Blue Diatoms: Global Phenomenon of ″Greening″ in Shellfish and Record of Planktonic *Haslea* Species in the South Adriatic Sea

Plave dijatomeje: globalni fenomen ″zelenih″ školjkaša i nalaz planktonske dijatomeje roda Haslea u južnom Jadranskom moru

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Summary

Diatoms are unicellular, photoautotrophic eukaryotic microorganisms, often forming colonies and can be found in most aquatic and moist terrestrial habitats. All known diatoms today have specific golden-brown pigment fucoxanthin that masks chlorophylls in diatom plastid, but one genus represents an exception, having the additional specific pigment marennine due to whom the cells appear blue. Blue diatoms from genus *Haslea* cause a global phenomenon of ″greening″ in shellfish (mostly oysters) affecting them in both positive and negative ways. In this study, historical and recent review regarding blue diatoms and physiological and behavioural effect of marennine as well as challenges in shellfish farming from diatom perspective is addressed. This study is also a first record of blue *Haslea* diatom in the South Adriatic Sea during BIOTA (Bio-tracing Adriatic Water Masses) cruise in March 2016. Investigated blue diatom was cultured in laboratory and morphologically analysed with light microscopy. Diatom investigations are very important for better understanding of the ecology of specific marine area, but also for the economy, aquaculture and tourism. The emergence of green coloured flesh of shellfish in the Adriatic Sea has not been recorded yet, but this finding of the blue diatom from genus *Haslea* does not rule out this possibility in the future.

KEY WORDS
blue diatoms
marennine
shellfish
Haslea
South Adriatic Sea

Sažetak

Dijatomeje su jednostanični, često kolonijalni, fotoautotrofni, eukariotski mikroorganizmi koje nalazimo u gotovo svim vodenim i vlažnim kopnenim staništima. Većina poznatih dijatomeja je karakterističnog zlatno-smeđeg obojenja zbog pigmenta fukoksantina, dok se jedan rod izdvaja jer sadrži još i pigment marenin koji stanice čini plavo obojenima. Plave dijatomeje iz roda Haslea uzrokuju globalni fenomen ″ozelenjavanja″ mesa školjkaša (uglavnom kamenica), a sam fenomen ima i pozitivne i negativne utjecaje na školjkaše. U ovom radu prikazan je povijesni i sadašnji pregled plavih dijatomeja te fizioloških i bihevioralnih učinaka marenina na komercijalno važne školjkaše, a uz plave dijatomeje dan je i pregled izazova u uzgoju školjkaša iz općenite perspektive dijatomeja. Ovaj rad je ujedno i prvi nalaz plave dijatomeje roda Haslea u južnom Jadranskom moru tijekom BIOTA (Bio-tracing Adriatic Water Masses) istraživanja u ožujku 2016. godine. Jadranska plava dijatomeja uzgojena je u laboratoriju, a njezina morfologija je analizirana uz pomoć svjetlosnog mikroskopa. Istraživanja dijatomeja su, osim za bolje razumijevanje ekologije specifičnih morskih područja, izuzetno značajna također i za privredu, uzgoj školjkaša i turizam. Pojava zeleno obojenog mesa školjkaša u Jadranskom moru dosad nije zabilježen, no ovaj nalaz plave dijatomeje roda Haslea tu mogućnost u budućnosti ne isključuje.

KLJUČNE RIJEČI
plave dijatomeje
marenin
školjkaši
Haslea
južno Jadransko more

1. BEHIND THE SCENES: DIATOMS / *Zakulisno: dijatomeje*

Plankton includes organisms that are carried by water currents and are distinguished as phytoplankton (photoautotrophic, mixotrophic or heterotrophic organisms), zooplankton and bacterioplankton depending on their trophic preferences. Photoautotrophic phytoplankton includes microscopic algae that use sunlight and CO_2 to perform photosynthesis and create simple sugar molecules (glucose) and O_2 as a by-product. Diatoms (Bacillariophyta) are mostly photoautotrophic organisms that are usually single-celled but can often form colonies. They are usually called golden-brown microalgae

