

TAXONOMIC SURVEY OF BENTHIC DIATOMS IN NEUM BAY, SOUTHEASTERN ADRIATIC

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The objective of this work was to contribute to the knowledge of microphytobenthos in the Adriatic Sea, by studying for the first time the benthic diatom communities in Neum Bay in Bosnia and Herzegovina. For this purpose, the benthic diatoms naturally growing on stones and macroalgae were identified at one station in the small semi-enclosed oligotrophic Neum Bay during 2010 and 2011. A total of 24 samples were collected at two different depths, 0.5 m and 8 m, and analysed with light and scanning electron microscopy. Altogether, 425 pennate and 58 centric taxa (species and infraspecific taxa) belonging to 60 families and 115 genera were noted. Genera with the greatest number of taxa were: *Mastogloia* (46 taxa), *Navicula* (36), *Diploneis* (35), *Nitzschia* (34), *Amphora* (31), *Cocconeis* (27), *Achnanthes* (14), *Halamphora* (12), *Lyrella* (11), and *Surirella* and *Licmophora* (10 each). *Amphora bigibba* var. *interrupta* and *Cocconeis scutellum* were the most frequent taxa, being present in 87.5% of the samples. In total, 142 taxa were found only once (sporadic taxa). Although benthic diatom richness is high in the bay, taxa are apparently not distributed evenly temporally. Consistent quantitative and qualitative data are still needed for a better determination of the seasonal and spatial changes of the epilithic assemblages in the region.

Keywords: Bacillariophyta, shallow oligotrophic bay, species identification, biodiversity, Bosnia and Herzegovina.

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Cilj rada bio je doprinijeti poznavanju mikrofitobentosa u Jadranskom moru istražujući po prvi put bentoske dijatomejske zajednice u Neumskom zaljevu u Bosni i Hercegovini. Bentoske dijatomeje su određene na jednoj postaji u poluzatvorenom oligotrofnom Neumskom zaljevu 2010. i 2011. Sakupljeno je 24 uzoraka na dubinama 0,5 i 8 m, a analizirani su s pomoću svjetlosnog i skenirajućeg elektronskog mikroskopa. U uzorcima je utvrđeno 425 penatnih i 58 centričnih svojiti (vrsta i nižih taksonomskih kategorija) dijatomeja unutar 60 porodica i 115 rodova. Najveći broj svojiti nađen je unutar sljedećih rodova: *Mastogloia* (46 svojiti), *Navicula* (36), *Diploneis* (35), *Nitzschia* (34), *Amphora* (31), *Cocconeis* (27), *Achnanthes* (14), *Halamphora* (12), *Lyrella* (11) te *Surirella* i *Licmophora* (svaki po 10 svojiti). Najčešće svojite su *Amphora bigibba* var. *interrupta* i *Cocconeis scutellum* s 87,5% učestalosti u ukupnom broju uzoraka. Utvrđene su 142 sporadične svojite (utvrđene u samo jednom uzorku). Iako je broj svojiti relativno visok, nije utvrđena pravilnost u njihovoj vremenskoj raspodjeli. Radi preciznijeg utvrđivanja sezonskih i prostornih promjena strukture epilitskih dijatomejskih zajednica, potrebni su detaljniji kvalitativni i kvantitativni podaci.

Ključne riječi: Bacillariophyta, plitki oligotrofni zaljev, taksonomska istraživanja, bogatstvo svojiti, Bosna i Hercegovina

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INTRODUCTION

Benthic diatoms are colonial or unicellular diatoms, free living or attaching to the substratum by gelatinous extrusion. They are mostly associated with substrata throughout their life cycles. Some of them move actively in sediments and sometimes resuspend in the water column. Benthic diatoms are the most abundant and diversified part of marine microphytobenthos playing an important role in marine ecosystems (FALKOWSKI *et al.*, 2004). These organisms are used as water quality indicators as well as in palaeoecological reconstructions due to their ecophysiological features (CIBIC & BLASUTTO, 2011; STEVENSON & PAN, 1999). In addition, knowledge of the structure of the benthic diatom community and the autoecology of individual taxa is a unique source of information in the study of the dynamics of the marine microphytobenthos.

