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 concerns over 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 struc-
ture; 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 organisms 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...
and Parsons (1972). Subsamples (50 mL) for \(\text{NH}_4^+\) 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/\text{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; \([\log_{10}(\text{Chl } a \times D\%O \times \text{DIN} \times \text{TP} + k)/m]\) was calculated according to factors which represent a variable reflected in the trophic state: Chl \(a\)=chlorophyll \(a\) concentration (\(\mu\text{gL}^{-1}\)), \(D\%O\)=dissolved oxygen (absolute deviation from 100 % oxygen saturation), dissolved inorganic nitrogen DIN and TP=total phosphorus (\(\mu\text{gL}^{-1}\)) (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\(_2\)O\(_2\). 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), SNOEIJIS (1993, 1999), SNOEIJIS & POTAPAHOVA (1995), SNOEIJIS & KASPEROVICENÉ (1996), SNOEIJIS & BALASHLOV A (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 Pi*elou’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

| 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 NO₃⁻, 0.89 μM NO₂⁻, 1.47 μM NH₄⁺, 0.36 PO₄³⁻, and 8.74 μM SiO₄⁴⁻. 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 NO₃⁻. 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 (O₂/O₂') 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 (Ardissonnea, 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 characterized 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 paradoxo (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) Simonson, 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 paludos (W.Smith) Reimer, H. coffeiformis, N. salinicola, and Nitzschia sigma (Kützing) W.Smith.
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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 = *Halimphora coffeiformis* (C.Agardh) Levkov; Hahy = *Halimphora 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    |          |          |     |             |        |     |     |
| S     |       |       |          |          |          |     |             | 0.99   |     |     |
| NO$_3^-$ |       | -0.97 |          |          |          |     |             |        |     |     |
| NO$_2^-$ |       |       |          |          |          |     |             | -0.95  |     |     |
| NH$_4^+$ |       |       |          |          |          |     |             |        |     |     |
| TIN   |       |       |          |          |          |     |             |        |     |     |
| PO$_4^{3-}$ |       |       |          |          |          |     |             |        |     |     |
| Si    |       |       |          |          |          |     |             |        |     |     |
| CHL   | 0.99  | -0.95 |          |          |          |     |             |        |     |     |
| SAT   |       |       |          |          |          |     |             |        |     |     |
| Acbr  |       |       |          |          |          |     |             |        |     | 0.99|
| Acps  |       |       |          |          |          |     |             |        |     |     |
| 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, *Halimphora 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*, *Halimphora 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 cofeformis (C.Agardh) Levkov; Hayh = 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$_3^-$ 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-level classification (sensu Witkowski et al. 2020, Guiry & Guiry, 2020), weekly distribution in samples of different substrates (G – glass, R – rock, P – Padina sp.), absolute (n), percentage (%) frequency of appearance and average relative abundance (Avg. RA %).

