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Phytoplankton size structure and species composition as an indicator of trophic status in transitional ecosystems: the case study of a Mediterranean fjord-like karstic bay*

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Abstract

The species composition and size-structure of the phytoplankton community in the Boka Kotorska Bay (SE Adriatic Sea) were analysed with respect to abundance and carbon biomass, together with the physico-chemical parameters, with the aim of evaluating the predefined oligo-mesotrophic status of this transitional water ecosystem. Three stations located in the inner part of the Bay were sampled with seasonal frequency in 2008/2009. Picophytoplankton cells were counted using flow cytometry; nanophytoplankton and microphytoplankton were identified and counted by light microscopy. The relative importance of the picoplankton in the Bay, in terms of both abundance and biomass, during all the investigated seasons emphasized their significance in the phytoplankton community. Picocyanobacteria (*Synechococcus*) constituted a significant part of the summer assemblages with regard to both abundance (up to 3.38×10^8 cells L^{-1}) and carbon biomass (up to 73% of total phytoplankton carbon). The contribution of the nanophytoplankton was found to be generally low ($< 20\%$ of the total phytoplankton carbon) in all seasons, and was dominated by autotrophic/mixotrophic flagellates. Species with a preference towards nutrient-enriched conditions, like the diatom *Skeletonema marinoi*, dominated the microphytoplankton fraction. *S. marinoi* was the most abundant in spring/winter (up to 2.86×10^6 cells L^{-1}) above the halocline (making a 96% contribution to the microphytoplankton). The potentially toxin-producing diatom *Pseudo-nitzschia pseudodelicatissima* was recorded at abundances greater than 10^5 cells L^{-1}, together with *Thalassionema frauenfeldii*, as well as the dinoflagellates *Prorocentrum micans* and the potentially harmful *P. minimum.* The higher values of phytoplankton biomass and the dominance of phytoplankton species or groups with preferences for nutrient-enriched conditions appear to be consistent with the oligo-mesotrophic status of this specific ecosystem.

1. Introduction

The size distribution of phytoplankton assemblages is a crucial biological factor determining the direction and magnitude of energy and carbon fluxes in marine pelagic food webs (Riegman et al. 1993, Legendre & Rassoulzadegan 1995), consequently affecting ecosystem productivity. It is generally considered that communities dominated by larger cells are responsible for phytoplankton biomass accumulation and dominate eutrophic coastal systems, while small cells are typical of oligotrophic systems (Siokou-Frangou et al. 2009, Šolić et al. 2010). However, there are examples in the literature representing exceptions to this general rule, as reported by Zingone et al. (2011), where a high phytoplankton biomass was coupled with small-sized cells. The phytoplankton size-structure, productivity and species composition are subject to environmental forcings such as the vertical mixing regime, light and temperature fluctuations, turbulence, salinity and nutrient availability. The phytoplankton responses to fluctuations under different environmental conditions are rapid and very complex.

Coastal waters are characterized by a high degree of spatial and temporal variability of environmental parameters. These ecosystems face increasing anthropogenic influences, mainly due to the increasing human population density in coastal areas, and are described as 'critical transition zones' because of their position at terrestrial, freshwater and marine interfaces (Levin et al. 2001). Therefore, in any evaluation of the ecological consequences of human activities, such as urbanization and tourism, on the functioning of coastal ecosystems (Cloern 1999) it is essential to determine the basic structural properties of phytoplankton assemblages in these marine areas.

The Adriatic Sea, the northernmost part of the Mediterranean, can be generally described as a marine system with an across-shelf and longitudinal trophic gradient resulting in an asymmetric distribution of the phytoplankton composition, abundance and biomass (Polimene et al. 2007). The ecosystem's trophic levels range from shallow and nutrient-enriched in the north-west to extremely oligotrophic in the south-east. There are only a few studies that take into consideration all the phytoplankton size fractions in the different areas of the Adriatic (Vanucci et al. 1994, Caroppo 2000, Bernardi Aubry et al. 2006, Paoli et al. 2007, Pugnetti et al. 2008, Cerino et al. in press). Most show that the main fraction of the autotrophic biomass consists of picophytoplankton. The phytoplankton communities of the south-eastern Adriatic Sea have been widely investigated in recent decades, not only in offshore waters (Viličić 1989, Viličić et al. 1995, Socal et al. 1999, Šilović et al. 2011), but also in coastal waters (Saracino & Rubino 2006, Mangoni et al. 2010, Moscatello et al. 2010). These studies all confirm the fact that the whole area, including the coastal zone, is highly oligotrophic. In the oligotrophic environment, it is the microbial food web that predominates in the circulation of organic matter and energy through the ecosystem (Siokou-Frangou et al. 2009).

The Boka Kotorska Bay represents a unique karstic coastal environment in the south-eastern Adriatic Sea, described by Krivokapić et al. (2011) as an oligo-mesotrophic system. We chose this transitional area as a case study area for the evaluation of an ecosystem with a predefined higher trophic status. For a better biological quality assessment of the ecosystem, a trophic evaluation based solely on physico-chemical parameters and phytoplankton biomass expressed as chlorophyll *a* concentration must be supplemented with information on the phytoplankton size structure and the taxonomic composition and abundance (Toming & Jaanus 2007, Jaanus et al. 2009). Bays are transitional systems, i.e. boundary environments between land and sea, characterized by the presence of diverse interfaces resulting in a distinct specificity of the biological communities within them, different

from those found in adjacent marine and continental biomes (Sarno et al. 1993). Although human influence in the Boka Kotorska area has become more evident in recent years, e.g. as a result of the accelerating urbanization of the coastal zone and increasing tourist activities, the Bay is considered to be a system where natural eutrophication still prevails over anthropogenic eutrophication (Krivokapić et al. 2011). Apart from studies focusing on the temporal distribution of phytoplankton biomass (chl *a*) (Krivokapić et al. 2009), the spatial distribution of chl *a* and microphytoplankton abundance in relation to organic matter and environmental parameters (Campanelli et al. 2009), information on the structural properties of the phytoplankton community in the investigated area is lacking.

The aims of this study were (i) to define the dynamics and size structure of the autotrophic carbon biomass with particular focus on the contribution of the picoplankton fraction as an indicator of the ecosystem's trophic status, (ii) to determine the dominant phytoplankton taxa and evaluate their significance in an assessment of the trophic status, and (iii) to identify the phytoplankton species that have the potential to form harmful algae blooms (HAB).

2. Material and methods

2.1. Study area

Boka Kotorska Bay is the largest bay of the Adriatic Sea and is located on its south-eastern coast. It is often described as 'Europe's southernmost fjord' because of the steep and high slopes that surround it, but it is in fact a drowned river valley. The total surface area is 87.3 km^2 and the maximum depth is 60 m. The Bay area can be divided into four, smaller, interconnected bays (Herceg Novi Bay, Tivat Bay, Risan Bay and Kotor Bay). Kotor Bay, the area investigated in this study, is located in the innermost part of Boka Kotorska Bay around the city of Kotor, encompassing approximately 30% of the Boka Kotorska Bay area. The freshwater influx from five small rivers, numerous streams and karstic submarine springs greatly affects the hydrological and chemical properties of the water column (Milanović 2007). Previous studies have shown that the annual rainfall pattern has a significant influence on nutrient-loading seasonality in the area (Krivokapić et al. 2009), since the Bay is surrounded by the high (above 1800 m) steep limestone mountains of the Dinaric Alps, which have one of the highest levels of precipitation (4584 mm per year) in Europe (Magaš 2002).

The small rivers entering Boka Kotorska Bay are not seriously impacted by humans, and the source of organic matter is primarily from in situ

biological production (Campanelli et al. 2009). The human impact on eutrophication in the area is still generally considered less than that from natural sources, but anthropogenic influences from urbanization and tourism have become more evident in recent years. Regarding mariculture, there are 16 shellfish farms cultivating mostly mussels, and two fish farms rearing seabass/seabream registered in Boka Kotorska Bay (FAO 2011).

