ISSN: 0001-5113 AADRAY	ACTA ADRIAT., 62 (1): 21 - 44, 2021	ORIGINAL SCIENTIFIC PAPER

Comparison of benthic diatom community structures on natural and artificial substrates in marine lake (Adriatic Sea)

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In this study, the diatoms of three alternative habitats (epilithon, epiphyton and artificial substrate) were compared to understand the differences in composition on artificial and natural substrates. For this purpose, the samples were collected weekly between 11th August and 2nd September 2016 at a sampling site in a shallow marine lake, Mrtvo More (Dead Sea) on Lokrum Island near Dubrovnik (South Adriatic, Croatia).

In addition to detailed light microscopic analysis, ultrastructural analysis of benthic diatoms from Lake Mrtvo More was performed for the first time using scanning electron microscopy (SEM). A total of 97 taxa were identified in 12 samples. Cocconeis scutellum Ehrenberg and Halamphora coffeiformis (C.Agardh) Levkov were the most frequent taxa in the samples. Shannon-Wiener diversity index (H') values varied from 1.78 (in September on Padina sp.) to 4.52 (in August on glass). According to non-metric multidimensional scaling ordination, there were two groups: epilithon and artificial glass substrate as Group1 and macroalgae as Group2.

The results of the analysis showed that the diatom communities developing on artificial substrates accurately corresponded to the diatom community of a rock substrate and thus can be used as a representative alternative tool for studies of epilithic diatoms in further experiments.

Key words: Bacillariophyta; shallow marine lake; species identification; biodiversity; NE Mediterranean

INTRODUCTION

The marine lake Mrtvo More is located on Lokrum Island near Dubrovnik. Since 1948, Lokrum Island has been a special reserve of forest vegetation and today the island (72 ha) and the sea-belt are also a Natura 2000 site (CRNČEVIĆ *et al.*, 2017). As a geomorphological phenomenon, the Mrtvo More with its pit hole and the channel connecting the marine lake to the open sea is a Natura 2000 habitat of the type 'Submerged or partially submerged sea cave'.

Benthic diatoms are unicellular or colonial organisms that are free-living or attached to the substrate by gelatinous extrusion and play an important role in primary production in marine ecosystems (FALKOWSKI *et al.*, 2004). They are used as water quality indicators as well as in paleoecological reconstructions due to their ecophysiological features (CIBIC & BLASUTTO, 2011; STEVENSON & PAN, 1999). Knowledge of the structure of the benthic diatom community and the ecology of individual taxa is a unique source of information in the study of the dynamics of marine microphytobenthos.

Some of the potential advantages of using artificial substrates in diatom studies include reduced effort and cost of sampling and processing, less habitat disruption, and substantially improved sampling precision (LAMBERTI & RESH, 1985; LANE *et al.*, 2003). The greatest benefit of using an artificial substrate over sampling natural habitats is the consequent standardization between replicates. Additionally, the use of artificial substrates for monitoring purposes does not compromise the algal settlements and artificial substrates can be used globally as they are not limited by the natural lifecycle and distribution range of the macroalgae (CARREIRA-FLORES *et al.*, 2020).

Although artificial substrates have been used in diatom studies for almost 100 years (NAUMANN, 1915; cited in TUCHMAN & STEVENSON 1980; HOAGLAND *et al.*, 1986; BARBIERO, 2000), there are still concern whether diatom communities developing on artificial substrates accurately correspond to communities developing on natural substrates (LANE *et al.*, 2003). Ideally artificial substrates should support a community composition and abundance that is representative of natural substrates at the same site (TUCHMAN & STEVENSON, 1980; LAMBERTI & RESH, 1985; LANE *et al.*, 2003). It may be, for example, that diatom communities developing on artificial substrates more closely represent the diatom community of a particular natural substratum (LANE *et al.*, 2003). Hence the need exists for further comparative research examining diatom community structure on artificial and various natural substrates.

In the Adriatic Sea, benthic diatoms from natural sediment samples and artificial substrates have been reported from various areas, including: the Gulf of Trieste (BARTOLE *et al.*, 1991-94; SDRIGOTTI *et al.*, 1999; MUNDA, 2005), the Venice Lagoon (TOLOMIO & ANDREOLI, 1989; TOLOMIO *et al.*, 1999; FACCA *et al.*, 2002; TOLOMIO *et al.*, 2002; FACCA & SFRISO, 2007), the Northwestern Adriatic coast (TOTTI 2003; TOTTI *et al.*, 2007; FRANZO *et al.*, 2015, and references therein), and the Eastern Adriatic Sea coast (BURIĆ *et al.*, 2004; MIHO & WITKOWSKI, 2005; CAPUT *et al.*, 2008; LEVKOV *et al.*, 2010; CAR *et al.*, 2012, 2019a,b, 2020; NENADOVIĆ *et al.*, 2015; MEJDANDŽIĆ *et al.*, 2015; HAFNER *et al.*, 2018a,b; KANJER *et al.*, 2019). Nevertheless, knowledge about the composition and spatial distribution of marine benthic diatoms around the coast of the South Adriatic remains limited.

The objective of this work was to contribute to the knowledge of microphytobenthos in the Adriatic Sea, by studying benthic diatom communities on an immersed artificial substrate and natural substrates with various physicochemical properties in the shallow marine lake Mrtvo More (Dead Sea) on Lokrum Island near Dubrovnik (South Adriatic, Croatia) in a period of intense anthropogenic influence due to tourist activities.

The main goal of this work was to investigate the potential for using artificial substrates for benthic diatom assemblage monitoring as an alternative to natural epiphyton and epilithon samples. Two hypotheses were proposed and tested: (1) that natural rocks and glass artificial substrates had similar diatom community structure; and (2) macroalgae were sheltering different assemblages of benthic diatoms.

MATERIAL AND METHODS

Study area

The study was carried out at one station (42°37'21"N; 18° 7'14"E) in the roughly circular-shaped marine lake Mrtvo More (Croatian: 'Dead Sea') situated in the southern part of the island of Lokrum near Dubrovnik (South Adriatic), Croatia (Fig. 1).

The island of Lokrum has a typical Mediterranean climate. The average annual air temperature of the Dubrovnik area is 16 °C. The average temperature of the warmest months (July and August) is about 25 °C and of the coldest (January and February) about 9 °C. The rainiest and cloudiest month is November, and the driest and clearest is July. The average annual precipitation on Lokrum is 1360 mm, while during 2016 a yearly rainfall of 1054 mm was recorded (meteorological data for the Dubrovnik area for 1961-2017, Croatian Meteorological and Hydrological Service; Fig. S1). Lokrum is directly exposed to sea currents from the south and the Strait of Otranto, which influences the distribution of benthic organms and plankton (BATISTIĆ *et al.*, 2014; GARIĆ & BATISTIĆ, 2016).

Sampling strategy and analyses

Physical-chemical parameters

Water samples for analysis of physicochemical variables were taken weekly (Table 1) from 11th August to 2nd September 2016, at the same place where diatom sampling was carried out, i.e. near the bottom (1 m depth) at the investigated station located in the southern part of the Island of Lokrum (Fig. 1). All the samples were taken at the same time of the day (from 10 till 11 am). Temperature (T) and salinity (S) were measured using a WTW Multiline P4 multiparametric sounding lineprobe. Seawater samples were taken with a 5 L Niskin bottles and kept cold until analysis. Analyses of measured nutrients [nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), total inorganic nitrogen (TIN = NO_3^- + NO_2^- + NH_4^+), orthophosphate (PO_4^{3-}) and orthosilicate (SiO_4^{4-})] and chlorophyll *a* (Chl *a*) were performed following the standard procedures (APHA, 2005). Samples for NO_3^-, NO_2^-, PO_4^{3-}, and SiO_4^{4-} were frozen (-22 °C) and analysed in laboratory according to Strickland

