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DISTRIBUTION OF PHYTOPLANKTON ON MUSSEL FARMS IN BOKA KOTORSKA BAY

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ABSTRACT

Distribution of phytoplankton on mussel farms in small semi-enclosed Boka Kotorska Bay were presented.

In this study, samplings were performed during 2011 with seasonal frequency: winter (January), spring (April) and summer (July). Samples were taken on ten positions in winter and eleven positions in spring and summer period, in two parts of the Bay: inner, closer Kotor Bay and middle part of Boka Kotorska Bay (Tivat Bay). Maximum of phytoplankton was noticed in spring period, on the mussel farm position Morinj which belongs to Kotor Bay (5.87 x 10^5 cells/L*). Diatoms prevailed almost during all investigation period, except summer period when dinoflagellates were more abundant. Maximum of diatoms was recorded in spring (5.79 x* 10^5 *cells/L). Domination of nutrients preferred species was noticed: Thalassionema nitzschioides, Pseudo-nitzschia spp., Chaetoceros affinis, Navicula spp.*

There is a lack of information concerning distribution of phytoplankton on mussel farms in Boka Kotorska Bay. Due to the anticipated increase of human impact in the area and important of phytoplankton on quality of seafood, this study can serve as a base for future studies of phytoplankton on mussel farms in Boka Kotorska Bay.

Keywords: Phytoplankton distribution, mussel farms, Boka Kotorska Bay.

INTRODUCTION

Aquaculture is the fastest growing food-producing sector in the world and is the only means of filling the growing gap between consumer demand and seafood production from traditional capture fisheries. While there is a need for the continued worldwide expansion of aquaculture to fill this gap, industry development needs to be promoted and managed in a manner that minimizes negative environmental impacts (FAO, 2008).

Environmental concerns are related primarily to how the cultured mussels interact within the ecosystem. Mussels live in dense colonies and have an exceptional capacity to filter large volumes of water to extract food (phytoplankton and other suspended particulate matter). Filter feeding by mussels naturally results in some local reduction (depletion) of their phytoplankton food supply. However, if the mussel culture is consuming phytoplankton faster than they can be replaced by tidal flushing and phytoplankton growth, then the mussels will become food limited and production will be less than maximal for that site. This is referred to as exceeding “production carrying capacity” (Cranford *et al.* 2008).

Monitoring of phytoplankton is important for successful operation of mussel farms because it helps not only to evaluate the food resources in the investigated area but also to reveal and forecast changes in the marine ecosystems (Pautova 1990; Selina 1992; Senicheva 1989; Arzul *et al.* 2001).

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. Monitoring system is going on as part of programme which is financed by the Ministry of agriculture and rural development of Montenegro. The aim is to get useful information which can be used to prevent negative effect on environment, foods and consumers. Phytoplankton analyses are included in the monitoring as an important factor, as mussels are filter feeders which accumulate phytoplankton. The problem can occur if there is a presence of toxic phytoplankton that can caused negative human health consequences.

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).

The purpose of this study is to analyze phytoplankton assemblages and to estimate possible changes that can cause negative effect on humans health through consuming of infected mussels.

MATERIALS AND METHODS

Investigated area was Boka Kotorska Bay, which consists of four small Bays: Kotor Bay, Risan Bay, Tivat Bay and Herceg Novi Bay. The total surface area of Boka Kotorska Bay which comprises 87.3 km^2 is divided into three parts: inner, middle and external. This study was conducted in inner and middle part of the Bay (Kotor and Tivat Bay) (Figure 1, Table 1). Both investigated Bays are under high influence of fresh water inputs (undersea springs, rivers, streams and precipitations) compared to opener external part (Herceg Novi Bay) which is under the influence of the open sea. The Kotor Bay, where are mostly sampling stations, is located in the innermost part encompassing around 30% of the Boka Kotorska Bay. 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). In the area of the Kotor Bay, there are two rivers: Škurda and Ljuta. Škurda is active during the whole year, while Ljuta only during the late fall, winter and early spring. In Tivat Bay there are also two rivers Široka and Gradiošnica and only Široka is active during the whole year. In the inner part waters dynamic are less then in the middle which caused higher phytoplankton growth in the inner part.