2.2. Sampling

Sampling was carried out four times: on 2 April (spring), 3 July (summer), 5 October (autumn) in 2008 and 3 March 2009 (winter) at three stations BK1, BK2 and BK3, situated in Kotor Bay, where the water depths are 18 m, 30 m and 30 m respectively (Figure 1). 76 water samples were collected with 5 l Niskin bottles at station BK1 at five depths (surface, 2 m, 5 m, 10 m and 15 m), and at BK2 and BK3 at seven depths (surface, 2 m, 5 m, 10 m, 15 m, 20 m and 25 m). Temperature and salinity were measured in situ with a universal meter (Multiline P4; WTW). Subsamples for the determination of dissolved nutrients – dissolved inorganic nitrogen (DIN), phosphate (PO_4) and silicate (SiO_4) – were analysed on a Seal AutoAnalyser 3 using conventional automated methods (Grasshoff 1976). The DIN concentrations were calculated as the sum of ammonia, nitrite and

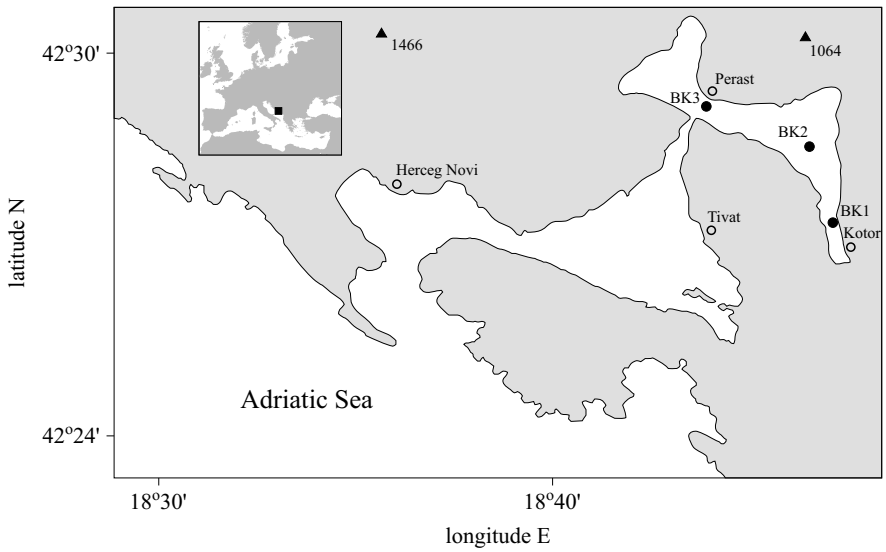

Figure 1. The Boka Kotorska Bay study area showing the position of the sampling stations

nitrate concentrations. Subsamples (1 l) for the determination of chlorophyll *a* were filtered onto Whatman GF/F (47 mm) filters and levels determined by high-performance liquid chromatography following the method of Barlow et al. (1997).

2.3. Phytoplankton

Phytoplankton abundance was determined using an inverted light microscope (LM) and a flow cytometer. The cells were attributed to pico- (0.2–2 μm), nano- (2–20 μm) and microphytoplankton (>20 μm) size classes (Sieburth et al. 1978) after measurements of the maximum cellular linear dimension (MLD) and the equivalent spherical diameter (ESD) (Table 2). In the case of the colony-forming diatom taxa (e.g. *Skeletonema marinoi*, *Chaetoceros diversus*), the chain length was considered rather than single cell dimensions, and these species were allocated to the micro-size-class. Picophytoplankton cell counts were obtained using flow cytometry (FC). 4 ml of samples were treated with 0.5% glutaraldehyde for 10 minutes, frozen in liquid nitrogen, stored at $-80°$C and analysed using a PAS III flow cytometer (Partec) equipped with an argon laser (488 nm). Data were collected in listmode files using red fluorescence (FL3) as a trigger parameter and processed with FloMax software (Partec). The final abundance of each subgroup was obtained instrumentally, which enabled true volumetric absolute counting. The different subpopulations of phytoplankton were distinguished by the autofluorescence of the cell chlorophyll content (FL3) and the phycoerythrin content of the cells (FL2), which the instrument provides, as well as by the cells' side-angle light scatter (SSC) as a proxy of their size. This allowed differentiation of picocyanobacteria *Synechococcus* and picoeukaryotic cells. For the biomass calculations of picophytoplankton, cell counts of each analysed group were converted to carbon units (μg C L^{-1}) using the following factors: 200 fg C cell^{-1} for *Synechococcus* (Charpy & Blanchot 1998) and 1500 fg C cell^{-1} for picoeukaryotes (Zubkov et al. 1998). For the micro- and nanophytoplankton cell counts, 200 ml samples were preserved with hexamine-buffered formaldehyde (1.4% final concentration). At each station, samples were taken with plankton tows (mesh sizes 20 μm and 5 μm), preserved with glutaraldehyde (2%), and used for additional taxonomic analyses. Cells were identified and counted using an Zeiss Axiovert 200 inverted microscope operating with phase contrast and bright-field optics in sub-samples of 50 ml after >24 h of sedimentation (Lund et al. 1958, Utermöhl 1958). For nanophytoplankton one transect along the counting chamber bottom was scanned at 400x magnification, and microphytoplankton were counted along two transects at 200x magnification. Abundant species were counted on a variable number

of random fields (5–20) at 200x or 400x magnification depending on their size. In addition, the bottom half of the chamber was also examined at a magnification of 100x, to obtain a more correct evaluation of less abundant microphytoplankton taxa. The minimum concentration of microphytoplankton cells that can be detected by this method is 20 cells L^{-1}. The identification of selected species was confirmed at 1000x magnification or by electron microscopy. Microalgae that could not be identified to specific or generic level were assigned to suprageneric groups. Transmission electron microscope (TEM) observations were made by deposition of acid-cleaned (H_2NO_3 and H_2SO_4) material onto Formvar carbon-coated grids and examined under a Zeiss EM10A microscope. Preserved samples not subjected to cleaning were filtered on 3 μm polycarbonate filters, dehydrated, mounted on stubs, sputter-coated with gold and examined with a Phillips XL30 scanning electron microscope (SEM).

We used the following references for phytoplankton identification: Bérard-Therriault et al. (1999), Hasle et al. (1996), Hasle & Syvertsen (1997), Kraberg et al. (2010) and Sarno et al. (2005). Cell volumes were calculated for 104 photosynthetic taxa and groups out of a total of 115 taxa identified in this study. A distinction between photosynthetic and non-photosynthetic species was made using the information available in the literature (Hoppenrath et al. 2009). Small, unidentified nanoplankton flagellates and dinoflagellates were always included, despite the probable presence of heterotrophic species. Cell sizes were measured after image analysis and processing using a Zeiss MRc digital camera and the AxioVision 4.8.2 digital system. Cell sizes were determined on more than ten specimens for rare species and more than 50 specimens for abundant species. Cell biovolumes were calculated by assigning the cells to geometric bodies and applying standard formulae (Hillebrand et al. 1999). The phytoplankton carbon content was calculated from mean cell biovolumes using the formula introduced by Menden Deuer & Lessard (2000).

2.4. Data analyses

The Primer 6 statistical package (Clarke & Gorley 2006) was used for Principal Component Analysis (PCA) of physical and chemical variables between samples with superimposed bubble plots representing different abundances of dominant phytoplankton taxa. A logarithmic transformation $[\log_{10}(x+1)]$ was used on the data prior to the statistical analyses in order to obtain a normal distribution. A standard Pearson correlation using the Statistica program, version 8.0 (Statsoft), was used to quantify direct correlations between phytoplankton abundance and environmental

parameters. The Grapher 7.0 program (Golden Software) was used for the preparation of the figures.

3. Results

3.1. Environmental parameters

The physico-chemical properties measured in Kotor Bay during the research period are shown in Table 1. Temperature and salinity values indicated intensive water column stratification throughout the study. Halocline depth was generally at 2 m in all seasons, but the salinity difference between the layers varied depending on the freshwater discharge, as the surface salinity minimum ranged between 5.2 in spring and 17.4 in summer. The mean salinity of the upper layer varied between 14.6 and 28.0, while values below the halocline were >34 m in all seasons. In addition, during the summer, the thermocline contributed to the haline stratification due to the extensive heating of the surface layer. The mean temperature decreased from 27.9 to 20.1°C between the upper and the bottom layers. In spring, the temperature distribution was uniform throughout the water column, and there was an inverse temperature gradient in the autumn and winter, when the surface layer was colder than the rest of the water column. Nutrient concentrations were generally elevated above the halocline in all seasons with the highest mean values for total inorganic nitrogen (17.70 μmol L^{-1}) and silicate (22.86 μmol L^{-1}) recorded in the autumn and for phosphate (0.36 μmol L^{-1}) in the spring.

