing. Phosphate maximal concentration ranged from 0.18  $\mu\text{mol/l}$  in April to 0.76  $\mu\text{mol/l}$  in December. For silicate concentration, minimal and maximal values were noticed in February 2015 (0.37  $\mu\text{mol/l}$ ) and December 2014 (23.17  $\mu\text{mol/l}$ ). Oxygen concentration varied slightly with maximal and minimal values were in December 2014 (11.12 mg/l and 5.40 mg/l) (Table 1).

Maximal value of chlorophyll *a* concentration was 2.33 mg  $\text{m}^{-3}$  in December. Minimum concentration of chlorophyll *a* was in April 0.09 mg  $\text{m}^{-3}$  (Table 1).

Nitrates concentration during investigation showed significant negative correlation with temperature ( $r = -0.538$ ;  $P < 0.05$ ) and salinity ( $r = -0.443$ ;  $P < 0.05$ ), while phosphates showed no significant positive correlation. Silicates concentration showed significant negative correlation with temperature ( $r = -0.484$ ;  $P < 0.05$ ) and salinity ( $r = -0.512$ ;  $P < 0.05$ ). Nitrates had a significant positive correlation with phosphates ( $r = +0.246$ ;  $P < 0.05$ ), silicates ( $r = +0.811$ ;  $P < 0.05$ ), with oxygen concentration ( $r = +0.508$ ;  $P < 0.05$ ) and with concentration of chlorophyll *a* ( $r = +0.363$ ;  $P < 0.05$ ). Concentration of phosphates had a significant positive correlation with total phosphorus ( $r = +0.552$ ;  $P < 0.05$ ), nitrates ( $r = +0.246$ ;  $P < 0.05$ ) and nitrites ( $r = +0.363$ ;  $P < 0.05$ ). Silicates concentration had a significant positive correlation with oxygen concentration ( $r = 0.606$ ;  $P < 0.05$ ) and chlorophyll *a* concentration ( $r = 0.270$ ;  $P < 0.05$ ) (Table 2.)

### Microplankton and Microplankton Fractions

Microplankton abundance reached the highest value in November 2014, after which the abundance decreased. From December 2014 to April 2015 values of microplankton were in order  $10^4$  cells/l, while in November 2014 value was in order  $10^5$  cells/l. Medians values of microplankton abundance were higher in December 2014 and January 2015, while in November 2014, March 2015 and April 2015 were lower. Spatial distribution of microplankton abundance showed higher means values on positions IBM and COGI and lower on Žanjic. The highest microplankton abundance was noticed at the location IBM (Figure 2).

Diatoms maximal abundance, too, was recorded in November 2014, while in other months abundance decreased. The lowest abundance was in April with the value of 800 cells/l. Medians values of diatoms abundance were higher in December 2014 and

**Table 1.** Values of physical, chemical and biological parameters during investigation period on three locations in the Boka Kotorska Bay (Max-maximum, Min-minimum, AVG- average, SD- standard deviation)

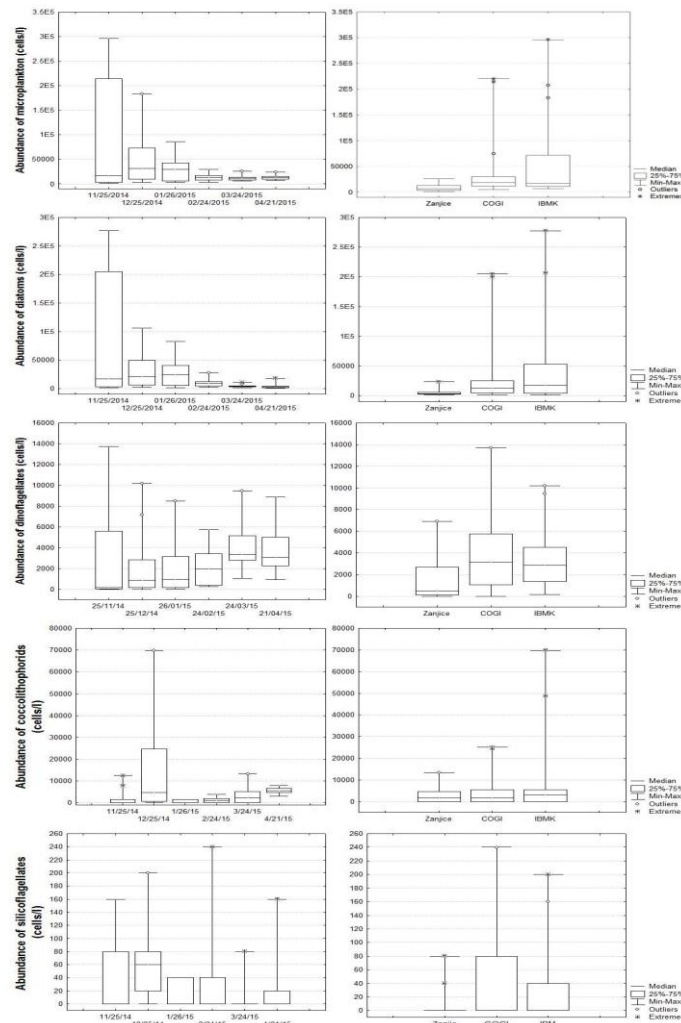
Parameters	Nov			Dec			Jan			Feb			Mar		
	Max	Min	AVG± SD	Max	Min	AVG± SD	Max	Min	AVG± SD	Max	Min	AVG± SD	Max	Min	AVG± SD
Temp (°C)	19.90	11.10	17.4±3.15	18.60	10.40	15.97±2.62	14.80	9.80	13.14±1.58	15.50	12.30	13.85±1.1	16.70	15.70	16.2±0.29
Sal (psu)	37.80	5.60	28.9±12.54	38.10	10.40	30.68±10.15	36.80	2.00	26.68±11.81	37.70	13.90	29.54±8.91	37.40	27.90	33.22±3.78
Oxy (mg/l)	9.74	5.52	7.20±1.60	11.12	5.40	7.93±1.73	10.40	6.72	7.91±1.20	10.56	7.54	8.79±0.98	9.40	7.22	8.44±0.61
NTOT (µmol/l)	81.01	1.78	29.39±30.82	294.39	29.07	117.12±77.05	1046.79	37.32	192.57±286.30	1077.24	128.60	537.37±301.68	53.51	46.95	51.01±2.27
NO <sub>3</sub> <sup>-</sup> (µmol/l)	11.52	0.44	4.06±3.78	11.75	0.54	3.87±3.73	14.75	0.87	5.09±4.02	9.93	0.66	3.77±3.41	1.77	0.23	0.97±0.52
NO <sub>2</sub> <sup>-</sup> (µmol/l)	0.41	0.08	0.23±0.10	0.55	0.08	0.24±0.13	0.51	0.10	0.28±0.12	0.46	0.06	0.15±0.10	0.14	0.02	0.07±0.04
PTOT(µmol/l)	1.03	0.19	0.52±0.26	0.85	0.19	0.48±0.21	0.61	0.23	0.36±0.14	0.74	0.00	0.26±0.28	0.61	0.14	0.31±0.14
PO <sub>4</sub> <sup>3-</sup> (µmol/l)	0.27	0.13	0.19±0.04	0.76	0.04	0.22±0.19	0.27	0.04	0.14±0.68	0.30	0.03	0.13±0.08	0.18	0.01	0.09±0.06
SiO <sub>4</sub> <sup>-</sup> (µmol/l)	17.95	1.64	6.90±5.56	23.17	1.35	8.19±6.62	13.61	2.06	6.29±3.88	19.45	2.18	7.29±5.39	6.96	2.07	4.29±1.53
Chl. <i>a</i> (mg m <sup>-3</sup> )	1.24	0.10	0.40±0.34	2.33	0.10	0.85±0.76	1.21	0.10	0.45±0.38	1.99	0.15	0.80±0.67	0.67	0.09	0.29±0.14