In the Mediterranean Sea, ÇOLAK SABANCI & KORAY (2010), ÇOLAK SABANCI (2012) and ÁLVAREZ-BLANCO & BLANCO (2014) recently investigated the structure and taxonomic composition of benthic diatom communities. In the Adriatic Sea, the diatom taxa from natural sediment samples and artificial substrata of the Gulf of Trieste (BARTOLE *et al.*, 1991-94, SDRIGOTTI *et al.*, 1999; MUNDA, 2005), the Venice lagoon (TOLOMIO & ANDREOLI, 1989, TOLOMIO *et al.*, 1999; FACCA *et al.*, 2002; TOLOMIO *et al.* 2002; FACCA & SFRISO, 2007), the northwestern Adriatic coast (TOTTI, 2003; TOTTI *et al.*, 2007; FRANZO *et al.*, 2015, and references therein) were reported. Several studies on epibiontic diatom communities on marine hydroids in the Ligurian Sea have also been reported (ROMAGNOLI *et al.*, 2007, 2014, and references therein). Finally, a checklist on microphytobenthos of the Italian seas, including benthic diatom taxa, was compiled by CIBIC & FACCA (2010).

On the other hand, to the best of our knowledge, the structure and taxonomic composition of benthic diatoms have been only rarely taken into consideration in the eastern Adriatic Sea (BURIĆ *et al.*, 2004; MIHO & WITKOWSKI, 2005; CAPUT *et al.*, 2008; LEVKOV *et al.*, 2010; CAR *et al.*, 2012; NENADOVIĆ *et al.*, 2015; MEJANDŽIĆ *et al.*, 2015). Diatoms were investigated synoptically and mostly related to estuaries or coastal wetlands.

The objective of this work was to contribute to the knowledge of microphytobenthos in the Adriatic Sea, by studying for the first time the benthic diatom communities in Neum Bay in Bosnia and Herzegovina.

MATERIAL AND METHODS

Study area

Bosnia and Herzegovina has a total sea area of ca. 8 km² with only 21.2 kilometers of coastline (Fig. 1). The coast is low and rocky, built up of karstified carbonates and represents only 0.26% of the total length of the Adriatic coastline (BLAKE & TOPALOVIĆ, 1996; PIKELJ & JURAČIĆ, 2013). In fact, the sea area of the country is represented by the small, semi-enclosed Neum Bay, located between mainland and the 7 km-long Klek Peninsula (DUPČIĆ-RADIĆ *et al.*, 2013).

Neum Bay is part of the larger Mali Ston Bay, which is enclosed on the seaward side by the 62 km long Pelješac peninsula. It expands to the northwest and connects with the Neretva River channel which is linked with the open sea. Due to its ecological and economic importance, this area with a centuries-long mariculture tradition was in 1983 proclaimed the Mali Ston Bay and Malo More Special Marine Reserve, including Neum Bay. The most important factors that affect the ecological conditions in Mali Ston Bay,

The sampling points were located at the distance of 1 m for the shallow samples and 25 m for the deeper samples from the coastline due to the small bathymetric gradients. The diatom samples were obtained by scraping of the submersed stones and collecting attached macroalgae [mostly *Cystoseira* spp., *Codium vermilara* (Olivi) Delle Chiaje, *Ceramium* spp., *Dictyota dichotoma* (Hudson) J.V.Lamouroux]. To identify epilithic diatoms, stones of 15-20 cm in diameter were collected. Deeper stones were collected by scuba diving. Stones were collected as randomly as possible amongst those that are not completely covered by filamentous algae and on which the diatom biofilm was visible. Stones were put into a plastic bag of 1 L in which 200 mL of distilled water was added. The upper parts of the stones were rubbed with a toothbrush and the mixture was decanted into 250 mL polyethylene bottles (WINTER & DUTHIE, 2000). The samples were preserved with a final concentration of 4% formaldehyde.