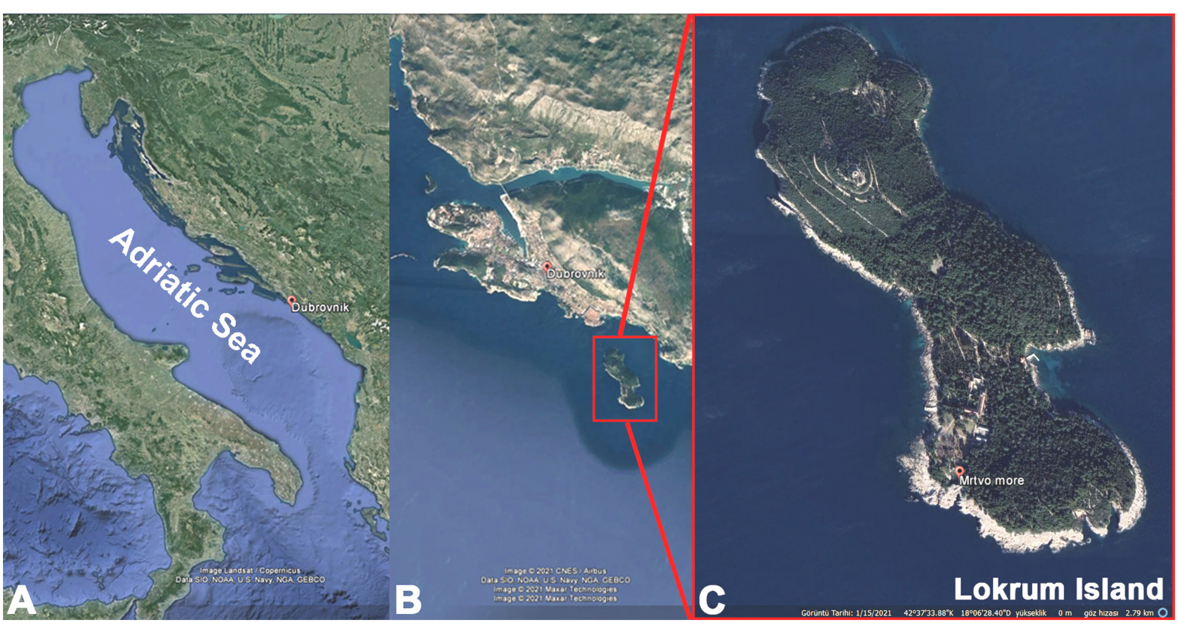


Fig. 1. Map of the study area. A) Location of sampling site in Adriatic Sea (●); B) Close up map of Dubrovnik area; C) position of Mrtvo More on southern side of Island of Lokrum (Google Earth, 6 June 2021)

and Parsons (1972). Subsamples (50 mL) for NH_4^+ were fixed immediately after collection with 2 mL of 1 molL^{-1} phenol/EtOH, kept at 4 °C and later analysed according to IVANČIĆ & DEGOBBIS (1984). Chl *a* was determined from 1 L sub-samples filtered through Whatman GF/F glass-fiber filters and stored at -20 °C for a period of 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).

Dissolved oxygen was determined by the Winkler method and oxygen saturation (O_2/O_2') was calculated from the 100% solubility of oxygen (O_2) in seawater as a function of temperature and salinity (WEISS, 1970; UNESCO, 1973). Trophic status (TRIX index; [$\log_{10}$(Chl *a*×D%O×DIN×TP)+k]/m) was calculated according to factors which represent a variable reflected in the trophic state: Chl *a*=chlorophyll *a* concentration (μgL^{-1}), D%O=dissolved oxygen (absolute deviation from 100 % oxygen saturation), dissolved inorganic nitrogen DIN and TP=total phosphorus (μgL^{-1}) (VOLLENWEIDER *et al.*, 1998; GIOVANARDI & VOLLENWEIDER, 2004; KARYDIS, 2009; PRIMPAS & KARYDIS, 2011). The parameters k=1.5 and m = 1.2, are scale coefficients, introduced to fix the lower limit value of the Index and the extension of the related Trophic Scale, from 0 to 10 TRIX units (0–4 oligotrophic, 4–5 mesotrophic, 5–6 eutrophic, 6–10 extremely eutrophic).

Experimental setup and diatom analysis

In order to test two proposed hypotheses, diatom samples were taken from the rocks, from the autochthonous brown alga *Padina* sp. and from standard glass microscope slides measuring about 75 mm x 25 mm x 1 mm used as a substrate for biofilm formation from the same locality to compare the diatom community on the artificial substrate (glass) with diatom communities from natural substrates. As an artificial substrate, microscope glass slides were fixed on the upper side of a plexiglass sheet. On 19 April 2016, the plexiglass sheet was submerged horizontally with four diving weights at a depth of approximately 1 m (i.e. on the bottom of Lake Mrtvo More) about 2 m offshore. Every week the plexiglass sheet was hauled up and another microscopic slide for diatom analysis was taken out and gently plunged into filtered seawater (Millipore, acetate cellulose 0.22 μm). For this survey, samples were collected at weekly intervals from the 11th of August to 2nd of September 2016.

For a quantitative biofilm assay a microscopic glass surface of 1 cm^2 was scraped using a razor blade, and the microalgae were collected in Falcon tubes. Samples were preserved by adding a known amount (3 mL) of solution (3%) of formaldehyde-filtered seawater. Quantitative analysis of homogenized samples was determined with an inverted microscope (Olympus IX 71) equipped with phase contrast. Results are expressed as number of cells per cm^2.

The natural epilithic diatom communities were obtained by scraping off the randomly collected submerged rocks of 5-10 cm^2 on which the diatom biofilm was visible. The upper parts of the rocks were rubbed with a toothbrush in a plastic bag of 1 L in which 200 mL of sterile freshly filtered seawater was added and the mixture decanted into 250 mL polyethylene bottles (WINTER & DUTHIE, 2000). All samples were preserved with 4% formaldehyde. Over a period of one month 12 diatom samples were collected: 4 diatom samples from artificial substrates together with 4 diatom samples from *Padina* sp. and 4 diatom samples from the rocks. There were no replicate samples for diatom analyses.

After a quantitative biofilm assay, the glass slides were treated with 10% hydrochloric acid (HCl) to remove carbonates and cleaned of organic material by boiling with 30% H_2O_2. They were then rinsed with deionized water, pipetted onto ethanol-cleaned cover-slips and left to air dry before mounting in Naphrax®. Detailed light microscopy (LM) analysis was performed on permanent slides of processed material (hydrogen peroxide treated) with a Nikon E600 microscope at a magnification of

1000 x. The abundances of the species were expressed as percentages of the total number of frustules counted (relative abundances in %). In total, 400 valves per each sample were counted. Permanent slides were deposited in the diatom collection of the Institute for Marine and Coastal Research, University of Dubrovnik, Dubrovnik, Croatia [no. AC-MM-517-528].

For scanning electron microscopy (SEM) a drop of the cleaned sample was air-dried on aluminium stubs and coated with gold using Emitech Quorum K550X. SEM observations were made at the Eskisehir Osmangazi Technical University (Turkey) using a Zeiss ULTRA Plus.

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), HARTLEY (1986), SNOEIJS (1993, 1999), SNOEIJS & POTAPOVA (1995), SNOEIJS & KASPEROVICIENÉ (1996), SNOEIJS & BALASHLOVA (1998), HARTLEY *et al.* (1996), WITKOWSKI *et al.* (2000) and KOCIOLEK *et al.* (2020). Nomenclature follows AlgaeBase (GUIRY & GUIRY, 2020).

Statistical analysis

The data were analysed using the Primer v.6 software (CLARKE & GORLEY, 2006) and Statistica 7.0 (StatSoft, Inc. 2004).

The diatom community diversity and structure were investigated for each diatom sample. The Shannon-Wiener Biodiversity Index, the Margalef index (KWANDRANS, 2007) and the Pielou's evenness (PIELOU, 1966) were computed.

Raw diatom counts were expressed as relative abundance and transformed by square root to normalize the data. CLUSTER (using the group average mode and the SIMPROF test for significance) and non-metric multidimensional scaling (nMDS) analyses based on the Bray–Curtis dissimilarity matrix (LEGENDRE & LEGENDRE, 1983; CLARKE & GORLEY, 2006) of the relative abundance data of 97 taxa over 12 samples on square-root transformed density data, were used to define the benthic diatom abundance with respect to sampling dates. The significant differences among samples were determined using SIMPROF test at the 0.05 level (SIMPROF; $p < 0.05$) (ZHANG *et al.,* 2012; YUANYUAN *et al.*, 2014). Similarity percentage analyses (SIMPER, CLARKE & WARWICK, 1994) were used to identify the percentage contribution of each taxon to the Bray–Curtis dissimilarity between the averages of groups observed in the nMDS plot. ANOSIM randomization (CLARKE & WARWICK, 1994) was used to test for significant differences in species composition of diatoms growing on various substrates over the sampling period and for clusters that were significantly different in the cluster analysis. Canonical analysis of principal coordinates (CAP) was used to summarize the structure of diatom assemblages over the substrates and to determine which diatom taxa were considered important and directly responsible for the variations observed in the groups.

The relationship between the most abundant species and the main physico-chemical parameters was analysed by correlation matrices using Statistica 7.0. A total of 10 taxa with frequency

Table 1. Weekly values of the physical–chemical parameters in the Mrtvo More in 2016. T - temperature (°C); S - salinity (psu); O2/O2' - oxygen saturation; Chl a - chlorophyll-a concentrations (μg/L); SiO44- - silicate (μM); PO43- - phosphate (μM); NO3- - nitrate (μM); NO2- - nitrite (μM); NH4+ - ammonium (μM); TIN - total inorganic nitrogen [(TIN) = (NO3-) + (NO2-) + (NH4+)] (μM); TRIX=[log10(Chl a×D%O×DIN×TP)+k]/m, the range of the TRIX scale from 0 to 10 (0–4 oligotrophic, 4–5 mesotrophic, 5–6 eutrophic, 6–10 extremely eutrophic).

