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Colonization of bacteria and diatoms on an artificial substrate in a marine lake (eastern Adriatic Sea, NE Mediterranean)

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Abstract – The initial colonization of bacteria and diatoms on a immersed artificial substrate and the development of diatom assemblages in relation to physico-chemical parameters were investigated on a weekly basis at one station in the marine Lake Mrtvo More, South Croatia, from April to October 2016. According to TRIX trophic index, lake showed different trophic character: (i) oligotrophic (at the beginning and the end of the study), (ii) mesotrophic (the end of June to mid-July), (iii) eutrophic (the end of July to mid-September). Heterotrophic bacteria increased to peak abundances (69,268 cells cm^{-2}) at the beginning of June when the diatoms abundances start to increase. The lake has high diatom species richness (285 diatom taxa within 72 genera), with the highest species diversity index in August. Among diatoms, adnate were the primary colonizers, particularly *Cocconeis dirupta W.Gregory* var. *flexella* (Janisch and Rabenhorst) Grunow and *Cocconeis scutellum* Ehrenberg var. *scutellum*, while motile taxa joined the fouling communities from July to September. This study showed close relationship between diatom species composition and changes of physico-chemical parameters, particularly the nutrient concentrations.

Keywords: Bacillariophyta, benthos, biodiversity, Croatia, environmental parameters, heterotrophic bacteria, TRIX index

Introduction

Any permanently exposed, unprotected surface will eventually become fouled. The adsorption of macromolecules to a surface starts within seconds after immersion, bacterial colonization beginning after ca. an hour, and colonization by unicellular eukaryotes (e.g., diatoms, yeasts, and protozoa) usually starts several days after immersion (Wahl 1989). Raphid diatoms are generally among the earliest and most abundant primary colonizers of natural and artificial surfaces (Hoagland et al. 1986). The presence of bacteria and unicellular algae in the biofilm can promote further colonization of the substrate by plants and animals (Totti et al. 2007, and references therein). However, the intensity of fouling pressure varies with season, latitude, depth and local ecological factors (Wahl 1989). Biological, physical and chemical factors may regulate abundance, distribution and species composition of diatom communities. Amongst these, substrate characteristics, sampling site location and depth, grazing pressure, and stage of season have been identified as important factors influencing the shallow water communities (Majewska et al. 2016).

Diatom assemblages are widely used as indicators of ecological change in aquatic environments (Ulanova and Snoeijs 2006). Diatoms are ideal environmental indicators (Dixit et al. 1992) as they are sensitive to a range of environmental parameters, including salinity (Roberts and McMinn 1998, Cunningham and McMinn 2004). The influence of increased nutrient concentration on benthic diatoms became a subject of scientific investigations when the eutrophication problem became acute. It became evident that benthic microalgae exerted a strong influence on the nutrient flux between sediment and overlying water (Agatz et al. 1999, and references therein). Sundbäck and Snoeijs (1991) detected

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significant changes in the diatom flora in nutrient enrichment experiments after only 14 days. Results of a study of diatom diversity at multiple scales in urban reservoirs by Marra et al. (2018) highlight the key role of nutrient availability by showing that diatoms grow under very specific physical and chemical conditions, and eutrophication may cause community variation.

Marine debris is listed among the major perceived threats to biodiversity and is cause for particular concern due to its abundance, durability and persistence in the marine environment (Gall and Thompson 2015). The material types most commonly found in marine debris are glass, metal, paper and plastic (OSPAR 2007). An extensive literature search has reviewed the current state of knowledge on the effects of marine debris on marine organisms (Gall and Thompson 2015, and references therein).

Colonization of artificial substrates differs from that of natural substrates (see Mejdandžić et al. 2015). Comparative studies have shown that while living (macrophytes) and organic (wood, leaves) substrates act as additional sources of nutrients for attached communities, the advent of newly introduced inorganic artificial substrates (e.g. glass, plastic) in the marine environment provides an opportunity to monitor the initial development and the succesion of diatoms in the periphyton (Nenadović et al. 2015).

Previous studies of fouling by diatoms on artificial substrates have been conducted in the northern Adriatic and estuaries (e.g. Tolomio and Andreoli 1989, Tolomio et al. 1991, Bartole et al. 1991–1994, Burić et al. 2004, Munda 2005, Totti et al. 2007, Caput Mihalić et al. 2008, Levkov et al. 2010, etc.). Although Mejdandžić et al. (2015) and Nenadović et al. (2015) were studied the development of periphytic diatoms on different artificial substrates (plexiglass, asbestos, painted iron, wood, concrete, glass, plastic, etc.), their result were mostly based at the generic level. In the Venice lagoon benthic diatoms were investigated from the surface sediment layer to investigate a possible relation of epipelic diatoms with the water quality of shallow coastal areas affected by marked physical and chemical gradients and high anthropogenic impact (Facca and Sfriso 2007).

In this study, the initial colonization of diatoms in the periphytic community and the development of diatom assemblages on a immersed artificial substrate with physicochemical properties were examined in a shallow marine lake during the warmer part of the year, when the ecosystem is under significant anthropogenic influence. We present a qualitative and quantitative data of the marine benthic diatom communities in order to derive a better understanding of the multiple interactions that occur between them and the environment.

The objectives of this study were (i) to determine the abundances of diatom and bacteria on an artificial glass substrate, (ii) to demonstrate their succession through the investigated period of six months, (iii) to determine the weekly temporal changes in diatom population structure, and (iv) to determine the effect of some environmental variables on the diatom colonization rate in a semi-enclosed marine lake.

Materials and methods

The study area

The experiment was carried out at one station in the marine Lake Mrtvo More (English: 'Dead Sea', 42°37'21" N, 18°07'14" E) on the island of Lokrum located in front of the Old City of Dubrovnik, South Croatia (Fig. 1). The island of Lokrum (72 ha) is a nature reserve and NATURA 2000-Ecological network-site (Site of Community Importance, code: HR 4000017). Lake Mrtvo More (surface area 1310 m^2, perimeter 150 m, max. depth 6 m and average of 2 m) is linked to the open sea by 15–20 m long underwater tunnel. The lake is a favourite swimming spot for many visitors during the summer (On-line Suppl. Fig. 1A).

The region experiences a typical Mediterranean climate: Summers are warm and dry, and winters are mild and rainy. Annually, average air temperature is 16 °C and precipitation 1308 mm (data from Dubrovnik meteorological sta-

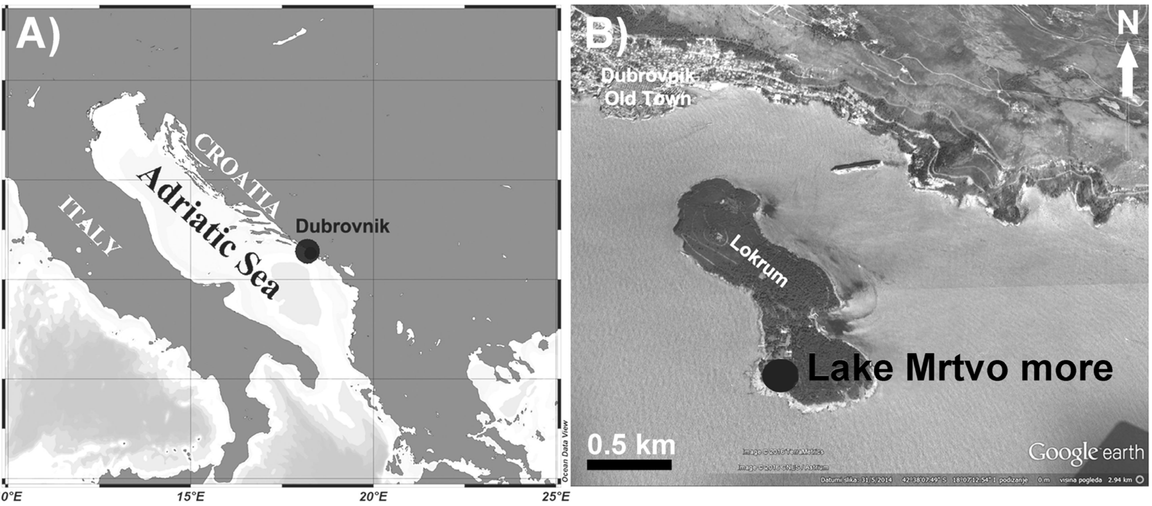

Fig. 1. Marine Lake Mrtvo More on the island of Lokrum. A – position of the study area on the eastern Adriatic coast, B – location of Lake Mrtvo More on the island of Lokrum (derived and adapted from Google earth).

tion for 1961–2018, Croatian Meteorological and Hydrological Service). Average temperature during the coldest month (January) was 9.1 °C, and during the warmest (August) 25.2 °C. The highest rainfall is from October to March. During the dry season (June–August) total rainfall is only 155.4 mm. Average annual wind speed is 3.33 m s^{-1}, with the dominant southerly winds (SE, SSE) blowing from April to September. Annual potential evapotranspiration is around 1500 mm $year^{-1}$ with maximum values in vegetation period (April-September) (Orešić and Čanjevac 2020). In the area, the range of diurnal sea-level oscillations is close to 19 cm (Mihanović et al. 2006). Seawater surrounded the island is under the direct influence of incoming currents from the Ionian Sea (Garić and Batistić 2016).

Sampling

The experiment was carried out over a period of 25 weeks from April to October 2016. Water samples for analysis physico-chemical parameters were taken weekly (On-line Suppl. Tab. 1) from 19th April to 12th October, 2016 at the same place where diatom sampling was carried out, i.e. at the bottom (1 m depth). Temperature (T) and salinity (S) were measured using a WTW Multiline P4 multiparametric sounding lineprobe. Seawater samples for nutrient analyses (Strickland and Parsons 1972, Ivančić and Degobbis 1984) and chlorophyll *a* concentrations (Chl *a*, Holm-Hansen et al. 1965) were taken by 5 L Niskin bottles. Measured nutrients included 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-}).