Temp (°C): Temperature; Sal (psu): Salinity; Oxy (mg/l): Oxygen concentration; Chl. *a* (mg m<sup>-3</sup>): Chlorophyll *a* concentration; NTOT (µmol/l): Total nitrogen concentration; NO<sub>3</sub><sup>-</sup> (µmol/l): Nitrate concentration; NO<sub>2</sub><sup>-</sup> (µmol/l): Nitrite concentration; PTOT (µmol/l): Total phosphorus concentration; PO<sub>4</sub><sup>3-</sup> (µmol/l): Phosphate concentration; SiO<sub>4</sub><sup>-</sup> (µmol/l): Silicate concentration

**Table 2.** Spearman Rank Order Correlation of physico-chemical parameters on three investigation locations in the investigated area (\*P<0.05)

	Tem	Sal	Oxy	Chl <i>a</i>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	SiO <sub>4</sub> <sup>-</sup>	PTOT	NTOT
Tem	1.000									
Sal	0.686*	1.000								
Oxy	-0.481*	-0.508*	1.000							
Chl <i>a</i>	-0.219	-0.279*	0.351*	1.000						
NO <sub>3</sub> <sup>-</sup>	-0.538*	-0.443*	0.508*	0.363*	1.000					
NO <sub>2</sub> <sup>-</sup>	0.063	0.221	-0.445*	0.131	0.136	1.000				
PO <sub>4</sub> <sup>3-</sup>	0.098	0.029	-0.072	0.144	0.246*	0.363*	1.000			
SiO <sub>4</sub> <sup>-</sup>	-0.484*	-0.512*	0.606*	0.270*	0.811*	-0.127	0.104	1.000		
PTOT	0.193	-0.058	-0.004	0.176	0.190	0.231	0.552*	0.061	1.000	
NTOT	-0.294*	-0.001	-0.149	0.138	-0.114	0.023	-0.235	-0.168	-0.270*	1.000

Temp: Temperature; Sal: Salinity; Oxy: Oxygen concentration; Chl. *a*: Chlorophyll *a* concentration; NO<sub>3</sub><sup>-</sup>: Nitrate concentration; NO<sub>2</sub><sup>-</sup>: Nitrite concentration; PO<sub>4</sub><sup>3-</sup>: Phosphate concentration; SiO<sub>4</sub><sup>-</sup>: Silicate concentration; NTOT: Total nitrogen concentration; PTOT: Total phosphorus concentration.



**Figure 2.** Abundance of microplankton, diatoms, dinoflagellates, coccolithophorids and silicoflagellates during investigation period on left side and on locations on right side.

January 2015, while in November 2014 was lower, and in March and April 2015 means values of diatom abundance were the lowest. Diatoms abundance was the highest at the IBM location with the value of

$\times 10^5$  cells/l. Median values of diatoms abundance were similar for IBM and COGI, while in Žanjica location it was lower (Figure 2).

Dinoflagellates were present in lower

abundances in comparison with diatoms. The highest abundance of dinoflagellates was at the location COGI and the lowest at the Žanjic location. Median values were also higher at the location COGI in comparison with the other two locations. Regarding temporal distribution, dinoflagellates abundance was the highest in November 2014 ( $1.37 \times 10^4$  cells/l) and then decreased until March 2015 when a slow increase of abundance was noted. Medians values of dinoflagellates abundance were higher in March and April, while from November to February they were lower.

The highest coccolithophores abundance was in December 2014, while mean value was the highest in April 2015. Regarding spatial distribution, the highest value was recorded at the location IBM, also the mean values were the highest at the IBM location.

The highest silicoflagellates abundance was in February 2015 (240 cells/l). Regarding spatial distribution, the highest value was recorded at the COGI location.

