Oceanologia (2016) 58, 302—316

Available online at www.sciencedirect.com

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ORIGINAL RESEARCH ARTICLE

Eutrophication influence on phytoplankton community composition in three bays on the eastern Adriatic coast

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Received 31 December 2015; accepted 6 May 2016
Available online 20 May 2016

KEYWORDS
Phytoplankton;
Chlorophyll *a*;
Biodiversity;
The eastern Adriatic coast

Summary This study shows the influence of eutrophication pressure on the phytoplankton community structure, abundance and biodiversity in the investigated bays with different hydromorphological features. Šibenik Bay is a highly stratified estuary of the karstic river Krka; Kaštela Bay is a semi-enclosed coastal bay, which is influenced by the relatively small river Jadro; and Mali Ston Bay is located at the Neretva River estuary, the largest river on the eastern part of the Adriatic Sea. All of the areas are affected by urban pressure, which is reflected in the trophic status of the waters. The greatest anthropogenic influence was found in Kaštela Bay while the lowest influence was found in Mali Ston Bay. In this study, the highest biomass concentration and maximum abundance of phytoplankton were recorded at the stations under the strongest anthropogenic influence. Those stations show a dominance of abundance compared to the biomass and a dominance of opportunistic species, which is reflected in the lower biodiversity of phytoplankton community. Diatoms were the most represented group of the phytoplankton community in all three bays, followed by the dinoflagellates. Diatoms that were highlighted as significant for the difference between the bays were *Skeletonema marinoi* in Šibenik Bay, *Leptocylindrus minimus* in Kaštela Bay and the genus *Chaetoceros* spp. in Mali Ston Bay. Dinoflagellates were more abundant at the stations under the strongest anthropogenic influence, and most significant were *Prorocentrum triestinum* in Kaštela Bay and *Gymnodinium* spp. in Šibenik Bay and Mali Ston Bay.

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Peer review under the responsibility of Institute of Oceanology of the Polish Academy of Sciences.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.oceano.2016.05.003
0078-3234/

1. Introduction

Phytoplankton biomass and community composition were analyzed in three bays on the eastern Adriatic coast, with different hydrological and trophic statuses. Phytoplankton is very sensitive to changes in its environment and, therefore, provides good insight into water quality before it becomes visible on higher trophic levels and the excessive eutrophication of certain areas commences (Brettum and Andersen, 2005). Eutrophication is an enrichment of water with nutrients, primarily nitrogen and phosphorus, which stimulates primary production. In some cases, that leads to visible blooms and accumulation of submerged and floating organic material in the water (Vollenweider, 1992). Eutrophication can have natural and anthropogenic origins. A natural one occurs due to substrate remineralization, upwelling and increase of rivers inflow. Resuspension of particulate matter can enhance primary production because of the intrusion of the pore water rich with nutrients from the sediments into the bottom layer and consequentially into the whole water column (Guinder et al., 2015; Su et al., 2015). A previous study of the investigated areas shows that sediment resuspension is one of the sources of ammonia, nitrate and phosphate regeneration (Barić et al., 2002). Upwelling brings nutrient rich waters from the deeper layers, and rivers' inflow bring the bulk of total nitrogen to the sea. Anthropogenic eutrophication occurs due to various human activities in the vicinity of the coastal area, such as the inflow of urban and industrial wastewaters, rinsing of agricultural land and atmospheric pollution. A causal link between anthropogenic sources of nutrients and the eutrophication of the system is generally accepted (McQuatters-Gollop et al., 2009; Smith, 2006), although it is very important to take into account systems-specific features of a certain area to distinguish changes in the ecosystem resulting from natural seasonal and interannual dynamics. Given the scale, the eutrophication process could be beneficial for the ecosystem, but it could have adverse effects depending on the different characteristics of each ecosystem (Crossetti et al., 2008; Marasović and Pucher-Petković, 1985; Skejić et al., 2014; Su et al., 2015). The beginning of eutrophication causes an increase in phytoplankton biomass, but the composition of the phytoplankton community becomes more uniform. Certain species disappear, while at the same time, opportunistic species of phytoplankton begin to dominate (McQuatters-Gollop et al., 2009). Species diversity is reduced because of the competitive exclusion between species, whereas with a slight increase of eutrophication, competition is relaxed, thus resulting in increased diversity. With a further increase in eutrophication, diversity drops again because of species reduction due to stress (Spatharis et al., 2007). Eutrophication tends to favour small and fast-growing organisms, which usually means that the proportion of the dominant taxa to the total biomass is relatively low, meaning that the biodiversity values are higher than when large-sized taxa dominate (Uusitalo et al., 2013).

In highly eutrophicated systems, the trophic chain is lacking higher links, and autotrophic processes exceed heterotrophic, which significantly affects the balance of the system (Richardson et al., 1998). The responses of phytoplankton to the eutrophication process have been reported mostly through chlorophyll *a* concentrations (Edwards et al., 2003; Gowen et al., 1992; Vollenweider, 1976). The phytoplankton biomass (chl *a*) is a common indicator of eutrophication because it provides consistent insights of a certain area, but it should be monitored with the compositional changes of the community structure (McQuatters-Gollop et al., 2009; Ninčević Gladan et al., 2015). The Water Framework Directive (WFD) (European Commission, 2008) states that phytoplankton and its biodiversity are one of the crucial biological elements in the assessment of the ecological status of the sea. Previous studies of researched bays along the eastern Adriatic coast, revealed that these are the areas with the highest nutrient concentration and primary productivity. These are the semi-enclosed areas and salt-wedge estuaries with a high urban nitrogen and phosphorus loading, and a high natural nitrate and silicate loading by the rivers' inflow (Barić et al., 1992; Legović et al., 1994). In previous studies, the trophic status of investigated bays has been determined using the phytoplankton abundance and volume (Čalić et al., 2013; Marasović and Ninčević, 1997; Viličić, 1989).

The aim of this study is to determine a difference between the stations considering the specific nutrients, to quantify the potential anthropogenic pressures and to determine the relationship between abiotic parameters and biomass. In addition, we determine the similarity of the phytoplankton community regarding the abundance of species and define how much individual species affect the diversity observed between the investigated stations. The aim is to establish the relationship between biomass and phytoplankton abundance and use it as a way to define the level of disturbance in the investigated areas. Overall, the aim of this study is to investigate the impact of anthropogenic pressures on phytoplankton community structure and biodiversity.

2. Material and methods

2.1. Study area

Šibenik Bay is a highly stratified estuary of the karstic river Krka, with small tidal amplitudes and permanently brackish surface water (Svensen et al., 2007). The Krka River is one of the most pristine European rivers, characterized by low concentrations of nutrients and extremely low input of terrigenous material (Legović et al., 1994). Freshwater discharge from the Krka River has been systematically monitored since 1947 (Bonacci and Ljubenkov, 2005), and it can vary between 5 and 565 $m^3\ s^{-1}$ with an annual average discharge of 52.9 $m^3\ s^{-1}$ (1950—1998). The Krka River estuary is a typical salt-wedge, highly stratified estuary (Žutić and Legović, 1987) that is 25 km long and relatively narrow except for two wider parts, Prokljan Lake and Šibenik harbour. The depth gradually increases from 5 to 43 m at the mouth. The town of Šibenik is located in the estuary's middle reach, and it is the only source of direct anthropogenic eutrophication (Gržetić et al., 1991; Legović et al., 1994). Šibenik harbour has reduced exchange with the waters of the open sea, and it is under a direct anthropogenic influence (Kušpilić, 2005). The phytoplankton community in the estuary is dependent on seasonal cycles of temperature and salinity (winter—spring and summer—autumn), and on the degree of eutrophication, which can

