Research Article

Relationship between marine epilithic diatoms and environmental variables in oligotrophic bay, NE Mediterranean

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Handling Editor: Antonia Giannakourou

Received: 4 July 2017; Accepted: 1 December 2017; Published on line: 8 June 2018

Abstract

The taxonomic composition and structure of a marine epilithic diatom community were sampled from the bottom of the two sites at monthly intervals from January to December 2011 in the small semi-enclosed oligotrophic Neum Bay in Bosnia and Herzegovina (Middle Adriatic). Altogether, 264 diatom taxa (species and infraspecific taxa) within 69 genera were identified. Among them, 149 and 203 taxa occurred in samples from the shallow (0.5 m depth) and deep (8 m depth) sites, respectively. The monthly distribution of most of the diatoms was irregular and high numbers of sporadic taxa were found. SIMPER analysis indicated that the difference between shallow and deep sites could be largely attributed to the frequently recorded diatom taxa and those with high percentage abundances. They were *Halamphora coffeiformis*, *Caloneis excentrica*, *Cocconeis scutellum* var. *scutellum*, *Licmophora flabellata*, *Licmophora gracilis*, *Licmophora* sp., *Navicula abunda*, *Rhabdonema adriaticum*, and *Striatella unipunctata*. Canonical Correspondence Analysis (CCA) showed that temperature, oxygen saturation ($O_2/O'_2$), silicate concentration ($SiO_4$), and salinity were the most important factors influencing diatom community structure in the bay.

Keywords: Bacillariophyta; Bosnia and Herzegovina; diversity; eastern Adriatic; environmental factors; shallow-water habitat.

Introduction

Diatoms are the most important photosynthetic unicellular eukaryotes and aquatic primary producers on Earth, accounting for approximately 40% (Falkowski et al., 2004). The benthic diatoms are of particular value in the understanding of present ecosystems in shallow coastal areas and very important tools for the monitoring and interpretation of environmental conditions of both the past and the present (Stevenson & Pan, 1999). They have become increasingly studied in European countries and many other parts of the world (Delgado et al., 2010; Lavoie et al., 2014; Tan et al., 2015).

Generally, there is only limited literature available dealing with the composition of epilithic assemblages grown on either natural or artificial substrates in marine coastal waters (Brandini et al., 2001; Hillebrand & Sommer, 2000; MacLulich, 1987). This may be explained chiefly by the existence of methodological difficulties in the processing of samples and of a lack of taxonomic knowledge regarding marine populations (Agatz et al., 1999). In the Mediterranean, several studies, in both heavily urbanized and undisturbed areas, have dealt with the composition of diatom populations grown on artificial (Munda, 2005; Totti et al., 2007) or natural substrates (Facca et al., 2002; Facca & Sfriso, 2007; Çolak Sabanci & Koray, 2010; Çolak Sabanci, 2011, 2012; Cibic & Facca, 2010 and references therein). Many more studies have been reported on diatom epibenthic communities on macroalgae and seagrasses (epiphyton) (Car et al., 2012; De Stefano et al., 2000) or animals (epizoon) (Romagnoli et al., 2007, 2014 and references therein). Most recently, Álvarez-Blanco & Blanco (2014) contributed to knowledge on benthic diatom taxa in Mediterranean, including some Adriatic sites. On the other hand, there are only a few studies on benthic diatoms in the eastern Adriatic, mostly focused on the ecology and taxonomy of brackish periphytic diatoms in the Croatian estuaries (Buric et al., 2004; Caput Mihalić et al., 2008; Levkov et al., 2010; Munda, 2005) or Albanian coastal wetlands (Miho & Witkowski, 2005). In addition,
Mejdandžić et al. (2015) and Nenadović et al. (2015) reported on the development of periphytic diatoms on different artificial substrates, based mostly at the generic level.

There is no consensus on the main factors that determine diatom species composition and distribution with regard to environmental conditions, particularly in the shallow marine or lagoon systems. Therefore, the results from different studies may prove contradictory (Çolak Sabanci, 2011; Jasprica et al., 2012; Sullivan & Currin, 2000, etc.). This paper considers the taxonomic composition and seasonality of marine epilithic diatoms in relation to some key environmental variables such as temperature, salinity, nitrate (NO$_3$), nitrite (NO$_2$), ammonium (NH$_4$), phosphate (PO$_4$), silicate (SiO$_4$), total inorganic nitrogen (TIN), oxygen saturation (O$_2'/O_2$), and chlorophyll $a$ concentrations in a shallow marine bay in Bosnia and Herzegovina, an area not yet investigated. The present study addresses this deficiency and contributes to a better knowledge of benthic algal communities and their ecology in the Mediterranean in general and the Adriatic in particular.

**Materials and Methods**

**The study area**

Bosnia and Herzegovina (NW Balkan Peninsula) has only 21.2 km of coastline and represents only 0.26% of the total Adriatic coastline length (Pikelj & Juračić, 2013). The maritime area consists of the Neum Bay with total sea surface area of ca. 8 km$^2$. The coast is low and rocky, built up of karstified carbonates. The average and maximum sea depth in the bay are 17 and 27 m, respectively. The bottom is mostly covered by rocky sediments. The bay is located between the mainland and the 7 km long Klek Peninsula, and it is treated as a ‘closed sea’ (mare clausum). This semi-enclosed bay is part of the larger Mali Ston Bay, which is enclosed on the seaward side by the 62 km long Pelješac peninsula, Croatia. Mali Ston Bay expands to the northwest and connects with the Neretva River channel which is linked with the open sea. Due to its ecological and economical importance, this area with a centuries long mariculture tradition was proclaimed as the Special Marine Reserve of Mali Ston Bay and Malo More, including the Neum Bay, in 1983. The town of Neum (estimated population 3,236 in 2013) is the only coastal settlement in Bosnia and Herzegovina and an important national tourism destination. The region experiences a typical Mediterranean climate: summers are warm and dry, winters are mild and rainy (Jasprica et al., 2005, 2015). Detailed data on the hydrology, climate, geology, and vegetation of the area have been presented by Jasprica et al. (2012, 2015) and Čalić et al. (2013).