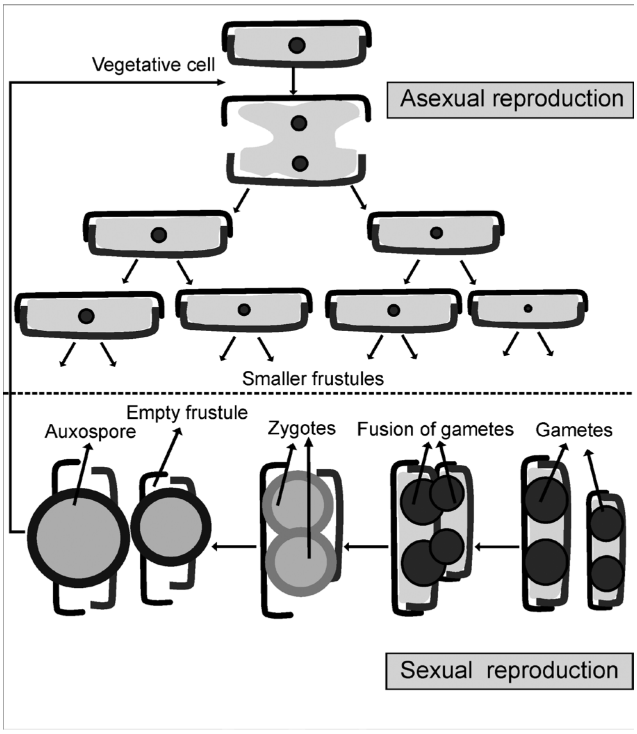

Figure 1 Reproduction in pennate diatoms.
Slika 1. Razmnožavanje penatnih dijatomeja

due to their pigmentation -chlorophyll *a* and *c*, masked by fucoxanthin, diadinoxanthin and diatoxanthin. Concerning phytoplankton, diatoms are most successful group obtaining more than 20% of world's carbon fixation which in total exceeds carbon uptake by rain forests. The unique hallmark of diatoms is the specially silicified cell wall, called frustule, which consists of two halves unequal in size, the epitheca and the hypotheca, that are held in place by silicified girdle bands, and which present a great variety of size and shapes [33]. Diatoms reproduce by mitosis, and when a cell undergoes mitosis, each daughter cell receives one of the two valves of the frustule from the parent cell. The inherited valve is used as the epitheca of the frustule, leaving daughter cell to synthesise its own hypotheca. As a consequence, one daughter cell is identical in size to parental cell, while the other one is smaller, a phenomenon that usually leads to a reduction in the average cell size of the population and to its die-out. After the reduction in the average cell size, diatom cell usually undergoes a sexual phase of reproduction in which zygote turn into auxospore that expands forming the initial cell and restoring the maximal specific cell size (Figure 1). Conventionally, diatoms are divided into two groups based on valve symmetry and their mode of sexual reproduction: centric forms which are radial symmetric and oogamus (i.e. they produce small motile male gametes and large non-motile female gametes) and pennate forms which are boat-shaped bilaterally symmetric and aplanogamus (they do not release flagellate gametes) (Figure 1). Today, taking into account molecular data, we distinguish three major groups of diatoms: Coscinodiscophyceae (radial centrics), Mediophyceae (multipolar centrics) and Bacillariophyceae (pennate diatoms) [21] (Figure 2).

2. GREENING OYSTERS: FINGERPRINT OF BLUE PENNATE DIATOMS / *Zelene kamenice: karakteristični otisak plavih penatnih dijatomeja*

Oysters (Bivalvia, Osteridae), as a shellfish filter feeders, have several important roles by which they help marine ecosystem preserve its balance. Possibly the most important role of oysters is that they are considered to be foundation species of oyster reefs. Also, asoysters are filter feeders, they can greatly influence nutrient cycling in estuarine systems and maintain the stability of the ecosystem. Oysters are economically very important, as a part of mariculture and food industry worldwide.