In the laboratory, the material collected was cleaned of organic material for light (LM) and scanning electron microscopy (SEM) observations. Material was treated with sulfuric acid-potassium permanganate, decolorized with oxalic acid, and acid residues were removed by repeated decantation. The cleaned material was pipetted onto ethanol-cleaned cover-slips and left to air-dry, before mounting in Canada balsam when they were ready for light microscopic examination as permanent slides (HASLE & FRYXELL, 1970). In order to register all diatom taxa, the entire surface area of each permanent slide has been surveyed. Whenever possible, diatoms were identified to species rank and counted at 1000 × magnification by phase-contrast optics with a Microstar binocular microscope (AO Scientific Instruments), 100 × PlanApo oil immersion objective. Slides have been deposited in the diatom collection of the Institute for Marine and Coastal Research, University of Dubrovnik, Dubrovnik, Croatia.

Ultrastructural analysis was performed using SEM. A drop of the cleaned sample was air-dried overnight on aluminium stubs and coated with gold-palladium or osmium. SEM observations were made at the Warsaw University of Technology, Faculty of Materials Science and Engineering, using a Hitachi S-3500, SU-70 and SEM/STEM S-5500.

Identifications were made following PERAGALLO & PERAGALLO (1897–1908), HENDEY (1964), RICARD (1974, 1975, 1977), POULIN *et al.* (1984, 1990), BÉRARD-TERRIAULT *et al.* (1986, 1987), HARTLEY (1986), SNOEIJIS (1993, 1999), SNOEIJIS & POTAPOVA (1995), SNOEIJIS & KASPEROVICIENÉ (1996), SNOEIJIS & BALASHLOVA (1998), HARTLEY *et al.* (1996), and WITKOWSKI *et al.* (2000). Nomenclature and data on general environments of taxa follow AlgaeBase (GUIRY & GUIRY, 2017), and only partially ÁLVAREZ-BLANCO & BLANCO (2014).

RESULTS

Environmental conditions

In 2011, surface water temperature varied between 10.0 and 27.1°C, and maximum was recorded in September, while the minimum was noted in January (Tab. 2). The salinity ranged between 32.0 in April and 38.4 in December. Warm summer months were characterized by lower salinity, while in the cooler months of winter and spring salinity was mostly related to the precipitation regime and the extremely dynamic water flow from the underwater karstic springs (“vruljas”) that feed the bay.

Tab. 2. Monthly distribution of the temperature and salinity on the surface (0.5 m depth) in Neum Bay in 2010 and 2011.

Years	2010			2011											
Months	3	5	7	1	2	3	4	5	6	7	8	9	10	11	12
Temperature (°C)	10.4	15.9	25.2	10.0	10.9	10.2	15.9	15.5	22.5	24.9	26	27.1	23.1	.	15.7
Salinity	36.7	37.2	36.4	34.2	33.5	36.6	32.0	36.9	34.9	35.6	34.7	35.2	.	.	38.4

Taxonomic composition of the benthic diatom community

During this study, a total of 483 specific and infraspecific diatom taxa were identified in Neum Bay (Tab. 3). The list of taxa included 425 pennate and 58 centric diatoms belonging to all three recognized diatom classes: Coscinodiscophyceae, Mediophyceae and Bacillariophyceae (MEDLIN & KACZMARSKA, 2004), and 60 families (Tab. 3). Among them, two taxa were included in Bacillariophyta *incertae sedis* (*sensu* GUIRY & GUIRY, 2017). Ten families were included in Coscinodiscophyceae, 11 in Mediophyceae and 39 in Bacillariophyceae.

Families with the greatest number of genera were: Naviculaceae (8 genera), Bacillariaceae (6), Achnanthesiaceae (6), Surirellaceae (6), Fragilariaceae (6), Eupodiscaceae (6) and Plagiogrammaceae (4). Additionally, the highest number of species and infraspecific taxa belonged to Naviculaceae (56).

