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Dinoflagellate assemblages in the Boka Kotorska Bay

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ABSTRACT

This study presents distribution of dinoflagellates in the semi-enclosed Boka Kotorska Bay. Samplings were performed twice per month from the June 2009 to the June 2010. Samples were taken on five positions in two parts of the Bay: inner, closer Kotor Bay and middle part of Boka Kotorska Bay (Tivat Bay). Maximum of dinoflagellates abundance was noticed in summer period, on position IMB (1.05 x 10^5 cells/l). Among the dinoflagellates, eight species that produce toxins were recorded: Dinophysis acuminata, D. acuta, D. caudata, D. fortii, D. hastata, D. sphaerica, Phalacroma rotundatum *and* Prorocentrum minimum.

These are the basic information concerning distribution of dinoflagellates in Boka Kotorska Bay. Knowledge that increase of human impact in the area and appeareance of toxic dinoflagellates can cause serious problems to marine ecosystem could be useful for prevention.

Keywords: Dinoflagellates assemblages, toxic dinoflagellates, Boka Kotorska Bay.

INTRODUCTION

Reactions of phytoplankton organisms on fluctuations under different environmental conditions are rapid and very complex. Coastal area are characterized by high spatial and temporal variability of environmental parameters. Mainly due to the increasing of impact of human activities on the functioning of coastal ecosystems, it is essential to determine the basic phytoplankton assemblages in these areas (Cloern 1999).

In marine ecosystems, dinoflagellates, along with diatoms, are important components of the phytoplankton. Approximately 90% of all dinoflagellates species are marine, most of them distributed in temperate waters and most prevalent in summer months (Taylor 1987).

Since dinoflagellates display very diverse ecophysiological characteristics, generalizations about their roles in ecosystems are not easily made. Photosynthetic species play a role in marine primary production, but most species are heterotrophic (Loeblich III 1984), and may be important grazers on the larger phytoplankton. Some species may cause red tides and some species produce potent neuro-toxins which may accumulate in the food chain (Hallegraeff 1993).

Investigations of dinoflagellates populations were performed in Adriatic Sea, with emphasis on their toxicity (Boni *et* al., 1992; Honsell *et* al., 1996) and consequences on shellfish farms (Marasović *et al*., 1998; Marasović *et al*., 2007; Ninčević *et al*., 2008).

In Boka Kotorska Bay mussel farming began in the 1980s and today there are 16 farms using the system of the floating buoys and ropes. As mussels are filter feeders which accumulate phytoplankton, the problem can occur if there is a presence of toxic phytoplankton, mostly are dinoflagellates that can caused negative human health consequences.

Several previous studies related to the Boka Kotorska Bay referred to phytoplankton assemblages and include data of dinoflagellates composition and density (Drakulović *et al*. 2011, 2012). Usually outbreaks of harmful and toxic microalgea are observed in areas of the sea occupied by mariculture installations (Probyn *et al.* 2001; Rhodes *et al.* 2001; Ventilla 1982).

This study aimed at forming a species list of dinoflagellates and showing their abundances. The purpose is to identify the dinoflagellates species and to emphasize species which have the potential to form harmful algae blooms and to get chance to estimate possible changes that can cause negative effect on humans' health through consuming of infected mussels.

MATERIALS AND METHODS

Boka Kotorska Bay was investigated area in this study. Bay is situated in southeastern part of Adriatic Sea and it consists of four small Bays: Kotor Bay, Risan Bay, Tivat Bay and Herceg Novi Bay. Verige strait is the narrowest section of the Bay, which separate the inner Bay which belongs to the natural and culturo- historical region of Kotor, a World Heritage Site, from the rest of the Bay. The total surface area of Boka Kotorska Bay comprises 87.3 km^2. It is divided into three parts: inner, middle and external. Current study was conducted in inner and middle part of Boka Kotorska Bay (Kotor and Tivat Bay) (Figure 1, Table 1). Climate is of the Mediterranean type, and the precipitation regime is heavily influenced by mount Orjen which receives Europe's heaviest precipitation with rain occurring seasonally (Magaš, 2002).