The first literature record of green oysters dates back to the 17th century when Thomas Sprat in 1667. observed greening of an oyster growing pond near Colchester, England [35]. He also observed that oyster's gills turn green after the pond turned green, and have hypothesised how combined action of sun and earth led to a green colouration of pond sediment. Later on, other studies mentioned more abiotic factors as possible explanations for greening oysters: disease of shellfish similar to obesity [27], ˝liver malfunction˝ [37], oyster ingestion of Priestley's green matter (aggregation of algae firstly described as ˝vegetable˝ responsible for production of oxygen)[38], presence of specific metallic ions in pond sediment, especially copper and zinc [11]. No matter which reason for greening effects took place at that time, green oysters were gastronomically very famous in France and have been celebrated as a delicacy fit to a

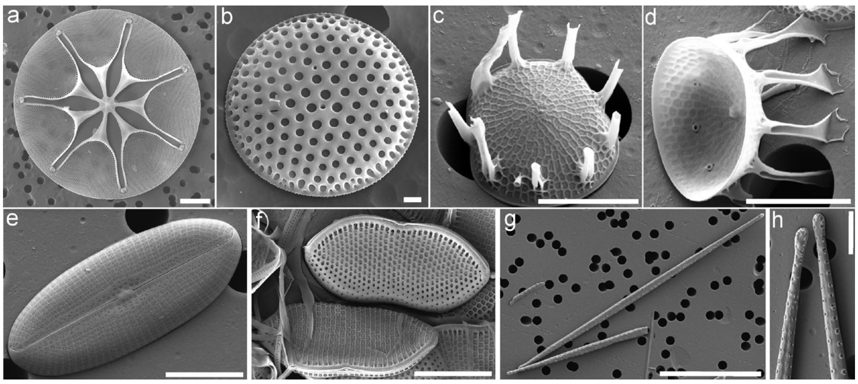

Figure 2 SEM micrographs of three major groups of diatoms: a, b - Coscinodiscophyceae (radial centrics); c, d - Mediophyceae (multipolar centrics); e, f, g, h - Bacillaryophyceae (pennate diatoms). Scale bars: 30 µm (g), 10 µm (a), 5 µm (e, f, h), 3 µm (c, d), 1 µm (b) Photos credit: Carlos E. Wetzel and Sunčica Bosak.

Slika 2. SEM mikrofotografije triju glavnih grupa dijatomeja : a, b - Coscinodiscophyceae (radijalne centrice); c, d - Mediophyceae (multipolarne centrice); e, f, g, h - Bacillaryophyceae (penatne dijatomeje). Mjerilo: 30 µm (g), 10 µm (a), 5 µm (e, f, h), 3 µm (c, d), 1 µm (b). Fotografije izradili: Carlos E. Wetzel i Sunčica Bosak.

king – at the very last for the ˝Sun King˝; it was one of the Louis XIV's favourite meals [18].

First actual experimental work on the green oysters did Benjamin Gaillon, an officer of French Customs during the Restoration and the early July Monarchy [14]. He scraped shells and microscopically observed, as he said, little green motile worms, and consequently, named them *Vibrio ostrearius*. Almost at the same time, controversially, French botanist, explorer and Dragoons cavalry officer during Napoleonic wars – Bory de Saint-Vincent, classified Gaillon's ˝green worms˝ into his ˝psychodaire˝ kingdom which contained all organisms whose position between animal and plant was unclear [3]. He thus proposed a different name for those organisms – *Navicula ostrearia*, because the shape of organisms on the shells reminded him on naviculoid diatoms. After the introduction of electron microscopy in morphological diatom studies, Simonsen transferred the ˝blue navicula˝ from the genus *Navicula* to *Haslea*, a new genus he created for this purpose, based on specific morphological features of the frustule, and he used *Haslea ostrearia* as a type species [34]. *Haslea ostrearia* (Gaillon) Simonsen 1974. is a tychopelagic diatom species, standing for an organism that can be either benthic or epiphytic but also planktonic [33]. *H. ostrearia* is also a euryhaline species (broad tolerance to salinity changes) and can thrive in high light environments (such as shallow ponds exposed to high UV intensity throughout most of the day). One specific physiological feature that distinguishes *H. ostrearia* from other diatoms is the production of *Haslea*-specific pigment called marennine [30]. Marennine is a water-soluble pigment and based on some biophysical and chemical characteristics is possibly a polyphenolic compound existing in two forms; one intracellular and one extracellular, which differ in their spectral characteristics (UV-visible spectrophotometry, Raman spectroscopy) and molecular weight (10.7 and 0.9 kDa, respectively). Based on pH value, marennine can differ in colour from acidic violet-blue to basic green. Cells of *H. ostrearia* actively secret marennine in surrounding water, eventually colouring it in greenish colour, turning shellfish gills and flesh in green (Figure 3). The natural occurring greening phenomenon of oysters besides in England and France was observed in Denmark, United States, Canada and Australia (Moreton Bay, Great Oyster Bay) [16]. Reporting these greening phenomenon, *H. ostrearia* was thought to be a one, cosmopolite species (Figure 4). However, today there are three known blue *Haslea* diatoms: *H. ostrearia*, *H. karadagensis* (Davidovich, Gastineau & Mouget) described from Karadag Natural Reserve, Crimea (Ukraine) and *H. provincialis* Gastineau, Hansen & Mouget described from the area of Boulouris, France [15, 16, 17]. Nevertheless, new blue diatom species from genus *Haslea* are likely to be found worldwide.