Season	Summer			Autumn
Date	11-Aug	17-Aug	24-Aug	2-Sep
T	26,60	23,70	21,70	24,00
S	36,67	34,07	37,07	36,77
NO_3^-	3,74	8,34	2,69	3,93
NO_2^-	1,22	0,70	0,65	1,00
NH_4^+	0,95	0,69	1,10	3,14
TIN	5,91	9,73	4,44	8,06
PO_4^{3-}	0,48	0,18	0,29	0,48
SiO_4^{4-}	6,93	11,35	7,22	9,43
Chl *a*	3,50	0,66	3,39	3,17
O_2/O_2'	0,86	0,84	0,73	0,87
TRIX	5,54	4,91	5,58	5,65

of occurrence ≥ 30% and relative abundance ≥ 5.9% from 12 samples collected in Mrtvo More in August and September 2016 were selected for correlation analysis. Spearman-Rank correlations were performed after the Kolmogorov-Smirnov test was used for testing normality of the data distribution. Environmental data were first transformed [log(x+1)] (CASSIE, 1962) to enable the correlation tests between variables. Only significant ($p < 0.05$) values are reported.

RESULTS

Environmental conditions

In the investigated period, the water temperature in Lake Mrtvo More varied between 21.7 °C and 26.6 °C, with an average of 24 °C (Table 1). The average salinity was 36.149 psu. Average nutrient concentrations were: 4.67 µM NO_3^-, 0.89 µM NO_2^-, 1.47 µM NH_4^+, 0.36 PO_4^{3-}, and 8.74 µM SiO_4^{4-}. The concentrations of total inorganic nitrogen (TIN) ranged from 4.44 (24th August) to 9.73 (17th August) µM and mostly follows the distribution of NO_3^-. While the minimum chlorophyll *a* concentration at the Mrtvo More site in this study was recorded on the 17th of August (0.66 µg/L), the maximum concentration of 3.50 µg/L was recorded on 11th of August. Average chlorophyll *a* concentration for the investigated period was 2.68 µg/L. Oxygen saturation (O_2/O_2') ranged from 0.73 to 0.87 (average 0.83). The average value of trophic index TRIX was 5.42, indicating eutrophic state according to Vollenweider's scale (VOLLENWEIDER *et al.*, 1998).

Taxonomic composition of the benthic diatom community

During this study, a total of 97 specific and infraspecific diatom taxa were identified in the Mrtvo More (Table S1). A total of 42 genera were found. Genera with the greatest number of taxa were: *Nitzschia* (14 taxa), *Mastogloia* (8), *Achnanthes* (7), *Cocconeis* (6), *Halamphora* (5), *Navicula* (5), *Amphora* (4), *Licmophora* (4), *Diploneis* (3), and *Grammatophora* (3). In total, 4 genera (*Ardissonea*, *Caloneis*, *Haslea*, *Tabularia*) were represented with two taxa each, while 28 were composed of one taxon only.

Altogether, 23 taxa were found in at least 50% or more of the total number of samples and could be characterised as taxa with a higher frequency of occurrence. *Cocconeis scutellum* var. *scutellum* Ehrenberg and *Halamphora coffeiformis* (C.Agardh) Levkov were the most frequent taxa, being present in all samples. Other taxa with high frequencies (75-92%) were: *Cocconeis costata* W.Gregory (92%), *Cocconeis pseudomarginata* W.Gregory (92%), *Nitzschia valdestriata* Aleem & Hustedt (83%), *Grammatophora oceanica* Ehrenberg (75%), and *Licmophora paradoxa* (Lyngbye) Agardh (75%). In total, 37 taxa were found only once (sporadic taxa) during the investigated period (Table S1).

Regarding the substrate type, 47 diatom taxa have been characterized as exclusive; 26 were found only on glass, 16 were only on rock, while only 5 have been characterized as exclusively *Padina* sp. diatoms. Altogether, 27 taxa were found on all three investigated substrates.

Regarding the habitat type (*sensu* GUIRY & GUIRY, 2020), the greatest number of diatom taxa (64) have been characterized as exclusively marine (Table S1). Among truly marine diatoms, two *Cocconeis* taxa (*Cocconeis costata* W.Gregory and *C. pseudomarginata* W.Gregory) showed a high frequency of appearance and were found in more than 92% of the total number of samples. Three exclusively freshwater species were observed in the diatom composition (*Aulacoseira granulata* (Ehrenberg) Simonsen, *Amphora gracilis* Ehrenberg, *Placoneis flabellata* (F.Meister) Kimura, H.Fukushima & Ts.Kobayashi) with an average abundance of less than 1.5%, whereas 10 species were characterized with marine-brackish habitat preference. Amongst these taxa, *Achnanthes brevipes* C.Agardh, *C. scutellum* var. *scutellum*, *H. coffeiformis*, *Navicula salinicola* Hustedt, and *Synedra fulgens* (Greville) W.Smith were present with a frequency over 58%. Species with a broad habitat preference (marine to freshwater) observed in the study were *Entomoneis paludosa* (W.Smith) Reimer, *H. coffeiformis*, *N. salinicola*, and *Nitzschia sigma* (Kützing) W.Smith.

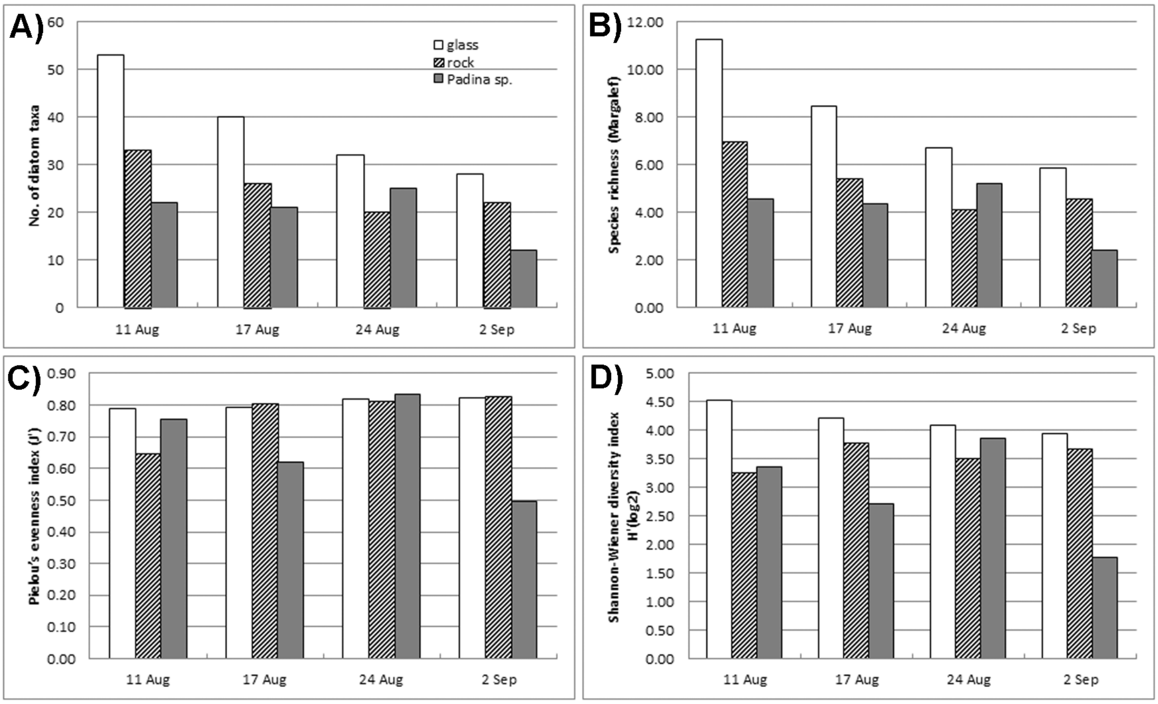


Fig. 2. Number of diatom taxa (A), Margalef's diversity index (B), Pielou's evenness index (C) and the Shannon-Wiener diatom diversity index (D) on glass, rock and Padina sp. during the period from 11 August to 2 September 2016 in Mrtvo More.

Table 2. Results of the ANOSIM test performed on species relative abundance data.

	Type of substrate (artificial/natural)	**Type of benthic diatoms (epilithic/epiphytic) = Simprof Groups 1 & 2**	**Substrate (glass, rock, *Padina* sp.)**	**Substrate (glass, rock)**	**Substrate (glass, *Padina* sp.)**
p	>0.05	0.001	0.001	>0.05	0.001
Global R	0.042	0.693	0.676	0.375	0.958

In general, the number of taxa per sample ranged from 12 (2nd September, *Padina* sp.) to 53 (11th August, glass), with an average of 28 (Fig. 2A). An average number of diatom taxa for glass, rock, and *Padina* sp., were 38, 25, and 20, respectively. Margalef species richness index was calculated as 8.09, 5.27, and 4.13 for glass, rock and *Padina* sp. respectively (Fig. 2B). For glass samples, a decrease in species richness index from the middle of August was noted and the minimum occurred in September. Pielou's species evenness ranged from 0.50 to 0.83 (the average 0.75) with the minimum occurring in September on *Padina* sp. (Fig. 2C). The species diversity index (H', log2 based) varied from 1.78 to 4.52, with an average of 3.56 (Fig. 2D). The minimum value was recorded in September on *Padina* sp. The average abundance of diatom taxa on the glass artificial substrate over the study period was 275 856 cells/cm^2 (data not shown) with a peak value of 333 076 cells/cm^2 observed on 11th of August.