Sampling was carried out twice per month from the June 2009 to the June 2010. Materials for phytoplankton analysis was collected from five positions in the Boka Kotorska Bay. Three positions were in the Kotor Bay and two in the Bay of Tivat.

In Kotor Bay, samples were taken from IMB and Orahovac on three depths (0 m, 5 and 10 m), and from Kotor-central on five depths (0m, 5, 10, 20 and 30m). In Tivat Bay, sampling were performed on position Sveta Nedelja on three depths (0 m, 5m and 10 m) and from site Tivat- central on five depths (0 m, 5m, 10m, 20m and 30 m).

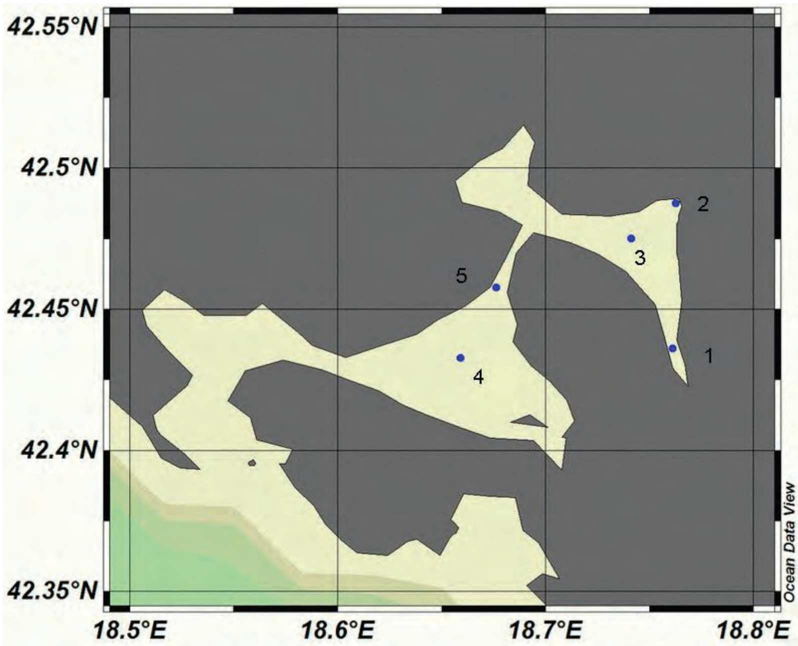


Figure 1. Investigated area

Table 1. Investigated positions

	POSITION	SOUTH	EAST
KOTOR BAY	1. Institute of Marine Biology-IMB	42° 26' 20.7"	18° 45' 65.5"
	2. Orahovac	42° 29' 23.6"	18° 45' 64.2"
	3. Kotor-central	42° 28' 43.6"	18° 44' 40.8"
TIVAT BAY	4. Tivat-central	42° 25' 91.0"	18° 39' 40.8"
	5. Sveta Nedelja	42° 27'39.3"	18° 40' 50.5"

Samples were collected with Niskin sampler of 5 l. Physical parameters such as temperature, salinity and dissolved oxygen concentration were measured *in situ* using a universal meter (Multiline P4; WTW). Nutrient (nitrates, nitrites, silicates and phosphates) concentrations were determined by standard colorimetric method (Strickland *et al*. 1972) using a spectrophotometer type *Perkin Elmer* χ *2*. Phytoplankton samples were preserved in a 3 % neutralized formaldehyde solution. After 24 h of sedimentation in sediment chambers, cells were enumerated using inverted microscope according to Utermöhl (1958). For determination of phytoplankton species, it was used appropriate key for the specified field of investigation (Hustedt 1959, Hasle & Syvertsen 1997, Round *et al*. 1990, and Throndsen *et al*. 2007).

RESULTS AND DISCUSSIONS

During the study, as we expect, in the summer period temperature showed maximum peak (26.83 °C), while in winter period it was noticed minimum temperature 7.2 ^{o}C (Table 2.).

Salinity varied during the study period, decreasing to a minimum value of 1‰ at surface layer (Table 2.). The minimum value was due to the impact of rainfall, as recorded in the winter, but also the impact of the influx of fresh water through rivers that are present at the position Orahovac (the inner part of the Bay, Kotor Bay), where it was measured the lowest salinity.