3. MARENNINE: APPLICATIONS AND PHYSIOLOGY OF THE BLUE PIGMENT / *Marenin: primjena i fiziologija plavog pigmenta*

Ever since Edwin Ray Lankester in 1886. discovered a new pigment and named it marennine according to Marennes-Oléron area in western France, marennine is being studied in a wide context: as an autotoxin (associated with cell pathological states); as an allelopathy chemical (inhibiting the growth of some algal species encountered in oyster ponds and modifying inter-specific competition among phytoplankton); as antibacterial and antiviral, antioxidant or anti-proliferative agent [20, 26, 30]. A role in the protection against metals such as copper has also been suggested. The exact structure of marennine is still unknown, although it has been hypothesised to be a polyphenolic compound [30]. Additionally, the observation of the ultrastructure of blue cells demonstrated the abundance of vesicles with a diameter of 5 µm in the cytoplasm along with the accumulation of marennine, which suggests that this pigment is synthesised or stored in these vesicles [24]. Marennine plays a significant role in photosynthesis acting as a physical barrier by modifying light quality and intensity as it passes through the water column. On the other hand, global ˝greening˝ phenomenon in shellfish which is a direct consequence of marennine (in most cases the extracellular form of mareninne) has both positive (green shellfish as a delicacy) and negative impact on this important aquaculture branch [32]. Piveteau (1999) demonstrated that oysters fed with *H. ostrearia* in artificial seawater ponds grow slower compared to those fed with the diatom *Skeletonema costatum* over a long period of time (e.g. 8 weeks) [28]. Yet, the reason for the delay of growth in oysters remains unclear; whether it is due to the poor quality of *Haslea* given or biological activity from the marennine released into the ponds. Prasetiya et al. (2015) investigated changes in the clearance rate (CR - volume of water cleared of suspended particles per unit of time) of *Crasostrea gigas* when is fed with cells of *H. ostrearia* and extracellular form of marennine, and concluded that CR significantly decreases (51 %) when compared to control cell suspension without marennine in water [32]. Prasetiya et al. (2016) also showed that two economically important shellfish – *Mytilus edulis* and *Crasostrea virginica* have the behavioural response to higher concentrations of marennine and both species displayed curtailed valve opening compared to control groups that were not exposed to high concentrations of marennine [31]. This is of an extreme importance for shellfish – when valve openings are curtailed, shellfish cannot completely close its shell, leaving it more fragile for predators. Next important effect of marennine on these shellfish is the scope for growth which was 58% lower in *M. edulis* and 85% lower in *C. virginica* under long-term (8 weeks) exposure to marennine [32]. Oxygen uptake in *C. virginica* is also affected by the higher concentration

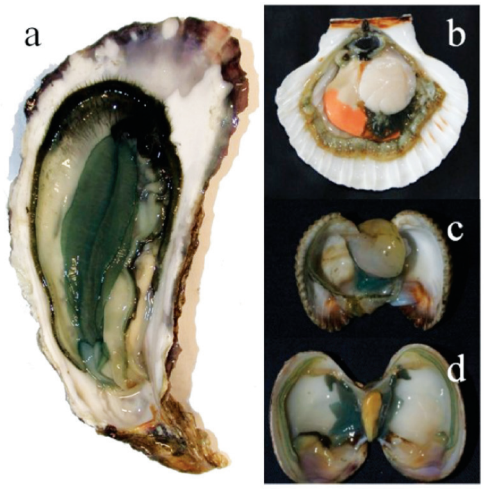

Figure 3 Greening effect of marennine on shellfish. (a) pacific oyster, (b) scallop, (c) cockle, (d) clam.
Adapted from Gastineau et al. 2014.

