

Epilithic diatom communities from areas of invasive *Caulerpa* species (*Caulerpa taxifolia* and *Caulerpa cylindracea*) in the Adriatic Sea, NE Mediterranean

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Abstract

Epilithic diatom community structures are compared among three locations of the eastern Adriatic Sea coast (Croatia), in the presence of two different invasive *Caulerpa* species, *C. taxifolia* and *C. cylindracea*, which have spread rapidly in the Mediterranean in the last decades. Forty samples were taken seasonally at three sites (island of Hvar, island of Mljet, and Dubrovnik) between November 2008 and October 2010. Light and electron microscopy examinations were used for qualitative analysis. Altogether 310 epilithic taxa belonging to 65 genera were identified. The highest number of these taxa belonged to *Mastogloia* (48) and *Amphora* (32), followed by *Diploneis* (24), *Nitzschia* (23), *Navicula* (22), and *Cocconeis* (19). A similar seasonal shift in growth form structure was observed for epilithic communities at the three sampling sites, where the number of erect group diatoms doubled (Hvar, Mljet) and tripled (Dubrovnik) in spring. Apart from erect forms, Dubrovnik and Mljet were characterised by adnate forms, while Hvar was characterised by tube-dwelling forms. The highest values of the Shannon-Wiener Diversity Index were recorded in autumn and ranged from 5.26 to 5.34. ANOSIM tests performed on species relative abundance data confirmed that the diatom communities differed significantly between the sampling sites, and that the differences were correlated with the presence of the two species of invasive macroalgae (Hvar – area of *C. taxifolia*; Mljet and Dubrovnik – areas of *C. cylindracea*). As revealed by SIMPER analyses, the group of taxa contributing the most to variance between diatom assemblages from sites of different invasive alga included *Cocconeis scutellum* var. *scutellum*, *Rhopalodia pacifica*, *Navicula ramosissima*, and *Berkeleya rutilans*. Although the spatial variation in communities may also reflect the effects of unmeasured environmental variables, the results suggest the possible influence of invasive seaweeds of the genus *Caulerpa* which may affect the habitat by competing with autochthonous algae. This study contributes to the knowledge of diatom diversity both at regional and at broader scales in these challenged environments.

Keywords: Adriatic Sea; allochthonous species; Bacillariophyta; benthos; biodiversity; Croatia; environmental parameters.

Introduction

Diatom communities are widely used as indicators of ecological changes in aquatic environments (Ulanova & Snoeijjs, 2006). Benthic diatoms are the most abundant and diversified part of the marine phytobenthos and play an important role in marine ecosystems. Marine ecosystems have been exposed to serious anthropogenic impacts including habitat loss and degradation, fishing, pollution, climate change, eutrophication, and the introduction and establishment of alien species (Álvarez-Blanco & Blanco, 2014).

Abundance and composition of marine benthic diatom communities are reported from several areas around

the world, e.g., the Baltic Sea (Hillebrand & Sommer, 1997, 2000; Hillebrand *et al.*, 2000; Vilbaste *et al.*, 2000), Antarctica (Cunningham & McMinn, 2004; Majewska *et al.*, 2016), and Brazil (Brandini *et al.*, 2001). In the Mediterranean Sea, Álvarez-Blanco & Blanco (2014) provided a comprehensive overview of benthic diatom taxa.

The composition of diatom populations grown on natural substrates has also been described, in both heavily anthropised and undisturbed areas (Facca *et al.*, 2002a, b; Facca & Sfriso, 2007; Çolak Sabancı & Koray, 2010; Çolak Sabancı, 2011, 2012a; 2012b, 2013; Çolak Sabancı *et al.*, 2011; Cibic & Facca, 2010 and references therein). Furthermore, studies have been conducted on epiphytic diatom communities on macroalgae and seagrasses (Car

et al., 2012; De Stefano *et al.*, 2000) and epizoic diatoms on animals (Romagnoli *et al.*, 2007, 2014 and references therein). Among the epiphytic diatoms, communities on *Posidonia oceanica* (Linnaeus) Delile are among the most analysed (Mazzella, 1983; Mazzella & Spinoccia, 1992; Mazzella *et al.*, 1994; De Stefano *et al.*, 2000; Majewska *et al.*, 2014). In the Adriatic Sea, previous studies have been conducted in the northern coastal areas and estuaries, while seasonal fouling by diatoms has been studied mainly on artificial substrates (e.g., Burić *et al.*, 2004; Munda, 2005; Totti *et al.*, 2007; Caput Mihalić *et al.*, 2008; Levkov *et al.*, 2010; Mejdandžić *et al.*, 2015; Nenadović *et al.*, 2015).

Benthic diatom communities of the Italian North Adriatic have been well studied on an artificial hard substrates at two stations, one in the Gulf of Trieste (Bartole *et al.*, 1991-94) and the other in Venice lagoon (Tolomio & Andreoli, 1989; Tolomio *et al.*, 1991), and in natural sediment samples from the Gulf of Trieste (Sdrigotti *et al.*, 1999; Welker *et al.*, 2002; Cibic *et al.*, 2007; 2009; 2012; Franzo *et al.*, 2014; Rogelja *et al.*, 2018), the Venice lagoon (Tolomio *et al.*, 1999, 2002; Facca *et al.*, 2002a, b; Tolomio, 2004; Facca & Sfriso, 2007), the coast from Ancona to the Po delta (Totti, 2003), between Rimini and Pesaro (Franzo *et al.*, 2015), and in the Lesina lagoon (Gambi *et al.*, 2003). While an extensive literature on microphytobenthic species of the Italian seas exists (see Cibic & Facca, 2010 and references therein), only limited information is available on the composition of epilithic assemblages grown on either natural or artificial substrates in marine coastal waters of the eastern Middle and South Adriatic, e.g., epilithic diatoms from stones in the oligotrophic Neum Bay in Bosnia and Herzegovina (Hafner *et al.*, 2018) and along Albanian coastal wetlands (Miho & Witkowski, 2005). The only study on benthic diatoms from areas with the invasive species *C. taxifolia* in the Middle Adriatic was focused on morphology and valve ultrastructure of a newly described marine epiphytic diatom, *Cocconeis caulerpacola* (Car *et al.*, 2012). In general, studies on the diatom communities associated with the *Caulerpa* spp. are inadequate. Diatoms epiphytic on *Caulerpa cylindracea* were analysed along the Pacific coast of Japan, where the alga is not an invasive species, with a focus primarily on the morphology of taxa of genus *Cocconeis* (Suzuki *et al.*, 2001). From the rich literature available on epilithic microalgae in general, it is clear that benthic diatom communities must play a particularly important role in the functioning of the Mediterranean Sea coastal ecosystems whilst some aspects of their ecology, taxonomy, distribution, and biodiversity remain unexplained.

Two tropical / subtropical *Caulerpa* species, *C. taxifolia* (Vahl) C. Agardh and *C. cylindracea* (Sonders), spread surprisingly quickly across the Mediterranean in the last decades, causing intense degradation of indigenous biota (Musco *et al.*, 2014). The green feather-like *C. taxifolia*, widely used as a decorative plant in the marine aquarium trade and accidentally released from the Monaco Aquarium in 1984 (Meinesz & Hesse, 1991), rapidly spread, primarily by means of fishing nets and anchors,

across the Mediterranean basin (Meinesz *et al.*, 2001). Paradoxically, *C. cylindracea* generated much less public interest although it was more invasive than *C. taxifolia* and spread much further and faster, primarily by sea currents (Nizamuddin, 1991). These invasive species are characterised by the presence of secondary metabolites such as caulerpenyne (CYN), the main function of which is chemical defence against herbivores and epiphytes (Box *et al.*, 2008; Sureda *et al.*, 2008, 2009). Concentrations of CYN for the “aquarium-Mediterranean” strain of *C. taxifolia* were observed to be at a maximum in autumn and a minimum in spring, reaching much higher values than those observed in other *Caulerpa* species (Dumay *et al.*, 2002).

In the eastern Adriatic, the invasive alga *C. taxifolia* was observed for the first time in 1994 in Stari Grad Bay (island of Hvar) on hard, sandy, and muddy substrates without vegetation or within meadows of the endemic Mediterranean seagrass *Posidonia oceanica* (Žuljević & Antolić, 2002). The invasive *C. cylindracea* was first recorded in the autumn of 2000 and the number of locations with this species increased very rapidly, in contrast with the single site with *C. taxifolia* (Žuljević *et al.*, 2003). Predominantly dispersed by sea currents, by the end of 2006 *C. cylindracea* was found at more than 50 locations along the eastern Adriatic coast, most of them in the Middle and South Adriatic. The impacts of *C. cylindracea* in the eastern Adriatic Sea have been described in the studies of Antolić *et al.* (2008), Kružić *et al.* (2008), and Žuljević *et al.* (2011).

The diatom community composition in areas invaded by *Caulerpa* species in the Adriatic has not been thoroughly examined, in spite of the potentially important role of diatoms in the functioning of the ecosystems influenced by *Caulerpa*. Based on the results of previous studies on the seasonality of diatoms (e.g., Munda, 2005; Totti *et al.*, 2007; Çolak Sabancı, 2012b), it was expected that the taxonomic composition of epilithic diatoms would vary across the seasons. The main objectives of this study were: i) to investigate the taxonomic composition of marine epilithic diatoms collected from areas influenced by invasive macroalgae *Caulerpa taxifolia* and *Caulerpa cylindracea* during a two-year period, ii) to compare the structure of the epilithic diatom assemblages between sites, and iii) to compare the structure of the epilithic diatom assemblages among seasons.

Materials and Methods

Study Area

Samples for studies on epilithic diatom communities were taken during a two-year period from November 2008 to October 2010 by SCUBA divers at three sites in the Middle and South Adriatic Sea coast, Croatia (Table 1, Fig. 1):

1. The Bay of Stari Grad, Island of Hvar (43° 10' 54" N, 16° 35' 00" E); an area affected by *Caulerpa taxifolia* at depths ranging from 5 m to 8 m,

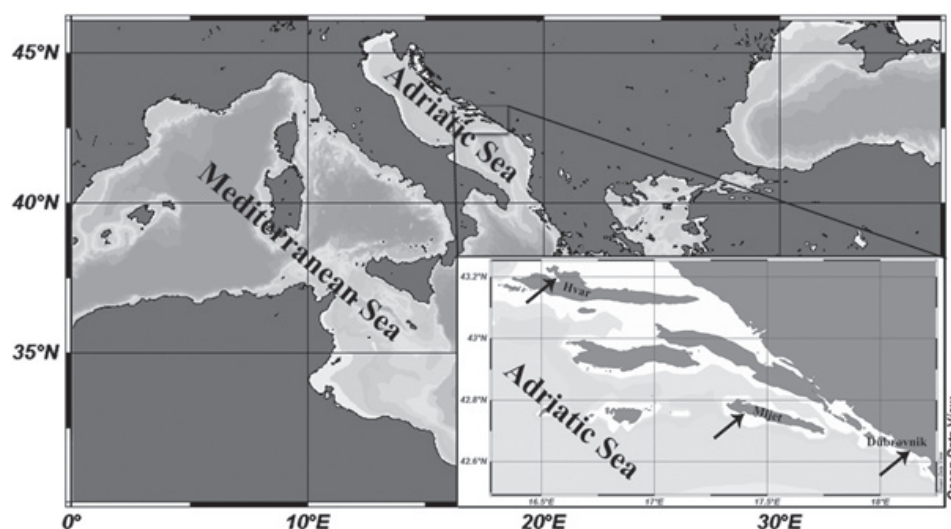


Fig. 1: The locations of sampling sites.

Table 1. Samples taken at each sampling site throughout the seasons in 2008, 2009, and 2010.

| Season / Sampling site | Winter | Spring | Summer | Autumn | Total number of samples |
|------------------------------------|---------------|------------|-------------|----------------|----------------------------|
| Hvar | January 2009 | April 2009 | June 2009 | November 2008 | 18 |
| | January 2010 | May 2009 | July 2009 | September 2009 | |
| | February 2010 | March 2010 | August 2009 | October 2009 | |
| | | April 2010 | August 2010 | November 2009 | |
| | | May 2010 | | September 2010 | |
| | | | | October 2010 | |
| Mljet | February 2009 | March 2009 | July 2010 | September 2009 | 14 |
| | January 2010 | April 2009 | | October 2009 | |
| | February 2010 | May 2009 | | November 2009 | |
| | | March 2010 | | September 2010 | |
| | | April 2010 | | | |
| | | May 2010 | | | |
| Dubrovnik | December 2009 | March 2010 | August 2009 | September 2010 | 8 |
| | | April 2010 | July 2010 | | |
| | | May 2010 | August 2010 | | |
| Total number of samples | 7 | 14 | 8 | 11 | 40 |

2. National Park Mljet, Gonoturska Bay (42° 45' N, 17° 23' E); an area affected by *Caulerpa cylindracea* at depths ranging from 5 m to 10 m,
3. Location Orsula near Dubrovnik (42° 37' 50" N, 18° 8' 2" E); an area affected by *Caulerpa cylindracea* at depths ranging from 5 m to 10 m.

