Epiphytic diatom assemblages on invasive *Caulerpa taxifolia* and autochthonous *Halimeda tuna* and *Padina* sp. seaweeds in the Adriatic Sea – summer/autumn aspect

by

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Abstract

This study focuses on the taxonomy of epiphytic diatoms in the area of invasive macroalgae from the genus *Caulerpa*. *Caulerpa* species are characterized by the presence of secondary metabolites, such as caulerpenyne (CYN), the main function of which is a chemical defense mechanism against herbivores and epiphytes. Epiphytic diatoms were studied on fronds of *Caulerpa taxifolia* ("killer seaweed") and, for comparison, on autochthonous macroalgae *Padina* sp. and *Halimeda tuna* at the eastern Adriatic Sea coast (Island of Hvar) in the summer and autumn of 2010. The qualitative analysis was performed with the use of light and scanning electron microscopy. The Shannon–Wiener Diversity Index determined for *Caulerpa taxifolia* showed a wide range of values (3.11–4.88), with a maximum in August and a minimum in October. While the number of taxa on *Caulerpa taxifolia* fronds increased from June (41) to August (88), it declined in autumn due to the high relative abundance of *Cocconeis caulerpacola*, which is a diatom typical for *Caulerpa*. On the other hand, the largest number of taxa on *Padina* sp. was observed in September (82). The detailed composition of epiphytic diatoms and seasonal dynamics in the area affected by the invasive macroalga *Caulerpa taxifolia* have been determined for the first time.

Key words: macroalgae, epibiotic, *Cocconeis*, *Mastogloia*, Mediterrranean, diatom community, succession

Introduction

The marine green macroalga *Caulerpa taxifolia* (M.Vahl) C.Agardh has pinnate, fern-like fronds (up to 25 cm long, 2 cm wide) that extend upward from horizontal stolons (Meinesz et al. 1995; Fig. 1). This alga outcompetes native seaweeds and seagrasses in the Mediterranean by forming dense carpets, leading to a loss of biodiversity. It is indigenous to tropical and subtropical seas worldwide, including Australia (Phillips & Price 2002) and has been widely used as a decorative plant in the marine aquarium trade. It was accidentally released from the Monaco Aquarium in 1984 (Meinesz & Hesse 1991), rapidly spread across the western Mediterranean basin (Meinesz et al. 2001) and became one of the most invasive species. In the eastern Adriatic, *C. taxifolia* was observed for the first time in 1994 in the Bay of Stari Grad (43°10′54″N, 16°35′00″E) on a hard, sandy and muddy substrate without vegetation or within meadows of *Posidonia oceanica* (L.) Delile (Žuljević & Antolić 2002).

Caulerpa species are characterized by the presence of secondary metabolites, such as caulerpenyne (CYN), the main function of which is as a chemical defense mechanism against herbivores and epiphytes (Box et al. 2008; Sureda et al. 2009). The maximum concentrations of CYN for the "aquarium-Mediterranean" strain of *C. taxifolia* were recorded in autumn and the minimum in spring, reaching values that are much higher than those observed in other *Caulerpa* species (Dumay et al. 2002). *Caulerpa taxifolia* has a marked seasonal biomass cycle with higher biomass corresponding to higher water temperatures (Meinesz et al. 1995). In summer and autumn, *C. taxifolia* can grow by nearly 2 cm per day (Meinesz 2002) and during this period fronds of *C. taxifolia* reach their maximum length (Meinesz & Hesse 1991; Meinesz et al. 1993; 1995). As the growth and toxicity of *C. taxifolia* vary greatly throughout the year (Amade & Lemée 1998; Thibaut et al. 2004), the chemical defense of this species may affect the settlement and development of different sessile organisms in invaded systems to varying degrees (Prado & Thibaut 2008). As the species turnover is known to be strictly controlled by seasonal variables of the host plant, such as leaf growth (e.g. Wittmann et al. 1981; Prado & Thibaut 2008), it is important to analyze epiphytic diatom assemblages throughout different seasons, which in the case of *C. taxifolia* means particularly in summer and autumn.

The specific composition of diatom communities of *C. taxifolia* has not been thoroughly examined, despite the potentially important role of epiphytic diatoms in the functioning of ecosystems influenced by *C. taxifolia*. The only studies of benthic diatoms from areas of invasive *C. taxifolia* in the Adriatic were those related to epiphytic diatoms of *C. taxifolia* and focused on the morphology and description of valve ultrastructure of a new marine diatom, *Cocconeis caulerpacola* (Car et al. 2012), and the study of epilithic diatom communities from areas of invasive *Caulerpa* species (Car et al. 2019). The analysis of epiphytic diatoms of another taxa from the genus *Caulerpa*, *Caulerpa racemosa*, on the Pacific coast
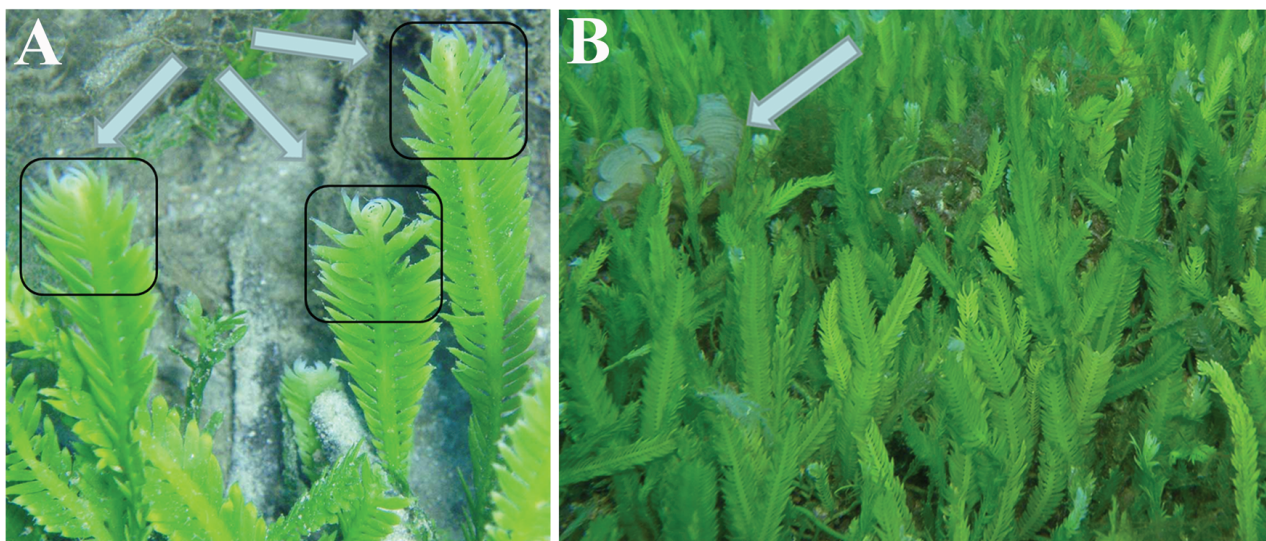
Figure 1

A) *Caulerpa taxifolia*, the Bay of Stari Grad, the Island of Hvar, 2 cm long cut-off upper part (photo by Tonči Dulčić). B) *Padina* sp. (arrow) in a dense patch of *Caulerpa taxifolia*, the Bay of Stari Grad, the Island of Hvar (photo by Tonči Dulčić)

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of Japan was primarily focused on the morphology of taxa from genus *Cocconeis* (Suzuki et al. 2001). In the Mediterranean Sea, the genus *Cocconeis* was thoroughly researched when a fine-scale analysis of diatoms associated with *Posidonia oceanica* was carried out to investigate the composition and diversity of epiphytic communities, with special reference to the most common and abundant genus – *Cocconeis* (Majewska et al. 2014). The epiphytic diatom communities of the endemic Mediterranean seagrass *Posidonia oceanica* are among the most frequently analyzed (Mazzella et al. 1994; De Stefano et al. 2000; Majewska 2014). Unlike the *Posidonia* communities, there are few studies of diatom communities associated with the green macroalga *Padina* sp. and the brown macroalga *Halimeda tuna* (J. Ellis & Solander) J. V. Lamouroux in the Mediterranean (e.g. Belegratis & Economou-Amilli 2002).