Samples for NO_3^-, NO_2^-, PO_4^{3-} and SiO_4^{4-} were frozen (−22 °C) and analysed in a laboratory (Strickland and Parsons 1972). Subsamples (50 mL) for NH_4^+ were fixed immediately after collection with 2 mL of 1 mol L^{-1} phenol/EtOH, kept at 4 °C and later analysed according to Ivančić and Degobbis (1984). Chl *a* was determined from 1 L subsamples 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). Due to the exceptionally high Chl *a* value of 39 µg L^{-1} on 20th of July, this record was removed from further analysis.

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 (Weiss 1970, UNESCO 1973). Trophic status was characterized by the TRIX index (Vollenweider et al. 1998), commonly used to classify coastal marine areas in the Mediterranean (see Primpas and Karydis 2011): TRIX = $[\log_{10}(\text{Chl } a \times D\%O \times DIN \times TP) + k]/m$. Each of the factors represents a variable reflected in the trophic state: Chl *a* – chlorophyll *a* concentration (µg L^{-1}), D%O – dissolved oxygen (absolute deviation from 100 % oxygen saturation), dissolved inorganic nitrogen DIN and TP – total phosphorus (µg L^{-1}). The parameters k = 1.5 and m = 1.2 set the range of the TRIX scale from 0 to 10: 0–4 oligotrophic, 4–5 mesotrophic, 5–6 eutrophic, 6–10 extremely eutrophic.

Glass slides were used as a substrate for biofilm formation because of their convenience compared to a natural substrate. The dimensions of a standard microscope slide for bacteriological and for algological sampling are the same, measuring about 75 mm by 25 mm and about 1 mm thick. Microhabitats were made of 33 microscope glass slides which were arranged in three rows at a distance of approximately 1 cm and fixed on the upper side of a Plexiglas sheet which was then submerged horizontally with four diving weights at one station in the lake at a depth of approximately 1 m (i.e. on the bottom of the Lake Mrtvo More) about 2 m offshore on 19 April 2016 (On-line Suppl. Fig. 1B). After three weeks, the Plexiglas sheet was hauled up and the first microscopic slide for diatom analysis was removed. Every week another microscope slide was taken out and gently plunged into filtered seawater (Millipore, acetate cellulose 0.22 µm). In total, there were 21 diatom samples (On-line Suppl. Tab. 1). For bacteriological analysis, 12 glass slides were collected in a period from 20th May to 6th September (On-line Suppl. Tab. 1). All samples were preserved with 4% formaldehyde.

Bacteriological analysis

The total number of heterotrophic bacteria was determined by using a direct counting method counting under epifluorescent microscopy (Hobbie et al. 1977). All samples were analyzed within five days, and before processing were stored in the dark in a refrigerator at a temperature of about 5 °C. Glass slides were gently brushed and washed with sterile freshly filtered seawater (Millipore, acetate cellulose 0.22 µm) and the biofilm was dispersed. For bacteria colouring a 0.01% solution of acridine orange was used and the 2 mL subsamples were filtered through Nucleopore filters (pore diameter of 0.2 µm). Bacterial cells were counted using a Jenalumar Zeiss fluorescent microscope under 1500× magnification. These values are expressed as cells per cm^2.

Diatom analysis

A microscopic glass surface of 1 cm^2 was scraped using a razor blade, and the microalgae were collected in Falcon tubes preserved by adding a known amount (3 mL) of solution (3%) of formaldehyde-filtered seawater (Millipore, acetate cellulose 0.22 µm). Quantitative analysis of homogenized samples was determined with an inverted microscope (Olympus IX 71) equipped with phase contrast. In these samples, taxa were not determined. Results are expressed as total number of diatom cells per cm^2.

A detailed diatom analysis was performed on permanent slides of processed material (hydrogen peroxide treated before mounting in Naphrax® as reported by Car et al. 2019) with a Nikon E600 microscope at a magnification of 1000×. The species abundances 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 of light microscopy (LM) have been deposited in the diatom collection of the Institute for Marine and Coastal Research, University of Dubrovnik, Dubrovnik, Croatia. Identifications were made following keys and guides reported by Hafner et al. (2018). Nomenclature follows AlgaeBase (Guiry and Guiry 2019).

Statistical analysis

Cluster analysis was used to determine the similarity level among physico-chemical parameters in samples (Clarke et al. 2008). A hierarchical clustering algorithm based on Euclidean distances on log(x+1)-transformed, normalized data and the average group linkage method were used. The similarity profile routine (SIMPROF, $P < 0.05$) were used to define the significantly different clusters, and analysis of similarities (ANOSIM) was applied to evaluate a differences among seasons/months (Clarke and Warwick 1994, Clarke et al. 2008).

Nonmetric multidimensional scaling (NMDS) was used for analysis of the community composition variability, i.e. to define the diatom abundance with relation to sampling dates. In order to normalize data, diatom abundances expressed as relative percentages were square root transformed. The Bray Curtis matrix included 285 taxa over 21 samples. In this case, SIMPROF ($P < 0.05$), SIMPER and ANOSIM randomization were also used: (i) to define the significantly different clusters, (ii) to identify the taxa making the greatest contribution to differences among clusters, (iii) to test differences in diatom community over the sampling period.

To investigate the community diversity in the diatom samples, the Shannon-Wiener Biodiversity Index and the Margalef index was computed (Kwandrans 2007). As the diversity index is not completely effective in describing community structure, the evenness of benthic diatom assemblages was also computed using both Pielou's, and Smith and Wilson's evenness values (Pielou 1966, Smith and Wilson 1996, Beisel et al. 2003).

Canonical analysis of principal coordinates (CAP) was used to summarize the structure of diatom assemblages over the months and to determine which physico-chemical parameters were directly responsible for the variations observed in diatom abundances.

The relationship between the most abundant species and physico-chemical parameters was analysed by Spearman-Rank correlation coefficient. Data were transformed [log(x+1)] to enable the correlation tests among variables (Cassie 1962). The Kolmogorov-Smirnov test was used for testing normality of the data distribution. Only significant values (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) were reported.

Statistical analyses were performed using the PRIMER v6 software (Clarke and Gorley 2006) and Statistica 7.0 (StatSoft, Inc. 2004).

Results

Physico-chemical parameters

Over the study period seawater temperature ranged from 16 °C to 27.3 °C, and salinity ranged from 26.6 to 37.3 (average 35.5) (Fig. 2A, B). TIN ranged from 0.96 to 10.02 μM and mostly follow the distribution of NO_3^-. PO_4^{3-} varied from 0.066 μM to 0.578 μM and $SiO_4^{4-}$from 3.23 to 13.02 μM. The highest value both PO_4^{3-} and SiO_4^{4-} was recorded on 20th of July. During whole study period, average nutrient concentrations were: 3.14 μM NO_3^-, 0.58 μM NO_2^-, 0.97 μM NH_4^+, 0.24 PO_4^{3-} and 7.65 μM SiO_4^{4-}. Oxygen saturation (O_2/O_2') ranged from 0.57 to 1.39 (average 0.92).

In May-June period the average Chl *a* was 0.3 μg L^{-1}. During the whole study period, minimum Chl *a* (0.12 μg L^{-1}) was on 31st May and maximum (39 μg L^{-1}) on 20th July. Average Chl *a* in August and September was 2.5 μg L^{-1} and 2 μg L^{-1}, respectively (Fig. 2D).

The trophic index (TRIX) was lower than 4 (oligotrophic character of the lake) during the initial sampling period (up to 24th June) and towards the end (from the mid-October). Lake showed mesotrophic character (4.03–4.76) in the period the end of June-mid-July, and again at the end of September-beginning of October. The lake was mostly eutrophic (5.54–6.02) in the period the end of July–mid-September, and under highly eutrophic conditions (6.44) on the 20th July.

Physico-chemical parameters varied significantly (ANOSIM, $P < 0.05$) among months, seasons (spring, summer, autumn), and between samples collected before the 18th June (Group 1) and afterwards, with exception on 7th June (On-line Suppl. Fig. 3, On-line Suppl. Tabs. 1, 2).

Bacteria

On 20th May 2016, heterotrophic bacteria reached values of 35,479 cells cm^{-2} of the glass slide (Fig. 3A). The average number of bacteria during the study was 42,114 cells cm^{-2} with the peak (69,268 cells cm^{-2}) at the beginning of June. During the second part of study a decline in the number of bacteria was observed.

Diatoms

A peak value of 333,076 cells cm^{-2} was observed in August. The average abundance over entire study period was 165,946 cells cm^{-2} (Fig. 3B).

A total of 285 diatom taxa belonging to 72 genera were found in samples (Appendix). Genera with the greatest number of taxa were: *Mastogloia* (36), *Nitzschia* (29), *Navicula* (20), *Amphora* (13), *Diploneis* (17), *Achnanthes* (13) and *Cocconeis* (12). The most abundant taxa were *Cocconeis scutellum* Ehrenberg var. *scutellum* and *Cocconeis dirupta W.Gregory* var. *flexella* (Janisch and Rabenhorst) Grunow which occurred in all samples with average relative abundance of 30% and 25%, respectively. The maximum abundance of *C. scutellum* var. *scutellum* (90%) was recorded on 7th June, while the maximum abundance of *C. dirupta* var. *flexella* (65%) was recorded one month later (7th July) (see

On-line Suppl. Tab. 4). In total, 48 taxa were found only once (sporadic) in all samples (Appendix).