The island of Hvar (H) belongs to the sunniest areas of Croatia with more than 2700 hours of insolation a year, or an average of more than 7 hours per day (Zaninović & Matzarakis, 2007). It belongs to a group of middle Adriatic islands, influenced by the open sea in the west and south and by the proximity of the coast in the east and north (Koletić *et al.*, 2012). Stari Grad Bay is a semi-enclosed bay, with the prevailing cyclonic currents mainly

driven by seasonal winds (Cvitković *et al.*, 2017). More information on the sampling site H is available in the reports of Žuljević *et al.* (1998), Antolić *et al.* (2001), Žuljević (2001), Žuljević & Antolić (2001, 2002), Cvitković *et al.* (2017), and references therein.

The Mljet National Park (M) was established in 1960 at the westernmost part of Mljet Island, south Adriatic Sea. The NW Mljet coast is exposed to waves generated by the NE wind (bora), S wind (sirocco), and NW wind (mistral). Detailed information on the M area investigated is available in Bralić (1990), Bogнар & Curić (1995), Riđanović & Šimunović (1995), Benović *et al.* (2000), Zavodnik (2003), Dell'Angelo & Zavodnik (2004), Kružić *et al.* (2008), and Hrutić & Bobanović-Čolić (2017).

The Dubrovnik site (D) is located two miles SE from the Old Town. The whole South Adriatic is under the influence of incoming currents from the Ionian Sea which are known to modify Adriatic plankton community composition and to bring alien species of Atlantic/Western Mediterranean or Eastern Mediterranean/Lessepsian origin into the basin on a pluriannual scale (Batistić *et al.*, 2014; Garić & Batistić, 2016; Čalić *et al.*, 2018). This is notably controlled by the Bimodal Oscillating System (BiOS), which refers to the circulation regime in the North-Ionian gyre (Civitarese *et al.*, 2010), whilst the primary productivity is positively related to the winter convective mixing events (Ljubimir *et al.*, 2017).

All three sampling sites have a Mediterranean climate and are exposed to anthropogenic impact (tourism) limited to the summer season.

The key variables of interest from our monthly-conducted samplings were gathered according to seasons: winter (December-February), spring (March-May), summer (June-August), and autumn (September-November).

Physical-chemical parameters

In order to determine the relationships between diatom communities and environmental variables, water samples for physical-chemical analyses were taken monthly from November 2008 to October 2010 at 8-10 m depth in the same area as where diatom sampling was carried out. While at the site D samples were collected monthly throughout the whole study, at the site M physical-chemical variables were measured monthly from October 2009 to September 2010. At the site H, due to technical problems, only temperature (T) was measured by using a reversing thermometer (Richter und Wiese, Germany). Measurements were not performed in replicates.

At sites D and M, temperature (T) and salinity (S) values were obtained by using a CTD multiparametric probe (Seabird Seacat 19plus). Seawater samples for the nutrients and chlorophyll *a* (Chl *a*) analysis were taken with 5-L Niskin bottles. Measured nutrients included total inorganic nitrogen (TIN), where $TIN = \text{nitrate (NO}_3^-) + \text{nitrite (NO}_2^-) + \text{ammonium (NH}_4^+)$, 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 in accord with Strickland and Parsons (1972). Subsamples (50 mL) for NH_4^+ were fixed immediately after collection on board with 2 mL of 1 mol L⁻¹ phenol/EtOH, kept at 4°C and later analysed in accord with Ivančić and Degobbi (1984). Chl *a* was determined from 1-L sub-samples filtered through Whatman GF/F filters and stored at -20°C for a period of less than a month. Filtered samples were homogenised and extracted in 90% acetone for 24 hours at room temperature (Holm-Hansen *et al.*, 1965). Chl *a* was determined fluorometrically by using a Turner TD-700 Laboratory Fluorometer (Sunnyvale, CA, USA) calibrated with pure Chl *a* (Sigma). Dissolved oxygen (O_2) was determined by the Winkler method and oxygen saturation (O_2/O_2') was calculated from the 100% solubility of oxygen (O_2) in seawater as a

function of temperature and salinity (Weiss, 1970; UNESCO, 1973).

Diatom analysis

The biofilm was removed from at least three randomly selected stones at each site using a knife and a hard toothbrush to scrape off the diatoms. Samples were obtained from the upper parts of the stones, which comprised approximately 100 cm² of surface, and then placed into a sampling tube. All samples were preserved by 4% final concentration of neutralised formaldehyde. In total, there were 40 samples (Table 1) without replications.

For light (LM) and electron microscopy (EM) observations all traces of organic material were removed from samples by boiling them with 30% H_2O_2 and adding 10% HCl to remove CaCO_3 . Samples were then rinsed with deionised water, pipetted onto ethanol-cleaned cover-slips, and left to air dry before mounting in Naphrax®. Permanent slides and prepared material have been deposited in the diatom collection (SZCZ) of the Institute of Marine Sciences, University of Szczecin, Szczecin (Poland). The abundances of the species were expressed as percentages of the total number (relative abundances, RA in %) of at least 300 diatom valves counted per sample. Identifications were made following Witkowski *et al.* (2000). Terminology follows Round *et al.* (1990). Nomenclature of recorded taxa follows AlgaeBase (Guiry & Guiry, 2018).

For a structural analysis of the diatom assemblages, identified taxa were divided into growth form groups: adnate (cells strongly adhering to surface), erect (cells attached to the substrate by stalks, pads or peduncles), motile (biraphid cells moving on the substrate surface), and tube-dwelling (cells producing mucilage tubes) (Majewska *et al.*, 2014; Majewska *et al.*, 2016; Round *et al.*, 1990; Totti *et al.*, 2007).

Data analysis

In order to analyse the diversity of epilithic diatom assemblages at different sampling sites and in different seasons, Shannon-Wiener Diversity Index (SWDI) was computed (Krebs, 1999) along with Pielou's Evenness Index (Pielou, 1966).

As a complete environmental data set was not available, only the differences between environmental conditions at sites D and M for the second year of the study were analysed with a Student's *t*-test. This was performed on T, S, O_2/O_2' , NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} , SiO_4^{4-} , TIN and Chl *a*. One-Way ANOVA was performed for the environmental data obtained from all three sites, testing for significant differences between T.

Analysis of similarities (ANOSIM) was used to determine whether significant difference in diatom community growth form structure and species composition occurred among the selected sampling sites and seasons. Multidimensional scaling (MDS) ordination and hierarchical clustering (Cluster) were used to display difference

in communities associated with the sampling sites and were based on standardised RA data of all recorded diatom taxa. Data used to build the MDS ordination to assess differences among sampling sites and sampling seasons were organised in a matrix of 310 taxa over 40 samples collected during the period of two years (November 2008 – October 2010). A dissimilarity percentage analysis (SIMPER, Clarke & Warwick, 1994) was used to identify the taxa making the greatest contributions to differences between the clusters observed in the MDS plot. Data used to build the second Cluster and MDS ordination and to assess differences between sampling sites (areas of *C. cylindracea* and *C. taxifolia*) were organised in a matrix of the 10 diatom taxa which contribute the most, according to SIMPER analysis, to dissimilarities between epilithic diatom assemblages over 40 samples collected during the two-year period (November 2008 – October 2010). A SIMPROF test which highlights significantly ($p < 0.05$) different groups was superimposed on the MDS. The data used for ANOSIM test performed on growth form relative abundance to assess differences among sampling sites and sampling seasons were organised in a matrix of 202 taxa over 40 samples collected during the two-year period.

Adnate diatom taxa belong to the genera *Cocconeis*, *Amphora*, and *Halamphora*, while erect diatoms belong to the genera *Grammatophora*, *Licmophora*, *Ardissonia*, *Striatella*, *Synedra*, *Fragilaria*, *Tabularia*, and *Achnanthes*, the motile diatoms belong to the genera *Navicula*, *Nitzschia*, *Tryblionella*, and *Pleurosigma*, and the tube-dwelling diatom taxa to genera *Berkeleya* and *Parlibellus*.

In order to determine the relationship between the 22 most abundant diatom taxa ($> 35\%$ frequency and $> 1\%$ average RA) of all three sampling sites and physical-chemical parameters, Spearman-Rank correlations were performed after the Kolmogorov-Smirnov test was used for testing normality of the data distribution. Data were first transformed [$\log(x+1)$] (Cassie, 1962) to enable the correlation tests between variables. Only the statistically significant correlations ($p < 0.05$) are shown. All statistical analyses were performed using the PRIMER v6 software (Clarke & Gorley, 2006) and Statistica 7.0 (StatSoft, Inc. 2004).

Results

Physical-chemical parameters

Dubrovnik

T ranged from 12°C (February 2010) to 26°C (July 2009) (Table S1). Low T for this location were recorded also in March (12.7°C) and April (13.2°C) of 2009 and in January (13.7°C) and March (13.2°C) of 2010. During the two sampling years, the average S was 37.65 with the lowest (35.15) value in January 2010 and the highest

(38.38) value in September 2010.

Average concentrations of inorganic nitrogen species for the whole study period were as follows: $0.99\ \mu\text{M}\ \text{NO}_3^-$, $0.33\ \mu\text{M}\ \text{NH}_4^+$, and $0.05\ \mu\text{M}\ \text{NO}_2^-$. TIN generally followed the distribution of the most dominant inorganic nitrogen species in our study (NO_3^-). In 2010, NO_3^- and TIN ($0.30\ \mu\text{M}$ and $0.75\ \mu\text{M}$, respectively) were below the mentioned averages for the whole study.

PO_4^{3-} ranged from $0.01\ \mu\text{M}$ (April 2010) to $0.09\ \mu\text{M}$ (August 2009). SiO_4^{4-} ranged from $0.73\ \mu\text{M}$ (April 2010) to $8.06\ \mu\text{M}$ (January 2010). Oxygen saturation (O_2/O_2') ranged from 0.94 (December 2009) to 1.17 (July 2010). An average O_2/O_2' was close to 100% and in line with well-balanced production and degradation of the organic matter, reflecting the oligotrophic character of the site (Table S2). Chl *a* ranged from 0.01 (August 2010) to $0.49\ \mu\text{g L}^{-1}$ (January 2010) which is also in line with the oligotrophic character of the site.

Mljet

Comparatively low T values ($< 14^{\circ}\text{C}$) were recorded in February and March 2010. Salinity varied from 36.93 (January 2010) to 38.50 (September 2010). Average inorganic nitrogen concentrations during the whole study period were: $0.47\ \mu\text{M}\ \text{NO}_3^-$, $0.06\ \mu\text{M}\ \text{NO}_2^-$ and $0.17\ \mu\text{M}\ \text{NH}_4^+$. The average concentration of TIN was $0.70\ \mu\text{M}$ while the average concentration of PO_4^{3-} was $0.09\ \mu\text{M}$. SiO_4^{4-} ranged from $1.24\ \mu\text{M}$ (April 2010) to $8.64\ \mu\text{M}$ (February 2010). O_2/O_2' (range 0.92–1.12, average 1.01) and average Chl *a* concentration ($0.12\ \mu\text{g L}^{-1}$) also indicated oligotrophic conditions (Table S2). The maximum Chl *a* concentration of $0.48\ \mu\text{g L}^{-1}$ was recorded in December 2009. In May, August and September Chl *a* was below the detection limit ($0.01\ \mu\text{g L}^{-1}$).

Hvar

In Stari Grad Bay, T $> 20^{\circ}\text{C}$ were recorded from June till October in both years (2009 and 2010), while during the winter T was 12°C .