In general, the majority of previous studies on benthic diatoms in the Adriatic were conducted in the northern Adriatic and estuaries, and seasonal fouling by diatoms was studied on artificial substrates (e.g. Burić et al. 2004; Totti et al. 2007; Caput Mihalić et al. 2008; Levkov et al. 2010; Mejdandžić et al. 2015; Nenadović et al. 2015). Limited information is available on the composition of diatom assemblages growing on either natural or artificial substrates in marine coastal waters of the eastern Middle and South Adriatic, e.g. diatoms from stones in the oligotrophic Bay of Neum in Bosnia and Herzegovina (Hafner et al. 2018a,b) and along the Albanian coastal wetlands (Miho & Witkowski 2005).

The main objective of this study was to describe the dynamics of diatoms on the invasive macroalga *Caulerpa taxifolia* during a monthly sampling over summer and autumn and to compare the structure of epiphytic diatom assemblages of the investigated invasive macroalgae with the taxonomic composition of epiphytic diatoms on autochthonous brown (*Padina* sp.) and green (*Halimeda tuna*) algae in the area inhabited by *C. taxifolia* in the Adriatic. Due to differing thallus architecture, it was expected that the taxonomic composition of epiphytic diatoms on the investigated macroalgae would vary. In addition, the diversity of dominant diatom genera found in this area is thoroughly described. The results of the analysis of the epiphytic diatom community and its succession on macroalgae on a fine time scale in an area affected by invasive *C. taxifolia* in the Adriatic Sea are described for the first time.

Materials and methods

Study Area

Thalli of *Caulerpa taxifolia* were sampled during summer and autumn 2010 by SCUBA diving in the Bay of Stari Grad, the Island of Hvar, the Central Adriatic, Croatia (Fig. 2). The Bay of Stari Grad is a semi-enclosed bay with the prevailing cyclonic currents driven mainly by seasonal winds (Cvitković et al. 2017). The sampling site is one of the sunniest areas in Croatia (Zaninović & Matzarakis 2007). It is characterized by Mediterranean climate and is exposed to anthropogenic impact
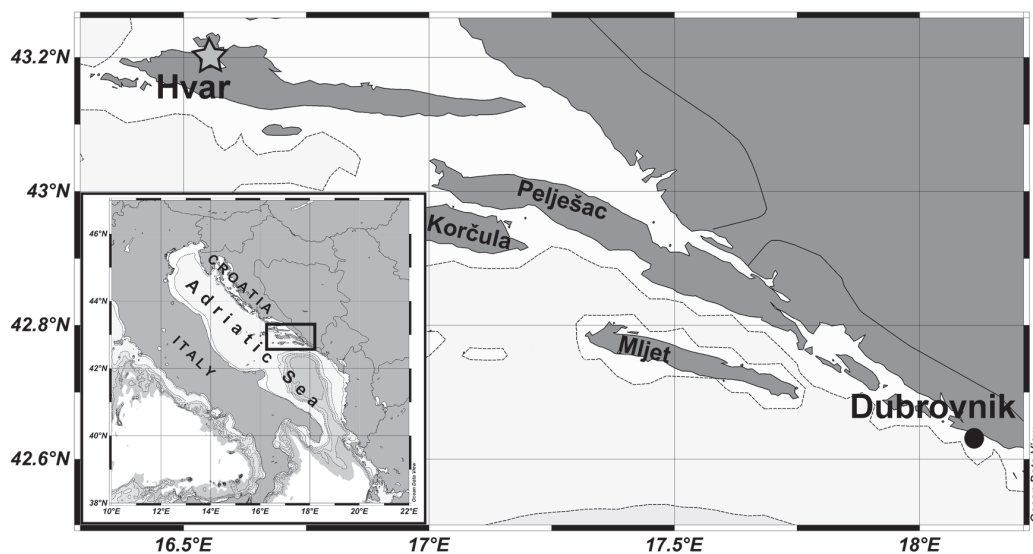
Figure 2

Map of the study site

(tourism) limited to the summer season. The sampling site of *C. taxifolia* was quite shallow (maximum depth of 8 m) and water temperature ranged from 20 to 25°C. Specimens of *C. taxifolia* were carefully detached from the seafloor at a depth of ca. 5–8 m in three replicates without damaging the fronds and avoiding the dispersal of fragments. In order to conduct a detailed analysis of epiphytic diatom assemblages occurring on fronds of *C. taxifolia* and to describe their dynamics on a fine time scale, the younger 2 cm tips of *C. taxifolia* fronds were cut and prepared for diatom analysis. Sampling was carried out from June to October 2010.

To compare the epiphytic diatom community on *Caulerpa taxifolia* with communities reported in previous studies and to provide information about host dependence, particularly due to the existence of toxins characteristic for *Caulerpa* species, sampling of the autochthonous brown alga *Padina* sp. and the autochthonous green alga *Halimeda tuna* was conducted at the same time from the same localities. Replicates of three different specimens of each macroalga collected simultaneously were selected. While all five samples of *Padina* sp. were collected in three replicates between June and October 2010, only three samples of *H. tuna* were collected in three replicates in June, July and September.

Sample preparation

Immediately after the macroalgal samples were collected, they were fixed in 4% formaldehyde solution in seawater. Organic material was removed from samples prior to light (LM) and electron microscopy (EM) observations by boiling with 30% H_2O_2 and adding 10% HCl to remove $CaCO_3$. They were then rinsed with deionized water, pipetted onto ethanol-cleaned coverslips and left to air dry before mounting in Naphrax®.

Light microscopy

Slides and the prepared material were deposited in the diatom collection (SZCZ) of the Institute of Marine Sciences, University of Szczecin (Poland). The abundance of diatoms was calculated from three replicate slides per sampling date and substrate to confirm the average relative abundance (RA) of diatoms. The RA of individual taxa and taxa richness in the assemblages were estimated on the basis of at least 300 diatom valves counted per glass slide. As the surface (or weight) of thalli fragments was not measured before the cleaning procedure, the actual abundance (cells cm^{-2} or cells g^{-1}) could not be calculated. Our method was designed for qualitative

analysis and the abundance of diatom taxa expressed as RA can be found in many papers dealing with diatom communities (Vilbaste et al. 2000; Cunningham & McMinn 2004; Çolak Sabanci 2011; 2012; Çolak Sabanci et al. 2011 etc.).

Identifications were carried out following Witkowski et al. (2000). Terminology follows Round et al. (1990). Nomenclature of the identified taxa follows AlgaeBase (Guiry & Guiry, 2018). Some taxa reported here could not be clearly assigned to the species level (assigned as "sp.") and they will be subjected to further taxonomic investigations.

For the structural analysis of the diatom communities, the identified taxa were categorized according to their growth form into one of the following groups: adnate (cells growing with the valve face strongly adherent to the substrate and having a limited motility), erect (cells attached to the substrate by stalks, pads or peduncles), motile (biraphid cells moving on the substrate surface), and tube-dwelling (raphid forms living in mucilage tubes produced by themselves; Majewska et al. 2013; 2014; 2016; Round et al. 1990; Totti et al. 2007).

The results of the analysis of epiphytic diatoms were gathered according to the seasons: summer (June, July, August) and autumn (September, October).

Electron microscopy

Ultrastructural analysis was performed with the use of scanning electron microscopy (SEM). A drop of a cleaned sample was air-dried overnight on aluminum stubs and coated with Au/Pd using Precision Etching Coating System Model 682 (Gatan, USA), a coating thickness of 10 nm. SEM observations were primarily conducted at the Warsaw University of Technology, the Faculty of Materials Science and Engineering, using Hitachi S-3500, SU-70 and SEM/ STEM S-5500 (Hitachi, Tokyo, Japan).

Statistical analysis

To analyze the diversity of epiphytic diatom assemblages from different substrates over different months, the Shannon–Wiener Diversity Index (SWDI) was computed (Krebs 1999).

Multidimensional scaling (MDS) ordination and hierarchical clustering (CLUSTER) together with the SIMPROF test, which highlights significantly different ($p < 0.05$) groups, were used to display differences in communities associated with the substrates and were based on the standardized RA data of all recorded diatom taxa. Data used to perform MDS and Cluster analyses to assess differences between invasive

C. taxifolia and autochthonous *Padina* sp. and *H. tuna*, and between sampling months, were organized in a matrix of 254 taxa over 13 samples collected during the period of 5 months (June–October 2010). Species abundance data were square root transformed prior to the analysis to normalize the data. A resemblance matrix of the data was generated using Bray Curtis analysis. The dissimilarity percentage analysis (SIMPER; Clarke & Warwick 1994) was used to identify the taxa that contributed most to the differences between the observed clusters.