Oxygen concentration ranged from 6.85 to 10.43 mg/l, which shows that the water of Boka Kotorska Bay is rich in oxygen (Table 2.). Sufficient oxygen concentration was throughout the study period, but it was lower in the summer and higher in the colder, winter period.

Concentration of nutrients in the Boka Kotorska Bay was generally high during the investigated period and favorable for phytoplankton development. The maximum value of nitrate was 25.88 µmol/l and it was recorded in April 2010 when the phytoplankton abundance was lower. Peak of phosphate concentration was noticed in January (0.97 µmol/l), while peak of silicate concentration was 75.08 µmol/l in January 2010 (Table 2). Statistica 7and Primer 5 programs were used for statistical analyses and graphical presentations of physical, chemical, and biological data.

Dinoflagellates abundance varied during the investigated period. Maximum abundance of dinoflagellates ranged from 1.76 x 10^3 cells/l in June 2009, on Tivat position, to 1.05 x 10^5 cells/l in September 2009, on the position of IMB (Table 3).

Table 2. Maximum (Max.), minimum (Min.), AVG (mean) values and standard deviation (SD) of temperature (Temp.), salinity (Sal.), oxygen concentration (Oxy. Conc.), nitrate (NO_3^-), phosphate (PO_4^{3-}) and silicate (SiO_4^-) from June 2009 to June 2010.

	Temp. (°C)	**Sal. (°/∘∘)**	**Oxy conc. (mg/l)**	**NO_3^- (µmol/l)**	**PO_4^{3-} (µmol/l)**	**SiO_4^- (µmol/l)**
Max	26.83	39.9	10.43	25.88	0.97	75.08
Min	7.2	1	6.85	0	0	0
AVG	17.63	31.29	8.93	2.59	0.18	5.82
SD	4.03	9.29	0.65	3.16	0.15	8.63

A peak of dinoflagellate in September 2009, on the surface layer (1.05 x 10^5 cells/l) can be explained by decreasing of concentration of available nutrients. That resulting with stratification which causes reduction of the mixing of nutrients regenerated in the sediments with the warmer, upper layers of the water column. Important is that the reduced supply of nutrients favored the development of dinoflagellates in summer (Burić *et al*., 2007). Polat (2002) reported the dominance of dinoflagellates in summer period, when dinoflagellates were done more than 60% of the total plankton. Mainly after the spring "blooms" of diatoms, when the waters were poor with nutrients, favoring the development of dinoflagellates, which have lower requirements for nutrients (Thingstad & Sackshang, 1990). These are recorded by Bernardi-Aubry *et a*l. (2004), when the maximum dinoflagellates occurred in June-July, after the development of diatoms. Noticed value of dinoflagellates differ from data quoted by Fanuko and Valčić (2011) for the Stella Maris Lagoon, where the maximum number of dinoflagellates was in the spring and the value does not exceed 10^4 cells /l. Svensen *et al.* (2007) for eastern Adriatic sea found a highest number of dinoflagellates than was observed in this study.

Table 3. Mean value, minimum (Min), maximum (Max.), standard deviation (SD) of the dinoflagellates in the investigated period.

	Month	**Mean (cells/l)**	**Min.(cells/l)**	**Max.(cells/l)**	**SD**
2009	June	544	0	1.76×10^3	5.09×10^2
	July	2.76×10^3	40	1.25×10^4	2.98×10^3
	August	2.49×10^3	0	1.88×10^4	4.59×10^3
	September	5.16×10^3	200	1.05×10^5	1.72×10^4
	October	930	80	7.09×10^3	1.33×10^3
	November	3.20×10^3	0	5.13×10^4	8.51×10^3
	December	2.17×10^3	40	1.38×10^4	3.19×10^3
2010	January	985	0	2.48×10^3	7.15×10^2
	February	792	0	2.37×10^3	7.28×10^2
	March	939	80	4.67×10^3	9.89×10^2
	April	1.98×10^3	0	2.16×10^4	4.29×10^3
	May	1.17×10^3	0	8.17×10^3	1.75×10^3
	June	6.68×10^3	40	3.98×10^4	1.13×10^4

Dinoflagellate abundance varied depending on the depths and the largest value was at the surface layer (5.1×10^3 cells/l) and then started to decrease. Duncan test run on abundance of dinoflagellates showed a statistically significant difference between surface and deeper layers (10m, 20 and 30 m) and between depth of 5 m and remaining deeper layers (Figure 2).