The number of taxa per sample ranged from 9 (25th May and 7th June 2016) to 52 (11th August 2016), with an average of 25 (Fig. 3C). The Shannon-Wiener Biodiversity Index varied from 0.74 to 4.51, with an average of 2.93 (Fig. 3D). Pielou's species evenness ranged from 0.23 to 0.86 (the average 0.63) with the minimum occurring in June and the maximum at the end of September (Fig. 3E). Smith and Wilson species evenness ranged from 0.06 to 0.45 (the average 0.21) with the minimum at the end of May and the maximum in August 2016 (Fig. 3G).

Diatom assemblages differed significantly (NMDS, ANOSIM, $P < 0.05$) between the samples collected up to the middle of July (Group 1) and afterwards (Group 2). Additionally, sample from the 12th October (Group 3) differed significantly from all the others (Fig. 4, On-line Suppl. Tab. 3). *Cocconeis scutellum* var. *scutellum*, *C. dirupta* var. *flexella*, *Opephora mutabilis* (Grunow) Sabbe et Wyverman, *Navicula salinicola* Hustedt, *Cocconeis costata* W.Gregory, *Halamphora hyalina* (Kützing) Rimet et R.Jahn, *Licmophora paradoxa* (Lyngbye) Agardh, *Licmophora flabellata* (Greville) C.Agardh, *Halamphora coffeiformis* (C.Agardh) Levkov and *Psammodictyon rudum* (Cholnoky) D.G.Mann contributed the most (cumulatively 70%) to the variance between assemblages from Group 1 (10th May-13th July) and 2 (20th July-3rd October, SIMPER, Tab. 1). Within Group 1, *C. scutellum* var. *scutellum* and *C. dirupta* var. *flexella* contributed the most (cumulatively 95%) to the similarity among diatom assemblages from the 10 samples.

Diatom assemblages also differed significantly (ANOSIM, $P < 0.05$) between the samples collected before the end of June and samples collected afterwards so the first group contained sub-groups A and B with similarity of 40%. *Cocconeis scutellum* var. *scutellum*, *C. dirupta* var. *flexella*, *L. flabellata*, *P. rudum*, *C. costata*, *Navicula flagellifera* Hustedt, *Nitzschia frustulum* (Kützing) Grunow, *Cocconeis pseudomarginata* W.Gregory and *Mastogloia cuneata* (Meister) R.Simonsen contributed the most (cumulatively 81%) to the variance between assemblages from these two su-bgroups.

Diatom assemblages varied significantly (ANOSIM, $P < 0.05$) among months (On-line Suppl. Tabs 3, 4). The pioneer colonization diatom taxa observed after one month (20th May) of exposure of the glass slides were *Cocconeis scutellum* var. *scutellum* and *C. dirupta* var. *flexella* which occured with average relative abundance of 73% and 15% respectively. These taxa were recorded in all 21 samples. In May, they contributed the most (cumulatively 90%) to the similarity among diatom assemblages. *Cocconeis scutellum* var. *scutellum* had the highest average relative abundances

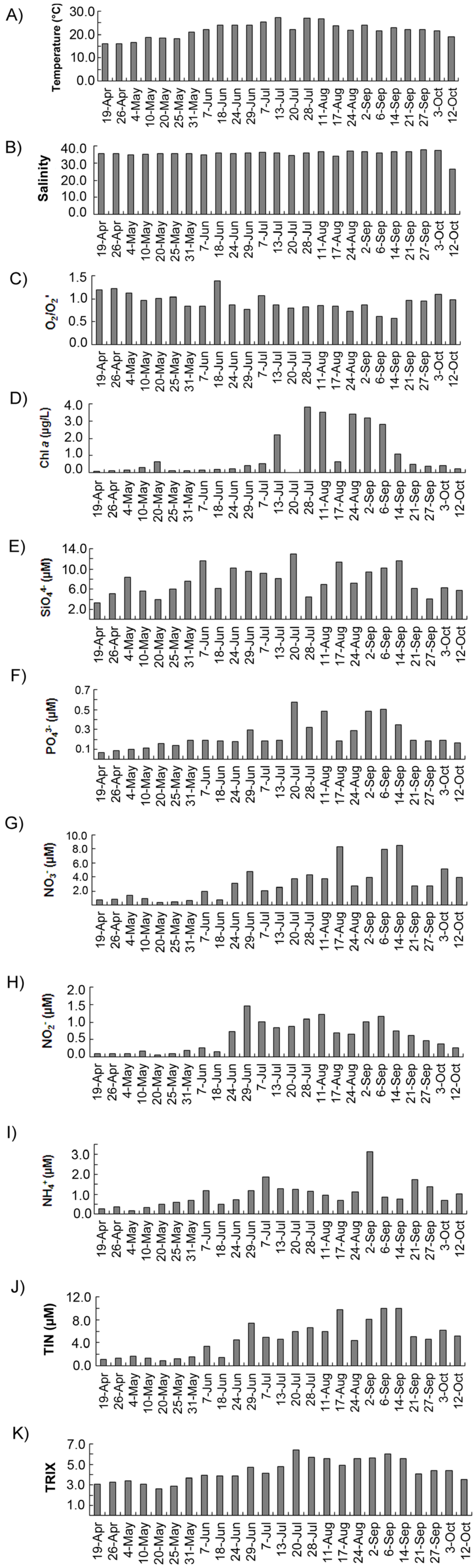

Fig. 2. Distribution of the physico-chemical parameters in Lake Mrtvo More in 2016. A – temperature, B – salinity, C – oxygen saturation (O_2/O_2'), D – chlorophyll-*a* concentrations, E – silicate (SiO_4^{4-}), F – phosphate (PO_4^{3-}), G – nitrate (NO_3^-), H – nitrite (NO_2^-), I – ammonium (NH_4^+), J – total inorganic nitrogen (TIN), K – TRIX trophic index.

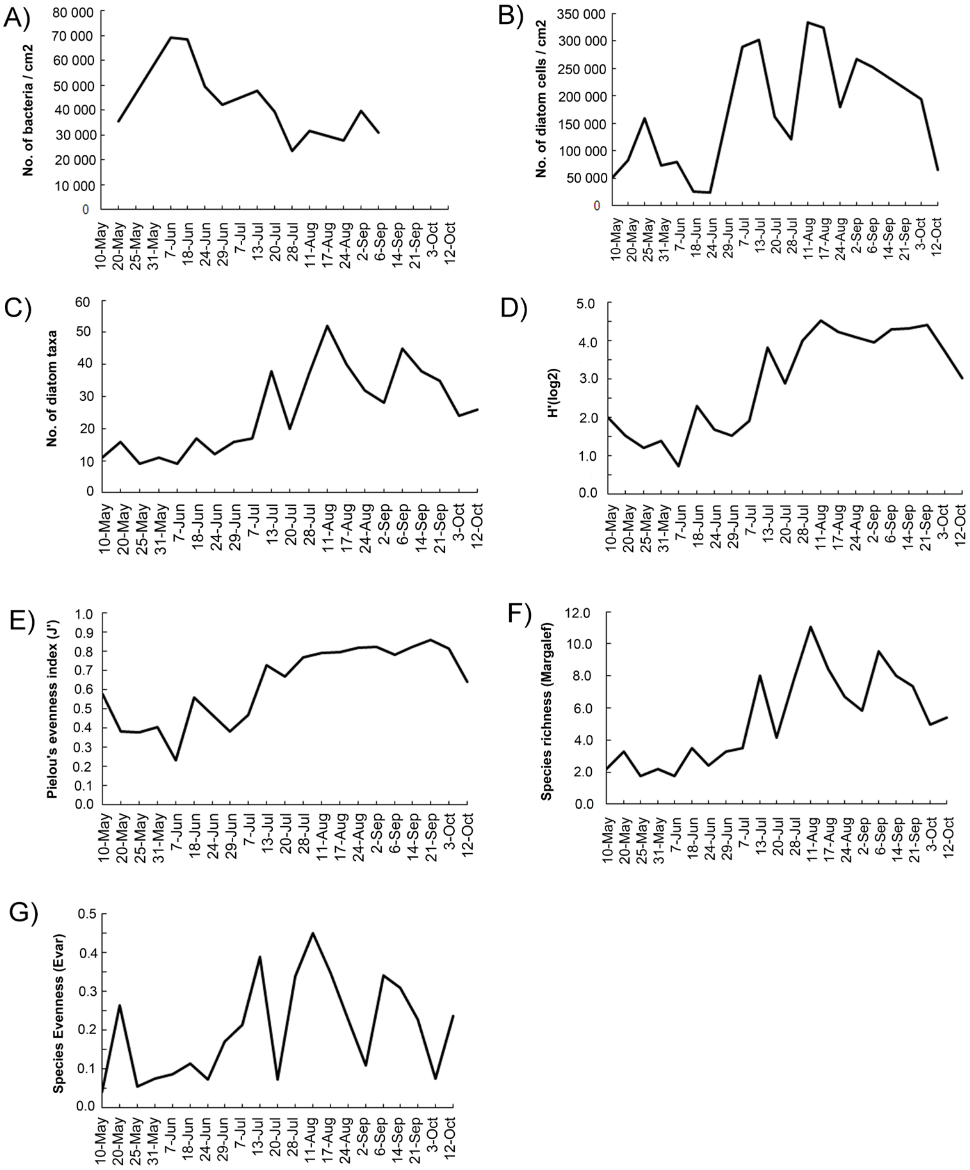

Fig. 3. Distribution of bacteria and diatom abundances, number of diatom taxa, diversity and evenness indicies in Lake Mrtvo More in 2016. A – number of bacteria cells cm^{-2}, B – number of diatom cells cm^{-2}, C – number of diatom taxa, D – Shannon-Wiener diatom diversity index, E – Pielou's evenness index, F – Margalef's diversity index, G – Smith and Wilson's evenness index.

in May (72%) and June (58%), while *C. dirupta* var. *flexella* dominated (42%) in July. The abundance of *C. dirupta* var. *flexella* was 17% and 14% in August and September, respectively. *Halamphora hyalina* occurred in 50% of the samples with an average abundance of 6% and maximum of 14% recorded on 24th August. In 15 samples, *L. flabellata* and *H. coffeiformis* were observed with average relative abundance of 3% with the maximum of 13.5% on 7th July and 2nd September, respectively.

