NAT. CROAT. | VOL. 28 | No 1 | 1-20 | ZAGREB | June 30, 2019

original scientific paper / izvorni znanstveni rad

DOI 10.20302/NC.2019.28.1

EPIPHYTIC DIATOMS ON *POSIDONIA OCEANICA* (L.) DELILE LEAVES FROM EASTERN ADRIATIC SEA

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Kanjer, L., Mucko, M., Car, A. & Bosak, S.: Epiphytic diatoms on *Posidonia oceanica* (L.) Delile leaves from eastern Adriatic Sea. Nat. Croat. Vol. 28, No. 1., 1-20, Zagreb, 2019.

This research provides a description of the epiphytic diatom community living on the blades of the marine seagrass *Posidonia oceanica* in the coastal area of the eastern Adriatic Sea. Samples were collected in May 2017, from a meadow located in Tetevišćica Bay, on the western side of Dugi otok island (43° 58′ 22′′ N; 15° 03′ 36′′ E) at three depths: 10, 15 and 20 m. Each blade sample was divided into three segments along the leaf axis (basal meristemal, middle and apical) to explore the possible community zonation pattern. Twenty-one samples were analyzed by light microscope and the epiphytic diatom community structure was determined. A total of 68 taxa belonging to 30 genera were found: 43 from 10 m depth, 41 from 15 m depth and 39 from 20 m depth. All identified species are commonly recorded from other marine benthic periphytic and epiphytic habitats. The genus *Cocconeis* was dominant in all samples, which is typical for epiphytic biofouling of *P. oceanica* leaves. Although no changes in the community structure related to the position on *P. oceanica* blade were observed, ANOSIM tests ($p<0.05$) performed on species' relative abundances confirmed that the diatom communities differed significantly according to their depths. This study contributed to the knowledge on biodiversity associated with Adriatic *P. oceanica* meadows, which are threatened by human activities and invasive species. Additionally, this research provided important information on the identification of the taxa-specific epiphytic diatom community on seagrasses in the eastern Adriatic Sea.

Keywords: Bacillariophyta, Neptune grass, Mediterranean Sea, *Cocconeis*, species identification, light microscopy

Kanjer, L., Mucko, M., Car, A. & Bosak, S.: Epifitske alge kremenjašice na listovima *Posidonia oceanica* (L.) Delile u istočnom Jadranu. Nat. Croat. Vol. 28, No. 1., 1-20, Zagreb, 2019.

U ovom je radu istraživan epifitski obraštaj algi kremenjašica (dijatomeja) na listovima morske cvjetnice *Posidonia oceanica* u obalnom području istočnog Jadrana. Uzorci su sakupljeni u svibnju 2017, s livade morske cvjetnice u uvali Tetevišćica (43° 58′ 22′′ N; 15° 03′ 36′′ E) koja se nalazi na zapadnoj obali Dugog Otoka s ukupno tri dubine: 10, 15 i 20 m. Svaki uzorak lista podijeljen je duž lisne osinatri dijela: bazalni, srednji te apikalni dio. Analiziran je 21 uzorak pomoću svjetlosnog mikroskopa te određena struktura epifitskih dijatomejskih zajednica. Identificirano je ukupno 68 svojti podijeljenih u 30 rodova, 43 svojte s dubine 10 m, 41 s dubine 15 m i 39 s dubine 20 m. Sve zabilježene svojte su tipične za morska bentička, perifitonska i epifitska staništa. Rod *Cocconeis* bio je dominantan u zajednicama na svim dubinama, što je i karakteristika epifitskih obraštaja na listovima *P. oceanica*. Unatoč tome što nije zabilježena razlika u zajednicama dijatomeja s obzirom na položaj na listu, ANOSIM test ($p<0.05$) na temelju relativne brojnosti vrsta potvrdio je da se zajednice dijatomeja značajno razlikuju između dubina. Ovo istraživanje doprinosi poznavanju bioraznolikosti jadranskih naselja *P. oceanica* koje su trenutno ugrožene ljudskim aktivnostima i širenjem invazivnih vrsta. Također, ovo je istraživanje važno za poznavanje zajednice epifitskih dijatomeja na morskim cvjetnicama u Jadranskom moru.

Ključne riječi: Bacillariophyta, oceanski porost, Mediteransko more, *Cocconeis*, identifikacija vrsta, svjetlosna mikroskopija

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INTRODUCTION

Five species of seagrasses can be found in the Adriatic Sea, four species native to European waters (Borum & Greve, 2004): *Posidonia oceanica* (L.) Delile, *Zostera marina* L., *Zostera noltei* Hornemann, *Cymodocea nodosa* (Ucria) Ascherson, and one Mediterranean alien species *Halophila stipulacea* (Forssk.) Asch (Kashta & Pizzuto, 1995). Seagrass meadows are ecologically and economically of extreme importance to marine coastal environments around the world (Ruiz-Frau *et al.*, 2017). The most important meadows in the Adriatic Sea are formed by the species *Posidonia oceanica* (Neptune grass), an endemic Mediterranean species that occurs along the eastern part (the coastline of Slovenia, Croatia, Montenegro and Albania) as well as along the south-western Italian coast, with a small patch recorded in the north-west (Telesca *et al.*, 2015). *Posidonia oceanica* grows from shallow waters down to 50 – 60 m of depth in areas with very clear waters, which is much deeper than other seagrass species (Borum & Greve, 2004). The meadow can occupy large areas, displaying high biomass and productivity, preserving coastal areas from sediment erosion and building shelters, feeding areas, hatchery and nursery for various marine organisms. Although they have big impact on coastal ecosystems, these beds are endangered by human activities (trawlers, anchoring, coastal development etc.) and the introduction of invasive species such as green algae *Caulerpa cylindracea* Sonder and *Caulerpa taxifolia* (M.Vahl) C.Agardh that can grow and spread very fast over the *P. oceanica* meadows. Because of its importance, *P. oceanica* is a strictly protected species in Croatia (Bakran-Petricioli, 2011).

Posidonia oceanica is a very slow growing species that forms thick meadows with an average leaf lifespan of approximately one year (Gobert *et al.*, 2006), 50 days being needed for a leaf to grow to its full length (Duarte, 1991; Marba *et al.*, 2004). *Posidonia oceanica* leaves are a suitable habitat for diverse epizoic organisms: bacteria, micro- and macroalgae, and invertebrates such as bryozoans, annelids or sponges (Borowitzka *et al.*, 2006). Various organisms associate with *P. oceanica* leaves for numerous reasons including the provision of shelter, illumination, or constant oxygen and sugar flow as a product of seagrass photosynthesis reactions (Ugarelli *et al.*, 2017). One of the important microalgae groups in *P. oceanica* biofilms are diatoms (Orth *et al.*, 1982). Biofouling on seagrasses is the main food source for higher trophic levels in these ecosystems because herbivores rarely consume leaves of *P. oceanica* (Mazzella & Spinoccia, 1982).