Comparison of sampling sites

Over the study period, at all three sampling sites T (Table S1) ranged from 12°C to 26°C . The ANOVA test, performed on T associated to all samples, indicated no significant differences ($p > 0.05$) among three sampling sites ($F(2, 58) = 0.15420$, $p = 0.85745$). Salinity was even less variable, with an average value (over sampling sites and sampling periods) of 37.77 (ranged from 35.15 to 38.50). In winter and spring S was mostly dependant on the precipitation regime. The only significant ($p < 0.05$) difference between sites D and M was recorded for ammonium concentration (Table S2).

Diatom assemblage analyses

Altogether, 310 diatom taxa belonging to 65 genera were identified from 40 samples. Among them, 248 taxa were found at site H, 216 were found at site M and 154 taxa occurred at site D. Among 310 diatom taxa, 68 taxa were found exclusively at the H site, 32 were found exclusively at site M and 15 occurred only at site D (Appendix 1).

In total, 85 taxa were found only once (sporadic). Among them, 49, 23, and 13 taxa were found in samples from H, M, and D, respectively (Appendix 1). The average RA of these sporadically found taxa was 0.6%. Only 15 taxa were found exclusively at the *Caulerpa cylindracea* sampling sites D and M and not found at *Caulerpa taxifolia* H site (Appendix 1). Among these 15 taxa, 5 belonged to *Mastogloia* (*M. adriatica*, *M. braunii*, *M. laminaris*, *M. mauritiana*, *M. peragalli*). However, during the study, there were 112 taxa common to all three sampling sites.

The average number of taxa per sample was 70, while the maximum was 110 (M; May 2009) and the minimum 35 (D; March 2010). Considering all collected samples from sites M and D, the average number of taxa was 64 per sample (Fig. 2). The average number of taxa at H was 77 per sample, with a minimum of 46 observed in August 2010.

In the winter, spring, and summer, considering all three sites during the two-year period (autumn 2008 – autumn 2010), the average number of taxa amounted to 66 per sample. In autumn the average number of taxa on the rocks increased to 79 per sample, with the highest in October 2009 (92) and the lowest in November 2009 (72) (Fig. 3).

The genera with the highest number of taxa were *Mastogloia* (48) and *Amphora* (32), followed by *Diploneis* (24), *Nitzschia* (23), *Navicula* (22), and *Cocconeis* (19) (see Appendix 1). Although *Mastogloia* and *Amphora* had the highest number of taxa, they occurred with an average RA of less than 1%. The most abundant genera were *Hyalosynedra*, *Rhopalodia*, *Navicula*, *Cocconeis*, *Berkeleya*, *Tabularia*, *Nitzschia*, and *Grammatophora*.

Regarding form structure, D and M were characterised by a higher percentage of adnate forms, while tube-dwelling forms dominated the H site. At D, epilithic communities that developed in winter contained a higher percentage (55%) of adnate forms (e.g., *Cocconeis* spp., *Amphora* spp.) followed by the (25%) motile ones (e.g., *Navicula* spp., *Nitzschia* spp.). In spring, the percentage of adnate forms remained almost constant (48%) while erect group diatoms (e.g., *Grammatophora* spp., *Licmophora* spp., *Ardissonia* spp., *Striatella* sp.) increased their number by 3.4 fold. Moreover, maximum RA of 43% (the highest RA value observed during the study period) was recorded in spring for site D for erect group diatom (*Grammatophora oceanica*) (Table 2). A similar seasonal shift in growth form structure was observed for epilithic communities for M and H, where the number of erect group diatoms doubled in spring. However, epilithic communities for site H contained a lower percentage of both

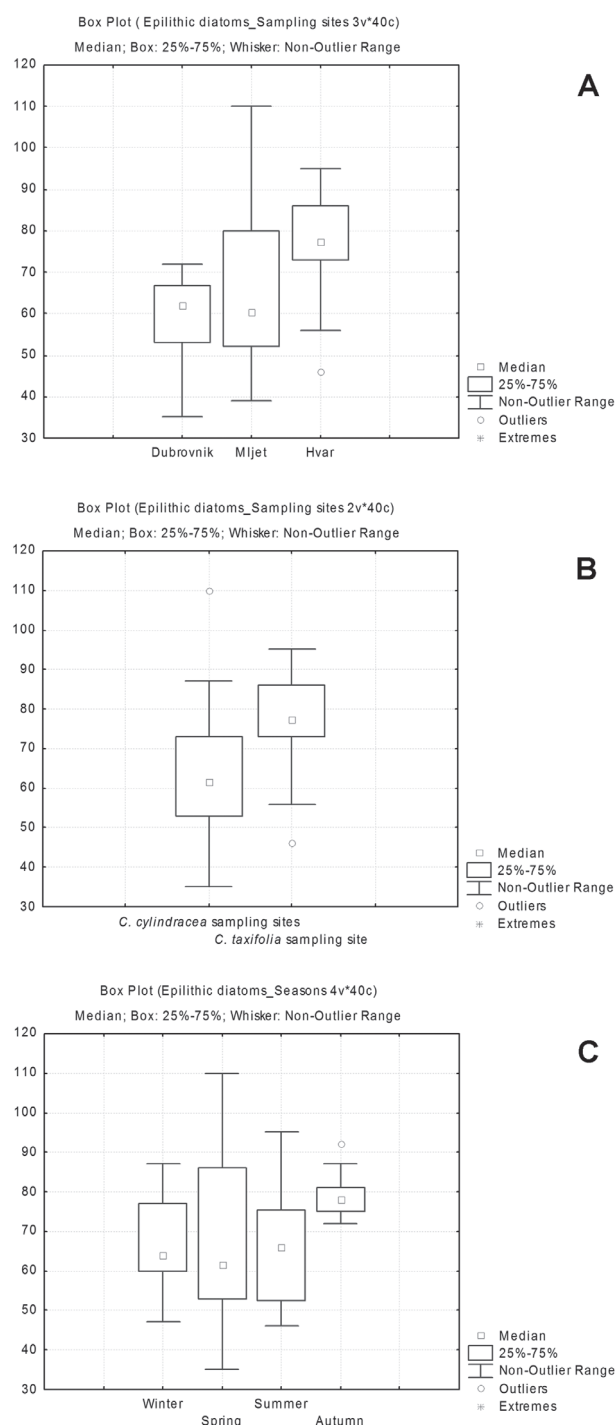


Fig. 2: Box and Whisker Plot showing differences in number of taxa: A) on sampling sites Dubrovnik, Mljet, and Hvar; B) on *C. cylindracea* sampling sites and on *C. taxifolia* sampling site; C) during different seasons.

adnate (43%) and erect (15%) forms than found in the D and M samples (at both, D and M sites in spring the same percentage of adnate, 48%, and erect, 23%, were found). Another characteristic of H was the high abundance of tube-dwelling diatoms (*Berkeleya* spp., *Parlibellus* spp.), particularly during winter and spring. In spring, maximum RA of 22% was recorded for tube-dwelling group diatom (*B. rutilans*) at H (Table 2). While at H adnate

Table 2. Dominant diatom taxa on rocks collected at all sampling sites with frequency of appearance (Freq.) > 35% and average relative abundance (Avg. RA) > 1%. N = 40. Seasonal and spatial distribution of dominant diatom taxa collected at sampling sites: Dubrovnik (D), Mljet (M) and Hvar (H) during winter (W), spring (Sp), summer (S) and autumn (A). Mean values were obtained from all the analysed samples (40 samples) across the whole sampling period (e.g., over all seasons and sampling sites). Mean values for winter were obtained from 7 analysed winter samples over all three sampling sites, for spring from 14 samples, for summer from 8, and for autumn from 11 samples collected at all sampling sites. Mean values for Dubrovnik were obtained from 8 samples, and for Mljet from 14 samples, and for Hvar from 18 samples over all seasons.

| Taxa | Freq. (%) | Avg. RA (%) | Max. RA (%) | Sampling date of Max. RA | W | Sp | S | A | D | M | H |
|---|--------------|-------------------|-------------------|-----------------------------|-------|-------|------|------|-------|-------|------|
| <i>Ardissonia formosa</i> (Hantzsch) Grunow | 42.50 | 1.03 | 7.84 | M; Mar 2009 | 0.26 | 1.68 | 0.56 | 0.67 | 0.85 | 1.59 | 0.48 |
| <i>Berkeleya rutilans</i> (Trentepohl) Grunow | 75.00 | 3.44 | 21.57 | H; Mar 2010 | 2.09 | 4.69 | 1.91 | 3.21 | 0.52 | 0.69 | 5.57 |
| <i>Berkeleya scopulorum</i> (Brébisson ex Kützing) E.J.Cox | 67.50 | 2.06 | 10.06 | H; Jan 2009 | 4.65 | 2.19 | 0.94 | 0.82 | 0.72 | 0.63 | 3.18 |
| <i>Cocconeis costata</i> Gregory var. <i>costata</i> | 62.50 | 3.16 | 21.41 | M; Jul 2010 | 1.70 | 2.73 | 6.85 | 1.52 | 5.16 | 4.21 | 0.45 |
| <i>Cocconeis molesta</i> var. <i>crucifera</i> Grunow in Van Heurck | 90.00 | 4.03 | 17.97 | H; Apr 2009 | 3.04 | 6.02 | 4.46 | 2.03 | 7.25 | 4.63 | 2.13 |
| <i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i> | 100.00 | 8.69 | 25.47 | M; Jul 2010 | 12.55 | 10.25 | 8.71 | 4.24 | 12.84 | 13.41 | 3.17 |
| <i>Fragilaria investiens</i> (W. Smith) Cleve-Euler | 77.50 | 2.01 | 8.31 | D; May 2010 | 1.86 | 3.00 | 1.35 | 0.88 | 3.03 | 2.66 | 1.01 |
| <i>Grammatophora oceanica</i> (Ehrenberg 1854 pro parte) Grunow | 60.00 | 3.82 | 43.19 | D; Mar 2010 | 2.78 | 6.81 | 3.31 | 0.56 | 6.56 | 2.97 | 0.23 |
| <i>Halamphora kolbei</i> (Aleem) Álvarez-Blanco & S.Blanco | 45.00 | 1.53 | 7.97 | D; Dec 2009 | 2.49 | 1.52 | 2.25 | 0.50 | 4.20 | 0.94 | 0.46 |
| <i>Hyalosynedra laevigata</i> (Grunow) Williams & Round | 100.00 | 3.87 | 8.14 | M; Nov2009 | 2.95 | 3.56 | 4.47 | 4.41 | 2.71 | 3.65 | 4.56 |
| <i>Licmophora gracilis</i> (Ehrenberg) Grunow var. <i>gracilis</i> | 52.50 | 1.64 | 8.63 | M; Feb 2009 | 3.71 | 0.93 | 0.27 | 2.01 | 0.73 | 2.59 | 0.81 |
| <i>Licmophora paradoxa</i> (Lyngbye) Agardh | 75.00 | 2.17 | 10.69 | M; Feb 2010 | 2.53 | 2.51 | 2.49 | 1.03 | 3.68 | 2.43 | 0.88 |
| <i>Mastogloia binotata</i> (Grunow) Cleve | 82.50 | 2.80 | 12.71 | D; Aug2009 | 1.55 | 1.96 | 4.97 | 3.18 | 4.69 | 3.43 | 1.00 |
| <i>Navicula ramosissima</i> (Agardh) Cleve | 95.00 | 8.42 | 34.67 | M; Apr 2009 | 9.26 | 9.23 | 6.77 | 8.19 | 7.31 | 11.04 | 6.95 |
| <i>Nitzschia angularis</i> W. Smith | 90.00 | 3.59 | 7.14 | D; Dec 2009 | 4.16 | 2.52 | 3.58 | 4.52 | 3.66 | 2.93 | 4.02 |
| <i>Nitzschia fusiformis</i> Grunow | 82.50 | 2.41 | 7.19 | H; Apr 2010 | 2.02 | 2.75 | 2.49 | 2.18 | 1.65 | 1.76 | 3.07 |
| <i>Nitzschia lanceolata</i> var. <i>minima</i> Grunow | 87.50 | 3.60 | 12.04 | H; Mar 2010 | 1.80 | 3.57 | 4.60 | 4.16 | 2.39 | 2.58 | 4.57 |
| <i>Nitzschia macilenta</i> W.Gregory | 35.00 | 7.25 | 21.81 | H; Jan 2010 | 18.71 | 2.08 | 2.18 | 3.94 | 2.24 | 10.93 | 8.10 |
| <i>Nitzschia panduriformis</i> Gregory var. <i>panduriformis</i> | 90.00 | 1.60 | 7.45 | H; Aug2010 | 1.47 | 1.16 | 2.09 | 1.83 | 1.01 | 1.01 | 2.19 |
| <i>Opephora pacifica</i> (Grunow) Petit | 72.50 | 1.95 | 8.83 | H; Aug 2009 | 0.62 | 1.31 | 3.19 | 2.59 | 0.26 | 0.68 | 2.78 |
| <i>Rhopalodia pacifica</i> Krammer | 82.50 | 4.21 | 25.56 | H; Sep 2009 | 2.55 | 1.24 | 4.04 | 7.65 | 0.49 | 1.15 | 6.99 |
| <i>Tabularia ktenoides</i> M.Kuylenstierna | 92.50 | 2.53 | 8.40 | M; Apr 2010 | 2.10 | 4.45 | 1.35 | 0.77 | 2.74 | 3.08 | 1.89 |