The analysis of similarities (ANOSIM) was used to determine whether there were any significant differences in the growth form structure and species composition of diatom communities among the selected substrates (macroalgae) and seasons. Adnate diatom taxa belong to the genera *Cocconeis, Amphora,* and *Halamphora*, while erect diatoms belong to the genera *Grammatophora, Licmophora, Ardissonea, 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 the genera *Berkeleya* and *Parlibellus*.

Canonical analysis of principal coordinates (CAP) was used to summarize the structure of diatom assemblages and to characterize epiphytic communities along the months and substrates.

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

Results

Altogether, 137 diatom taxa belonging to 42 diatom genera were identified as epiphytes on the upper 2 cm part of *C. taxifolia* fronds. The genus with the largest number of taxa was *Mastogloia* (29), followed by *Amphora* (18), *Nitzschia* (14), *Cocconeis* (9) and *Navicula* (7).

The number of taxa on *C. taxifolia* fronds increased from June to August, with the smallest number in June (41) and the largest one in August (88). The largest number of taxa on *Padina* sp. was observed in September (82; Fig. 3a).

The widest (3.11–4.88) range of the Shannon–Wiener Diversity Index was determined for *C. taxifolia*, with the maximum in August and the minimum in October (Fig. 3b). The reason for the low value of the Shannon–Wiener Diversity Index in June and October 2010 was the high RA (> 40%) of the genus *Cocconeis* that occurred with a small number of taxa. *Cocconeis molesta* var. *crucifera* and *Cocconeis scutellum*
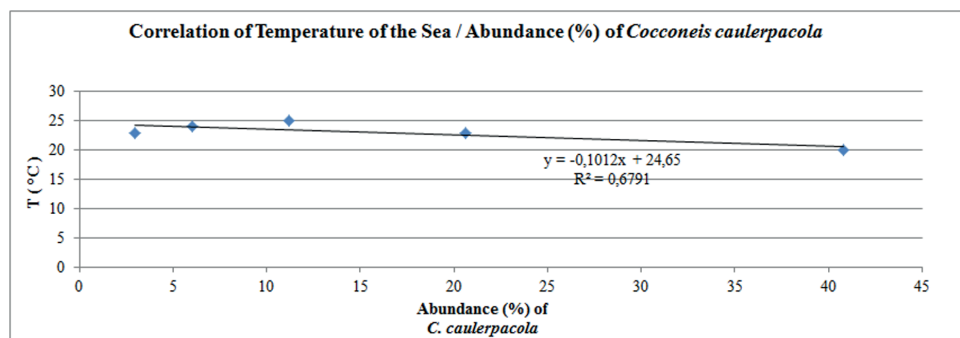

Figure 3

A) The number of epiphytic diatom taxa on *Caulerpa taxifolia* fronds, *Padina* sp. and *Halimeda tuna* from June to October 2010. B) Values of the Shannon–Wiener Diversity Index for epiphytic diatom samples on *Caulerpa taxifolia, Padina* sp. and *Halimeda tuna* in summer and autumn 2010 on the Island of Hvar. C) Contribution of individual *Cocconeis* taxa to the epiphytic diatom community on *Caulerpa taxifolia* in summer and autumn 2010

var. *scutellum*, which were initially the main fouling components occurring as patches, declined in July to be replaced by *Cocconeis caulerpacola*, the latter species being dominant in autumn (Fig. 3c). Due to the high RA of *C. caulerpacola* in September, the maximum abundance of taxa observed on *C. taxifolia* that month was replaced by the maximum abundance of taxa on *Padina* sp. The maximum RA of *C .caulerpacola* of over 40% was observed in October. No correlation between the abundance of *C. caulerpacola* and sea water temperature was confirmed (Fig. 4).

In June, the taxa richness on *Padina* sp. and *H. tuna* was higher than on *C. taxifolia* and in both cases the value of the Shannon–Wiener Diversity Index was almost the same and amounted to about 4.5. In July, the taxa richness of both autochthonous algae declined (Fig. 3b). While the taxa richness on *H. tuna* remained unchanged throughout the autumn, the taxa richness on *Padina* sp. increased in August and the Shannon–Wiener Diversity Index remained at the highest level observed in this study (4.95–4.96) over the next months.

Correlation of Temperature of the Sea / Abundance (%) of *Cocconeis caulerpacola*

$y = -0,1012x + 24,65$
$R^2 = 0,6791$

Figure 4

Average abundance of *Cocconeis caulerpacola* on *Caulerpa taxifolia* from the Island of Hvar is presented in relation to sea water temperature. N = 5

Table 1

Dominant diatom taxa on upper 2 cm of *Caulerpa taxifolia* fronds with average relative abundance (Avg. RA) > 1% and frequency of occurrence (Freq.) > 40% in summer and autumn 2010. N = 5

Caulerpa taxifolia fronds – dominant taxa:	Freq. (%)	Avg. RA (%)
Amphora helenensis Giffen	60.00	2.05
Ardissonea fulgens (Greville) Grunow	100.00	1.54
Berkeleya scopulorum (Brébisson) Cox	80.00	1.13
Cocconeis caulerpacola Witkowski, Car & Dobosz	100.00	16.31
Cocconeis cf. *scutellum* Ehrenberg	80.00	6.25
Cocconeis molesta var. *crucifera* Grunow in Van Heurck	100.00	9.01
Cocconeis scutellum Ehrenberg var. *scutellum*	100.00	5.47
Hyalosynedra laevigata (Grunow) Williams & Round	100.00	14.44
Licmophora remulus Grunow	80.00	1.88
Mastogloia crucicula (Grunow) Cleve var. *crucicula*	100.00	2.59
Mastogloia crucicula var. *alternans* Zanon	100.00	2.02
Mastogloia cuneata (Meister) Simonsen	80.00	1.69
Mastogloia pseudolatecostata Yohn & Gibson	80.00	3.23
Mastogloia spec. (Ico.Diat.Vol.7, Pl. 75 Figs 7-9)	80.00	1.52
Navicula arenaria Donkin var. *arenaria*	80.00	3.38
Navicula ramosissima (Agardh) Cleve	60.00	1.55
Navicula subagnita Proschkina-Lavrenko	40.00	1.88
Nitzschia angularis W. Smith	100.00	1.37
Nitzschia lanceolata var. *minima* Grunow	100.00	2.96
Nitzschia panduriformis Gregory var. *panduriformis*	100.00	2.61
Opephora pacifica (Grunow) Petit	60.00	1.57
Tabularia ktenoides Kuylenstierna	40.00	1.21
Toxarium undulatum Bailey	100.00	1.57

Table 1 lists 22 dominant taxa, which were most abundant in the epiphytic diatom assemblage on *C. taxifolia* fronds. They dominated in terms of both frequency of occurrence (> 40%) and RA in the total number of samples (> 1%). When combined together in a single sample, these 22 dominant taxa contribute from 70 to 87% of RA. The following taxa, characteristic of summer and autumn on *C. taxifolia* fronds, occurred in all five samples with an average RA > 1%: *Cocconeis caulerpacola*, *Hyalosynedra laevigata*, *Cocconeis molesta* var. *crucifera*, *Cocconeis scutellum* var. *scutellum*, *Nitzschia lanceolata* var. *minima*, *Nitzschia panduriformis* var. *panduriformis*, *Mastogloia crucicula* var. *crucicula*, *Mastogloia crucicula* var. *alternans*, *Toxarium undulatum*, *Ardissonea fulgens* and *Nitzschia angularis*.

Colonization of diatoms on *C. taxiofolia* fronds in summer indicated, however, that taxa of the genus *Mastogloia* belong to the dominant taxa in the fouling community. In July and August 2010, *Mastogloia* spp. occurred with high RA (> 20%) and with a large number of taxa, thus contributing to the high value of the Shannon–Wiener Diversity Index. Of the 19 *Mastogloia* taxa identified in July 2010, the most abundant was *Mastogloia crucicula* var. *crucicula*, followed by *Mastogloia crucicula* var. *alternans* and *Mastogloia cuneata* (Table 2, Fig. 5). Although *Mastogloia binotata* occurred in all five samples of *Caulerpa taxifolia* fronds, with the highest RA (1%) observed in July, the average abundance was only 0.6% and therefore *M. binotata* is not included on the list of the dominant taxa of *C. taxifolia* fronds. In June 2010, only three taxa of *Mastogloia*, i.e. *M. crucicula* var. *crucicula*, *M. crucicula* var. *alternans* and *M. cuneata*, together accounted for 73% of the total abundance of *Mastogloia* taxa, thus contributing to the low value of the Shannon–Wiener Diversity Index.