Among epiphytic diatoms, the communities on *P. oceanica* are some of the most investigated (e.g. Mazzella, 1983; Novak, 1984; Mazzella & Spinoccia, 1992; Mazzella *et al.*, 1994; De Stefano *et al.*, 2000; Majewska *et al.*, 2014). The research by Car *et al.* (2012) into epiphytic diatom assemblages within meadows of *P. oceanica* was focused on the diatom community on the thalli of invasive *Caulerpa* species that are out-competing native seagrasses in the Mediterranean. In addition, although there have been numerous studies on the role of *P. oceanica* as a habitat for epiphytic diatoms, most of them have been focused on the composition of the epiphytic diatoms (Mazzella, 1983; Mazzella *et al.*, 1994; De Stefano *et al.*, 2000; Majewska *et al.*, 2014, Car *et al.* 2012), while zonation along the leaves has received scarce attention (Novak, 1984; Mazzella & Spinoccia, 1992); no such study has ever been conducted in the eastern Adriatic Sea area. Previous studies have shown that diatom community structure and abundance depend on the position on the leaf that is associated with the leaf age. The younger, meristematic part of the leaf is near the rhizome and the apex is the older part of the leaf (Mazzella & Spinoccia, 1992). Salinity and nutrients also play a role in shaping the diatom community (Frankovich & Wachnicka, 2015).

Because of the anthropogenic destruction of *P. oceanica* meadows in the Adriatic Sea, it is of the utmost importance to understand these ecosystems in their full complexity, for only then can we try to truly protect them (Ugarelli *et al.*, 2017). The aim of this study was to compare diatom communities growing on different parts of *P. oceanica* leaves collected from different depths of the same meadow. We expected the taxonomic composition of epiphytic diatoms communities to be more diverse in the apical parts than in the basal part of the leaves, thus confirming the results obtained by previous studies (e.g. Mazzella & Spinoccia, 1992). These authors found that the crucial factor in structuring the diatom community is the position along the leaf blade of *P. oceanica* that represents the age gradient. Additionally, we tested the hypothesis that diatom communities would differ depending on the sampling depth.

MATERIALS AND METHODS

Samples of *P. oceanica* leaves were collected on 10th May 2017 by scuba divers at one location in Teteviščica Bay (43° 58′ 22′′ N; 15° 03′ 36′′ E) on the western side of Dugi Otok island situated in the northern part of the eastern Adriatic Sea, Croatia (Fig. 1). A total of 9 leaves were collected from one sampling site (one meadow at three different depths: 10, 15, and 20 m, for each depth, 3 different leaf samples

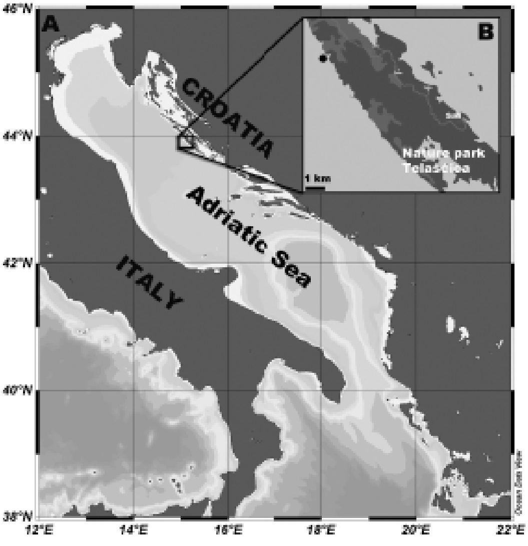

Fig. 1. Map of the study area. A) Location of sampling site in Adriatic Sea; B) Close up map of Teteviščica Bay, western side of Dugi otok. The exact position of sampling location is indicated with black dot (Ocean Data View; https://odv.awi.de).

(Tab. 1). *Posidonia oceanica* leaves were cut with scissors underwater: the complete leaf from its base to its apex was collected and put into a 50 mL Falcon tube filled with seawater. Immediately after collection, samples were preserved with 4% formaldehyde (final concentration) and kept at 4°C upon arrival at the laboratory.

Tab. 1. List of subsamples collected in Teteviščica Bay on 10th of May 2017 at three different depths in *Posidonia oceanica* meadow; Y – young (basal) leaf part, M – middle leaf part, O – old (apical) leaf part. n.o. (not observed) designates subsamples without diatom frustules.

Depth	*Posidonia oceanica* leaf age			Number of samples
	Basal (Y)	Middle (M)	Apical (O)	
10 m	X	X	X	7
	n.o.	X	X	
	n.o.	X	X	
15 m	X	X	X	9
	X	X	X	
	X	X	X	
20 m	X	n.o.	X	5
	n.o.	n.o.	X	
	X	n.o.	X	
Number of samples	6	6	9	21

Subsequently, each leaf sample was cut into three subsamples (area between 2–5 cm^2): a basal, middle and apical part (Fig. 2); in total 21 subsamples (Tab. 1). In order to remove the organic matter from diatom frustules, each subsample was treated with 5 mL of saturated $KMnO_4$ solution, left overnight, to which was added 5 mL of HCl (37%) and heated on a spirit lamp for several seconds. All subsamples were then washed with dH_2O and sedimented in a centrifuge (2000 rpm, 10 min) at least 5 times to remove the remaining acid. Permanent slides were prepared by pipetting the clean material on glass coverslips, air-drying and mounted in Naphrax (Brunel Microscopes Ltd., UK).

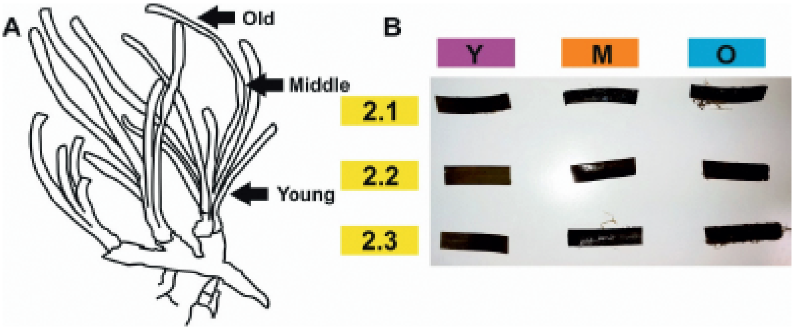

Fig. 2. A) Scheme of *Posidonia oceanica,* adapted and redrawn from Borum J. & Grevet T.M. (2004); B) Samples of P. oceanica from the depth of 15 m: 2.1, 2.2 and 2.3 are sample's ID, Y – the youngest (basal) part of the leaf, M – the middle part of the leaf, O – the oldest (apical) part of the leaf.

A light microscope (LM), Olympus BX51 (Olympus Optical, Tokyo, Japan), was used for species identification and counting diatom valves. Due to sampling and methodology problems, some subsamples did not contain any diatom frustules and are therefore left out of the statistical analysis (Tab. 1). Diatoms were identified to species level when possible, otherwise, the identification was made at the genus level. The whole surface of a slide was examined at 1000× magnification. Quantitative analysis was done by counting at least 400 valves in linear transect on each permanent slide. The abundances of the species were expressed as relative abundances (RA in %) of at least 400 diatom valves counted per sample. Taxa were identified following Alvarez-Blanco & Blanco (2014), Witkowski *et al*., (2000) and Al-Yamani & Saburova (2011). Nomenclature of recorded taxa follows AlgaeBase (Guiry & Guiry, 2018). Permanent slides and prepared material have been deposited in the diatom collection of Department of Biology, Faculty of Science, University of Zagreb, Zagreb (Croatia).