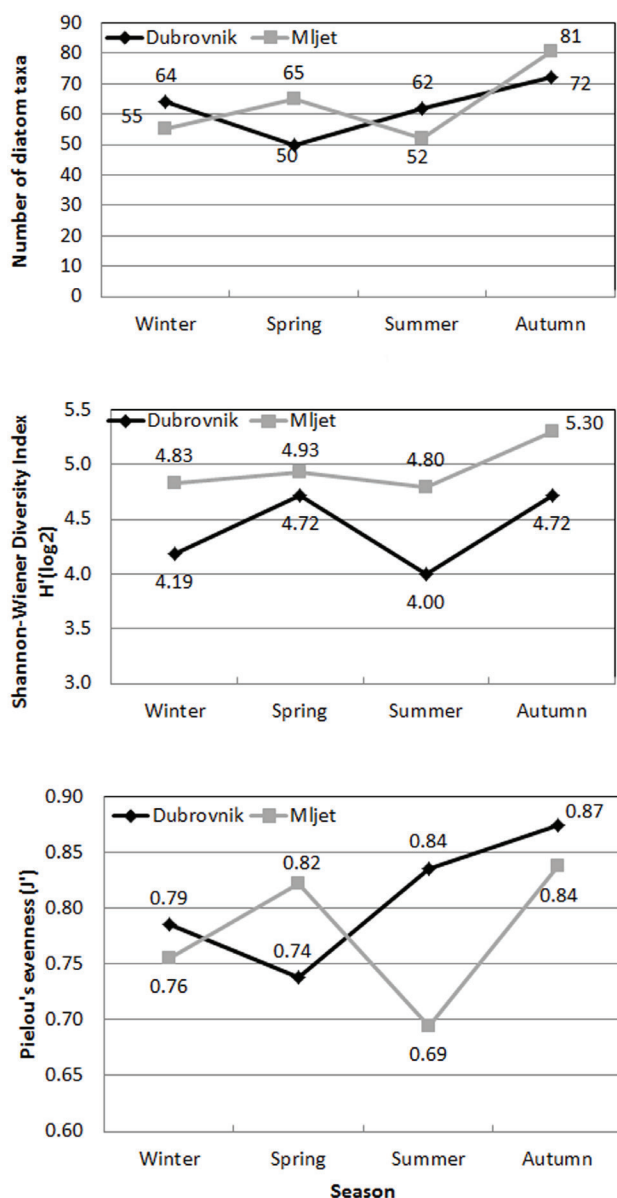


Fig. 3: Number of diatom taxa, the Shannon-Wiener Diversity Index, and Pielou's Evenness Index at *C. cylindracea* sampling sites of Dubrovnik and Mljet, throughout seasons. N = 22.

forms remained almost constant through all seasons, at M adnate forms (*Cocconeis scutellum* var. *scutellum* and *Cocconeis costata* var. *costata*) dominated during summer.

Table 2 lists the 22 dominant taxa (frequency >35%, average RA >1%) for all sites. Of the 22 dominant taxa, 5 belonged to *Nitzschia*. *Nitzschia macilenta* was the dominant taxon of the winter samples (Table 2), occurring with RA of 21% at H both in January 2009 and 2010 and at M in January 2010 (data not shown). *Berkeleya* occurred with two species, *B. rutilans* and *B. scopulorum*. While a higher abundance of *B. scopulorum* was observed during winter, *B. rutilans* was a characteristic taxon of spring samples particularly for March 2010 at H when the maximum occurrence of 22% was recorded (Table 2). Additionally in March 2010 at H, approximately 63% of

species had RA of less than 1%. Also in March 2010, at D the highest RA (43%) of *Grammatophora oceanica* in our study was recorded. Approximately 69% of species in a sample from D in March 2010 had an RA of less than 1%. Only 12 taxa (*G. oceanica*, *Navicula ramosissima*, *Rhopalodia pacifica*, *Cocconeis scutellum* var. *scutellum*, *N. macilenta*, *B. rutilans*, *C. costata* var. *costata*, *C. molestata* var. *crucifera*, *Mastogloia binotata*, *N. lanceolata* var. *minima*, *Licmophora paradoxa*, *B. scopulorum*) occurred with an RA above 10% (Table 2).

Although the most abundant taxon *C. scutellum* var. *scutellum* (RA = 8.7%) was recorded in all samples, it exhibited some differences in its spatial distribution in relation to sampling periods and sites (Table 2, Table 3). At H, this species was one of the dominant taxa, together with *N. macilenta* and *B. scopulorum*, but only in winter, whereas during the other seasons it was not even in the top 10 dominant species. At M, *C. scutellum* var. *scutellum* predominated in diatom communities during winter, spring and summer with RA of 16%, 14% and 26%, respectively (Table 3), while in autumn, along with *C. scutellum* var. *scutellum* (RA 7%), *N. ramosissima* (11%) contributed the most to total diatom abundance (Table 3). The diatom community at D was also dominated by *C. scutellum* var. *scutellum* during winter (19%), spring (15%), and summer (11%) and, like in M, *N. ramosissima* had the highest RA (13%) in autumn. During spring at D, along with *C. scutellum* var. *scutellum*, the diatom community was also dominated by *G. oceanica* (15%), resulting in the lowest value of the Pielou's Evenness Index (0.74) for that area (Fig. 3). Some other diatom species sometimes strongly dominated the epilithic community (e.g., joint RA of *C. scutellum* var. *scutellum* and *C. costata* var. *costata* at M was 47% in July 2010; RA of *N. macilenta* at H was 18% in winter), resulting in low evenness values (0.69 at M in summer; 0.80 at H in winter) (Figs 3 & 4). Regarding all the sampling sites, the lowest evenness value was measured for M in summer (0.69), due to the dominance of *C. scutellum* var. *scutellum* (Fig. 3, Table 3).

Generally, the lowest Shannon-Wiener Diversity Index (SWDI) was recorded at D (Fig. 3). SWDI had slightly wider range (4.45-5.26) at *C. cylindracea* sampling sites D and M (together) in comparison to H (4.97-5.34). Generally, during winter, spring and summer, higher SWDI values were observed at H, while the lowest SWDI occurred at D and M. In autumn, SWDI values were greatest (5.26-5.34) independently of the presence of the invasive macroalgae (Fig. 4).

Regarding seasonality, during winter the diatom community in H samples was dominated by *N. macilenta* (18%) (Table 3). In spring, *B. rutilans* and *N. ramosissima* had the highest RA (in total 19%). While *N. ramosissima* (9%) and *R. pacifica* (8%) contributed the most to total diatom abundance in summer, *R. pacifica* dominated with RA of 13% in autumn. Generally, *R. pacifica* was a characteristic taxon of summer and autumn samples (Table 2, Table 3), but particularly in September for H, where RA of 26% and 20% were recorded in September 2009 and September 2010, respectively.

Table 3. List of diatom taxa and their percentage contribution to total diatom community composition (taxa with relative abundances, RA, $\geq 4\%$ are only shown) from Dubrovnik, Miljet, and Hvar throughout the seasons in 2008, 2009 and 2010.

| Season / Sampling site: | Winter | Spring | Summer | Autumn |
|----------------------------|--|---|---|--|
| | RA (%) | RA (%) | RA (%) | RA (%) |
| Hvar | <i>Nitzschia macilenta</i> W.Gregory | <i>Berkeleya rutilans</i> (Trentepohl) Grunow | <i>Navicula ramosissima</i> (Agardh) Cleve | <i>Rhopalodia pacifica</i> Krammer |
| | 18.10 | 10.62 | 8.87 | 12.77 |
| | <i>Berkeleya scopulorum</i> (Brébisson ex Kützing) E.J.Cox | <i>Navicula ramosissima</i> (Agardh) Cleve | <i>Rhopalodia pacifica</i> Krammer | <i>Halamphora acutiuscula</i> (Kützing) Levkov |
| | 6.99 | 8.51 | 7.82 | 6.39 |
| | <i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i> | <i>Cocconeis molesta</i> var. <i>crucifera</i> Grunow in Van Heurck | <i>Hyalosynedra laevigata</i> (Grunow) Williams & Round | <i>Navicula ramosissima</i> (Agardh) Cleve |
| | 6.74 | 4.93 | 6.29 | 5.58 |
| | <i>Nitzschia angularis</i> W. Smith | <i>Hyalosynedra laevigata</i> (Grunow) Williams & Round | <i>Nitzschia lanceolata</i> var. <i>minima</i> Grunow | <i>Nitzschia distans</i> var. <i>distans</i> Gregory |
| | 4.60 | 4.87 | 6.06 | 5.26 |
| | <i>Navicula ramosissima</i> (Agardh) Cleve | <i>Nitzschia lanceolata</i> var. <i>minima</i> Grunow | <i>Nitzschia distans</i> var. <i>distans</i> Gregory | <i>Nitzschia angularis</i> W. Smith |
| | 4.53 | 4.62 | 5.13 | 4.67 |
| Miljet | <i>Berkeleya rutilans</i> (Trentepohl) Grunow | | <i>Rhopalodia musculus</i> (Kützing) Otto Müller | <i>Nitzschia lanceolata</i> var. <i>minima</i> Grunow |
| | 4.43 | | 5.13 | 4.51 |
| | <i>Hyalosynedra laevigata</i> (Grunow) Williams & Round | | <i>Opephora pacifica</i> (Grunow) Petit | <i>Berkeleya rutilans</i> (Trentepohl) Grunow |
| | 4.17 | | 4.46 | 4.18 |
| | | | <i>Nitzschia angularis</i> W. Smith | |
| | | | 4.22 | |
| | <i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i> | <i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i> | <i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i> | <i>Navicula ramosissima</i> (Agardh) Cleve |
| | 16.31 | 13.99 | 25.47 | 10.95 |
| | <i>Navicula ramosissima</i> (Agardh) Cleve | <i>Navicula ramosissima</i> (Agardh) Cleve | <i>Cocconeis costata</i> Gregory var. <i>costata</i> | <i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i> |
| | 14.42 | 11.14 | 21.41 | 7.35 |
| Miljet | <i>Nitzschia macilenta</i> W.Gregory | <i>Cocconeis molesta</i> var. <i>crucifera</i> Grunow in Van Heurck | <i>Mastogloia binotata</i> (Grunow) Cleve | <i>Licmophora flabellata</i> (Car-michael) Agardh |
| | 10.39 | 7.31 | 8.40 | 6.25 |
| | <i>Licmophora gracilis</i> (Ehrenberg) Grunow var. <i>gracilis</i> | <i>Grammatophora oceanica</i> (Ehrenberg 1854 pro parte) Grunow | <i>Grammatophora oceanica</i> (Ehrenberg 1854 pro parte) Grunow | <i>Hyalosynedra laevigata</i> (Grunow) Williams & Round |
| | 4.87 | 6.89 | 8.13 | 5.41 |
| | <i>Licmophora paradoxa</i> (Lyngbye) Agardh | <i>Tabularia ktenoeides</i> M.Kuylenstierna | | <i>Mastogloia binotata</i> (Grunow) Cleve |
| | 4.65 | 4.89 | | 4.86 |
| Miljet | <i>Grammatophora oceanica</i> (Ehrenberg 1854 pro parte) Grunow | | | <i>Nitzschia angularis</i> W. Smith |
| | 4.37 | | | 4.35 |
| | | | | <i>Nitzschia lanceolata</i> var. <i>minima</i> Grunow |
| | | | | 4.11 |