Table 2

Occurrence of different taxa from the genus *Mastogloia* on *Caulerpa taxifolia* fronds from June to October 2010 ("+" indicates the presence and " " the absence of a taxon in the sample)

Mastogloia species:	June 2010	July 2010	August 2010	September 2010	October 2010
Mastogloia baldjikiana Grunow			+		
Mastogloia binotata (Grunow) Cleve	+	+	+	+	+
Mastogloia borneensis Hustedt			+	+	
Mastogloia cf. *cyclops* Voigt				+	
Mastogloia cf. *laminaris* Grunow				+	+
Mastogloia cf. *varians* Hustedt				+	
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 delicatissima Hustedt			+		
Mastogloia emarginata Hustedt		+		+	+
Mastogloia erythraea Grunow var. *erythraea*		+			
Mastogloia fimbriata (Brightwell) Cleve		+	+	+	
Mastogloia hovarthiana Grunow	+	+	+	+	
Mastogloia ignorata Hustedt		+	+	+	
Mastogloia inaequalis Cleve	+				
Mastogloia linearis Simonsen	+				
Mastogloia pisciculus Cleve				+	
Mastogloia pseudoexigua Cholnoky		+			
Mastogloia pseudolatecostata Yohn & Gibson		+	+	+	+
Mastogloia pumila (Grunow) Cleve		+			
Mastogloia pusilla (Grunow) Cleve var. *pusilla*		+	+	+	+
Mastogloia regula Hustedt		+			
Mastogloia similis Hustedt		+	+	+	
Mastogloia spec. 1 (Ico.Diat.Vol.7, Pl. 75 Fig. 7-9)	+	+	+	+	
Mastogloia spec. 2 (Ico.Diat.Vol.7, Pl. 82 Fig. 13, 14)			+		
TOTAL :	8	19	18	17	10

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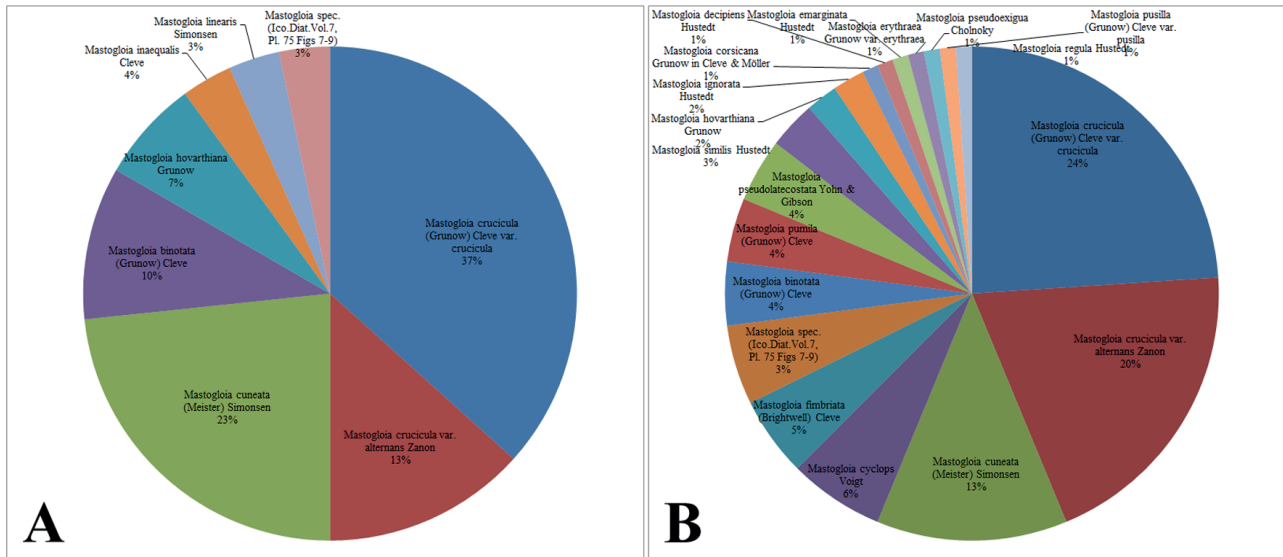
Figure 5

A) Relative percentage contribution of individual *Mastogloia* taxa on *Caulerpa taxifolia* fronds in June 2010. B) Relative percentage contribution of individual *Mastogloia* taxa on *Caulerpa taxifolia* fronds in July 2010

The distribution of the relative abundance of the dominant genera over time showed that the majority of these genera reached the high RA in July and August (Figs 6a, 7). The exception was *Cocconeis* with the lowest RA in July and August and the highest value in October (Fig. 6c). The average abundance of *Hyalosynedra* declined from 25% in June to 8% in October, whereas *Amphora* showed an increase in the abundance at the beginning of autumn. No significant difference in RA of *Navicula* taxa was observed during this study period.

The analysis of the succession of diatoms colonizing *C. taxiofolia* fronds in summer and autumn by LM and SEM (Fig. 8) showed that colonial tube dwelling forms, e.g. *Berkeleya* taxa, belonged to the dominant taxa of the fouling community. In the case of the *Berkeleya* taxa, an interesting switch in the species composition was observed in August and September. The relative abundance of *Berkeleya scopulorum*, initially the only fouling component representing *Berkeleya*, decreased in late summer and was replaced by *Berkeleya rutilans* in early autumn.

The most striking differences in the species composition of the epiphytic diatom community were observed between invasive *C. taxifolia* on the one hand and the autochthonous green and brown macroalgae on the other (Fig. 9). The cluster analysis performed on the species RA data revealed two groups of significantly different assemblages of epiphytic diatoms inhabiting different hosts (group 1 – *Halimeda tuna* and *Padina* sp. and group 2 – *Caulerpa taxifolia*).

The similarity between the two groups, one group consisting of samples of invasive *Caulerpa* and the other group consisting of autochthonous algae, was less than 40%.

As revealed by the cluster analysis, the type of macroalgae has a greater impact on the diatom community structure than monthly changes.
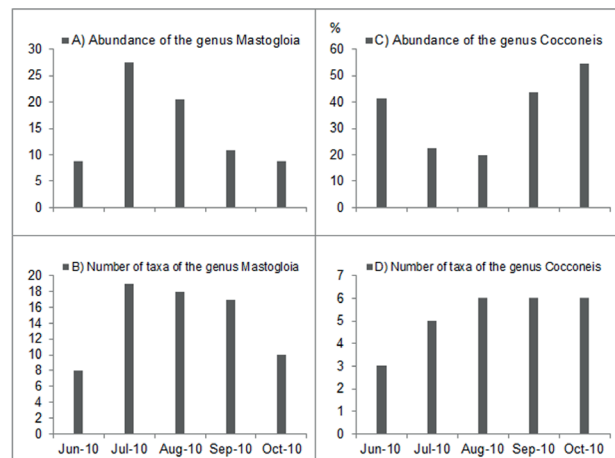
Figure 6