Figure 1. Investigated area

Table 1. Investigated positions

Number	Position	N	E
1	IMB	42° 26′ 10.9“	18° 45′ 50.3“
2	Ljuta	42° 29′ 01.2“	18° 45′ 52.3“
3	Orahovac	42° 29′ 20.3“	18° 45′ 51.0“
4	Guskić	42° 29′ 09.3“	18° 44′ 37.1“
5	Morinj	42° 29′ 17.5“	18° 39′ 05.4“
6	Lipci	42° 29′ 36.4“	18° 39′ 06.9“
7	Sveta nedelja	42° 27′ 33.5“	18° 40′ 21.0“
8	Obala Đuraševića	42° 23′ 40.8“	18° 42′ 03.1“
9	Uvala Kukuljina	42° 24′ 48.6“	18° 42′ 41.4“
10	Ostrvo cvijeća	42° 24′ 17.4“	18° 42′ 23“
11	Stoliv	42° 28' 38.69"	18° 41' 39.4"

Samples were taken with seasonal frequency: winter (January), spring (April) and summer (July) in 2011, on ten positions during the winter and eleven positions in spring and summer period (Figure 1). Most investigated positions are located in Kotor Bay (7 positions) and less in the middle part-Tivat Bay (4 positions). Water samples were collected from one depth, from 0.5m with 5l Niskin bottles.

Samples were taken for analyses of physico-chemical parameters and phytoplankton organisms. Physical parameters such as temperature, salinity, dissolved oxygen concentration and oxygen saturation were measured *in situ* using a universal meter (Multiline P4; WTW). Transparency was determined using a Secchi disc (30 cm). 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*.

Samples for phytoplankton investigation were taken also with 5 l Niskin bottles and preserved in a 3% neutralized formaldehyde solution. In laboratory after sedimentation of 24h in sediment chambers, cells were enamurated using a Leica DMI 4000 B inverted microscope according to Utermöhl (1958). Determination of phytoplankton species was done using the keys for phytoplankton identification such as: Hustedt (1959), Hasle & Syvertsen (1997), Round *et al*. (1990), and Throndsen *et al*. (2007). Small individuals (nanoplankton) could not be detected under light microscope and were not included in the taxonomic list. They were classified to taxonomic category: green nanoflagellates, small dinoflagellates and small coccolithophorids.

RESULTS AND DISCUSSIONS

In current study, maximum temperature was 24.6°C and maximum salinity was 37.7 ‰ (Table 4), on the position Ostrvo Cvijeća in Tivat Bay. Lowest values of temperature and salinity were in colder period, in winter. Minimum temperature was 7.2°C, on Guskić position and salinity minimum was 1.05 ‰, on Orahovac position (Table 2). In spring, temperature varied from minimum of 14.8°C to maximum of 17.4 °C (Table 3). In spring salinity maximum was 35.8 ‰, while minimum was 10.2 ‰ (Table 3).

Dissolved oxygen concentration was highest on the position Lipci in winter period (10.9 mg/L), where is noticed temperature of 10.5 °C (Table 2). In spring and summer period dissolved oxygen concentration was lower as a result of increased temperature and lower oxygen dissolution (Table 3, 4). Oxygen saturation as dissolved oxygen concentration was highest in winter period and reached value of 121% on position Lipci and minimum in summer (77%) (Table 2, 4).

Transparency was maximum (10m) in summer period and minimum (4m) in winter period but in spring was also recorded lowest value of 5m (Table 2, 3, 4).