continued

Table 3 continued

| Season / Sampling site: | Winter | Spring | Summer | Autumn | |
|----------------------------|---|--------|---|---|--|
| | RA (%) | RA (%) | RA (%) | RA (%) | |
| Dubrovnik | <i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i> | 18.68 | <i>Grammatophora oceanica</i> (Ehrenberg 1854 pro parte) Grunow | <i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i> | 12.83 |
| | <i>Cocconeis molesta</i> var. <i>crucifera</i> Grunow in Van Heurck | 8.24 | <i>Cocconeis scutellum</i> Ehrenberg var. <i>scutellum</i> | <i>Cocconeis molesta</i> var. <i>crucifera</i> Grunow in Van Heurck | 6.91 |
| | <i>Navicula ramosissima</i> (Agardh) Cleve | 7.97 | <i>Cocconeis costata</i> Gregory var. <i>costata</i> | <i>Mastogloia binotata</i> (Grunow) Cleve | 5.59 |
| | <i>Halamphora kolbei</i> (Aleem) Álvarez-Blanco & S. Blanco | 7.97 | <i>Navicula ramosissima</i> (Agardh) Cleve | <i>Navicula ramosissima</i> (Agardh) Cleve | 4.28 |
| | <i>Nitzschia angularis</i> W. Smith | 7.14 | <i>Cocconeis molesta</i> var. <i>crucifera</i> Grunow in Van Heurck | <i>Licmophora paradoxa</i> (Lyngbye) Agardh | |
| | <i>Cocconeis costata</i> Gregory var. <i>costata</i> | 6.59 | <i>Fragilaria investiens</i> (W. Smith) Cleve-Euler | <i>Cocconeis costata</i> Gregory var. <i>costata</i> | |
| | | | <i>Licmophora paradoxa</i> (Lyngbye) Agardh | | |
| | | | <i>Cocconeis</i> cf. <i>irregularis</i> (Schulz) Witkowski | | |
| | | | <i>Tabularia ktenoeides</i> M.Kuylenstierna | | |
| | | | <i>Halamphora kolbei</i> (Aleem) Álvarez-Blanco & S. Blanco | | |
| | | | <i>Navicula subagnita</i> Proschkina-Lavrenko | | |
| | | | | | <i>Navicula ramosissima</i> (Agardh) Cleve |

Dubrovnik

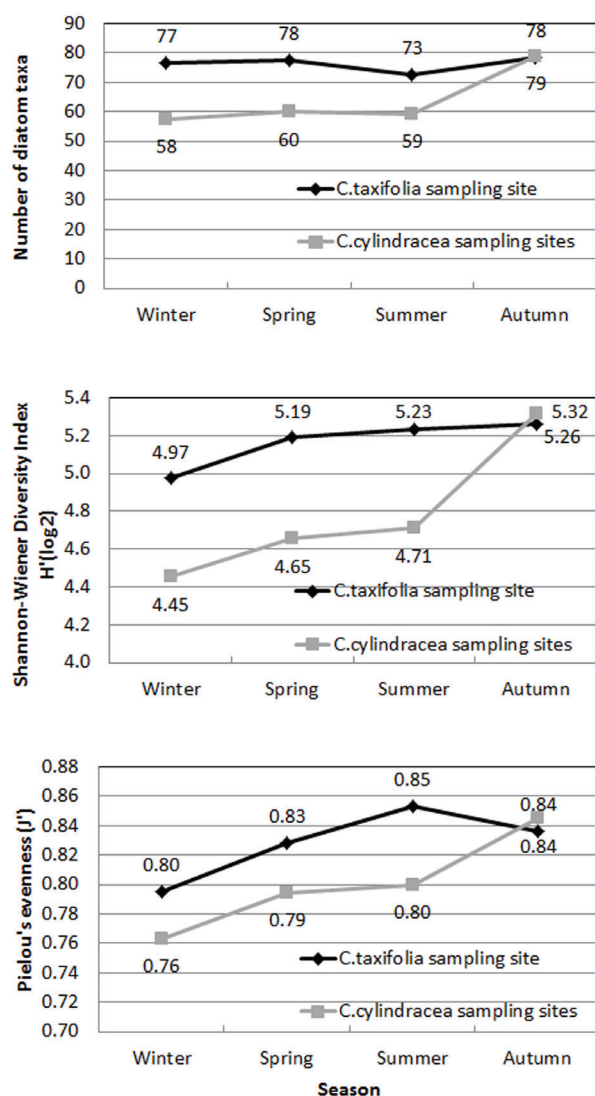


Fig. 4: Number of diatom taxa, the Shannon-Wiener Diversity Index and Pielou's Evenness Index of epilithic diatom community at the *C. taxifolia* and *C. cylindracea* sampling sites throughout seasons. N = 40.

The diatom communities differed significantly ($p < 0.05$) among the sampling sites (D, M, H) in terms of both species composition and growth form structure (Table 4). The highest Global R (0.601) value was obtained in the analysis of similarity among diatom communities associated with sites of different invasive macroalgal species. The same test performed on species data indicated that the differences between diatom communities at different sampling seasons were not significant ($p > 0.05$) for all three sampling sites. Additionally, the lowest Global R

(0.182) value was obtained in the analysis of similarity among diatom communities associated with T.

The Cluster analysis performed on species RA data revealed three groups of significantly different assemblages of epilithic diatoms. Difference in species composition of the epilithic diatom communities was observed between the H samples and samples from areas of invasive *C. cylindracea* (Fig. 5). Similarity between two groups (group 1 - site H and group 2 - sites M and D) amounted to 30%. Additionally, two spring samples from M and D (group 3) showed significant ($p < 0.05$) differences from the others. Some taxa were notably responsible for differences among sampling sites and seasons (e.g., *G. oceanica* with RA of 43% in the sample from D in March 2010).

As revealed by SIMPER analyses, the group of taxa contributing the most (cumulatively 37%) to variance between diatom assemblages from groups 1 and 2 (sites of different invasive alga) included *C. scutellum* var. *scutellum*, *R. pacifica*, *N. ramosissima*, *B. rutilans*, *C. molesta* var. *crucifera*, *N. macilenta*, *G. oceanica*, *N. lanceolata* var. *minima*, *C. costata* var. *costata*, and *M. binotata* (Table S3). The MDS performed on the RA data of these 10 species revealed two groups of significantly different assemblages of epilithic diatoms (data not shown) and was generally in agreement with previous result of MDS performed on species RA data of all 310 recorded taxa (data not shown). Besides clusters 1 and 2, SIMPROF analysis also detected sub-clusters A, B, C, and D (data not shown). Diatom samples collected exclusively from H were forming sub-cluster A. Additionally, at site H samples from July till October significantly differed from other months. Cluster 2 included diatom samples collected exclusively from *C. cylindracea* sites and sub-clusters C and D. Sub-cluster D included two spring samples from M and D (Fig. 5, group 3) together with sample from M from July 2010.

None of the 49 taxa that were found exclusively at H (Appendix 1, marked as [*H]) belonged to the list of main diatom taxa that contributed (cumulative 70%) to dissimilarities between epilithic diatom assemblages from areas associated to groups 1 and 2 (Fig. 5, Table S3). Additionally, none of the 15 taxa which were found only at the *C. cylindracea* sampling sites (Appendix 1, marked as [Cc]) belonged to the list of main diatom taxa which contributed (cumulative 70%) to dissimilarities between epilithic diatom assemblages from areas associated to groups 1 and 2 (Table S3).

Significant negative correlations were identified between diatom RA and T for *C. scutellum* var. *scutellum* at D. A significant positive correlation between diatom RA and T was observed for *B. scopulorum* and *H. laevi-*

Table 4. Results of ANOSIM test performed on species and growth form relative abundance data.
S – species; GF – growth form.

| | Sampling site | | Invasive alga | | Season | | Sea Temperature | |
|----------|---------------|-------|---------------|-------|--------|-------|-----------------|-------|
| | S | GF | S | GF | S | GF | S | GF |
| <i>p</i> | 0.001 | 0.001 | 0.001 | 0.001 | >0.05 | >0.05 | >0.05 | >0.05 |
| Global R | 0.574 | 0.448 | 0.601 | 0.44 | 0.097 | 0.063 | 0.182 | 0.157 |

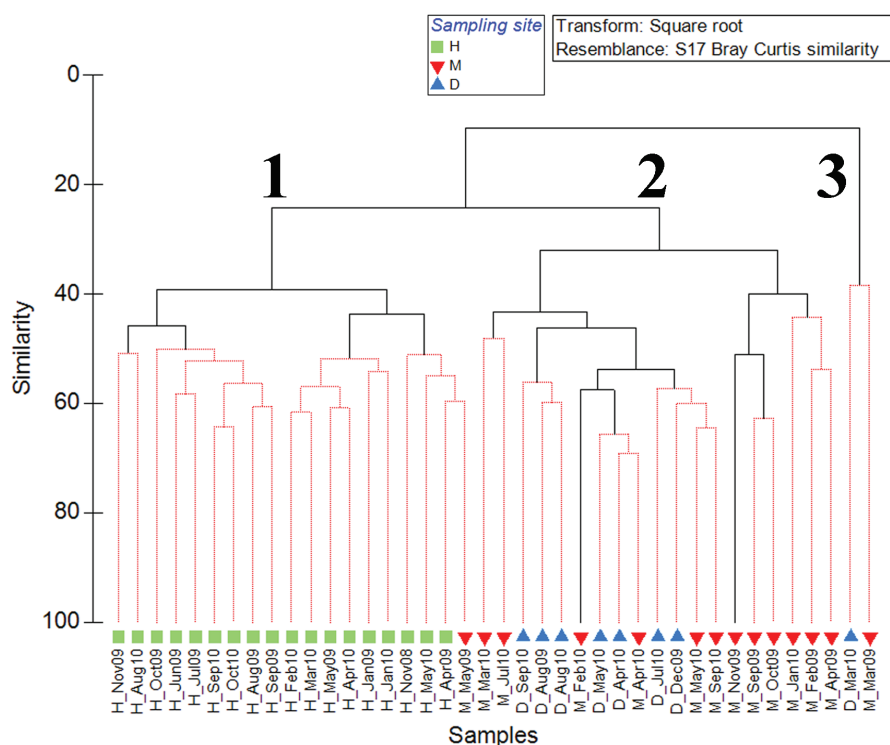


Fig. 5: Cluster analysis of the epilithic diatom assemblages from all three sampling sites (Dubrovnik, Mljet, Hvar) during a two year time period from November 2008 to October 2010 (taxa relative abundance data). For the ordination analysis all recorded diatom taxa were used. Red lines indicated taxa homogeneous clusters detected by SIMPROF. Symbols H (Hvar); M (Mljet); D (Dubrovnik). N(D) = 8; N(M) = 14; N(H) = 18.

gata at D and for *R. pacifica* at M. Regarding H, a significant negative correlation between diatom RA and T was observed for *B. scopulorum*, *C. costata* var. *costata*, *C. molesta* var. *crucifera*, *C. scutellum* var. *scutellum*, *N. macilenta*, and *Tabularia ktenooides*, and a significant positive correlation for *Opephora pacifica* and *R. pacifica* (Table 5).

Discussion

The present study confirms differences in the taxonomic composition of epilithic diatoms among the selected sites in the Middle and South Adriatic Sea. The results show that the benthic diatom communities of the coastal rocks appear to be affected by multiple factors reflecting site-specific conditions possibly caused by invasive macroalgae. Our results reveal a higher variability of the epilithic diatom community structure among sampling sites than among seasons and highlight the necessity of further diatom research for understanding Mediterranean ecosystems dominated by invasive macroalgae.

A few genera made a significant contribution to the assemblage structure of epilithic diatoms from areas of invasive *Caulerpa* species: *Hyalosynedra*, *Navicula*, *Cocconeis*, *Berkeleya*, *Nitzschia*, and *Grammatophora*. In our study, the genus *Amphora* was one of the richest in taxa. The results of our study are in agreement with the observations by Álvarez-Blanco and Blanco (2014), who reported that the genera *Cocconeis* and *Amphora* (including *Halamphora*) had very high species richness

in samples taken from coastal rocks at seven locations in the Mediterranean (Spain, Italy, Greece, Turkey and the coasts of the Adriatic and Aegean Sea) in the spring of 2010. Álvarez-Blanco and Blanco (2014) also found a very high species richness for the genus *Licmophora*, but that observation was not confirmed in our results retrieving only 7 *Licmophora* species.