**Diatom sampling design and analyses**

Samples for epilithic diatom analysis were collected in the southern part of Neum Bay from the substrate of the two sites (Sites 1 and 2; Fig. 1). Site 1 was situated at 1 m from the coastline with the maximum depth of 0.5 m and was marked as ‘shallow site’. Site 2 was situated at 25 m from the coastline with the maximum depth of 8 m and was marked as ‘deep site’. Samples were collected at monthly intervals from January to December 2011. In January, February, and March 2011 only samples from the shallow site were taken, and a total of 21 samples were analyzed. However, due to site conditions, technical problems and complexities (scuba-diving gear), samples from the deep site could not be collected during period January to March 2011. For defining the species composition of epilithic diatoms, stones of 15-20 cm in diameter were collected. Stones from deeper site were collected by scuba diving. Stones were collected randomly as possible amongst those that are not smothered with filamentous algae and in which the diatom film was evident. Stones were taken into a plastic bag of 1-L in which 200 mL.
of filtered seawater was added. The upper parts of the stones were rubbed with a toothbrush and the mixture was decanted into the 250 mL polyethylene bottles (Winter & Duthie, 2000). The samples were then preserved with 4% formaldehyde.

Collected material was cleaned of organic material for light microscopy (LM) and scanning electron microscopy (SEM) observations utilizing sulphuric acid, potassium permanganate, and oxalic acid (cf. Hasle & Fryxell, 1970). They were then rinsed with distilled water, pipetted onto ethanol-cleaned cover-slips and left to air dry before mounting in Canada balsam. Samples entirely cleaned from organic material were made ready as permanent preparations. Whenever possible, diatoms were identified and counted to species level at x1000 magnification by phase-contrast optics with Microstar binocular microscope (AO Scientific Instruments) and a x100 Planapo oil immersion objective. Some taxa reported here could not be clearly assigned to species level (assigned as “sp.”) and they will be subject of further taxonomic investigations.

The abundances of the species were expressed as percentages of the total number of frustules counted (relative abundances, in %). In total, 400 valves per sample were counted. Slides have been deposited in the diatom collection of the Institute for Marine and Coastal Research, University of Dubrovnik, Dubrovnik, Croatia [no. DHB-1-21]. Identiﬁcations were made following Peragallo & Peragallo (1897–1908), Hendrey (1964), Ricard (1974, 1975, 1977), Poulin et al. (1984, 1990), Bérard-Therriault et al. (1986, 1987), Snoeijis (1993), Snoeijis & Potapova (1995), Snoeijis & Kasperoviciené (1996), Snoeijis & Balashlova (1998), Hartley (1986), Hartley et al. (1996), and Witkowski et al. (2000). Nomenclature follows AlgaeBase (Guiry & Guiry, 2017) and Álvarez-Blanco & Blanco (2014).

Physical-chemical parameters

In order to determine the relationships between diatom communities and environmental variables, water samples were taken near the substrate for chemical analysis, i.e., at the same place as where diatom sampling was carried out. Water samples were collected in 5-L Niskin bottles, and sub-samples were taken for the nutrients (Strickland & Parsons, 1972; Ivančić & Degobbis, 1984) and chlorophyll-a concentrations (Chi a; Holm-Hansen et al., 1965). Measurements were performed in single time (no replicates were made). Samples were collected from February to December 2011 for most physical-chemical parameters except in November. Due to technical problems and sample handling on the boat, temperature, salinity, and oxygen saturation were not measured in October on both sites, and Chl a was not measured at the deeper site in December.

Chemical variables (nutrients) included total inorganic nitrogen (TIN) [TIN = nitrate (NO3⁻) + nitrite (NO2⁻) + ammonium (NH4⁺)], phosphate (PO4³⁻) and silicate (SiO₄²⁻). Temperature was measured with an inverted thermometer. Salinity was determined by argentometric titration (Grasshoff et al., 1983). Dissolved oxygen was determined by the Winkler method and oxygen saturation (O2/ O2') was calculated from solubility of oxygen in seawater as a function of temperature and salinity (Weiss, 1970; UNESCO, 1973).

Chl a was determined from 500 mL sub-samples filtered through Whatman GF/F glass-ﬁbre filters and stored at -20°C for a period less than a month. Filtered samples were homogenized and extracted in 90% acetone for 24 hours at room temperature (Holm-Hansen et al., 1965). Chl a was determined fluorometrically using a Turner TD-700 Laboratory Fluorometer (Sunnyvale, CA) calibrated with pure Chl a (Sigma).

Trophic status was characterized by the TRIX index, commonly used to classify coastal marine areas in the Mediterranean (Vollenweider et al., 1996; Karydis, 2009).

Statistical analysis

Non-metric multidimensional scaling ordination (NMDS) was applied to deﬁne the benthic diatom abundance with respect to sampling dates and depth distribution. Ordination was based on the Bray-Curtis similarity matrix (Legendre & Legendre, 1983; Clarke & Warwick, 2001). For this purpose, a matrix of 264 taxa over 21 samples was constructed. Cell abundance data were square-root transformed. ANOSIM randomization (Clarke & Warwick, 1994) was used to test for significant differences in benthic community structure between shallow and deeper site across seasons.

The dissimilarity percentage program (SIMPER, Clarke & Warwick, 1994) was used to identify the taxa making the greatest contribution to differences between clusters observed in the NMDS plot.

As diversity indices provide more information than simply the number of taxa present (i.e., they account for some taxa being rare and others being common) and serve as valuable tools to quantify diversity in a community, in order to examine the taxa richness of epilithic diatom assemblages at different depths, Shannon-Wiener Biodiversity Index values were computed (Krebs, 1999):

$$H = -\sum_{i=1}^{s} \rho_i \log_2 \rho_i$$

where $H$ is the Shannon-Wiener diversity, $s$ is the total number of species and $\rho_i$ is the proportional abundance of $i$-th species. This index is commonly used in ecology of benthic diatom communities (cf. Kwandrans, 2007).

These statistical analyses were performed using PRIMER v5 software (Clarke & Gorley, 2001; Wilkinson, 1986).

As diversity index is not completely effective in de-
scribing community structure species evenness of epilithic diatom assemblages at different depths was computed. According to Beisel et al. (2003) any evenness index should not be used alone so Pielou’s evenness values and Smith and Wilson’s evenness values were computed (Pielou, 1966; Smith & Wilson, 1996). While Pielou’s evenness has a weak sensitivity to variations on rare taxa, Smith and Wilson’s evenness has the weak sensitivity to variations on dominant taxa.