According to nMDS, diatom assemblages differed significantly (ANOSIM, $p < 0.05$) between the epilithic diatom samples collected from natural rock samples and artificial glass substrates (group 1) and samples of epiphytic diatoms from *Padina* sp. (group 2) (Fig. 4). Additionally, four samples from the artificial glass substrates did not differ significantly from each other (Fig. 2, Tables S1, 2). While the diatom composition of artificial glass substrate did not differ significantly from that of rock substrate ($p > 0.05$), it differed significantly from that of macroalgae (Table 2).

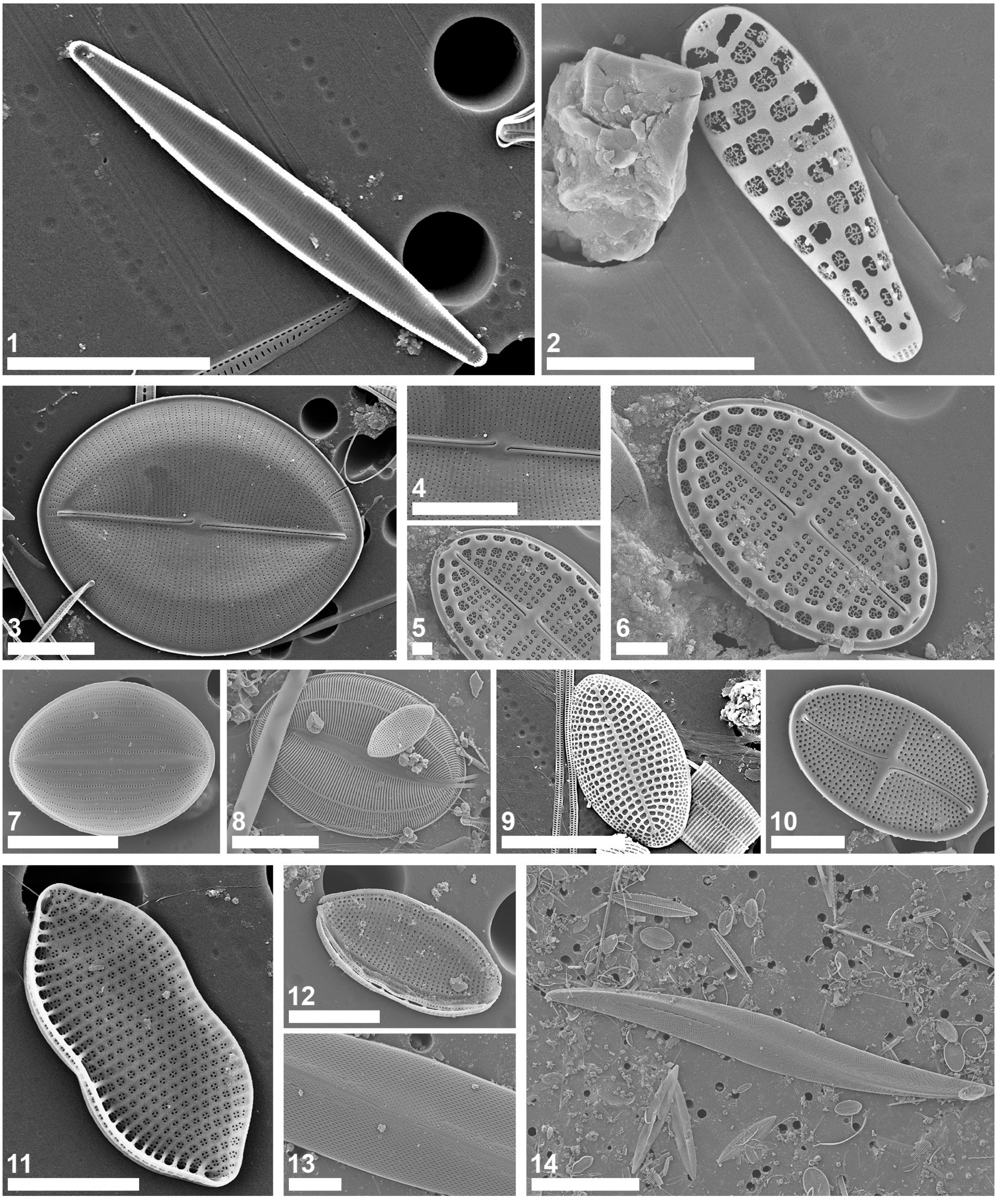


Fig. 3. Scanning electron microscope (SEM) micrographs of benthic diatoms in the Mrtvo More: Scanning electron microscope (SEM) micrographs of benthic diatoms in the Mrtvo More; 1) Tabularia fasciculata (C.Agardh) D.M.Williams & Round; 2) Gedaniella mutabilis (Grunow) Chunlian Li & Witkowski; 3, 4, 8) Cocconeis pseudomarginata W.Gregory; 5, 6) Cocconeis stauroneiformis H.Okuno; 7) Cocconeis convexa M.H.Giffen; 9) Cocconeis scutellum var. scutellum Ehrenberg; 10) Cocconeis dirupta var. flexella (Janisch & Rabenhorst) Grunow; 11) Tryblionella coarctata (Grunow) D.G.Mann; 12) Psammodictyon rudum (Cholnoky) D.G.Mann; 13, 14) Pleurosigma formosum W. Smith. Scale bar: (5): 1 µm, (6) 2 µm, (2, 10, 11, 12) 5 µm, (1, 3, 4, 7, 8, 9, 13) 10 µm, (14) 50 µm

Table 3. Correlation between 10 environmental variables and 10 diatom taxa [only significant ($p < 0.05$) values are reported]. A dataset of 10 diatom taxa (with frequency of appearance ≥ 33% and average relative abundance ≥ 5.9%) was selected. Abbreviations: Si – SiO_4^{4-}, silicate, TIN – total inorganic nitrogen, NO_3^- – nitrate, NO_2^- – nitrite, NH_4^+ – ammonium, PO_4^{3-} – phosphate, SAT – oxygen saturation (O_2/O_2'), S – salinity, CHL – chlorophyll *a* concentrations, T – temperature. Codes for diatom taxa are: Acbr = *Achnanthes brevipes* C.Agardh; Acps = *Achnanthes pseudogroenlandica* Hendey; Coco = *Cocconeis costata* W.Gregory; Cofl = *Cocconeis dirupta* var. *flexella* (Janisch & Rabenhorst) Grunow; Codi = *Cocconeis dirupta* W.Gregory; Cosc = *Cocconeis scutellum* var. *scutellum* Ehrenberg; Haco = *Halamphora coffeiformis* (C.Agardh) Levkov; Hahy = *Halamphora hyalina* (Kützing) Rimet & R.Jahn; Nasa = *Navicula salinicola* Hustedt; Rhad = *Rhabdonema adriaticum* Kützing.

	T	**S**	**NO_3^-**	**NO_2^-**	**NH_4^+**	**TIN**	**PO_4^{3-}**	**Si**	**CHL**	**SAT**
T										
S			**-0.97**						**0.99**	
NO_3^-		**-0.97**							**-0.95**	
NO_2^-										
NH_4^+										
TIN										
PO_4^{3-}										
Si										
CHL		**0.99**	**-0.95**							
SAT										
Acbr										
Acps					**0.99**					
Coco										
Cofl				**0.99**						
Codi										
Cosc										
Haco										
Hahy										
Nasa							**-0.97**			
Rhad									**-0.96**	

SIMPER analysis showed that *Cocconeis dirupta* W.Gregory, *Cocconeis dirupta* var. *flexella* (Janisch & Rabenhorst) Grunow, *Navicula salinicola* Hustedt, *Cocconeis costata* W.Gregory, *Halamphora coffeiformis* (C.Agardh) Levkov, *Achnanthes pseudogroenlandica* Hendey, and *Achnanthes brevipes* C.Agardh contributed the most (cumulatively 60%) to the variance between assemblages from groups 1 and 2. According to SIMPER analysis, *C. dirupta*, *C. costata*, *C. scutellum* var. *scutellum*, *H. coffeiformis*, *C. pseudomarginata*, and *Gedaniella mutabilis* (Grunow) Chunlian Li & Witkowski contributed the most (cumulatively 90%) to the similarity between diatom assemblages from the four *Padina* sp. samples of group 2.