Slika 3. Zeleni efekt marenina na školjkašima a) pacifičkoj kamenici, b) kapici, c) čančici, d) lisanki Prilagođeno iz Gastineau et al. 2014.

Figure 4 World distribution of *Haslea ostrearia* according to the literature. Each point indicates a site where the presence of *Haslea ostrearia* was assessed from observation of diatom with blue apices, or deduced from the occurrence of green oysters. Map adapted from Gastineau et al. 2014.

Slika 4. Globalno rasprostranjenje dijatomeje Haslea ostrearia prema literaturi. Svaka točka prikazuje mjesto nalaza dijatomeje Haslea ostrearia, direktnim nalazom dijatomje ili posredno nalazovom zeleno obojenih kamenica. Karta preuzeta iz Gastineau et al. 2014.

of marennine - 31.8% lower oxygen uptake while exposed to 2 mg L^{-1} of marennine [32]. Finally, marennine is proved to be negative interactor in the accumulation of energy reserves in shellfish, as both of these economically important species have less ω-3-unsaturated fatty acids accumulated in digestive glands [32]. Future applications of intrinsic blue pigment, marennine, are numerous: food industry (as edible coloration agent), textile industry (as textile or paper paint), antimicrobial or antiviral compound in aquaculture, cosmetic industry (facial lotions with UV protection factor), etc.

4. *HASLEA* IN THE ADRIATIC SEA / *Haslea u Jadranskom moru*

The Adriatic Sea, the northernmost part of the Mediterranean, is a semi-enclosed oligotrophic basin bathymetrically divided into three area: i) the shallow northern Adriatic basin (maximum depth 50 m), ii) central/middle Adriatic basin with depressions up to 280 m and iii) the southern Adriatic basin characterized by a deep Southern Adriatic Pit (SAP) (maximum depth 1230 m). General circulation shaped with two main currents – East Adriatic Current (EAC) which brings highly saline and low-nutrient waters from Ionian and Levantine Seas and Western Adriatic Current (WAC) which carries out large amounts of high-nutrient freshwater from the Po River describes Adriatic Sea as quite heterogeneous marine system with the across-shelf and longitudinal trophic gradient resulting in the asymmetric distribution of the phytoplankton composition, abundance and biomass [29]. In the Adriatic Sea, as well as in world oceans, diatoms are the most abundant counterpart of microphytoplankton. Viličić et al. (2002.) listed 504 species of diatoms in the Adriatic Sea, but we can presume that number is much higher as the discovery of new species happens at a constant rate [40]. Spring phytoplankton bloom is mostly composed of diatoms, especially in the northern Adriatic Sea where they do dominate phytoplankton community all over the year [2]. Due to the oligotrophy of the southern Adriatic Sea, the most abundant primary producers are nanophytoplankton and picophytoplankton, while the higher contribution of diatoms is recorded in closed bays as in Mali Ston Bay or Boka Kotorska Bay [8, 9, 39]. Phytoplankton, especially diatoms are being studied intensively for last two decades in the Adriatic Sea, while genus *Haslea* was documented only two times [23, 25]. Munda (2005.) recorded *H. ostrearia* in the northern Adriatic Sea (Trieste, Italy) during summer period (July and August) and labelled the species as abundant/common on permanent concrete plates that were permanently exposed to fouling, and rare/extremely rare on concrete plates that were scraped and sampled monthly [23]. In the middle Adriatic Sea, *Haslea* spp. was reported on various substrates such as iron, painted iron, wood and concrete [25].

In order to determine bio traces of the Adriatic Sea Water masses and optimise a method of their fast detection, two winter cruises (February/March 2015. and March 2016.) with research vessel ˝Naše more˝ were performed in the South Adriatic Sea. Water samples were collected at selected stations (transect from Dubrovnik to isobath of 1000 m) with 5 L Niskin bottles and phytoplankton net at depths determined *in situ* based on respective CTD profile (Figure 5). Whole phytoplankton community was analysed quantitatively with Utermöhl method. In both years the microphytoplankton community was largely dominated by diatoms, with maximal abundances slightly higher in 2016 than in 2015, 1.8×10^{4}, cells L^{-1} and 1.2×10^{4} cells L^{-1}, respectively. In 2015 we observed a somewhat unusual number of diatom cells at greater depths, up to 500 m, probably