A) Abundance (%) of *Mastogloia* taxa on *Caulerpa taxifolia* fronds from June to October 2010. B) The number of *Mastogloia* taxa on *Caulerpa taxifolia* fronds. C) Abundance (%) of the genus *Cocconeis* on *Caulerpa taxifolia* fronds from June to October 2010. D) The number of *Cocconeis* taxa on *Caulerpa taxifolia* fronds
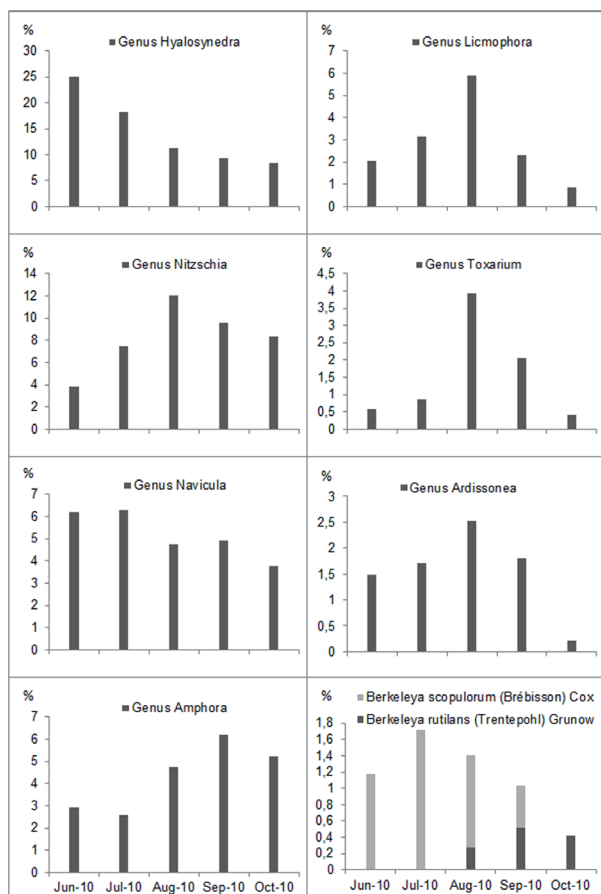
Figure 7

Abundance (%) of the genera *Hyalosynedra, Nitzschia, Navicula, Amphora, Licmophora, Toxarium, Ardissonea* and *Berkeleya* taxa on *Caulerpa taxifolia* fronds from June to October 2010

According to the cluster analysis, the shorter initial period (June–July) was clearly separated from the later sampling (August–September–October) for invasive *C. taxifolia* and *Padina* sp. No separation related to the month of sampling has been demonstrated for *H. tuna*.

In addition to clusters 1 and 2, SIMPROF analysis also distinguished sub-clusters A, B, C, and D. Sub-cluster A contained diatom samples of *H. tuna*, sub-cluster B contained samples of *Padina* sp. Cluster 2 contained *C. taxifolia* samples from June and July (sub-cluster C) and samples from August, September and October (sub-cluster D). The highest similarity was observed between samples of *Padina* sp. from August, September and October.

The SIMPER analysis identified the group of taxa contributing the most (cumulatively 51%) to the variance between invasive and autochthonous algae: *Cocconeis caulerpacola, Navicula ramosissima, Hyalosynedra laevigata, Nitzschia fusiformis, Cocconeis molesta* var. *crucifera, Cocconeis scutellum* var. *scutellum, Cocconeis* cf. *scutellum, Nitzschia angularis, Navicula arenaria* var. *arenaria, Mastogloia decipiens* and *Mastogloia pusilla* var. *pusilla* (Table 3).

Altogether, 158 taxa from 46 genera were identified as epiphytes on the investigated brown macroalga *Padina* sp. between June and October 2010. Genera with the largest number of taxa were *Mastogloia* (39), *Amphora* (21), *Nitzschia* (13), *Diploneis* (12), *Licmophora* (7) and *Cocconeis* (7). The diatom assemblage of the brown alga was characterized by high RA of taxa belonging to *Mastogloia* (Table 4). Of the 30 dominant taxa, 12 taxa represented *Mastogloia*. The average abundance of *M. binotata, M. crucicula* var. *alternans, M. cuneata, M. decipiens, M. ignorata, M. pseudolatecostata* and *M. pusilla* var.

Table 3

SIMPER analysis of diatom taxa contributing (% cumulative = 51%) to dissimilarities between invasive *C. taxifolia* and coexisting autochthonous (*Padina* sp. + *Halimeda tuna*) macroalgae

Taxa	Invasive alga Av. Abund	Autochthonous algae Av. Abund	Av. Diss	Contrib%	Cum.%
Cocconeis caulerpacola Witkowski, Car & Dobosz	16.31	0.03	8.23	11.87	11.87
Navicula ramosissima (Agardh) Cleve	0.93	11.45	5.30	7.64	19.51
Hyalosynedra laevigata (Grunow) Williams & Round	14.44	5.75	4.36	6.29	25.80
Nitzschia fusiformis Grunow	0.33	6.85	3.31	4.77	30.57
Cocconeis molesta var. *crucifera* Grunow in Van Heurck	9.01	3.27	3.16	4.56	35.13
Cocconeis scutellum Ehrenberg var. *scutellum*	5.47	6.16	3.00	4.33	39.46
Cocconeis cf. *scutellum* Ehrenberg	5.00	0.00	2.53	3.65	43.11
Nitzschia angularis W. Smith	1.37	4.96	1.82	2.63	45.74
Navicula arenaria Donkin var. *arenaria*	2.70	0.16	1.31	1.89	47.63
Mastogloia decipiens Hustedt	0.16	2.34	1.16	1.67	49.30
Mastogloia pusilla (Grunow) Cleve var. *pusilla*	0.35	2.50	1.13	1.64	50.94

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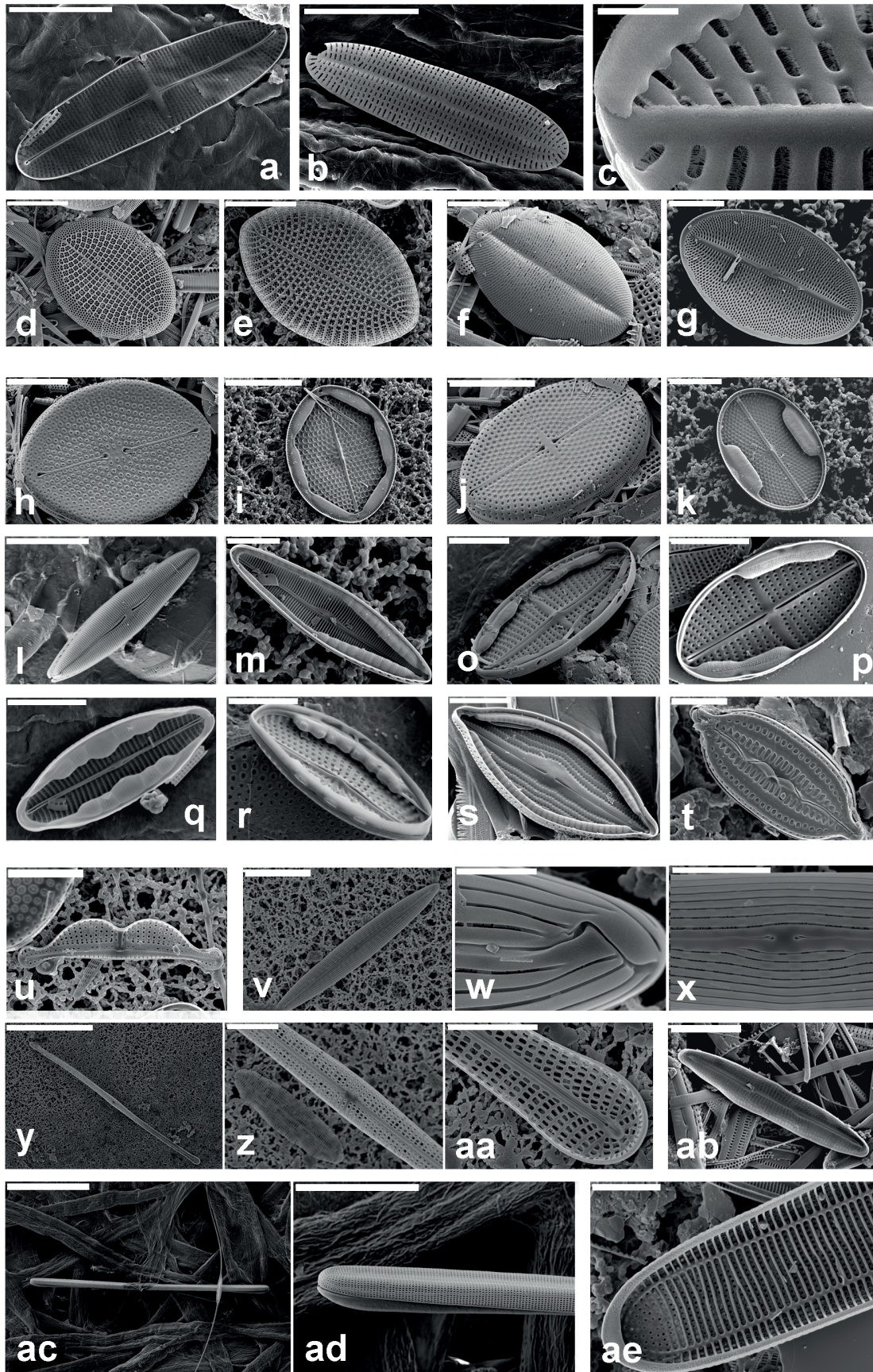
Figure 8