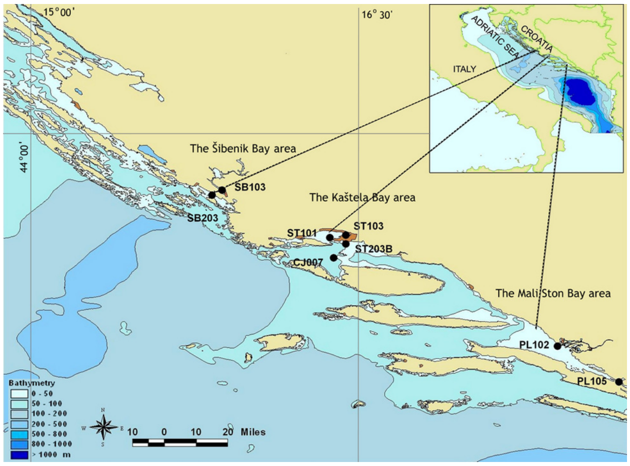

Figure 1 The investigated areas with sampling stations.

be of natural or anthropogenic origin. Decomposition of freshwater phytoplankton greatly contributes to natural eutrophication and the regeneration of nutrients in the upper reaches of the estuary, whereas in the lower parts, the eutrophication favours anthropogenic sources (Legović et al., 1994; Svensen et al., 2007; Viličić et al., 1989). Previous studies of this area show that the main source of nitrates and orthosilicates is the Krka River, while the total phosphorus is mainly of anthropogenic origin from the Šibenik urban area (Legović et al., 1994). In this area, samples were taken at two stations, SB103 and SB203, which were 35 m and 25 m deep, respectively (Fig. 1).

Kaštela Bay is a semi-enclosed coastal bay, the largest in the middle part of the eastern Adriatic coast, 15 km long and 6 km wide, with an average depth of 23 m. It communicates with the adjacent channel through an inlet that is 1.8 km wide and 40 m deep. The most important fresh water source is the Jadro River, a relatively small river with an average annual discharge of 8 $m^3 s^{-1}$, which discharges into the eastern part of the bay (Ljubenkov, 2015). The discharge of several submarine springs is of a lower intensity. Kaštela Bay is under the strong impact of untreated municipal and industrial effluents. Previous studies show that anthropogenic eutrophication and nutrient inflow from the Jadro River cause frequent summer algal blooms with the development of toxic dinoflagellates (Marasović et al., 1991). Regularity in spring—autumn maximum abundances was found, but there was also evidence that algal biomass and community structure changed over time. An increase of abundance and phytoplankton biomass have been recorded in mid-1980 to mid-1990's period, following the decrease (Ninčević Gladan et al., 2009). Previous studies also show a regularity of layout in the size fractions. Smaller pico and nano fractions contributed more to community composition in the outer more open part of the bay, while larger micro fractions occurred in the inner and more eutrophicated part (Marasović and Ninčević, 1997).

In this area, samples were taken at four stations, two located within the bay (ST101, ST103) and the other two just outside of the bay (ST203B, CJ007) (Fig. 1). The outer stations were included in the research to determine the reach of anthropogenic influence to the surrounding area. The depth of the investigated stations ST101, ST103, ST203B and CJ007 were 37 m, 12 m, 35 m and 50 m, respectively.

Mali Ston Bay is deeply cut between the mainland and the Pelješac Peninsula, and it is located at the end of the Neretva Channel. The area is influenced by a considerable freshwater discharge from the Neretva River, the largest river on the eastern part of the Adriatic Sea, and several submarine springs situated inside Mali Ston Bay. The average annual discharge of the Neretva River is 332 $m^3 s^{-1}$ (Orlić et al., 2006). Neretva River estuary is classified as the salt-wedge type, where due to small tidal currents, the advection of the river water is much larger than the introduction of seawater through tidal mixing. The bay generates estuarine circulation with a brackish water output current on the surface, while saline water enters below the halocline from the open sea. This area is sparsely populated, so the anthropogenic impact on the bay is low. Previous studies show that the principal regulator of production conditions in Mali Ston Bay is the specific, constant and strong exchange of water within the bay and the open sea, the strong impact of the Neretva River and the karstic submarine springs (Viličić, 1989). According to the nutrient concentration, the transparency of the water column and the quantity of phytoplankton, Mali Ston Bay may be classified as a moderate natural eutrophicated system (Jasprica and Carić, 1994; Jasprica et al., 2012; Skejić et al., 2015; Viličić, 1989; Viličić et al., 1998). Thanks to the hydrographic features and favourable primary production, the bay has been well known for cultivated mussels since ancient times, and today it presents one of the most important places for shellfish farming in Croatia. In this area, samples were taken at two stations PL102 and PL105, 21 m and 8 m deep, respectively (Fig. 1).

2.2. Sampling methods

Sampling was performed during 2005, with the intent of capturing the seasonal cycle of phytoplankton in a certain area. In Kaštela Bay, sampling was performed monthly in a period from January to December, while in Šibenik and Mali Ston Bay, the study covers 8 months of sampling (January, May, June, July, August, September, November, December). Physical and chemical parameters (salinity, temperature, nutrients) were sampled simultaneously with phytoplankton samples to make a more complete ecological characterization of the study area. Sampling and determination of phytoplankton, physical and chemical parameters were conducted using standard oceanographic methods (Strickland and Parsons, 1972). Temperature and salinity were measured with a Seabird-25 CTD probe. Dissolved oxygen was determined by the Winkler method of thiosulphate titration. Dissolved inorganic nutrient concentrations were measured photometrically with an AutoAnalyzer III system (Bran + Luebbe), using modified automated methods according to Grasshoff (1976). Chlorophyll *a* concentrations were measured using the fluorometric method from 90% acetone extracts (Strickland and Parsons, 1972), and the results were expressed as mg chl *a* m^{-3}. Phytoplankton abundance and community composition have been determined according to the Utermöhl method (Utermöhl, 1958). Water samples (250 mL) were collected with Nansen bottles and preserved with formaldehyde to a final concentration of 2% formaldehyde-sea water solution. Subsamples of 25 mL were settled in counting chambers for at least 24 h. Counting was performed in one transect of the sedimentation chamber, using an inverted microscope with magnifications of $\times 200$, and $\times 400$ for different species, depending on their respective sizes. In the case of blooms or a high abundance of some species, counting was done in several randomly selected fields.

2.3. Data analysis

Analysis of variance (ANOVA) was used to determine the difference between the stations considering the specific nutrients.

The impact assessment of anthropogenic influence on land in the study area was determined by calculating the LUSI index (Land Uses Simplified Index), using the Croatian CORINE (Coordination of Information on the Environment) digital database that assesses the environmental pressure. CORINE has been accepted and evaluated by the European Union as a fundamental reference data set for spatial and territorial analysis. The LUSI index is quantifying potential anthropogenic pressures according to the percent of land used in various anthropogenic activities such as urbanization, industrialization, and proximity to large urban and agricultural areas. In this study, the LUSI index was calculated in accordance with Flo et al. (2011) with a small modification, which relates to a radius being taken into account. A radius of 5 km from the investigated points was taken to insure that all the stations are included in the calculation.

The biological data was tested for normality by the Kolmogorov—Smirnov and Liliefor tests, which showed that it does not have a normal distribution and was analyzed with nonparametric methods.

The Bray—Curtis similarity coefficient was used to determine the similarity of the phytoplankton community structure regarding the abundance of species (Bray and Curtis, 1957). Total abundances were used, and the input data were transformed using a logarithm (Clarke, 1993; Field et al., 1982). The obtained data gave the similarity matrix, and using the method of cluster analysis, the dendrogram of average similarity between stations was made. For a graphical representation, MDS (Multidimensional Scaling) was used with vectors of phytoplankton groups (Clarke and Warwick, 2001).

SIMPER (Similarity Percentage analysis) was used to determine how much individual species affect the diversity observed between the investigated stations. This analysis identifies species that are typical in terms of regularity of occurrence in a constant for most samples. The analysis was used to compare the phytoplankton community at the stations that are in areas of highest anthropogenic pressure in this investigation.

Non-parametric Spearman rank order correlations were used to determine the relationship between biomass and abiotic parameters.

The relationship between biomass and phytoplankton abundance was determined through ABC-plots, a simple graphic way to determine the level of disturbance in the investigated area.

Biodiversity was presented through the curves of cumulative dominance of species with k-dominance plots and different types of diversity indexes, of which the most significant proved to be Menhinick's diversity index (*D*).