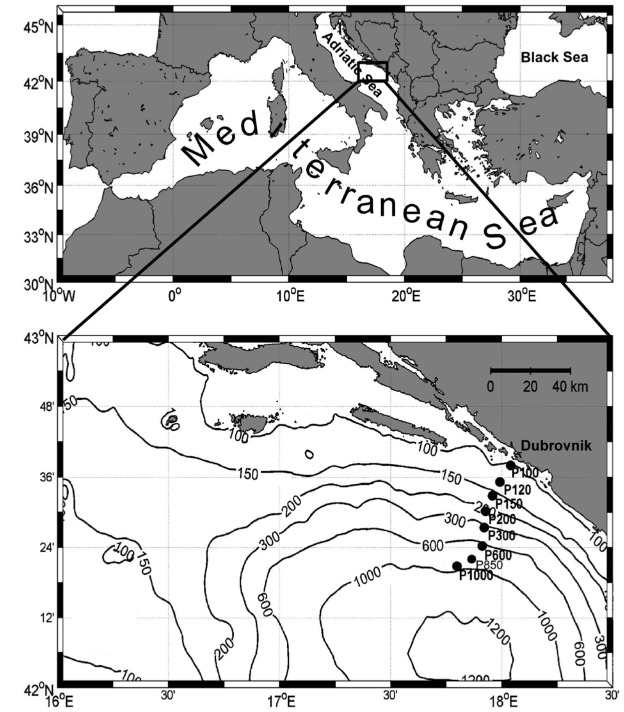

Figure 5 Map of investigated area with stations.
Slika 5. Karta istraživanog područja sa postajama

due to the phenomenon of vertical convection, while the following year the diatoms were only present in the euphotic layer [5]. The finding of particular blue *Haslea* species occurred only in 2016. despite the fact that genus *Haslea* represented by other species was documented in both years.

In addition to standard phytoplankton methods, we took live net samples for establishing diatom laboratory cultures. Immediately upon an arrival in the laboratory, samples were inoculated in Guillard's f/2 Marine Water Enrichment Solution (Sigma-Aldrich, United Kingdom), and after acclimatisation, individual cells were manually isolated in monoclonal cultures. Cells of *Haslea* sp. were at first recognised as belonging to genus *Navicula* due to the naviculoid shape of frustules, but after two weeks of growth in monoclonal culture, the cultures appeared blue-green in colour. Investigated *Haslea* clone BIOTAII-43 was isolated from coastal station P150 (42°32' N; 17°59' E). After more detailed microscopic observation, it was observed that cells in culture have blue apices indicating genus *Haslea* and synthesis of marennine (Figure 6). During a frequent examination of Adriatic *Haslea* sp. cultures, it was observed that with the maturation of cells more marennine was synthesised and accumulated at cells apices. Additionally, aggregation of cells in blue-green floccules appeared at the bottom of the flasks approx. every two weeks. In order to identify Adriatic clone, further morphological examinations were done: light microscopy on live and cleaned diatom material. Cleaning of diatom frustules from organic matter is crucial for the morphological examination due to the necessity of measuring and observing different morphological features of the frustule (i.e. striae, areolae, raphe, central and apical nodes, helictoglossa…). The samples were firstly rinsed with distilled water, followed at the addition of the equal amount (approx. 5 mL) of saturated $KMnO_4$ (or diluted 50%) for oxidation of organic matter and left for 24 hours. The next day an equal amount of concentrated HCl was added, gently heated over a flame and then rinsed again with distilled water five times. Permanent slides were prepared by drying cleaned material on coverslips and mounting in Naphrax. Light microscopy observations were performed with Zeiss AxioVert 200 inverted microscope equipped with DIC and phase contrast (for cleaned material) and Olympus BX51 light microscope (for live material). Minimally 30 frustules were examined for morphometric analyses. The average cell length and width of Adriatic *Haslea* sp. were 89.60 ±1.05 µm and 11.23 ± 0.3 µm, respectively. Compared with other *Haslea* species, Adriatic *Haslea* sp. is larger, as *H. ostrearia* (the largest recorded blue diatom) is 71.8 ± 1.7 µm long and 7.3 ± 0.1 µm wide. Other two blue diatoms – *H. karadagensis* and *H. provincialis* are also smaller than Adriatic *Haslea* sp. - 52.5 ± 0.1 µm and 65.8 ± 0.1 µm in length and 8.0 ± 0.03 µm and 7.4 ± 0.1 µm in width, respectively [17]. After all morphological analyses, the Adriatic clone of genus *Haslea* could only be identified to the genus as *Haslea* sp. (Figure 6). More detailed morphological studies (examination with scanning electron microscopy) and molecular analyses are needed for identification to the species level.