Diatom communities and environmental parameters

Spearman-Rank correlation coefficient displays positive correlation between diatom relative abundance and temperature in case of *C. dirupta* var. *flexella* and *P. rudum* (Tab. 2). *Psammodictyon rudum* also had positive correlation with salinity. In the case of nutrients, the species *C. costata*, *O. mutabilis*, *R. adriaticum* and *Seminavis* sp. correlated with PO_4^{3-} and NO_3^-. Six diatom taxa (*A. kuwaitensis*, *C. costata*, *N. salinicola*, *Navicula* sp., *O. mutabilis* and *Seminavis* sp.) correlated negatively with oxygen saturation. Interestingly, *Nitzschia frustulum* was not influenced by any of these 10 environmental variables. In addition, none of diatom taxa correlated with SiO_4^{4-} (Tab. 2).

Canonical analysis of principle coordinates (CAP) showed that the samples collected in May and June are more related with abundance of adnate diatoms, particularly *C. dirupta* var. *flexella* and *C. scutellum* var. *scutellum*, while motile forms were better related in the samples from July, August and September (Fig. 5). Taxa presented in the samples collected from July to September were associated with higher seawater temperature and higher nutrient concentrations (e.g. *P. rudum*, *R. adriaticum*, *T. coarctata*). Erect diatoms (e. g. *L. paradoxa*, *L. flabellata*, Fig. 6) appeared in October, when salinity was low.

Tab. 1. Diatom taxa with their average abundances contributing to dissimilarities (cumulative = 90%) between diatom assemblages from Group 1 and Group 2, according to SIMPER analysis. Av.abund. – average abundance, Av.diss. – average dissimilarity, Contrib. – contribution to differences (%), Cum. – cumulative contribution to differences (%).

Taxon	Group 1	Group 2		Contrib.	Cum.
	Av.abund.	Av.abund.	Av.diss.	(%)	(%)
Cocconeis scutellum var. *scutellum*	52.53	6.33	23.23	32.17	32.17
Cocconeis dirupta var. *flexella*	29.52	18.96	8.87	12.29	44.46
Opephora mutabilis	0	7	3.5	4.85	49.31
Navicula salinicola	0.35	5.87	2.84	3.94	53.24
Cocconeis costata	1.3	6.23	2.58	3.57	56.82
Halamphora hyalina	0.05	5.11	2.54	3.52	60.33
Licmophora paradoxa	0.25	3.86	1.83	2.53	62.87
Licmophora flabellata	2.48	1.95	1.69	2.34	65.21
Halamphora coffeiformis	1.3	3.64	1.6	2.22	67.43
Psammodictyon rudum	1.13	2.71	1.4	1.95	69.38
Achnanthes kuwaitensis	0	2.61	1.31	1.81	71.19
Rhabdonema adriaticum	0	2.53	1.26	1.75	72.94
Tryblionella coarctata	0	2.39	1.2	1.66	74.59
Fragilaria sp. 2	0	2.03	1.01	1.4	75.99
Halamphora kolbei	0	1.95	0.97	1.35	77.34
Amphora sp. 1	1.8	0.53	0.93	1.28	78.63
Seminavis sp.	0.05	1.85	0.91	1.26	79.88
Navicula flagellifera	0.62	1.65	0.83	1.15	81.03
Cocconeis pseudomarginata	0.82	2.32	0.82	1.13	82.16
Nitzschia laevis	0	1.6	0.8	1.11	83.27
Navicula sp.1	0.18	1.49	0.78	1.08	84.35
Navicula directa	0.15	1.25	0.62	0.86	85.21
Halamphora subangularis	0	1.22	0.61	0.85	86.06
Striatella unipunctata	0.48	1.02	0.6	0.84	86.89
Mastogloia cuneata	1.1	0.2	0.53	0.74	87.63
Diploneis crabro	0	0.65	0.32	0.45	88.08
Pinnularia sp.	0	0.65	0.32	0.45	88.53
Grammatophora oceanica	0.58	0.65	0.31	0.43	88.95
Nitzschia frustulum	0.6	0	0.3	0.42	89.37
Haslea duerrenbergiana	0	0.57	0.29	0.4	89.77
Pinnularia quadratarea var. *cuneata*	0	0.5	0.25	0.35	90.12

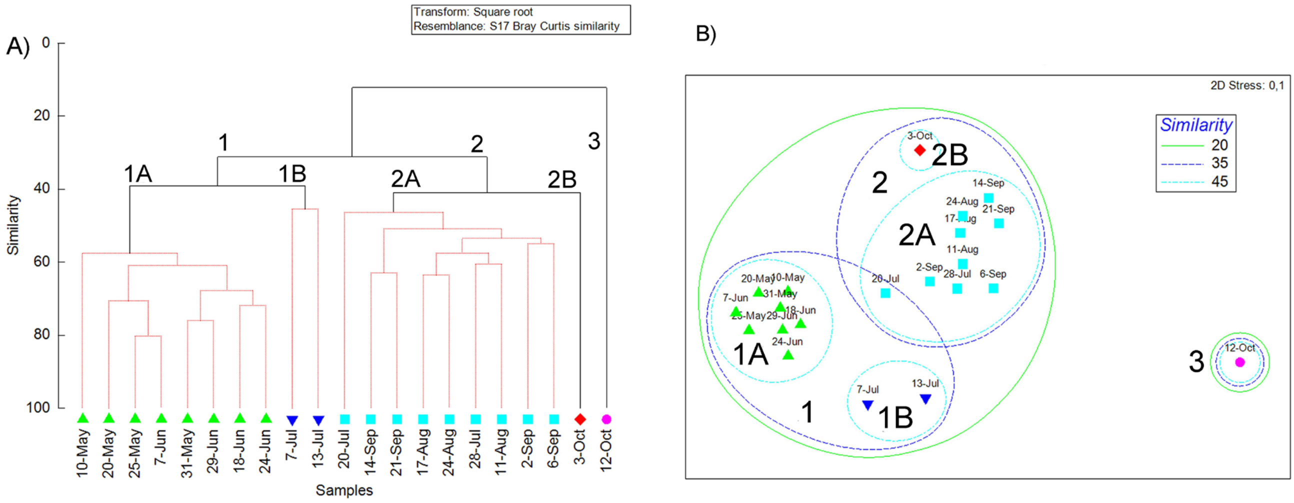

Fig. 4. Cluster analysis and non-metric multidimensional scaling (NMDS) ordination on Bray-Curtis similarities matrices from square root transformed species-relative abundance data of periphytic diatom communities in 21 samples on artificial substrate (glass slides) at depth of 1 m in the marine Lake Mrtvo More in 2016, showing the colonization dynamics of the diatom communities in abundance. For the ordination analysis all recorded diatom taxa were used. Group average similarity values of clusters with significant differences from CLUSTER analysis were overlaid on the NMDS plot (SIMPROF, $P < 0.05$). A) Cluster analysis. Red dotted lines showing no significant difference among samples and indicating taxa homogeneous clusters detected by SIMPROF. B) NMDS. Numbers correspond to the same main clusters detected by SIMPROF. Letters A and B indicate sub-clusters within main clusters. N = 21.