Scanning electron microscope (SEM) micrographs of epiphytic diatoms on the invasive *Caulerpa taxifolia* and autochthonous *Halimeda tuna* and *Padina* sp. macroalgae from the Adriatic Sea. Figs a–c. *Cocconeis caulerpacola* Witkowski, Car & Dobosz. Figs d, e. *Cocconeis scutellum* Ehrenberg var. *scutellum*, sternum valves. Fig. d. External view. Fig. e. Internal view. Figs f, g. *Cocconeis molesta* var. *crucifera* Grunow in Van Heurck, Sternum valves. Fig. f. External view; Fig. g. Internal view. Figs h, i. *Mastogloia fimbriata* (Brightwell) Cleve. Fig. h. External valve view. Fig. i. Internal valve view. Figs j, k. *Mastogloia binotata* (Grunow) Cleve. Fig. j. External valve view. Fig. k. Internal valve view. Figs l, m. *Mastogloia cuneata* (Meister) Simonsen. Fig. l. External valve view. Fig. m. Internal valve view. Fig. o. *Mastogloia crucicula* (Grunow) Cleve var. *crucicula*; internal view. Fig. p. *Mastogloia crucicula* var. *alternans*; internal view. Fig. q. *Mastogloia pumila* (Grunow) Cleve; internal view. Fig r. *Mastogloia ovalis* A. Schmidt; internal view. Fig. s. *Mastogloia cyclops* Voigt; internal valve view. Fig. t. *Mastogloia corsicana* Grunow in Cleve & Möller; external valve view. Fig. u. *Amphora kolbei* Aleem; internal view. Figs v, w, x. *Navicula subagnita* Proshkina-Lavrenko. Figs y, z, aa. *Berkeleya scopulorum* (Brébisson) Cox; internal views. Fig. ab *Berkeleya rutilans* (Trentepohl) Grunow; internal view. Figs ac, ad. *Ardissonea fulgens* (Greville) Grunow (SEM); external views. Fig. ae. *Ardissonea crystallina* (C.A. Agardh) Grunow; internal view. Scale bars = 100 μm (Fig. ac); 50 μm (Fig. y); 30 μm (Fig. ad); 20 μm (fig. i); 10 μm (Figs d, e, h, j, k, l, s, v); 5 μm (Figs a, b, f, g, m, o, p, q, r, t, u, z, ab, ae); 4 μm (Fig. aa); 3 μm (Fig. x); 1 μm (Fig. w); 500 nm (Fig. c)

Table 4

Dominant diatom taxa on *Padina* sp. with average relative abundance (Avg. RA) > 1% and frequency of occurrence (Freq.) > 20% in summer and autumn 2010. N = 5

Padina sp. – dominant taxa:	Freq. (%)	Avg. RA (%)
Amphora helenensis Giffen	100.00	1.54
Ardissonea crystallina (C.A. Agardh) Grunow	80.00	1.34
Auricula sp. 1	20.00	1.93
Berkeleya rutilans (Trentepohl) Grunow	100.00	2.22
Berkeleya scopulorum (Brébisson) Cox	100.00	1.66
Cocconeis molesta var. *crucifera* Grunow in Van Heurck	100.00	3.17
Cocconeis scutellum Ehrenberg var. *scutellum*	100.00	2.12
Cyclophora tenuis Castracane 1878	40.00	1.12
Hyalosynedra laevigata (Grunow) Williams & Round	100.00	5.20
Mastogloia binotata (Grunow) Cleve	100.00	2.16
Mastogloia corsicana Grunow in Cleve & Möller	100.00	1.05
Mastogloia crucicula (Grunow) Cleve var. crucicula	100.00	1.87
Mastogloia crucicula var. *alternans* Zanon	100.00	2.30
Mastogloia cuneata (Meister) Simonsen	100.00	3.60
Mastogloia decipiens Hustedt	80.00	4.50
Mastogloia ignorata Hustedt	100.00	3.12
Mastogloia inaequalis Cleve	60.00	1.40
Mastogloia ovalis A. Schmidt	80.00	1.17
Mastogloia pseudolatecostata Yohn & Gibson	100.00	2.09
Mastogloia pusilla (Grunow) Cleve var. *pusilla*	100.00	3.16
Mastogloia spec. 1 (Ico.Diat.Vol.7, Pl. 75 Figs 7-9)	100.00	1.50
Navicula ramosissima (Agardh) Cleve	100.00	9.71
Nitzschia angularis W. Smith	100.00	3.81
Nitzschia fusiformis Grunow	100.00	10.41
Nitzschia lanceolata var. *minima* Grunow	100.00	3.14
Nitzschia panduriformis Gregory var. *panduriformis*	100.00	2.51
Nitzschia scalpelliformis Grunow	20.00	1.47
Opephora pacifica (Grunow) Petit	100.00	1.05
Rhopalodia pacifica Krammer	100.00	2.79
Toxarium undulatum Bailey	80.00	1.06

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pusilla was higher than 2%. The average abundance of *M. decipiens* on *Padina* sp. was 4.5%, reaching up to 8.4% in August 2010. The diatom assemblage on *Padina* sp. in June and July 2010 was dominated by *Nitzschia fusiformis* with RA of 13.48% and 26.25%, respectively. In August, September and October, diatom assemblages were dominated by *Navicula ramosissima* with RA of 11%, 7.5% and 13% respectively.

In general, epiphytic diatom assemblages of *Halimeda tuna* are different from those hosted by *Padina* sp. As revealed by the SIMPER analysis, *Cocconeis scutellum* var. *scutellum*, *Nitzschia fusiformis*, *Navicula ramosissima*, *Navicula* sp.1, *Nitzschia angularis*, *Mastogloia decipiens*, *Amphora acutiuscula*, *Mastogloia pusilla* var. *pusilla*, *Mastogloia cuneata*, *Mastogloia ignorata*, *Rhopalodia pacifica*, *Tabularia ktenoides*, *Hyalosynedra laevigata* and *Berkeleya rutilans* contributed the most (cumulatively 51%) to the variance between assemblages (Table 5).

Altogether, 115 diatom taxa representing 34 genera were recorded as epiphytes on *H. tuna* from the Hvar sampling site. *H. tuna* hosted diatom assemblages dominated by taxa belonging to *Mastogloia* (20),

(*C. taxifolia*, *Padina* sp., *Halimeda tuna*). The highest Global R (0.85) value was obtained in the analysis of similarity between diatom communities associated with the type of different macroalgal species (invasive/autochthonous; Table 7). The same test performed on the species RA data indicated that the difference between diatom communities over different sampling seasons was not significant ($p > 0.05$). In terms of growth forms, no significant differences were found between the diatom communities on different macroalgal host species (Table 7).

Canonical Analysis of Principal Coordinates CAP (Fig. 10) showed that the samples from sub-cluster A (*H. tuna*) correlated with *Navicula ramosissima* and *Nitzschia angularis*, samples from sub-cluster B (*Padina* sp.) with taxa of the genus *Mastogloia* (*M. decipiens* and *M. pusilla* var. *pusilla*) together with *Nitzschia fusiformis*, while samples from sub-cluster D (*C. taxifolia*; August, September and October) correlated with the abundance of *Cocconeis caulerpacola* and *Cocconeis* cf. *scutellum*.