Canonical Correspondence Analysis (CCA) was used to relate the abundance of diatom taxa to environmental variables. CCA extracts synthetic gradients from the biotic and environmental matrices, which are quantitative-ly represented by arrows in graphical biplots (ter Braak & Verdonschot, 1995). CCA ordination biplot was used to show relation of taxa and environmental variables. A dataset included eight samples from shallow site and eight samples from deeper site from April to December 2011. Missing data from November were excluded in the analysis. Neither transformation (e.g., square root or log) of species data nor down-weighting of rare species was performed. The data were centered and standardized before analyses as they were measured on different scales. A Monte Carlo permutation test (reduced model - 499 permutations) was used to test the significance of each variable. Eigenvalues calculated measure the importance of each of the ordination axes (0-1). Species-environment correlation measures the strength of the relationship between taxa and the environment for particular axes. The analysis was carried out using CANOCO for Windows 4.52 software (ter Braak & Šmilauer, 2002).

Results

Physical-chemical parameters

Variations in the physical and chemical parameters in shallow and deeper site are presented in Fig. 2. There were statistically significant differences (Student’s t-test, P<0.05) in all environmental parameters, except Chl a, between samples from shallow and deeper site. Temperature and concentrations of TIN, PO₄ and SiO₂ showed higher values on shallower site. On the contrary, salinity and at least some Chl a had higher values at the deep site.

Water temperature (Fig. 2) ranged from 10.2°C (March) to 27.1°C (September) in shallow depth and from 11.1°C (March) to 24.5°C (September) at the deep site. On both sites it rose steadily from March to September, and began to decrease in October. Salinity (Fig. 2) varied from 36.7 (April) to 38.4 (December) in samples from the shallow site. It was higher in samples from the deep site, varying from 36.7 (April) to 38.4 (December). Warm summer months were characterized by lower salinity, while in the cooler months of winter and spring salinity was mostly dependent on the precipitation regime and the extremely dynamic water flow from the karstic springs that feed the bay. Oxygen saturation (O₂/O₃) of both depths indicated good aeration (range 0.95–1.36, average 1.12).

The nutrient concentrations in samples from shallow site – especially TIN and SiO₂ – oscillated much more than those in samples from deeper site. TIN ranged from 0.98 (February) to 2.79 (April) µM and from 0.35 (February) to 1.72 (October) µM, respectively, in samples from shallow and deeper site. In this study, TIN generally followed the distribution of NO₃ with exception in August on shallow site and in October on deeper site when NH₄ significantly contributed (1.42 and 0.63 µM, respectively). Phosphate (PO₄) ranged from 0.03 (March) to 0.22 (July) µM in shallow samples and from 0.03 (February, April) to 0.13 (December) µM in deeper samples. Silicate (SiO₂) ranged from 1.49 (July) to 9.59 (August) µM in shallow samples and from 0.51 (February) to 5.02 (December) µM in samples from deeper site. Chl a ranged from 0.1 (May) to 0.34 µg L⁻¹ (September) on the shallow site and from 0.11 (June and July) to 0.54µg L⁻¹ (October) on the deep site (Fig. 2).

Secchi disc transparency extended to the bottom of the Site 2 (8 m depth) throughout all the year. According to the TRIX index (annual average 3.23±0.48 TRIX units), Neum Bay can be characterized as oligotrophic.

Species richness and the Shannon-Weiner diatom diversity index

Altogether, 264 taxa (species and infraspecific taxa) within 69 genera were identified in Neum Bay in 21 samples originating from the bottom of the shallow (0.5 m) and deep (8 m) site. Genera with the greatest number of taxa were: Mastogloia (35 taxa), Diploneis (26), Nitzschia (19), Amphora (19), Navicula (16), and Cocconeis (16) (see Appendix 1). Over the entire study, 149 and 203 taxa were found on the shallow and deep site, respectively. Among them, 42 taxa were found exclusively on the shallow and 96 occurred only on the deep site (Appendix 1). However, during the study, 119 taxa were common to both sampling depths.

In total, 100 taxa were found only once (sporadic) in all samples (Appendix 1). Among them, 47 and 53 taxa have been found on the shallow and deep sites, respectively. All of these taxa had relative abundances lower than 1%.

The number of taxa per sample in the shallow samples ranged from 16 (April) to 62 (August), with an average of 39.2. At the deep site, the range of taxa per sample varied from 27 (September) to 78 (April), the average being 65.2. The species diversity index varied from 1.40 to 3.62 in samples from shallow site, and 3.06 to 3.93 in samples from deeper site (Fig. 3). Generally, these vary either with depth or with season. An increase in species diversity index in the warm summer months was noted in the shallow samples. On the deep site the lowest index was found in September, while the highest was in October.

Pielou’s species evenness ranged from 0.47 to 0.88 (the average 0.71) on shallow site and from 0.86 to 0.93 (the average 0.89) on deeper site (Fig. 3). Smith and Wil-
son species evenness ranged from 0.14 to 0.60 (the average 0.36) in the samples from shallow site and from 0.06 to 0.62 (the average 0.49) in the samples from deeper site.

**Relative abundances and distribution of diatom assemblages**

According to NMDS, epilithic diatom assemblages were significantly different (ANOSIM, P<0.05) between the shallow and deeper site (Fig. 4, Table 1). SIMPER analysis showed that *Striatella unipunctata*, *Cocconeis scutellum* var. *scutellum*, *Licmophora gracilis*, *Halimphora coffeiformis*, *Rhabdonema adriaticum*, *Tryblionella compressa*, *Dimeregramma minus*, *Grammatophora oceanica*, *Caloneis liber* var. *linearis*, *Actinocyclus subtilis*, *Achnanthes brevipes*, *Rhopalodia pacifica*, *Licmophora* sp., *Mastogloia binotata*, and *Trachyneis aspera* contributed the most (cumulatively 25%) to the variance between assemblages from the shallow and deeper site.