We can hypothesise why blue *Haslea* species haven't been reported in the South Adriatic Sea yet: use of phase-contrast in light microscopy (specific marennine colour remains unrecognised), low number of *Haslea* diatoms in field samples, sampled cells not mature enough to start producing marennine, etc. Giving in mind that previous documentations of *Haslea* in the Adriatic Sea were from northern and middle Adriatic Sea, this study is even more valuable as this is the first record of *Haslea* sp. in the South Adriatic Sea. Studies regarding marine phytoplankton, especially diatoms are important for better understanding of ecosystem in general. Diatoms are good bio-indicators that can inform us about the trophic state

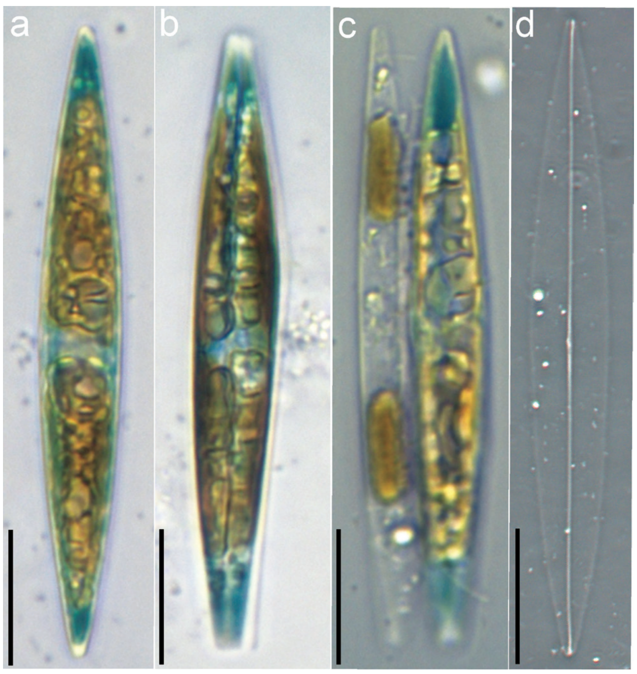

Figure 6 Light micrographs of the Adriatic clone BIOTAII-43 *Haslea* sp. a, b, c - live cells with marennine; b, c – cells in vegetative division; d – light micrograph of cleaned *Haslea* sp. frustule. Scale bar = 20 µm

Slika 6. Svjetlosne mikrofotografije Jadranskog klona BIOTAII-43 Haslea sp. a, b, c - žive stanice s mareninom b, c –stanice u vegetativnom dijeljenju; d – svjetlosna mikrofotografija očišćene Haslea sp. frustule. Mjerilo = 20 µm of the environment, primary production rate, and indirectly, enable estimation of ecosystem sustainability. Understanding the link between primary producers (e.g. diatoms) and higher trophic levels (e.g. shellfish) can generate better economic and environmental outcome. At last, from the scientific point of view, studying diatoms is of an extreme importance worldwide, as these organisms have a high impact on carbon fixation and carbon injection in deeper oceans, which is today burning question of climate change that we all want to reduce.

5. CHALLENGES IN SHELLFISH FARMING FROM DIATOMS PERSPECTIVE / *Izazovi u uzgoju školjkaša iz perspektive dijatomeja (algi kremenjašica)*