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

Shannon-Wiener Diversity Index (H’) and Pielou’s evenness index (J’) were calculated for each diatom sample to investigate the diatom community diversity and structure (Krebs, 1999; Pielou, 1966). Raw diatom counts expressed as relative abundance were square root transformed and resemblance matrix of the data was generated using Bray Curtis analysis. The Bray-Curtis similarity matrix of the relative abundance data of 68 taxa over 21 samples was constructed. Hierarchical clustering, CLUSTER, (using the group average mode and the SIMPROF test for significance) and multidimensional scaling (MDS) analyses were used to display the difference in communities associated with the sampling depths and leaf age. A SIMPROF test that highlights significantly ($p < 0.05$) different groups was superimposed on the MDS.

Analysis of similarities (ANOSIM) was used to determine whether there were significant differences in the diatom community at the selected depths and *Posidonia* leaf segments representing leaf age. A dissimilarity percentage analysis (SIMPER, Clarke & Warwick, 1994) was used to identify the taxa making the greatest contribution to the differences at selected depths.

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

RESULTS

Altogether, 68 taxa belonging to 30 genera were identified from the epiphytic assemblage on *Posidonia oceanica* leaves (Tab. 2). Among them, 43, 41 and 39 taxa were found on leaves collected at 10, 15 and 20 m, respectively. The most common genera (found in >50% samples with relative abundances >1%) were *Cocconeis* Ehrenberg (Fig. 6a-d), *Mastogloia* Thwaites ex W. Smith (Fig. 7a), *Navicula* Bory (Fig. 7b), *Grammatophora* Ehrenberg (Fig. 8d and e), *Licmophora* C. Agardh (Fig. 8c) and *Toxarium* Bailey. Relative abundances of the most common genera are shown in Fig. 3.

Tab. 2. Species list and relative abundances of identified taxa on *Posidonia oceanica* leaf samples; Y – young (basal) leaf part, M – middle leaf part, O – older (apical) leaf part; 1,2,3 – sample number.

#	Taxon	10 m							15 m									20 m				
		Y1	**M1**	**O1**	**M2**	**O2**	**M3**	**O3**	**Y1**	**M1**	**O1**	**Y2**	**M2**	**O2**	**Y3**	**M3**	**O3**	**Y1**	**O1**	**O2**	**Y3**	**O3**
1	*Actinocyclus* spp.													<1%								
2	*Amphora graeffeana* Hendey			1%																		
3	*Amphora* spp.	<1%	1%	1%				<1%	<1%	<1%					<1%							
4	*Ardissonea crystalina var. dalmatica* (Kützing) Mills		<1%			1%		2%			<1%										<1%	1%
5	*Ardissonea formosa* (Hantzsch) Grunow	<1%	2%		<1%		<1%	<1%													1%	1%
6	*Bidulphia* sp.								<1%													
7	*Brachysira aponina* Kützing										<1%	40%										
8	*Campylodiscus neofastuosus* Ruck & Nakov							<1%														
9	*Cocconeis britannica* Naegeli	20%	3%	4%	1%	11%	3%	3%	26%	5%	2%	3%	4%	<1%	7%	3%	<1%	5%	1%	<1%	3%	<1%
10	*Cocconeis krammeri* Lange-Bertalot & Metzeltinmeri	3%		5%			<1%		<1%			1%	<1%	3%	15%	13%	9%	3%	1%	9%	4%	1%
11	*Cocconeis neothumensis* var. *marina* De Stefano, Marino, & Mazzella	1%		22%		<1%	4%	5%	3%	5%	12%	1%	4%	12%	25%	33%	4%	4%		2%	43%	29%
12	*Cocconeis peltoides* Hustedt						<1%									<1%						
13	*Cocconeis scutellum* Ehrenberg	28%	34%	36%	62%	26%	44%	32%	31%	75%	39%	21%	42%	53%	45%	44%	61%	32%	49%	6%	15%	17%
14	*Cocconeis* sp. 1										25%											
15	*Cocconeis* sp. 2																	46%	23%	1%	5%	3%
16	*Cocconeis* sp. 3								15%						<1%							
17	*Cocconeis* spp.	22%	14%		31%	19%	35%	11%	15%	6%			45%	26%								
18	*Cyclotella* spp.					1%		<1%				4%										

Tab. 2. Species list and relative abundances of identified taxa on *Posidonia oceanica* leaf samples; Y – young (basal) leaf part, M – middle leaf part, O – older (apical) leaf part; 1,2,3 – sample number (continued).

#	Taxon	10 m							15 m									20 m				
		Y1	M1	O1	M2	O2	M3	O3	Y1	M1	O1	Y2	M2	O2	Y3	M3	O3	Y1	O1	O2	Y3	O3
20	*Diploneis aestuari* Hustedt		<1%																			
21	*Diploneis bombus* (Ehrenberg) Ehrenberg		1%	1%		1%					<1%											
22	*Diploneis incurvata* (Gregory) Cleve					<1%																
23	*Diploneis smithii* (Brébisson) Cleve																				<1%	
24	*Diploneis* spp.	<1%						1%	1%										<1%	<1%		<1%
25	*Grammatophora macilenta* Smith				<1%																	
26	*Grammatophora marina* (Lyngbye) Kützing	6%	16%	6%	3%	19%	<1%	6%	1%		1%		1%		<1%	<1%			<1%			
27	*Grammatophora oceanica* Ehrenberg	8%	5%	2%	1%		1%	23%	1%	2%	7%		1%	2%	<1%		4%	1%	3%	14%	2%	4%
28	*Gyrosigma* sp.								<1%													
29	*Halamphora cejudoae* Álvarez-Blanco & Blanco			<1%																		
30	*Halamphora cymbifera* (Gregory) Levkov				<1%		1%															
31	*Halamphora* spp.			1%		<1%		<1%	1%					1%	<1%	1%	<1%			1%	3%	1%
32	*Hyalosira interrupta* (Ehrenberg) J.N.Navarro					5%		1%														
33	*Licmophora abbreviata* C. Agardh																			<1%		
34	*Licmophora dalmatica* (Kützing) Grunow																			1%	6%	16%

Tab. 2. Species list and relative abundances of identified taxa on *Posidonia oceanica* leaf samples; Y – young (basal) leaf part, M – middle leaf part, O – older (apical) leaf part; 1,2,3 – sample number (continued).