3.2. Phytoplankton community structure

The contribution of size-classes to the total phytoplankton carbon biomass indicated different distributions between the upper and lower layers as well as between seasons (Figure 2). In the spring, microphytoplankton was dominant at all three stations in the layer above the halocline, accounting for $>70\%$ of the total biomass, with the maximum total phytoplankton biomass of 144.02 μg C L^{-1} recorded at station BK1. Below the halocline, total biomasses were lower (<40 μg C L^{-1}) and the pico size-class was predominant, accounting for $>80\%$ of the total biomass. In the summer, picophytoplankton was dominant in both layers with a mean contribution of 73% in the whole water column. The total biomass values were higher in the upper part of the water column and especially at station BK1, where they reached 173.02 μg C L^{-1} owing to the contribution of both micro- and picophytoplankton size fractions. In the autumn, the total biomass was generally low, with values <20 μg C L^{-1} and the

Table 1. Minimum, maximum, mean values and standard deviation of environmental parameters measured during the sampling period. The values represent averaged value for above halocline (surface – 2 m) and below halocline (2 m – bottom layer). DIN – dissolved inorganic nitrogen, PO_4^{3-} – phosphate, SiO_4^{4-} – silicate. Number of samples per season = 19

Parameters	Spring			Summer			Autumn			Winter		
	min	max	avg ± sd	min	max	avg ± sd	min	max	avg ± sd	min	max	avg ± sd
temperature [°C]												
above	14.1	15.4	15.0 ± 0.5	26.7	27.9	27.2 ± 0.5	14.0	17.0	15.4 ± 1.2	10.7	14.7	12.0 ± 0.4
below	14.5	15.1	14.8 ± 0.2	17.4	24.1	20.1 ± 2.3	17.5	18.9	18.4 ± 0.4	12	13.3	12.7 ± 0.4
salinity												
above	5.2	27.2	14.6 ± 9.0	24.0	30.8	28.0 ± 2.6	6.2	25.3	14.6 ± 7.6	10	31.5	21.6 ± 9.9
below	30.8	36.0	34.3 ± 1.7	33.8	36.2	35.3 ± 0.8	31.3	36.6	35.0 ± 1.7	33	37	35.6 ± 1.2
DIN [μmol L^{-1}]												
above	2.28	8.58	5.98 ± 2.71	1.03	2.37	1.59 ± 0.51	1.03	36.20	17.70 ± 18.40	0.10	2.68	1.30 ± 1.34
below	0.64	3.79	1.53 ± 0.91	0.89	2.88	1.69 ± 0.74	0.58	3.89	1.66 ± 0.92	0.81	2.43	1.30 ± 0.42
PO_4^{3-} [μmol L^{-1}]												
above	0.17	0.36	0.26 ± 0.09	0.05	0.14	0.10 ± 0.03	0.05	0.27	0.14 ± 0.08	0.05	0.34	0.14 ± 0.11
below	0.02	0.16	0.12 ± 0.04	0.01	0.10	0.05 ± 0.03	0.02	0.24	0.09 ± 0.06	0.02	0.11	0.06 ± 0.03
SiO_4^{4-} [μmol L^{-1}]												
above	12.53	33.31	22.78 ± 9.16	1.42	2.26	4.61 ± 0.85	11.75	44.30	22.86 ± 12.19	2.06	19.67	8.72 ± 7.10
below	2.53	6.47	4.33 ± 1.43	0.44	2.68	1.42 ± 0.69	3.59	11.47	6.39 ± 2.24	1.16	2.34	1.56 ± 0.31
chlorophyll *a* [μg L^{-1}]												
above	0.23	0.50	0.39 ± 0.10	0.30	1.80	0.70 ± 0.60	0.30	3.60	1.40 ± 1.40	0.50	1.90	1.10 ± 0.50
below	0.40	1.10	0.74 ± 0.24	0.09	0.23	0.14 ± 0.05	0.10	1.10	0.30 ± 0.30	0.30	0.80	0.50 ± 0.20

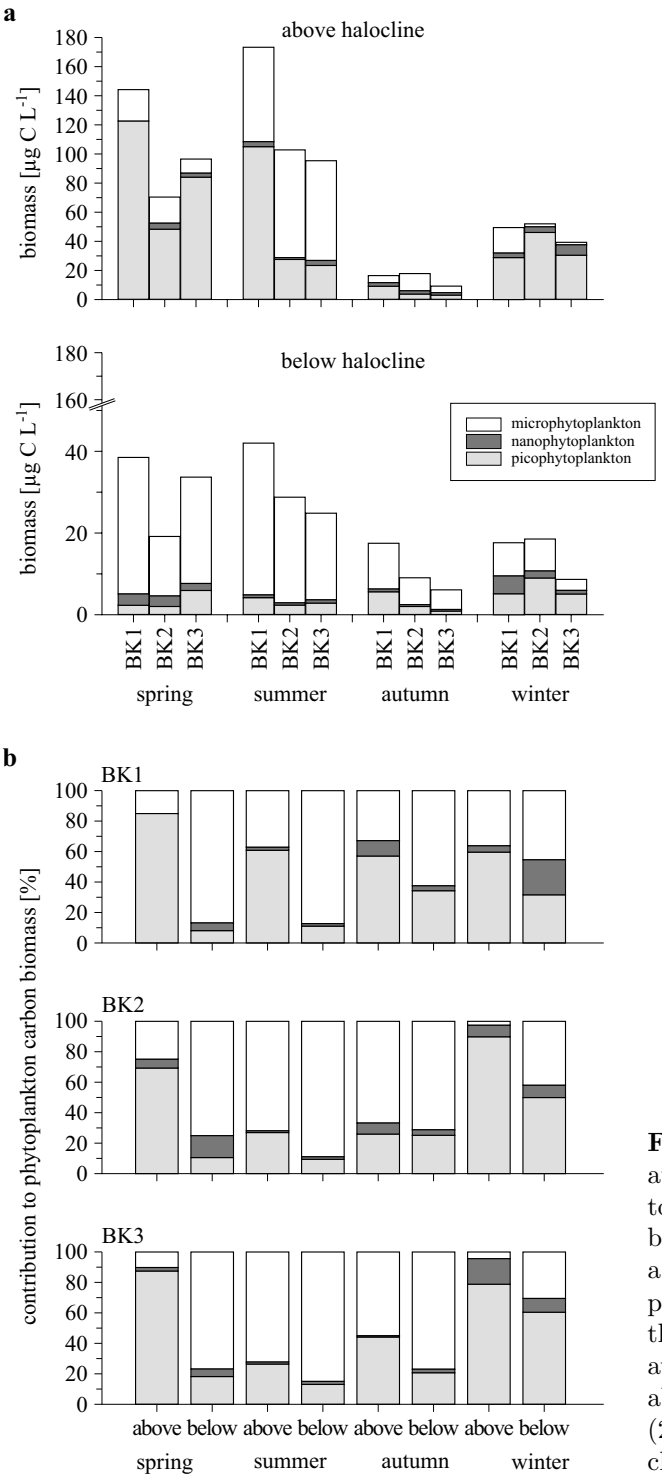

Figure 2. Seasonal variation of the phytoplankton size-distributed carbon biomass in terms of a) absolute values and b) percentage contribution at the three stations. Values averaged for the layer above (0–2 m) and below (2 m-bottom) the halocline

pico size-class predominated, accounting on average for 61% of the total biomass in the whole water column. The exception was at station BK1, where the micro size-class contributed to 60% of the total biomass. In the winter, microphytoplankton predominated throughout the water column at all stations, while the largest contribution of the pico size-class (40%) was recorded at station BK1 above the halocline, where it also contributed to the highest biomass values of 51.34 μg C L^{-1}. The highest contribution of the nanophytoplankton biomass (24%) was recorded in the winter at station BK1 below the halocline, but their contribution was generally $< 20\%$ in all seasons and layers.