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*Fig. 2:* Monthly distribution of the physical and chemical parameters in the shallow (0.5 m) and deep (8 m) site in Neum Bay in 2011. A) temperature; B) salinity; C) oxygen saturation (O$_2$/O$_2^\prime$); D) total inorganic nitrogen (TIN); E) phosphate (PO$_4$); F) silicate (SiO$_4$); G) chlorophyll-a concentrations.
Fig. 3: Number of diatom taxa (A), the Shannon-Wiener diatom diversity index (B), Pielou’s evenness index (C), and Smith and Wilson’s evenness index (D) in the shallow (0.5 m) and deep site (8 m) in Neum Bay in 2011.

Fig. 4: NMDS ordination based on the epilithic diatom relative abundance (%) data in the shallow (▲ - 0.5 m) and deep (▼ - 8 m) sites in Neum Bay in 2011.
Table 1. List of diatom taxa and their percentage contributions to total diatom community composition (taxa with relative abundances ≥ 4%, RA, only are shown) for the shallow (0.5 m) and deep (8 m) site in Neum Bay in 2011.

| Season / Site (Depth): | Month | Shallow Site (0.5 m) | Deep Site (8 m) |
|------------------------|-------|----------------------|-----------------|
|                        |       | RA (%)               | RA (%)          |
| Winter                 | January | Striatella unipunctata (Lyngbye) C. Agardh | 25.50 |
|                        |        | Cocconeis scutellum Ehrenberg var. scutellum | 18.25 |
|                        |        | Achnanthes brevipes C. Agardh | 11.50 |
|                        |        | Rhabdonema adriaticum Kützing | 9.50 |
|                        |        | Achnanthes longipes C. Agardh | 9.00 |
|                        |        | Licmophora flabellata (Grev.) C. Agardh | 4.50 |
|                        | February | Striatella unipunctata (Lyngbye) C. Agardh | 30.50 |
|                        |        | Cocconeis scutellum Ehrenberg var. scutellum | 17.50 |
|                        |        | Licmophora flabellata (Grev.) C. Agardh | 13.50 |
|                        |        | Rhabdonema adriaticum Kützing | 7.50 |
|                        |        | Licmophora pfannkuckae Giffen | 5.00 |
|                        | March | Cocconeis scutellum Ehrenberg var. scutellum | 24.50 |
|                        |        | Halamphora coffeiformis (C.Agardh) Levkov | 16.25 |
|                        |        | Rhabdonema adriaticum Kützing | 6.50 |
|                        |        | Striatella unipunctata (Lyngbye) C. Agardh | 5.50 |
|                        |        | Achnanthes longipes C. Agardh | 4.75 |
|                        | April | Licmophora gracilis (Ehrenberg) Grunow | 55.00 |
|                        |        | Cocconeis scutellum Ehrenberg var. scutellum | 10.00 |
|                        |        | Striatella unipunctata (Lyngbye) C. Agardh | 16.25 |
|                        |        | Paralia sulcata (Ehrenberg) Cleve | 8.75 |
|                        |        | Cocconeis scutellum Ehrenberg var. scutellum | 15.25 |
|                        |        | Halamphora coffeiformis (C. Agardh) Levkov | 7.00 |
|                        |        | Navicula flagelliforma Hustedt | 4.50 |
|                        | May | Licmophora sp. | 59.50 |
|                        |        | Cocconeis scutellum Ehrenberg var. scutellum | 21.75 |
|                        |        | Grammatophora oceanica Ehrenberg | 7.00 |
|                        |        | Coloneis liber var. linearis Cleve | 5.25 |
|                        |        | Petrodictyon gemma (Ehrenberg) D. G. Mann | 4.50 |
|                        | June | Licmophora gracilis (Ehrenberg) Grunow | 32.50 |
|                        |        | Halamphora coffeiformis (C. Agardh) Levkov | 13.25 |
|                        |        | Cocconeis scutellum Ehrenberg var. scutellum | 19.25 |
|                        |        | Nitzschia coarctata Grunow | 7.00 |
|                        |        | Striatella unipunctata (Lyngbye) C. Agardh | 14.00 |
|                        |        | Coloneis liber var. linearisCleve | 6.75 |
|                        |        | Licmophora paradoxa (Lyngbye) C. Agardh | 8.75 |
|                        |        | Grammatophora oceanica Ehrenberg | 6.00 |