According to SIMPER analysis, *C. dirupta*, *C. dirupta* var. *flexella*, *Halamphora hyalina* (Kützing) Rimet & R.Jahn, *N. salinicola*, *H. coffeiformis*, *A. pseudogroenlandica*, *A. brevipes,* and *C. costata* contributed the most (cumulatively 55%) to the variance between assemblages from artificial and natural (rock + *Padina* sp.) substrates. While average dissimilarity between these substrates was 66%, the average dissimi-

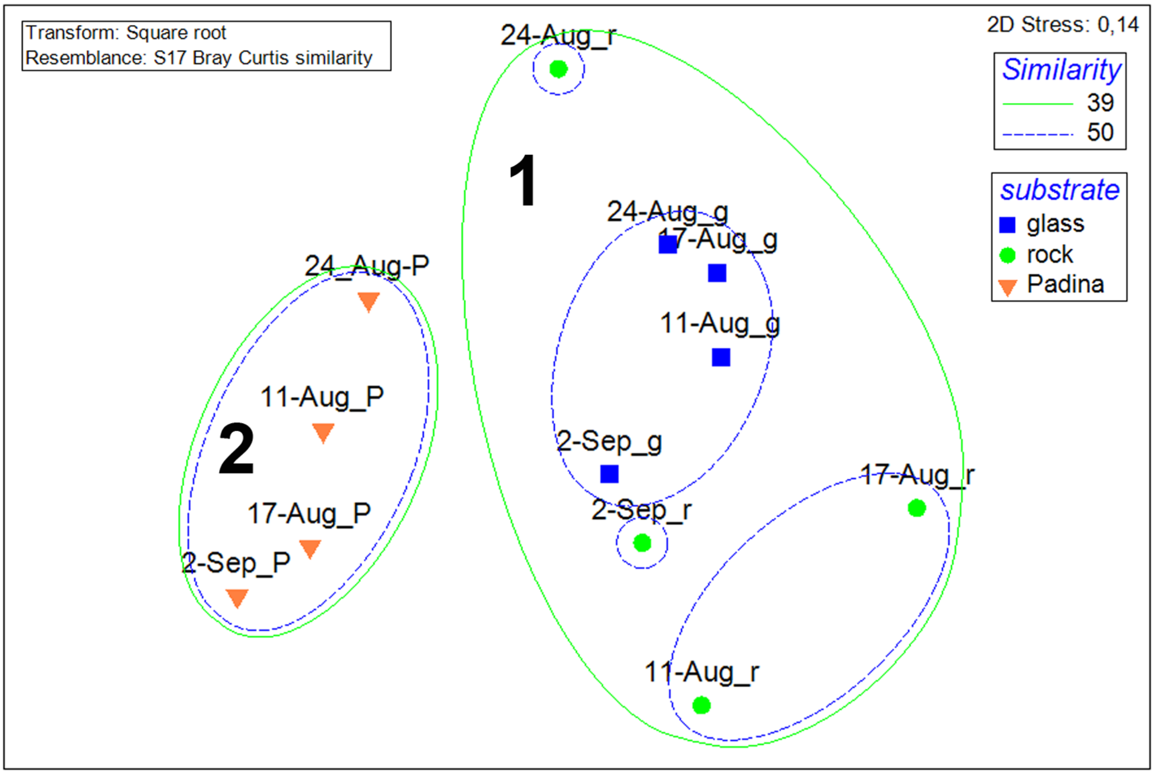


Fig. 4. Non-metric multidimensional scaling (nMDS) ordination on Bray-Curtis similarities matrices from square root transformed species-relative abundance data of periphytic diatom communities in 12 samples [4 of artificial substrate (glass slides); 4 of rock samples and 4 of Padina sp.] collected at depth of 1 m in the marine lake Mrtvo More in August-September 2016. For the ordination analysis all recorded diatom taxa were used. Numbers 1 and 2 indicate main clusters. N = 12.

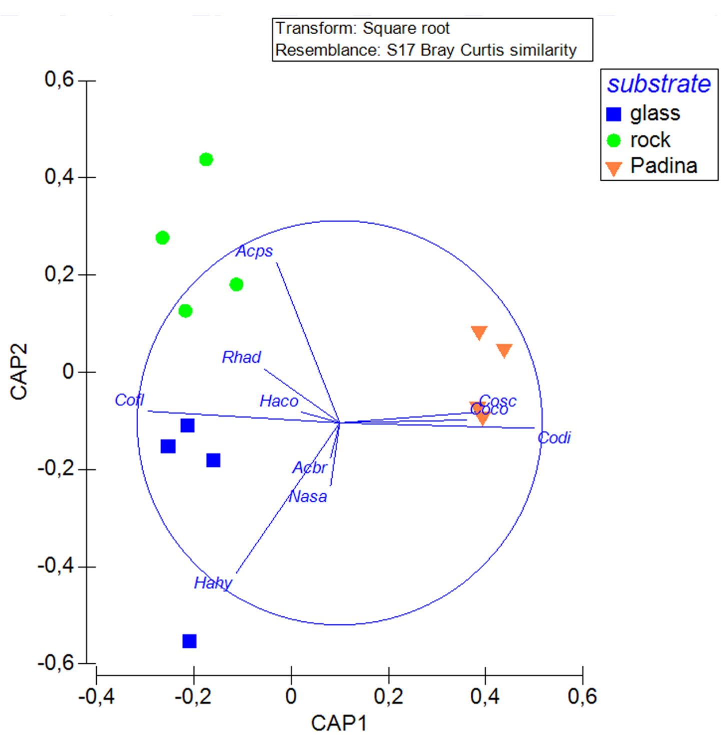


Fig. 5. Canonical analysis of Principle coordinates (CAP; Primer+PERMANOVA, U.K.). CAP biplot showing substrates and vectors of diatom relative abundance (%) data (arrows) based on 12 samples. A dataset of 10 diatom taxa (with frequency of appearance ≥ 33% and average relative abundance ≥ 5.9%) was selected. Codes for diatom taxa are: Acbr = Achnanthes brevipes C.Agardh; Acps = Achnanthes pseudogroenlandica Hendey; Coco = Cocconeis costata W.Gregory; Cofl = Cocconeis dirupta var. flexella (Janisch & Rabenhorst) Grunow; Codi = Cocconeis dirupta W.Gregory; Cosc = Cocconeis scutellum var. scutellum Ehrenberg; Haco = Halamphora coffeiformis (C.Agardh) Levkov; Hahy = Halamphora hyalina (Kützing) Rimet & R.Jahn; Nasa = Navicula salinicola Hustedt; Rhad = Rhabdonema adriaticum Kützing.

larity between groups 1 and 2 (i.e. epilithic and epiphytic diatom assemblages) was 74%.

Canonical analysis of principle coordinates (CAP) showed that the samples collected from *Padina* sp. are more related with abundance of adnate diatoms, particularly *C. dirupta, C. costata, C. scutellum var. scutellum* (Fig. 5).

Significant ($p < 0.05$) and positive correlation was observed between diatom relative abundance and NO_2^- for *C. dirupta* var. *flexella* and between diatom relative abundance and NH_4^+ for *A. pseudogroenlandica*. A significant negative correlation between diatom relative abundance and PO_4^{3-} were identified for *N. salinicola* and between diatom relative abundance and Chl *a* for *Rhabdonema adriaticum* Kützing (Table 3).

DISCUSSION

This study compares the diatom communities colonising glass slides in a marine lake to the naturally occurring communities in the epilithon and epiphyton. For the first time the ultrastructural analysis of benthic diatoms from Lake Mrtvo More was performed using scanning electron microscopy (SEM).

The average number of diatom taxa was higher on artificial substrates (38) than on natural substrates (23). Differences in the number of diatoms colonizing the different substrates emphasize the care needed in selecting a substratum on which to study the settlement of organisms, especially if the experiments are to be used for subsequent prediction (EDYVEAN *et al.*, 1985). Although the number of diatom taxa recorded varied substantially between the different habitat types, for the one-month study period, both glass micro slides and natural rock substrates showed similar diatom community compositions, which indicates that diatom communities developing on artificial substrates accurately represent communities developing on natural substrates. The results of this study show that glass micro slides are suitable artificial substrates for providing representative samples of the natural epilithic diatom community composition in the studied lake.

Our results are in accordance with NENADOVIĆ *et al.* (2015) showing high colonization of glass artificial substrates by benthic diatoms. Previous studies have shown that newly introduced inorganic artificial substrates (e.g. glass) in a marine environment provide an opportunity to monitor the initial development and succession of diatoms in the periphyton (NENADOVIĆ *et al.*, 2015; CAR *et al.*, 2020). In contrast, DEDIĆ *et al.* (2015) investigated artificial and natural substrates in a karstic spring and reported that artificial substrates include fewer diatom taxa. However, this might be related to the differences between marine and freshwater ecosystems, whereas generally marine environments provide a greater diatom biodiversity compared to freshwater ecosystems.

