

## Mediterranean Marine Science

Vol 19, No 2 (2018)



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doi: [10.12681/mms.14151](https://doi.org/10.12681/mms.14151)

#### To cite this article:

HAFNER, D., CAR, A., JASPRICA, N., KAPETANOVIĆ, T., & DUPČIĆ RADIĆ, I. (2018). Relationship between marine epilithic diatoms and environmental variables in oligotrophic bay, NE Mediterranean. *Mediterranean Marine Science*, 19(2), 223–239. <https://doi.org/10.12681/mms.14151>

## 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

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### 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<sub>2</sub>/O<sub>2</sub><sup>'</sup>), silicate concentration (SiO<sub>4</sub>), 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.

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### 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 (Burić *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<sub>3</sub>), nitrite (NO<sub>2</sub>), ammonium (NH<sub>4</sub>), phosphate (PO<sub>4</sub>), silicate (SiO<sub>4</sub>), total inorganic nitrogen (TIN), oxygen saturation (O<sub>2</sub>/O<sub>2</sub>'), 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

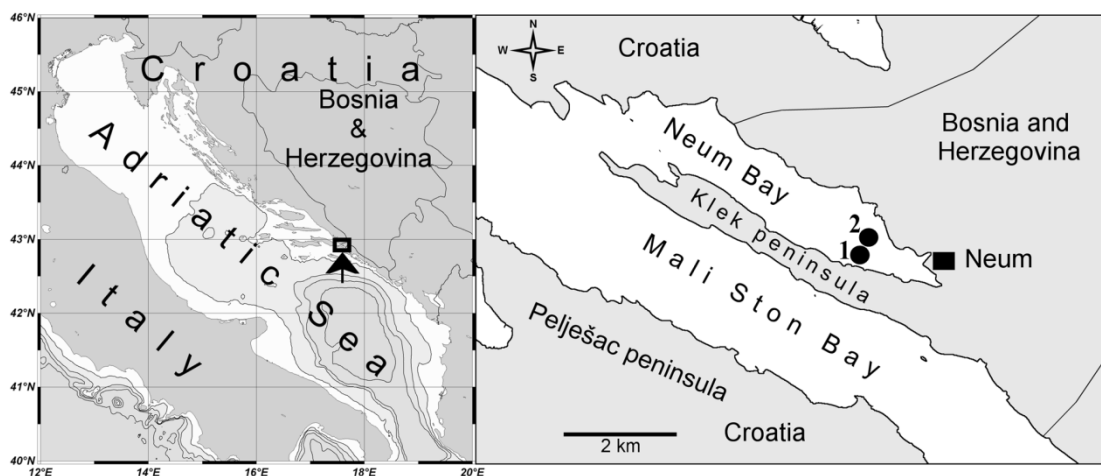
### The study area

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<sup>2</sup>. 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



**Fig. 1:** Sampling sites (●) in Neum Bay, Bosnia and Herzegovina. Site 1 – Shallow site (0.5 m depth); Site 2 – Deep site (8 m depth).

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 polythene 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. DH-NB-1-21]. Identifications were made following Peragallo & Peragallo (1897–1908), Hendey (1964), Ricard (1974, 1975, 1977), Poulin *et al.* (1984, 1990), Bérard-Therriault *et al.* (1986, 1987), Snoeijs (1993), Snoeijs & Potapova (1995), Snoeijs and Kasperoviciené (1996), Snoeijs & 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 & Degobbi, 1984) and chlorophyll-*a* concentrations (Chl *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 (NO<sub>3</sub>) + nitrite (NO<sub>2</sub>) + ammonium (NH<sub>4</sub>)], phosphate (PO<sub>4</sub>) and silicate (SiO<sub>4</sub>).

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 (O<sub>2</sub>/O<sub>2</sub><sup>'</sup>) 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.*, 1998; 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):

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

where *H* is the Shannon-Wiener diversity, *s* is the total number of species and *p<sub>i</sub>* is the proportional abundance of *i*-th species. This index is commonly used in ecology of benthic diatom communities (cf. Kwadrans, 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 quantitatively 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,  $\text{PO}_4$  and  $\text{SiO}_4$  showed higher values on shallow 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 32.0 (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 ( $\text{O}_2/\text{O}_2'$ ) 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  $\text{SiO}_4$  – oscillated much more than those in samples from deeper site. TIN ranged from 0.98 (February) to 2.79 (April)  $\mu\text{M}$  and from 0.35 (February) to 1.72 (October)  $\mu\text{M}$ , respectively, in samples from shallow and deeper site. In this study, TIN generally follows the distribution of  $\text{NO}_3$  with exception in August on shallow site and in October on deeper site when  $\text{NH}_4$  significantly contributed (1.42 and 0.63  $\mu\text{M}$ , respectively). Phosphate ( $\text{PO}_4$ ) ranged from 0.03 (March) to 0.22 (July)  $\mu\text{M}$  in shallow samples and from 0.03 (February, April) to 0.13 (December)  $\mu\text{M}$  in deeper samples. Silicate ( $\text{SiO}_4$ ) ranged from 1.49 (July) to 9.59 (August)  $\mu\text{M}$  in shallow samples and from 0.51 (February) to 5.02 (December)  $\mu\text{M}$  in samples from deeper site. Chl *a* ranged from 0.1 (May) to 0.34  $\mu\text{g L}^{-1}$  (September) on the shallow site and from 0.11 (June and July) to 0.54  $\mu\text{g L}^{-1}$  (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  $3.23 \pm 0.48$  TRIX units), Neum Bay can be characterized as oligotrophic.