A diatom community similar to that presented in our study has been reported also for different hard substrates from the Adriatic (Munda, 2005; Totti *et al.*, 2007). Munda (2005), who studied seasonal fouling by diatoms on artificial substrates in the heavily polluted area near Piran in the Gulf of Trieste, reported that genera *Berkeleya*, *Navicula*, and *Licmophora* were dominant and covered most of the experimental surfaces. The community structures of epilithic diatoms reported in our study are in agreement with results from the northern Adriatic in the study of Totti *et al.* (2007) which was performed on artificial hard substrates and showed a dominance of motile taxa (*Navicula* spp., *Nitzschia* spp.), followed by erect (*Grammatophora marina*, *G. oceanica*), adnate (*Halamphora coffeiformis* and *C. scutellum*) and tube-dwelling diatoms (*B. rutilans*). Although, Totti *et al.* (2007) reported a high seasonal variability of epilithic microalgal communities, our data indicate the variation of community structure in terms of relative abundances of diatom taxa to be associated with the sampling sites and possibly with the presence of the two invasive macroalgae considered, rather than linked to seasonal effects.

Epilithic diatoms composition of M and D with *C. cylindracea*, were more similar between them than to that

Table 5. Correlations at $p < 0.05$ between dominant diatom taxa and physical-chemical parameters; sea temperature (T), salinity (S), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), total inorganic nitrogen (TIN), oxygen saturation (O_2/O_2'), and chlorophyll *a* concentrations (Chl *a*). “/” represent samples not analysed. Asterisks represent levels of significance: $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$. Key to species: **ARFO** *Ardissonia formosa* (Hantzsch) Grunow, **BERU** *Berkeleya rutilans* (Trentepohl) Grunow, **BESC** *Berkeleya scopulorum* (Brébisson ex Kützing) E.J.Cox, **COCO** *Cocconeis costata* Gregory var. *costata*, **COMO** *Cocconeis molesta* var. *crucifera* Grunow in Van Heurck, **COSC** *Cocconeis scutellum* Ehrenberg var. *scutellum*, **FRIN** *Fragilaria investiens* (W. Smith) Cleve-Euler, **GROC** *Grammatophora oceanica* (Ehrenberg 1854 pro parte) Grunow, **HAKO** *Halamphora kolbei* (Aleem) Alvarez-Blanco & S.Blanco, **HYLA** *Hyalosynedra laevigata* (Grunow) Williams & Round, **LIGR** *Licmophora gracilis* (Ehrenberg) Grunow var. *gracilis*, **LIPA** *Licmophora paradoxo* (Lyngbye) Agardh, **MABI** *Mastogloia binotata* (Grunow) Cleve, **NARA** *Navicula ramosissima* (Agardh) Cleve, **NIAN** *Nitzschia angularis* W. Smith, **NIFU** *Nitzschia fistiformis* Grunow, **NILA** *Nitzschia lanceolata* var. *minima* Grunow, **NIMA** *Nitzschia macilenta* W.Gregory, **NIPA** *Nitzschia panduriformis* Gregory var. *panduriformis*, **OPPA** *Opephora pacifica* (Grunow) Petit, **RHPA** *Rhopalodia pacifica* Krammer, **TAKT** *Tabularia ktenoideis* M.Kuylenstierna

| Code | Site/Variables | | | | | | | | | | | | | | | | | | | | | |
|----------------------------------|----------------|-------|----------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|--------------------------------|--------|--------------|-------|---|----------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|--------------------------------|--------|--------------|--------|---------|
| | Dubrovnik | | | | | | | | | | Mljet | | | | | | Hvar | | | | | |
| | T | S | O ₂ /O ₂ ' | NO ₃ ⁻ | NO ₂ ⁻ | NH ₄ ⁺ | PO ₄ ³⁻ | SiO ₄ ⁴⁻ | TIN | Chl <i>a</i> | T | S | O ₂ /O ₂ ' | NO ₃ ⁻ | NO ₂ ⁻ | NH ₄ ⁺ | PO ₄ ³⁻ | SiO ₄ ⁴⁻ | TIN | Chl <i>a</i> | T | |
| TEMP | | 0.78* | | | | | | | -0.78* | | | | | | -0.79* | | | | | | -0.77* | |
| SAL | 0.78* | | | | | | | | | | | | | | | | | | | | | / |
| O ₂ /O ₂ ' | | | | -0.83* | | | | | -0.85* | | | | | | -0.82* | | | | | -0.89** | | / |
| NO ₃ ⁻ | | | -0.83* | | | | | | 0.93** | | | | | | | | | | | 0.91** | | / |
| NO ₂ ⁻ | | | | | | | | | | -0.79* | | | | | | | | | | | / | / |
| NH ₄ ⁺ | | | | | | | | | | | | | | | | | | | | | / | / |
| PO ₄ ³⁻ | | | | | | | | | | | | | | | | | | | | | / | / |
| SiO ₄ ⁴⁻ | | | | | | | | | | | | | | | | | | | | | / | / |
| TIN | -0.78* | | | | | | | | 0.77* | | | | -0.89** | 0.91** | | | | | | | / | / |
| Chl <i>a</i> | | | -0.85* | 0.93* | | | | 0.77* | | -0.77* | | | | | | | | | | | | / |
| ARFO | | | | | | | | | | | | | | | | | | | | | | |
| BERU | | | | | | | 0.82* | | | | | | | | | | | | | | 0.76* | |
| BESC | 0.76* | | | | | | | | | | | | | | | | | | | | | -0.66** |
| COCO | | | | | | | | | | | | | | | | | | | -0.82* | | | -0.55* |
| COMO | | | | | | | | | | | | | | | | | | | | | | -0.65** |
| COSC | -0.83* | | | | -0.80* | | | | | | | | | | | 0.79* | | | | | | -0.56* |
| FRIN | | | | | | | | | | | | | | | | | | | | | | |
| GROC | | | | | | | 0.89** | | | | | | | | | | | | | | | |
| HAKO | | | | | | | | | | | | | | | | | | | | | | |
| HYLA | 0.76* | | | | | | | | | | | | | | | | | | | | | |
| LIGR | | | | | | | | | | | | | | | | | | | | | | |
| LIPA | | | | | | | | | | | | | | | | | | | | | | |
| MABI | | | | | | | | | | | | | | | | | | | | | | |

continued

Table 5 continued

| Code | Site/Variables | | | | | | | | | | | | | | | | | | | | | | | |
|------|----------------|---|----------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|-----|--------------|---|-------|----------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|-----|--------------|---|--|------|--|
| | Dubrovnik | | | | | | | | | | | Mljet | | | | | | | | | | | Hvar | |
| | T | S | O ₂ /O ₂ ' | NO ₃ ⁻ | NO ₂ ⁻ | NH ₄ ⁺ | PO ₄ ³⁻ | SiO ₄ ⁺ | TIN | Chl <i>a</i> | T | S | O ₂ /O ₂ ' | NO ₃ ⁻ | NO ₂ ⁻ | NH ₄ ⁺ | PO ₄ ³⁻ | SiO ₄ ⁺ | TIN | Chl <i>a</i> | T | | | |
| NARA | | | | | | | | | | | | | | | | | | | | | | | | |
| NIAN | | | | | | | | | | | | | | | | | | | | | | | | |
| NIFU | | | | | | | | | | | | | | | | | | | | | | | | |
| NILA | | | | | | | | | | | | | | | | | | | | | | | | |
| NIMA | | | | | | | | | | | | | | | | | | | | | | | | |
| NIPA | | | | | | | | | | | | | | | | | | | | | | | | |
| OPPA | | | | | | | | | | | | | | | | | | | | | | | | |
| RHPA | | | | | | | | | | | | | | | | | | | | | | | | |
| TAKT | | | | | | | | | | | | | | | | | | | | | | | | |

H with the invasive *C. taxifolia*. Sites M and D are both exposed to the inflow of the oligotrophic Eastern Adriatic Current (EAC) (Orlić *et al.*, 1992) and were observed to be similar in our study except for ammonium concentration. The H site is instead located in a relatively sheltered bay without the direct influence of EAC and more protected from the open sea influence than D/M sites, resulting in lower disturbance by waves at H than at D/M sites. We estimate that wave energy at D/M sites is significantly higher than at H site according to the maximal height of the waves at H (up to 2.5 m) and D/M (up to 9 m) throughout the year (pers. comm. Srđan Čupić, Hydrographic Institute of the Republic of Croatia, Split). In addition to the greater number of samples collected at H in comparison to the two other sites (Table 1), this could also be one of the reasons why at site H a higher number of taxa and generally a greater diatom diversity occurred.

The minimum values of Shannon-Wiener Diversity Index and Pielou's Evenness Index at *C. cylindracea* sampling sites M and D during winter reflected the lowest number of diatom taxa observed for those sites, with a prevalence of *Cocconeis scutellum* var. *scutellum* (Table 4). Our data on the distribution of *C. scutellum* var. *scutellum* agree with a study from Neum Bay in Bosnia and Herzegovina in which, although no strong seasonal variation of diatom taxa was observed, *C. scutellum* var. *scutellum* showed high contributions in the winter-spring diatom communities (Hafner *et al.*, 2018). These results contrast those of the study of benthic diatoms on natural substrata from the Aegean Sea (Turkey) by Çolak Sabancı (2012b) where *C. scutellum* did not show any kind of seasonality. In our study *C. scutellum* var. *scutellum* was registered in all samples although with some differences in spatial distribution in relation to sampling periods. Generally, *C. scutellum* is indeed the most frequently recorded diatom taxon on all (epiphytic, epilithic, epizotic) different substrata (Car *et al.*, 2012; Çolak Sabancı, 2012b; De Stefano *et al.*, 2000, 2008; Hafner *et al.*, 2018; Sullivan, 1984; Totti *et al.*, 2007).

In the Mediterranean Sea the genus *Cocconeis* was thoroughly investigated by a fine-scale analysis of diatoms associated with *Posidonia oceanica* at 19 sampling sites in order to give a spatial overview and to reflect differences between epiphytic diatom communities in various parts of the Mediterranean Sea (Majewska *et al.*, 2014). These authors emphasised that, despite the salinity and temperature gradients and the various bio-chemo-physical factors affecting diatom communities in different Mediterranean basins, those factors may be less important in the case of the distribution of epiphytes of *Posidonia*. Moreover, Majewska *et al.* (2014) highlighted that especially coastal zones are strongly exposed to sudden short-term changes in temperature or salinity, but it should not be assumed that diatom species were particularly sensitive to changes of these factors. Other authors (Mazzella, 1983; Mazzella & Spinoccia, 1992; Mazzella *et al.*, 1994) working on epiphytes of *Posidonia* reported that diatom microflora presents well-defined morphological features and its species composition, with the genus *Cocconeis* as its most abundant component, is very sim-

ilar on all seagrass species, regardless of geographical zone. This may indicate that the substrate is the primary factor in determining the composition of associated diatom flora (Majewska *et al.*, 2014).

Autumn, as the period of the greatest species diversity, appears to coincide with the maximum toxicity (Dumay *et al.*, 2002) observed for *C. taxifolia*. Hafner *et al.* (2018) observed for the Adriatic an increase in species diversity index in the samples from the depth of 0.5 m in the warm summer months, while the highest diversity was associated to the samples from the depth of 8 m in October.

In spite of the high RA of *Grammatophora oceanica* in March 2010 at site D, based on other spring samples from 2009 and 2010, this species did not show any apparent preference for a given season. Therefore, the March 2010 results should be considered as some special and sporadic event, which is in agreement with results from the Aegean Sea (Turkey) that suggested no seasonal preference for *G. oceanica* (Çolak Sabancı, 2012b).