3. Results and discussion

3.1. Environmental parameters in the bays

During this study in Šibenik Bay area, the sea temperature ranged from 7.35 to 23.47°C. Both extreme values were observed in the surface layer at station SB103, where the minimum temperature was recorded in January and the maximum in September. The salinity of this area ranged from 4.42 to 38.71 in December at the surface and in August at the bottom, respectively. Both extremes were found at the same station (SB103), indicating the strong influence of the freshwater inflow of the Krka River at this station. This assertion is confirmed by the maximum values of orthosilicate (9.59 mmol m^{-3}) and nitrate (TIN 57.93 mmol m^{-3}) recorded at that station. Orthophosphate concentrations in this area ranged from 0.002 to 0.12 mmol m^{-3} recorded at SB203 and SB103, respectively. The oxygen saturation of this area ranged from 78 to 125%.

The sea temperature in the Kaštela Bay area ranged from 9.45 to 26.93°C, recorded at the surface at stations ST101 in March and ST103 in July, respectively. The salinity ranged from 34.16 to 38.71, and both extremes were recorded at station ST101, in May at the surface and in July at 5 m of depth, respectively. The maximum values of orthosilicate and nitrate were recorded at station ST103, which is under direct influence of the Jadro River. The highest value of orthosilicates (12.32 mmol m^{-3}) was recorded in December on the surface, and the maximum value of nitrates was measured in August at a depth of 5 m (TIN 24.90 mmol m^{-3}). These findings have confirmed the influence of the freshwater inflow of the Jadro River at this station. Orthophosphate concentrations in this area had the highest range of all investigated areas, from 0.002 mmol m^{-3} at station CJ007, which is under the lowest influence from the land, to 1.5 mmol m^{-3} at station ST103, which is closest to land and under the direct influence of urban wastewaters. Oxygen saturation in the Kaštela Bay area ranged from 84 to 133%.

In the Mali Ston Bay area, the sea temperature ranged from 10.54 to 23.65°C at station PL105 in January and PL102 in August, respectively. Both extremes were recorded at the surface. Salinity in this area ranged from 26.47 to 38.62. These values were recorded at station PL102, strongly influenced by land, submarine springs and freshwater inflow of the Neretva River, which is confirmed by the large range of recorded values of orthosilicates (0.37—21.93 mmol m^{-3}) and TIN (0.34—38.85 mmol m^{-3}). The maximum value of orthosilicates was recorded in December at the surface and of nitrates in November at a depth of 5 m. Orthophosphate concentrations in this area had the lowest range of all investigated areas, from 0.01 to 0.11 mmol m^{-3}, indicating a week anthropogenic influence. Oxygen saturation in this area ranged between 94 and 127%.

The seasonal distribution of temperature and salinity at the surface layer at all investigated stations is presented in Fig. 2. It is evident that the influence of the seasons and freshwater inflow were great at stations closest to the mouth of the rivers and land. The abiotic parameters of the investigated areas were studied in detail in earlier research and have been presented in Bužančić et al. (2012).

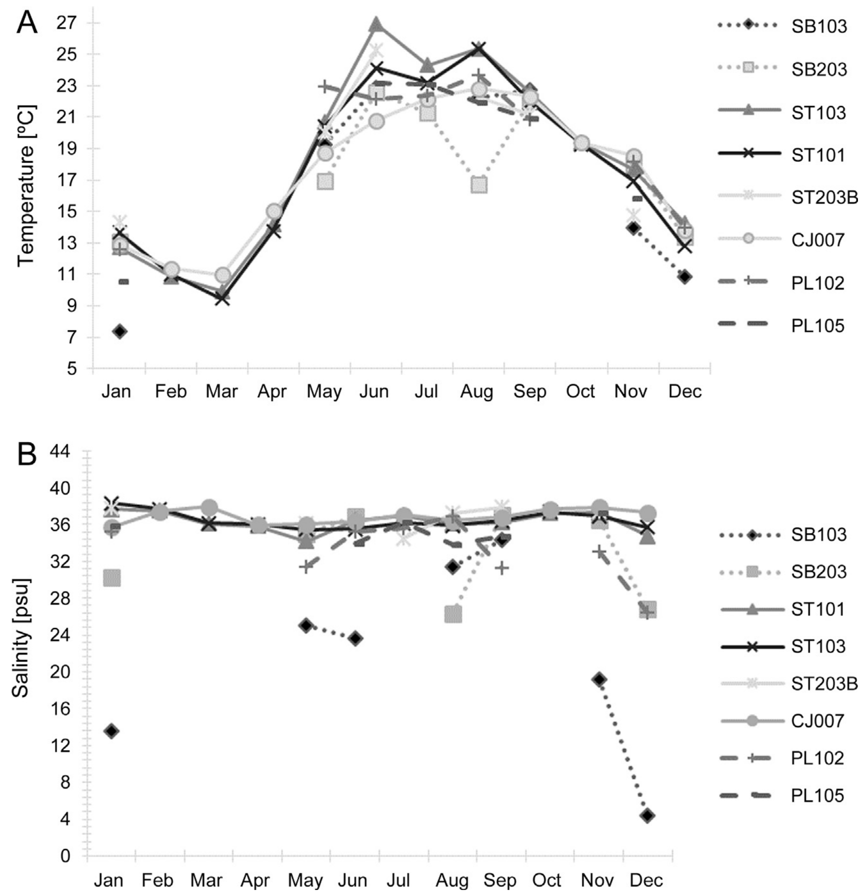

Figure 2 Seasonal distribution of temperature [°C] (A) and salinity [psu] (B) in the surface layer at all investigated stations.

The difference between the stations considering the specific nutrients was determined by an analysis of variance (ANOVA).

A statistically significant difference ($p < 0.001$) with respect to salinity was observed between stations SB103 and all other investigated ones. This station, given the very low salinity, describes the transitional waters while all other stations belong to the coastal waters (Kušpilić et al., 2011; Ninčević Gladan et al., 2015). At the stations in the coastal waters, the salinity was lowest in the Šibenik Bay area (outer station), followed by Mali Ston Bay and Kaštela Bay (inner stations), while the Kaštela Bay area (outer stations) was characterized by higher salinity. The difference in salinity between Kaštela Bay (inner stations) and the Šibenik Bay area (outer station) is statistically significant.

The highest nitrate concentration was recorded at station SB103 where the fresh water inflow from the Krka River is the greatest. Statistically significant ($p < 0.05$) higher concentrations of nitrate were found at station SB103, in contrast to stations CJ007 and ST203B, which were weakly influenced by fresh water inflow. At the other investigated stations, the nitrate concentration was lower in comparison to station SB103, but this difference was not statistically significant. The reason for this is the influence of the fresh water inflow of the Jadro and Neretva Rivers and the influence of submarine springs.

The highest concentration of orthosilicates was observed in the transitional waters of Šibenik Bay and the inner part of Mali Ston Bay, which is under a stronger influence of submarine springs. Statistically significant ($p < 0.01$) higher concentrations of orthosilicates were observed at stations SB103 and PL105 in relation to all other stations (ST103, SB203 and PL102). The lowest statistically significant correlations were observed at stations CJ007, ST101 and ST203B, which are located furthest from the river mouth.

A statistically significant ($p < 0.001$) higher concentration of orthophosphates was recorded at station ST103, which is under the strongest anthropogenic influence, compared to all other investigated stations.

Regarding the concentration of ammonium salts, a significant difference ($p < 0.05$) was observed between stations SB103, characterized by the highest concentration, and stations CJ007, ST203B, SB203 and PL102, characterized by the lowest concentration of ammonium salts. The remaining stations (ST102, ST103, PL105) had increased concentrations of ammonium ions but were not statistically different.

The proximity and intensity of the rivers' inflow and the influence of activities from the land mainly condition the physical and chemical characteristics of the investigated areas. The impact of agricultural activities on water quality in most European rivers is manifested through increased concentrations of nitrates (Crouzet et al., 1999; Ludwig et al., 2009). Rivers and groundwater bring the bulk of total nitrogen to the sea (nitrates, nitrites, ammonium salts), as confirmed by our research. The maximum concentrations of TIN were recorded at all stations in the surface layer through

Table 1 LUSI index of the investigated stations.