| Taxon                                      | Genus           | Family         | GE  |
|--------------------------------------------|-----------------|----------------|-----|
| 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 moniligerah Ehrenberg       | Climacosphenia   | Climacospheniaceae | . | + |
| Cocconeis convexa M.H.Giffen               | Cocconeis       | Achnanthiaceae | .   |
| Cocconeis costata W.Gregory                | Cocconeis       | Achnanthiaceae | .   |
| Cocconeis dirupta var. flexella (Janisch & Rabenhorst) Grunow | Cocconeis       | Achnanthiaceae | .   |
| Cocconeis dirupta W.Gregory                | Cocconeis       | Achnanthiaceae | .   |
| Cocconeis pseudomarginata W.Gregory        | Cocconeis       | Achnanthiaceae | .   |
| Cocconeis scutellum var. scutellum Ehrenberg | Cocconeis      | Achnanthiaceae | +   |
| Coronia decora (Brébisson) Ruck & Guiry    | Coronia          | Surirellaceae  | +   |
| Craspedostauros decipiens (Hustedt) E.J.Cox | Craspedostauros | Mastogloiaeace | .   |
| Diploneis crabro (Ehrenberg) Ehrenberg     | Diploneis       | Diploneidaeace | .   |
| Diploneis nitescens (W.Gregory) Cleve       | Diploneis       | Diploneidaeace | .   |
| Diploneis splendida Cleve                  | Diploneis       | Diploneidaeace | .   |
| Entomoneis paludosa (W.Smith) Reimer        | Entomoneis      | Entomoneidaeace| +   |
| Fallacia ny (Cleve) D.G.Mann                | Fallacia        | Sellaphoraceae | +   |
| Fragilaria sp.1                            | Fragilaria      | Fragilariaeace | .   |
| Gedaniella mutabilis (Grunow) Chunlian Li & Witkowski | Gedaniella     | Staurosiaceae  | .   |
| 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 | +   |
| Date       | B F G R P | G R P | G R P | G R P | n  | Freq. (%) | Avg. RA (%) |
|------------|-----------|-------|-------|-------|----|-----------|-------------|
| 11 August  | + + + + . | . + + | + . + | . + + | 7  | 58,33     | 6,07        |
| 17 August  | + + + . . | + . . | . . . | . . . | 1  | 8,33      | 1,00        |
| 24 August  | . . . . . | . . . | . . . | . . . | 1  | 8,33      | 2,50        |
|            | + + . . + | + . + | . + . | . + . | 6  | 50,00     | 3,11        |
| 2 September| . . . . . | . . . | . . . | . . . | 1  | 8,33      | 0,75        |
|            | + + + + + | + + + | + + + | + + + | 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        |
|            | . . . . + | . . . | . . . | . . . | 1  | 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        |