In order to assess the influence of a set of environmental and chemical variables in abundance of phytoplankton groups a redundancy analysis (RDA) was performed. RDA calculations were based on log-transformed phytoplankton abundance in order to reduce the effect of uneven density distributions. The arrows representing explanatory variables indicated the direction of maximum change of these variables across the diagram and cosine of the angle between the arrows gave the correlation between the corresponding explanatory variables (Figure 3.). Additionally, the non-parametric Spearman correlation coefficient was used to confirm correlations of phytoplankton groups with environmental and chemical conditions (Table 3).

The two first axes of the ordination analysis explained 31.97% of the phytoplankton species abundance. The contributions of the axes were not equal as indicated by the eigenvalues (7.087 and 1.749 for phytoplankton groups). The analysis captured 100% of the phytoplankton groups variations that could be explained by considered variables.

In regards to axis 1, nutrients showed positive association, especially  $\text{NO}_3^-$ . Salinity showed strong negative association with axis 1, while temperature was slightly negatively associated. Concentration of oxygen and chl *a* showed strong explanatory power according to axis 2. Coccolithophores was in a strong negative correlation with axis 1, indicating a positive correlation with temperature and salinity and inversely with nutrients. Diatoms correlated slightly positively with axis 1, indicating a positive correlation with most of nutrients and a negative correlation with temperature and salinity. According to axis 2 coccolithophores showed negative correlation with oxygen concentration and chlorophyll *a*.

In regards to axis 2, dinoflagellates showed strong negative association, indicating a positive correlation with oxygen concentration, chl *a* and  $\text{SiO}_4^-$

and  $\text{NO}_3^-$  and negative with temperature and salinity. Silicoflagellates did not show significant distribution in regard to axis 1 and axis 2. These results were confirmed by the non-parametric Spearman correlation coefficient (Table 3).

### Taxa Abundances and Composition

Total number of 100 taxa was noticed during investigation from November 2014 to April 2015. Recorded were 43 taxa of diatoms, 50 taxa of dinoflagellates, 5 of coccolithophores and 2 of silicoflagellates.

From 100 taxa, 9 potentially toxic and toxic taxa were noted. Among diatoms, species from genus *Pseudo-nitzschia* were noted. Among toxic dinoflagellates, eight species from four genera were noted: *Dinophysis*, *Lingulodinium*, *Phalacroma* and *Prorocentrum*.

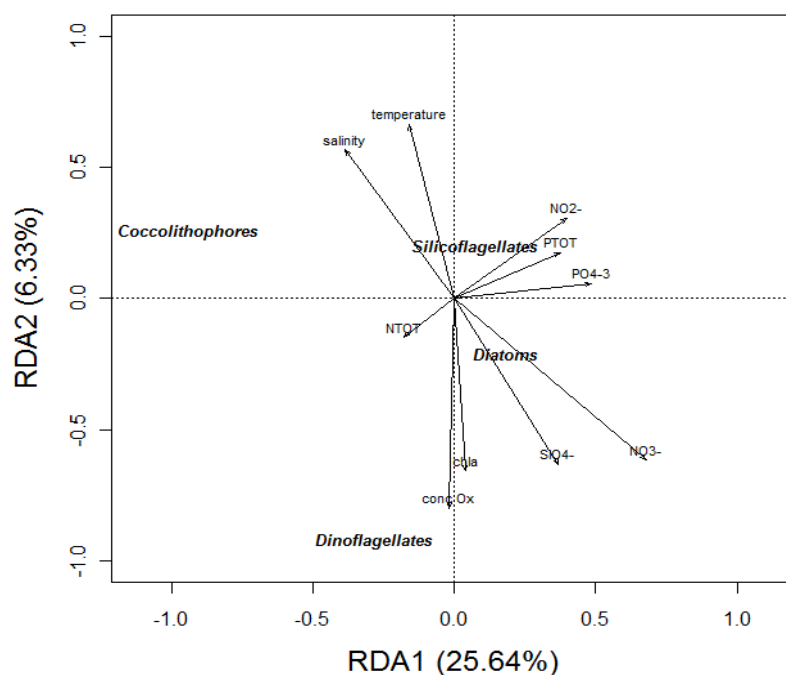
Potentially toxic species from genus *Pseudo-nitzschia* spp. was present in all three locations (Table 4.). As regards *Dinophysis* genus, all toxic species were noticed in two locations, while species *Lingulodinium polyedra*, *Prorocentrum cordatum* and *Phalacroma rotundatum* were present at all three locations.

During the research, the most frequently identified was a potentially toxic species from diatoms genus *Pseudo-nitzschia* with percentage of appearance of 88.40%. Among toxic dinoflagellates the most frequent was potentially toxic species *Dinophysis acuminata*. Species *D. fortii* and *Prorocentrum cordatum* also occurred frequently. *Dinophysis tripos* was a less present species during research (Table 4). The highest abundance of *D. acuminata* was 320 cells/l, while *D. fortii* reached the highest value of 160 cells/l (Table 5).

Of toxic dinoflagellates, the highest abundance was reached by the species *Prorocentrum cordatum* ( $4.7 \times 10^3$  cells/l), while diatoms species that belong to genus *Pseudo-nitzschia* reached the highest abundance of  $1.85 \times 10^5$  cells/l (Table 5.).

Species from genus *Pseudo-nitzschia* were the most represented in diatoms abundance, while as regards dinoflagellates, *Prorocentrum cordatum* were most represented, followed by the second most frequent and abundant species from the genus *Dinophysis* (Figure 4). *Pseudo-nitzschia* spp. was the most abundant in location IMB at the depth of 7m. *Prorocentrum cordatum* was also the most abundant at the position IBM in the surface layer while *Dinophysis* species was the most abundant at the location COGI in the surface layer (Figure. 4).

The abundance of phytoplankton toxic and potentially toxic species was partly explained by the hydrographic and chemical variables considered in the RDA analysis (Figure 5). Species *Prorocentrum cordatum*, *Dinophysis acuminata* and diatoms from genus *Pseudo-nitzschia* showed positive associations with axis 1, indicating positive correlation with



**Figure 3.** Redundancy analysis (RDA) showing bi-dimensional ordination of phytoplankton groups abundance. Superimposed vectors represent explanatory variables: temperature, salinity, oxygen concentration, nutrients concentration, Chl *a* concentration, diatoms, dinoflagellates, coccolithophorids and silicoflagellates. In order to make their scales comparable, both biological and hydrological variables were scaled to unit variance.