Altogether, 115 genera were found in all samples. Thirteen genera were included in Coscinodiscophyceae, 17 in Mediophyceae and 87 in Bacillariophyceae. Genera with the greatest number of taxa were: *Mastogloia* (46 taxa), *Navicula* (36), *Diploneis* (35), *Nitzschia* (34), *Amphora* (31), *Cocconeis* (27), *Achnanthes* (14), *Halamphora* (12), *Lyrella* (11) and *Surirella* and *Licmophora* (10 each). In total, 24 genera had two taxa each, while 60 were composed of one taxon only. Regarding the habitat type (*sensu* GUIRY & GUIRY, 2017), the greatest number of diatom taxa (152) have been characterized as exclusively marine, 15 are brackish and 29 exclusively freshwater diatoms, while one (*Mastogloia exigua*) has been characterized as exclusively a soil diatom. Among truly marine diatoms, three *Amphora* taxa (*Amphora bigibba* var. *interrupta*, *A. hyalina* and *A. laevissima*) showed high frequency of appearance and were found in more than 50% of the samples.

However, *Amphora bigibba* var. *interrupta* and *Cocconeis scutellum* were the most frequent taxa, being present in 87.5% of the samples. Taxa with high frequencies (79.1-83.3%) were: *Achnanthes brevipes* (83.3%), *Toxarium undulatum* (79.1%) and *Licmophora remulus* (79.1%), while others (centric diatoms *Actinocyclus subtilis*, *Biddulphia biddulphiana*; and pennates *Grammatophora oceanica*, *Halamphora coffeiformis*, *Brebissonia lanceolata*, *Coronia decorus*, *Synedra fulgens*, *Trachyneis aspera* and *Surirella fastuosa*, Fig. 2) were found from 62.5% to 66.7% of the samples. Altogether, 34 taxa were found in at least 50% or more of the samples and could be characterised as taxa with higher frequency of appearance. In total, 142 taxa were found only once (sporadic taxa) during the investigated period (Tab. 3).

Some taxa appeared only in particular seasons and/or depths, but the determination of the relationship between their appearance and abundances, and the ecological conditions in the bay falls outside of the scope of the present paper.