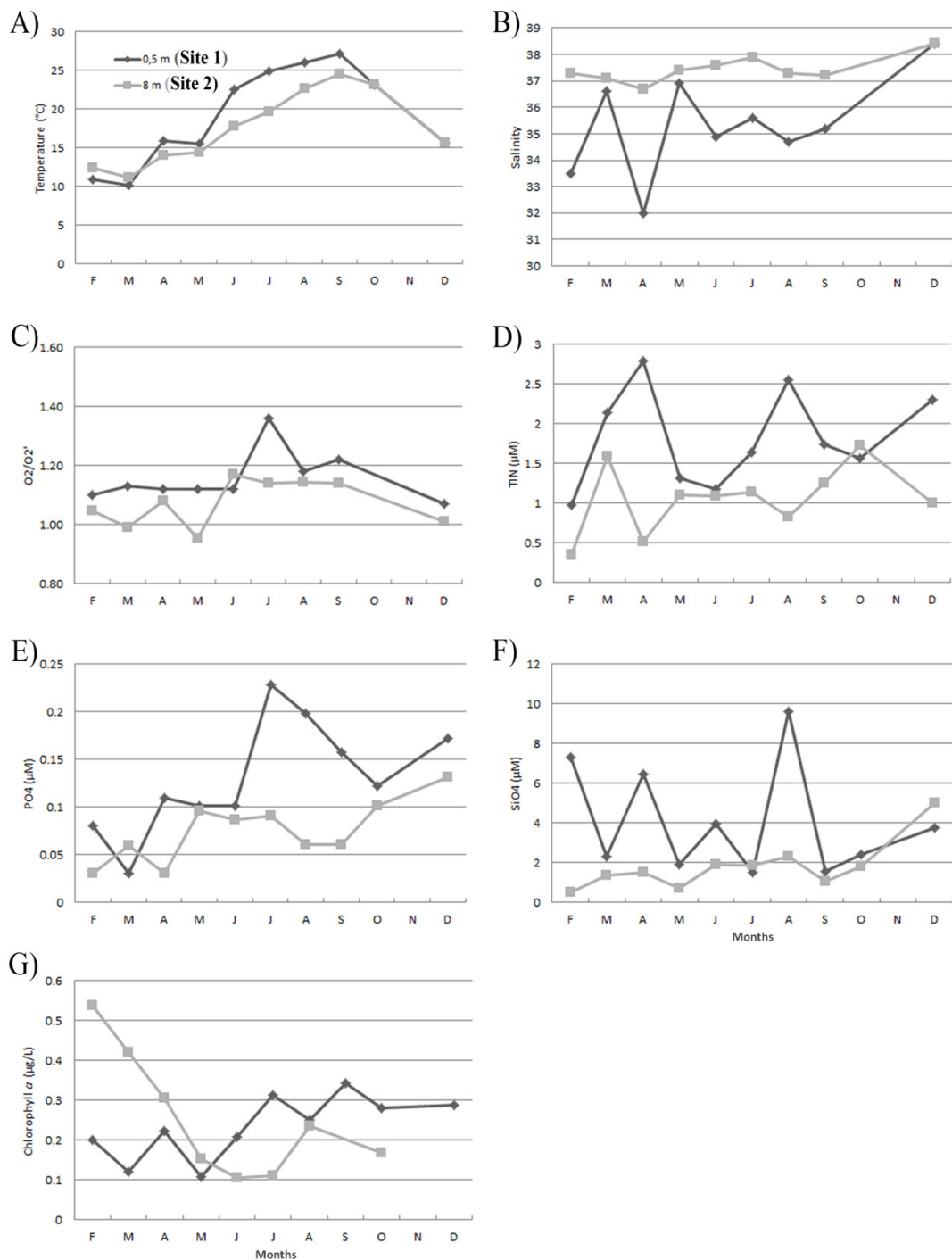
### *Species richness and the Shannon-Weiner diatom diversity index*