Over the last 60 years, world aquaculture production has greatly increased from about 20 million tonnes in 1950. to 150 million tonnes in 2010. while production of marine shellfish presently accounts for 75% of global marine aquaculture [13]. Probably the most important factor that needs to be considered that when defining a link between phytoplankton and filter feeders is that shellfish are non-selective filter feeders, meaning they filter particles just based on their size, not type. Consequently, all shellfish use phytoplankton as their main food source while phytoplankton's primary production and growth depends on various environmental factors such as nutrient availability, light (turbidity) and temperature. This is particularly important as many studies showed when shellfish are grown under similar conditions at various sites; up to 85% of any difference in growth observed can be attributed to water temperature and primary production [19]. Additionally, shellfish can exert ˝top-down˝ grazer control on phytoplankton and in the process of raising turbidity, thereby increase the amount of light reaching the sediment surface and sustain favourable growth conditions for seagrass or benthic algae. In some situations, shellfish can also exert ˝bottom-up˝ control on phytoplankton production by changing nutrient regeneration processes within the sediment [19]. Therefore, continuous monitoring of shellfish farms is necessary to monitor environmental factors such as temperature, oxygen concentrations and nutrient availability and composition and variability of phytoplankton.

In most studies, diatoms have been recognised as the main component of phytoplankton as the available food source for shellfish. An example is the study of Mediterranean Thau Lagoon, where *Ostreococcus tauri*, a minute picoeukaryote (cells smaller than 1µm) is responsible for most of the primary production in the summer, but such small pico particle is not efficiently retained by the gills of shellfish, particularly oysters, so oysters mainly use micro-fraction (diatoms) for food [12]. Generally, diatom species are considered to be a highly nutritious class of microalgae and many species have been shown to promote survival and growth of shellfish [12]. Commercially important *M. edulis* has been shown to capture diatoms from the genus *Phaeodactylum* in preference to smaller and larger natural particles, and similarly, oyster *C. gigas* captures the diatom, *Nitzschia closterium*, preferentially when compared to larger phytoplankton [1, 5]. This can be explained by differences in cell shape or flexibility; in particular, elongated or tri-radiated cells (such as diatoms from genus *Nitzschia* or *Phaeodactylum*) may be more efficiently retained in the shellfish gills than spherical particles of the same volume. Another possibility is that actively swimming cells interact with the ctenidium of some shellfish species in a fundamentally different way. Bricelj et al. (1998) used video endoscopy to study capture and transport of toxic and non-toxic phytoplankton by the ctenidia of two shellfish species [7]. One interesting finding was the difference in how the diatom, *Thalassiosira weissflogii* (11 µm diameter) and toxic and non-toxic strains of the dinoflagellate (*Alexandrium* spp.; 35 µm length) were handled by the ctenidium of *O. edulis* [7]. In contrast to diatoms, dinoflagellates were not retained on the frontal surface of the ctenidium [7]. Not only planktonic diatoms are important food for shellfish - Cognie et al. (2001.) fed four pennate benthic diatom species to the oyster, *C. gigas*, and found that oysters filtered a significantly higher proportion of two intermediate size diatoms (35 – 45 µm length) compared to the smallest (22 µm length) and largest (60 µm length) diatom [12]. This can be particularly important at coastal areas where benthic diatom flora is more diverse than in water column, and therefore can explain preferable ingestion of naviculoid diatoms such as *Haslea* which are in most cases epipelic or epiphytic diatoms.

Shellfish aquaculture in Croatia has more than 50 registered farmers of different shellfish species, but mainly mussels and oysters. Only three breeders have more than 100 tonnes of annual increase, while the rest of them are small farmers, possibly due to the collection of larvae from natural populations to collectors (bypassing larvae controlled production), which has been main breeding method [6]. Regarding only oyster aquaculture, unfortunately it does not even sustain needs for this delicacy during a touristic season (we produce approximately 150 tonnes per year). Most recognisable and prominent nursery on east Adriatic shore is Mali Ston Bay in which oyster farming started a long time ago (the first record is from XVI. century) and where more than 50 farmers are registered [36]. According to the phytoplankton abundance values and phosphate concentrations, the bay has been qualified as moderately/naturally eutrophicated ecosystem [39].

So far, there has been no record of the greening of shellfish in any of the published monitoring programs and studies on shellfish nurseries in the eastern Adriatic Sea. The reason for the lack of data for *Haslea* occurrence could be that *Haslea* species can easily be mistaken with *Navicula* species due to many of morphological similarities. It could be hypothesised that their abundance had never reached values sufficient for the greening effect to take place. Nevertheless, this neglecting of blue *Haslea* diatoms does not need to be amiss since greening phenomenon is not harmful and *Haslea* species are not toxic.

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