Impact	Station							
	SB103	SB203	ST101	ST103	ST203B	CJ007	PL102	PL105
Urban	1	1	2	2	3		1	
Industrial				1			1	
Agricultural	1	1	1	1			1	1
Harbour	1		1	1	1		1	
LUSI index	3	2	4	5	4	0	4	1

the winter—spring season, at the time of increased freshwater inflow and precipitation. The urban influence is manifested through an increased gradient of orthophosphate and ammonium ions. Previous research in the northwestern Mediterranean Sea shows the same association between the physical and chemical parameters (Flo et al., 2011).

3.2. Anthropogenic influence on the investigated stations

The Land Uses Simplified Index (LUSI) was used to determine the anthropogenic influence on the investigated stations (Table 1).

The greatest anthropogenic influence on the body of water was found in Kaštela Bay at ST103 situated in the eastern part. A relatively high LUSI index was established at station ST203B, which is under strong urban pressure, but as it is located in the channel area with strong current dynamics, there is no pronounced impact of human activity. At the referent station, CJ007 is located outside of Kaštela Bay, furthest from the coast and anthropogenic influence, and LUSI was 0. The lowest index was found in Mali Ston Bay at PL105. At station PL102, which is under a strong influence of agricultural land in the valley of the Neretva River, harbour and urban pressures from the city Ploče, the LUSI index was high. At the same station, the anthropogenic influence was not expressed as in Kaštela Bay, which is probably due to the hydro-geomorphological characteristics of its location. The Šibenik Bay area had a moderate anthropogenic pressure on the body of water.

3.3. Phytoplankton biomass

The highest phytoplankton biomass expressed as chlorophyll *a* concentration was recorded in Šibenik Bay at SB103 (4.73 mg m^{-3}), followed by Kaštela Bay at ST103 (2.79 mg m^{-3}), as both stations are directly influenced by freshwater inflow and high anthropogenic pressure. At all other stations, the recorded values of chlorophyll *a* were generally below 1 mg m^{-3} (Fig. 3).

In the Šibenik Bay area, the range of chl *a* data varied from 0.07 to 4.73 mg m^{-3}. The highest value was measured in May at SB103 on the surface layer, and the minimum one was recorded in July at SB203 in the bottom layer. In this area, the highest values of chl *a* have been recorded in the surface layer during the whole investigated period at both stations. The seasonal distribution of the phytoplankton biomass has shown a big difference between these two stations, where much higher concentrations were recorded at SB103 due to the positive impact of freshwater inflows and anthropogenic eutrophication (Fig. 3).

In the Kaštela Bay area, the phytoplankton biomass varied from 0.01 to 2.79 mg m^{-3} (Fig. 3). The maximum values of chl *a* were recorded at ST103 in September throughout the water column (2.17—2.79 mg m^{-3}), while the minimum one (0.01 mg m^{-3}) was recorded at station CJ007. Higher biomasses were recorded at stations inside of Kaštela Bay (ST101, ST103) opposed to stations just outside of the bay (ST203B, CJ007). The seasonal distribution of chl *a* in this area followed the distribution of nutrients with a marked increase in the autumn—winter period. Such regularity was much more pronounced at stations inside of the bay. Higher values at the surface in relation to the bottom and the vertical distribution of the biomass during the summer period indicate an inflow of nutrients at the surface layer due to the anthropogenic influence on stations placed in Kaštela Bay.

The maximum value of chlorophyll *a* (1.26 mg m^{-3}) in the Mali Ston Bay area was recorded at station PL105 in November, and the minimum value (0.04 mg m^{-3}) was recorded at PL102 in July. A strong anthropogenic influence on PL102 was not reflected on the phytoplankton biomass, probably due to the hydro-geomorphological characteristics of this area (Viličić, 1989). The seasonal distribution in the Mali Ston Bay area was in accordance with the distribution of nutrients and has shown that maximum values of chl *a* occurred in the autumn—winter period (Fig. 3).

According to the seasonal distribution of biomass, there were well pronounced differences between bays. Šibenik Bay is characterized by the highest values of chl *a* and the significantly more frequent appearance of higher biomasses compared to other areas. Although the greatest human impact was recorded in the eastern part of Kaštela Bay, the largest biomass was recorded in Šibenik Bay, largely due to natural eutrophication by the strong influence of the Krka River along with anthropogenic load. The eutrophication of this area was determined in earlier research (Cetinić et al., 2006; Legović et al., 1994; Svensen et al., 2007; Viličić et al., 1989) and confirmed by this study. Besides Šibenik Bay, Kaštela Bay was also characterized by a greater incidence of high concentrations of chl *a*, opposed to Mali Ston Bay, which was characterized by small concentrations of chl *a*. This finding confirms the significantly lower trophic level of this area, determined in earlier research studies (Jasprica et al., 2012; Skejić et al., 2015; Viličić et al., 1998).

3.4. Phytoplankton community composition

With the qualitative analysis of phytoplankton composition at the investigated area of Šibenik Bay, 114 phytoplankton taxa