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-



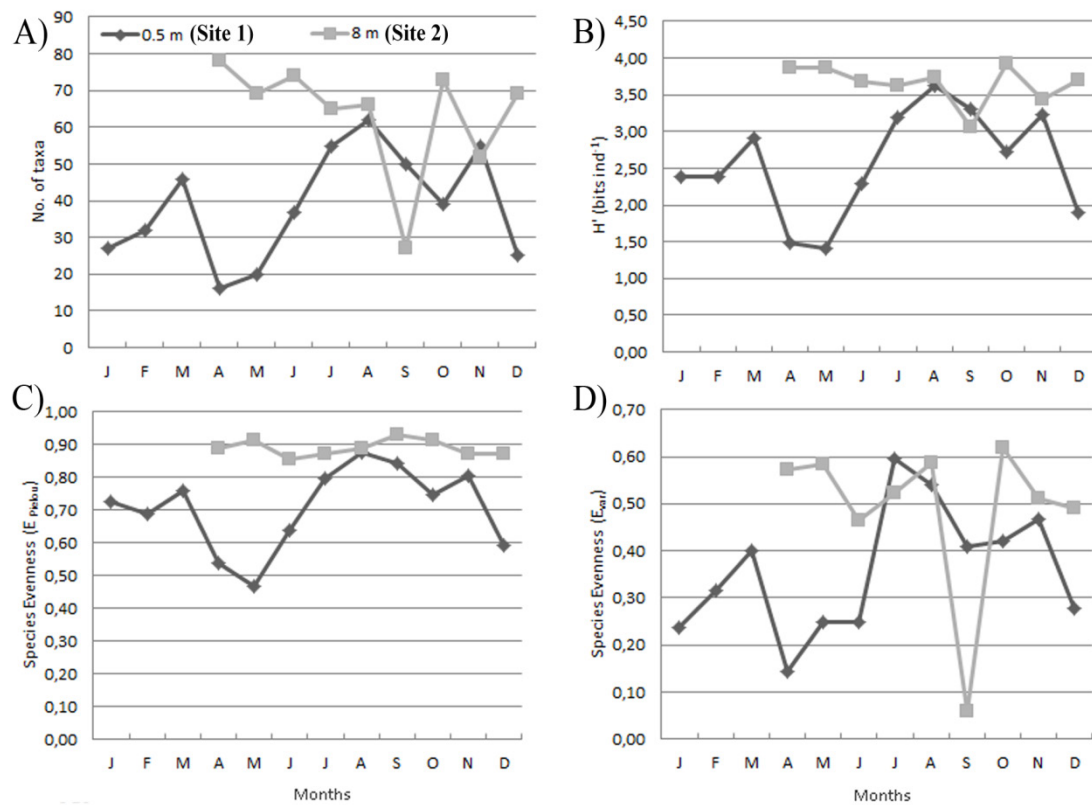
**Fig. 2:** Monthly distribution of the physical and chemical parameters in the shallow (0.5 m) and deep (8 m) site in Neum Bay in 2011. A) temperature; B) salinity; C) oxygen saturation (O<sub>2</sub>/O<sub>2</sub>’); D) total inorganic nitrogen (TIN); E) phosphate (PO<sub>4</sub>); F) silicate (SiO<sub>4</sub>); G) chlorophyll-*a* concentrations.

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.

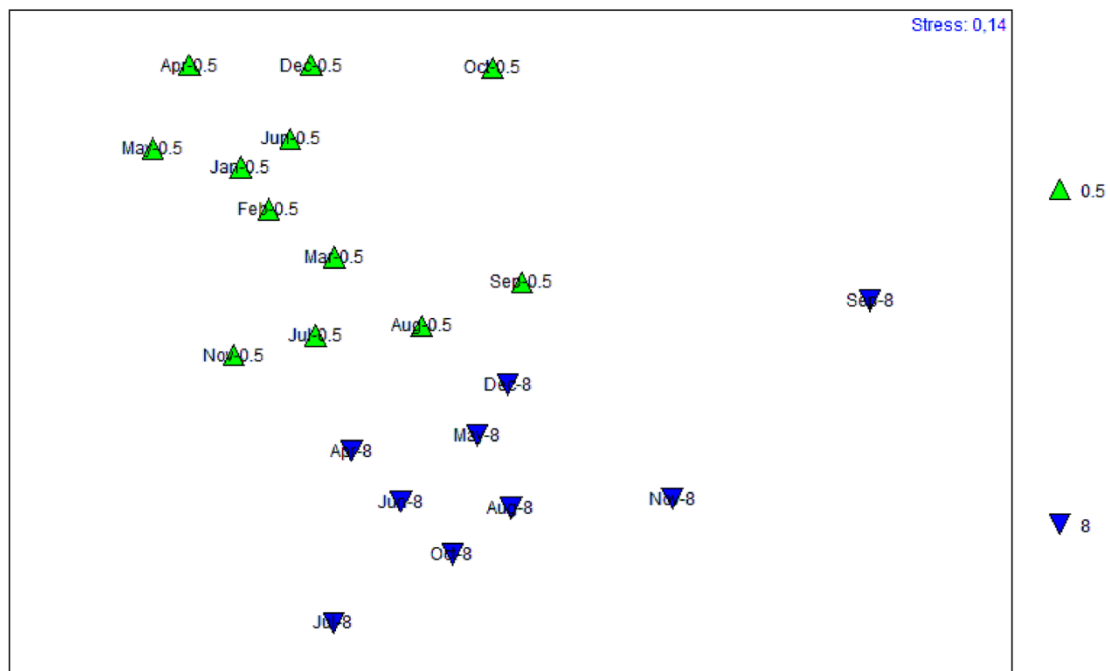
**Relative abundances and distribution of diatom assemblages**

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*, *Halamphora 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.



**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  $\geq 4\%$ , RA, only are shown) for the shallow (0.5 m) and deep (8 m) site in Neum Bay in 2011.