As far as abundance and biomass are concerned, the picophytoplankton was dominated in all seasons by phycoerythrin-rich unicellular cyanobacteria of the *Synechococcus* type (Figure 3). Cell abundances ranged from 6.17×10^6 to 3.38×10^8 cells L^{-1} and the picocarbon biomass ranged from 1.23 to 74.36 μg C L^{-1} with the minima recorded in the winter and the maxima in the summer. The highest *Synechococcus* abundances occurred in the summer in the layer above the halocline at all three stations, with the maximum reaching 3.38×10^8 cells L^{-1} at the surface at station BK2, which corresponds to a biomass of 74.36 μg C L^{-1}. Picoeukaryotes were present

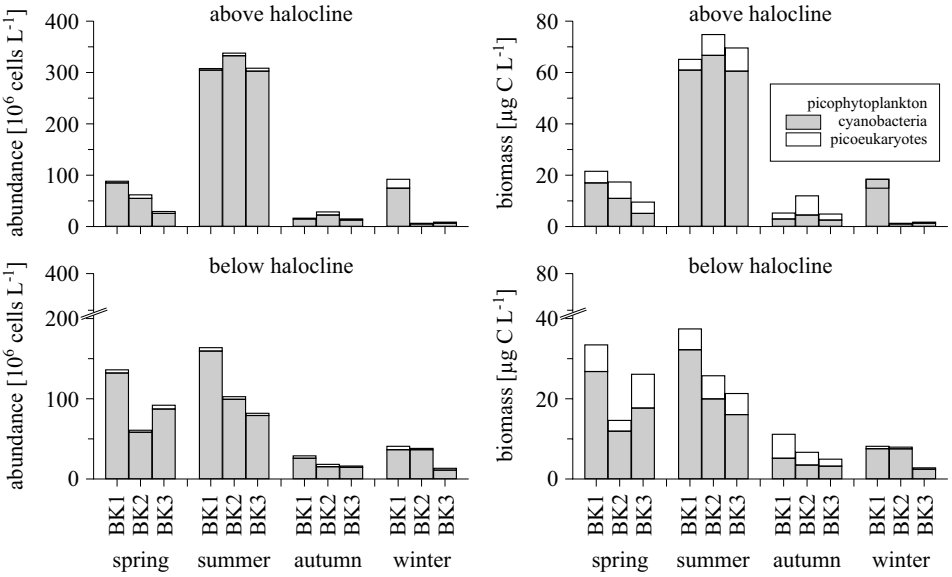

Figure 3. Seasonal variation of the picophytoplankton abundance (left panel) and biomass (right panel) at the three stations. Values are averaged for the layer above (0–2 m) and below (2 m-bottom) the halocline

in low abundances in the water column in all the seasons investigated: their cell numbers did not exceed 5.89×10^6 cells L^{-1}, and their biomass was no greater than 8.53 μg C L^{-1}.

A total of 104 micro- and nanophytoplankton taxa and taxonomic groups, corresponding to 61 diatoms, 24 dinoflagellates, 10 coccolithophores and 9 phytoflagellates, were identified in Boka Kotorska Bay; the complete list is given in Table 2.

The nanophytoplankton was composed of diatoms, dinoflagellates, coccolithophores and 'others' (Figure 4). Cell abundances ranged from 2.84×10^3 to 3.02×10^5 cells L^{-1} and the nanocarbon biomass from 0.06 to 6.86 μg C L^{-1}, with the minima recorded in the autumn and the maxima in the winter. Nanoplankton diatoms encompassed mostly small-sized single cell diatoms like *Chaetoceros throndsenii* or *C. tenuissimus*. Their abundance and contribution to the biomass was low, with respective maxima up to 2.48×10^4 cells L^{-1} and 0.34 μg C L^{-1} in the spring. Nanoplankton dinoflagellates comprised mostly unidentified gymnoid athecate forms. They reached the highest abundance of 1.65×10^4 cells L^{-1} and a biomass of 1.50 μg C L^{-1} in the spring below the halocline. In the autumn, the

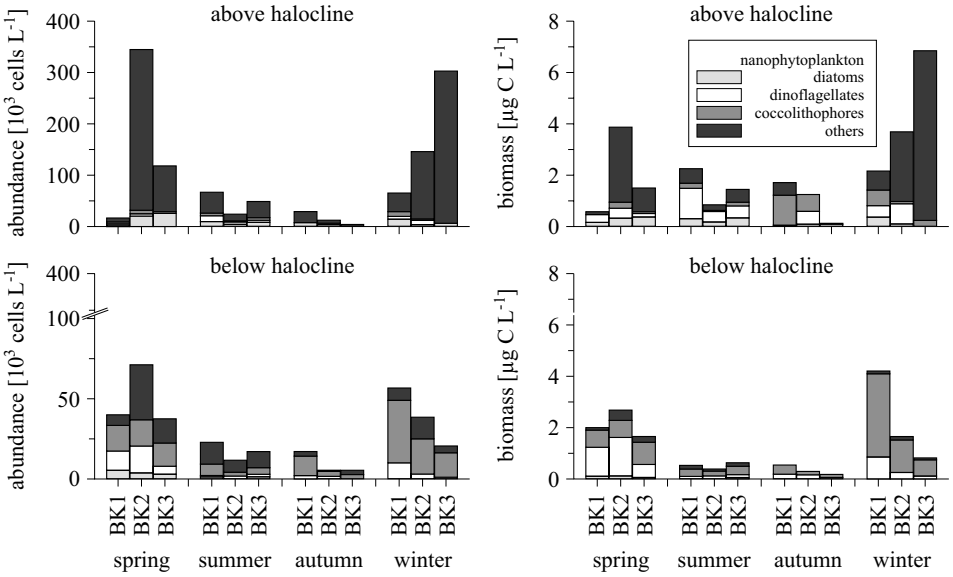

Figure 4. Seasonal variation of the nanophytoplankton abundance (left panel) and biomass (right panel) at the three stations. Values are averaged for the layer above (0–2 m) and below (2 m-bottom) the halocline

Table 2. List of phytoplankton taxa and taxonomic groups identified in the Boka Kotorska Bay during 2008/2009 with mean cellular volumes; ESD – equivalent spherical diameter, MLD – maximum linear dimension and carbon content; N – nanophytoplankton; M – microphytoplankton; P – picophytoplankton; ND – not determined

Species	Size-class	Volume [μm^3]	ESD [μm]	MLD [μm]	Carbon [pg]
Diatoms					
Achnanthes sp.	N	704	11.04	16.09	59
Asterionellopsis glacialis (Castracane) Round	M	2229	16.21	70.96	150
Bacillaria sp.	M	13390	29.47	156.83	640
Bacteriastrum hyalinum Lauder	M	18242	32.67	28.55	822
Bacteriastrum sp.	M	8311	25.14	23.78	435
Cerataulina pelagica (Cleve) Hendey	M	37672	41.60	100.10	1481
Chaetoceros affinis Lauder	M	1793	15.07	19.23	125
Chaetoceros contortus Schütt	M	2068	15.81	16.56	141
Chaetoceros costatus Pavillard	M	3924	19.57	22.81	237
Chaetoceros curvisetus Cleve	M	2930	17.76	20.59	187
Chaetoceros danicus Cleve	M	5546	21.96	18.37	313
Chaetoceros decipiens Cleve	M	5919	22.45	23.02	330
Chaetoceros densus (Cleve) Cleve	M	15700	31.07	40.02	728
Chaetoceros diversus Cleve	M	397	9.12	8.13	37
Chaetoceros peruvianus Brightwell	M	9082	25.89	24.04	352
Chaetoceros simplex Ostenfeld	N	410	9.22	8.50	38
Chaetoceros sp.	M	301	8.31	8.18	29
Chaetoceros tenuissimus Meunier	N	149	6.58	6.73	17
Chaetoceros throndsenii Marino, Montresor et Zingone	N	61	4.90	10.00	8
Chaetoceros wighamii Brightwell	M	955	12.22	11.50	75
Cylindrotheca closterium (Ehrenberg) Reihmann et Lewin	M	291	8.22	26.21	29
Cocconeis scutellum Ehrenberg	M	2298	16.38	33.42	153
Coscinodiscus sp.	M	162245	67.68	68.71	4840
Cyclotella sp.	N	510	9.92	10.21	45
Dactyliosolen fragilissimus (Bergon) Hasle	M	13247	29.36	75.25	634
Detonula pumila (Castracane) Gran	M	5595	22.03	22.43	315
Diploneis bombus Ehrenberg	M	1885	15.33	28.34	131
Diploneis sp.	M	10134	26.85	40.56	511
Eucampia cornuta (Cleve) Grunow	M	12920	29.12	52.85	622

Table 2. (*continued*)