Significant differences were found between the diatom assemblages colonizing *Padina* sp. and glass artificial substrates, showing that microscopic slides cannot be used as a representative alternative tool for epiphytic diatom analysis in further diatom studies. These differences in the structure of diatom assemblages could be the result of the interactions of several significant drivers. Comparative studies have shown that colonization of artificial substrates differs from that of natural substrates and that living substrates (e.g. macrophytes) act as additional sources of nutrients for attached communities (HAMILTON & DUTHIE, 1984; SABATER *et al.*, 1998). However, while artificial glass substrates could show some resemblance in terms of diatom communities, possibly similar surfaces (epilithon) yield diatoms so as to create a biofilm in which common taxa could grow as suggested by our findings. In addition, the observed differences that occurred are probably due to the structural complexity of macrophytes. Several studies have reported that the diatom composition of macrophytes could differ from epilithon and species such as *Cocconeis* spp. show abundance in the community which could attach to the macrophyte (CAR *et al.*, 2012; MAJEWSKA *et al.*, 2014). Our results confirmed that species of *Cocconeis costata*, *C. dirupta*, *C. pseudomarginata* and *C. scutellum* var. scutellum were present on *Padina* sp., accompanied by *Gedaniella mutabilis* and the frequent taxa *Halamphora coffeiformis* adapted to all three substrates. In general, adnate taxa (e.g. *C.*

Table S1. Species and infraspecific taxa of benthic diatoms in the Mrtvo More in August-September 2016, including data on their family-l
et al. 2020, Guiry & Guiry, 2020), weekly distribution in samples of different substrates (G – glass, R – rock, P – Padina sp.), absol

Taxon	Genus	Family		GE
			S	M
Achnanthes brevipes C.Agardh	Achnanthes	Achnanthaceae	+	+
Achnanthes brevipes var. intermedia (Kützing) Cleve	Achnanthes	Achnanthaceae	.	+
Achnanthes groenlandica (Cleve) Grunow	Achnanthes	Achnanthaceae	.	+
Achnanthes hyperboreoides A.Witkowski, Metzeltin & Lange-Bertalot	Achnanthes	Achnanthaceae	.	+
Achnanthes kuwaitensis Hendey	Achnanthes	Achnanthaceae	.	+
Achnanthes pseudogroenlandica Hendey	Achnanthes	Achnanthaceae	.	+
Achnanthes separata Hustedt	Achnanthes	Achnanthaceae	.	+
Amphora bigibba var. interrupta (Grunow) Cleve	Amphora	Catenulaceae	.	+
Amphora gracilis Ehrenberg	Amphora	Catenulaceae	.	.
Amphora laevissima W.Gregory	Amphora	Catenulaceae	.	+
Amphora sp. 1	Amphora	Catenulaceae	.	.
Ardissonea crystallina (C.Agardh) Grunow	Ardissonea	Ardissoneaceae	.	.
Ardissonea formosa (Hantzsch) Grunow	Ardissonea	Ardissoneaceae	.	+
Aulacoseira granulata (Ehrenberg) Simonsen	Aulacoseira	Aulacoseiraceae	.	.
Bacillaria socialis (Gregory) Ralfs	Bacillaria	Bacillariaceae	.	+
Caloneis bicuneata (Grunow) Boyer	Caloneis	Naviculaceae	.	+
Caloneis liber var. linearis Cleve	Caloneis	Naviculaceae	.	+
Climacosphenia moniligera Ehrenberg	Climacosphenia	Climacospheniaceae	.	+
Cocconeis convexa M.H.Giffen	Cocconeis	Achnanthidiaceae	.	+
Cocconeis costata W.Gregory	Cocconeis	Achnanthidiaceae	.	+
Cocconeis dirupta var. flexella (Janisch & Rabenhorst) Grunow	Cocconeis	Achnanthidiaceae	.	+
Cocconeis dirupta W.Gregory	Cocconeis	Achnanthidiaceae	.	+
Cocconeis pseudomarginata W.Gregory	Cocconeis	Achnanthidiaceae	.	+
Cocconeis scutellum var. scutellum Ehrenberg	Cocconeis	Achnanthidiaceae	+	+
Coronia decora (Brébisson) Ruck & Guiry	Coronia	Surirellaceae	.	+
Craspedostauros decipiens (Hustedt) E.J.Cox	Craspedostauros	Mastogloiaceae	.	+
Diploneis crabro (Ehrenberg) Ehrenberg	Diploneis	Diploneidaceae	.	+
Diploneis nitescens (W.Gregory) Cleve	Diploneis	Diploneidaceae	.	+
Diploneis splendida Cleve	Diploneis	Diploneidaceae	.	+
Entomoneis paludosa (W.Smith) Reimer	Entomoneis	Entomoneidaceae	.	+
Fallacia ny (Cleve) D.G.Mann	Fallacia	Sellaphoraceae	.	+
Fragilaria sp.1	Fragilaria	Fragilariaceae	.	.
Gedaniella mutabilis (Grunow) Chunlian Li & Witkowski	Gedaniella	Staurosiraceae	.	+
Grammatophora angulosa var. islandica (Ehrenberg) Grunow	Grammatophora	Grammatophoraceae	.	+
Grammatophora marina (Lyngbye) Kützing	Grammatophora	Grammatophoraceae	.	+
Grammatophora oceanica Ehrenberg	Grammatophora	Grammatophoraceae	+	+
Halamphora coffeiformis (C.Agardh) Levkov	Halamphora	Amphipleuraceae	.	+
Halamphora hyalina (Kützing) Rimet & R.Jahn	Halamphora	Amphipleuraceae	+	+

evel affiliations, general environment (GE: S – soil taxa, M – marine, B – brackish, F – freshwater, (sensu Witkowski et al. 2000, Kociolek ate (n), percentage (%) frequency of appearance and average relative abundance (Avg. RA %).

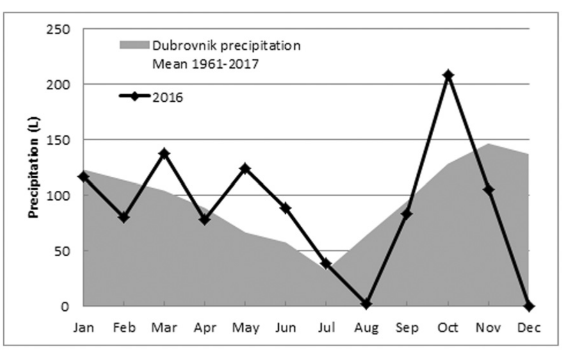
			Date														
E			11 August			17 August			24 August			2 September			n	Freq. (%)	Avg. RA (%)
	B	F	G	R	P	G	R	P	G	R	P	G	R	P			
	+	.	.	+	+	+	.	.	+	+	.	+	+	.	7	58,33	6,07
	+	+	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	1,00
	.	.	.	.	.	.	.	.	.	.	.	.	+	.	1	8,33	2,50
	.	.	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	0,75
	.	.	+	.	.	+	+	.	+	.	+	.	+	.	6	50,00	3,11
	.	.	+	+	+	.	+	+	.	+	.	.	+	+	8	66,67	6,34
	.	.	.	+	.	.	.	.	.	.	.	.	+	.	2	16,67	0,38
	.	.	+	.	.	+	+	.	.	.	+	+	.	.	5	41,67	0,95
	.	+	+	+	+	.	.	+	.	.	+	+	+	+	8	66,67	1,44
	.	.	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	0,75
	.	.	+	+	.	.	.	.	.	.	.	+	.	.	3	25,00	1,67
	+	.	.	+	.	.	.	.	.	.	.	.	.	.	1	8,33	0,25
	.	.	+	+	.	.	.		.	.	.	.	.	.	2	16,67	0,25
	.	+	.	+	.	.	.	.	.	.	.	.	.	.	1	8,33	0,25
	+	.	+	.	.	.	.	+	+	.	.	+	.	.	4	33,33	0,62
	.	.	.	.	.	.	+	.	.	.	.	.	.	.	1	8,33	1,00
	.	.	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	0,25
	.	.	.	.	.	+	.		+	.	.	.	.	.	2	16,67	0,25
	.	.	.	.	.	.	.	.	+	.	+	.	+	.	3	25,00	1,582
	.	.	+	+	+	+	.	+	+	+	+	+	+	+	11	91,67	8,15
	.	.	+	+	.	+	+	.	+	+	.	+	+	.	8	66,67	17,61
	.	.	.	.	+	.	.	+	.	.	+	.	.	+	4	33,33	40,88
	.	.	+	+	+	+	+	+	+	.	+	+	+	+	11	91,67	2,61
	+	.	+	+	+	+	+	+	+	+	+	+	+	+	12	100,00	5,94
	.	.	.	.	.	+	.	.	+	.	.	.	.	.	2	16,67	0,25
	.	.	.	.	.	.	.	.	.	+	+	.	.	.	2	16,67	4,13
	.	.	.	+	.	.	+	.	.	.	.	.	.	.	2	16,67	0,50
	.	.	.	+	.	.	+	.	.	.	.	.	.	.	2	16,67	1,13
	.	.	.	.	.	.	.	+	.	.	.	.	.	.	1	8,33	0,25
	+	+	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	0,75
	.	.	.	.	+	.	.	.	.	.	.	.	.	.	1	8,33	1,00
	.	.	.	.	.	+	.	.	.	.	.	.	.	.	1	8,33	1,00
	.	.	+	.	+	+	.	+	+	+	+	+	+	+	10	8,33	1,25
	.	.	.	+	.	.	.	.	.	.	.	.	.	.	1	8,33	0,25
	.	.	.	+	+	+	+	.	.	.	.	+	.	.	5	41,67	0,55
	+	.	+	+	+	+	+	+	.	.	+	+	.	+	9	75,00	1,17
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	12	100,00	8,04
	.	.	+	.	.	+	+	+	+	.	.	+	.	.	6	50,00	6,77