(continued)
### Table 1

| Season / Site (Depth): | Shallow Site (0.5 m) | Deep Site (8 m) |
|------------------------|----------------------|-----------------|
| **RA (%)**             | **RA (%)**           |
| **July**               |                      |                 |
| Cocconeis scutellum    | 26.00                | Rhopalodia musculus (Kützing) Otto Müller |
| Ehrenberg var. scutellum | 10.00                |                 |
| Navicula abunda Hustedt | 10.00                | Coloneis liber var. linearis Cleve |
| Halamphora coffeiformis (C. Agardh) Levkov | 7.50   | Cocconeis scutellum Ehrenberg var. scutellum |
| Rhabdonema adriaticum Kützing | 7.25   | Halamphora coffeiformis (C. Agardh) Levkov |
| Achnanthes pseudogroenlandica Hendey | 7.00   |                 |
| **August**             |                      |                 |
| Mastogloia similis Hustedt | 11.00               | Halamphora coffeiformis (C. Agardh) Levkov |
| Plagiotorpis tayrecta T. B. B. Paddock | 9.75   | Coloneis liber var. linearis Cleve |
| Trachyneis aspera (Ehrenberg) Cleve | 8.25   | Rhopalodia pacifica Krammer |
| Actinocyclus subtilis (W. Gregory) Raifs | 5.00   |                 |
| Striatella unipunctata (Lyngbye) C. Agardh | 4.75   |                 |
| Caloneis liber var. linearis Cleve | 4.75   |                 |
| Mastogloia ignorata Hustedt | 4.75   |                 |
| Licmophora gracilis (Ehrenberg) Grunow | 4.50   |                 |
| **September**          |                      |                 |
| Actinocyclus subtilis (W. Gregory) Raifs | 13.00  | Caloneis excentrica (Grunow) Boyer |
| Rhopalodia pacifica Krammer | 12.00  | Nitzschia pararosstrata (Lange-Bertalot) Lange-Bertalot |
| Licmophora sp. | 6.50 | Trachyneis aspera (Ehrenberg) Cleve |
| Trachyneis aspera (Ehrenberg) Cleve | 6.25   | Grammatophora oceanica Ehrenberg |
| Halamphora coffeiformis (C. Agardh) Levkov | 4.25   | Lyrella lyra (Ehrenberg) Karajeva |
| **October**            |                      |                 |
| Actinocyclus subtilis (W. Gregory) Raifs | 23.75  | Halamphora coffeiformis (C. Agardh) Levkov |
| Licmophora fannkuckae Giffen | 14.25  | Rhopalodia pacifica Krammer |
| Striatella unipunctata (Lyngbye) C. Agardh | 12.75  | Dimeregramma minus (Gregory) Raifs |
| Rhabdonema adriaticum Kützing | 10.00  | Caloneis liber (W. Smith) Cleve |
| Rhopalodia pacifica Krammer | 7.00  |                 |
| **November**           |                      |                 |
| Striatella unipunctata (Lyngbye) C. Agardh | 25.00  | Tryblionella compressa(J.W.Bailey) Poulin |
| Halamphora coffeiformis (C. Agardh) Levkov | 7.50   | Mastogloia binotata (Grunow) Cleve |
| Cocconeis scutellum Ehrenberg var. scutellum | 5.25   | Mastogloia splendida (Gregory) H. Pergallo |
| Nitzschia ventricosa Kitton | 5.00   | Actinopycthus splendens (Shadbolt) Raifs |
| Psammodictyon panduriforme (W. Gregory) D.G.Mann | 6.75   | Trachyneis aspera (Ehrenberg) Cleve |
| **December**           |                      |                 |
| Striatella unipunctata (Lyngbye) C. Agardh | 42.50  | Halamphora coffeiformis (C. Agardh) Levkov |
| Cocconeis scutellum Ehrenberg var. scutellum | 22.50  | Dimeregramma minus (Gregory) Raifs |
| Achnanthes brevipes C. Agardh | 9.00   | Rhabdonema adriaticum Kützing |
| Licmophora fabellata (Grev.) C. Agardh | 7.50   | Striatella unipunctata (Lyngbye) C. Agardh |
| Ardissonea crystallina (C. Agardh) Grunow | 4.50   | Achnanthes brevipes var. intermedia (Kützing) Cleve |

**Notes:**
- RA: Relative Abundance
- The species names are followed by their respective authors or references.
Altogether, only five taxa were presented in all samples: Cocconeis scutellum var. scutellum, Grammatophora oceanica, Halamphora coffeiformis, Licmophora remuluus, and Trachyneis aspera.

The taxa with the highest relative abundances were: Halamphora coffeiformis, Caloneis excentrica, Cocconeis scutellum var. scutellum, Licmophora flabellata, Licmophora gracidis, Licmophora sp., Navicula abunda, Rhabdonema adriaticum, and Stratiella unipunctata (Table 1). In general, these taxa were dominated in the diatom community in the shallow site. On the contrary, Halamphora coffeiformis showed opposite relative abundance patterns. For some taxa with lower relative abundance (<1%; e.g., Achnanthes septa var. incurvata, Diploneis papula, Diploneis vacillans, Mastogloia inaequalis, Navicula directa, etc.), no particular difference in abundances between the shallow and deeper site was observed.

Regarding the seasonality, diatom community was dominated by Stratiella unipunctata, Cocconeis scutellum var. scutellum, and Achnanthes brevipes (in total 55.25%) in shallow site in January (Table 1). In February, Stratiella unipunctata, Cocconeis scutellum var. scutellum, and Licmophora flabellata had highest relative abundance (in total 61.5%), while Cocconeis scutellum var. scutellum (24.5%) and Halamphora coffeiformis (16.25%) contributed the most to total diatom relative abundance in March. Licmophora gracidis (55%) and Licmophora sp. (59.5%) dominated in relative abundances in April and May, respectively. Like in February, Licmophora flabellata, Cocconeis scutellum var. scutellum and Stratiella unipunctata dominated (in total 65.75%) in the shallow site in June. In July, the taxa with highest relative abundance in diatom community were Cocconeis scutellum var. scutellum (26%) and Navicula abunda (10%). In August, Mastogloia similis (10%) prevailed in diatom community, and other seven taxa had relative abundances between 4.5 and 5.75% each (Table 1). Actinoecysis subtilis dominated in September and October with relative abundances of 13% and 23.75%, respectively. Diatom community was dominated by Stratiella unipunctata in November (25%) and December (42.5%), respectively.

On deeper site, Cocconeis scutellum var. scutellum (10%), Grammatophora oceanica (7%), and Halamphora coffeiformis (13.25%) had the highest relative abundances in April, May, and June, respectively. Rhopalodia musculus (10%) and Halamphora coffeiformis (9.75%) were dominant in the diatom community in July and August, respectively. Caloneis excentrica (11.5%) and Nitzschia pararosstrata (11.25%) had the highest relative abundances in September. Halamphora coffeiformis was dominated in diatom community in October (8.5%) and December (13%), while Tryblionella compressa (10.5%) prevailed in November.

In addition, some taxa appeared only in particular seasons: in winter 10, spring 20, summer 57, and autumn 33 (Appendix 1). Among them, taxa with the highest relative abundances were: in winter Halamphora exigua (1.5%), spring Amphora glacialis (2%), summer Navicula abunda (10%), and autumn Caloneis excentrica (11.5%). All of the taxa in this study were recorded for the first time in Bosnia and Herzegovina.

Relationships between epilithic diatoms and environmental parameters

In total, 33 taxa with relative abundance (in %) ≥ 2.5% and frequency of occurrence ≥ 8.33% from 16 samples collected from shallow and deep sites between April and December 2011 (excluded November) were selected for this analysis. Eigenvalues from the CCA analysis for the first four axes were 0.472, 0.350, 0.335, and 0.276 (Fig. 5). The first two axes explain 37.9% of variance of species–environment relationship. Temperature, oxygen saturation (O2/O3), silicate concentration (SiO2), and salinity were the most important factors influencing diatom community structure in the bay and they accounted for approximately 55% of the total variability. NO3, PO4, and NH4 showed the lowest significance. For illustration purposes, all environmental variables have been included in the graph (Fig. 5). Halamphora coffeiformis was associated with higher temperature values. The salinity and oxygen saturation vectors are found within the same quadrant as the most included taxa (e.g., Licmophora paradoxa, Grammatophora oceanica, Psammodictyon panduriforme, etc.). In the case of nutrients (TIN, NO3, and SiO2), Licmophora gracilis and Paralia sulcata s.l. were associated with higher concentrations, at the higher left quadrant. The most abundant taxa Licmophora sp. appeared in May, when salinity was high and nutrient concentrations were low. The most frequently occurring taxa Stratiella unipunctata, among others (e.g., Psammodictyon panduriforme, Tryblionella compressa, Caloneis liber var. linearis, Trachyneis aspera, etc.), are principally situated in the center of the plot, which means that they were not strongly influenced by any of considered variables.