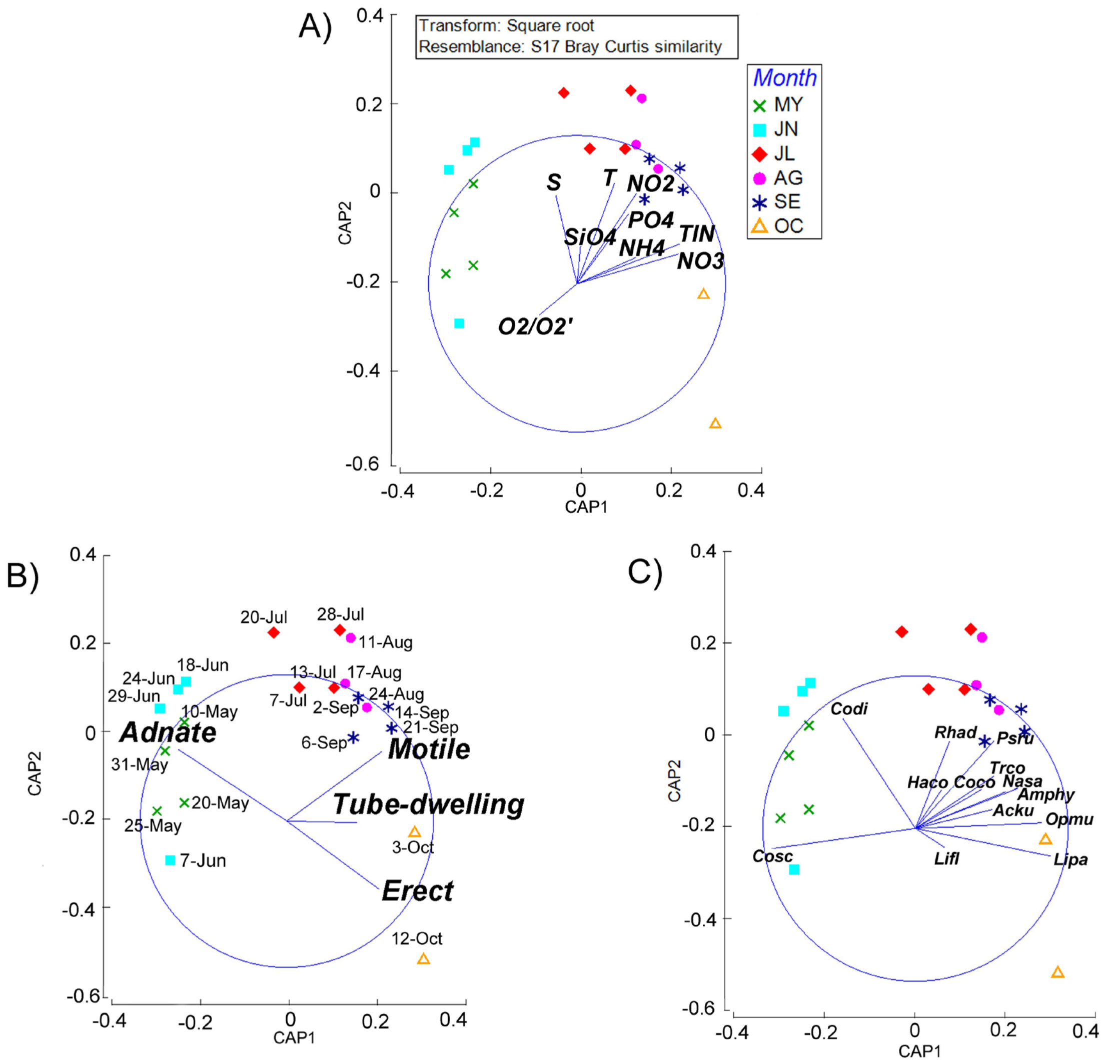

Fig. 5. Canonical analysis of Principle coordinates (CAP) biplot based on 21 samples in Lake Mrtvo More in 2016 showing: A – diatom relative abundance (%) data and vectors of the nine physico-chemical parameters (arrows), B – diatom relative abundance (%) data and vectors of the diatom growth forms (arrows), C – months and vectors of diatom relative abundance (%) data (arrows). S – salinity, T – temperature, O_2/O_2' – oxygen saturation, TIN – total inorganic nitrogen, NO_3^- – nitrate, NO_2^- – nitrite, NH_4^+ – ammonium, PO_4^{3-} – phosphate, SiO_4^{4-} – silicate. A dataset of 13 diatom taxa with frequency of appearance ≥ 17% and average relative abundance ≥ 2% was selected: Acku – *Achnanthes kuwaitensis*, Amhy – *Halamphora hyalina*, Coco – *Cocconeis costata*, Codi – *Cocconeis dirupta* var. *flexella*, Cosc – *Cocconeis scutellum* var. *scutellum*, Haco – *Halamphora coffeiformis*, Lifl – *Licmophora flabellata*, Lipa – *Licmophora paradoxa*, Nasa – *Navicula salinicola*, Opmu – *Opephora mutabilis*, Psru – *Psammodictyon rudum*, Rhad – *Rhabdonema adriaticum*, Trco – *Tryblionella coarctata*.

Discussion

This study confirms that glass surfaces in a marine environment are susceptible to biofouling and the biofilm is mostly composed of bacteria and diatoms. Although glass is a high-energy hydrophilic surface and, as reported by many studies, diatoms adhere more successfully to hydrophobic surfaces such as plastic panels, glass has been widely used as artificial substrate for the settlement of diatoms in both marine and freshwater environments (Nenadović et al. 2015).

In this study, within 30 days of contact a brownish-green film of periphyton appeared on the glass substrate surface, consisting mostly of diatoms dominated by genus *Cocconeis*. These results are in agreement with previous observations of Romagnoli et al. (2007) who reported that a well-developed community, characterized by the presence of adnate living forms, is established after 3–5 weeks (the "mature phase"). Our findings are also similar to a investigation of Yuanyuan et al. (2014) where the colonization periods of 10 days or more might be considered sufficient for the mature communities of periphytic diatoms. Additionally, results of this study confirm that the sampling strategy at 1 m is effective in detecting the ecological features for bioassessment of marine ecosystems (Yuanyuan et al. 2014).

The relationships between diatom communities and substrate are mediated by the presence of the bacterial biofilm that first covers the substrate in succession phases (Totti et al. 2007). The presence of bacterial biofilm on artificial substrates may reduce any selective preference displayed by substrates as the presence of organic biofilm makes the substrate

Tab. 2. Correlation matrix composed of 10 physico-chemical parameters and 20 diatom taxa with frequency of appearance ≥ 17% and average relative abundance ≥ 2% (n = 25). Only significant correlations are reported (*P < 0.05, **P 0.01, ***P < 0.001). T – temperature, S – salinity, TIN – total inorganic nitrogen, PO_4^{3-} – phosphate, SiO_4^{4-} – silicate, CHL – chlorophyll *a* concentrations, O_2/O_2' – oxygen saturation, NO_3^- – nitrate, NO_2^- – nitrite, NH_4^+ – ammonium, Acku – *Achnanthes kuwaitensis*, Amhy – *Halamphora hyalina*, Amko – *Halamphora kolbei*, Coco – *Cocconeis costata*, Codi – *Cocconeis dirupta* var. *flexella*, Cosc – *Cocconeis scutellum* var. *scutellum*, Haco – *Halamphora coffeiformis*, Hasu – *Halamphora subangularis*, Lifl – *Licmophora flabellata*, Lipa – *Licmophora paradoxa*, Nafl – *Navicula flagellifera*, Nasa – *Navicula salinicola*, Nasp – *Navicula* sp., Nifr – *Nitzschia frustulum*, Nila – *Nitzschia laevis*, Opmu – *Opephora mutabilis*, Psru – *Psammodictyon rudum*, Rhad – *Rhabdonema adriaticum*, Sesp – *Seminavis* sp., Trco – *Tryblionella coarctata*.

	T	S	TIN	PO_4^{3-}	SiO_4^{4-}	CHL	O_2/O_2'	NO_3^-	NO_2^-	NH_4^+
T	.	.	.	.	.	.	.	.	**0.548	.
S	.	.	.	.	.	.	.	.	.	.
TIN	.	.	.	*0.687	*0.511	*0.550	**–0.620	***0.963	***0.759	.
PO_4^{3-}	.	.	*0.687	.	.	**0.686	*–0.706	*0.644	**0.678	.
SiO_4^{4-}	.	.	*0.511	.	.	.	*–0.529	*0.544	.	.
CHL	.	.	*0.550	**0.686	.	.	.	*0.548	*0.591	.
O_2/O_2'	.	.	**–0.620	*–0.706	*–0.529	.	.	**–0.690	.	.
NO_3^-	.	.	***0.963	*0.643	*0.544	*0.548	**–0.690	.	*0.707	.
NO_2^-	**0.548	.	***0.759	**0.678	.	*0.591	.	*0.707	.	*0.538
NH_4^+	.	.	.	.	.	.	.	.	*0.538	.
Acku	.	.	*0.478	.	.		*–0.509	*0.498	.	.
Amhy	.	.	*0.557	.	.	*0.599	.	*0.555	.	.
Amko	.	.	*0.516	.	.		.	*0.514	.	.
Coco	.	.	**0.684	***0.775	.	*0.550	*–0.542	**0.660	.	.
Codi	*0.532	.	.	.	.	.	.	.	.	.
Cosc	.	*–0.525	**–0.631	*–0.494	.	*–0.587	.	**–0.577	.	.
Haco	.	.	.	.	.	.	.	.	.	.
Hasu	.	.	.	.	.	.	.	.	.	.
Lifl	.	.	.	.	.	.	.	.	.	*0.478
Lipa	.	.	*0.523	*0.567	.	*0.662	.	.	.	.
Nafl	.	.	.	.	.	.	.	.	.	.
Nasa	.	.	*0.548	.	.	*0.516	*–0.474	*0.589	.	.
Nasp	.	.	.	.	.	.	*–0.479	.	.	.
Nifr	.	.	.	.	.	.	.	.	.	.
Nila	.	.	.	.	.	.	.	.	.	.
Opmu	.	.	**0.672	*0.664	.	*0.641	*–0.584	**0.656	.	.
Psru	**0.601	*0.475	.	.	.	*0.648	.	.	.	.
Rhad	.	.	*0.599	*0.518	.	*0.663	.	*0.588	*0.512	.
Sesp	.	.	*0.557	***0.738	.	*0.675	*–0.501	*0.528	.	.
Trco	.	.	*0.504	.	.	*0.572	.	*0.552	.	.

uniform (Korte and Blinn 1983) or may enhance or inhibit the growth of different diatom species (Peterson and Stevenson 1989). Most of the research done so far has focused on the first hours of the experimental periods. Cviić (1953) showed that the rapidity of attachment depends on the quantity of organic material in ambient water and that the first film on the slides is formed by bacteria and following them the most numerous attachments are provided by diatomeae. Similar results have also been reported and showed that in a eutrophic environment bacteria rapidly reach maximum capacity on the slide (Zobell and Allen 1935, Cviić 1953).

The direct microscopy method of counting includes all visible bacterial cells of which some could not form colonies on agar plate, or would take a long time to incubate while spread on the agar plate method, traditionally used in microbiology, has its limits both in qualitative and quantitative sense because it yields counts of less that 1% of the total bacterial numbers (Simu et al. 2005). Because of that fact the number of attached heterotrophic bacteria in our experiments could not be compared with results in the north Adriatic Sea counted on agar plate in the initial stages of experiments (Mejdandžić et al. 2015). In addition, Mejdandžić et al. (2015) investigated colonization of bacteria on plexiglass (polymer of methyl methacrylate) plates set vertically above the bottom at a depth of 5 m.