Concentration of nitrites and nitrates was highest in winter period, which is result of higher precipitation and higher input of nutrients by rivers. Maximum of nitrites concentration was in winter period, on the mussel farm position Ostrvo Cvijeća and was 0.851µmol/L, while nitrates concentration was highest on the mussel farm position Ljuta, also in winter (33.957 µmol/L) (Table 5). Nitrites concentration was minimum in spring, on position Stoliv (0.020 µmol/L), which can be result of nitrification as nitrates concentration on same position was high (11.125 µmol/L) (Table 6). Minimum concentration of nitrates was in summer period (0.227 µmol/L) (Table 7). Maximum of phosphates was 0.892 µmol/L, on the mussel farm on position Ostrvo Cvijeća, in winter (Table 5) and concentration of phosphates was also high in summer period when reached value of 0.535 µmol/L (Table 7).

Table 2. Values of temperature, salinity, dissolved oxygen concentration, oxygen saturation and transparency in winter period on investigation positions.

Winter 2011					
Positions	Temp.(˚C)	Sal.(‰)	O_2(mg/L)	O_2(%)	Trans.(m)
IMB	8.3	5.96	10.15	113	5
Ljuta	8.09	8.97	9.32	112	5
Orahovac	10.4	1.05	10.5	117	4
Guskić	7.2	4.7	9.97	114	5
Morinj	9.9	10.01	10.2	113	5
Lipci	10.5	11.61	10.9	121	7
Sveta Nedjelja	12.1	23.5	9.05	109	6
Obala Đuraševića	11.6	33.5	9.17	110	6
Uvala Kukuljina	10.8	33.9	7.9	93	5
Ostrvo Cvijeća	9.9	25.1	8.5	108	5

Table 3. Values of temperature, salinity, dissolved oxygen concentration, oxygen saturation and transparency in spring period on investigation positions.

Spring 2011					
Positions	Temp.(˚C)	Sal.(‰)	O_2(mg/L)	O_2(%)	Trans.(m)
IMB	15.7	22.8	8.31	94	6
Ljuta	17.4	22.6	7.7	89	5
Orahovac	14.8	22.6	8.88	105	5
Guskić	17.0	27.6	8.56	96	7
Morinj	16.5	10.2	10.08	118	6
Lipci	15.0	13.9	10.3	116	5.5
Sveta Nedjelja	16.1	34.6	6.5	78	7
Obala Đuraševića	16.4	35.6	7.11	83	7
Uvala Kukuljina	15.5	35.8	7.18	85	6
Ostrvo Cvijeća	15.9	35.8	7.18	86	6
Stoliv	16.5	29.1	8.3	95	7

In the Adriatic Sea (Burić *et al*. 2007, Viličić *et a*l. 2008), phosphate was detected as a limiting factor all year round and nitrogen sporadically in the summer. This agrees with this study where generally nitrates were lowest in summer period and highest in winter. Silicates were highest in winter period (17.417 µmol/L) and also were higher in spring, while the lowest silicates concentration was noticed in summer period (1.001 µmol/L). In winter period as result of increased precipitation and strong wind activity, concentration of nutrients was highest (Table 5, 6, 7).

Table 4. Values of temperature, salinity, dissolved oxygen concentration, oxygen saturation and transparency in summer period on investigation positions

Summer 2011					
Positions	Temp.(˚C)	Sal.(‰)	O_2(mg\L)	O_2(%)	Trans.(m)
IMB	21.8	27.2	7.5	83	6.5
Ljuta	23.2	31.4	7.3	82	7
Orahovac	20.8	36.5	8.26	94	7
Guskić	23.4	33.2	7.43	82	8
Morinj	19	11.3	8.68	97	6
Lipci	21	31.5	7.81	85	8
Sveta Nedjelja	22.1	35	7.78	89	9
Obala Đuraševića	23.4	37.1	6.67	78	9
Uvala Kukuljina	24.3	37.6	6.53	77	8
Ostrvo Cvijeća	24.6	37.7	7.09	80	9
Stoliv	21.8	30.9	8.34	96	10

If as the criteria for assessing the trophic level is taken the maximum of nutrients in winter period, according to UNEP (1994), this area is classified as eutrophic. Even in winter when water was richer with nutrients, algal growth wasn't noticed and negative influence can't be caused for mussel farms and seafoods. In spring and summer period, according the same criteria of UNEP (1994), this area was mesoeutrophic. In spring period, when was present mesoeutrophic conditions maximum of phytoplankton growth was noticed. This nutrients depletion can be explained by higher phytoplankton consumed of nutrients.