#	Taxon	10 m							15 m									20 m				
		Y1	M1	O1	M2	O2	M3	O3	Y1	M1	O1	Y2	M2	O2	Y3	M3	O3	Y1	O1	O2	Y3	O3
36	*Licmophora hyalina* (Kützing) Grunow																			1%		
37	*Licmophora paradoxa* (Lyngbye) C.Agardh	2%	2%	3%		4%	3%	3%	2%	1%	3%	3%	<1%	1%	2%	1%	3%	3%	3%	21%	1%	1%
38	*Licmophora probosciodea* Mereschkowsky																			11%	1%	1%
39	*Licmophora remulus* (Grunow) Grunow																				<1%	1%
40	*Mastogloia arabica* Hendey																<1%					
41	*Mastogloia binotata* (Grunow) Cleve	<1%		1%					1%		<1%				<1%	1%	<1%			1%	<1%	<1%
42	*Mastogloia cuneata* (Meister) R.Simonsen																			<1%	1%	1%
43	*Mastogloia emarginata* Hustedt																				1%	
44	*Mastogloia fimbriata* (T.Brightwell) Grunow		5%	1%		<1%			1%		<1%			1%	<1%	<1%	1%	1%	1%	1%		1%
45	*Mastogloia ovulum* Hustedt		2%							1%	3%			1%		1%	7%	<1%	3%		<1%	1%
46	*Mastogloia* sp.	2%	1%				3%			<1%								<1%	1%		1%	
47	*Microtabella delicatula* (Kützing) Round			<1%																		
48	*Nagumoea* sp.											1%										
49	*Navicula capitatoradiata* H.Germain																		1%			
50	*Navicula normalis* Hustedt						<1%						<1%									
51	*Navicula veneta* Kützing		<1%																			
52	*Navicula* spp.	1%	2%	9%	<1%		3%	2%	2%	3%	4%	9%	1%	1%	1%	1%	6%	2%	8%		6%	8%

Tab. 2. Species list and relative abundances of identified taxa on *Posidonia oceanica* leaf samples; Y – young (basal) leaf part, M – middle leaf part, O – older (apical) leaf part; 1,2,3 – sample number (continued).

#	Taxon	10 m							15 m									20 m				
		Y1	M1	O1	M2	O2	M3	O3	Y1	M1	O1	Y2	M2	O2	Y3	M3	O3	Y1	O1	O2	Y3	O3
54	*Nitzschia sigma* (Kützing) W.Smith		<1%																			
55	*Nitzschia* spp.	<1%				1%						1%										
56	*Petroneis arabica* (Grunow ex Schmidt) D.G.Mann											3%										
57	*Podocystis adriatica* (Kützing) Ralfs						<1%	1%			<1%		<1%		2%	<1%	<1%		<1%			
58	*Psammodictyon panduriforme* (W.Gregory) D.G.Mann																					<1%
59	*Pteroncola inane* (Giffen) F.E.Round		4%	<1%				1%			1%		<1%		<1%		<1%			<1%		
60	*Rhopalodia musculus* (Kützing) Otto Müller			<1%																		
61	*Striatella unipunctata* (Lyngbye) C.Agardh																			8%	<1%	2%
62	*Tabularia fasciculata* (C.Agardh) D.M.Williams & Round												<1%									
63	*Tabularia investiens* (W.Smith) D.M.Williams & Round			2%																		
64	*Tabularia* spp.	1%	<1%	<1%				<1%			2%	9%		<1%	<1%	<1%	<1%	<1%	3%		1%	1%
65	*Thalassionema* sp.												<1%									
66	*Toxarium undulatum* Bailey	3%	7%	2%	1%	10%	2%	9%		1%			<1%		<1%		<1%	<1%	2%	3%	1%	3%
67	*Trachyneis aspera* (Ehrenberg) Cleve					1%		<1%		<1%			<1%			<1%	<1%		<1%			
68	*Tryblionella coarctata* (Grunow) D.G.Mann									<1%									<1%	<1%	<1%	

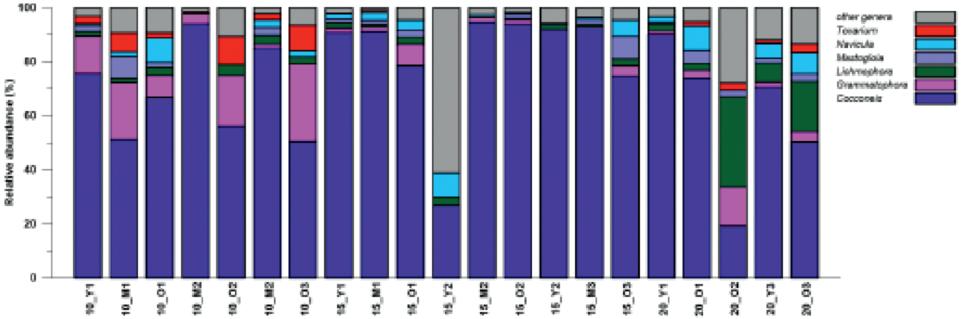

Fig. 3. Relative abundance of 6 most common genera: *Cocconeis, Grammatophora, Licmophora, Mastogloia, Navicula* and *Toxarium.*

The genus *Cocconeis* had the highest relative abundance in the majority of samples (in 85% of samples, species of the genus *Cocconeis* represented more than 50 % of the diatom community). The most dominant species in samples from all depths and leaf positions was *Cocconeis scutellum* Ehrenberg (Fig. 4, 6a). *Cocconeis scutellum* was also the most frequent taxa, being present in all the samples. *Halamphora* sp. (Fig. 7c), *Striatella unipuncata* (Lyngbye) C.Agardh (Fig. 7c) and *Podocystis adriatica* (Kützing) Ralfs (Fig. 7b) are some of the species that were not very abundant but were frequently present in the samples.

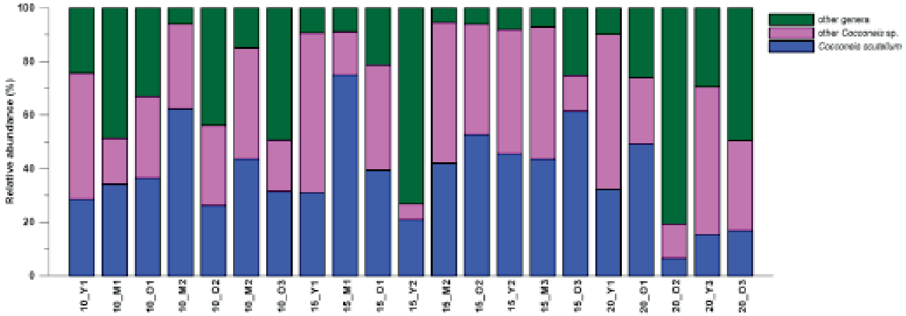

Fig. 4. Relative abundance of *Cocconeis scutellum* and other species of *Cocconeis* genus.

Genera with the greatest number of taxa were *Cocconeis* (9) *Licmophora* and *Mastogloia* (7 each) and *Diploneis* Ehrenberg ex Cleve (5).

In the samples collected from the depth of 10m, the number of taxa ranged from 10 to 23 for each sample, with a median of 18 (Fig. 5), while at the depth of 15 m, the range of taxa per sample varied from 13 to 18 (median 16), and at the 20 m depth, the highest median of 21 taxa was recorded and the range varied from 14 to 26.

Generally, the lowest Shannon-Wiener Diversity Index (2.54) is exhibited by the sample from the middle of *P. oceanica* leaves collected at the depth of 10 m (Fig. 5). The species diversity index varied from 3.04 to 3.58 in samples from 15 m depth. An increase in species diversity index was noted in the samples from the depth of 20 m (3.25-4.30), and in general, the highest index (4.30) was found in a sample from older (apical) part of *P. oceanica* leaves. SWDI had a slightly wider range (2.54-4.13) at 10 m depth than at 15 m. Regarding the age of *P. oceanica* leaves, the average SWDI was 3.61, 3.25 and 3.77 for basal (young), middle and the old (apical) part, respectively.

Pielou's species evenness ranged from 0.76 to 0.93 (the average 0.87) (Fig. 5). Regarding the age of *P. oceanica* leaves, the highest average Pielou species evenness was on apical parts of leaves (the average 0.89), while regarding depths was at 20 m (the average 0.90).