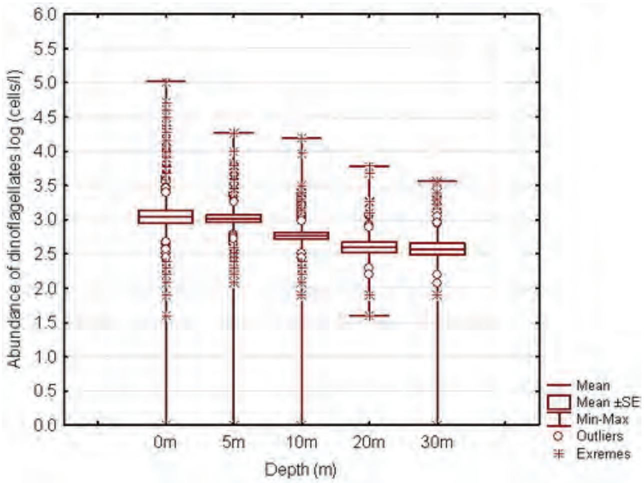
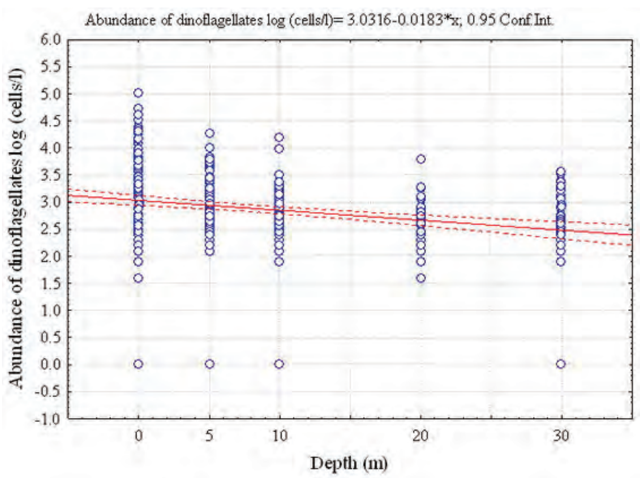


Figure 2. Mean abundance of dinoflagellates by depths

Decreased number of dinoflagellates was observed, going to the deeper layers, as was shown with negative correlation (Figure 3).



Depth (m):Abundance of dinoflagellates log (cells/l): r = -0,2458; p = 0,0000004

Figure 3. Correlation of dinoflagellates abundance and depths

The highest mean abundance of dinoflagellates was recorded at the position of IMB, while at other position was lower. Duncan's test run on dinoflagellates abundance showed statistically significant differences between IMB position and other positions (Figure 4).

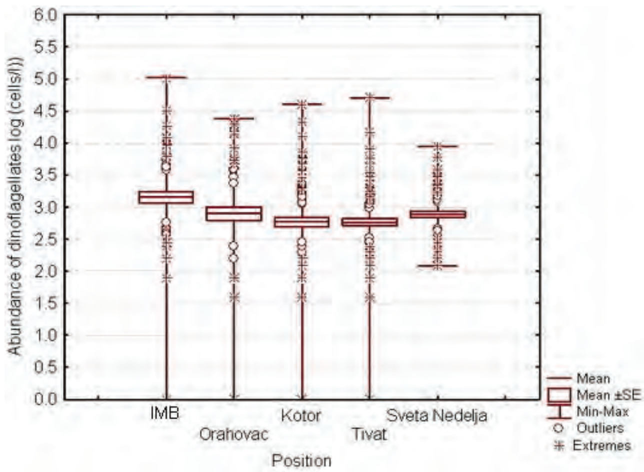
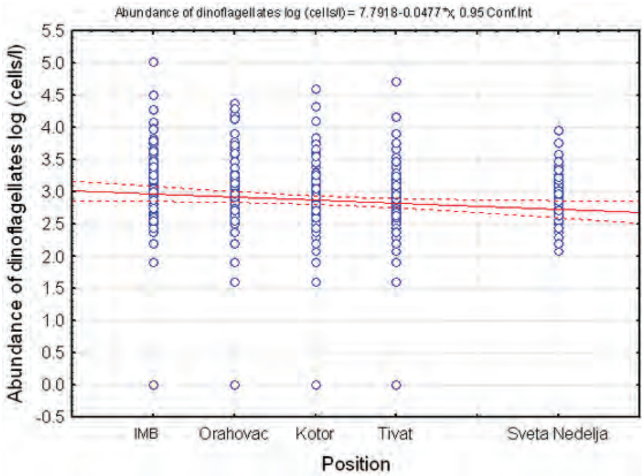