Table 5. Concentration of nutrients (nitrites, nitrates, phosphates, total P and silicates) in winter period on investigation positions.

Winter 2011					
Position	NO_2^- µmol/L	NO_3^- µmol/L	PO_4^{3-} µmol/L	Total P µmol/L	SiO_2^- µmol/L
IMB	0.218	12.941	0.580	0.188	12.412
Ljuta	0.218	33.957	0.580	0.141	12.412
Orahovac	0.574	14.068	0.401	0.282	15.015
Guskić	0.356	9.313	0.312	0.235	10.310
Morinj	0.277	7.926	0.267	0.470	11.812
Lipci	0.317	11.488	0.580	0.470	12.112
Sveta Nedjelja	0.277	3.962	0.357	0.987	10.611
Obala Đuraševića	0.297	1.527	0.535	0.611	1.201
Uvala Kukuljina	0.336	1.138	0.267	0.611	1.502
Ostrvo Cvijeća	0.851	5.655	0.892	0.188	17.417

Table 6. Concentration of nutrients (nitrites, nitrates, phosphates, total P and silicates) in spring period on investigation positions.

Spring 2011					
Position	NO_2^- µmol/L	NO_3^- µmol/L	PO_4^{3-} µmol/L	Total P µmol/L	SiO_2^- µmol/L
IMB	0.099	10.913	0.490	0.517	12.913
Ljuta	0.079	11.819	0.267	0.189	4.705
Orahovac	0.139	9.883	0.267	0.329	4.004
Guskić	0.178	12.493	0.357	0.282	4.204
Morinj	0.079	12.624	0.357	0.189	13.113
Lipci	0.040	12.897	0.134	0.235	6.206
Sveta Nedjelja	0.119	10.405	0.401	0.189	3.704
Obala Đuraševića	0.139	19.611	0.178	0.470	15.215
Uvala Kukuljina	0.079	10.958	0.267	0.376	9.610
Ostrvo Cvijeća	0.059	14.063	0.089	0.235	15.115
Stoliv	0.020	11.125	0.134	0.188	5.405

Table 7. Concentration of nutrients (nitrites, nitrates, phosphates, total P and silicates) in summer period on investigation positions.

Position	Summer 2011 NO_2^- µmol/L	NO_3^- µmol/L	PO_4^{3-} µmol/L	Total P µmol/L	SiO_2^- µmol/L
IMB	0.099	7.671	0.535	0.564	10.911
Ljuta	0.238	1.730	0.446	0.517	9.109
Orahovac	0.198	0.227	0.223	0.329	1.301
Guskić	0.317	1.262	0.401	0.658	12.412
Morinj	0.198	2.403	0.490	0.376	7.708
Lipci	0.158	0.603	0.446	0.611	1.001
Sveta Nedjelja	0.297	0.363	0.401	0.517	3.704
Obala Đuraševića	0.178	3.560	0.401	0.517	2.703
Uvala Kukuljina	0.079	2.704	0.138	0.282	3.403
Ostrvo Cvijeća	0.198	3.918	0.357	0.517	1.602
Stoliv	0.119	2.384	0.357	0.517	12.813