**Table 3.** Spearman Rank Order Correlation of physico-chemical and biological parameters during investigation period on three locations in the Boka Kotorska Bay.

	Temp	Sal	Oxy	Chl <i>a</i>	NTOT	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	PTOT	PO <sub>4</sub> <sup>3-</sup>	SiO <sub>4</sub> <sup>-</sup>
Diat.	-0.171	-0.175	0.051	0.461*	-0.070	0.489*	0.357*	0.177	0.187	0.316*
Dino.	-0.243*	-0.369*	0.453*	0.424*	0.044	0.101	-0.281*	-0.183	-0.306*	0.303*
Cocco.	0.286*	0.157	-0.007	-0.101	0.001	-0.397*	-0.253*	-0.186	-0.277*	-0.094
Silic.	0.191	0.004	-0.114	0.068	-0.057	0.071	0.088	0.059	0.159	0.067
<i>Pseudo.</i>	-0.018	-0.196	0.065	0.276*	-0.225	0.371*	0.214	0.290*	0.262*	0.373*
<i>D. acuminata</i>	-0.170	-0.497*	0.447*	0.311*	-0.099	0.123	-0.264*	0.081	-0.149	0.318*
<i>D. acuta</i>	-0.255*	-0.293*	0.246*	0.269*	0.059	0.213	-0.095	0.059	-0.069	0.226
<i>D. caudata</i>	0.100	0.005	-0.019	-0.178	-0.090	-0.121	0.067	0.036	-0.158	-0.084
<i>D. fortii</i>	-0.226	-0.405*	0.326*	0.280*	-0.040	0.251*	-0.094	-0.188	-0.086	0.308*
<i>D. tripos</i>	0.078	-0.035	-0.160	-0.098	0.195	-0.191	-0.072	0.154	0.148	-0.243*
<i>L. polyedra</i>	0.117	0.084	-0.121	0.087	0.196	-0.240*	0.006	0.117	0.076	-0.333*
<i>Ph. rotundatum</i>	-0.152	-0.131	0.146	0.108	-0.020	0.269*	0.156	-0.011	-0.056	0.248*
<i>P. cordatum</i>	-0.050	-0.049	0.208	0.175	-0.109	0.037	0.018	0.059	-0.070	0.217

Temp: Temperature; Sal: Salinity; Oxy: Oxygen concentration; Chl. *a*: Chlorophyll *a* concentration; NTOT: Total nitrogen concentration; NO<sub>3</sub><sup>-</sup>: Nitrate concentration; NO<sub>2</sub><sup>-</sup>: Nitrite concentration; PTOT: Total phosphorus concentration; PO<sub>4</sub><sup>3-</sup>: Phosphate concentration; SiO<sub>4</sub><sup>-</sup>: Silicate concentration; Diat: diatoms; Dino: dinoflagellates; Cocco: coccolithophores; Silic: silicoflagellates; *Pseudo*: *Pseudo-nitzschia* spp; *D. acuminata*: *Dinophysis acuminata*; *D. acuta*: *Dinophysis acuta*; *D. caudata*: *Dinophysis caudata*; *D. fortii*: *Dinophysis fortii*; *D. tripos*: *Dinophysis tripos*; *L. polyedra*: *Lingulodinium polyedra*; *Ph. rotundatum*: *Phalacroma rotundatum*; *P. cordatum*: *Prorocentrum cordatum* (\*P<0.05)

nitrites, silicates, chlorophyll *a* and oxygen concentration. These results were confirmed by the nonparametric Spearman correlation coefficient.

## Discussion

The period of research included autumn and winter period, when streams and springs have an influence on the physical, chemical and biological dynamics of the seawater. This region is different

from the northern part of the Adriatic Sea, which receives significant freshwater inputs throughout the year that have a pronounced positive impact on productivity, while in the Bay in the summer period, inflow of waters was lower (Degobbis, Gilmartin, & Revelante, 1986; Degobbis & Gilmartin, 1990). Therefore, remineralization processes and sewage discharge are considered the most important nutrient sources in this system.