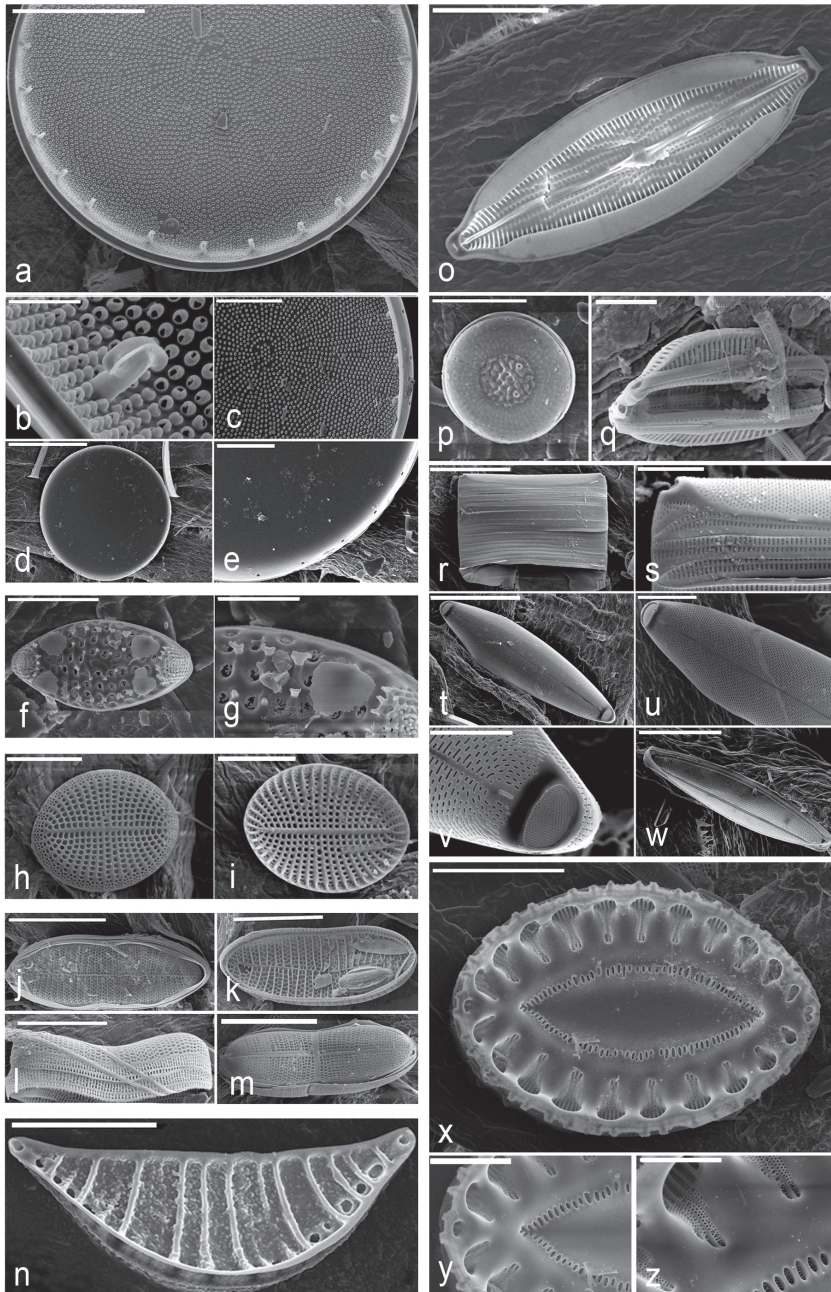


Fig. 2. Scanning electron microscope (SEM) micrographs of benthic diatoms in the Neum Bay: a-e) *Actinocyclus subtilis* (W.Gregory) Ralfs; f-g) *Dimeregramma minus* (W.Gregory) Ralfs; h-i) *Cocconeis scutellum* Ehrenberg; j-m) *Achnanthes brevipes* C.Agardh; n) *Rhopalodia pacifica* Krammer; o) *Mastogloia ignorata* Hustedt; p) *Cyclotella meneghiniana* Kützing; q) *Halamphora coffeiformis* (C.Agardh) Levkov; r-w) *Striatella unipunctata* (Lyngbye) C.Agardh; x-z) *Surirella fastuosa* (Ehrenberg) Ehrenberg. Scale bars = 40 μm (d), 30 μm (j, m, r, t), 20 μm (a, k, l, w, x), 10 μm (c, e, h, i, n, o, u, y), 5 μm (f, p, q, s, v, z), 2 μm (b, g).