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

(continued)



Table 1 Continued

Season:	Month / Site (Depth):	Shallow Site (0.5 m)		Deep Site (8 m)		
			RA (%)		RA (%)	
Summer	July	<i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i>	26.00	<i>Rhopalodia musculus</i> (Kützing) Otto Müller	10.00	
		<i>Navicula abunda</i> Hustedt	10.00	<i>Caloneis liber</i> var. <i>linearis</i> Cleve	7.50	
		<i>Halumphora coffeiformis</i> (C. Agardh) Levkov	6.25	<i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i>	7.25	
		<i>Rhabdonema adriaticum</i> Kützing	6.00	<i>Halumphora coffeiformis</i> (C. Agardh) Levkov	7.00	
		<i>Achnanthes pseudogroenlandica</i> Hendeby	5.25	<i>Amphora pseudohyalina</i> Simonsen	4.50	
					<i>Dimeregramma minus</i> (Gregory) Ralfs	4.25
	August	<i>Mastogloia similis</i> Hustedt	11.00	<i>Halumphora coffeiformis</i> (C. Agardh) Levkov	9.75	
		<i>Plagiotropis tayrecta</i> T. B. B. Paddock	5.75	<i>Caloneis liber</i> var. <i>linearis</i> Cleve	9.25	
		<i>Trachyneis aspera</i> (Ehrenberg) Cleve	5.00	<i>Rhopalodia pacifica</i> Krammer	8.25	
		<i>Actinocyclus subtilis</i> (W. Gregory) Ralfs	5.00			
		<i>Striatella unipunctata</i> (Lyngbye) C. Agardh	4.75			
		<i>Caloneis liber</i> var. <i>linearis</i> Cleve	4.75			
		<i>Mastogloia ignorata</i> Hustedt	4.75			
		<i>Licmophora gracilis</i> (Ehrenberg) Grunow	4.50			
	Autumn	September	<i>Actinocyclus subtilis</i> (W. Gregory) Ralfs	13.00	<i>Caloneis excentrica</i> (Grunow) Boyer	11.50
			<i>Rhopalodia pacifica</i> Krammer	12.00	<i>Nitzschia pararostrata</i> (Lange-Bertalot) Lange-Bertalot	11.25
			<i>Licmophora</i> sp.	6.50	<i>Trachyneis aspera</i> (Ehrenberg) Cleve	7.25
<i>Trachyneis aspera</i> (Ehrenberg) Cleve			6.25	<i>Grammatophora oceanica</i> Ehrenberg	6.00	
<i>Halumphora coffeiformis</i> (C. Agardh) Levkov			4.25	<i>Lyrella lyra</i> (Ehrenberg) Karajeva	5.00	
				<i>Dimeregramma minus</i> (Gregory) Ralfs	5.00	
			<i>Diploneis crabro</i> (Ehrenberg) Ehrenberg	5.00		
			<i>Actinocyclus ochotensis</i> A. P. Jousé	4.50		
October		<i>Actinocyclus subtilis</i> (W. Gregory) Ralfs	23.75	<i>Halumphora coffeiformis</i> (C. Agardh) Levkov	8.50	
		<i>Licmophora pfannkuckae</i> Giffen	14.25	<i>Rhopalodia pacifica</i> Krammer	5.25	
		<i>Striatella unipunctata</i> (Lyngbye) C. Agardh	12.75	<i>Dimeregramma minus</i> (Gregory) Ralfs	5.00	
		<i>Rhabdonema adriaticum</i> Kützing	10.00	<i>Caloneis liber</i> (W. Smith) Cleve	4.50	
	<i>Rhopalodia pacifica</i> Krammer	7.00				
November	<i>Striatella unipunctata</i> (Lyngbye) C. Agardh	25.00	<i>Tryblionella compressa</i> (J.W.Bailey) Poulin	10.50		
	<i>Halumphora coffeiformis</i> (C. Agardh) Levkov	7.50	<i>Mastogloia binotata</i> (Grunow) Cleve	8.25		
	<i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i>	5.25	<i>Mastogloia splendida</i> (Gregory) H. Pergallo	7.75		
	<i>Nitzschia ventricosa</i> Kitton	5.00	<i>Actinoptychus splendens</i> (Shadbolt) Ralfs	6.75		
			<i>Psammodictyon panduriforme</i> (W. Gregory) D.G.Mann	6.50		
		<i>Trachyneis aspera</i> (Ehrenberg) Cleve	6.00			
Winter	December	<i>Striatella unipunctata</i> (Lyngbye) C. Agardh	42.50	<i>Halumphora coffeiformis</i> (C. Agardh) Levkov	13.00	
		<i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i>	22.50	<i>Dimeregramma minus</i> (Gregory) Ralfs	5.75	
		<i>Achnanthes brevipes</i> C. Agardh	9.00	<i>Rhabdonema adriaticum</i> Kützing	5.00	
		<i>Licmophora flabellata</i> (Grev.) C. Agardh	7.50	<i>Striatella unipunctata</i> (Lyngbye) C. Agardh	4.50	
		<i>Ardissonea crystallina</i> (C. Agardh) Grunow	4.50	<i>Achnanthes brevipes</i> var. <i>intermedia</i> (Kützing) Cleve	4.50	

Altogether, only five taxa were presented in all samples: *Cocconeis scutellum* var. *scutellum*, *Grammatophora oceanica*, *Halamphora coffeiformis*, *Licmophora remulus*, and *Trachyneis aspera*.