Concentration of nutrients in the Boka Kotorska



**Table 4.** List of phytoplankton taxa identified in the Boka Kotorska Bay. Total number is 69 samples

Taxa	IBM	COGI	Žanjic
Atoms			
<i>Achnanthes brevipes</i> C. Agardh	+	+	+
<i>A. longipes</i> C. Agardh	+		
<i>Amphora ostrearia</i> Brébisson ex Kützing	+	+	+
<i>Amphiprora gigantea</i> var. <i>sulcata</i> (O' Meara) Cleve	+		+
<i>Asterionellopsis glacialis</i> (Castracane) Round		+	
<i>Bacteriastrium delicatulum</i> Cleve	+	+	
<i>B. hyalinum</i> Lauder	+	+	
<i>Cerataulina pelagica</i> (Cleve) Hendeby			+
<i>Chaetoceros affinis</i> Lauder	+	+	
<i>C. messanensis</i> Castracane		+	
<i>Chaetoceros</i> spp.	+		+
<i>Cocconeis scutellum</i> Ehrenberg	+	+	+
<i>Coscinodiscus perforatus</i> Ehrenberg	+	+	+
<i>Diploneis bombus</i> (Ehrenberg) Ehrenberg	+	+	+
<i>Entomoneis pulchra</i> (Bailey) Reimer	+		+
<i>Grammatophora oceanica</i> Ehrenberg	+		+
<i>Hemiaulus hauckii</i> Grunow ex Van Heureck			+
<i>H. chinensis</i> Greville		+	+
<i>Leptoylindrus mediterraneus</i> (H. Peragallo) Hasle	+	+	+
<i>Licmophora flabellata</i> (Greville) C. Agardh		+	+
<i>L. paradoxa</i> (Lyngbye) C. Agardh	+	+	+
<i>Lioloma pacificum</i> (Cupp) Hasle	+	+	+
<i>Lithodesmium undulatum</i> Ehrenberg	+	+	+
<i>Melosira nummuloides</i> C. Agardh	+	+	+
<i>Navicula</i> spp.	+	+	+
<i>Neocalyptrella robusta</i> (G. Norman ex Ralfs) Hernández- Becerril & Meave del Castillo		+	
<i>Nitzschia closterium</i> (Ehrenberg) W. Smith			+
<i>Nitzschia incerta</i> (Grunow) M. Peragallo	+	+	+
<i>Nitzschia longissima</i> (Brébisson) Ralfs	+	+	+
<i>Paralia sulcata</i> (Ehrenberg) Cleve		+	
<i>Pleurosigma angulatum</i> (J. T. Quekett) W. Smith	+	+	+
<i>P. elongatum</i> W. Smith	+	+	+
<i>P. formosum</i> W. Smith	+	+	+
<i>Proboscia alata</i> (Brightwell) Sundström	+	+	+
<i>Pseudosolenia calcar avis</i> (Schultze) B. G. Sundström	+	+	+
<i>Pseudo-nitzschia</i> spp.	+	+	+
<i>Rhizosolenia imbricata</i> Brightwell			+
<i>Rhizosolenia setigera</i> Brightwell	+	+	
<i>Synedra crystallina</i> (C. Agardh) Kützing		+	+
<i>Thalassionema nitzschioides</i> (Grunow) Mereschkowsky	+	+	+
<i>T. frauenfeldii</i> (Grunow) Tempère & Peragallo	+	+	+
<i>Thalassiosira eccentrica</i> (Ehrenberg) Cleve	+	+	+
<i>Trieres mobiliensis</i> (J.W. Bailey) Ashworth & Theriot		+	
Dinoflagellates			
<i>Ceratoperidinium falcatum</i> (Kofid & Swezy) Reñéde Salas	+	+	+
<i>Dinophysis acuminata</i> Claparède & Lachmann	+	+	
<i>D. acuta</i> Ehrenberg	+	+	
<i>D. caudata</i> Saville-Kent	+	+	
<i>D. fortii</i> Pavillard	+	+	
<i>D. tripos</i> Gourret	+		+
<i>Diplopsalis lenticula</i> Bergh		+	+
<i>Gonyaulax digitale</i> (Pouchet) Kofoid	+	+	+
<i>Gonyaulax polygramma</i> Stein	+	+	+
<i>Gonyaulax</i> spp.	+	+	+
<i>Gonyaulax verior</i> Sournia	+		
<i>Gymnodinium</i> spp.	+	+	+
<i>Gyrodinium fusiforme</i> Kofoid & Swezy	+	+	+
<i>Gyrodinium</i> spp.			
<i>Hermesinium adriaticum</i> O. Zacharias		+	+
<i>Lingulodinium polyedra</i> (F. Stein) J. D. Dodge	+	+	
<i>Ornithocercus quadratus</i> Schütt			+
<i>O. steinii</i> Schütt			+

Table 4. Continued.

Taxa	IBM	COGI	Žanjic
<i>Atoms</i>			
<i>Oxytoxum sceptrum</i> (F. Stein) Schröder	+	+	+
<i>O. scolopax</i> Stein	+		
<i>Phalacroma rotundatum</i> (Claparede & Lachmann) Kofoid & Michener	+	+	+
<i>Podolampas elegans</i> Schütt		+	
<i>P. palmipes</i> Stein	+		
<i>Prorocentrum cordatum</i> (Ostenfeld) J. D. Dodge	+	+	+
<i>P. micans</i> Ehrenberg	+	+	+
<i>P. triestinum</i> J. Schiller			
<i>Protoperidinium conicum</i> (Gran) Balech		+	
<i>P. crassipes</i> (Kofoid) Balech	+	+	
<i>P. diabolus</i> (Cleve) Balech	+	+	+
<i>P. divergens</i> (Ehrenberg) Balech	+	+	
<i>P. globulus</i> (F. Stein) Balech	+	+	
<i>P. oceanicum</i> (Vanhöffen) Balech			+
<i>P. pallidum</i> (Ostenfeld) Balech	+	+	+
<i>P. pellucidum</i> Bergh		+	+
<i>P. steinii</i> (Jørgensen) Balech	+	+	
<i>P. tuba</i> (Schiller) Balech		+	+
<i>Pyrocystis elegans</i> Pavillard	+		
<i>P. lunula</i> (Schütt) Schütt	+		
<i>Scrippsiella</i> sp.		+	+
<i>Triadinium polyedricum</i> (Pouchet) Dodge	+	+	+
<i>Tripos azoricus</i> (Cleve) F. Gómez			+
<i>T. furca</i> (Ehrenberg) F. Gómez	+	+	+
<i>T. fusus</i> (Ehrenberg) F. Gómez	+	+	+
<i>T. horridus</i> (Cleve) F. Gómez	+	+	
<i>T. kofoidii</i> (Jørgensen) F. Gómez			+
<i>T. macroceros</i> (Ehrenberg) F. Gómez	+		
<i>T. pentagonus</i> (Gourret) F. Gómez	+		
<i>T. muelleri</i> Bory de Saint-Vincent	+	+	+
<i>T. teres</i> (Kofoid) F. Gómez			+
<i>Tryblionella compressa</i> (J. W. Bailey) Poulin	+	+	
<i>Coccolithophorids</i>			
<i>Calciosolenia brasiliensis</i> (Lohmann) J. R. Young	+	+	+
<i>Calyptrosphaera oblonga</i> Lohmann	+	+	+
<i>Helicosphaera walichii</i> (Lohmann) Okada & McIntyre	+	+	+
<i>Rhabdosphaera tignifer</i> Schiller	+	+	+
<i>Syracosphaera pulchra</i> Lohmann	+	+	+
<i>Silicoflagellates</i>			
<i>Dictyocha fibula</i> Ehrenberg	+	+	+
<i>Octactis octonaria</i> (Ehrenberg) Hovasse	+	+	+

Bay was generally lower during the investigated period, if we compare with the previous data available for the Boka Kotorska Bay (Drakulović, Pestorić, Cvijan, Krivokapić, & Vuksanović, 2012). Generally, in the Adriatic Sea (Burić *et al.*, 2007; Viličić *et al.*, 2008), limited nutrient is phosphate almost throughout the year, while nitrogen availability is sporadically limited in the summer.