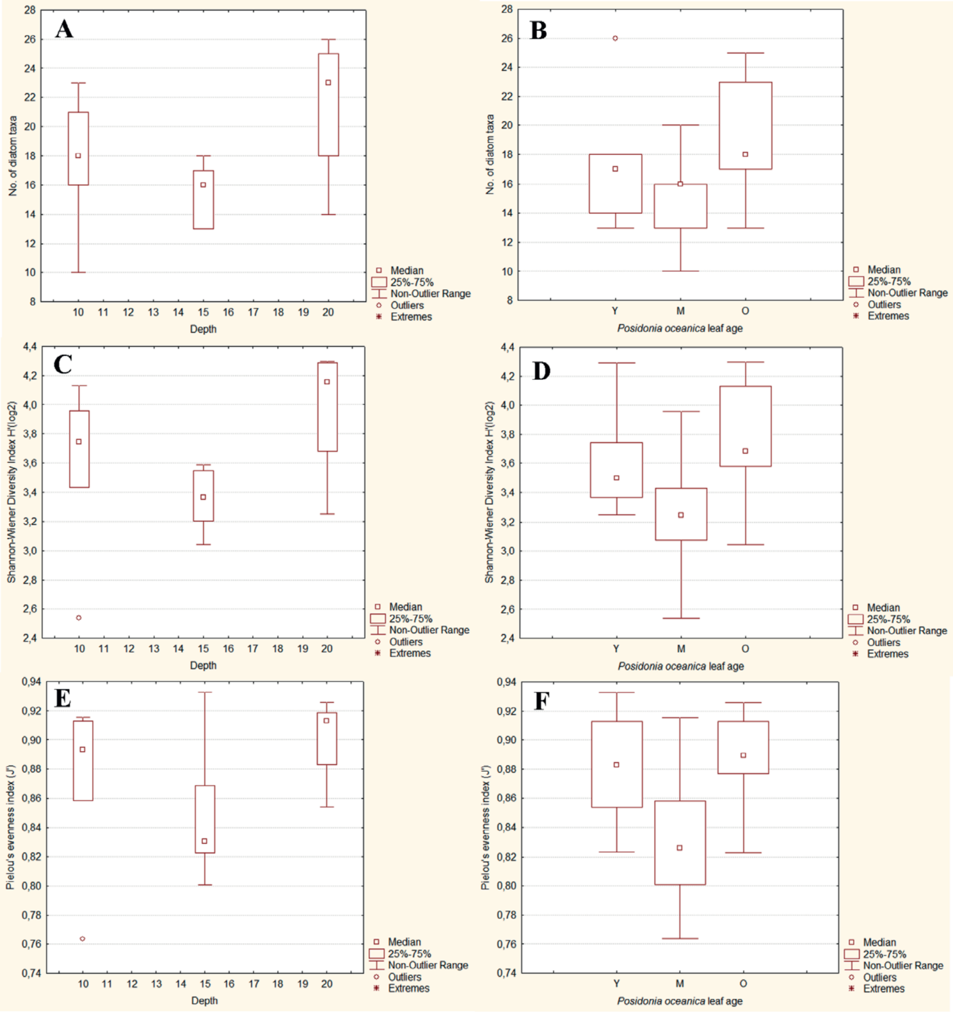

Fig. 5. Box and Whisker Plot showing differences in number of taxa, the Shannon-Wiener Diversity Index and Pielou's evenness index at different depths (A, C, E) and on different parts of *Posidonia oceanica* leaves (B, D, F); Y – youngest (basal) part of the leaf, M – middle part of the leaf, O – oldest (apical) part of the leaf.

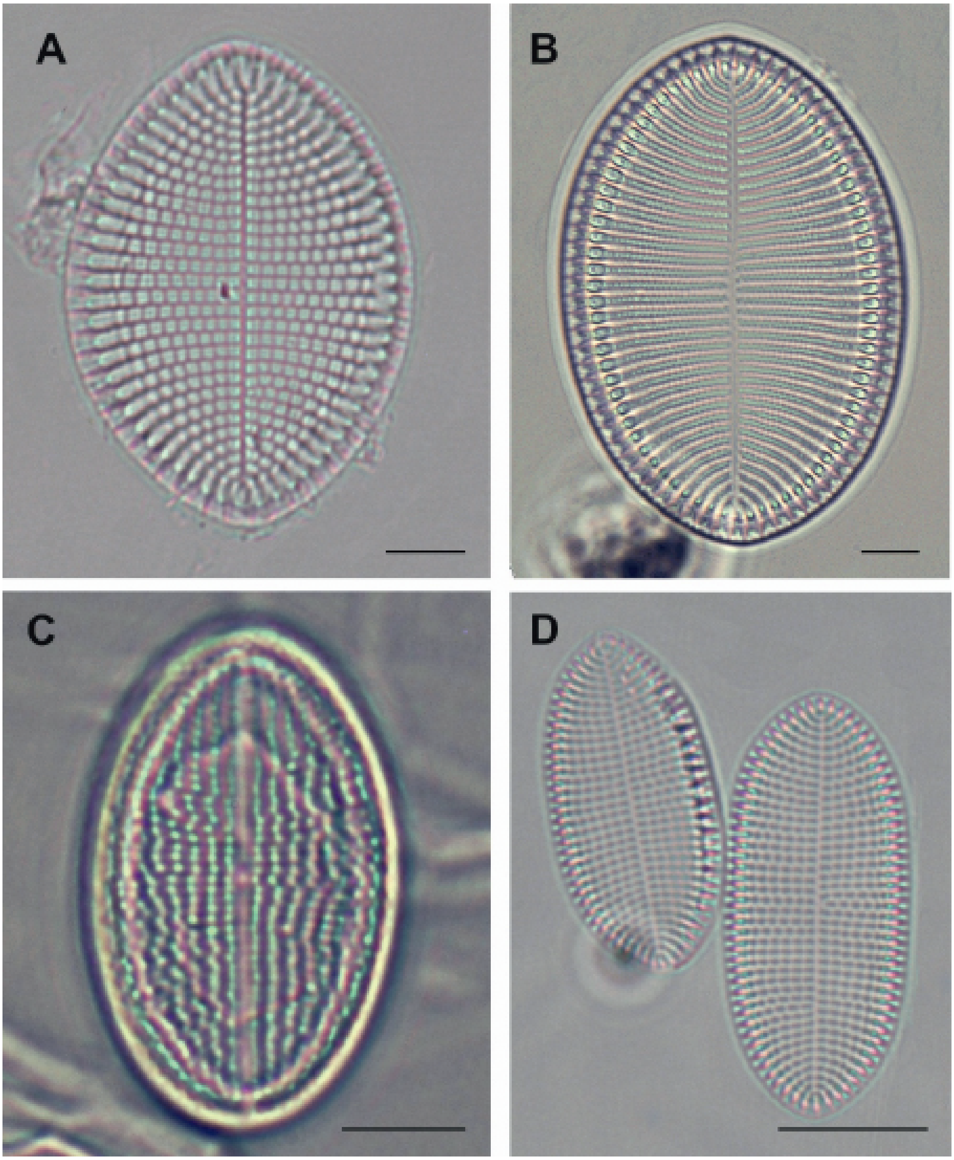

Fig. 6. Light microscopy images:
A – *Cocconeis scutellum*, B – *Cocconeis britannica*, C – *Cocconeis neothumensis*,
D – *Cocconeis* sp. 2; scale bars = 5 μm.