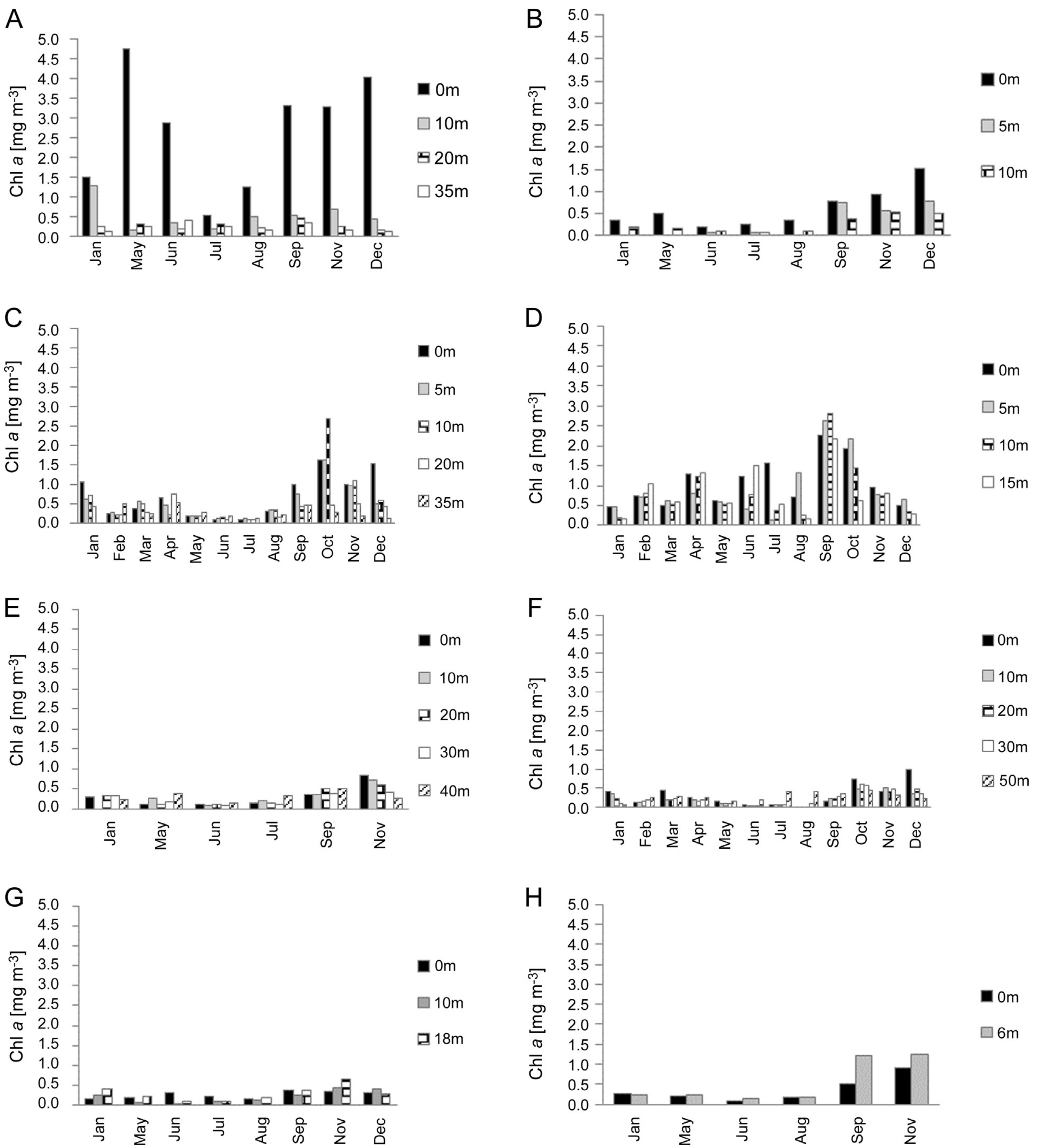

Figure 3 Seasonal distribution of chlorophyll *a* concentrations [mg m^{-3}] at the station SB103 (A), the station SB203 (B), the station ST101 (C), the station ST103 (D), the station ST203B (E), the station CJ007 (F), the station PL102 (G) and the station PL105 (H).

have been determined. The most diverse functional groups were diatoms (61) and dinoflagellates (37). Coccolithophorids contributed with 6, cryptophytes with 3, and silicoflagellates, euglenophytes, chlorophytes with 2 taxa each. The diverse flagellate group of phytoplankton, which is placed by size in the microflagella group, was present in the whole area with a high frequency of findings (96%). The most diverse station in this area with 100 recorded taxa was SB103, located in the Šibenik harbour, which is under a strong influence of urban waste waters and the city port and marina. At station SB203, 66 phytoplankton taxa were found.

In the investigated area of Kaštela Bay, a total of 193 phytoplankton taxa have been recorded. The dinoflagellate functional group was the most diverse one with 92 taxa, followed by diatoms (80), coccolithophorids (9), silicoflagellates and euglenophytes (4), cryptophytes (2) and chlorophyte (1). The microflagella group also had a high frequency of findings in this area (99%). The station with the most

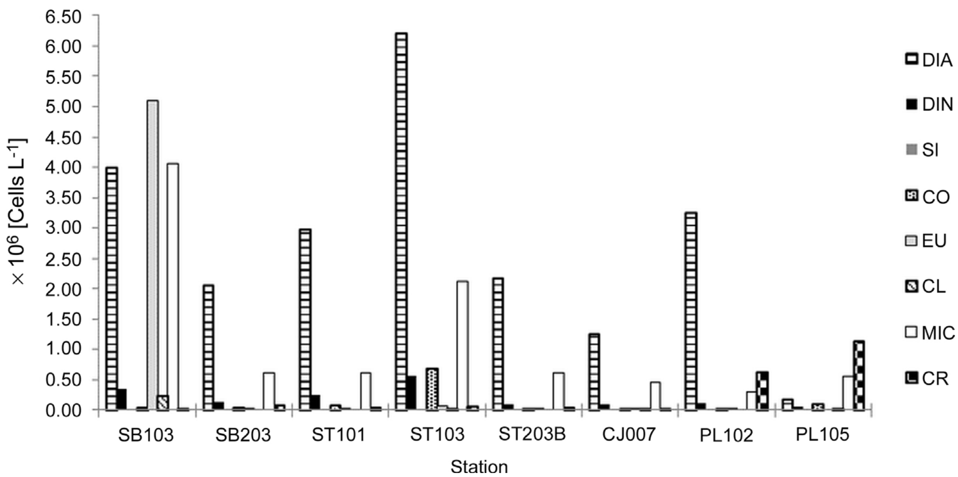

Figure 4 Total abundance of phytoplankton groups [cells L^{-1}] in the surface layer at all investigated stations (DIA, diatoms; DIN, dinoflagellates; SI, silicoflagellates; CO, coccolithophorids; EU, euglenophytes; CL, chlorophytes; MIC, microflagellates; CR, cryptophytes).

recorded phytoplankton taxa was ST103 (140), situated deepest in the bay and under a direct influence of the Jadro River and strong anthropogenic pressure. A somewhat smaller number of taxa were recorded at station ST101 (108), located further away from the freshwater influence but under strong anthropogenic pressure. Stations ST203B with 84 and CJ007 with 82 recorded taxa had the lowest diversity in this area, presumably because they are influenced by canal waters and currents, preventing nutrient retention at these stations.

In the Mali Ston Bay area, a total of 88 phytoplankton taxa have been found: 39 diatoms, 36 dinoflagellates, 4 coccolithophorids, 3 chrisophyte, 2 silicoflagellates and euglenophytes and 1 chlorophyte taxa. A diverse microflagella group had the highest frequency of findings in this area (100%). Station PL102, under the direct influence of the Neretva River, was the more diverse one, with 72 taxa recorded, compared to PL105 where 49 taxa were found.

The complete list of all phytoplankton taxa determined in the investigated areas with their frequency of findings was presented in Bužančić et al. (2012).

Diatoms were the most abundant component of the phytoplankton community at all stations except SB103, where euglenophytes prevailed and PL105, where cryptophytes were the most significant group (Fig. 4). Dinoflagellates were a second significant component of the phytoplankton community, whose contribution was most evident at stations SB103 and ST103 under the highest trophic load, followed by SB203, ST101 and PL102. The lowest contribution was at the stations under the lowest trophic load: ST203B, CJ007 and PL105. A diverse microflagella group was represented with great abundance in all areas with an increase in the spring—summer period.

The maximum abundance in the diatom group was reached by *Leptocylindrus minimus* (4,480,000 cells L^{-1}) in Kaštela Bay at ST103, followed by *Skeletonema marinoi* (3,870,000 cells L^{-1}) in Šibenik Bay, at SB103, and *Chaetoceros* spp. taxa (2,770,000 cells L^{-1}) in the Mali Ston Bay area at PL102. The maximum abundance of the dinoflagellate group was reached by *Prorocentrum triestinum* (489,000 cells L^{-1}) in Kaštela Bay at station ST103, followed by *Gymnodinium* spp. taxa with 88,100 cells L^{-1} and 42,600 cells L^{-1}, in Šibenik Bay (SB103) and Mali Ston Bay (PL102), respectively.

Dinoflagellates were most abundant at the stations that were under the greatest anthropogenic influence and generally more abundant in the warmer part of the year, which is in accordance with previous studies (Ninčević Gladan et al., 2009; Skejić et al., 2012; Smayda, 2000). Previous research indicates that diatoms are positively correlated with nitrates and silicates, and dinoflagellates with phosphates (Svensen et al., 2007).

It is also noteworthy to indicate the monospecific bloom of euglenophyta *Eutreptia lanowii* (5,090,000 cells L^{-1}) at station SB103, which occurred after a sudden rise in temperature of the surface layer. That is characteristic for diluted, warm and eutrophicated waters, and this taxon is used as a biological indicator of organic pollution (Ninčević Gladan et al., 2015; Stonik and Selina, 2001). Blooming of euglenophyta was replaced by blooming of diatom *S. marinoi* at the beginning of July, characterized by strong growth due to the increase of nitrate (DeManche et al., 1979; Ninčević Gladan et al., 2015). This makes it a better competitor among diatoms in eutrophicated conditions, so it is a good indicator species for eutrophication (Collos et al., 1997; Lagus et al., 2004).

It is interesting to point out that the Mali Ston Bay area, in contrast to the other two investigated bays, was characterized by a greater abundance of cryptophytes, which is according to many authors (Pick and Caron, 1987; Skejić et al., 2015; Watson et al., 1997; Willen et al., 1990) common in oligotrophic systems. In addition, coccolithophorids contributed more to community composition in the Mali Ston Bay area than in all other areas of research.