Table 5

SIMPER analysis of diatom taxa contributing (% cumulative = 51%) to dissimilarities between *Padina* sp. and *Halimeda tuna* habitats

Taxa	*Padina* sp. Av. Abund	*Halimeda tuna* Av. Abund	Av. Diss	Contrib. %	Cum. %
Cocconeis scutellum Ehrenberg var. *scutellum*	2.12	12.89	5.40	9.65	9.65
Nitzschia fusiformis Grunow	10.41	0.90	4.76	8.50	18.15
Navicula ramosissima (Agardh) Cleve	9.71	14.37	2.60	4.65	22.80
Navicula sp.1	0.00	4.64	2.32	4.15	26.95
Nitzschia angularis W. Smith	3.81	6.86	1.91	3.42	30.37
Mastogloia decipiens Hustedt	3.60	0.24	1.73	3.09	33.46
Amphora acutiuscula Kützing	0.70	3.72	1.51	2.70	36.17
Mastogloia pusilla (Grunow) Cleve var. *pusilla*	3.16	1.39	1.29	2.30	38.47
Mastogloia cuneata (Meister) Simonsen	3.60	1.25	1.28	2.29	40.76
Mastogloia ignorata Hustedt	3.12	0.65	1.27	2.27	43.02
Rhopalodia pacifica Krammer	2.79	0.33	1.27	2.26	45.29
Tabularia ktenoides Kuylenstierna	0.76	2.88	1.09	1.94	47.23
Hyalosynedra laevigata (Grunow) Williams & Round	5.20	6.68	1.02	1.82	49.05
Berkeleya rutilans (Trentepohl) Grunow	2.22	0.50	1.01	1.81	50.87

Amphora (19), *Nitzschia* (13), *Cocconeis* (11) and *Navicula* (8). Of the 30 dominant taxa, six taxa represented *Amphora* (Table 6), of which *Amphora acutiuscula* showed the maximum RA of 6% in July. The diatom assemblage on *H. tuna* in July 2010 was also dominated by *N. ramosissima* and *C. scutellum* var. *scutellum* with RA of 10% and 23%, respectively. In September, diatom assemblages were dominated by the genus *Navicula*.

ANOSIM indicated that the diatom communities differed significantly depending on the substrate

Discussion

Due to the scarcity of information on benthic diatoms in the Adriatic, particularly from the area affected by the invasive macroalga *Caulerpa taxifolia*, the results of this study were compared with the literature data from different seas worldwide and the comparison was not limited to the investigated host (macroalga).

Table 6

Dominant diatom taxa on *Halimeda tuna* with average relative abundance (Avg. RA) > 1% and frequency of occurrence (Freq.) > 33% in summer and autumn 2010. N = 3

Halimeda tuna - dominant taxa:	Freq. (%)	Avg. RA (%)
Amphora acutiuscula Kützing	100.00	3.72
Amphora cf. *marina* Witkowski, Lange-Bertalot & Metzeltin	100.00	2.02
Amphora helenensis Giffen	100.00	1.48
Amphora kolbei Aleem	66.67	1.69
Amphora sp.1	33.33	1.54
Amphora sp. 2	33.33	1.10
Ardissonea crystallina (C.A. Agardh) Grunow	100.00	1.18
Bacillaria socialis (Gregory) Ralfs	33.33	1.28
Berkeleya scopulorum (Brébisson) Cox	33.33	2.56
Cocconeis cf. *krammeri* Lange-Bertalot & Metzeltin	33.33	3.09
Cocconeis costata Gregory var. *costata*	66.67	1.12
Cocconeis molesta var. *crucifera* Grunow in Van Heurck	100.00	3.45
Cocconeis scutellum Ehrenberg var. *scutellum*	100.00	12.89
Cyclophora tenuis Castracane 1878	33.33	2.31
Diploneis vacillans (A. Schmidt) Cleve var. *vacillans*	100.00	1.66
Fragilaria investiens (W. Smith) Cleve-Euler	100.00	2.16
Hyalosynedra laevigata (Grunow) Williams & Round	100.00	6.68
Licmophora remulus Grunow	33.33	1.03
Mastogloia crucicula var. *alternans* Zanon	100.00	2.40
Mastogloia cuneata (Meister) Simonsen	100.00	1.25
Mastogloia pusilla (Grunow) Cleve var. *pusilla*	66.67	2.09
Navicula ramosissima (Agardh) Cleve	100.00	14.37
Navicula sp.1	66.67	6.96
Navicula subagnita Proschkina-Lavrenko	100.00	1.81
Nitzschia angularis W. Smith	100.00	6.86
Nitzschia lanceolata var. *minima* Grunow	66.67	2.99
Nitzschia panduriformis Gregory var. *panduriformis*	100.00	1.34
Nizschia lanceolata var. *minima* Grunow	33.33	1.69
Seminavis sp. 1	100.00	1.12
Tabularia ktenoides Kuylenstierna	100.00	2.88

Table 7

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

|	Substrate (*C. taxifolia*, *Padina* sp., *H. tuna*)		Type of macroalga (invasive/autochthonous)		Months		Season (summer/autumn)		Sea temperature	
	S	GF	S	GF	S	GF	S	GF	S	GF
p	0.001	> 0.05	0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Global R	0.717	0.518	0.850	0.463	−0.251	−0.335	0.049	−0.022	−0.214	−0.138

The data in this study confirmed the expected differences in the taxonomic composition and abundance of epiphytic diatoms among the investigated macroalgae. This may be explained, at least partly, by different physical, chemical and biological requirements of hosts and their ecological conditions.

In this study, *Cocconeis* taxa were the dominant epiphytic diatoms on *C. taxifolia* fronds. This is consistent with the findings of Majewska et al. (2014) for *Posidonia oceanica* leaves in the Mediterranean. In the case of the latter host, *C. scutellum* was the dominant taxon. Tanaka (1986) found that diatom taxa having higher adhesive strength (*Cocconeis*, *Achnanthes*) may be abundant on various macroalgae and tolerate adverse hydrodynamic conditions such as wave action.

In addition, Al-Yamani & Saburova (2011) showed that the epiphytic diatom assemblage on intertidal macroalgae, including *Padina* sp. from Kuwait, was

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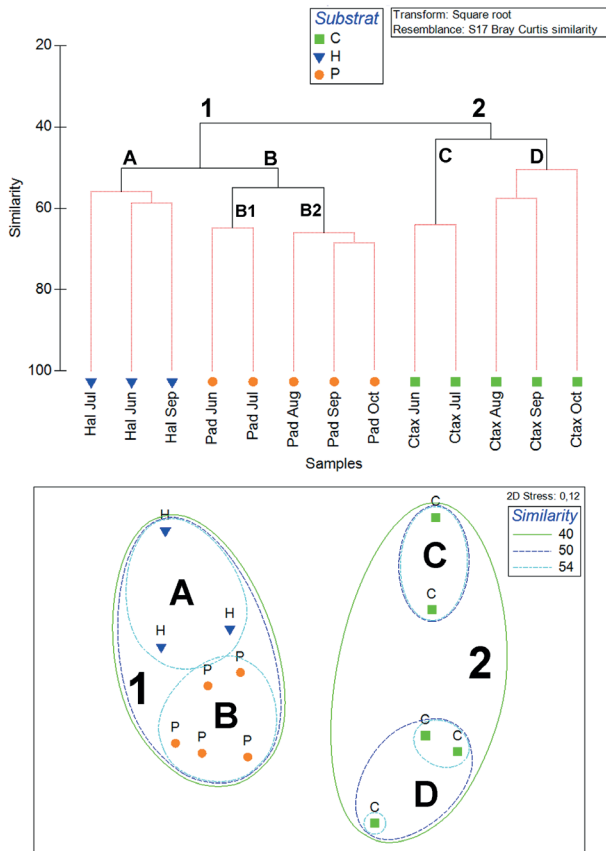
Figure 9

Cluster analysis and multidimensional scaling (MDS) ordination on Bray–Curtis similarity matrices of square root transformed species-abundance data of epiphytic diatom assemblages collected from all three substrates (*Caulerpa taxifolia*, *Halimeda tuna*, *Padina* sp.) during a period of 5 months from June to October 2010 (taxa relative abundance data). All recorded diatom taxa were used in the ordination analysis. Group average similarity values of clusters with significant differences from CLUSTER analysis were superimposed on the MDS plot (SIMPROF; *p* < 0.05). Top: Cluster analysis. Red lines indicate homogeneous clusters of taxa detected by SIMPROF. Bottom: MDS. Numbers correspond to the same main clusters detected by SIMPROF. Letters A, B, C and D indicate sub-clusters within the main clusters. Symbols Ctax (*Caulerpa taxifolia*), Hal (*Halimeda tuna*), Pad (*Padina* sp.); Jun (June); Jul (July); Aug (August); Sep (September); Oct (October). N(Ctax) = 5; N(Hal) = 3; N(Pad) = 5
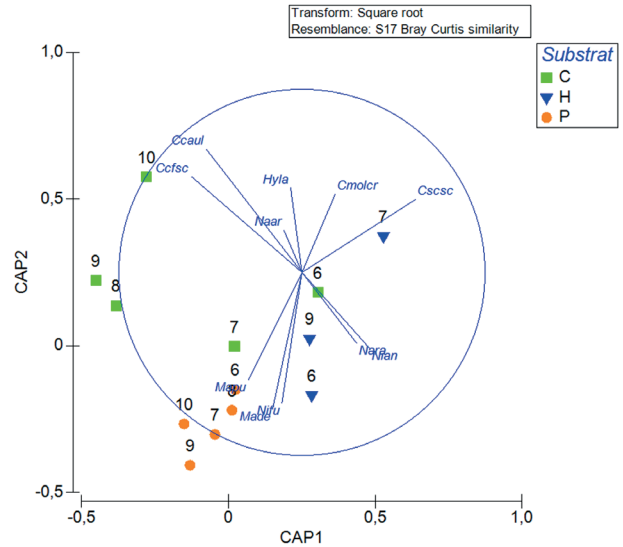
Figure 10