Figure 4. Mean abundance of dinoflagellates by positions

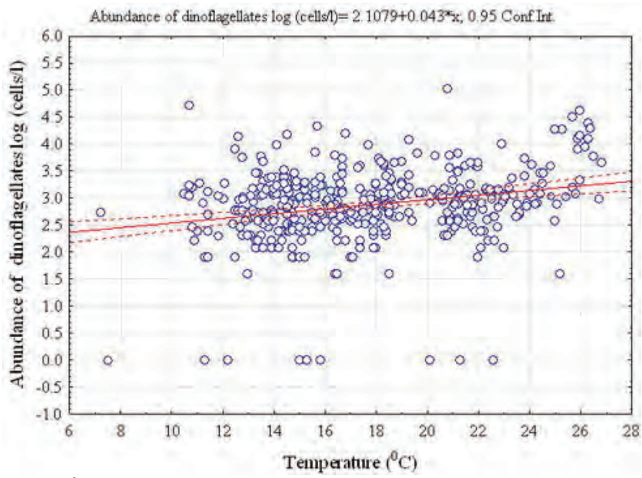
Dinoflagellates abundance increased in the summer period, at higher water temperature, which was confirmed with positive correlation between these two parameters (Figure 6.)

Dinoflagellate abundance was negatively correlated with nitrate and silicate, and positively with phosphate (Figure 7, 8, 9). This coincided with the data noticed by Viličić *et al*. (2007) in the Lim Bay for silicates, and Svensen *et al*. (2006) in the eastern Adriatic for phosphate .



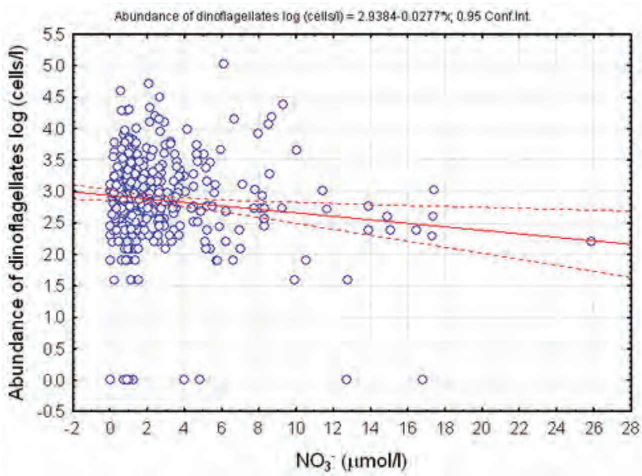
Position:Abundance of dinoflagellates log (cells/l): r=-0,1079; p=0,0275