Halamphora kolbei (Aleem) Álvarez-Blanco & S.Blanco	Halamphora	Amphipleuraceae	.	+
Halamphora pseudohyalina (Simonsen) J.G.Stepanek & Kociolek	Halamphora	Amphipleuraceae	.	+
Halamphora subangularis (Hustedt) Levkov	Halamphora	Amphipleuraceae	.	+
Haslea spicula (Hickie) Bukhtiyarova	Haslea	Naviculaceae	.	+
Haslea duerrenbergiana (Hustedt) F.A.S.Sterrenburg, nom. inval.	Haslea	Naviculaceae	+	+
Hyalosynedra laevigata (Grunow) D.M.Williams & Round	Hyalosynedra	Ulnariaceae	.	+
Licmophora flabellata (Greville) C.Agardh	Licmophora	Licmophoraceae	.	.
Licmophora paradoxa (Lyngbye) Agardh	Licmophora	Licmophoraceae	.	+
Licmophora pfannkuckae Giffen	Licmophora	Licmophoraceae	.	.
Licmophora tincta (C.Agardh) Grunow	Licmophora	Licmophoraceae	.	+
Mastogloia binotata (Grunow) Cleve	Mastogloia	Mastogloiaceae	.	+
Mastogloia cuneata (Meister) R.Simonsen	Mastogloia	Mastogloiaceae	.	+
Mastogloia erythraea Grunow	Mastogloia	Mastogloiaceae	.	+
Mastogloia exilis Hustedt	Mastogloia	Mastogloiaceae	.	+
Mastogloia fimbriata (T.Brightwell) Grunow	Mastogloia	Mastogloiaceae	.	+
Mastogloia ignorata Hustedt	Mastogloia	Mastogloiaceae	.	+
Mastogloia ovalis A.Schmidt	Mastogloia	Mastogloiaceae	.	+
Mastogloia pseudolatecostata T.A.Yohn & R.A.Gibson	Mastogloia	Mastogloiaceae	.	+
Nanofrustulum sopotense (Witkowski & Lange-Bertalot) E.Morales, C.E.Wetzel & Ector	Nanofrustulum	Staurosiraceae	.	+
Navicula directa (W.Smith) Ralfs	Navicula	Naviculaceae	.	+
Navicula flagellifera Hustedt	Navicula	Naviculaceae	+	+
Navicula salinicola Hustedt	Navicula	Naviculaceae	.	+
Navicula sp.	Navicula	Naviculaceae	.	.
Navicula sp.1	Navicula	Naviculaceae	.	.
Nitzschia agnita Hustedt	Nitzschia	Bacillariaceae	.	+
Nitzschia compressa (Bailey) Boyer var. compressa	Nitzschia	Bacillariaceae	.	+
Nitzschia compressa var. elongata (Grunow) Lange-Bertalot	Nitzschia	Bacillariaceae	.	+
Nitzschia distans W.Gregory	Nitzschia	Bacillariaceae	.	+
Nitzschia fusiformis Grunow	Nitzschia	Bacillariaceae	.	+
Nitzschia grossestriata Hustedt	Nitzschia	Bacillariaceae	.	+
Nitzschia insignis W.Gregory	Nitzschia	Bacillariaceae	.	+
Nitzschia laevis Frenguelli	Nitzschia	Bacillariaceae	.	+
Nitzschia macilenta W.Gregory	Nitzschia	Bacillariaceae	.	+
Nitzschia marginulata var. didyma Grunow	Nitzschia	Bacillariaceae	+	+
Nitzschia reversa W.Smith	Nitzschia	Bacillariaceae	.	+
Nitzschia sigma (Kützing) W.Smith	Nitzschia	Bacillariaceae	+	+
Nitzschia subconstricta Desikachary & Prema	Nitzschia	Bacillariaceae	.	+
Nitzschia valdestriata Aleem & Hustedt	Nitzschia	Bacillariaceae	.	.
Opephora sp.	Opephora	Staurosiraceae	.	.
Parlibellus delognei (Van Heurck) E.J. Cox	Parlibellus	Berkeleyaceae	.	+
Placoneis flabellata (F.Meister) Kimura, H.Fukushima & Ts.Kobayashi	Placoneis	Gomphonemataceae	.	.
Pleurosigma formosum W. Smith	Pleurosigma	Pleurosigmataceae	.	+
Pleurosigma sp. 1	Pleurosigma	Pleurosigmataceae	.	.
Psammodictyon rudum (Cholnoky) D.G.Mann	Psammodictyon	Bacillariaceae	.	+

.	.	+	.	.	+	.	.	.	+	+	.	.	.	4	33,33	0,69
.	.	.	.	+	.	.	+	.	.	+	.	.	+	4	33,33	2,25
.	.	.	.	.	+	.	.	.	.	+	.	.	.	2	16,67	0,88
+	.	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	0,25
+	.	.	+	+	+	.	+	+	.	+	.	.	.	6	50,00	1,957
.	.	.	.	.	.	.		.	.	.	+	.	.	1	8,33	0,50
+	.	+	.	.	.	.	.	+	.	.	+	+	.	4	33,33	1,56
.	.	+	+	+	+	+	.	+	+	.	+	+	.	9	75,00	1,91
+	.	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	0,25
.	.	.	.	.	.	.	.	.	+	.	.	.	.	1	8,33	0,25
.	.	+	.	.	+	.	.	.	.	.	.	.	.	2	16,67	0,38
.	.	+	.	.	+	+	.	+	.	.	.	.	.	4	33,33	0,75
.	.	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	0,50
.	.	.	.	.	.	+	.	.	.	.	.	.	.	1	8,33	0,50
.	.	+	+	.	+	.	.	.	.	.	.	.	.	3	25,00	0,42
.	.	+	.	.	+	.	.	.	.	.	.	.	.	2	16,67	0,25
.	.	.	.	.	.	.	.	+	.	.	.	.	.	1	8,33	0,50
.	.	+	.	.	.	.	+	.	.	.	.	.	.	2	16,67	0,25
+	.	.	.	.	+	.	.	.	.	.	.	.	.	1	8,33	2,75
.	.	+	.	+	+	.		+	+	.	+	+	.	7	58,33	1,03
.	.	+	.	+	+	.	+	+	.	.	+	.	.	6	50,00	2,25
+	+	+	.	+	+	+	+	+	+	+	.	.	.	8	66,67	8,55
.	.	.	.	.	.	.	.	.	.	.	.	+	.	1	8,33	2,50
.	.	.	.	.	.	.	.	+	.	.	.	.	.	1	8,33	7,67
+	+	.	.	.	.	.	.	.	+	.	.	.	.	1	8,33	2,50
.	.	+	+	.	.	+	+	+	.	.	.	.	.	5	41,67	0,55
.	.	.	+	.	.	.	.	.	.	.	.	.	.	1	8,33	0,50
.	.	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	1,00
.	.	+	.	.	.	.	.	.	+	.	.	.	.	2	16,67	1,63
.	.	+	.	.	+	.	.	+	.	.	.	.	.	3	25,00	0,83
.	.	.	.	.	+	.	+	.	.	.	.	.	.	2	16,67	0,25
.	.	+	.	+	+	.	.	+	+	+	+	.	.	7	58,33	2,46
.	.	.	.	.	.	.	.	+	.	.	.	.	.	1	8,33	0,25
.	.	.	.	.	+	.		.	.	.	.	.	.	1	8,33	0,50
+	+	+	.	.	.	.	.	.	.	.	.	.	.	1	8,33	0,50
+	+	.	+	.	.	.		.	+	+	.	.	.	3	25,00	1,58
.	.	.	+	.	.	.	.	.	.	.	.	.	.	1	8,33	0,50
+	.	+	.	.	.	.	.	.	+	.	.	.	.	2	83,33	4,04
.	.	+	.	.	.	.	.	.	.	+	.	.	.	2	16,67	3,13
.	.	.	.	+	+	+	.	+	.	.	.	.	.	4	33,33	0,93
.	+	.	.	.	.	.	.	.	.	+	.	.	.	1	8,33	1,50
.	.	+	.	.	+	.	.	.	.	.	+	.	.	3	25,00	0,42
.	.	.	.	+	.	.		.	.	.	.	.	.	1	8,33	0,25
.	.	+	+	+	+	.	.	+	+	+	+	.	.	8	66,67	2,03