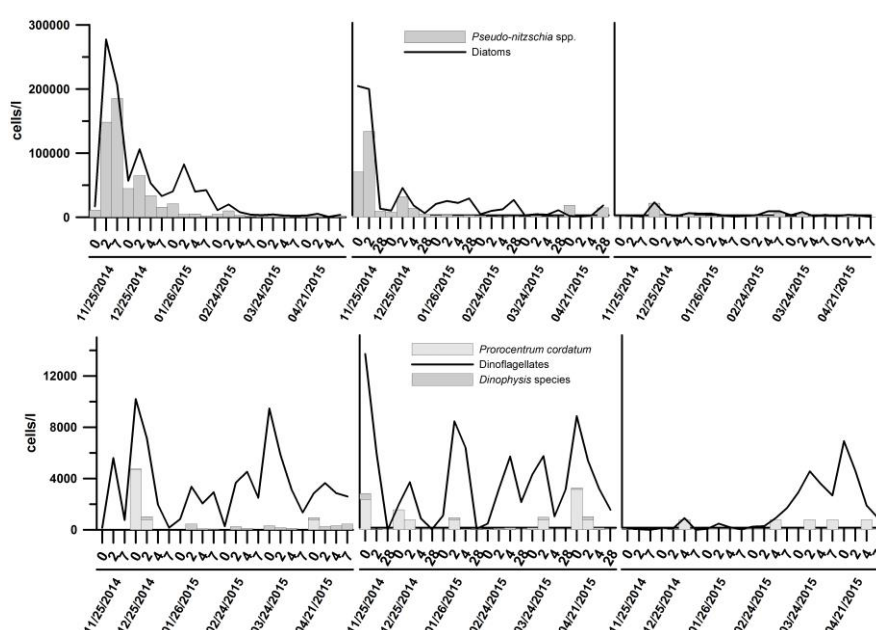
Negative significant correlation between nitrate and silicate on one side and salinity on other indicates an influence of river runoff during periods of high precipitation. The same correlation was noticed in other Adriatic estuaries (Burić *et al.*, 2007; Aubry, Berton, Bastianini, Socal, & Aciri, 2004), while in previous study of the Bay correlation was positive (Drakulović *et al.*, 2012).

Seasonality of phytoplankton biomass (as

indicated by chlorophyll *a* concentration) is generally characterized by the highest values in winter and minimum in summer (Krivokapić *et al.*, 2009, Ninčević & Marasović, 1998). According to our part of research, which includes autumn and winter period, chlorophyll *a* was the highest in the early winter period. The same situation was noticed in the coastal north-western Mediterranean (Cermenio *et al.*, 2006) and in the eastern Adriatic Sea (Buzančić, Ninčević-Gladan, Marasović, Kušpilić, & Grbec, 2016) where chl *a* increases in the autumn—winter period. The present result is slightly different from the results recorded in the Zrmanja estuary (Burić *et al.*, 2007) and in the south-eastern Adriatic (Krivokapić *et al.*, 2011), where a chlorophyll *a* peak was noticed in late winter and spring when the water column is rich with regenerated nutrients and solar radiation is increasing.

**Table 5.** List of toxic and potentially toxic phytoplankton taxa identified in the Boka Kotorska Bay. Total number is 9 samples. Abundances were expressed as cells/l. MAX: maximum abundance; FR%: frequency of appearance; AVG: average abundance; SD: standard deviation

Taxa	Max (cells/l)	FR%	AVG	SD
<b>Diatoms</b>				
<i>Pseudo-nitzschia</i> spp.	1.85 x 10 <sup>5</sup>	88.40	14062.46	33612.98
<b>Dinoflagellates</b>				
<i>Dinophysis acuminata</i> Claparede & Lachmann	320	33.33	44.64	79.57
<i>D. acuta</i> Ehrenberg	80	10.14	6.38	20.14
<i>D. caudata</i> Seville-Kent	40	5.80	2.32	9.42
<i>D. fortii</i> Pavillard	160	23.19	19.13	40.94
<i>D. tripos</i> Gourret	40	2.90	1.16	6.76
<i>Lingulodinium polyedra</i> (Stein) Dodge	40	5.80	2.35	9.48
<i>Phalacroma rotundatum</i> (Claparede & Lachmann) Kofoid & Michener	40	7.25	2.90	10.45
<i>Prorocentrum cordatum</i> (Ostenfeld) Dodge	4.7 x 10 <sup>3</sup>	21.74	295.80	774.56



**Figure 4.** A. Contribution of *Pseudo-nitzschia* spp., in total abundance of diatoms on three locations B. Contribution of *Dinophysis* species and *Prorocentrum cordatum* in total abundance of dinoflagellates on three locations.