Figure 5.Correlation of dinoflagellates abundance and position



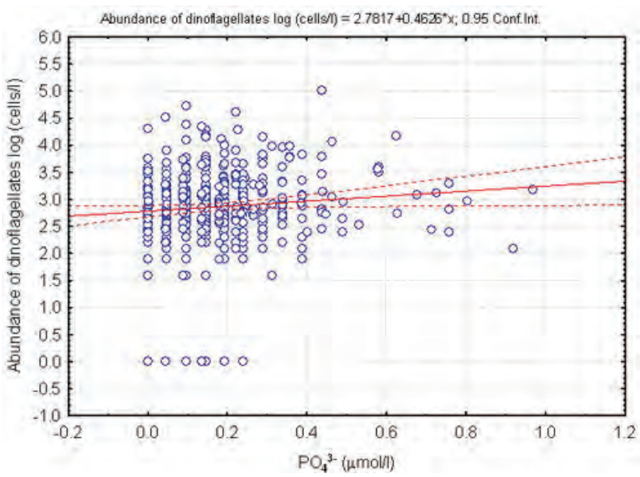
Temperature (^{0}C):Abundance of dinoflagellates log (cells/l): r = 0,2524; p = 0,0000002

Figure 6. Correlation of dinoflagellates abundance and temperature



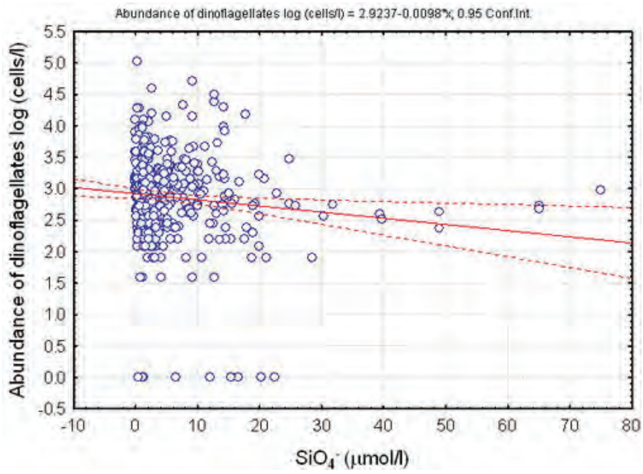
NO_3^-(µmol/l):Abundance of dinoflagellates log (broj cells./l): r = -0,1278; p = 0,0089

Figure 7. Correlation of dinoflagellates abundance and nitrates concentration



PO_4^{3-}(µmol/l):Abundance of dinoflagellates log (cells/l): r = 0,1019; p = 0,0374

Figure 8. Correlation of dinoflagellates abundances and phosphates concentration



SiO_4^- (μmol/l): Abundance of dinoflagellates log (cells/l): r = -0,1231; p = 0,0117

Figure 9. Correlation of dinoflagellates abundance and silicates concentration

Dinoflagellates are the most important group of marine phytoplankton, which produce biotoxins and harmful algal "blooms". A total of 83 species of dinoflagellates were recorded.

The dominant species (of which the number is greater than 10^3 cells/l or which are present in more than 10% of the samples) were: *Dinophysis fortii* (22,97%), *Diplopsalis lenticula* (16,51%), *Gonyaulax* spp. (30,38%), *Gymnodinium* spp. (69,38%), *Gyrodinium fusiforme* (22,97%), *Neoceratium furca* (16,03%), *N. fusus* (11,96%), *N. horridum* (15,55%), *N. tripos* (15,79%), *Oxytoxum sceptrum* (11,00%), *Prorocentrum compressum* (12,92%), *P. micans* (62,92%), *P. minimum* (16,03%), *Protoperidinium crassipes* (17,22%), *P. diabolum* (13,88%), *P. globulum* (11,48%), *Protoperidnium* spp. (24,64%) i *Scrippsiella* sp. (20,10%).

Table 3. Lists of dinoflagellates determined in Boka Kotorska Bay from June 2009 to June 2010. (MAX. – maximum value; FR. –frequency of species; AVG. –mean value SD. –standard deviation;).