Taxon	Family	2010												2011												n	Freq. (%)		
		GE			July	January	February	March	April	May	June	July	August	September	October	November	December												
		S	M	B	F	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5									
<i>Amphora copulata</i> (Kützing) Schoeman & R.E.M.Archibald	Catenulaceae	.	.	.	+	1	4.17	
<i>Amphora copuleritina</i> var. <i>capitata</i> Tempere & Peragallo	Catenulaceae	1	4.17
<i>Amphora crassa</i> W.Gregory	Catenulaceae	1	4.17
<i>Amphora delticatissima</i> Krasske	Catenulaceae	1	4.17
<i>Amphora cf. gacialis</i> W.Smith	Catenulaceae	5	20.83
<i>Amphora grae ilis</i> Ehrenberg	Catenulaceae	4	16.67
<i>Amphora graffiana</i> Hervey	Catenulaceae	2	8.33
<i>Amphora grevilliana</i> Gregory	Catenulaceae	2	8.33
<i>Amphora hughiana</i> Kützing	Catenulaceae	14	58.33
<i>Amphora immarginata</i> Nagumo	Catenulaceae	1	4.17
<i>Amphora lacis</i> Gregory	Catenulaceae	2	8.33
<i>Amphora lacissima</i> W.Gregory	Catenulaceae	12	50.00
<i>Amphora lineolata</i> Ehrenberg	Catenulaceae	11	45.83
<i>Amphora lunata</i> Ostrup	Catenulaceae	9	37.50
<i>Amphora obtusa</i> W.Gregory	Catenulaceae	1	4.17
<i>Amphora ovalis</i> (Kützing) Kützing	Catenulaceae	4	16.67
<i>Amphora pediculus</i> (Kützing) Grunow	Catenulaceae	2	8.33
<i>Amphora proteus</i> W.Gregory	Catenulaceae	2	8.33
<i>Amphora pseudohughiana</i> Simonsen	Catenulaceae	13	54.17
<i>Amphora robusta</i> Gregory	Catenulaceae	1	4.17
<i>Amphora cf. securula</i>	Catenulaceae	1	4.17
<i>Amphora sibacuthiusula</i> Schoeman	Catenulaceae	1	4.17
<i>Amphora</i> sp.1	Catenulaceae	4	16.67
<i>Amphora</i> sp.2	Catenulaceae	1	4.17
<i>Anorthoneis hughiana</i> Hustedt	Achnanthesiaceae	2	8.33
<i>Avidissonea formosa</i> (Hantzsch) Grunow	Ardissonaceae	9	37.50
<i>Avidissonea robusta</i> (Ralls ex Pritchard) De Notaris	Ardissonaceae	11	45.83
<i>Astartella</i> sp.1	Achnanthesiaceae	1	4.17
<i>Asteromphalus hookeri</i> Ehrenberg	Asterolampraceae	3	12.50
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	Aulacoseiraceae	3	12.50
<i>Auliscus sulptus</i> (W.Smith) Brightwell	Eupodisceae	6	25.00
<i>Auricula amphitritis</i> Castracane	Auriculaceae	2	8.33
<i>Auricula</i> sp.1	Auriculaceae	2	8.33
<i>Azeplita nodulifera</i> (A.W.F.Schmidl) G.A.Fryxell & P.A.Sims	Heliopeleaceae	1	4.17
<i>Bacillaria paxillifera</i> (O.F.Müller) T.Marsson	Bacillariaceae	8	33.33

DISCUSSION

In the present study, small forms belonging to some of the most taxonomically complex genera (e.g. *Amphora*, *Navicula* and *Nitzschia*), characterized by a considerable morphological variability, were recognized (SULLIVAN & CURRIN, 2000). A similar diatom community has been reported for different hard substrates from the Adriatic Basin (MUNDA, 2005; TOTTI *et al.*, 2007) and for coastal rocks of the Mediterranean (cf. ÁLVAREZ-BLANCO & BLANCO, 2014). Regarding their ecology, the genus *Navicula* includes taxa with a very wide ecological range (KRAMMER & LANGE-BERTALOT, 1991), while *Amphora* and some *Achnanthes* taxa are mostly typical of nutrient-poor regions (AGATZ *et al.*, 1999). The difficulty in their identification was a major problem in this study. As a matter of fact, more than 40 taxa in Tab. 3 are not fully determined to species level. Additionally, it has been pointed out that some taxa may exhibit more than one growth form, reflecting the different strategies of spatial utilization (cf. ROMAGNOLI *et al.*, 2014, and references therein) adopted for the competitive advantage they provide. In this study, although the samples contained a mixture of forms growing on the stones and some epiphytic forms, we refer to this collective community as epilithon (*sensu* ÇOLAK SABANCI, 2012).