Species	Size-class	Volume [μm^3]	ESD [μm]	MLD [μm]	Carbon [pg]
Guinardia flaccida (Castracane) Peragallo	M	169193	68.63	170.56	5007
Guinardia striata (Stolterfoth) Hasle	M	66527	50.28	166.44	2349
Haslea wawrikae (Hustedt) Simonsen	M	13686	29.68	531.53	647
Hemiaulus hauckii Grunow	M	21563	34.54	64.33	942
Leptocylindrus danicus Cleve	M	2833	17.56	60.36	182
Leptocylindrus mediterraneus (Peragallo) Hasle	M	9421	26.21	69.18	481
Leptocylindrus minimus Gran	M	1134	12.94	44.38	86
Licmophora sp.	M	21006	34.24	93.25	922
Lioloma pacificum (Cupp) Hasle	M	9650	26.42	397.04	469
Lithodesmium undulatum Ehrenberg	M	35101	40.63	36.92	1398
Melosira nummuloides (Dillwyn) C. Agardh.	M	2056	15.78	17.38	140
Navicula sp.	M	16673	31.70	83.75	765
Nitzschia cf. *incerta* (Grunow) Peragallo	M	1972	15.56	76.43	135
Nitzschia longissima (Brébisson) Ralfs	M	795	11.50	145.24	65
Nitzschia sp.	M	180	7.01	40.04	19
Pleurosigma sp.	M	119571	61.13	226.82	3779
Psammodictyon panduriforme (Gregory) Mann	N	293	8.24	16.91	29
Proboscia alata (Brightwell) Sundström	M	37197	41.42	68.27	1466
Pseudo-nitzschia pseudodelicatissima (Hasle) Hasle species complex	M	340	8.66	85.38	33
Pseudo-nitzschia seriata group	M	1652	14.67	100.70	117
Pseudosolenia calcar-avis (Schultze) Sundström	M	1447836	140.38	714.88	28559
Rhizosolenia decipiens Sundström	M	113070	60.01	386.69	3611
Rhizosolenia imbricata Brightwell	M	44153	43.86	297.50	1.684
Rhizosolenia sp.	M	23550	35.57	300.23	1.012
Skeletonema marinoi Sarno et Zingone	M	443	9.46	15.72	40
Striatella unipunctata (Lyngbye) C. Agardh	M	61230	48.91	65.37	2.196
Thalassionema nitzschioides Mereschkowsky	M	371	8.92	44.12	35

Table 2. (*continued*)

Species	Size-class	Volume [μm^3]	ESD [μm]	MLD [μm]	Carbon [pg]
Thalassionema frauenfeldii (Grunow) Halleagraef	M	2142	16.00	80.20	145
Thalassiosira rotula Meunier	M	25312	36.43	38.43	1073
Thalassiosira sp.	M	12334	28.67	33.09	599
undetermined pennate diatoms >20 μm	M	1176	13.10	42.07	89
undetermined pennate diatoms <20 μm	N	40	4.24	14.23	6
Dinoflagellates					
Dinophysis acuminata Claparède et Lachmann	M	28704	37.99	54.78	3315
Dinophysis sacculus Stein	M	18417	32.77	51.18	2185
Diplopsalis group	M	118526	60.96	60.96	12554
Gonyaulax sp.	M	13599	29.62	32.60	1644
Gymnodinium sp.	M	2832	17.56	22.50	377
Gyrodinium sp.	M	4439	20.39	46.02	574
Mesoporos perforatus (Gran) Lillick	M	2910	17.72	18.35	386
Neoceratium furca (Ehrenberg) Gómez, Moreira et López-Garcia	M	34137	40.25	249.36	3901
Neoceratium fusus (Ehrenberg) Gómez, Moreira et López-Garcia	M	12197	28.56	327.90	1484
Neoceratium hexacanthum (Gourret) Gómez, Moreira et López-Garcia	M	201305	72.73	379.71	20643
Neoceratium massiliense (Gourret) Gómez, Moreira et López-Garcia	M	76546	52.69	392.28	8326
Neoceratium pentagonum (Gourret) Gómez, Moreira et López-Garcia	M	71189	51.43	330.15	7778
Neoceratium sp.	M	71806	51.58	326.01	7841
Neoceratium trichoceros (Ehrenberg) Gómez, Moreira et López-Garcia	M	26536	37.01	322.91	3079
Neoceratium tripos (Müller) Gómez, Moreira et López-Garcia	M	97035	57.02	279.77	10403
Oxytoxum sp.	M	2577	17.01	27.54	345
Phalacroma rotundatum (Claparéde et Lachmann) Kofoid et Michener	M	14719	30.41	50.03	1770
Prorocentrum gracile Schütt	M	9611	26.38	47.26	1187
Prorocentrum micans Ehrenberg	M	10504	25.50	45.96	1290
Prorocentrum minimum (Pavillard) Schiller	N	1247	13.36	18.33	174

Table 2. (*continued*)

Species	Size-class	Volume [μm^3]	ESD [μm]	MLD [μm]	Carbon [pg]
Pyrocystis lunula (Schütt) Schütt	M	32500	39.60	168.56	3725
Scrippsiella sp.	M	8861	25.68	32.46	1099
undetermined dinoflagellates > 20 μm	M	10884	27.50	40.69	1333
undetermined dinoflagellates < 20 μm	N	621	10.59	11.53	91
Prymnesiophyceans					
Acanthoica quattrospina Lohmann	N	533	10.06	10.06	78
Calciosolenia brasiliensis (Lohmann) Young	M	1202	13.19	105.30	168
Calciosolenia murrayi Gran	M	220	7.49	32.29	34
Calyptrosphaera oblonga Lohmann	N	924	12.09	12.09	132
Emiliania huxleyi (Lohmann) Hay et Mohler	N	129	6.27	6.27	21
Helicosphaera carteri (Wallich) Kamptner	N	1988	15.60	15.60	270
Ophiaster sp.	N	99	5.75	5.75	16
Rhabdosphaera stylifera Lohmann	N	523	10.00	10.00	77
Syracosphaera pulchra Lohmann	N	2246	16.25	15.06	303
undetermined coccolithophores N (< 20 μm)	268	8.00	8.00	41	
Chrysochromulina sp.	N	351	8.75	15.47	53
Dictyochophyceans					
Dictyocha fibula Ehrenberg	M	19438	33.37	33.37	2299
Octactis octonaria (Ehrenberg) Hovasse	M	7180	23.94	23.94	902
Chrysophyceans					
Dinobryon sp.	N	99	5.75	8.32	16
Cryptophyceans					
undetermined cryptophyceans < 20 μm	N	55	4.71	13.33	9
Prasinophyceae					
Pseudoscourfieldia marina (Throndsen) Manton	N	10	2.66	3.57	2
Chlorophyceae					
Pediastrum sp.	M	125143	62.07	89.28	16321

Table 2. (*continued*)

Species	Size-class	Volume [μm^3]	ESD [μm]	MLD [μm]	Carbon [pg]
uncertain					
Meringosphaera mediterranea Lohmann	N	155	6.66	6.66	25
other flagellates					
undetermined phytoflagellates < 20 μm	N	142	6.47	7.59	23

potentially toxic nanodinoflagellate *Prorocentrum minimum* (Figure 8f) was recorded among the dominant species in the phytoplankton assemblage, with a maximum abundance reaching 3.97×10^4 cells L^{-1}. Coccolithophores were also an important component of the nano-assemblages, especially below the halocline, reaching a maximum abundance in the winter of 3.94 cells L^{-1}, which corresponds to a biomass of 3.26 μg C L^{-1}. *Ophiaster* sp. was recognized as a dominant species in the phytoplankton in the autumn, reaching a maximum abundance of 1.85×10^4 cells L^{-1}. The greatest contribution to the nanoplankton size class was from various autotrophic/mixotrophic flagellates with diverse taxonomic affiliations belonging to the group 'others'. Their abundance and biomass was highest in the spring and winter above the halocline. The spring peak at station BK2, corresponding to a biomass of 2.96 μg C L^{-1}, was due mostly to the mixotrophic cryptophytes (6.07×10^5 cells L^{-1}) and the chrysophyte *Dinobryon* sp. (1.15×10^5 cells L^{-1}). The winter maximum corresponded to the somewhat lower abundance of 5.63×10^5 cells L^{-1}. As it was dominated by phytoflagellates (green flagellates belonging mostly to Prasinophyceae and Chlorophyceae) that have a greater contribution of carbon per cell, the winter maximum at station BK3 had a higher biomass (6.85 μg C L^{-1}).