Phytoplankton abundance (microplankton), in winter period reached maximum of 3.49 x 10^4 cells/L, while minimum of microplankton was 1.16 x 10^3 cells/L. In winter period value of phytoplankton wasn't high although nutrients were highest. That can be explained with increased nutrients supply, but lower other factors necessary for phytoplankton growth such as sun light in winter period. Abundance of nanoplankton, in same period was highest on the position IMB and reached value of 3.94 x 10^5 cells/L (Figure 2). In spring period, abundance of phytoplankton was highest (10^5 cells/L). Maximum abundance of microplankton was 5.87 x 10^5 cells/L on the mussel farm position Morinj. The lowest abundance of microplankton was 1.90 x 10^3 cells/L on the mussel farm position Orahovac (Figure 2). This maximum in spring is result of favorable environmental conditions which are necessary for higher phytoplankton growth (temperature, dissolved oxygen, light and nutrients). Highest value of microplankton was recorded in Kotor Bay, which is closer and with less water dynamics. Values of 10^4 and 10^5 cells/L, recorded in winter and spring, were already noticed in Boka Kotorska Bay (Drakulović *et al*. 2011) and coincided with data from northeastern Adriatic Sea (Viličić *et al*. 2009, Bosak *et al.* 2009). Mandić *et a*l. (2012) were investigated distribution of phytoplankton on mussel farms in Boka Kotorska Bay and founded values of 10^5 cells/L.

In summer period of stratification, abundance of microplankton was lower, but higher comparing to abundance in winter period. Abundance varied from 1.2 x 10^3 cells/L to 5.89 x 10^5 cells/L. Nanoplankton maximum was on the same mussel farm position as nanoplankton maximum in winter and spring period on IMB and reached value of 1.65 x 10^5 cells/L. On position Morinj, nanoplankton abundance was also 1.65 x 10^5 cells/L (Figure 2). Lower values can be result of slower water dynamics and lower precipitation in warmer period. Noticed abundances were higher then abundances in winter period as result of intense sun light and higher nutrients supply caused by increased number of tourists. Results are in contrast with data that gives Drakulović *et al.* (2010, 2011) also for area of Boka Kotorska Bay, when maximum values were in summer period. It is important to emphasize that our data comprise winter, spring and summer and for autumn period there are no data so it is not suitable to compare.

If the criteria for assessing the trophic level is taken the phytoplankton abundance, according to Yamada *et al*. (1980), this area was eutrophic.

Bosak *et al*. (2012) investigated area of Boka Kotorska Bay and found high phytoplankton abundances which were similar with values found in this work. These values were higher than values noticed in the outer coastal (Socal *et al*. 1999, Saracino & Rubino 2006) and offshore (Viličić *et a*l. 1995) waters of the south-eastern Adriatic.