The taxa with the highest relative abundances were: *Halamphora coffeiformis*, *Caloneis excentrica*, *Cocconeis scutellum* var. *scutellum*, *Licmophora flabellata*, *Licmophora gracilis*, *Licmophora* sp., *Navicula abunda*, *Rhabdonema adriaticum*, and *Striatella 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 *Striatella unipunctata*, *Cocconeis scutellum* var. *scutellum*, and *Achnanthes brevipes* (in total 55.25%) in shallow site in January (Table 1). In February, *Striatella 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 gracilis* (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 *Striatella 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). *Actinocyclus subtilis* dominated in September and October with relative abundances of 13% and 23.75%, respectively. Diatom community was dominated by *Striatella 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 pararostrata* (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.

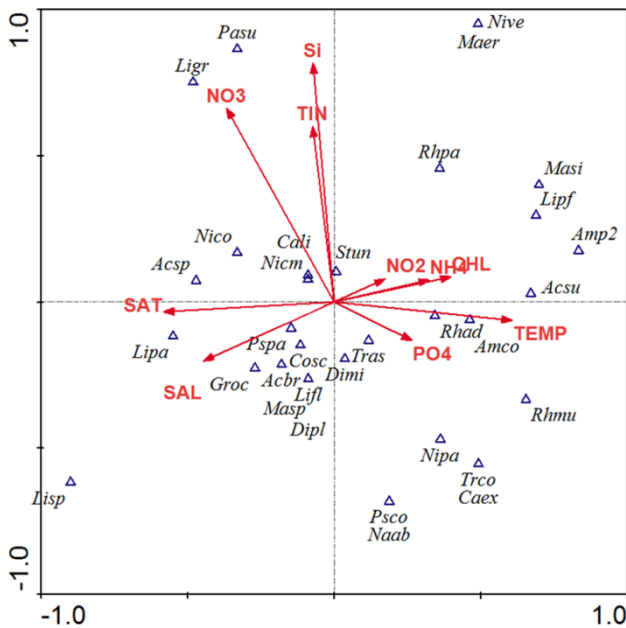
### **Relationships between epilithic diatoms and environmental parameters**

In total, 33 taxa with relative abundance (in %)  $\geq 2.5\%$  and frequency of occurrence  $\geq 8.33\%$  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 ( $O_2/O_2'$ ), silicate concentration ( $SiO_4$ ), and salinity were the most important factors influencing diatom community structure in the bay and they accounted for approximately 55% of the total variability.  $NO_2$ ,  $PO_4$ , and  $NH_4$  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*, *Psammodyctyon panduriforme*, etc.). In the case of nutrients (TIN,  $NO_3$ , and  $SiO_4$ ), *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 *Striatella unipunctata*, among others (e.g., *Psammodyctyon 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 characters of the bay (Vollenweider *et al.*, 1998; Karydis, 2009). The bay is characterized by low chlorophyll *a* concentrations, while concentrations of  $SiO_4$  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 (Čalić *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  $\geq 2.5\%$  and frequency of appearance  $\geq 8.33\%$ ) was selected. Abbreviations: Si -  $\text{SiO}_4$ , TIN - total inorganic nitrogen,  $\text{NO}_3$  - nitrate,  $\text{NO}_2$  - nitrite,  $\text{NH}_4$  - ammonium,  $\text{PO}_4$  - phosphate, SAT - oxygen saturation ( $\text{O}_2/\text{O}_2'$ ), SAL - salinity, CHL - chlorophyll *a* concentrations, TEMP - temperature. Codes for diatom taxa are:

- Acbr = *Achnanthes brevipes*,
- Acsp = *Actinopterychus splendens*,
- Acsu = *Actinocyclus subtilis*,
- Amco = *Halamphora coffeiformis*,
- 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*,
- Lifl = *Licmophora flabellata*,
- Ligr = *Licmophora gracilis*,
- Lipa = *Licmophora paradoxa*,
- Lipf = *Licmophora pfannkuckae*,
- Lisp = *Licmophora* sp.,
- Maer = *Mastogloia erythraea* var. *grunowii*,
- Masi = *Mastogloia similis*,
- Masp = *Mastogloia splendida*,
- Naab = *Navicula abunda*,
- Nicm = *Tryblionella compressa*,
- Nico = *Nitzschia coarctata*,
- Nipa = *Nitzschia parastrotrata*,
- Nive = *Nitzschia ventricosa*,
- Pasu = *Paralia sulcata* s.l.,
- Psc = *Psammodyctyon panduriforme* var. *continuum*,
- Pspa = *Psammodyctyon panduriforme*,
- Rhad = *Rhabdonema adriaticum*,
- Rhmu = *Rhopalodia musculus*,
- Rhpa = *Rhopalodia pacifica*,
- Stun = *Striatella unipunctata*,
- Tras = *Trachyneis aspera*,
- Trco = *Tryblionella coarctata*.

hard substrates from the Adriatic Basin (Munda, 2005; Totti *et al.*, 2007) and for coastal rocks of the Mediterranean (cf. Álvarez-Blanco & Blanco, 2014). Taxa of *Mastogloia*, one of the largest diatom genera (Pennesi *et al.*, 2011 and references therein), can be found within different biotopes (Çolak Sabanci, 2013), but in general high abundances of *Mastogloia* species have been reported in epipelagic and epiphytic flora (Martinez-Goss & Evangelista, 2011). In our study, the most abundant *Mastogloia* was the epilithic *M. similis*, found in the shallow sample during the summer. On the contrary, this taxon was found by Çolak Sabanci (2013) in shallow brackish habitats during winter. *Navicula* includes species with a very wide ecological range (Krammer & Lange-Bertalot, 1991), while *Amphora* and *Achnanthes* are typically recorded in the more nutrient-poor regions (Agatz *et al.*, 1999).