Dinoflagellates	**MAX.**	**FR.**	**AVG.**	**SD.**
Amphidinium acutissimum Schiller	160	2,87	2,01	12,99
A. lanceolatum Schröder	40	0,48	0,19	2,76
Amphidinium sp.	785	2,15	6,59	66,83
Corythodinium constrictum (Stein) Taylor	40	0,72	0,29	3,38
C. tesselatum (Stein) Loeblich Jr & Loeblich III	40	0,96	0,38	3,90
Dinophysis acuminata Clap. et Lachm.	600	5,26	5,07	35,16
D. acuta Ehrenb.	40	0,24	0,10	1,96
D. caudata Seville-Kent	240	6,22	4,31	20,37
D. fortii Pav.	960	22,97	18,76	61,51
D. hastata Stein	40	0,48	0,19	2,76
D. sphaerica Stein	40	0,24	0,10	1,96
Dinophysis sp.	40	0,24	0,10	1,96
Diplopsalis lenticula Bergh	480	16,51	18,46	54,98
Diplopsalis sp.	400	6,94	6,89	32,40
Dissodinium elegans (Pav.) Matz.	120	1,91	1,15	9,12
Ebria tripartita (Schumann) Lemmermann	40	0,48	0,19	2,76
Goniodoma polyedricum (Pouchet) Jørg.	200	7,66	4,59	19,04
Gonyaulax digitale (Pouchet) Kof.	80	1,91	0,86	8,93
G. hyalina Ostenf.& Schmidt	40	0,24	0,10	1,96
G. polygramma Stein	1.200	7,89	9,67	72,04
G. spinifera (Clap. et Lachm.) Diesing	40	0,24	0,10	1,96
Gonyaulax spp .	3.925	30,38	79,72	280,53
G. verior Sournia	120	1,20	0,86	8,93
Gymnodinium cucumis Schütt	40	0,24	0,10	1,96
Gymnodinium spp.	28.286	69,38	578,14	1.990, 76
Gyrodinium fusiforme Kof. et Sw.	2.560	22,97	34,74	146,37
Gyrodinium spp.	160	3,59	2,39	13,78
Heterodinium milneri (Murr.&Whitt.) Kof.	40	0,24	0,10	1,96
Neoceratium candelabrum (Ehrenb.) Gomez , Moreira and Lopez-Garcia	80	0,48	0,29	4,37
N. carriense Gourr.	80	1,91	0,96	7,27
N. contortum (Gourr.) Cleve	40	0,24	0,10	1,96
N. furca (Ehrenb.) Gomez , Moreira and Lopez-Garcia	1.240	16,03	13,21	67,70
N. fusus (Ehrenb.) Gomez , Moreira and Lopez-Garcia	520	11,96	9,09	41,15
N. gravidum (Gourr.)Gomez , Moreira and Lopez-Garcia	40	0,24	0,10	1,96
N. gibberum (Gourr.) Gomez , Moreira and Lopez-Garcia	40	0,24	0,10	1,96

Dinoflagellates	**MAX.**	**FR.**	**AVG.**	**SD.**
N. horridum (Gran) Gomez, Moreira and Lopez-Garcia	360	15,55	11,96	38,47
N. hexacantum (Gourr.) Gomez , Moreira and Lopez-Garcia	80	1,20	0,57	5,51
N. kofoidii (Jørg.) Gomez, Moreira and Lopez-Garcia	40	0,24	0,10	1,96
N. lineatum (Ehrenb.) Gomez Moreira and Lopez-Garcia	40	0,48	0,19	2,76
N.macroceros(Ehrenb.)Gomez,Moreira and Lopez-Garcia	40	0,48	0,19	2,76
N. massiliense (Gourr.) Gomez , Moreira and Lopez-Garcia	80	0,48	0,29	4,37
N. pentagonum (Gourr.) Gomez Moreira and Lopez-Garcia	240	2,39	1,82	16,17
Neoceratium sp.	40	0,96	0,38	3,90
N. trichoceros(Ehrenb.) Gomez,Moreira and Lopez-Garcia	640	7,18	7,27	43,98
N. tripos (Müller) Gomez, Moreira and Lopez-Garcia	2.080	15,79	26,70	159,20
Noctiluca scintillans (Macartney) Kof. et Sw.	200	5,50	3,73	18,93
Ornithocercus heteroporus Kof.	280	0,48	0,77	13,83
Oxytoxum adriaticum Schiller	80	0,96	0,57	6,17
O. caudatum Schiller	160	0,24	0,38	7,83
O. laticeps Schiller	360	1,44	1,82	20,37
O. sceptrum (Stein) Schröder	600	11,00	14,26	60,56
O. sphaeroideum Stein	360	2,63	2,87	22,99
O. scolopax Stein	480	1,20	1,53	23,78
Oxytoxum sp.	80	1,20	0,57	5,5
O. variabile Schiller	40	0,24	0,10	1,96
Phalacroma rotundatum (Clap.et Lachm.) Kof. et Michener	160	7,42	3,92	16,50
Podolampas elegans Schütt	120	2,87	1,63	10,05
Prorocentrum compressum (Bailey) Abé ex Dodge	2.080	12,92	16,46	110,79
P. micans Ehrenb.	93.122	62,92	669,66	4.913,47
P. minimum (Pav.) Schiller	78.540	16,03	487,74	4.708,6
P. scutellum Schröder	160	1,44	0,96	9,55
Prorocentrum sp.	80	0,48	0,29	4,37
P. triestinum Schiller	1.570	3,35	6,74	79,37
Protoperidinium crassipes (Kof.) Bal.	400	17,22	13,11	40,34
P. conicum (Gran) Bal.	160	1,91	1,44	11,5
P. depressum (Bailey) Bal.	40	0,24	0,10	1,96
P. diabolum (Cleve) Bal.	480	13,88	10,81	38,31