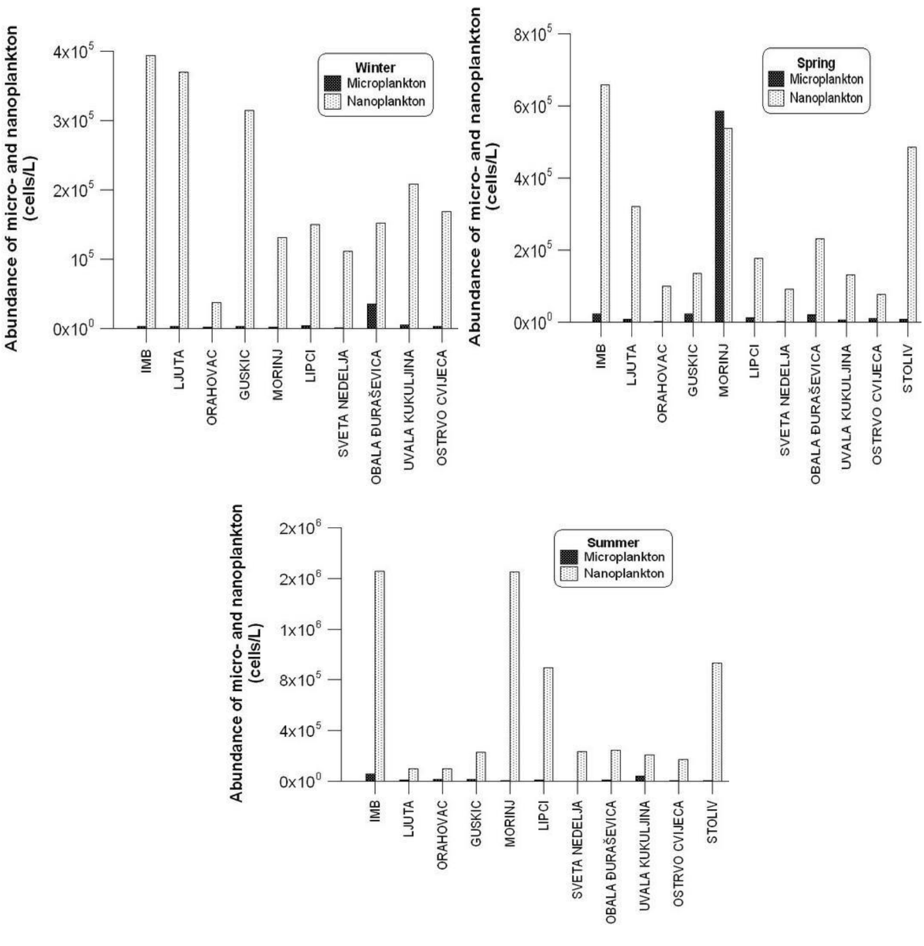

Figure. 2. Abundance of micro- and nanoplankton in winter, spring and summer period on investigated positions

In winter period, dominant phytoplankton group was diatoms, which were made mostly of microplankton. Maximum abundance of diatoms was 3.20 x 10^4 cells/L, on the same position where maximum of microplankton was noticed. Dinoflagellates were less abundant with maximum of 1.8 x 10^3 cells/L. Group others (coccolithophorids and silicoflagellates) was present only on three mussel farm positions: Lipci, Sveta Nedelja and Obala Đuraševića with maximum value of 613 cells/L. Concentration of silicates, was generally high in winter period, as can be result of domination of diatoms. Maximum of microplankton in spring period which was highest during all investigated period was made mainly of diatoms. Maximum value of diatoms was 5.79 x 10^5 cells/L on the same position as microplankton maximum, while maximum of dinoflagellates was 8.30 x 10^3 cells/L. Group others which consisted of coccolithophorids and silicoflagellates was noticed only on positions Sveta Nedelja and Uvala Kukuljina. In summer period, dinoflagellates were dominant on two positions. Maximum abundance of 4.56 x 10^4 cells/L was reached on the mussel farm position IMB. Maximum abundance of diatoms was 1.35 x 10^4 cells/L on the position Orahovac (Figure 3).

Domination of diatoms during almost all investigation period was already noticed in Boka Kotorska Bay (Drakulović *et al.* 2011, 2012). During investigation on phytoplankton structure, size and community in Boka Kotorska Bay, Bosak *et al*. (2012) also was founded domination of diatoms.

Dominant species in winter period were *Pseudo-nitzschia* spp., *Thalassionema nitzschioides*, *Navicula* spp., and *Chaetoceros affinis.* From dinoflagellates dominant species were *Prorocentrum micans* and *Gymnodinium* spp. In spring, dominant species were: *Pseudo-nitzschia* spp., *Thalassionema nitzschioides*, *Navicula* spp., *Licmophora paradoxa*.

Dominant dinoflagellates were *Prorocentrum micans* and *Neoceratium tripos*. Dominant species in summer period were: *Chaetoceros affinis, Pseudo-nitzschia* spp., *Thalassionema nitzschioides*, *Navicula* spp. and from dinoflagellates *Prorocentrum micans, Gymnodinium* spp., *Prorocentrum scutellum, Gonyaulax* spp. Potentially toxic species of genus *Pseudo-nitzschia* which dominated in this study, are also dominant component of the phytoplankton assemblages in the central (Burić *et al.* 2008) and southern Adriatic Sea (Caroppo *et al*. 2005, Burić *et al*. 2008). The dominant species in the phytoplankton assemblages (*Pseudo-nitzschia* spp., *Thalassionema nitzschioides* and *Chaetoceros affinis*) found in this study display preferences for nutrient-rich conditions (Pucher-Petković & Marasović 1980, Revelante & Gilmartin 1980).