3.4.1. Multi-dimensional scaling of the phytoplankton community structure

The similarity of the phytoplankton community structure in the investigated bays, regarding the abundance and distribution of species, is shown in a MDS diagram with vectors of the phytoplankton taxonomic groups (Fig. 5). The stress value of 0.01 confirms the statistical significance of the

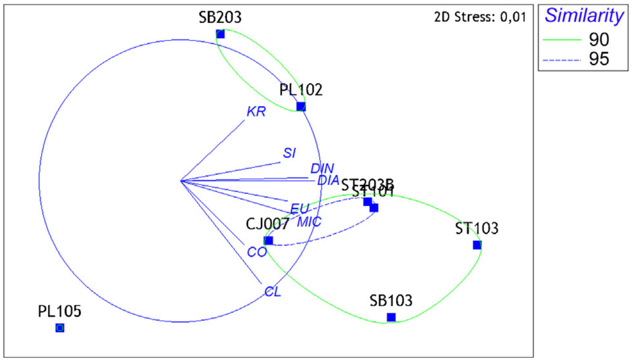

Figure 5 MDS diagram of the distance between the stations considering the groups of phytoplankton (DIA, diatoms; DIN, dinoflagellates; SI, silicoflagellates; CO, coccolithophorids; EU, euglenophytes; CL, chlorophytes; CR, cryptophytes; MIC, microflagellates).

MDS presentation. The similarity percentage of all stations, with regard to the identified groups, is over 80%.

This analysis connected stations in areas of high trophic levels, which are directly influenced by rivers' inflow and anthropogenic load (SB103, ST103), with more than 90% similarity (Fig. 6). There is a strong connection between stations ST101 and ST203B in the Kaštela Bay area with a similarity of over 95%, which are not directly influenced by river inflows, but are, according to the LUSI index, under a strong anthropogenic load. The analysis grouped stations SB203 and PL102, strongly influenced by river inflow and with high LUSI index, but because of their geomorphological features, the stations were not eutrophicated. Station PL105 was significantly different from all other investigated stations because it was not directly influenced by the Neretva River; it had the smallest pronounced anthropogenic eutrophication, and the smallest number of taxa was recorded. Diatoms, dinoflagellates and euglenophytes were mainly responsible for such grouping of the investigated stations.

3.4.2. SIMPER analysis of the phytoplankton community

The phytoplankton community was compared by SIMPER analysis at stations that have the highest value of LUSI index in the investigated areas (SB103, ST103, PL102). The analysis can point out those species that most affect the diversity at stations under a strong anthropogenic eutrophication. The phytoplankton community recorded at station ST103 in Kaštela Bay differs slightly more from the community recorded at PL102 (64.2%) rather than the community at station SB103 (61.63%). The species that contribute the most to the average differences between communities at these stations were proved to be *L. minimus* at station ST103, *S. marinoi* at SB103 and *Chaetoceros* spp. at PL102. Šibenik Bay and Kaštela Bay were characterized by a greater abundance of species *L. minimus* and *S. marinoi*, confirming the eutrophic characteristics of these areas and strong anthropogenic pressure.

3.5. Relationship between biomass and abiotic parameters

Non-parametric Spearman rank order correlations were used to determine the relationship between biomass and abiotic factors in the investigated areas. The analysis includes eight variables throughout the water column: temperature, salinity, nitrate, nitrite, ammonium salts, TIN, orthophosphates and orthosilicates. Results are shown in Table 2.

The negative correlation of phytoplankton biomass with salinity indicates the significant influence of freshwater inflow on the biomass in Šibenik Bay and at the stations located within Kaštela Bay. The stations located outside of Kaštela Bay and those in the Mali Ston Bay area do not exhibit such dependence. The phytoplankton biomass in the Mali Ston Bay area had a statistically significant and positive correlation with all nutrients except orthophosphates. This is associated with the lower trophic level of the area and the weaker anthropogenic influence so that any increase in nutrient concentrations has a positive effect on the biomass of phytoplankton. A similar correlation was observed in the Kaštela Bay area at stations located outside of the bay, where salinity along with nutrients and temperature, positively and significantly affected the growth of biomass. This indicates the lower impact of freshwater inflow to these stations and a greater dependence of biomass with nutrients. The negative correlation of biomass with orthophosphate is expected because as the development of phytoplankton spends orthophosphates, orthophosphate concentration decreases so the phytoplankton biomass increases.

3.6. Relationship between biomass and abundance: ABC-plots

The level of disturbance in the phytoplankton community, whether caused by natural process or anthropogenic load,

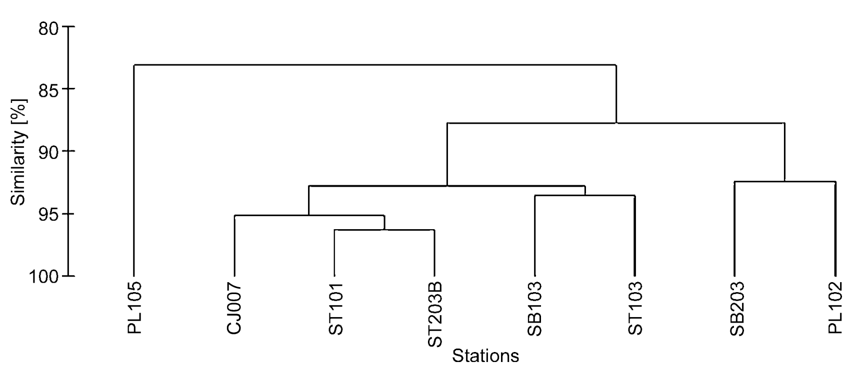

Figure 6 Dendrogram display of the relationship between the stations (similarity [%]) with respect to the phytoplankton groups.

Table 2 Spearman rank order correlations between phytoplankton biomass and abiotic factors (temperature, salinity, nitrates, nitrites, ammonium salts, TIN, orthophosphates and orthosilicates) in the investigated areas.

	Temperature	Salinity	NO_3^-	NO_2^-	NH_4^+	TIN	PO_4^{3-}	SiO_4^{4-}
The Šibenik Bay area SB103, SB203	0.06	−0.49[a]	0.17	0.16	0.16	0.19	−0.30[a]	0.44[a]
Kaštela Bay, inner stations ST101, ST103	0.09	−0.34[a]	−0.12	0.01	0.04	−0.02	−0.05	−0.08
Kaštela Bay, outer stations ST203B, CJ007	0.50[a]	0.42[a]	0.55[a]	0.59[a]	0.57[a]	0.61[a]	0.51[a]	0.53[a]
The Mali Ston Bay area PL102, PL105	−0.17	0.15	0.36[a]	0.53[a]	0.42[a]	0.58[a]	−0.33	0.55[a]

[a] Statistically significant.

was determined by the comparison method of phytoplankton abundance and biomass-abundance/biomass comparison plots (ABC-plots). The *W* index, visible on the graphs, is a practical measure of the trophic status of the particular area. Positive values of the *W* index, indicating the oligotrophic and unpolluted ecosystems, were recorded at the stations located outside Kaštela Bay (ST203B, CJ007) as well at station PL105 in Mali Ston Bay. Negative values of the *W* index, indicating the eutrophic or polluted ecosystems, were recorded at all other stations (SB103, SB203, ST101, ST103, PL102). In the graphic representation of these stations (Fig. 7), there is an evident difference between mesotrophic and eutrophic areas. At stations SB203 and ST101 abundance and biomass curves are closely matched and even intersected, indicating mesotrophic areas, while at stations SB103, ST103 and PL102, the abundance curve on the graph is located above the curve of the biomass, indicating eutrophic condition of these areas.