Dinoflagellates	**MAX.**	**FR.**	**AVG.**	**SD.**
P. divergens (Ehrenb.) Bal.	80	3,35	1,82	10,39
P. globulum (Stein) Bal.	400	11,48	8,99	35,41
P. leonis (Pav.) Bal.	40	0,24	0,10	1,96
P. oceanicum (Van Höffen) Bal.	40	1,44	0,57	4,76
P. pallidum (Ostenf.) Bal.	40	0,24	0,10	1,96
P. paulsenii (Pav.) Bal.	40	0,24	0,10	1,96
Protoperidinium spp.	9.429	24,64	170,26	773,68
P. steinii (Jørg.) Bal.	80	1,67	0,77	6,15
P. tuba (Schiller) Bal.	80	1,91	1,05	8,01
Pseliodinium vaubanii Sournia	200	6,94	4,11	18,44
Pyrocystis lunula (Schütt) Schütt	40	0,48	0,19	2,76
Pyrophacus steini (Schiller)Wall & Dale	40	0,24	0,10	1,96
Scrippsiella sp.	3.925	20,10	60,78	289,40
Torodinium robustum Kof. & Sw.	80	0,96	0,67	7,03
Torodinium sp.	40	0,24	0,10	1,96

Among the dinoflagellates, eight species that produce toxins were recorded: *Dinophysis acuminata, D. acuta, D. caudata, D. fortii, D. hastata, D. sphaerica, Phalacroma rotundatum, Prorocentrum minimum.* According to this research, the concentration of toxic dinoflagellates still isn't alarming, but there are certain findings where their number was increased. Regarding aquaculture, there are 16 shellfish farms cultivating mostly mussels, and 2 fish farms rearing seabass/seabream registered in the Boka Kotorska Bay. They are the main point for water filtration and potentially toxic substances. So the presence of the toxic phytoplankton species such as *Dinophysis* species indicate the importance of monitoring and research in the case of possible occurring of algal blooms in this area where is present active shellfish farming. This study revealed the toxic dinoflagellate *Dinophysis fortii* occurred at abundances 960 cells/l. This is serious because even at low concentrations of just over 1000 cells/l dinoflagellate *Dinophysis* spp. is harmful to humans (Marcaillou-Le Baut *et al.* 1993).

High value of dinoflagellates was already noticed in Boka Kotorska Bay by Drakulović *et a*l. 2012. They noticed abundance of *Prorocentrum micans* in order to 10^6 cells /l. Presence of toxic dinoflagellate *Prorocentrum minimum* was recorded in Boka Kotorska Bay among the dominant species in the phytoplankton assemblage, with a maximum abundance reaching 3.97×10^4 cells /l by Bosak *et al.* 2011. Finding of toxic species indicate the need for more intensive research and monitoring. Through more intensive monitoring of phytoplankton composition presence of toxic species and potential blooms of harmful algae can be confirmed. That can give us the possibility to react on time as these negative changes will affect shellfish farming activities.

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