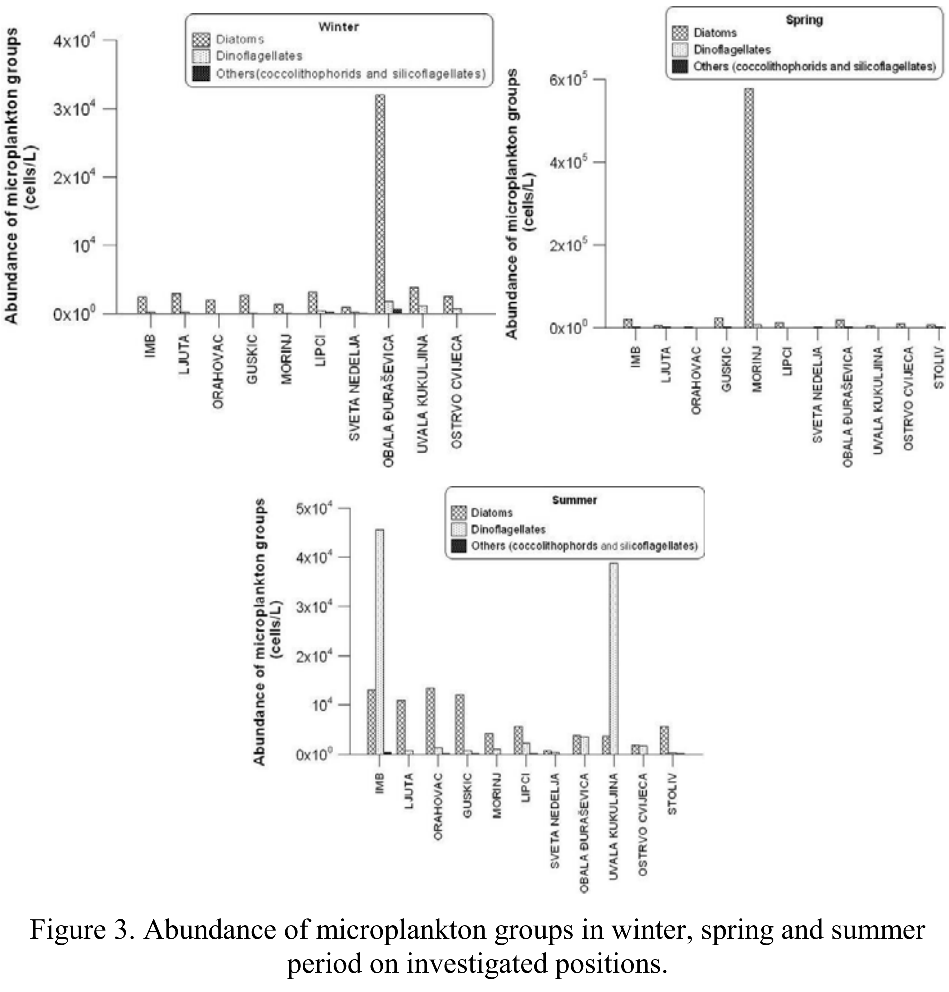

Figure 3. Abundance of microplankton groups in winter, spring and summer period on investigated positions.

Finding of potentially toxic phytoplankton species such as *Pseudo - nitzschia* spp. and toxic species *Prorocentrum micans* indicate the need for more intensive research and monitoring. Through more intensive monitoring of phytoplankton composition (on a monthly basis) presence of toxic species and potential blooms of harmful algae can be confirmed and data compared. That can give us the possibility to react on time as these negative changes will affect shellfish farming activities.

The contribution of the aquaculture in the Montenegrin national economy is 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 provide this potential.

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