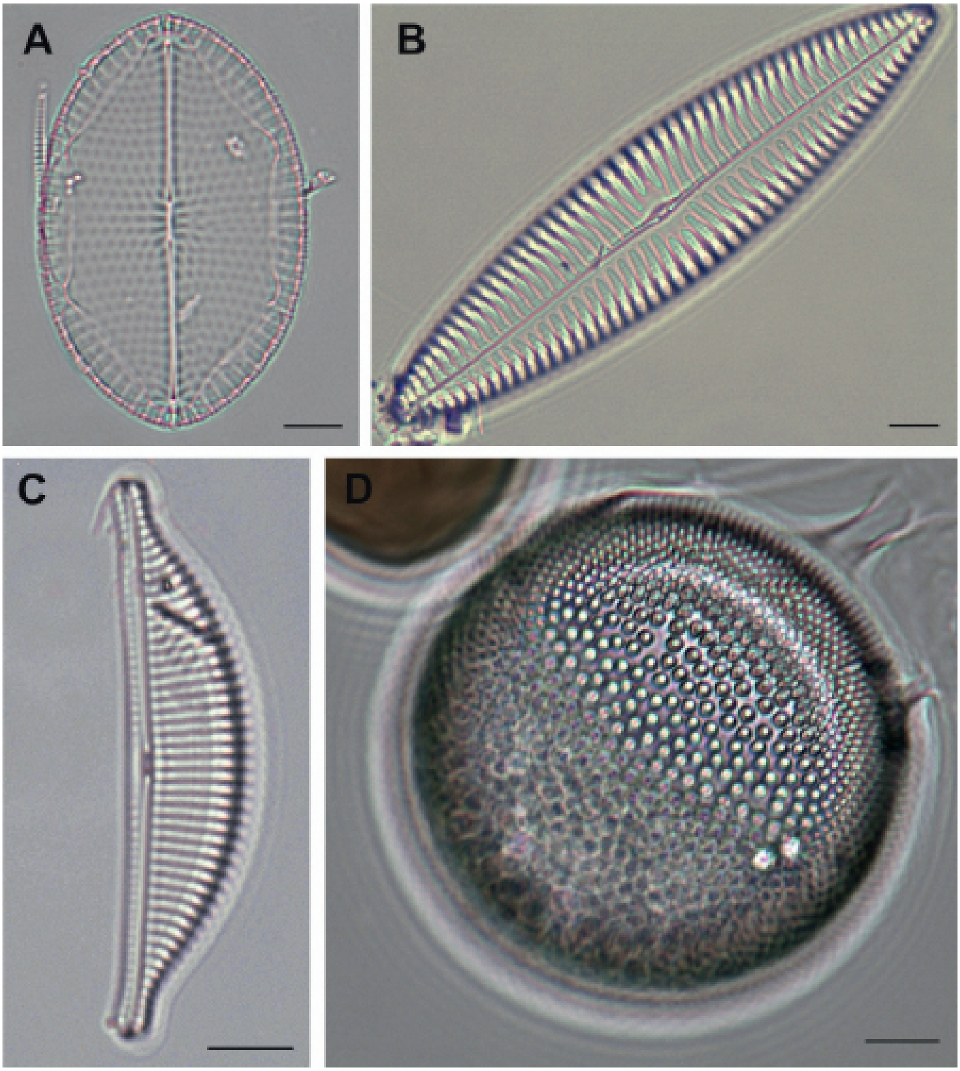

Fig. 7. Light microscopy images:
A – *Mastogloia fimbrata*, B – *Navicula normalis*, C – *Halamphora* sp., D – *Actinocyclus* sp.;
scale bar = 5 µm.

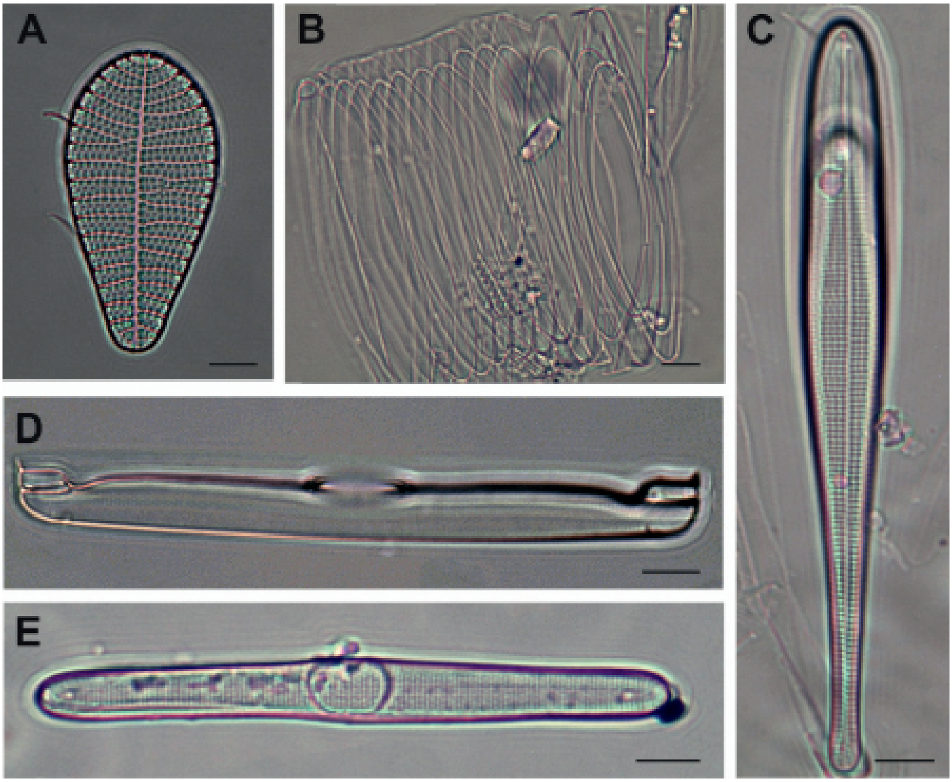

Fig. 8. Light microscopy images:
A – *Podocystis adriatica*, B – *Striatella unipunctata*, C – *Licmophora dalmatica*;
D – *Grammatophora oceanica* – girdle view, E – *Grammatophora oceanica* – valve view;
scale bars = 5 μm.

According to MDS, there were significant differences in the diatom assemblages (ANOSIM, $p < 0.05$) between the samples mostly collected at 10 m and 15 m depth (group 1) and samples collected at 20 m depth (group 2). Additionally, a sample from a basal part of *P. oceanica* leaf from 15 m depth (group 3) significantly differed from all the others (Fig. 9). SIMPROF analysis, however, detected apart from clusters 1 and 2, sub-clusters a, b, c and d (Fig. 9). Of 10 diatom samples forming sub-cluster a, 6 of them were collected at 10 m and 4 at 15 m depth. Only one sample from 10 m significantly differed from others from the same depth and formed sub-cluster b together with 4 samples from 15 m and 2 from 20 m depth. Cluster 2 included diatom samples collected only at 20 m depth and sub-clusters c and d (Fig. 9).