Kamenir and Dubinsky (2012) have also reported this comparison method of phytoplankton abundance and biomass. In unloaded ecological systems, the k-type species prevail because they are larger and have the capability of storing nutrients. They are slow growing, with abundance rarely dominant, but because of the cell size, those species form the bulk of the biomass. Altisan (2006) confirms a higher concentration of chlorophyll *a* in times when larger phytoplankton organisms prevailed in the community. With an increase of eutrophication, the r-type species are beginning to prevail in the phytoplankton community. Characterized by small size cells, which quickly respond to the increase in nutrient concentrations, they have a rapid growth so abundances become dominant and often create intense blooming. The results obtained in this study confirm the purpose of this method because the stations are under a strong natural or anthropogenic eutrophication (SB103, ST103, PL102), show a dominance of abundance compared to the biomass and a dominance of opportunistic species. The relationship between trophic levels, abundance of the community and cell size is well documented in many works (Cohen, 1991; Cohen et al., 2003; Kamenir and Dubinsky, 2012). In addition, the *W* index at station ST203B indicates an unpolluted condition although the station is under an anthropogenic influence according to the LUSI index (Table 1). That is a consequence of the distance from the coast, which is marked in previous studies (Flo et al., 2011; Ninčević Gladan et al., 2015).

3.7. Biodiversity in the investigated bays

3.7.1. Biodiversity indices

In this paper, we tried to define the characteristics of the community by using the ratio of number of taxa and the total abundance of phytoplankton in the investigated areas.

The maximum of total phytoplankton abundance was observed in Šibenik Bay, but the largest number of species in the total number of cells was noted in Kaštela Bay. The total phytoplankton abundance in the Šibenik and Kaštela Bay area was similar, while in the Mali Ston Bay area, it was considerably lower. We have calculated more types of diversity indices (Margalef's index (d), Menhinick's index (D), Pielou index (J'), Shannon's index (H') and Simpson index ($1 - \mathrm{Lambda}'$)) to better define biodiversity in the investigated areas (Table 3).

Spearman's correlation between the abundance of phytoplankton and various diversity indexes shows that Margalef's ($d = 0.574$*), Pielou ($J' = -0.385$*) and Menhinick's ($D = -0.637$*) diversity indexes had a statistically significant correlation with abundance ($p < 0.05$), while Shannon's ($H'(\log) = 0.010$) and Simpson's ($1 - \mathrm{Lambda}' = 0.019$) indexes did not. Although Shannon's diversity index is a commonly used index in biodiversity assessments, recent studies show that it is not linearly related to the trophic gradient (Spatharis and Tsirtsis, 2010). The most significant index is proved to be Menhinick's diversity index (D) due to its strong and significant correlation with the abundance of phytoplankton. The obtained negative correlation is expected because in the areas of higher trophic levels an increase in the number of phytoplankton cells is anticipated. A reduction in the diversity of the community is also expected due to opportunistic species that reproduce quickly and occupy the ecological niche, thus reducing the diversity of the phytoplankton community. The effectiveness of Menhinick's diversity index due to its consistent and linear change through the trophic gradient has been documented (Spatharis and Tsirtsis, 2010). Fig. 8 is presenting a seasonal distribution of Menhinick's index in the surface layer for all investigated sites. In the Šibenik and the Kaštela Bay areas, Menhinick's index was generally higher at stations located outside of the bays, in the areas of lower trophic levels, compared to stations located within the bays, areas of higher trophic levels. The Mali Ston Bay area, characterized as a moderate eutrophicated system, has a uniform distribution at both stations. These findings confirmed Menhinick's index

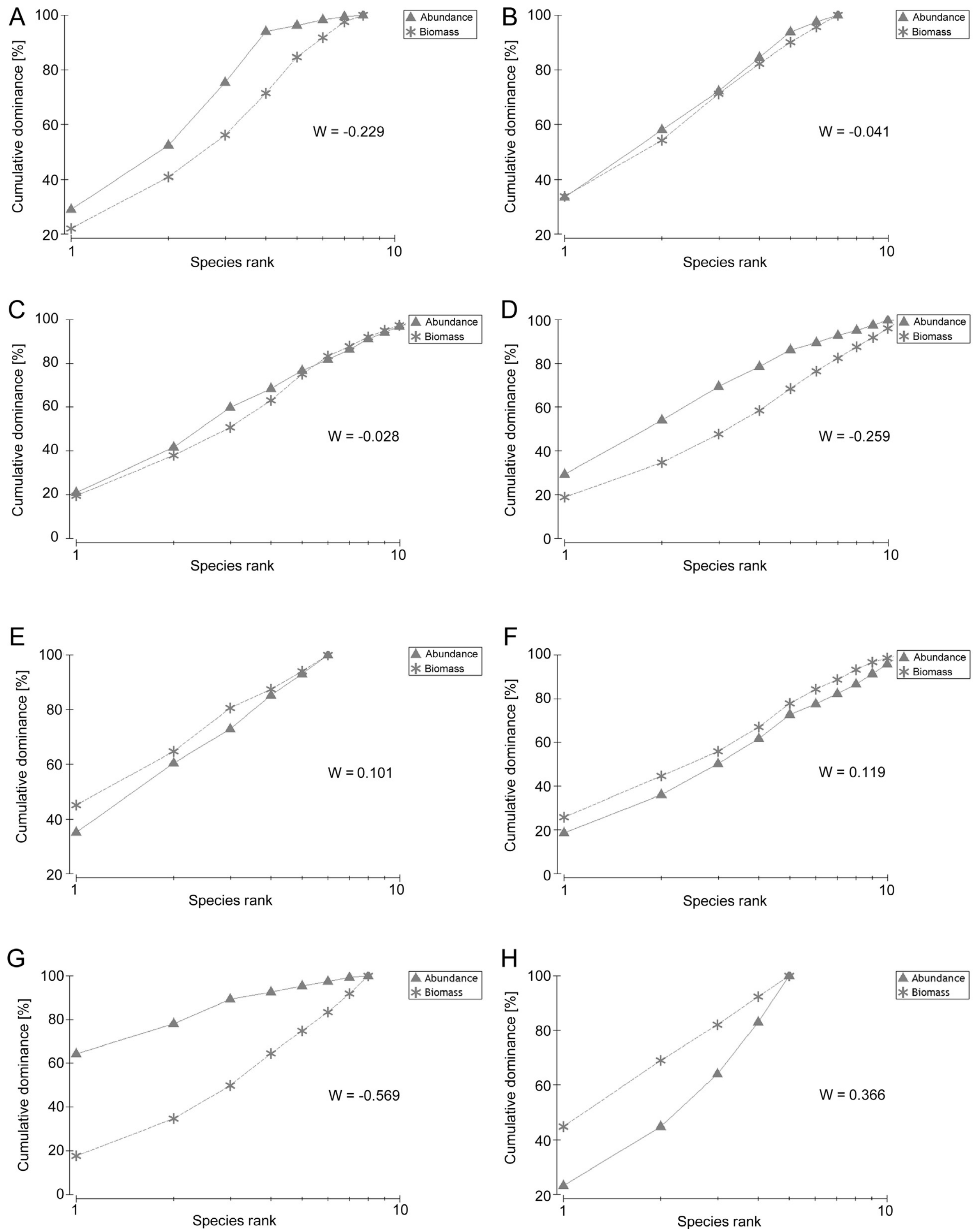

Figure 7 Abundance/biomass comparison plots of phytoplankton in the surface layer at the station SB103 (A), the station SB203 (B), the station ST101 (C), the station ST103 (D), the station ST203B (E), the station CJ007 (F), the station PL102 (G) and the station PL105 (H).

as a good indicator of biodiversity, which is highest in moderately eutrophic conditions because it is directly linked to the abundance of species that prove to be most numerous in moderately eutrophic areas. When assessing biodiversity, one must take into consideration that the phytoplankton community composition is highly sensitive to variation in their environment, which is reflected in the biodiversity assessment. The phytoplankton community consists of a very large

Table 3 Diversity indexes in the investigated areas (including number of species (*S*), the number of cells (*N*), Margalef's index (*d*), Menhinick's index (*D*), Pielou index (*J*′), Shannon's index (*H*′(log)) and Simpson index (1 − Lambda′)); their mean value (mean), the maximum (max) and minimum (min) values.