Discussion

The results presented herein deal with a shallow semi-enclosed bay. TRIX analysis has revealed oligotrophic characteristics of the bay (Vollenweider et al., 1998; Karydis, 2009). The bay is characterized by low chlorophyll a concentrations, while concentrations of SiO2 and TIN were higher than the half-saturation constants for the most of the year (Dupčić Radić et al., 2013). The ecological status of the bay is determined by inflow from Neretva River, submarine springs (“vruljas”), and precipitation. These factors, among others (e.g. shallowness), significantly caused frequent and rapid changes of environmental conditions in the bay. This was also found for the inner-part of the Mali Ston Bay (Ćalić et al., 2013) and the neighboring coastal lagoon (Jasprica et al., 2012).

The genera Mastogloia, Diploneis, Amphora, Nitzschia, Navicula, and Cocconeis were the richest taxa. A similar diatom community has been reported for different
Fig. 5: CCA biplot showing diatom taxa (triangle) and vectors of the ten environmental variables (arrows) based on 16 samples. A dataset of 33 diatom taxa (with relative abundance ≥ 2.5% and frequency of appearance ≥ 8.33%) was selected. Abbreviations: Si = SiO2, TIN = total inorganic nitrogen, NO3 - nitrate, NO2 - nitrite, NH4 - ammonium, PO4 - phosphate, SAT - oxygen saturation (O2/O2a), SAL - salinity, CHL - chlorophyll a concentrations, TEMP - temperature. Codes for diatom taxa are:

Achr = Achnanthes brevipes,
Acsn = Actinoptychus splendens,
Acsu = Actinocyclus subtilis,
Amco = Halamphora coffeformis,
Amp2 = Amphora sp.2,
Caex = Caloneis excentrica,
Cali = Caloneis liber var. linearis,
Cosc = Cocconeis scutellum var. scutellum,
Dimi = Dimeregramma minus,
Dipl = Diploneis sp.1,
Groc = Grammatophora oceanica,
Lift = Licmophora flabellata,
Ligr = Licmophora gracilis,
Lipa = Licmophora paradoxa,
Lipf = Licmophora pfanckuckae,
Lisp = Licmophora sp.,
Maer = Mastogloia erythraea var. grunowii,
Masi = Mastogloia similis,
Masp = Mastogloia splendida,
Naab = Navicula abunda,
Ncm = Trybionella compressa,
Nico = Nitzschia cocarcata,
Nipa = Nitzschia paranostrata,
Nive = Nitzschia ventricosa,
Pasa = Paralia sulcata s.l.,
Pscr = Psammodictyon panduriforme var. continuum,
Pspa = Psammodictyon panduriforme,
Rhad = Rhabdonema adriaticum,
Rhm = Rhopalodia musculus,
Rhip = Rhopalodia pacifica,
Stur = Striatella unipunctata,
Tras = Trachymeis aspera,
Toco = Trybionella coarctata.

Four environmental variables were mostly correlated to the distribution of diatom taxa. Of these, temperature, oxygen saturation, silicate concentration, and salinity were the most important factors influencing community structure. However, although no strong seasonal variation of diatom taxa was observed during the study, Cocconeis scutellum var. scutellum, Rhabdonema adriaticum, Striatella unipunctata, and Licmophora gracilis showed high percentage contributions in the winter-spring diatom communities. Many diatoms are superior competitors for nutrients at lower temperatures and their temperature dependence is one of the important mechanisms influencing taxon composition (Tilman et al., 1986). However, in our case, determination of the dominance of these major taxa to the supply-ratios falls outside of the scope of the present paper. Nevertheless, studies on the seasonal variations of microepilithic communities in the north-western Adriatic showed a marked seasonal variability with lower diatom abundances recorded during the winter (Totti et al., 2007). Conversely, diatoms were less abundant in warm summer months with the exception of Cocconeis scutellum var. scutellum. This, at least partially, contrasted with the findings of McIntyre & Moore (1977), who reported that C. scutellum, along with Striatella unipunctata and Grammatophora oceanica, are mostly restricted to the lower littoral regions protected from high light intensities, a factor which was not addressed in the present work.

In our study, C. scutellum var. scutellum is grouped in the direction of higher salinity. Although C. scutellum was analyzed on the artificial hard substrates and in plankton samples in the eastern Adriatic karstic Zrmanja Estuary (Burić et al., 2004), we could not compare temporal changes due to the exclusively summer sampling in that study. Generally C. scutellum, mostly accompanied by Halamphora coffeformis, were the most frequently recorded diatom taxa, not only on the natural hard substrata (Colak Sabanci, 2012), but on all different substrata (epiphytic, epilithic, epizoic) (Car et al., 2012;
The most abundant diatoms were represented chiefly by Licmophora taxa, and had their high relative abundances between winter and early summer, with a marked decrease in summer. Generally, erect diatoms have better access to light, although they are more exposed to grazing pressures (Hillebrand et al., 2000; Müller, 1999), and their capability of regulating stalk lengths makes them good competitors for light when dense benthic populations develop. In our case, their decrease in summer may be related either to the effect of the extremely high temperatures recorded in June and August of 2011 (Pandžić & Likso, 2013) or to the effect of increased grazing pressure. We consider that only prolonged series of observations, based on a more frequent sampling interval, can be expected to disclose a potential seasonality in temporal species-composition changes.