Microphytoplankton cell abundances ranged from 7.25×10^3 to 2.12×10^6 cells L^{-1} and the carbon biomass from 1.25 to 121.98 μg C L^{-1}, with the minima recorded in the autumn and the maxima in the spring. The micro size-class was almost exclusively dominated by diatoms in terms of abundance (Figure 5); as regards biomass, however, the situation was somewhat different. In the spring, at station BK1, the microchlorophytes (*Pediastrum* sp.) made a substantial contribution to the microphytoplankton carbon biomass – 81% (99.36 μg C L^{-1}). Among the diatoms, *Skeletonema marinoi* (Figures 8b,c,d) was the main component of the winter/spring bloom, contributing up to 96% of the microphytoplankton

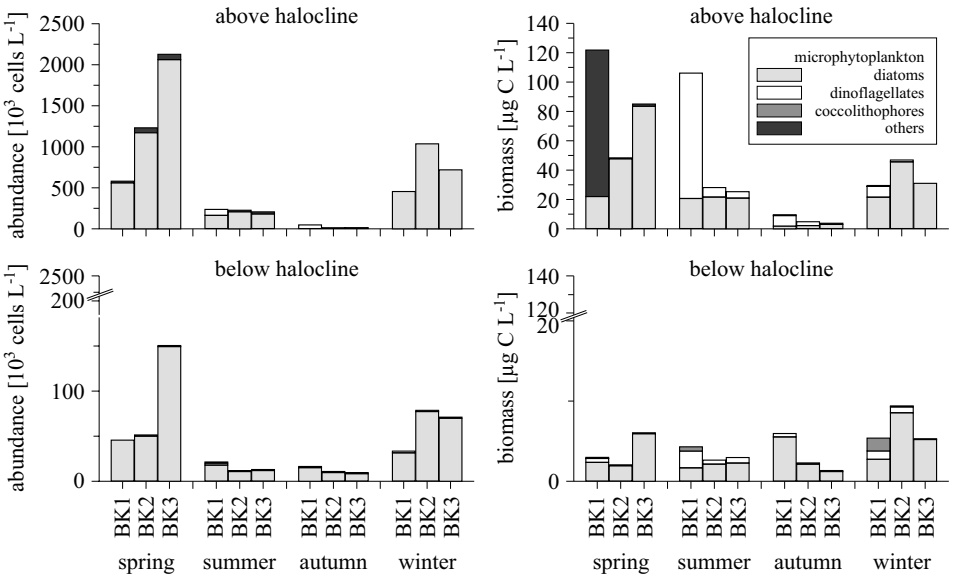

Figure 5. Seasonal variation of the microphytoplankton abundance (left panel) and biomass (right panel) at the three stations. Values are averaged for the layer above (0–2 m) and below (2 m-bottom) the halocline

abundance and achieving high cell concentrations above the halocline of 2.86×10^6 and 1.10×10^6 cells L^{-1} in spring and winter respectively. In the autumn, when cell numbers were low, *S. marinoi* was among the co-dominant species, constituting 15% of the microphytoplankton abundance (1.97×10^4 cells L^{-1}). In the summer, *Pseudo-nitzschia pseudodelicatissima* (Figures 8a,e) with maxima of 1.20×10^5 cells L^{-1} and *Thalassionema frauenfeldii* with maxima of 1.12×10^5 cells L^{-1} were the co-dominant diatom species, respectively contributing up to 45% and 30% of the total microphytoplankton cell concentration. Dinoflagellates were significant in the phytoplankton assemblages in the summer as well, especially at station BK1, where they reached values of 84.57 μg C L^{-1} or 80% of the microphytoplankton carbon, mostly due to the development of the species *Prorocentrum micans*.

3.3. Statistical analysis

The application of PCA to the environmental data revealed that the first three principal components (PCs) had eigenvalues > 0.05 and accounted for 97.6% of the total variance (Table 3), representing a good description the environmental structure. The first principal component (PC1)

Table 3. Variable loads of the environmental PCA

Variable	PC1	PC2	PC3
temperature	−0.055	−0.020	0.998
salinity	−0.359	0.390	−0.016
DIN	0.448	−0.725	0.008
PO_4^{3-}	0.042	0.012	0.002
SiO_4^{4-}	0.816	0.568	0.056

of accounted for 84.8% of the total variance, with nutrients representing the highest positive loads, whereas salinity loaded negatively. The second principal component (PC2) expressed 8.7% of the variation and was also related to nutrients. The samples from the layer above the halocline in the summer were related primarily to temperature. This was interpreted by the third principal component (PC3), which explained 4.1% of the variance. Abundances of dominant phytoplankton taxa were superimposed on the PCA scatter plot and their distributions indicated their preference for particular environmental conditions (Figures 6, 7). The correlation coefficients presented in Table 4 confirmed the statistically significant

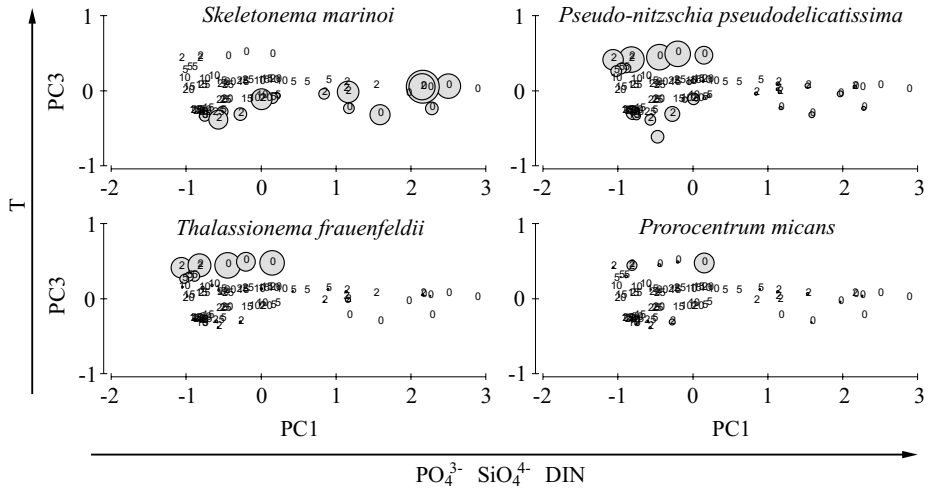

Figure 6. PCA of environmental parameters (denoted by the sampling depths) measured during the sampling period at all three stations with the abundances of dominant microphytoplankton taxa represented as superimposed bubbles increasing in size with increasing abundance. Sample distribution was interpreted as the mixing of nutrient-rich fresh water in the upper layer with nutrient-poorer saline water; the sampling depth of each sample lends support to this interpretation. Number of samples = 76

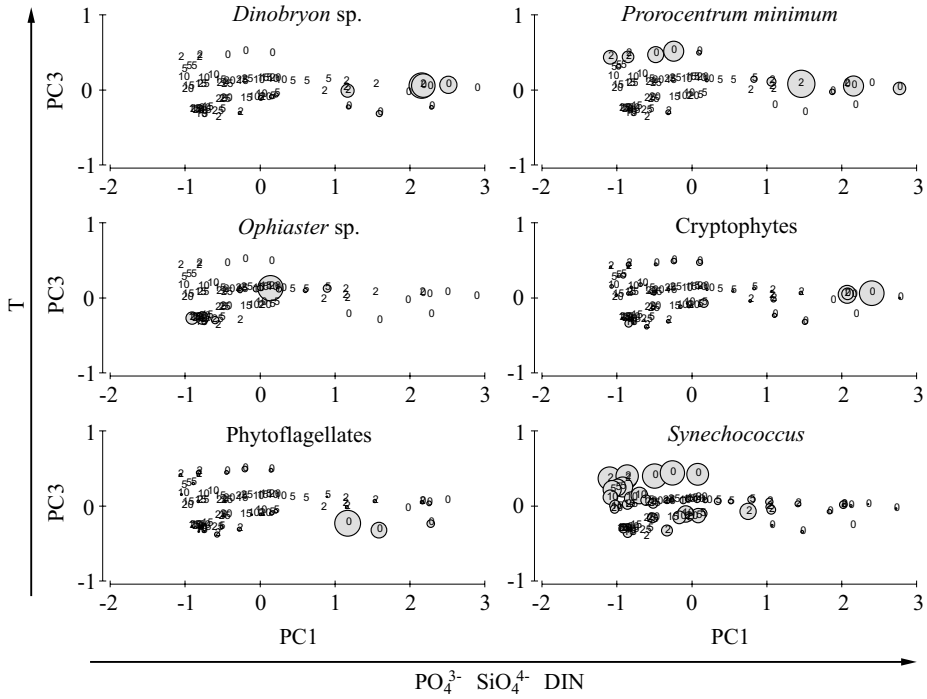

Figure 7. PCA of environmental parameters (denoted by the sampling depths) measured during the sampling period at all three stations with the abundances of dominant nanophytoplankton and picophytoplankton taxa and taxonomic groups represented as superimposed bubbles increasing in size with increasing abundance. Number of samples = 76

Table 4. Pearson's correlation coefficients between dominant phytoplankton taxa and environmental parameters

Phytoplankton taxa	Temperature	Salinity	DIN	PO_4^{3-}	SiO_4^{4-}
Skeletonema marinoi	**−0.24***	**−0.55****	**0.44****	**0.42****	**0.49****
Thalassionema frauenfeldii	**0.72****	−0.07	−0.09	0.02	−0.15
Pseudo-nitzschia pseudodelicatissima	**0.64****	−0.05	−0.11	−0.02	−0.18
Prorocentrum micans	**0.33****	−0.08	−0.02	0.02	−0.04
Prorocentrum minimum	**0.25***	**−0.32****	**0.23***	0.17	**0.29***
Ophiaster sp.	−0.01	0.12	−0.05	−0.07	−0.05
Dinobryon sp.	−0.09	**−0.48****	**0.42****	**0.36****	**0.52****
Cryptophytes	−0.05	**−0.42****	**0.35****	**0.43****	**0.49****
Phytoflagellates	−0.17	**−0.31****	**0.26***	0.11	0.10
Synechococcus	**0.47****	−0.05	−0.06	0.01	−0.09

Significant correlations in bold at $p < 0.01$ (**) and $p < 0.05$ (*).

relationships between species abundances and physico-chemical parameters.