Apart from erect forms, sites D and M were characterised by adnate forms, while site H was characterised by tube-dwelling forms. However, it must be noted that the unequal number of samples collected at each of the sampling sites may have had some influence on the results obtained as the sampling season also affected the communities. Romagnoli *et al.* (2007) studied the ecological succession of microalgal communities on different substrata, and reported that a well-developed community, characterised by the presence of adnate living forms, is established after 3-5 weeks. Due to their adhering mode on the host surface through the valve face, adnate taxa may easily benefit from a nutrient exchange with the host (Round, 1981; Sullivan, 1984; Romagnoli *et al.*, 2014). Additionally, adnate forms are strongly attached to the substratum and therefore they can cope with high hydrodynamism and are not easily swept away by currents and waves even when they are found in high current-velocity layers. Nearby the rocky outcrops of the northern Adriatic Sea, species able to attach to the sediment grains were mostly found in the areas where the bottom currents are more intense, whereas taxa that are loosely associated with the sediments proliferated in the areas characterised by relatively weak bottom currents (Cibic *et al.*, 2016). Not only adnate diatoms grow close enough to the substrate to lie within low current-velocity boundary layers and thereby avoid the shear stress of severe currents, but they are also the most resistant to grazing. However, they are easily overgrown by other organisms and may become light-limited by that overgrowth (McCormick & Stevenson, 1989). 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 movements on and through the biofilm (Round, 1971; Round, 1981; Romagnoli *et al.*, 2014). At the D sampling site, the epilithic communities that developed in winter contained a high number of adnate forms (*Cocconeis* spp.) followed by motile ones (*Navicula* spp., *Nitzschia* spp.). The higher RA of motile diatoms in winter is likely due to resuspension events caused by stronger

winds when motile diatoms inhabiting muddy sediments are first suspended in the water column, followed by the settling on a larger area and colonising the substrate on which they had settled. Further, a substantial change occurred at site D in spring: the number of erect diatoms tripled, which affected the community growth form structure. The occurrence of erect diatoms indicates a mature phase of the community. Erect diatoms have better access to light, although they are more exposed to grazing pressure (Hillebrand *et al.*, 2000) and their capability of regulating stalk lengths makes them competitors for light when dense benthic populations develop. Despite these observations, in our study the diatom communities did not differ significantly among seasons in terms of both growth form structure and species composition. Our data rather indicate significant differences in diatom community structure occurring among the three sampling sites in terms of RA (Table 4). Moreover, the differences were correlated with the presence of the two species of invasive macroalgae (Hvar – *C. taxifolia* area; Mljet and Dubrovnik – areas of *C. cylindracea*) in terms of both species composition and growth form structure.

Additionally, as revealed by Cluster and MDS ordinations, the site location (i.e., the presence of invasive macroalgae) has more impact on diatom community structure than the seasonal changes. Although as much as 28% of the total number of taxa exclusively were found only once with an RA less than 1%, these rare taxa do not contribute significantly to differences between stations, which is confirmed by an MDS made with abundances for just 10 of the most important species. Seasonal change affects abundances rather than the structure of diatom assemblages (i.e., dominant species). Thus, for example, one of the dominant species at site D through all seasons is *Cocconeis scutellum* var. *scutellum*, though with some RA differences across the seasons. The same species was also one of the dominant species for site M, also in this case with high but variable RA across seasons.

The highest RA levels of one of the most abundant taxa at site H, *Rhopalodia pacifica*, have been observed during the period of intensive growth of the invasive macroalgae (summer and autumn). Specifically, the RA of *R. pacifica* was 7% for site H, while it only reached approximately 1% at the *C. cylindracea* sampling sites. A Spearman-Rank correlation displayed significant positive correlations between *R. pacifica* abundance and T at sites H and M. As there was no significant difference between stations regarding T, the results imply that the epilithic diatom *R. pacifica* was more affected by local environmental factors than by T. However, without an assessment of the influence of the whole range of relevant environmental factors and a detailed analysis of the complex interaction occurring among all of the elements in the investigated system, the effect of invasive macroalgae on diatom communities remains still unconfirmed.

Diatom analyses of epiphytes of the invasive *Caulerpa* species were primarily focused on the morphology of new marine taxa of the genus *Cocconeis*, *C. caulerpacola* (Car, *et al.*, 2012). Interestingly, *C. caulerpacola* was not recorded in the investigated epilithic diatom community

although it was observed in samples of invasive macroalgae collected simultaneously from the same locations in the same period (Car *et al.*, 2012).

In the Venice lagoon, where benthic diatoms were studied in relation to the water quality in a highly impacted area, cell abundance and diversity fluctuations were observed to be more associated with locations than with seasons (Facca & Sfriso, 2007). These results from surface sediment diatom communities are in agreement with those of the present study concerning rocky shore communities, which appeared to be affected by some aspects of environmental conditions and showed no clear seasonality. The observed species distribution patterns in our study may partly be explained by the different sensitivity of benthic diatoms to dislodgement by current induced shear forces, the different energy of the sea waves at sampling sites or by grazing pressure (a preference for consumption of particular diatoms by macro-invertebrates). Additionally, as differences in nutrient availability are often reflected in the structure and species composition of algal communities (Sundback & Snoeijs, 1991; Underwood *et al.*, 1998; Snoeijs, 1999; Frankovich *et al.*, 2006), environmental conditions need to be thoroughly monitored and analysed in relation to diatom community structure. Finally, invasive algae as a relevant structuring factor could also contribute to determine differences in environmental conditions among sites and hence affect benthic diatom distribution.

In conclusion, this study showed for the first time the species composition of epilithic diatom communities and their variations over the seasons in areas affected by invasive macroalgae of the genus *Caulerpa* distributed along the coast of Croatia. The result of our study implies that epilithic diatoms are dependant more on the sampling location than on the season and are possibly exposed to important influence of some of the environmental variables. We assume that significantly lower energy of the waves at H in comparison to D and M sites throughout the year is at least partly responsible for our observations. A main feature of diatom assemblages is a greater species diversity index during autumn, but how this pattern relates to resource availability is not clear. Additionally, this study recorded a difference in terms of abundance and composition of diatom assemblages for the sampling sites that included the presence of two different invasive macroalgae. Multivariate analyses revealed that two groups of significantly different assemblages of epilithic diatoms may be affected by the influence of some environmental characteristic of the sampling site. It is unclear which variables were the most important for the observed differences between sampling sites. Although the spatial variation in communities may also reflect the effects of unmeasured environmental variables, the results of this study indicate that invasive macroalgae may have some effect on the composition of epilithic diatom species. We should emphasise, however, that this suggestion must be considered with some caution due to the lack of complete environmental data in this study, and since the ecological preferences of diatoms might also influence their distribution. Without an assessment of the influence of each of

the environmental factors and a detailed analysis of the complex interactions occurring between all elements of the investigated system, the effect of invasive macroalgae on the diatom communities should be considered as still uncertain. As diatom assemblages vary with the availability of nutrients and are widely used as indicators of ecological change in aquatic environments (Ulanova & Snoeijs, 2006) the results of this study should be verified by further research. Moreover, since we lack information on benthic diatoms from uncontaminated control sampling sites (free from any impact of invasive algae) in the Middle and South Adriatic, and as these are the first results on epilithic diatom assemblages in the mentioned regions affected by invasive macroalgae *C. taxifolia* and *C. cylindracea*, we have avoided speculation in offering explanations. However, the most significant result of this paper lies in the information about marine epilithic diatoms in a part of the Mediterranean Sea affected by invasive macroalgae, and it also contributes to our general knowledge of benthic diatoms in the Adriatic which can be useful for other studies in the temperate seas.

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Appendix

Appendix 1. List of marine epilithic diatom taxa from areas of invasive *Caulerpa* species (*Caulerpa taxifolia* and *Caulerpa cylindracea*) in the Adriatic Sea. Abbreviations: taxa found only once in all number of samples are indicated with asterisk (*); taxa found exclusively at Hvar (H), Dubrovnik (D), or Mljet (M) site are indicated; taxa found exclusively at *C. cylindracea* sites are indicated with (Cc).

- Achnanthes brevipes* C.Agardh [*M]
Achnanthes brockmannii Simonsen
Achnanthes cf. *javanica* Grunow [H]
Achnanthes danica (Flögel) Grunow [*M]
Achnanthes fimbriata (Grunow) R.Ross [*H]
Achnanthes longipes C.Agardh
Achnanthes sp.1 [*H]
Actinocyclus ochotensis A.P.Jousé [H]
Actinopterychus heliopelta Grunow [*H]
Actinopterychus senarius (Ehrenberg) Ehrenberg [*H]
Amphiprora pseudoduplex (Osada & Kobayasi) Hällfors [*H]
Amphora abludens R.Simonsen [H]
Amphora arenaria Donkin [Cc]
Amphora bigibba var. *interrupta* (Grunow) Cleve
Amphora cf. *amoena* Hustedt
Amphora marina W.Smith
Amphora cf. *ramsbottomi* M.M.Salah [*H]
Amphora delicatissima Krasske [H]
Amphora gracialis W. Smith
Amphora graeffeana Hendey
Amphora helenensis Giffen
Amphora holsaticoides T.Nagumo & H.Kobayasi
Amphora hyalina Kützing
Amphora immarginata Nagumo [*M]
Amphora jostesorum Witkowski. Lange-Bertalot & Metzeltin [*M]
Amphora laevis Gregory
Amphora mexicana A.W.F.Schmidt
Amphora ocellata Donkin [Cc]
Amphora pannucea M.H.Giffen
Amphora piper B.J.Cholnoky [*M]
Amphora praelata Hendey [*D]
Amphora proteus W.Gregory
Amphora pseudoholsatica T.Nagumo & H.Kobayasi
Amphora pseudohyalina Simonsen
Amphora spec. 161/1 (Ico.Diat.Vol.7. Pl. 161 Fig.20)
Amphora spec. 167/1 (Ico.Diat.Vol.7. Pl. 167 Fig.7) [*M]
Amphora spec. 168/2 (Ico.Diat.Vol.7. Pl. 161 Fig.9) [*D]
Amphora sp.1 [Cc]
Amphora sp.2
Amphora sp.3
Amphora sp.4
Amphora subacutiuscula Schoeman [M]
Amphora turgida var. *parallela* Heiden in Heiden & Kolbe [Cc]
Ardissonia crystallina (C.A. Agardh) Grunow
Ardissonia formosa (Hantzsch) Grunow
Ardissonia robusta (Ralfs) De Notaris
Auricula intermedia (F.W.Lewis) Cleve [*M]
Auricula sp.1
Auricula sp.2
Bacillaria socialis (Gregory) Ralfs
Berkeleya rutilans (Trentepohli) Grunow
Berkeleya scopulorum (Brébisson ex Kützing) E.J.Cox
Biddulphia pulchella Gray [*D]
Biremis ambigua (Cleve) D.G.Mann
Biremis sp.1 [*H]
Caloneis elongata (Grunow) Boyer [*D]
Caloneis excentrica (Grunow) Boyer [*M]
Caloneis fusoides (Grunow) Heiden & Kolbe [M]
Caloneis liber (W.Smith) Cleve
Campylodiscus sp.1 [*H]
Chamaepinnularia truncate (Dieter König) A.Witkowski. Lange-Bertalot & Metzeltin [*H]
Cocconeopsis sp.1
Cocconeis britannica Naegeli in Kützing
Cocconeis cf. *distantula* Giffen
Cocconeis cf. *irregularis* (Schulz) Witkowski [*D]
Cocconeis convexa Giffen
Cocconeis costata Gregory var. *costata*
Cocconeis dirupta Gregory var. *dirupta*
Cocconeis dirupta var. *flexella* (Janisch & Rabenhorst) Grunow [*D]
Cocconeis diruptoides Hustedt [*M]
Cocconeis distans Gregory
Cocconeis guttata Hustedt in Aleem & Hustedt
Cocconeis krammeri Lange-Bertalot & Metzeltin
Cocconeis molesta var. *crucifera* Grunow in Van Heurck
Cocconeis pelta A. Schmidt
Cocconeis peltoides Hustedt
Cocconeis pinnata Gregory ex Greville
Cocconeis pseudodiruptoides Foged [*D]
Cocconeis pseudomarginata Gregory [Cc]
Cocconeis scutellum Ehrenberg var. *scutellum*
Cocconeis speciosa Gregory [M]
Coronia decora (Brébisson) Ruck & Guiry
Cyclophora tenuis Castracane
Cylindrotheca closterium (Ehrenberg) Reimann & J.C.Lewin
Cymatosira lorenziana Grunow [H]
Delphineis minutissima (Hustedt) Simonsen [H]
Delphineis surirella (Ehrenberg) Andrews
Dimeregrammopsis furcigerum (Grunow) Ricard
Dimeregramma fulvum (Gregory) Ralfs in Pritchard
Dimeregramma minor (Gregory) Ralfs var. *minor*
Diploneis aestuarii Hustedt
Diploneis bombus Ehrenberg
Diploneis cf. *smithii* (Brébisson) Cleve [*H]
Diploneis chersonensis (Grunow) Cleve [Cc]
Diploneis crabro Ehrenberg [H]
Diploneis decipiens var. *parallela*
Diploneis divergens (A.W.F.Schmidt) Cleve [H]
Diploneis litoralis var. *clathrata* Østrup
Diploneis littoralis (Donkin) Cleve [*H]
Diploneis mirabilis D.König
Diploneis nitescens (W.Gregory) Cleve
Diploneis notabilis (Greville) Cleve
Diploneis papula (A.F.W.Schmidt) Cleve var. *papula* [H]
Diploneis parca (A. Schmidt) Boyer
Diploneis smithii (Brébisson) Cleve