Diversity indexes	The Šibenik Bay area			The Kaštela Bay area			The Mali Ston Bay area		
	mean	max	min	mean	max	min	mean	max	min
S	15	23	7	15	37	6	12	21	5
N	599,340	5,407,290	35,130	478,606	5,064,990	21,970	238,024	2,891,840	41,030
d	1.090	1.773	0.538	1.129	2.448	0.434	0.878	1.521	0.355
D	0.031	0.052	0.009	0.029	0.058	0.010	0.030	0.057	0.011
J′	0.666	0.894	0.107	0.620	0.887	0.147	0.597	0.801	0.091
H′(log)	1.739	2.382	0.331	1.655	2.528	0.306	1.426	2.150	0.263
1 − Lambda′	0.705	0.889	0.113	0.673	0.893	0.106	0.609	0.821	0.082

number of species, and many taxa simply cannot be identified to the species level by light microscopy of preserved samples (Ojaveer et al., 2010). Some of the taxa were identified at a higher taxonomic level than species, which may underestimate the biodiversity in cases where the genus or higher unit actually includes several species. Previous studies showed that the best option is to use all of the available data and accept the fact that the taxonomic units vary, since discarding parts of the data or aggregating those means losing information related to biodiversity (Uusitalo et al., 2013).

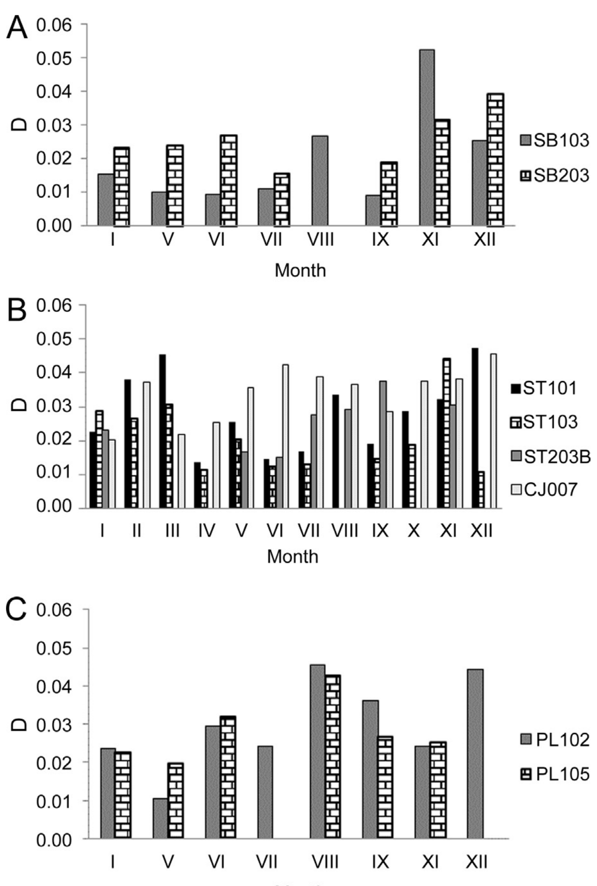

Figure 8 Seasonal distribution of Menhinick's diversity index (*D*) at the investigated stations in the surface layer of the Šibenik Bay area (A), the Kaštela Bay area (B) and the Mali Ston Bay area (C).

3.7.2. The cumulative dominance plots

In this paper, biodiversity is also presented through the curves of cumulative dominance of species (k-dominance plots) that represents the relationship between the number of organisms and the number of taxa found in the sample (Warwick and Clarke, 1991). The points on the graph indicate the percentage of the number of certain species in the phytoplankton community, and a curve that is placed highest on the graph has the lowest diversity.

The cumulative dominance curves in the surface layer for all investigated stations are presented in Fig. 9. There is a visible grouping of the stations placed in the area of higher trophic levels (SB103, ST101, ST103, PL102). These curves are placed higher on the chart, and they have a lower diversity from the stations (SB203, ST203B, CJ007, PL105) located further from the coast and away from sources of eutrophication, where the more diverse community was recorded.

Seasonal distribution of the k-dominance curves is a reflection of the seasonal distribution of the phytoplankton community, and the natural spring blooms are common and well documented in the subtropical seas (Carstensen et al., 2004; Spatharis et al., 2007; Zingone et al., 1995). Greater diversity in the winter period could be related to a moderate trophic condition caused by mixing of the water column and increasing concentrations of nutrients in the surface layer, which in addition to sufficient light during mild winters favour the grow of phytoplankton.

Previous studies show that a continuous inflow of nutrients results in communities dominated by species that are more competitive for limiting nutrients, while weaker competitor species are rare or non-existent in the community (Capblancq, 1990; Hardin, 1960; Sommer, 1985). The case of non-continuous inflow of nutrients increases the coexistence between species, leading to more species in the community (Harris, 1986; Margalef, 1978). However, by further eutrophication, the number of species drastically reduces, and the diversity rapidly decreases (Crossetti et al., 2008; Polishchuk, 1999), which is consistent with the hypothesis that the maximum number of species occurs in areas with moderate trophic levels (Connell, 1978). Spatharis et al. (2007) in the Aegean Sea and Aktan (2011) in the eastern Mediterranean obtained similar results. Crossetti et al. (2008) have also reported that biodiversity loss following trophic change was not a single dimension of a single factor, but rather a

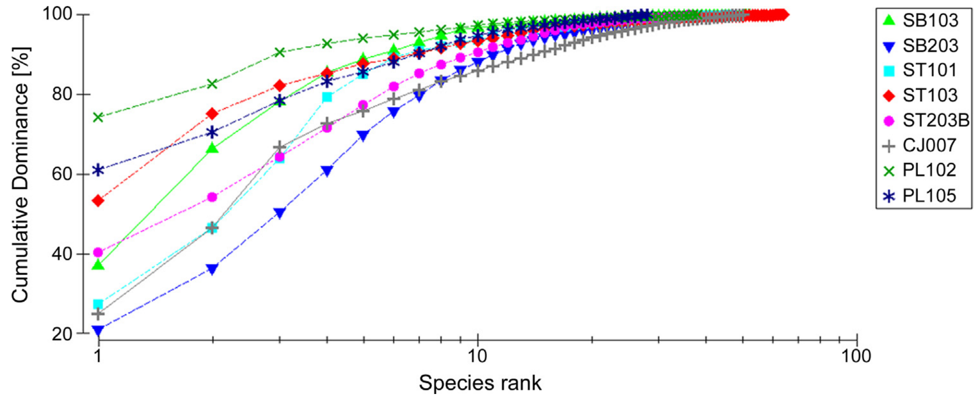

Figure 9 The k-dominance curves in the surface layer for all investigated stations.

template of factors co-varying in consequence of the larger levels of the biomass. Therefore, it is important to monitor the bays' area with a wide range of physical, chemical and biological parameters to make better ecological characterization of the study area.

4. Conclusion

This study confirms that biodiversity of the phytoplankton community is dependent on its spatial and temporal distribution in relation to environmental conditions, as well as its composition.

Diatoms were the most represented group of the phytoplankton community in all three bays. Species that were highlighted as significant for the specific area in this study were *S. marinoi* in Šibenik Bay, *L. minimus* in Kaštela Bay and the genus *Chaetoceros* spp. in Mali Ston Bay. Dinoflagellates were the second most significant group. A noticeably larger abundance of dinoflagellates was recorded in the Kaštela Bay area, characterized as the most influenced by anthropogenic pressure.

A MDS similarity analysis, based on the abundance of phytoplankton taxonomy groups, shows the close connection between stations strongly influenced by anthropogenic pressure. In addition, the k-dominance curves show the lowest biodiversity at these stations, and ABC curves with the negative *W* index at these stations indicate the conditions of stronger eutrophication.

Further monitoring of the bay areas is needed and an effort should be taken in order to keep the eutrophication at a moderate level, optimal for the phytoplankton community to maintain its great biodiversity, which is a good indicator of a balanced ecosystem.

Acknowledgements

The authors wish to thank H. Prelesnik bacc.ing. for analysing chlorophyll *a*, I. Pezo bacc.ing. for assistance with the fieldwork and all the people at the Laboratory of Plankton and Shellfish toxicity for their help and support.

This work has been fully supported/supported in part by Croatian Science Foundation under the project IP-2014-09-3606 (Marine plankton as a tool for assessment of climate and anthropogenic influence on the marine ecosystem).

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