Despite its small dimensions and a level of seasonal anthropogenic disturbance, Lake Mrtvo More had a high diatom species richness. In this study, the total number of diatom taxa (285) is comparable to some studies of epilithic diatoms in the south Adriatic (Hafner et al. 2018, Car et al. 2019) but higher than recorded in earlier studies of periphytic diatoms growing on artificial substrates in the north Adriatic (Mejdandžić et al. 2015, Nenadović et al. 2015) or in a study of surface sediment layer in the Venice lagoon

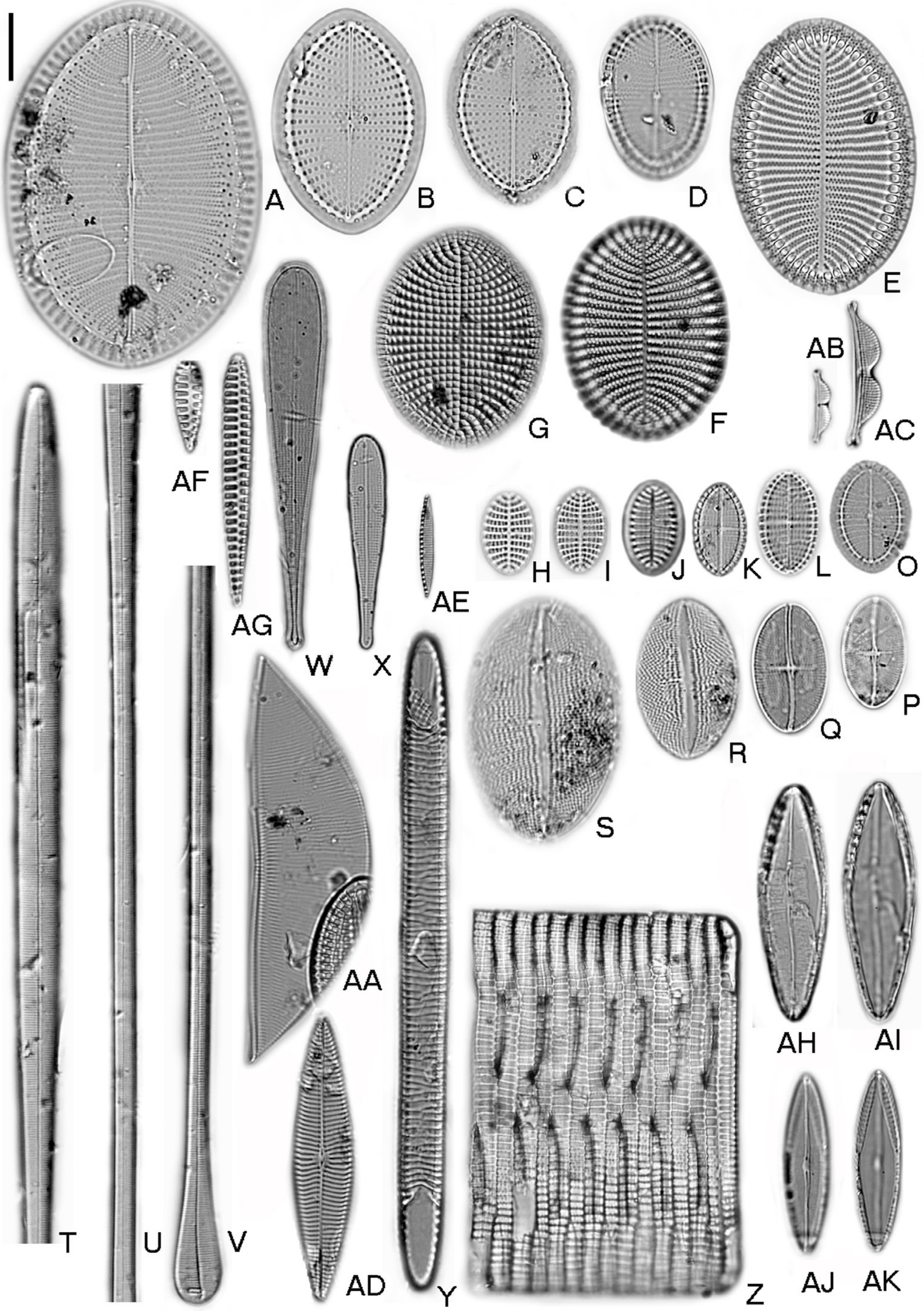

Fig. 6. Light micrographs of selected diatom taxa found in Lake Mrtvo More in 2016. A-G – *Cocconeis scutellum* var. *scutellum*, H-O – *Cocconeis costata*, P-S – *Cocconeis dirupta* var. *flexella*, T-V –*Licmophora flabellata*, W, X – *Licmophora paradoxa*, Y, Z – *Rhabdonema adriaticum*, AA – *Halamphora hyalina*, AB, AC – *Halamphora kolbei*, AD – *Navicula flagellifera*, AE – *Nitzschia frustulum*, AF, AG – *Opephora mutabilis*, AH, AI, AJ, AK – *Mastogloia cuneata*. Scale bar: 10 µm applies to all images.

(Facca and Sfriso 2007). Nevertheless, we believe that at least partly this can be caused by the differences in methodology used. Mejdandžić et al. (2015) determined 30 diatom taxa in the periphyton assemblage on plexiglass plates in a marine environment within 30 days of contact. Apart from the different artificial substrate used, the plates were set vertically rather than horizontally as in our study and the depth was 5 m. Nenadović et al. (2015) reported 41 diatom genera periphytic on 11 different artificial substrates, including glass, exposed to a marine environment in a coastal area of the Central Adriatic Sea for a period of 30 days. The iron substrate showed the greatest diversity (20 taxa), while the lowest diatom diversity was recorded on plastic (4 taxa), concrete (4 taxa) and rubber (2 taxa). While in this study 16 taxa were recorded after a period of one month, Nenadović et al. (2015) observed 10 diatom taxa associated with glass.

The differences in the number of diatom taxa detected were probably due to differences in methodology used as in the study of Nenadović et al. (2015) artificial substrates were exposed to the marine environment at the much greater depth of 12 m. Although Nenadović et al. (2015) concluded that the settling of diatoms on a substrate is greatly influenced by substrate characteristics and the preferences of a diatom communities and diatom species, Totti et al. (2007) found no significant difference in diatom abundance, composition and biomass values for the three artificial substrates examined (marble, quartzite and slate) and pointed out that beside the chemical composition of the substrate, its physical structure should also be considered. The greatest abundance (557,156 cells cm^{-2}) observed by Totti et al. (2007) were higher than those recorded in our study (333,076 cells cm^{-2}). Munda (2005) examined seasonal fouling by diatoms on vertical concrete plates as artificial substrate at different depths. In general, our findings lie within the results of Munda (2005). Caput Mihalić et al. (2008) also reported 50 diatom taxa on plexiglass plates after 4 weeks (July) during which the submerged artificial substrates exposed at depths of 0.5, 1, 1.5 and 2. Very similar observations in the number diatom taxa were found in a study of Hafner et al. (2018) who identified 264 diatom taxa within 69 genera in a marine epilithic diatom community of the small semi-enclosed oligotrophic bay in the Middle Adriatic. In addition, a comparable number of taxa (310 epilithic taxa, 65 genera) was observed by Car et al. (2019) in a study of epilithic diatom communities from areas of invasive *Caulerpa* species in the Adriatic.

In our study considerable fluctuation of diatom species number occurred. It is very likely that the set of algal taxa on the artificial substrate varies to some extent due to predation (as for example on 31st May when a snail was observed eating periphyton from the glass).

The composition of benthic diatoms throughout the exposure period was relatively consistent with the dominant taxa belonging to genus *Cocconeis* whose greatest abundance was observed after a month of exposure. As succession progressed, *Cocconeis* taxa were replaced by other genera of benthic diatoms the abundance of which increased, in particular, from the end of July. The second to appear on the newly available artificial habitats were motile taxa (e.g. *Nitzschia*, *Navicula*). The co-occurrence and dominance of motile diatoms is a further step since biraphid species are capable of finding the optimum light and nutrient conditions by active movement on and through the biofilm (Romagnoli et al. 2007).

The species assemblages present during early colonisation differed from those at later stages. Assemblages were found to be quite homogeneous up to the mid-July. An increase in species diversity index from mid-July was noted and the maximum occurred in August. In general, during summer diatom diversity increased, mostly due to fluctuations of taxa of the genera *Cocconeis*. Generally, abundance of diatom cells of genus *Cocconeis* decreased through the investigated period.

Similar values of the Shannon diversity index were found in Lake Mrtvo More as were recorded in a study of the benthic diatom abundance and taxonomic composition in the Venice lagoon (Facca and Sfriso 2007). Moreover, the seasonal variations of the Shannon diversity index in the Venice lagoon were not correlated with seawater temperature, although it varied between 6 and 29 °C, but rather with nutrient concentrations. A comparison is, however, difficult due to the different sampling design employed.