4. Discussion

Both phytoplankton abundances and carbon biomasses were generally higher in Boka Kotorska Bay than in the outer coastal (Socal et al. 1999, Saracino & Rubino 2006) and offshore (Viličić et al. 1995, Cerino et al. in press) waters of the south-eastern Adriatic. Most studies (e.g. Saracino & Rubino 2006) have focused only on the nano- and microphytoplankton size fractions and emphasize the dominance of the nanoplankton component (mostly phytoflagellates < 10 μm). However, the study by Cerino et al. (in press) encompassed the whole autotrophic compartment and showed the pico fraction as being a major component in the phytoplankton community. The reported abundances of picophytoplankton in the eastern Adriatic coastal area are in the 10^6–10^8 cells L^{-1} range, which lies within that found in our study, but the maximum values of both abundance and biomass in Kotor Bay were twice as high. The largest differences were found in the nano- and microphytoplankton abundances as well as in the biomass. For the nano size-class, they were about one order of magnitude lower in the bay than the values reported for offshore waters by the same authors. The opposite was found for the micro size-class: the range of 10^2–10^4 is reported for offshore waters, which is one order of magnitude less than the range reported in our study. As the studies from the nutrient-richer northern Adriatic (Totti et al. 2005, Bernardi Aubry et al. 2006) found similar trends in the distribution of the respective values of abundance and biomass per size compartment, we can conclude that the discrepancies between the findings of Cerino et al. (in press) and our study reflect the pronounced oligotrophy of the south-eastern Adriatic Sea in comparison to the higher trophic status of the Bay.

Although a seasonal sampling strategy cannot be exhaustive enough to appreciate the annual cycle of phytoplankton in the Bay, the collected data have nevertheless provided us with some new insights. The relative importance of the picophytoplankton in the Bay in terms of both abundance and biomass emphasizes their significance in the phytoplankton assemblages. The seasonal variation of the mean percentage contribution of picophytoplankton to the total phytoplankton carbon biomass showed that the smallest fraction was less important during the late winter/spring bloom, with a tendency to become more conspicuous during the summer and autumn. The contribution of picophytoplankton to the total carbon biomass during the summer period of intensive thermal stratification and low nutrient levels was as high as 73%, which is comparable to the 70%

pico-summer dominance reported from the more eutrophic coastal waters of the northern Adriatic (Bernardi Aubry et al. 2006). The smallest fraction was dominated by the picocyanobacteria *Synechococcus*. With respect to the other picocyanobacterial populations, *Prochlorococcus* cells were not detected in the samples. These results are in accordance with the findings of Šilović et al. (2011), who reported the absence of *Prochlorococcus* in a coastal area of the south-eastern Adriatic. The observed maximum abundance of *Synechococcus* (3×10^8 cells L^{-1}) in the Bay in the summer was close to the maximum observed in Mediterranean lagoon and estuarine systems (Caroppo 2000) and exceeded the maximum recorded (2.7×10^8 cells L^{-1}) in an estuarine area of the NW Adriatic during the summer (Bernardi Aubry et al. 2006). Picocyanobacteria play a substantial role in nutrient-richer transitional ecosystems, and they may even become the prevailing fraction of the phototrophic plankton at these sites (Paoli et al. 2007). This suggests the potential use of picophytoplankton as a functional biomarker of the higher trophic status of coastal marine environments. Analysis of phytoplankton size-spectra has already been used as a tool in the evaluation of transitional water bodies in the Adriatic Sea, but it was limited to taxa within the nano- and microphytoplankton size range (Sabetta et al. 2008). However, the study by Bec et al. (2011) found differences between the relative and absolute importance of the prokaryotic and eukaryotic components among the picoautotrophs along the trophic gradient in Mediterranean coastal lagoons. Those authors suggested that the numerical dominance of picocyanobacteria could reflect oligo-mesotrophic conditions in marine coastal waters. Because of their small size and high surface-to-volume ratios, these appear to be more competitive than picoeukaryotes and the larger phytoplankton in acquiring nutrients in resource-limited systems. This dominance could be related to the ability of *Synechococcus* to acquire phosphorus when concentrations are very low (Bec et al. 2011 and the references therein), because phosphorus is the limiting nutrient for phytoplankton growth in the whole Mediterranean Sea (Siokou-Frangou et al. 2009), including Boka Kotorska Bay (Krivokapić et al. 2011). Picoeukaryotic algae were recorded in the Bay in all seasons in small numbers, but as they contain more carbon per cell, their contribution was better reflected in terms of carbon biomass. The stable but negligible importance of the picoeukaryotic contribution has been demonstrated in other studies in coastal transitional areas of the Adriatic Sea (Vanucci et al. 1994). It has been reported that their relative importance with regard to abundance and biomass generally increases with increasing trophic status of the marine system, as they are the most competitive group among the pico- and nanophytoplankton (Bec et al. 2011). This was not the case in

our study, however; apparently, the Bay's trophic status is still not high enough to promote their greater development.

The dominant species in the phytoplankton assemblages found in this study display preferences for nutrient-rich conditions (Pucher-Petković & Marasović 1980, Revelante & Gilmartin 1980) and are found in higher abundances in only a few moderately eutrophic environments in the Adriatic Sea (Cetinić et al. 2006, Bosak et al. 2009). It should be noted that the Bay is primarily a marine ecosystem, so the phytoplankton species found here are exclusively of marine origin: there is no large river that continuously brings in freshwater organisms, as is the case in other semi-enclosed estuarine systems on the eastern Adriatic coast (Viličić et al. 1989, Cetinić et al. 2006, Burić et al. 2007). Nevertheless, freshwater phytoplankton species such as *Pediastrum* spp. were occasionally observed in the samples, probably due to local freshwater input from small rivers and springs, which is greater mainly in the winter and spring. The dominance of the diatom *S. marinoi* in the spring and winter resulted in microphytoplankton dominance in total carbon biomass above the halocline. *Skeletonema* blooms were a distinct feature of the Bay, clearly distinguishing its phytoplankton assemblages from those in adjacent waters. The species is reported to be one of the dominant species in the nutrient-richer areas (Revelante & Gilmartin 1976, Viličić et al. 2009), where it usually exhibits marked seasonal behaviour, forming blooms above the pycnocline in the late winter (Totti et al. 2005, Bernardi Aubry et al. 2006, Pugnetti et al. 2008). It is also found in other riverine water-influenced and nutrient-rich environments (Blanc et al. 1975, Thompson & Ho 1981, Spies & Parsons 1985, Morozova & Orlova 2005). In the waters surrounding the investigated Bay its presence is detected sporadically, but even then in very low abundances (Socal et al. 1999, Rubino et al. 2009). It has recently been discovered that different strains of *S. marinoi* can tolerate a wide range of salinity (Saravanan & Godhe 2010, Balzano et al. 2011), which is in accordance with our findings of the species' greatest abundance in surface samples (salinity < 5). Thus, its mass development in the surface waters of Boka Kotorska Bay can be attributed to the competitive advantages of this species over the other marine phytoplankton found in the water column in this period in view of its ability to flourish in conditions of low salinity and lower temperatures. In addition, bloom-forming species like *S. marinoi* are characterized by inherently high growth rates and can efficiently exploit nutrients, the levels of which are higher, especially in the layer above the halocline in the Bay (Smayda 1998).