Table S1. Species and infraspecific taxa of benthic diatoms in the Mrtvo More in August-September 2016, including data on general environment (GE: S – soil taxa, M – marine, B – brackish, F – freshwater; sensu Witkowski et al. 2000, Kociolek et al. 2020, Guiry & Guiry, 2020), weekly distribution in samples of different substrates (G – glass, R – rock, P – Padina sp.), absolute (n), percentage (%) frequency of appearance and average relative abundance (Avg. RA %).
| Species                               | Family       | Genera                              | Class      | Order     |
|---------------------------------------|--------------|-------------------------------------|------------|-----------|
| 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 | Ulvariaceae                          | .          | +         |
| Licmophora flabellata (Greville) C.Agardh | Licmophora   | Licmophoraceae                       | .          | .         |
| Licmophora paradoxo (Lyngbye) Agardh   | Licmophora   | Licmophoraceae                       | .          | +         |
| Licmophora pfannkuckiae Giffen         | Licmophora   | Licmophoraceae                       | .          | .         |
| Licmophora tincta (C.Agardh) Grunow    | Licmophora   | Licmophoraceae                       | .          | .         |
| Mastogloia binotata (Grunow) Cleve     | Mastogloia   | Mastogloia                           | .          | +         |
| Mastogloia cuneata (Meister) R.Simonsen | Mastogloia   | Mastogloia                           | .          | +         |
| Mastogloia erythrea Grunow             | Mastogloia   | Mastogloia                           | .          | +         |
| Mastogloia exilis Hustedt              | Mastogloia   | Mastogloia                           | .          | +         |
| Mastogloia fimbriata (T.Brightwell) Grunow | Mastogloia   | Mastogloia                           | .          | +         |
| Mastogloia ignorata Hustedt            | Mastogloia   | Mastogloia                           | .          | +         |
| Mastogloia ovalis A.Schmidt            | Mastogloia   | Mastogloia                           | .          | +         |
| Mastogloia pseudolatecostata T.A.Yohn & R.A.Gibson | Mastogloia   | Mastogloia                           | .          | +         |
| 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. 1                         | Navicula     | Naviculaceae                         | .          |         |
| Navicula sp.                           | 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 valdestrata 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                       | .          | .         |
| Species | Abundance | Importance |
|---------|-----------|------------|
| Pleurosigma sp. 1 | 4 | 33.33 |
| Pleurosigma formosum W. Smith | 1 | 100.00 |
| Placoneis flabellata (F.Meister) Kimura, H.Fukushima & Ts.Kobayashi | 1 | 50.00 |
| Opephora sp. | 1 | 100.00 |
| Nitzschia valdestriata Aleem & Hustedt | 1 | 83.33 |
| Nitzschia reversa W.Smith | 1 | 100.00 |
| Nitzschia marginulata var. didyma Grunow | 1 | 83.33 |
| Nitzschia macilenta W.Gregory | 1 | 100.00 |
| Nitzschia insignis W.Gregory | 1 | 83.33 |
| Nitzschia fusiformis Grunow | 1 | 50.00 |
| Nitzschia distans W.Gregory | 1 | 100.00 |
| Nitzschia agnita Hustedt | 1 | 50.00 |
| Navicula sp.1 | 2 | 100.00 |
| Navicula sp. | 1 | 100.00 |
| Navicula flagellifera Hustedt | 1 | 83.33 |
| Mastogloia ovalis A.Schmidt | 1 | 100.00 |
| Mastogloia ignorata Hustedt | 1 | 100.00 |
| Mastogloia fimbriata (T.Brightwell) Grunow | 1 | 83.33 |
| Mastogloia exilis Hustedt | 1 | 100.00 |
| Mastogloia cuneata (Meister) R.Simonsen | 1 | 50.00 |
| Mastogloia binotata (Grunow) Cleve | 1 | 100.00 |
| Licmophora tincta (C.Agardh) Grunow | 1 | 50.00 |
| Licmophora paradoxa (Lyngbye) Agardh | 1 | 50.00 |
| Haslea duerrenbergiana (Hustedt) F.A.S.Sterrenburg, nom. inval. | 1 | 100.00 |
| Halamphora kolbei (Aleem) Álvarez-Blanco & S.Blanco | 1 | 50.00 |
| Mastogloia | 1 | 100.00 |
| Placencia | 1 | 100.00 |
| Bacillariaceae | 1 | 100.00 |
| Pleurosigmataceae | 1 | 100.00 |
| Mastogloiaceae | 1 | 100.00 |
| Naviculaceae | 1 | 100.00 |
| Gomphonemataceae | 1 | 50.00 |
| Berkeleyaceae | 1 | 100.00 |
| Amphipleuraceae | 1 | 100.00 |
| Staurosiraceae | 1 | 100.00 |
| Nanofrustulum | 3 | 100.00 |

CAR et al: Comparison of benthic diatom community structures on natural and artificial substrates in marine lake
Rhabdonemataceae
Rhoicosphenia marina (Kützing) M.Schmidt
Rhopalodia pacifica Krammer
Seminavis sp.
Staurosira sp.
Striatella unipunctata (Lyngbye) C.Agardh
Surirella fastuosa (Ehrenberg) Ehrenberg
Synedra fulgens (Greville) W.Smith
Tabularia fasciculata (C.Agardh) D.M.Williams & Round
Tabularia investiens (W.Smith) D.M.Williams & Round
Toxarium undulatum J.W.Bailey
Trachyneis aspera (Ehrenberg) Cleve
Triceratium finnmarchicum Grunow
Trigonium sp. 1
Tryblionella coarctata (Grunow) D.G.Mann

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**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-
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.

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Usporedba struktura zajednica bentskih 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 sakupljeni tijekom kolovoza i rujna 2016. na jednoj lokaciji u plitkom morskom jezeru Mrtvo More na otoku Lokrum kod Dubrovnika (Južni Jadran, Hrvatska).

Osim detaljne analize svjetlosnim mikroskopom, po prvi put je provedena i ultrastrukturna analiza bentskih 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 bentskih dijatomeja: epiliton i umjetna staklena podloga kao Grupa 1 i makroalge kao Grupa 2. Rezultati analize pokazali su da zajednice bentskih 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