In our study, the monthly distribution of most diatom taxa was irregular, and a high number of sporadic taxa were recorded and statistically significant differences between the shallow and deep sites were found.

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 taxa 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 coffeiformis*, were the most frequently recorded diatom taxa, not only on the natural hard substrata (Çolak Sabanci, 2012), but on all different substrata (epiphytic, epilithic, epizoic) (Car *et al.*, 2012;



Chen *et al.*, 2010; De Stefano *et al.*, 2000, 2008; Korte & Blinn, 1983; Round *et al.*, 1961; Siqueiros-Beltrones *et al.*, 1985; Sullivan, 1978, 1984; Totti *et al.*, 2007, 2009; Wuchter *et al.*, 2003). Romagnoli *et al.* (2014) also suggest that these taxa do not seem to have a preference either for geographic region or for the type of substrate. *Cocconeis scutellum* was defined as typical epiphytic taxon (Ulanova & Snoeijs, 2006).

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<sub>4</sub> 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 (Snoeijs, 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 temporally 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 groenlandica* (Cleve) Grunow [B. W. \*]  
*Achnanthes longipes* C. Agardh  
*Achnanthes parvula* (Kützing) [S. Su. \*]  
*Achnanthes pseudogroenlandica* Hendey  
*Achnanthes septata* var. *incurvata* (Østrup) Cleve-Euler [A]  
*Achnanthes* sp. [S]  
***Actinocyclus gallicus*** F. Meister [B]  
*Actinocyclus ochotensis* A. P. Jousé  
*Actinocyclus splendens* J. Rattray [B. W. \*]  
*Actinocyclus subtilis* (W. Gregory) Ralfs  
***Actinoptychus senarius*** (Ehrenberg) Ehrenberg [S. A. \*]  
*Actinoptychus adriaticus* Grunow [S. Su. \*]  
*Actinoptychus octonarius* (Ehrenberg) Kützing [B. Sp]  
*Actinoptychus splendens* (Shadbolt) Ralfs [\*]  
***Amphora abludens*** R. Simonsen [S]  
*Amphora arenaria* Donkin  
*Amphora bigibba* var. *interrupta* (Grunow) Cleve  
*Amphora constricta* (Ehrenberg) W. Carruthers [S. Sp. \*]  
*Amphora delicatissima* Krasske [B. Su. \*]  
*Amphora gacialis* W. Smith [S. Sp]  
*Amphora graeffeana* 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]  
***Ardissonea crystallina*** (C. Agardh) Grunow  
*Ardissonea formosa* (Hantzsch) Grunow  
*Ardissonea robusta* (Ralfs ex Pritchard) De Notaris [\*]  
***Aulacoseira granulata*** (Ehrenberg) Simonsen [B. Sp. \*]  
***Azpeitia nodulifera*** (A. Schmidt) G. A. Fryxell & P. A. Sims [B. A. \*]  
***Bacillaria paxillifera*** (O. F. Müller) T. Marsson  
*Bacillaria socialis* (Gregory) Ralfs  
***Berkeleya scopulorum*** (Brébisson ex Kützing) E. J. Cox [B. Sp]  
***Biddulphia biddulphiana*** (J. E. Smith) Boyer  
*Biddulphia tuomeyi* (J. W. Bailey) Roper [B. A]  
***Brebissonia lanceolata*** (C. A. Agardh) R. K. Mahoney & Reimer [S]  
***Caloneis bicuneata*** (Grunow) Boyer [B. Su. \*]  
*Caloneis excentrica* (Grunow) Boyer [B. A]  
*Caloneis liber* (W. Smith) Cleve  
*Caloneis liber* var. *linearis* Cleve  
***Campylodiscus innominatus*** R. Ross & Abdin [B]  
***Cistula lorenziana*** (Grunow) Cleve [B]  
***Cocconeopsis orthoneoides*** (Hustedt) Witkowski [B. A]  
***Cocconeis costata*** Gregory [S. Su. \*]  
*Cocconeis costata* var. *hexagona* Grunow [S. A. \*]  
*Cocconeis dirupta* W. Gregory [S]  
*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. Schmidt [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. \*]  
***Coronia decora*** (Brébisson) Ruck & Guiry  
*Coscinodiscus* sp. [B]  
***Cyclotella meneghiniana*** Kützing [B. Su. \*]  
***Diatoma vulgare*** [B. Sp]  
***Dimeregramma fulvum*** (W. Gregory) Ralfs [B. W. \*]  
*Dimeregramma minus* (W. Gregory) Ralfs  
***Diploneis aestuari*** 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. *dubia* 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 rex* 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 splendida* Cleve [\*]  
*Diploneis stroemii* Hustedt [S. W. \*]  
*Diploneis vacillans* (A. Schmidt) Cleve [\*]  
*Diploneis vacillans* var. *renitens* (A. Schmidt) Cleve [\*]  
*Diploneis weissflogii* (A. W. F. Schmidt) Cleve [B]  
*Diploneis* sp.  
***Encyonema ventricosum*** (C. Agardh) Grunow [S. Su. \*]  
***Entomoneis paludosa*** (W. Smith) Reimer  
***Fallacia floriniae*** (M. Møller) Witkowski [B]  
*Fallaciaforcipata* (Greville) Stickle & D. G. Mann  
*Fallacia litoricola* (Hustedt) D. G. Mann [S. Su. \*]  
*Fallacia pygmaea* (Kützing) Stickle & D. G. Mann [\*]  
*Fallacia subforcipata* (Hustedt) D. G. Mann [B. Sp. \*]  
***Fragilaria sopotensis*** Witkowski & Lange-Bertalot [B. Su]  
*Fragilaria* sp. [A. \*]  
***Gomphonema olivaceum*** (Hornemann) Brébisson [B. Su. \*]  
***Grammatophora gibberula*** Kützing [S. W. \*]