The influence of the vertical salinity gradient in the phytoplankton distribution is also clearly perceptible in other phytoplankton groups.

Cryptophytes and *Dinobryon* sp. correlated positively with nutrients and negatively with salinity, confirming their preference for the upper, nutrient-rich and less saline layer. The mixotrophic chrysophyte *Dinobryon* sp. (McKenrie et al. 1995) and cryptophytes were found in high cell concentrations in the surface layer during spring. Their development was probably favoured by the higher inorganic nutrient concentrations as well by the release of organic matter by diatoms at this stage of the *Skeletonema marinoi* bloom.

The large contribution (60%) of the micro fraction to the phytoplankton carbon biomass in the summer was due mostly to the development of the microdinoflagellate *Prorocentrum micans*, constituting a large biomass of 84.57 μg C L^{-1} and reaching its maximum abundance in the surface layer. *Prorocentrum gracile* is a very similar species, which was observed together with *P. micans* in the summer bloom, but at much lower abundances (maximum 1.5×10^3 cells L^{-1}). The two species were distinguished mainly by their general shape, *P. gracile* cells being twice as long as wide, with a much longer spine, and possessing a mucron – a small tooth on the antapical part of the cell (Cohen-Fernandez et al. 2006). *P. micans* is a very common species in enclosed and semi-enclosed basins or estuarine waters, which may at times be heavily eutrophic, and where it often forms intensive blooms (Carstensen et al. 2007). It is generally reported as a typical component of summer and early autumn phytoplankton. For instance, in the Mediterranean coastal Fusaro lagoon, bloom concentrations of $>10^6$ cells L^{-1} have been reported, dominating up to 99% of the total phytoplankton carbon biomass (Sarno et al. 1993). In addition to *P. micans*, the diatoms *Thalassionema frauenfeldii* and *Pseudo-nitzschia pseudodelicatissima* were both present at all stations in the summer in relatively high cell concentrations ($>10^5$ cells L^{-1}). In the eastern Mediterranean *T. frauenfeldii* has been cited as the dominant and the most frequent species in the winter period (Gomez & Gorsky 2003), which is in contrast to our findings of its greatest development in the summer. Although it has been reported from the south-eastern and north-eastern Adriatic Sea (Saracino & Rubino 2006, Viličić et al. 2009), this study represents the first record of such high abundances of this particular species.

Diatoms of the potentially toxic genus *Pseudo-nitzschia* are a widespread and dominant component of the phytoplankton assemblages in the central (Burić et al. 2008) and southern Adriatic (Caroppo et al. 2005). Previous studies (Campanelli et al. 2009) recorded *Pseudo-nitzschia* spp. among the dominant diatoms in the early summer in Boka Kotorska Bay with maximum cell concentrations of 9.0×10^3 cells L^{-1}, which was less than what we recorded during the summer. Closer examination of the

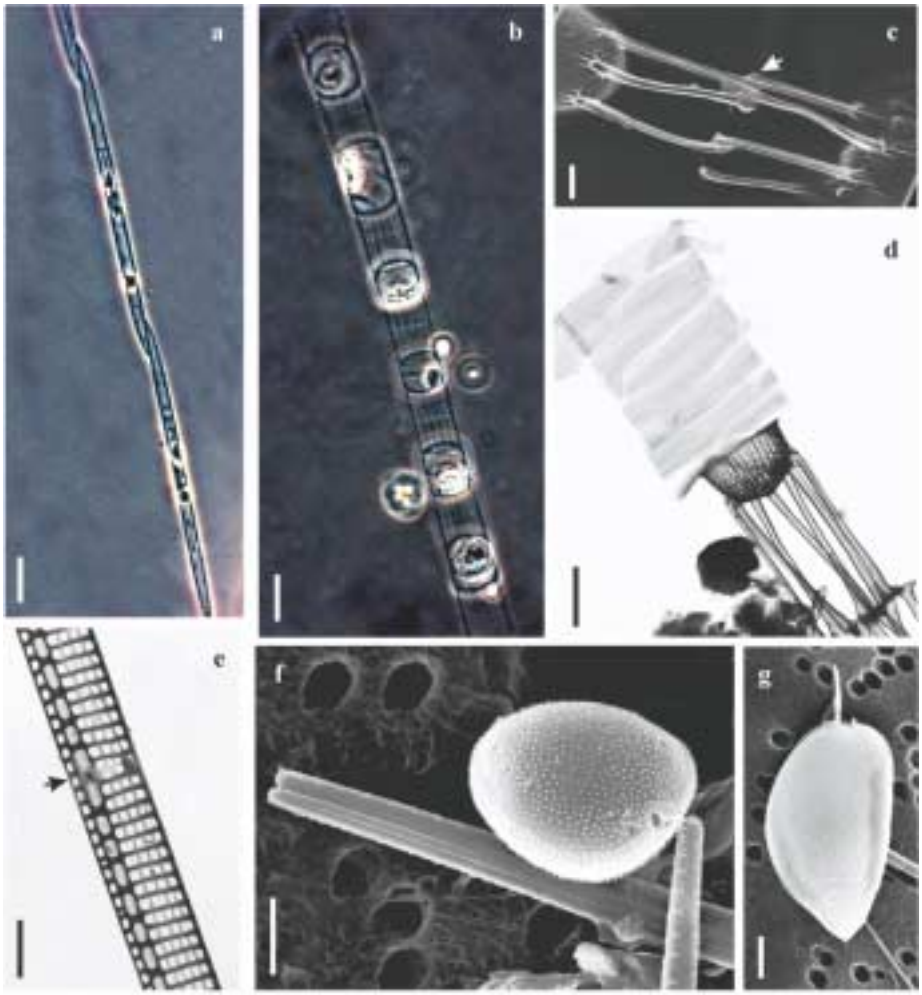

Figure 8. Images of the dominant phytoplankton taxa in Kotor Bay. Diatom *Pseudo-nitzschia pseudodelicatissima*: a) LM phase contrast image of a chain colony, e) TEM image of the central part of the valve showing the central nodule (arrow), mantle and stria structures. Diatom *Skeletonema marinoi*: b) LM phase contrast image of a chain colony. c) Intercalary valves with 1:2 junctions (arrow) of intercalary fultoportulae processes (SEM). d) Intercalary valve with junctions of intercalary fultoportulae processes and cingular bands (TEM). f) Dinoflagellate *Prorocentrum minimum*: spiny thecal ornamentation with visible apical spines and diatom *Thalassionema frauenfeldii*: part of the valve with the areolae externally crossed by simple unbranched silicified arches (SEM). g) Dinoflagellate *Prorocentrum gracile* right valve with prominent winged apical spine and visible mucron (small tooth in the antapical valve region) (SEM). Measurement scales: a, b, g – 10 μm; f – 5 μm; d, e – 2 μm, c – 1 μm

material collected during this study revealed the presence of three potentially toxin-producing species (Bosak et al. 2010). *P. calliantha* Lundholm, Moestrup & Hasle and *P. fraudulenta* Cleve (Hasle) were present at low abundances up to 10^4 cells L^{-1} in all seasons except the summer, when the maximum abundance of 10^5 cells L^{-1} was due to the species *P. pseudodelicatissima* (Figure 8a,e). The strains of this particular species isolated from the Mediterranean Sea have been shown to produce considerable quantities of the neurotoxin domoic acid (DA), the causative agent of amnesic shellfish poisoning (Moschandreou et al. 2010). In the area investigated, there are no records of any DA concentrations or toxicity events, so unfortunately, we cannot state definitively whether the high abundance of the species recorded in the study had any impact on shellfish farming activities. However, the probability of such events is rather high: there are previous records in a similar semi-enclosed system of higher DA concentrations, up to 6.55 μg g^{-1}, being measured in shellfish tissue, and which had been preceded by *Pseudo-nitzschia* blooms (Ujević et al. 2010). The presence of another potentially toxin-producing phytoplankton species, the dinoflagellate *Prorocentrum minimum* (Fig. 8f) has also been noted. The identity of the species has been confirmed by morphological examination of the flagellar pore complex (Monti et al. 2010). Since this is a red-tide species, known for its regular formation of summer blooms in the eutrophic areas in the Adriatic, we cannot rule out the potential occurrence of biomass peaks of this species in Boka Kotorska Bay. The discovery of potentially toxic phytoplankton species such as *P. pseudodelicatissima* and *P. minimum* point to the importance of more intensive research into and the monitoring of potential blooms of harmful algae occurring in the area, as these will affect active shellfish farming activities.

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