Rhabdonema adriaticum Kützing	Rhabdonema	Rhabdonemataceae	.	+
Rhoicosphenia marina (Kützing) M.Schmidt	Rhoicosphenia	Rhoicospheniaceae	.	+
Rhopalodia pacifica Krammer	Rhopalodia	Rhopalodiacae	.	+
Seminavis sp.	Seminavis	Naviculaceae	.	.
Staurosira sp.	Staurosira	Staurosiraceae	.	.
Striatella unipunctata (Lyngbye) C.Agardh	Striatella	Striatellaceae	.	+
Surirella fastuosa (Ehrenberg) Ehrenberg	Surirella	Surirellaceae	+	+
Synedra fulgens (Greville) W.Smith	Synedra	Fragilariaceae	.	+
Tabularia fasciculata (C.Agardh) D.M.Williams & Round	Tabularia	Ulnariaceae	.	+
Tabularia investiens (W.Smith) D.M.Williams & Round	Tabularia	Ulnariaceae	+	+
Toxarium undulatum J.W.Bailey	Toxarium	Toxariaceae	.	+
Trachyneis aspera (Ehrenberg) Cleve	Trachyneis	Naviculaceae	.	+
Triceratium finnmarchicum Grunow	Triceratium	Triceratiaceae	.	+
Trigonium sp. 1	Trigonium	Trigoniaceae	.	.
Tryblionella coarctata (Grunow) D.G.Mann	Tryblionella	Bacillariaceae	.	+

Fig. S1. Mean values of precipitation (L) in Dubrovnik for the period from 1961 to 2017 (provided by the Croatian Meteorological and Hydrological Service) together with precipitation (L) in Dubrovnik during 2016



scutellum var. *scutellum* and *C. dirupta*) adhere strongly horizontally to the substrate by means of their raphe valve and may easily benefit from nutrient exchange with the substrate due to their mode of adhesion over the valve face (ROUND, 1981; SULLIVAN, 1984; ROMAGNOLI *et al.*, 2014).

Diatom composition of the lake, in terms of genera, was dominated by mainly marine diatoms with a few freshwater and brackish taxa observed, as would be expected due to the connection between the lake and the open sea. The genus *Mastogloia*, one of the largest marine diatom genera (PENNESI *et al.*, 2011, and references therein), comprised of species which can be found within different biotopes (ÇOLAK SABANCI, 2013) was one of the richest in taxa number in our study. The most frequent *Mastogloia* species in our study was *Mastogloia cuneata* (Meister) R.Simonsen. Interestingly, *Mastogloia cyclops* Voigt, which has been characterized as a good indicator of coastal zones (WACHNICKA *et al.*, 2010) was not recorded during this one-month investigation.

The most frequent taxa in this study (*C. scutellum* var. *scutellum* and *H. coffeiformis*) were also found on different substrates and do not seem to have a preference either for a geographic region or for the substrate type (ROMAGNOLI *et al.*, 2014). Although in our study *C. scutellum* was recorded on all substrates, it is generally considered as a typical epiphytic taxon (ULANOVA & SNOEIJS, 2006). That is in accordance with results of this study as *C. scutellum* was recorded with the highest abundances on *Padina* sp.

Although precipitation in August 2016 was very low, (2.6 L, Fig. S1, data from Dubrovnik meteorological station for 1961-2017, Croatian Meteorological and Hydrological Service) the presence of taxa associated with brackish to freshwater habitats probably correlates with the precipitation regime as there is no other source of freshwater, such as a river or underground spring, that would feed the lake. The observed monthly diatom communi-

.	.	+	+	.	+	+	.	.	.	+	+	+	.	7	58,33	5,89
.	.	.	+	.	+	+	.	.	.	.	.	.	.	3	25,00	4,33
.	.	.	.	.	+	.	.	.	.	.	.	.	.	1	8,33	0,25
.	.	+	.	.	.	.	.	+	.	.	+	+	.	4	33,33	1,43
.	.	.	.	.	+	.	.	.	.	.	.	.	.	1	8,33	1,75
.	.	.	.	.	.	.	.	.	.	.	+	+	+	3	25,00	2,67
.	.	+	+	.	.	+	.	.	.	.	.	.	.	3	25,00	0,58
+	.	+	+	.	.	+	+	+	.	.	+	+	.	7	58,33	1,07
+	.	+	.	.	.	.		.	.	.	.	.	.	1	8,33	0,25
.	.	+	.	.	.	.	.	+	.	.	+	.	.	3	25,00	0,67
.	.	+	+	.	+	.	.	.	.	.	.	.	.	3	25,00	0,42
.	.	+	+	.	.	+	+	.	.	+	.	+	+	7	58,33	2,29
+	.	.	+	.	.	.	.	.	.	.	.	.	.	1	8,33	0,50
.	.	.	.	.	.	+	.	.	.	.	.	.	.	1	8,33	0,75
.	.	+	.	.	+	+	.	+	+	+	+	.	.	7	58,33	3,24

ties with the presence of brackish and freshwater species reveal that diatom composition can be affected by precipitation. A range of factors can naturally be expected to affect diatom development in the lake, especially when influenced by tourist activities, and this possibility will be investigated in-depth in the future.

Although similar diatom assemblages developed on glass artificial substrates and on rocks, there was no correlation observed between communities according to different sampling dates. This is most likely due to the short period of the study. The four months in the field might not be enough for the artificial substrate to reach a stable community similar to the natural rocks. Thus, if the immersion time had been longer, the assemblages may have been even more significantly similar to natural rock assemblages. Consistent quantitative and qualitative data are still needed to better determine the seasonal changes of the epilithic assemblages in the lake.

The results of the study of bacterial and diatom community in the same lake show a close relationship between diatoms and changes of physico-chemical parameters, especially nutrient concentrations (CAR *et al.*, 2020). Although CAR *et al.* (2020) investigated the initial colonization of bacteria and diatoms on an immersed artificial substrate in the marine Lake Mrtvo More, the observed variations in diatom composition and distribution demands further investigations if they are to be considered as potential indicator species of change.

The results of the present study focusing on a comparison between glass artificial substrate and two native habitat builder substrates (macroalgae and rocks) show that the initial hypotheses are supported and the observed diatom composition is shown to be influenced by the substrate. It can be concluded that glass artificial substrates are not suitable as an alternative for epiphytic but can be for epilithic diatom assemblage monitoring. Rocks (natural substrates) collected for comparison showed similar diatom community compositions to the artificial substrate analysed. Hence, diatom communities developing on artificial substrates accurately represent the diatom community of one particular natural substratum and can potentially be used as a representative alternative tool for studies of epilithic diatom analysis in further diatom studies. However, studies over long periods would show whether diatom assemblages associated with the artificial substrate are sensitive to local variation in environmental conditions and whether glass artificial substrates might be a valid standard replicable tool for monitoring purposes. In addition, the characterization of the biofilm for other locations and durations should be tested and the diatom assemblages on other natural substrates, such as different macroalgae, should be compared with those captured by the artificial substrate. However, the use of artificial habitat collectors as a method for epilithic diatom monitoring should be considered. This study is only the first step to find a standard methodology for benthic monitoring studies that can be used regardless of the geographic location.

ACKNOWLEDGEMENTS

The authors thank Steve Latham (UK) for improving the English, Dr. Nesil ERTORUN for his SEM documentation perform, and two anonymous reviewers whose helpful comments

This research was supported by Croatian Science Foundation (HRZZ, IP-2014-09-2945 & IP-2019-04-9043).

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Received: 6 April 2021
Accepted: 8 June 2021

Usporedba struktura zajednica bentoskih dijatomeja na prirodnim i umjetnim podlogama u morskom jezeru (Jadransko more)

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SAŽETAK

Kako bi se razumjele razlike između naseljavanja na umjetnim i prirodnim podlogama, u ovom istraživanju uspoređivani su sastavi dijatomeja s tri alternativna staništa (epiliton, epifiton i umjetni supstrat). U tu svrhu uzorci su sakupljani tjedno između kolovoza i rujna 2016. na jednoj lokaciji u plitkom morskom jezeru Mrtvo More na otoku Lokrumu kod Dubrovnika (Južni Jadran, Hrvatska).

Osim detaljne analize svjetlosnim mikroskopom, po prvi put je provedena i ultrastrukturna analiza bentoskih dijatomeja iz jezera Mrtvo More pomoću elektronske mikroskopije (SEM). U 12 uzoraka identificirano je ukupno 97 vrsta dijatomeja. Vrste *Cocconeis scutellum* Ehrenberg i *Halamphora coffeiformis* (C.Agardh) Levkov bile su najčešće vrste u uzorcima. Vrijednosti Shannon-Wiener (H') indeksa varirale su od 1,78 (u rujnu na vrsti *Padina* sp.) do 4,52 (u kolovozu na staklu).

Prema nMDS ordinaciji, razlikuju se dvije skupine zajednica bentoskih dijatomeja: epiliton i umjetna staklena podloga kao Grupa 1 i makroalge kao Grupa 2. Rezultati analize pokazali su da zajednice bentoskih dijatomeja koje se razvijaju na umjetnim podlogama, odgovaraju dijatomejskoj zajednici kamene podloge i da se stoga mogu koristiti kao reprezentativni alternativni alat za proučavanje epilitskih dijatomeja u daljnjim eksperimentima.

Ključne riječi: Bacillariophyta; plitko morsko jezero; identifikacija vrsta; bioraznolikost; Sjevero-istočno Sredozemlje