Relationships between physico-chemical parameters and benthic diatoms

Strong relationships between environmental variables and diatom assemblages were found in Lake Mrtvo More and shifts in dominance at the species level were recognized. In the first stage of the experiment, when generally the nutrient concentrations were low, the lowest number of diatom taxa was recorded and adnate diatoms appeared. In general, adnate taxa adhere strongly horizontally to the substrate by means of their raphe valve and may easily benefit from a nutrient exchange with the substrate due to their adhering mode through the valve face (Round 1981, Sullivan 1984, Romagnoli et al. 2014). Diatom species richness of Lake Mrtvo More was strongly correlated with TIN, constraints during which *C. scutellum* var. *scutellum* remained a common species in the diatom community. This taxon was the dominant in the assemblages during the first months of experiment but its relative abundance declined when seasonal anthropogenic disturbance started (July). It seems that the changes in nutrient concentrations induce changes in species diversity. This is in agreement with the results of Marcus (1980), who found differences in diversity between sites with varying levels of nitrogen concentration during investigation of periphytic communities using glass slide substrates when recording a greater algal growth downstream of a dam, which was attributed to nitrogen discharges from the reservoir. Marcus (1980) suggested that while *Cocconies* became dominant at the three downstream sites because of its greater efficiency in obtaining or incorporating limited nitrogen resources, species other than *Cocconies* dominated the diatom communities in which nitrogen concentrations were enriched apparently because of higher potential growth rates which could be realized with the elevated nutrient conditions. *Cocconeis* taxa clearly differ in their response to nutrient supply, leading to an altered community composition, which may be detected only if the species level is considered. As *C. dirupta* var. *flexella* was associated with higher temperature values, *C. dirupta* var. *flexella* remained a common species and characterized the benthic diatom assemblage of Mrtvo More during the warmer period of the year.

In this study *Nitzschia frustulum* was not influenced by any of these 10 physico-chemical parameters. This was showed in previous studies in which *N. frustulum* has been described as a highly tolerant diatom taxa which is resistant to organic pollution and is associated with areas affected by intensive agricultural and industrial activities (Tornés et al.

2007, and references therein). In addition, the genus *Navicula* has a high adaptability to all trophic status of ecosystem and appears to be tolerant of pollution (Agatz et al. 1999, Cunningham et al. 2005, Cibic and Blasutto 2011). Our findings are consistent with this observation, in particular for *N. salinicola*. Pollution tolerant genera like *Nitzchia* and *Navicula* occurred in abundance through the summer season. Generally, abundance of diatom taxa of *Nitzchia* and *Navicula* was low through the oligotrophic state of the lake.

The abundance of opportunistic species provided the possibility of distinguishing possible anthropogenic pressures on the ecosystem. Although the variability of the physico-chemical variables in Lake Mrtvo More suggests the presence of two distinct environmental contexts that enhance the proliferation of different benthic communities, it cannot be clearly connected with anthropogenic impact by visitors (swimmers) during the summer. Apart from an increase in nutrient concentrations during summer, sea temperature and salinity also rise. The increase in salinity is caused by the interaction of several factors, such as low precipitation and higher air temperature and evaporation. Moreover, along with salinity, seawater temperatures also seem to be very important for some species (e.g. *P. rudum*) as they were associated with higher temperature values. SiO_4^{4-} was measured and compared with benthic diatom abundance but no significant correlation was recorded. No relationships between SiO_4^{4-} in the water column and benthic diatom abundance have already previously been described (Facca and Sfriso 2007).

Conclusion

The study was based on a dataset collected from marine lake on the eastern Adriatic coast during the warmer period of the year. In sum, the data revealed the affinity of diatoms as a major fouling community to an artificial material. The results showed in particular the diatom colonization during increase in nutrient concentrations. Among physico-chemical parameters, temperature, salinity and NO_3^- had the greatest influence on diatom species abundance. An increase of species diversity was closely related to nutrient concentration enrichment.

The present study contributes to the knowledge of the taxonomy and ecology of benthic diatom communities in the Adriatic and Mediterranean as well. However, data obtained here needs to be extended with further investigation which will cover the whole year. These studies must include other important abiotic (e.g. irradiance) and biotic (e.g. grazing) factors not addressed in the present work. More practically, the quantification of diatom contribution to the flow of energy and cycling of material in the lake will be useful for a rational management of this important resource in the natural heritage.

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Author contributions

A.C. and N.J. designed the study. A.C. conducted the field sampling and led the writing. D.H. counted diatoms on slides using a light microscope. S.LJ. helped with experiment design, assisted the field sampling, made quantitative analyses of diatom samples and determined Chl *a* fluorometrically. I.D.R. analyzed physico-chemical parameters. S.B.Ć. made bacteriological analyses. All authors revised the manuscript.

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Appendix

List of 285 marine benthic diatom taxa recorded on artificial substrat (glass) in Lake Mrtvo More from May to October 2016. Taxa found only once in all number of samples (n = 21) with relative abundances lower than 1% are indicated with asterisk [*]. The genera at first mention are listed in boldface.

Achnanthes *brevipes* C.Agardh
Achnanthes brevipes var. *brevipes* Agardh
Achnanthes brevipes var. *intermedia* (Kützing) Cleve
Achnanthes cf. *ceramii* Hendey
Achnanthes cuneata Grunow
Achnanthes curvirostrum J.Brun
Achnanthes groenlandica (Cleve) Grunow
Achnanthes hyperboreoides A.Witkowski, Metzeltin & Lange-Bertalot [*]
Achnanthes javanica Grunow
Achnanthes kuwaitensis Hendey
Achnanthes longipes C.Agardh
Achnanthes pseudogroenlandica Hendey
Achnanthes separata Hustedt
Actinocyclus *roperi* (Brébisson) Grunow ex Van Heurck
Actinocyclus subtilis (W.Gregory) Ralfs [*]
Actinoptychus sp.
Amphicocconeis *disculoides* (Hustedt) Stefano & Marino
Amphitetras *subrotundata* Janisch
Amphora *abludens* R.Simonsen
Amphora bigibba var. *interrupta* (Grunow) Cleve
Amphora cingulata Cleve
Amphora crassa W.Gregory
Amphora delicatissima Krasske
Amphora exilitata M.H.Giffen
Amphora gracilis Ehrenberg
Amphora laevissima W.Gregory
Amphora lineolata Ehrenberg [*]
Amphora lunata Østrup
Amphora proteus W.Gregory [*]
Amphora pseudohyalina Simonsen [*]
Amphora sp.
Ardissonea *crystallina* (C.Agardh) Grunow
Ardissonea formosa (Hantzsch) Grunow
Ardissonea robusta (Ralfs ex Pritchard) De Notaris
Ardissonea sp. [*]
Asterolampra *marylandica* Ehrenberg
Aulacoseira *granulata* (Ehrenberg) Simonsen
Bacillaria paxillifera (O.F.Müller) T.Marsson
Bacillaria *socialis* (Gregory) Ralfs
Berkeleya sp.
Biddulphia *biddulphiana* (J.E.Smith) Boyer
Brachysira sp.
Brebissonia *lanceolata* (C.Agardh) R.K.Mahoney & Reimer
Caloneis *bicuneata* (Grunow) Boyer
Caloneis liber (W.Smith) Cleve
Caloneis liber var. *linearis* Cleve [*]
Caloneis sp.
Campylodiscus *innominatus* R.Ross & Abdin
Catacombas *gaillonii* (Bory) D.M.Williams & Round
Climacosphenia *moniligera* Ehrenberg
Cocconeis *convexa* M.H.Giffen
Cocconeis costata var. *hexagona* Grunow [*]
Cocconeis costata W.Gregory
Cocconeis dirupta var. *flexella* (Janisch & Rabenhorst) Grunow
Cocconeis dirupta W.Gregory
Cocconeis irregularis (P.Schulz) A.Witkowski in Witkowski
Cocconeis peltoides Hustedt
Cocconeis pseudomarginata W.Gregory
Cocconeis schmidtii Heiden
Cocconeis scutellum var. *scutellum* Ehrenberg
Cocconeis stauroneiformis (W.Smith) H.Okuno [*]
Cocconeis woodii Reyes [*]
Coronia *decora* (Brébisson) Ruck & Guiry
Craspedostauros *indubitabilis* (Lange-Bertalot & S.I.Genkal) E.J.Cox
Diploneis *bombus* (Ehrenberg) Ehrenberg
Diploneis cf. *parca* (A.W.F.Schmidt) Boyer
Diploneis chersonensis (Grunow) Cleve
Diploneis crabro (Ehrenberg) Ehrenberg [*]
Diploneis didyma (Ehrenberg) Ehrenberg
Diploneis incurvata var. *dubia* Hustedt [*]
Diploneis nitescens (W.Gregory) Cleve
Diploneis notabilis (Greville) Cleve
Diploneis smithii (Brébisson) Cleve
Diploneis smithii var. *recta* Peragallo
Diploneis sp.1 [*]
Diploneis sp.2
Diploneis sp.3
Diploneis splendida Cleve
Diploneis stroemii Hustedt
Diploneis vacillans (A.W.F.Schmidt) Cleve
Diploneis vacillans var. *renitens* A. Schmidt