We did not observe any significant difference according to the ANOSIM statistical test in the diatom community structure between the basal, middle and apical part of *P. oceanica* leaf blade. However, a significant difference in community structure was observed for the samples obtained at different depths in terms of RA of diatom taxa (Tab. 3). The highest dissimilarity of 66% occurred between 10 m and 20 m. As revealed by SIMPER analyses, the group of taxa contributing the most (cumulatively 91%) to variance between diatom assemblages from 10 m and 20 m included *Cocconeis scutellum*, *Cocconeis* spp. *Cocconeis* sp. 2, *Cocconeis neothumensis* var. *marina* De Stefano, Marino, & Mazzella, *Grammatophora marina* (Lyngbye) Kützing, *Grammatophora oceanica* Ehrenberg, *Cocconeis britannica* Naegeli, *Licmophora paradoxa* (Lyngbye) C.Agardh, *Dimeregramma acutum* Hustedt, *Licmophora dalmatica* (Kützing) Grunow, *Navicula* spp., *Toxarium undulatum* Bailey, *Cocconeis krammeri* Lange-Bertalot & Metzeltin, *Licmophora probosciodea* Mereschk. and *Striatella unipunctata*.

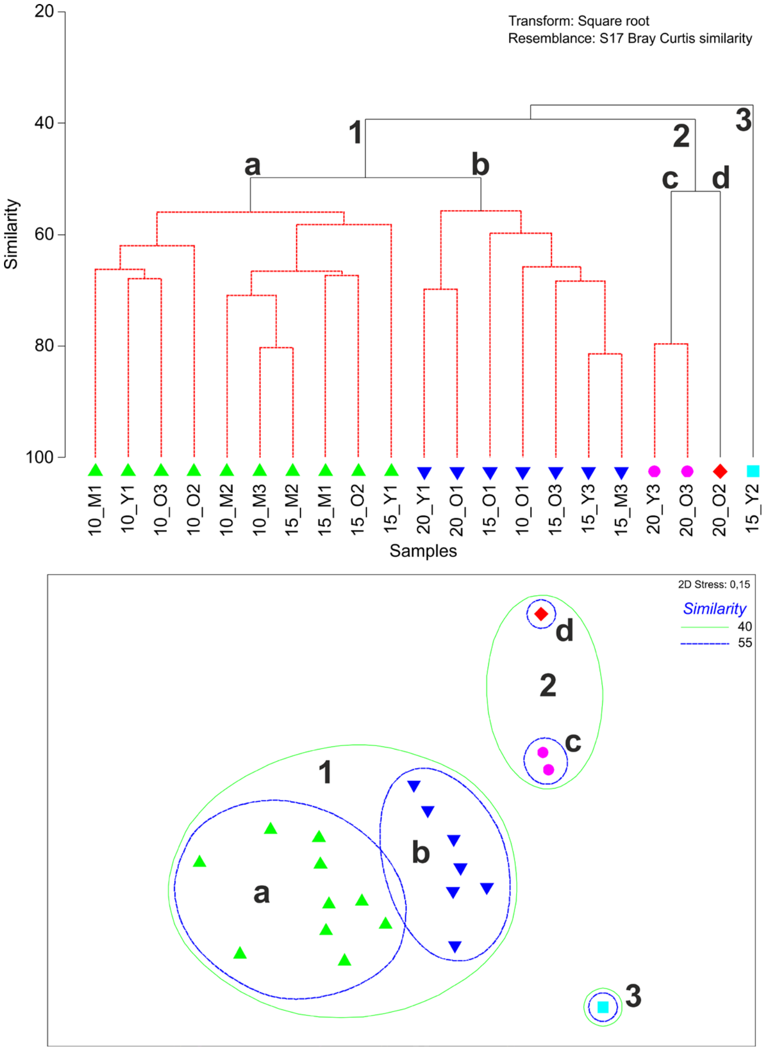

Fig. 9. Cluster and non-metric multi-dimensional scaling analysis (nMDS) of the epiphytic diatom assemblages from all three sampling depths (10 m, 15 m, 20 m) (taxa relative abundance data). For the ordination analysis all recorded diatom taxa were used. Top: Cluster analysis. Red lines indicated taxa homogeneous clusters detected by SIMPROF. Bottom: nMDS. Symbols correspond to the same main clusters detected by SIMPROF. N(10m) = 7; N(15m) = 9; N(20m) = 5.

Canonical analysis of principal coordinates (CAP) (Fig. 10.) showed that the samples collected from 10 m correlated with the abundance of *Grammatophora marina*, samples from 15 m with *Cocconeis scutellum* and *Cocconeis brittanica* while samples from 20 m correlated with other dominant species.