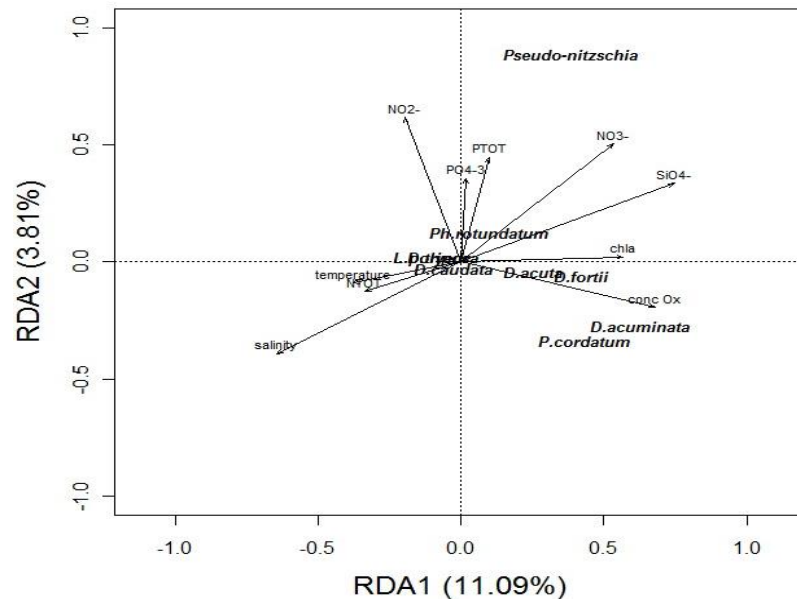
The chlorophyll *a* values in this study were significantly lower than those in the previous study of Boka Kotorska Bay (Drakulović *et al.*, 2012).

Numerous studies emphasize that that concentration of chl decreases as phytoplankton standing stocks increase (Desortova, 1981; Chow-Fraser, Trew, Findley, & Stainton, 1994; Sandu, Iacob, & Nicolescu, 2003; Kiss, Devai, Tothmeresz, & Szabo, 2006). This phenomenon may be caused by phytoplankton community structure (Nusch & Palme, 1975), the size frequency distribution of the algal cells (Watson & McCauley, 1988), and by seasonal shifts within the plankton community (Loth, 1985; Vanni *et al.*, 1993).

The maximum microplankton abundance in current research was lower than the values found in the Boka Kotorska Bay (Drakulović *et al.*, 2012; Bosak *et al.*, 2012) and similar with the data recorded in the northeastern Adriatic Sea (Lim Bay and

Zrmanja Bay) (Burić *et al.*, 2007; Bosak *et al.* 2009). Also the value was lower compared to with microplankton abundance in the northwestern Adriatic Sea (Aubry *et al.*, 2004), which is influenced by two important rivers (Po and Adige) that caused modification of local circulation with wind stress.

Diatoms were the most abundant component of the phytoplankton community in all locations and dinoflagellates were the second significant component of the phytoplankton community, while in terms of diversity, dinoflagellates were a bit higher. In this research, diatoms were present with 42 taxa, dinoflagellates with 50 taxa which is different from the result noted by Bosak *et al.* (2012) and similar with data noted by Drakulović *et al.* (2012). In the northwestern Adriatic Sea, as expected in a nutrient-enriched system, the community structure was dominated by diatoms over most of the year (Aubry *et al.*, 2004). Similar was noticed in the eastern Adriatic



**Figure 5.** Redundancy analysis (RDA) showing bi-dimensional ordination of phytoplankton species abundance. Superimposed vectors represent explanatory variables: temperature, salinity, oxygen concentration, nutrients concentration, chl *a* concentration, *Pseudo-nitzschia* spp., *Dinophysis acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. tripos*, *Lingulodinium polyedra*, *Phalacroma rotundatum*, *Prorocentrum cordatum*. In order to make their scales comparable, both biological and hydrological variables were scaled to unit variance.

by Bužančić, Ninčević-Gladan, Marasović, Kušpilić and Grebec (2016). In the middle Adriatic, on the western coastal area, Totti *et al.* (2000) noticed maximal value of diatoms due to the growth of diatoms species *Pseudo-nitzschia* spp., and also the similar finding is for southern Adriatic Sea (Caroppo, Congestri, Bracchini, & Albertano, 2005). In this study, maximal abundance of diatoms was in autumn, which is different when compared to previous researches by Drakulović *et al.* (2012) when the highest value was recorded in late winter –early spring.

Among the diatoms, potentially toxic species from genus *Pseudo-nitzschia* was the most frequent and with higher abundances in order up to  $10^5$  cells/l. Diatoms belonging to the genus *Pseudo-nitzschia* spp. are dominant in the phytoplankton of the Adriatic Sea. Ujević *et al.* (2010) state that *Pseudo-nitzschia* spp. was widely distributed across the Adriatic Sea of both warm and cold climate conditions within the phytoplankton community throughout the investigation period. Caroppo *et al.* (2005) and Viličić, Đakovac, Burić and Bosak (2009) noticed their appearance in late winter-spring which can be explained with their ability to survive relatively more turbulent conditions which are present during the cold part of the year. In this study the maximal abundance of *Pseudo-nitzschia* spp. was noticed in November 2014 ( $1.85 \times 10^5$  cells/l) (Figure 4), with diatoms abundance also the highest in November. These results were similar with the data noted by Drakulović *et al.* (2012) and Bosak *et al.* (2012) for the Boka Kotorska Bay, Totti *et al.* (2000) for the middle Adriatic.

The densities of *Pseudo-nitzschia* spp. negatively but not significantly correlated with temperature. Our results are similar with those of Caroppo *et al.* (2005), where this correlation was negative. Positive correlation with temperature was noticed by Bosak *et al.* (2012) for the Bay for the species *Pseudo-nitzschia pseudodelicatissima*. In this study, *Pseudo-nitzschia* showed positive correlation with total phosphorus and phosphates concentration as it was noticed for diatoms suggesting that phosphates limited productivity in the Bay. RDA results also suggest positive association of total phosphorus and phosphates concentration with abundance of *Pseudo-nitzschia* species (Figure 5).