The presence of taxa associated with fresh to brackish waters may be correlated with the precipitation regime and the dynamic water flow from the underground karstic springs ("vruljas") that feed the bay. These taxa were well adapted to this changing environment and these results also support the idea that some species could have different responses to the environmental conditions, resulting in a better or worst adaptation to them (UNDERWOOD *et al.*, 1998; RIBEIRO *et al.*, 2003; MIHO & WITKOWSKI, 2005).

In our study, the genus *Mastogloia* was the richest in taxa number, and the most frequent taxa were *Amphora bigibba* var. *interrupta*, *Cocconeis scutellum*, *Achnanthes brevipes*, *Toxarium undulatum* and *Licmophora remulus*. *Mastogloia* species, one of the largest diatom genera (PENNESI *et al.*, 2011, and references therein), can be found within different biotopes (ÇOLAK SABANCI, 2013). In our study, the most frequent *Mastogloia* was *M. cyclops* which has been characterized as good indicator of coastal zones (WACHNICKA *et al.*, 2010). Some taxa (e.g., *C. scutellum*, *Halamphora coffeiformis*) were found on different substrata and do not seem to have a preference either for geographic region or for the substrate type (ROMAGNOLI *et al.*, 2014). By contrast, *C. scutellum* is considered as typical epiphytic taxon (ULANOVA & SNOEIJIS, 2006).

It is difficult to determine the possible seasonal fluctuations in the assemblages based on qualitative data. Many taxa such as *Achnanthes brevipes*, *Actinocyclus subtilis*, *Grammatophora oceanica* and *Cocconeis scutellum* did not show any kind of marked seasonality. This was also found for several *Cocconeis* taxa (*C. molesta*, *C. placentula*, *C. scutellum*) in the Venice Lagoon (TOLMIO & ANDREOLI, 1989). Additionally, ÇOLAK SABANCI (2012) in the study based on the seasonal sampling, showed that some of the above mentioned taxa did not have any apparent preference for a given season in the Aegean lagoon in Turkey. In our case, a range of factors can naturally be expected to affect diatom development in the bay, and this research will be investigated in depth in the future.

In our study, the number of taxa was higher than found in floristic surveys conducted in other, similar habitats, in a broad sense and over a wide geographic area in Adriatic or Mediterranean (e.g., MIHO & WITKOWSKI, 2005; ÇOLAK SABANCI, 2011, 2012). Other authors obtained quite different results due to differences in the size of the studied areas, environmental conditions and seasons. In addition, special attention must be paid to the use of different cleaning and processing treatments of benthic marine diatom samples

from Mediterranean oligotrophic areas (VERMEULEN *et al.*, 2012); marine diatoms are likely to be very sensitive to common treatments used in studies of freshwater populations because of the lower availability of silicic acid and of the interference of salinity in silica fixation (CONLEY *et al.*, 1989).

To summarize, this study represents the first floristic list focused on benthic diatoms for Bosnia and Herzegovina. Our analysis shows that, although benthic diatom richness is high in the bay, taxa are apparently not distributed evenly temporally. This is most likely due to the discrete design of the surveys, as many of the rare taxa may occur commonly in unexplored localities of the Adriatic Basin. Also, consistent quantitative and qualitative data are still needed for a better determination of the seasonal and spatial changes of the epilithic assemblages in the region.

From a purely scientific standpoint, it will be intriguing to quantify their contribution to the flow of energy and cycling of material in the bay. More practically, these data will be useful for a rational management of this important regional resource. Namely, the bay is part of a complex ecosystem of the wider area of the Mali Ston Bay (Croatia), which is an important part of the region's natural heritage and they must in fact be protected according to international obligations. Thus, sustainable land management must ensure protection of the centuries-old cultivation of shellfish *Ostrea edulis* in whole of Mali Ston Bay (VILIČIĆ, 2017).

AUTHOR CONTRIBUTIONS

N.J. designed the study and wrote the manuscript, D.H. conducted the field sampling, prepared the samples for counting and analysed the samples using the light microscope, A.C. performed scanning electron microscopy analysis. All authors commented on the manuscript.

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