Entomoneis *paludosa* (W.Smith) Reimer [*]
Fallacia *floriniae* (M.Møller) Witkowski
Fallacia forcipata (Greville) Stickle & D.G.Mann
Fallacia ny (Cleve) D.G.Mann
Fallacia pygmaea (Kützing) Stickle & D.G.Mann
Fogedia *acuta* (Salah) Witkowski, Lange-Bertalot & Metzeltin
Fogedia christensenii A.Witkowski, Metzeltin & Lange-Bertalot
Fogedia finmarchica (Cleve & Grunow) A.Witkowski, Metzeltin & Lange-Bertalot
Fragilaria *capensis* Grunow
Fragilaria cf. *sopotensis* Witkowski & Lange-Bertalot [*]
Fragilaria sp.1
Fragilaria sp.2
Grammatophora *angulosa* Ehrenberg [*]
Grammatophora angulosa var. *islandica* [*]
Grammatophora macilenta W.Smith [*]
Grammatophora marina (Lyngbye) Kützing
Grammatophora oceanica Ehrenberg
Grammatophora oceanica var. *subtilissima* (J.W.Bailey) De Toni
Grammatophora serpentina Ehrenberg
Halamphora *acutiuscula* (Kützing) Levkov
Halamphora coffeiformis (C.Agardh) Levkov
Halamphora costata (W.Smith) Levkov
Halamphora cuneata (Cleve) Levkov
Halamphora exigua (W.Gregory) Levkov
Halamphora hyalina (Kützing) Rimet & R.Jahn
Halamphora kolbei (Aleem) Álvarez-Blanco & S.Blanco
Halamphora subangularis (Hustedt) Levkov
Halamphora subholsatica (Krammer) Levkov [*]
Halamphora turgida (Gregory) Levkov
Hantzschia cf. *distinctepunctata* Hustedt
Hantzschia cf. *marina* (Donkin) Grunow
Hantzschia sp.
Hantzschia virgata (Roper) Grunow
Hantzschia virgata var. *leptocephala* Østrup
Haslea *britannica* (Hustedt & Aleem) Witkowski, Lange-Bertalot & Metzeltin
Haslea crucigera (W.Smith) Simonsen
Haslea duerrenbergiana (Hustedt) F.A.S.Sterrenburg
Haslea spicula (Hickie) Bukhtiyarova
Hippodonta *caotica* Witkowski [*]
Hyalodiscus *radiates* (O'Meara) Grunow
Hyalosira *interrupta* (Ehrenberg) J.N.Navarro
Hyalosynedra *laevigata* (Grunow) D.M.Williams & Round
Licmophora *abbreviata* C.Agardh
Licmophora flabellata (Greville) C.Agardh
Licmophora gracilis (Ehrenberg) Grunow [*]
Licmophora paradoxa (Lyngbye) Agardh
Licmophora pfannkuckae Giffen [*]
Licmophora remulus (Grunow) Grunow
Licmophora sp. [*]
Licmophora tincta (C.Agardh) Grunow
Luticola sp.
Mastogloia *adriatica* Voigt
Mastogloia angulata F.W.Lewis
Mastogloia belaensis Voigt
Mastogloia binotata (Grunow) Cleve
Mastogloia biocellata (Grunow) G.Novarino & A.R.Muftah
Mastogloia borneensis Hustedt
Mastogloia braunii Grunow
Mastogloia cf. *affirmata* (Leudiger-Fortmorel) Cleve
Mastogloia corsicana Grunow
Mastogloia crucicula (Grunow) Cleve [*]
Mastogloia crucicula var. *alternans* Zanon [*]
Mastogloia cuneata (Meister) R.Simonsen
Mastogloia cyclops Voigt
Mastogloia decussata Grunow
Mastogloia emarginata Hustedt
Mastogloia emerginata (cf. *ovulum*)
Mastogloia erythraea Grunow
Mastogloia exigua F.W.Lewis
Mastogloia exilis Hustedt
Mastogloia fallax Cleve
Mastogloia fimbriata (T.Brightwell) Grunow
Mastogloia grunowii A.Schmidt
Mastogloia horvathiana Grunow
Mastogloia ignorata Hustedt
Mastogloia mauritiana Brun
Mastogloia obliqua Hagelstein
Mastogloia ovalis A.Schmidt [*]
Mastogloia ovulum Hustedt
Mastogloia pseudolatecostata T.A.Yohn & R.A.Gibson
Mastogloia pusilla Grunow
Mastogloia regula Hustedt
Mastogloia robusta Hustedt
Mastogloia similis Hustedt
Mastogloia splendida (Gregory) H.Pergallo
Mastogloia varians Hustedt
Mastogloia sp.1 [*]
Nanofrustulum *shiloi* (J.J.Lee, Reimer & McEnery) Round, Hallsteinsen & Paasche
Navicula *agnita* Hustedt
Navicula besarensis Giffen
Navicula borneoensis Hustedt
Navicula cincta (Ehrenberg) Ralfs
Navicula dehissa Giffen
Navicula directa (W.Smith) Ralfs
Navicula eidrigiana J.R.Carter
Navicula erifuga Lange-Bertalot
Navicula flagellifera Hustedt
Navicula frigida Grunow
Navicula gregaria Donkin [*]
Navicula grippii Simonsen
Navicula johanrossii Giffen
Navicula palpebralis Brébisson ex W.Smith
Navicula palpebralis var. *minor* (Gregory) Grunow
Navicula rostellata Kützing
Navicula salinarum var. *rostrata* (Hustedt) Lange-Bertalot
Navicula salinicola Hustedt
Navicula subagnita Proshkina-Lavrenko
Navicula sp.1
Neohuttonia *reichardtii* (Grunow) Hustedt
Nitzschia *agnewii* Choln
Nitzschia bulnheimiana (Rabenhorst) H.L.Smith
Nitzschia capitellata Hustedt, nom. inval.
Nitzschia carnicobarica Desikachary & Prema
Nitzschia compressa (Bailey) Boyer var. *compressa*
Nitzschia compressa var. *elongata* (Grunow) Lange-Bertalot
Nitzschia distans W.Gregory
Nitzschia frustulum (Kützing) Grunow
Nitzschia fusiformis Grunow
Nitzschia grossestriata Hustedt
Nitzschia improvisa Simonsen
Nitzschia incurvata var. *lorenziana* R.Ross
Nitzschia insignis W.Gregory
Nitzschia laevis Frenguelli
Nitzschia lanceolata var. *minima* Van Heurck
Nitzschia liebethruthii Rabenhorst
Nitzschia longissima (Brébisson) Ralfs [*]
Nitzschia macilenta W.Gregory
Nitzschia marginulata var. *didyma* Grunow [*]
Nitzschia panduriformis var. *continua* Grunow

Nitzschia pellucida Grunow
Nitzschia reversa W.Smith
Nitzschia sigma (Kützing) W.Smith
Nitzschia subconstricta Desikachary & Prema [*]
Nitzschia sp.1
Nitzschia sp.2 [*]
Nitzschia tryblionella Hantzsch
Nitzschia valdestriata Aleem & Hustedt [*]
Nitzschia ventricosa Kitton [*]
***Opephora** burchardtiae* Witkowski
Opephora guenter-grassii (Witkowski & Lange-Bertalot) Sabbe & Vyverman
Opephora mutabilis (Grunow) Sabbe & Wyverman
Opephora pacifica (Grunow) Petit [*]
Opephora sp.1 [*]
***Parlibellus** berkeleyi* (Kützing) E.J.Cox [*]
Parlibellus calvus A.Witkowski, Metzeltin & Lange-Bertalot
Parlibellus cf. *cruciculoides* (C.Brockmann) Witkowski, Lange-Bertalot & Metzeltin
Parlibellus delognei (Van Heurck) E.J.Cox
Parlibellus rhombicula (Hustedt) Witkowski
Parlibellus sp.
***Petrodictyon** gemma* (Ehrenberg) D.G.Mann
Pinnularia claviculus Schulz
***Pinnularia** quadratarea* var. *cuneata* Østrup [*]
Pinnularia sp. [*]
***Placoneis** flabellata* (F.Meister) Kimura, H.Fukushima & Ts. Kobayashi [*]
***Plagiogramma** staurophorum* (W.Gregory) Heiberg
***Plagiotropis** lepidoptera* (W.Gregory) Kuntze
Plagiotropis tayrecta Paddock
Planthotrix sp.1
***Pleurosigma** formosum* W.Smith
Pleurosigma sp.1
Pleurosigma sp.2
***Podocystis** adriatica* (Kützing) Ralfs [*]
***Protokeelia** cholnokyi* (M.H.Giffen) Round & Basson
***Psammodictyon** panduriforme* (W.Gregory) D.G.Mann
Psammodictyon rudum (Cholnoky) D.G.Mann
***Rhabdonema** adriaticum* Kützing
Rhabdonema arcuatum (Lyngbye) Kützing
***Rhoicosphenia** abbreviata* (C.Agardh) Lange-Bertalot [*]
Rhoicosphenia marina (Kützing) M.Schmidt
Rhoicosphenia sp.
***Rhopalodia** musculus* (Kützing) Otto Müller [*]
Rhopalodia pacifica Krammer [*]
Seminavis sp.1
***Stauroneis** plicata* C.Brockmann
Stauroneis undata Hustedt
***Stauronella** decipiens* (Hustedt) Lange-Bertalot [*]
Stauronella sp.1
Staurosira sp.1 [*]
***Stephanodiscus** hantzschii* Grunow
***Striatella** unipunctata* (Lyngbye) C.Agardh
***Surirella** fastuosa* (Ehrenberg) Ehrenberg
Surirella scalaris M.H.Giffen [*]
Surirella venusta Østrup
***Synedra** fulgens* (Greville) W.Smith
Synedra laevis Kützing
Synedra tabulata var. *obtusa* Pantocsek
***Tabularia** fasciculata* (C.Agardh) D.M.Williams & Round
Tabularia investiens (W.Smith) D.M.Williams & Round
***Tetramphora** decussata* (Grunow) Stepanek & Kociolek
Tetramphora sulcata (Brébisson) Stepanek & Kociolek
***Toxarium** hennedyanum* (Gregory) Pelletan
Toxarium undulatum J.W.Bailey
***Trachyneis** aspera* (Ehrenberg) Cleve
***Triceratium** pentacrinus* (Ehrenberg) Wallich
Triceratium reticulum Ehrenberg
Triceratium sp.1
***Trigonium** arcticum* (Brightwell) Cleve
Trigonium formosum (Brightwell) Cleve [*]
Trigonium sp.1
Trigonium sp.2
***Tryblionella** coarctata* (Grunow) D.G.Mann
Tryblionella didyma (Hustedt) D.G.Mann
Tryblionella navicularis (Brébisson) Ralfs
***Vikingea** promunturi* (Giffen) Witkowski, Lange-Bertalot & Metzeltin