*Grammatophora macilentata* W. Smith [B]  
*Grammatophora marina* (Lyngbye) Kützing  
*Grammatophora oceanica* Ehrenberg  
*Grammatophora oceanica* var. *subtilissima* (J. W. Bailey) De Toni [B. Su. \*]  
*Grammatophora pacifica* [S. A.]  
***Halamphora acutiuscula*** (Kützing) Levkov [B]  
*Halamphora coffeiformis* (C. Agardh) Levkov  
*Halamphora costata* (W. Smith) Levkov [B]  
*Halamphora exigua* (W. Gregory) Levkov [B. W.]  
*Halamphora holsatica* (Hustedt) Levkov [B. Sp. \*]  
*Halamphora kolbei* (Aleem) Álvarez-Blanco & S. Blanco  
*Halamphora subangularis* (Hustedt) Levkov [B. Su. \*]  
*Halamphora subholsatica* (Krammer) Levkov [\*]  
***Haslea duerrenbergiana*** (Hustedt) F. A. S. Sterrenburg [Su. \*]  
*Haslea spicula* (Hickie) Bukhtiyarova [B. Sp.]  
***Hyalosira interrupta*** (Ehrenberg) J. N. Navarro [\*]  
***Hyalosynedra laevigata*** (Grunow) D. M. Williams & Round  
***Licmophora abbreviata*** C. Agardh [\*]  
*Licmophora debilis* (Kützing) Grunow [Su. \*]  
*Licmophora ehrenbergii* (Kützing) Grunow [\*]  
*Licmophora flabellata* (Greville) C. Agardh  
*Licmophora gracilis* (Ehrenberg) Grunow  
*Licmophora paradoxa* (Lyngbye) C. Agardh  
*Licmophora pfannkuckae* Giffen  
*Licmophora remulus* (Grunow) Grunow  
*Licmophora* sp.  
***Lyrella abrupta*** (Gregory) D. G. Mann [\*]  
*Lyrella fogedii* Witkowski, Lange-Bertalot & Metzeltin [B]  
*Lyrella henedyi* (W. Smith) Stickle & D. G. Mann [S. Sp. \*]  
*Lyrella lyra* (Ehrenberg) Karajeva [\*]  
*Lyrella lyroides* (Hendey) D. G. Mann  
*Lyrella spectabilis* (Gregory) D. G. Mann  
***Mastogloia adriatica*** Voigt [S. A.]  
*Mastogloia binotata* (Grunow) Cleve  
*Mastogloia borneensis* Husted [S. Su. \*]  
*Mastogloia crucicula* (Grunow) Cleve [B]  
*Mastogloia crucicula* var. *alternans* Zanon [B. Sp.]  
*Mastogloia cuneata* (Meister) R. Simonsen [B. Su.]  
*Mastogloia cyclops* Voigt [Su. \*]  
*Mastogloia emarginata* Hustedt [S. Su. \*]  
*Mastogloia erythraea* Grunow [B. A.]  
*Mastogloia erythraea* var. *grunowii* Foged [B. Su. \*]  
*Mastogloia exigua* F. W. Lewis [B. A.]  
*Mastogloia exilis* Hustedt [B]  
*Mastogloia fallax* Cleve [S. Su.]  
*Mastogloia fimbriata* (T. Brightwell) Grunow [B]  
*Mastogloia grunowii* A. Schmidt [S. Su.]  
*Mastogloia horvathiana* Grunow [A.]  
*Mastogloia ignorata* Hustedt  
*Mastogloia inaequalis* Cleve [\*]  
*Mastogloia latecostata* Hustedt [B. Su. \*]  
*Mastogloia linearis* Simonsen [S. Su. \*]  
*Mastogloia macdonaldii* Greville [S. A. \*]  
*Mastogloia mauritiana* Brun [\*]  
*Mastogloia mediterranea* Hustedt [B]  
*Mastogloia ovata* Grunow [B]  
*Mastogloia ovulum* Hustedt [B. Su.]  
*Mastogloia paradoxa* Grunow [B. A.]  
*Mastogloia peragalloi* Cleve [B]  
*Mastogloia pseudolatecostata* T. A. Yohn & R. A. Gibson  
*Mastogloia pumila* (Grunow) Cleve [B. \*]  
*Mastogloia quinquecostata* Grunow [B. W. \*]  
*Mastogloia regula* Hustedt [B. Su. \*]  
*Mastogloia similis* Hustedt  
*Mastogloia smithii* Thwaites ex W. Smith [S. A. \*]  
*Mastogloia splendida* (Gregory) H. Pergallo [\*]  
*Mastogloia* sp.  
***Navicula abunda*** Hustedt [S. Su.]  
*Navicula agnita* Hustedt  
*Navicula arenaria* Donkin [Su. \*]  
*Navicula borneoensis* Hustedt [B. Su. \*]  
*Navicula cancellata* Donkin  
*Navicula cincta* (Ehrenberg) Ralfs [S. Su. \*]  
*Navicula digitoradiata* (W. Gregory) Ralfs [B. Su.]  