The impact of salinity in controlling diatom taxa needs to be stressed. Variations in salinity, usually a significant structuring factor (Weckström & Juggins, 2005), had no effect on the composition of the diatom communities of the Homa lagoon, in Turkey (Çolak Sabanci, 2011). In our study, CCA showed that Licmophora gracilis had the highest relative abundance during the lowest annual salinity values in April, when TIN and SiO$_2$ were high. Although L. gracilis was considered a marine taxon (Witkowski et al., 2000), it has been reported as an indicator of brackish waters and variable surface layers in the eastern Adriatic karstic Zrmanja Estuary and, in general, may be used as an indicator of environments with fluctuating salinities (Caput Mihalić et al., 2008). This highlights its ability to adapt to salinity changes (Snoeijis, 1999). In May, the pauci-specific community was mostly composed of Licmophora sp. (59.5%) and C. scutellum var. scutellum (21.75%), and, on the contrary, this may be related to high salinity and low nutrient concentrations. The increase in salinity is caused by the interaction of several factors, such as higher air temperature and evaporation, low precipitation, and lack of submarine activity in the area, while lower nutrient concentrations may be temporarily related to the low inflow of fresh water (Čalić et al., 2013). From a purely scientific standpoint, it will be intriguing to identify Licmophora sp. in sufficient detail to permit the provision of a precise reference in terms of taxa and elaborate on its ecology.

The results of the present study clearly show the appearance of some taxa that are not truly benthic. In shallow waters, benthic and planktonic communities are not clearly differentiated, mainly due to the continuous mixture of the shallow water column (Çolak Sabanci, 2011; Jasprica & Hafner, 2005). For example, Psammodictyon panduriforme, the taxon with a higher relative abundance (6.5%) in November in the deep site, previously was reported as planktonic (Sagan et al., 2000) but has also been found within epipelagic and epilithic communities (Çolak Sabanci, 2012; Çolak Sabanci et al., 2011). Diatom cells sink and living pelagic cells can also be found on the surface sediments (e.g., Nitzschia longissima), particularly when vertical mixing is low (Admiraal, 1984). Additionally, the centric diatom Paralia sulcata, which generally appears both in the benthos and plankton, has a competitive advantage under low light conditions (Margalef, 1969; McQuoid & Nordberg, 2003; Zong, 1997). However, our data on the distribution of P. sulcata s.l. are comparable with studies from the north-eastern Adriatic (Munda, 2005).

In conclusion, we believe that the most significant result of this paper lies in the information and quantitative data it provides about marine epilithic diatoms in this part of the Adriatic Sea. Although a monthly sampling strategy does not offer fine-grain resolution of the annual marine epilithic taxa cycles, a few taxa made significant contributions to the assemblage structure. Striatella unipunctata, Cocconeis scutellum var. scutellum and Halamphora coffeiformis were the most frequent occurring taxa, while some taxa (Licmophora gracilis, Licmophora sp.) sometimes strongly predominated over the others in the composition of the epilithic communities. A main feature of diatom assemblages is higher species diversity index at the deep site. High biodiversity in the bay is the result of balanced ecological conditions, but due to the increasing coastal development, it may become endangered. Due to the continual changing of ecological factors and interactions among them, it is difficult to understand which particular factor or factors affect the community structure in shallow systems. Clearly, further studies on benthic diatom communities in the area are required to increase the accuracy of predictions.

Acknowledgements

This research was supported by Croatian Ministry of Science, Education and Sports (project 275-0000000-3186) and by Croatian science foundation (HRZZ, IP-2014-09-2945). The authors thank Dr. Rade Garić for drawing Fig. 1, Steve Latham (UK) for improving the English, and two anonymous reviewers and editor whose observations improved the quality of the final version of the manuscript.

Author Contributions

N.J. designed the study and supervised the work. N.J. and A.C. led the writing of this manuscript. D.H. conducted the field sampling, prepared the samples for counting, and analyzed the samples using the light microscope. T.K. and A.C. performed the statistical analyses. I.D.R. analyzed the physical and chemical parameters.
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Appendix

Appendix 1. List of marine benthic diatom taxa found in Neum Bay in 2011. Abbreviations: Taxa found only in the shallow (S) and deep site (B). Taxa found only in a particular season are also indicated (Sp – spring. Su – summer. A – autumn. W – winter). Taxa found only once in all samples with relative abundances lower than 1% are indicated with asterisk (*).

Achnanthes brevipes C. Agardh
Achnanthes brevipes var. intermedia (Kützing) Cleve
Achnanthes greenlandica (Cleve) Grunow [B. W. *]
Achnanthes longipes C. Agardh
Achnanthes parva (Kützing) [S. Su. *]
Achnanthes pseudogreenlandica Hendey
Achnanthes septata var. incurvata (Østrup) Cleve-Euler [A]
Achnanthes sp. [S]
Actinocyclus gallicus F. Meister [B]
Actinocyclus ochotensis A. P. Jousé
Actinocyclus splendidus J. Rattray [B. W. *]
Actinocyclus subtilis (W. Gregory) Ralfs
Actinocyclus senarius (Ehrenberg) Ehrenberg [S. A. *]
Actinocyclus adriaticus Grunow [S. Su. *]
Actinocyclus octonarius (Ehrenberg) Kützing [B. Sp]
Actinocyclus splendidus (Shadbolt) Ralfs [*]
Amphora abhudsens R. Simonsen [S]
Amphora arenaria Donkin
Amphora bigibba var. interrupta (Grunow) Cleve
Amphora constictua (Ehrenberg) W. Carruthers [S. Sp.*]
Amphora delicatissima Krasske [B. Su. *]
Amphora gacialis W. Smith [S. Sp]
Amphora grzecefaena Hendey [B. A. *]
Amphora hyalina Kützing
Amphora laevis Gregory
Amphora laevissima W. Gregory
Amphora lineolata Ehrenberg
Amphora lunata E. V. Østrup
Amphora obtusa W. Gregory [B. A]
Amphora ovalis (Kützing) Kützing [B]
Amphora proteus W. Gregory [B. A. *]
Amphora pseudohyalina Simonsen
Amphora subacutiuscula Schoeman [B. A. *]
Amphora sp. 1
Amphora sp. 2 [B. A]
Aridiisonea crystallina (C. Agardh) Grunow
Aridiisonea formosa (Hantzsch) Grunow
Aridiisonea robusta (Ralfs ex Pritchard) De Notaris [*]
Aulacoseira granulata (Ehrenberg) Simonsen [B. Sp.*]
Azpeita nodulifera (A. Schmidt) G. A. Fryxell & P. A. Sims
Bacillaria paxillifera (O. F. Müller) T. Marsson
Bacillaria socialis (Gregory) Ralfs
Berkeleya scupororum (Brébisson ex Kützing) E. J. Cox [B. Sp]
Biddulphia biddulphiana (J. E. Smith) Boyer
Biddulphia tuomeyi (J. W. Bailey) Roper [B. A]
Brebiolosnia lanceolata (C. A. Agardh) R. K. Mahoney & Reimer [S]
Caloneis bicucueta (Grunow) Boyer [B. Su. *]
Caloneis excentrica (Grunow) Boyer [B. A.]
Caloneis liber (W. Smith) Cleve
Caloneis liber var. linearis Cleve
Campylocodiis implicatus R. Ross & Adbin [B]
Cistula lorenziana (Grunow) Cleve [B]
Cocconeis orthoneoides (Hustedt) Witkowski [B. A]
Cocconeis costata Gregory [S. Su. *]
Cocconeis costata var. hexagona Grunow [S. A. *]
Cocconeis distans W. Gregory [B. Sp]
Cocconeis fasciolata (Ehrenberg) N. E. Brown [B. Sp. *]
Cocconeis latecostata F. Hustedt [B]
Cocconeis molesta Kützing [S. W. *]
Cocconeis molesta var. crucifera Grunow [S. A. *]
Cocconeis notabilis A. W. F. Schmid [B]
Cocconeis pelta A. Schmidt [B. Su]
Cocconeis peltoides Hustedt [B. Sp]
Cocconeis pinnata W. Gregory ex Greville [B]
Cocconeis schmidtii Heiden; [*]
Cocconeis scutellum var. scutellum Ehrenberg
Cocconeis woodii Reyes [S. Su]
Cocconeis sp. [S. Su. *]