- Diploneis spec.* (Ico.Diat.Vol.7. Pl. 88 Figs 6-8)
- Diploneis splendida* Cleve
- Diploneis stroemii* Hustedt [Cc]
- Diploneis vacillans* (A. Schmidt) Cleve var. *vacillans*
- Diploneis vacillans* var. *renitens* (A.Schmidt) Cleve [*H]
- Diploneis vetula* (A.W.F.Schmidt) Cleve [*M]
- Diploneis weissflogii* (A. Schmidt) Cleve
- Diploneis* sp.1 [*H]
- Diploneis* sp.2 [*H]
- Entomoneis paludosa*** (W. Smith) Reimer var. *paludosa* [Cc]
- Entomoneis* sp.1 [*H]
- Fallacia aequorea*** (Hustedt) D.G.Mann [*H]
- Fallacia* cf. *vittata* (Cleve) D.G. Mann
- Fallacia florinae* (M.Møller) Witkowski [H]
- Fallacia forcipata* (Greville) Stickle & D.G.Mann
- Fallacia nummularia* (Grev.) D.G. Mann
- Fallacia ny* (Cleve) D.G.Mann [*H]
- Fallacia nyella* (Hustedt) D.G.Mann
- Fallacia versicolor* (Grunow) D.G.Mann
- Fragilaria capensis*** Grunow [*M]
- Fragilaria improbula* Witkowski & Lange-Bertalot [*H]
- Fragilaria investiens* (W. Smith) Cleve-Euler
- Fragilaria martyi* (Heribaud) Lange-Bertalot
- Fragilaria quebecensis* (Poulin. Hudon & Cardinal) A.Witkowski.
- Lange-Bertalot & Metzeltin [*H]
- Glyphodesmis distans*** (Gregory) Grunow in Van Heurck
- Grammatophora angulosa*** var. *angulosa* Ehrenberg [M]
- Grammatophora angulosa* var. *islandica* (Ehrenberg) Grunow
- Grammatophora arcuata* Ehrenberg
- Grammatophora macilentia* W. Smith
- Grammatophora marina* (Lyngbye) Kützing
- Grammatophora maxima* Grunow [*D]
- Grammatophora oceanica* (Ehrenberg 1854 pro parte) Grunow
- Grammatophora serpentina* (Ralfs) Kützing [Cc]
- Grammatophora undulata* Ehrenberg [M]
- Gyrosigma*** sp.1
- Halamphora acutiuscula*** (Kützing) Levkov
- Halamphora coffeiformis* (C.Agardh) Levkov
- Halamphora costata* (W.Smith) Levkov [D]
- Halamphora cuneata* (Cleve) Levkov
- Halamphora exigua* (W.Gregory) Levkov
- Halamphora granulate* (Gregory) Levkov
- Halamphora hybrida* (Grunow) Levkov [*H]
- Halamphora interrupta* (Heiden) Levkov [H]
- Halamphora kolbei* (Aleem) Álvarez-Blanco & S.Blanco
- Halamphora luciae* (Cholnoky) Levkov
- Halamphora staurophora* (Juhlin-Dannfelt) Álvarez-Blanco & S.Blanco
- Halamphora subholsatica* (Krammer) Levkov
- Halamphora turgida* (Gregory) Levkov
- Hantzschia*** sp.1 [*H]
- Haslea britannica*** (Hustedt & Aleem) Witkowski. Lange-Bertalot & Metzeltin [*D]
- Hyalosynedra laevigata*** (Grunow) Williams & Round
- Licmophora abbreviata*** Agardh
- Licmophora debilis* (Kützing) Grunow in Van Heurck
- Licmophora flabellata* (Carmichael) Agardh
- Licmophora gracilis* (Ehrenberg) Grunow var. *gracilis*
- Licmophora paradoxa* (Lyngbye) Agardh
- Licmophora remulus* Grunow
- Licmophora* sp.1
- Lyrella abrupta*** (W.Gregory) D.G.Mann
- Lyrella fagedii* Witkowski. Lange-Bertalot & Metzeltin
- Lyrella hennedyi* (W.Smith) Stickle & D.G.Mann [*M]
- Lyrella spectabilis* (Gregory) D.G.Mann [*H]
- Lyrella sulcifera* (Hustedt) Witkowski [*H]
- Lyrella* sp.1 [*H]
- Martyana schulzii*** (C.Brockmann) Snoeijs
- Mastogloia adriatica*** Voigt [Cc]
- Mastogloia affinis* Cleve
- Mastogloia angulata* Lewis [M]
- Mastogloia baldjikian* Grunow [H]
- Mastogloia binotata* (Grunow) Cleve
- Mastogloia biocellata* (Grunow) Novarino & Muftah
- Mastogloia braunii* Grunow [Cc]
- Mastogloia corsicana* Grunow in Cleve & Möller
- Mastogloia crucicula* (Grunow) Cleve var. *crucicula*
- Mastogloia crucicula* var. *alternans* Zanon
- Mastogloia cuneata* (Meister) Simonsen
- Mastogloia cyclops* Voigt
- Mastogloia decipiens* Hustedt
- Mastogloia emarginata* Hustedt
- Mastogloia erythraea* Grunow var. *erythraea*
- Mastogloia exilis* Hustedt
- Mastogloia fallax* Cleve [*H]
- Mastogloia fimbriata* (Brightwell) Cleve
- Mastogloia grunowii* A. Schmidt [*M]
- Mastogloia hovarthiana* Grunow [*H]
- Mastogloia ignorata* Hustedt
- Mastogloia inaequalis* Cleve
- Mastogloia laminaris* (Ehrenberg) Grunow [Cc]
- Mastogloia lanceolata* Thwaites in W. Smith [*M]
- Mastogloia linearis* Simonsen
- Mastogloia macdonaldii* Greville [M]
- Mastogloia manokwariensis* Cholnoky
- Mastogloia mauritiana* Brun in A. Schmidt Atlas [Cc]
- Mastogloia ovalis* A. Schmidt
- Mastogloia ovulum* Hustedt
- Mastogloia paradoxa* Grunow in Cleve & Möller
- Mastogloia parva* Hustedt
- Mastogloia peragalli* Cleve [Cc]
- Mastogloia pseudolatecostata* Yohn & Gibson
- Mastogloia pumila* (Grunow) Cleve [*H]
- Mastogloia pusilla* (Grunow) Cleve var. *pusilla*
- Mastogloia quinquecostata* Grunow
- Mastogloia recta* Hustedt [*H]
- Mastogloia regula* Hustedt [*H]
- Mastogloia robusta* Hustedt
- Mastogloia similis* Hustedt
- Mastogloia spec.* 75/1 (Ico.Diat.Vol.7. Pl. 75 Figs 7-9)
- Mastogloia spec.* 84/1 (Ico.Diat.Vol.7. Pl. 84 Figs 9-11) [*H]
- Mastogloia splendida* (Gregory) Cleve
- Mastogloia varians* Hustedt
- Mastogloia vasta* Hustedt
- Mastogloia* sp.1
- Mastogloia* sp.2 [*H]
- Navicula arenaria*** Donkin
- Navicula bipustulata* Mann [*M]
- Navicula cancellata* Donkin
- Navicula* cf. *fauta* Hustedt [H]
- Navicula directa* (W.Smith) Ralfs [*H]

- Navicula duerrenbergiana* Hustedt [*H]
Navicula kariana Grunow [*M]
Navicula longa (W.Gregory) Ralfs [*H]
Navicula lusoria Giffen
Navicula palpebralis Brébisson ex W.Smith
Navicula pavillardii Hustedt [*H]
Navicula ramosissima (Agardh) Cleve
Navicula recurvata Gran [M]
Navicula salinarum var. *rostrata* (Hustedt) Lange-Bertalot
Navicula salinicola Hustedt [*M]
Navicula spec. 130/1 (Ico.Diat.Vol.7. Pl. 130 Fig. 26)
Navicula subagnita Proschkina-Lavrenko
Navicula subrostellata Hustedt
Navicula zostereti Grunow [*H]
Navicula sp.1 [*D]
Navicula sp.2 [*H]
Navicula sp.3 [*H]
Neofragilaria nicobarica Desikachary. Prasad & Prema [*H]
Nitzschia angularis W. Smith
Nitzschia capitellata Hustedt [H]
Nitzschia carnicobarica Desikachary & Prema [*M]
Nitzschia cf. *bulnheimiana* (Rabenhorst) H.L.Smith [*H]
Nitzschia cf. *sigma* (Kützing) W. Smith
Nitzschia distans var. *distans* Gregory
Nitzschia fusiformis Grunow
Nitzschia grossestriata Hustedt [H]
Nitzschia hybrid Grunow
Nitzschia incurvata var. *lorenziana* R.Ross [*H]
Nitzschia lanceola Grunow [H]
Nitzschia lanceolata var. *minima* Grunow
Nitzschia lanceolata W. Smith [H]
Nitzschia liebethruthii Rabenhorst
Nitzschia lorenziana Grunow
Nitzschia macilenta W.Gregory
Nitzschia marginulata var. *didyma* Grunow [D]
Nitzschia ovalis H.J.Armott
Nitzschia panduriformis Gregory var. *panduriformis*
Nitzschia pellucida Grunow
Nitzschia scalpelliformis Grunow
Nitzschia sicula (Castracane) Hustedt
Nitzschia spec. 183/2 (Ico.Diat.Vol.7. Pl. 183 Fig. 11) [*M]
Opephora guenter-grassii (Witkowski & Lange-Bertalot) Sabbe & Vyverman
Opephora marina (W.Gregory) Petit [*H]
Opephora pacifica (Grunow) Petit
Paralia sulcata (Ehrenberg) Cleve [H]
Parlibellus bennikei Witkowski
Parlibellus berkeleyi (Kützing) E.J.Cox [*D]
Parlibellus delognei (Van Heurck) Cox
Pinnularia sp.1 [*H]
Pinnularia sp.2 [*M]
Plagiogramma interruptum (W.Gregory) Ralfs
Plagiogramma staurophorum (W.Gregory) Heiberg [*H]
Plagiogramma sp.1 [M]
Plagiotropis tayrecta Paddock
Planothidium aff. *engelbrechtii* (Cholnoky) Round & L.Bukhtiyarova [*H]
Planothidium cf. *campechianum* (Hustedt) Witkowski & Lange-Bertalot [*H]
Planothidium delicatulum (Kützing) Round & Bukhtiyarova
Planothidium quarnerensis (Grunow) Witkowski. Lange-Bertalot & Metzelin
Pleurosigma decorum W.Smith
Pleurosigma sp.1
Pleurosigma sp.2 [*H]
Pleurosigma sp.3 [*D]
Podocystis americana Bailey [*D]
Protokeelia cholnokiana (Giffen) Round et Basson
Psammodictyon roridum (M.H.Giffen) D.G.Mann
Rhabdonema adriaticum Kützing [Cc]
Rhaphoneis amphiceros (Ehrenberg) Ehrenberg [H]
Rhopalodia musculus (Kützing) Otto Müller
Rhopalodia pacifica Krammer
Rhopalodia sp.1 [*H]
Seminavis obtusiuscula (Grunow) Danieledis & D.G.Mann [*M]
Seminavis spec. 164/1 (Ico.Diat.Vol.7. Pl. 164 Fig. 17)
Seminavis sp.1
Striatella unipunctata (Lyngbye) C.Agardh
Surirella fastuosa (Ehrenberg) Ehrenberg
Synedra commutata Grunow
Tabularia fasciculata (C.Agardh) D.M.Williams & Round
Tabularia ktenooides M.Kuylensstierna
Tetramphora decussata (Grunow) Stepanek & Kociolek
Tetramphora securicula (Peragallo & Peragallo) Stepanek & Kociolek
Toxarium undulatum Bailey
Trachyneis aspera (Ehrenberg) Cleve
Triceratium reticulum Ehrenberg [*M]
Tryblionella acuminata W.Smith [*H]
Tryblionella apiculata W.Gregory
Tryblionella hungarica (Grunow) Frenguelli [*M]
Tryblionella persuadens (Cholnoki) K.P.Cavalcante. P.I.Tremarin & T.A.V.Ludwig