*Navicula directa* (W. Smith) Ralfs  
*Navicula eidrigiana* J. R. Carte [S. Su. \*]  
*Navicula flagellifera* Hustedt [\*]  
*Navicula longa* (W. Gregory) Ralfs [B. A.]  
*Navicula longa* var. *irregularis* Hustedt [B. A.]  
*Navicula pennata* A. Schmidt [B]  
*Navicula pinnata* Pantocsek [B. Su. \*]  
*Navicula subrostellata* Hustedt [B. Sp. \*]  
*Navicula* sp. [S]  
***Nitzschia agnita*** Hustedt [B. Su. \*]  
*Nitzschia angularis* W. Smith [\*]  
*Nitzschia bartholomei* Grunow [B. Su. \*]  
*Nitzschia distans* W. Gregory  
*Nitzschia distans* var. *tumescens* Grunow [B. Su.]  
*Nitzschia frustulum* (Kützing) Grunow [Su.]  
*Nitzschia fusiformis* Grunow [B]  
*Nitzschia incurvata* var. *lorenziana* R. Ross  
*Nitzschia insignis* W. Gregory [S. Sp. \*]  
*Nitzschia liebethuthii* Rabenhorst [\*]  
*Nitzschia longissima* (Brébisson) Ralfs [\*]  
*Nitzschia macilentata* W. Gregory  
*Nitzschia marginulata* var. *didyma* Grunow [B. A. \*]  
*Nitzschia normanii* Grunow [B. Su. \*]  
*Nitzschia pararostrata* (Lange-Bertalot) Lange-Bertalot  
*Nitzschia scalpelliformis* Grunow [A. \*]  
*Nitzschia sigma* (Kützing) W. Smith [\*]  
*Nitzschia ventricosa* Kitton [\*]  
*Nitzschia* sp. [\*]  
***Opephora marina*** (W. Gregory) Petit [S. A. \*]  
*Opephora mutabilis* (Grunow) Sabbe & Wyverman [S. Su.]  
*Opephora pacifica* (Grunow) Petit [Su.]  
***Pantocsekiella ocellata*** (Pantocsek) K. T. Kiss & E. Ács [\*]  
***Paralia sulcata*** (Ehrenberg) Cleve (defined as *P. s. s.l.*)  
***Petrodictyon gemma*** (Ehrenberg) D. G. Mann  
***Pinnularia clavicularis*** Schulz [B]  
***Plagiodiscus martensianus*** Grunow & Eulenstein [B]  
*Plagiodiscus nervatus* Grunow [B. A. \*]  
***Plagiogramma staurophorum*** (W. Gregory) Heiberg [B. W. \*]  
***Plagiotropis lepidoptera*** (W. Gregory) Kuntze  
*Plagiotropis tayrecta* T. B. B. Paddock  
***Planothidium delicatulum*** (Kützing) Round & Bukhtiyarova [B. Su. \*]  
*Planothidium quarnerensis* (Grunow) Witkowski, Lange-Bertalot & Metzeltin [B]  
***Platessa salinarum*** (Grunow) Lange-Bertalot [B. Su. \*]  
***Pleurosigma formosum*** W. Smith  
*Pleurosigma itium* Ricard [S. Su. \*]  
*Pleurosigma* sp. [Su.]  
***Podocystis adriatica*** (Kützing) Ralfs [S]  
***Psammodictyon panduriforme*** (W. Gregory) D. G. Mann  
*Psammodictyon panduriforme* var. *continuum* (Grunow) Snoeijis [B. Su.]  
*Psammodictyon rudum* (Cholnoky) D. G. Mann  
***Psammodiscus nitidus*** (W. Gregory) Round & D. G. Mann [Su.]  
***Rhabdonema adriaticum*** Kützing

*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-Bertalot [B. A]  
*Striatella unipunctata* (Lyngbye) C. Agardh  
*Surirella fastuosa* (Ehrenberg) Ehrenberg  
*Surirella scalaris* M. H. Giffen [B]  
*Synedra fulgens* (Greville) W. Smith  
*Tabularia investiens* (W. Smith) D. M. Williams & Round [\*]  
*Talaroneis furcigera* (Grunow) Sterrenburg

*Terpsinoë americana* (Bailey) Grunow [B]  
*Tetramphora decussata* (Grunow) Stepanek & Kociolek [B]  
*Tetramphora rhombica* (Kitton) Stepanek & Kociolek [S]  
*Thalassiosira* sp. [B]  
*Toxarium hennedyanum* (Gregory) Pelletan [B. Sp. \*]  
*Toxarium undulatum* J. W. Bailey  
*Trachyneis aspera* (Ehrenberg) Cleve  
*Trigonium formosum* (Brightwell) Cleve [S]  
*Tryblionella apiculata* W. Gregory [B. Su.\*]  
*Tryblionella coarctata* (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]