Ujević *et al.* (2010) reported from their study that *Pseudo-nitzschia* spp. were always present throughout the study period in the south Adriatic Sea and with no DA detected. It is important that not all *Pseudo-nitzschia* species are toxic and that even toxic ones do not always express toxicity. According to extensive data on the *Pseudo-nitzschia* spp. abundances in the study area of the Adriatic Sea, we can assume that if the abundance does not reach  $1.0 \times 10^5$  cells/l then the area can be considered safe with respect to ASP. If the abundance exceeds  $1.0 \times 10^6$  cells/l it might indicate the possible occurrence of ASP contamination. These results add to the previously published observations of domoic acid occurrence in shellfish found in many maritime countries of the European Union.

Dinoflagellates were less abundant phytoplankton group compared with diatoms but a bit more diverse than diatoms. Among dinoflagellates, 8 toxic dinoflagellates were recorded. The most

frequent genus is *Dinophysis*, represented by five species.

Higher abundance and frequency of *Dinophysis acuminata* was noticed in spring (Figure 4) which is similar to some published data (Sidari, Cok, Cabrini, Tubaro, & Honsell, 1995; Bernardy Aubry et al., 2000). In Maizuru Bay (Japan) *D. acuminata* occurred frequently in spring and autumn (Nishitani, Sugioka, & Imai, 2002). Correlation of *D. acuminata* and salinity was negative suggesting better growth when salinity is lower. This situation was also confirmed by RDA analysis (Figure 5).

*Dinophysis acuta* showed higher abundances and frequency in late winter (Figure 4) which differs from the data recorded by Caroppo, Congestri, and Bruno (2001) and Klöpper, Scharek, and Gerdtz (2003). Correlation of abundance of *D. acuta* and salinity was significantly negative which indicates better growth in the period of increased freshwater input which differs from the data recorded by Ninčević et al. (2008) where this species growth was higher when salinity is increased. This situation was also confirmed by RDA analysis (Figure 5). High numbers of *Dinophysis acuta* in the rias of Vigo and Pontevedra are typically found in autumn, whereas different *Dinophysis* species are recorded in very low concentrations throughout the winter months (Blanco et al., 1995).

In some exceptionally warm autumns, moderate to high concentrations of *D. acuminata* and *D. acuta* can be found until as late as mid-December (Blanco et al., 1995). In French Atlantic areas water stratification is considered the key environmental condition promoting the increase of *Dinophysis* cell density (Delmas, Herbland, & Maestrini, 1992)

Increased abundance of *D. fortii* in this study was noticed in late autumn early winter (Figure 4), which differs from the data noted by Caroppo et al. (2001) where this species was frequent and more abundant in spring. Correlation between abundance of *D. fortii* and salinity was negative (Figure 5), indicating their better growth when input of freshwater is higher. In Mali Ston, negative correlation was noted between *D. fortii* and temperature, similar to this study, but correlation was not significant, while correlation with salinity was positive (Ninčević et al., 2008).

Higher frequency of *Dinophysis caudata* in this study was noticed in January (Figure 4), different from the data noted by Caroppo et al. (2001) as this species was frequent and abundant in autumn. Ninčević et al. (2008) noticed a positive correlation of this species and temperature.

*Dinophysis tripos* was noticed in early and late winter (Figure 4). These results are different from the data of Sidari et al. (1995), Blanco et al. (1998), Bernardy Aubry et al. (2000) and coincided with the data noted by Ninčević et al. (2008).

Species *Phalacrocoma rotundatum* was noticed during the entire investigation period except in April.

This coincided with the data noted by Caroppo et al. (2001) where this species was abundant in autumn-winter period.

Species *Prorocentrum cordatum* was present during the entire investigation period with higher abundance ( $4.71 \times 10^3$  cells/l) in winter period (Figure 4). This value is lower than values noticed by Bosak et al. (2012) and Drakulović et al. (2012) for the same Bay, when abundance reached up to  $10^4$  cells/l. This species showed positive correlation with phosphate and silicate (Figure 5) which is similar to those found by Bosak et al. (2012).

*Lingulodinium polyedra* was noticed frequently in early and late winter. This species was in a slightly positive correlation with total nitrogen concentrations (Figure 5). Bellefeuille, Dorion, Rivoal & Morse (2014) found that this species reacts to nitrogen stress, as do most plants and microalgae, by stopping cell growth and diminishing levels of internal nitrogen, in particular in the form of protein and chlorophyll.

During research, toxic dinoflagellates were noticed in lower abundances except *Prorocentrum cordatum* which reached higher abundance. The main difference in comparison between *Prorocentrum* and *Dinophysis* is that *Dinophysis* blooms are rare, but they can induce poisoning even at low cell densities (Bruno et al., 1998).

The RDA results suggest that among the abiotic parameters, temperature and salinity and nitrates and silicates availability had greater influence on phytoplankton variability during our investigation.

## Conclusion

Ability of phytoplankton species to produce toxins may result in toxin accumulation throughout the food chain, and also impacts marine organisms, humans and ultimately the ecosystems and the economy. In current research, toxic and potentially toxic species were noticed. The presence of diatom *Pseudo-nitzschia* spp. is also important due to its possibility of producing domoic acid as abundance of this diatom was higher in comparison with other noticed toxic species. Among toxic dinoflagellates, *Prorocentrum cordatum* was noticed in higher abundance. Intensive growth of toxic species can lead to accumulation of toxins in the shellfish meat and through the food chain can reach to the final consumers, humans, causing serious shellfish poisoning that can seriously endanger human life. In the Boka Kotorska Bay, the investigated area, there are 19 shellfish farms cultivating mostly mussels, of which two are fish farms rearing seabass/seabream, registered in the Boka Kotorska Bay. The contribution of the aquaculture in the Montenegrin national economy is still insignificant. Although the economic value is currently very low, the aquaculture seems to have a great potential for future development. Therefore, modernization of the sector, diversification in production, and training and education, could all

develop this potential. The most important activity would be establishing of a continuous monitoring programme of biotoxins in shellfish meat and more intensive monitoring of toxic phytoplankton species in water on mussels farms in the Boka Kotorska Bay. These monitoring activities would enable timely reactions aimed at preventing the potential human poisoning and negative consequences for the ecosystem.

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