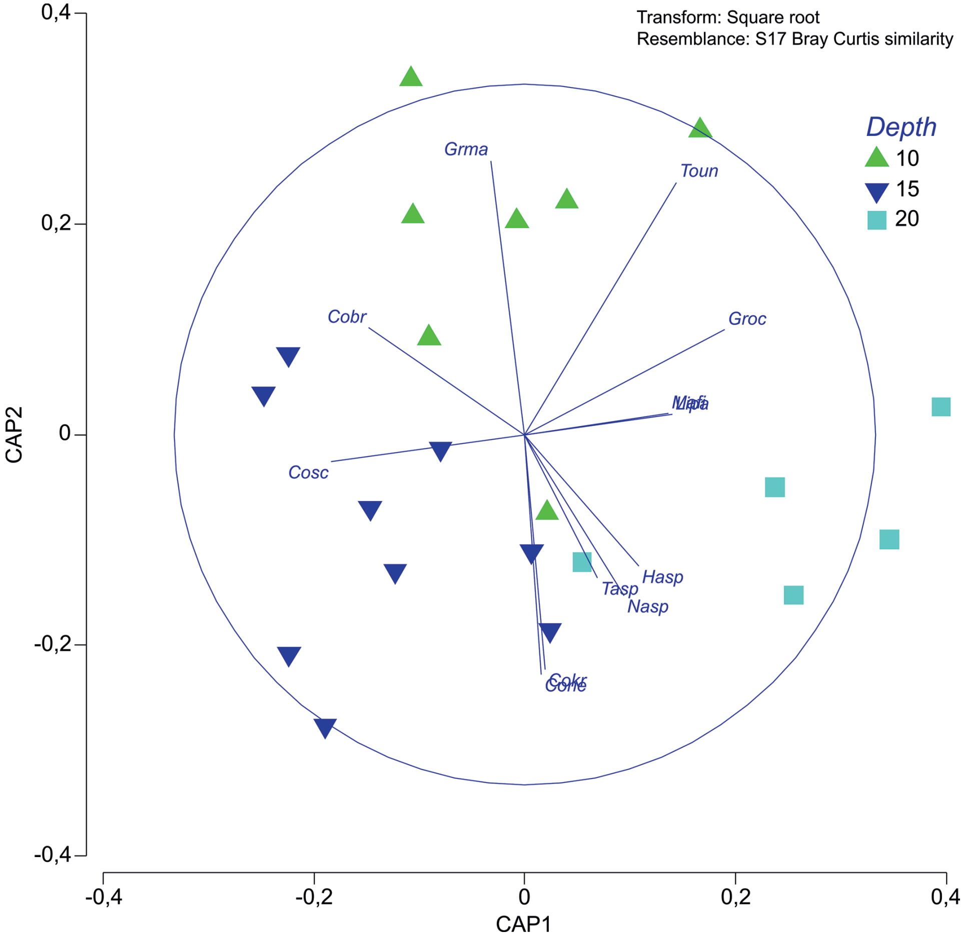

Fig. 10. Canonical analysis of Principle coordinates (CAP) biplot showing depths and vectors of diatom relative abundance (%) data (arrows) based on 21 samples. A dataset of 12 diatom taxa (with frequency of appearance > 50%) was selected. Codes for diatom taxa are: Cobr = *Cocconeis britannica Naegeli,* Cokr = *Cocconeis krammeri* Lange-Bertalot & Metzeltinmeri, Cone = *Cocconeis neothumensis* var. marina De Stefano, Marino, & Mazzella, Cosc = *Cocconeis scutellum* Ehrenberg, Grma = *Grammatophora marina* (Lyngbye) Kützing, Groc = *Grammatophora oceanica* Ehrenberg, Hasp = *Halamphora* spp., Lipa = *Licmophora paradoxa* (Lyngbye) C.Agardh, Mafi = *Mastogloia fimbriata* (T.Brightwell) Grunow, Nasp = *Navicula* spp., Tasp = *Tabularia* spp., Toun = *Toxarium undulatum* Bailey

Tab. 3. Results of ANOSIM test performed on species relative abundance data according to the depth (in general and between individual depths), and host leaf age associated with the position on the leaf; n.s. – not significant

	Depth	10m/ 15m	10m /20m	15m /20m	*Posidonia* leaf age	Simprof Group
p	0.001	n.s.	< 0.001	n.s.	n.s.	0.001
Global R	0.44	0.3	0.694	0.456	0.048	0.816

DISCUSSION

The diversity of epiphytic diatom communities associated with *P. oceanica* was previously described by several authors (Mazzella & Spinoccia, 1992; Mazzella *et al.*, 1994; De Stefano *et al.*, 2000; Majewska *et al.*, 2014) as composed of eight dominant genera (*Amphora*, *Cocconeis*, *Gomphonemopsis*, *Grammatophora*, *Lichmophora*, *Mastogloia*, *Nitzschia* and *Synedra*). Our results are congruent with these results, with an addition of two more genera, *Navicula* and *Toxarium*. All taxa identified are characteristic of benthic habitats in the Mediterranean (Alvarez-Blanco & Blanco, 2014) and Adriatic Seas (Hafner *et al.*, 2018). Considering communities' relative abundances, our samples are characteristic of epiphytic biofouling with the expected dominance of the genus *Cocconeis* (Mazzella & Spinoccia, 1992). The prevalence of the genus *Cocconeis* on *P. oceanica* leaves has already been reported in the Mediterranean Sea (Majewska *et al.*, 2014). Due to the limitation of LM and the taxonomical complexity of some genera (*Navicula*, *Amphora* Ehrenberg ex Kützing, *Halamphora* (Cleve) Levkov etc.) that have relatively small frustules, some taxa could not be identified at the species level. Likewise, biodiversity of the diatom community biofouling leaves of *Posidonia oceanica* in the Adriatic Sea could be considered much higher than revealed in our study. Some taxa appeared only in particular microhabitats on the parts of *P.* leaves and/or depths (eg. *Licmophora abbreviata* C. Agardh and *L. hyalina* (Kützing) Grunow occurred only at 20 m depth on apical parts of *Posidonia* leaves; *Amphora graeffeana* Hendey only at 10 m on apical parts; *Mastogloia emarginata* Hustedt only at 20 m depth on basal leaf parts), but the determination of the relationship between their occurrence and abundance, and the ecological conditions in the bay falls outside of the scope of the present paper.

The proposed hypothesis in this study is partly rejected, for although the highest diversity index was noted in the samples from the apical part there was no significant difference in diatom communities from *P. oceanica* leaves according to their different positions along the leaf axis. There are several reasons why this is the case. For example, in this study, due to the methodological reasons we used relative taxa abundances and it is possible if we measured the true abundances expressed as cells per cm^2, higher diatom abundance differences would have been detected as significant in the older apical part. Another possible reason would be that the taxa on *P. oceanica* leaves are still young in May when the samples were taken, and no stable community had yet been reached. Furthermore, *P. oceanica* is a species that change its leaves annually (Gobert *et al.*, 2006). On the other hand we detected significant differences between samples collected at 10m and 15m and samples collected at 20m. The diatom community in these samples was very diverse and dominated by taxa such as *Grammatophora oceanica, Mastogloia fimbriata, Tabularia* spp. reflecting the older, well established epiphytic biofilm, unlike samples from shallower depths that were mostly dominated by *Cocconeis* taxa. Species of the genus *Cocconeis* often grow very fast and dominate uninhabited surfaces like the young leaves of *P. oceanica* (Mazzella & Spinoccia, 1992). *Cocconeis scutellum* is extremely well adapted to these epiphytic habitats, and many varieties of the species have been described from *P. oceanica* leaves (De Stefano *et al.*, 2008). Mazzella & Spinoccia (1992) found that the diatom growth form is an important factor for the colonization of substrates such *P. oceanica* leaves as competition exists only among diatom species that have similar growth forms. Furthermore, Majewska *et al.* (2014) claimed that community composition depends on surface morphology. That could explain why the diatom communities from different samples were similar. *Posidonia oceanica* has a smooth and plain leaf surface which is suitable for the growth of adnate and motile species such as the species from the genus *Cocconeis*, which have a competitive advantage in these habitats and can dominate them. Adnate diatoms (eg. *Cocconeis*) adhere strongly horizontally to the substrate by means of the raphe valve and because of their limited motility. Due to their adhering mode on the host surface through the valve face, adnate taxa may easily benefit from nutrient exchange with the host (Round, 1981; Sullivan, 1984; Romagnoli *et al.*, 2014). 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 and are the most resistant to grazing. However, they are easily overgrown by other organisms and may become light-limited by that overgrowth (Mccormick & Stevenson, 1989). While adnate cells are closely appressed to the substratum, motile cells (eg. *Navicula, Nitzschia*) that were also recorded in our samples, have movement capability enabling them to glide along the substratum to find the optimum light and nutrient conditions (Round *et al.*, 1990).

CONCLUSIONS

Epiphytic biofoulings of *Posidonia oceanica* have great taxonomical, but also ecological importance. This study contributes to the knowledge of biodiversity associated with *P. oceanica* meadows in the Adriatic and the necessary identification of the taxa-specific epiphytic diatom community on seagrasses in the Adriatic Sea. Moreover, our results contribute to a better knowledge of epiphytic algal communities of the Mediterranean in general.

ACKNOWLEDGMENTS

The authors thank Vivien Tajmin and Hrvoje Čižmek for collecting the samples while scuba diving. Additionally, we thank to the Biology Students Association – BIUS, Ksenija Hocenski and Ivana Mišerić who organized research-educational project “Insula Tilagus 2017” during which this research took place.

This work has been supported in part by the Croatian Science Foundation under the project UIP-2017-05-5635 (TurtleBIOME) and project IP-2014-09-2945 (AdriaMedPLan). The authors thank anonymous reviewers for their helpful comments on the manuscript.

Received September 20, 2018

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