Canonical Analysis of Principal Coordinates (CAP; Primer + PERMANOVA, UK). CAP biplot showing months and vectors of diatom relative abundance (%) data (arrows). The group of 11 diatom taxa, which contribute the most (cumulatively 51%) to the variance between invasive and autochthonous algae, was selected according to SIMPER analysis. Codes for the diatom taxa are as follows:

Ccaul = *Cocconeis caulerpacola* Witkowski, Car & Dobosz

Ccfsc = *Cocconeis* cf. *scutellum* Ehrenberg

Cmolcr = *Cocconeis molesta* var. *crucifera* Grunow in Van Heurck

Cscsc = *Cocconeis scutellum* Ehrenberg var. *scutellum*

Hyla = *Hyalosynedra laevigata* (Grunow) Williams & Round

Made = *Mastogloia decipiens* Hustedt

Mapu = *Mastogloia pusilla* (Grunow) Cleve var. *pusilla*

Naar = *Navicula arenaria* Donkin var. *arenaria*

Nara = Navicula ramosissima (Agardh) Cleve

Nian = *Nitzschia angularis* W. Smith

Nifu = *Nitzschia fusiformis* Grunow

largely dominated by *Cocconeis* taxa and constituted a continuous, almost monospecific layer. In our case, despite the high taxa richness on *Padina* sp., the epiphytic diatom *C. caulerpacola* was absent on specimens of this taxon, while it was frequently found on fronds of *C. taxifolia*.

Epiphytic diatom assemblages on *Padina* sp. were different from those hosted by *Halimeda tuna*. Although this study showed the presence of a smaller number of diatom taxa growing on *H. tuna* compared to *Padina* sp., the number of the analyzed samples

(three on *H. tunavs* and five on *Padina*) could not be ignored. In the case of *H. tuna*, the *Amphora* taxa could be considered as frequent. The diversity of *Mastogloia* (> 39 taxa) on *Padina* was much higher than on the two other macroalgae studied, and with the high frequency of occurrence the taxon significantly contributed to the high value of the Shannon–Wiener Diversity Index.

In this study, the dominant taxa on *C. taxifolia* fronds, in addition to *Mastogloia* taxa, were those characterized by the high motility (e.g. *Navicula* and *Nitzschia*), capable of moving on the substrate to find optimal conditions. Totti et al. (2009) concluded that adnate diatoms, including mostly *Cocconeis* taxa, appear to be more affected by the structure of the host surface and their abundance increases on thalli that offer a more complex microarchitecture for colonization, while they were absent in soft thalli with smooth surface. Monoraphid *Cocconeis* tightly adhere along their raphe, but as organisms capable of slow motility they are also able to migrate to areas with the greatest availability of light and nutrients or the least chance of desiccation due to tidal fluctuations (Mitbavkar & Anil 2004), which are generally the areas that provide the most suitable and productive environmental conditions (Edgar & Pickett-Heaps 1984). The chemical defense of *C. taxifolia* may reduce the settlement rate and the development of sessile organisms in invaded systems (Prado & Thibaut 2008). Preliminary studies of secondary metabolites from macroalgae showed biological activity against epiphytes (Phillips & Towers 1982; de Nys et al. 1991; 1995). Organisms can produce different types and rates of metabolites such as wastes, nutrients and toxins, depending on the season, grazing pressure, developmental stage and biological cycles of individuals. Production and exudation may also vary among different organs of the same individual. Successful colonizers must have either a large tolerance range or settle during phases when – or on organs where – the composition and quantities of exudates are not harmful (Wahl 1989). For the Mediterranean strain of *C. taxifolia*, the maximum concentrations of caulerpenyne were recorded in autumn and the minimum in spring (Dumay et al. 2002). In our case, high summer and autumn values of toxic substances could be the cause of reduced diversity of diatoms and contribution of *C. caulerpacola*. In fact, this can be explained by the targeted chemical defense of macroalgae against particular taxa.

Defense and epibiosis may vary among different macroalgae parts. Jennings and Steinberg (1997) reported the greatest epiphyte abundance on the oldest tissue and the lowest on the youngest one. They also found that the epiphyte abundance was strongly correlated with the age of different parts of the thallus of the sublittoral kelp *Ecklonia radiata* (C.Agardh) J.Agardh. In the case of *C. taxifolia*, despite the fact that it grows nearly 2 cm per day throughout the summer and autumn (Meinesz 2002), it seems that *C. taxifolia* grows simultaneously with *C. caulerpacola.*

The research of Nenadović et al. (2015) on 11 different artificial substrates exposed to the marine environment in the eastern Middle Adriatic in spring and on shoots of *P. oceanica* showed differences in quantitative and qualitative composition of diatoms among newly colonized surfaces and implied the preference of diatoms for specific substrates. *Nitzschia*, *Cocconeis* and *Navicula* taxa, which can be considered pioneer epiphytic diatoms regardless of the colonized substrate (Railkin 2004; Nenadović et al. 2015), were frequently found on all substrates alongside the dominant diatom taxa, *Cylindrotheca closterium* and *Amphora*. Our results are consistent with those of Nenadović et al. (2015) and Navarro et al. (1989), who also found abundant taxa of the genera *Mastogloia*, *Navicula* and *Nitzschia* on diverse marine substrates (organic or inorganic) in the tropical region of Puerto Rico. In addition, research on *P. oceanica* leaves showed that the diatom community structure is also affected by the seasonal cycle of the plant, the depth of the meadow, the age of leaves, and the grazing pressure exerted by herbivores (Mazzella 1983; Mazzella & Spinoccia 1992; Mazzella et al. 1994; De Stefano et al. 2000).

The results of the presented study clearly showed differences in the diatom species distribution pattern between the selected autochthonous macroalgae and invasive *C. taxifolia*. This may be explained, at least partly, by varying thallus architecture of the studied species, given that *Padina* sp. and *H. tuna* have calcified thalli. In addition, multiple environmental factors reflecting the seasonal and site-specific conditions could also be manifested in the structure and species composition of algal communities. For this reason, the results of the present study imply only that an epiphytic diatom community is affected by macroalgae, especially those containing toxins. The lack of samples of younger parts cut from indigenous algae does not allow us to draw definite conclusions about succession and seasonal specificity or distribution of epiphytic diatoms on macroalgal hosts. It can be assumed that differences between invasive and autochthonous algae would also be noticeable at an early stage of life and exposure of macroalgae to benthic diatoms. Obviously, in order to increase the accuracy of predictions, further long-term

studies of dynamics of epiphytic diatoms on different macroalgae and other substrates, as well as effects of various environmental factors on the growth of epiphytic diatoms are necessary.

In summary, this study presents the epiphytic diatom species composition on the invasive macroalga *Caulerpa taxifolia* distributed along the coast of Croatia and the effects of lifespan and seasons on diatom assemblages. The diatom community on *C. taxifolia* fronds is dominated by *Cocconeis* taxa. The succession of diatom assemblages showed remarkable variations over the months of summer and autumn. We confirmed the hypothesis of host specificity of *Cocconeis caulerpacola* in relation to *Caulerpa* by showing the total absence of *C. caulerpacola* on *Halimeda tuna* and *Padina* sp. collected at the same time and at the same sites as in the case of the invasive *Caulerpa*. Knowledge of the diatom community structure in these areas is important for studies of toxic effects of the host and enables a better understanding of the functioning of ecosystems dominated by invasive *C. taxifolia*.

Acknowledgements

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