Corona decorana (Brébisson) Ruck & Guiry
Coscinodiscus sp. [B]
Cyclotella meneghiniana Kützing [B. Su. *]
Diatoma vulgaris [B. Sp]
Dimeregramma fulvum (W. Gregory) Ralfs [B. W. *]
Dimeregramma minus (W. Gregory) Ralfs
Diploneis aeniaruari Hustedt [B. A. *]
Diploneis bombus (Ehrenberg) Ehrenberg [*]
Diploneis chersonensis (Grunow) Cleve [B. Sp]
Diploneis coffaeiformis (Schmidt) Cleve [B]
Diploneis crabro (Ehrenberg) Ehrenberg
Diploneis didyma (Ehrenberg) Ehrenberg [B. Sp. *]
Diploneis hexagonum [B. A]
Diploneis incurvata (Gregory) Cleve [S. W. *]
Diploneis incurvata var. dubbia Hustedt [S. Su. *]
Diploneis litoralis (Donkin) Cleve [*]
Diploneis litoralis var. clathrata (Østrup) Cleve [*]
Diploneis nitescens (W. Gregory) Cleve [B]
Diploneis notabilis (Greville) Cleve
Diploneis papula (A. W. F. Schmidt) Cleve [Su]
Diploneis parca (A. W. F. Schmidt) Boyer [S. Su. *]
Diploneis rexy S. J. M. Droop [B]
Diploneis smithii (Brébisson) Cleve
Diploneis smithii var. dilatata (Peragallo) Terry [B. Sp. *]
Diploneis smithii var. hexagona [B]
Diploneis smithii var. recta Peragallo
Diploneis splendidula Cleve [*]
Diploneis stromii Hustedt [S. W. *]
Diploneis vacillans (A. Schmidt) Cleve [*]
Diploneis vacillans var. teniens (A. Schmidt) Cleve [*]
Diploneis weissflogii (A. W. F. Schmidt) Cleve [B]
Diploneis sp.
Encyonema ventricosum (C. Agardh) Grunow [S. Su. *]
Entomoneis paludosus (W. Smith) Reimer
Fallacia floriniae (M. Moller) Witkowski [B]
Fallaciaforcipata (Greville) Stickle & D. G. Mann
Fallacia litoricola (Hustedt) D. G. Mann [B. Su. *]
Fallacia pygmaea (Kützing) Stickle & D. G. Mann [*]
Fallacia subforcipata (Hustedt) D. G. Mann [B. Sp. *]
Fragilariopsis sopatus (W. Gregory) Lange-Bertalot [B. Su]
Fragilariopsis sp. [A. *]
Gomphonema olivaceum (Hornemann) Brébisson [B. Su. *]
Grammatophora gibberula Kützing [S. W. *]
Rhizosolenia styliformis T. Brightwell [B. Su. *]
Rhoicosphenia marina (Kützing) M. Schmidt
Rhopalodia acuminata Kramme
Rhopalodia musculus (Kützing) Otto Müller [B]
Rhopalodia pacifica Krammer
Seminavis barbara Witkowski [S. Su]
Staurosira punctiformis Witkowski, Metzeltin & Lange-Berta-
lot [B. A]
Striatella unipunctata (Lyngbye) C. Agardh
Surirella fastuosa (Ehrenberg) Ehrenberg
Surirella scalaris M. H. Giffen [B]
Synedra falcigens (Greville) W. Smith
Tabularia investiens (W. Smith) D. M. Williams & Round [*]
Talaronectis furcigera (Grunow) Sterrenburg
Terpsinoë americana (Bailey) Grunow [B]
Tetramphora decussata (Grunow) Stepanek & Kociolek [B]
Tetramphora rhombica (Kitton) Stepanek & Kociolek [S]
Thalassiosira sp. [B]
Toxarium kennedyanum (Gregory) Pelletan [B. Sp. *]
Toxarium undulatum J. W. Bailey
Trachyneis aspera (Ehrenberg) Cleve
Trigoniun formosum (Brightwell) Cleve [S]
Tryblionella apiculata W. Gregory [B. Su.*]
Tryblionella coarcta (Grunow) D. G. Mann [B.A]
Tryblionella compressa (J. W. Bailey) Poulin
Tryblionella didyma (Hustedt) D. G. Mann [B. Su. *]
Tryblionella hungarica (Grunow) Frenguelli [Su]