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EPIPHYTIC DIATOM COMMUNITY AND CALCIUM CARBONATE CRYSTALS CHARACTERISTICS ON THE SURFACE OF FRESHWATER *Ulva* THALLI[1]

Beata Messyasz, Marta Pikosz, Andrzej Rybak, Karolina Łepkowska

Department of Hydrobiology, Institute of Environmental Biology, Adam Mickiewicz University
Umultowska str. 89, 61-614 Poznań, e-mail: messyasz@amu.edu.pl

Summary. On the surface of the freshwater form of *Ulva* thalli the incrustations process takes place which resulted in the precipitate of calcium carbonate crystals ($CaCO_3$). At the same time, this area is also inhabited by large numbers of periphytic communities, mainly by diatoms. Siliceous frustules and calcite crystals are responsible for the thalli roughness in all stages of its growth. Diatom communities colonizing young, thin thallus of *Ulva* were dominated by *Cocconeis placentula* and *Melosira varians*, and mature thalli by *Cocconeis placentula* and *Navicula cryptocephala*. The obtained data show that the surface roughness of thalli depends on the participation of calcium carbonate in the dry matter of *Ulva* and diatoms content does not change significantly with age of the macroalga thallus.

Key words: thalli surface, macroalgae, *Ulva*, periphyton, calcium carbonate, roughness

INTRODUCTION

The level of settlement aquatic plants by epiphytic algae and diversity of periphyton communities depends on the type of surface [Straškraba and Pieczyńska 1970, Ondok 1978]. Periphytic diatoms could overgrown rocks, fragments of submerged plants and the surface layer of sediment [Kitner and Pouličková 2003, Pouličková *et al*. 2004, Messyasz and Kuczyńska-Kippen 2006, Messyasz *et al*. 2009]. Until now, only a few studies had been conducted about periphytic structure on the surface of macroalgae and mainly concerned on *Chara* [Messyasz and Kuczyńska-Kippen 2006, Messyasz *et al*. 2009] and *Cladophora* thalli [McSheffrey and McCafferty 1991]. Except diatoms, which dominate on

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the surface of macroalgae thalli, may also occurs green algae and cyanobacteria, which do not tent to attach to the substrate. One time research about species composition of epiphytic diatoms was conducted on the thalli surface of marine *Ulva* [Hasiuk-Krzak and Zgrundo 2008]. Freshwater populations of green algae from the *Ulva* genus may achieve a large size (up to 2 meters) and constitute a substrate for the development of many epiphytic species [Messyasz *et al*. 2009, Messyasz and Rybak 2010]. The development of particular groups of algae in shallow water bodies depend on not only from the intensity of solar radiation, but it is also related with habitat conditions in the water [Reynolds 2006]. Dense patches of *Ulva* thalli may limited the availability of light for planktonic algae, but floating in the surface layer of water provide enough light intensity to development of epiphytic diatoms.

A significant part of marine and some of freshwater algae performs calcification process – precipitation of calcite ($CaCO_3$), which makes the surface becomes less smooth and more fragile [Borowitzka 1984]. In freshwater ecosystems *Chara* thalli are the main producers of calcium carbonate, where degree of encrustation may exceed even 80% [Pentecost 1984]. Scanning electron microscopy (SEM) investigations show that on the surface of *Ulva* thalli crystal bands of $CaCO_3$ are present [Messyasz *et al*. 2009, 2010], moreover Starmach [1972] and Pliński [1988] recognized the roughness of *Ulva* thalli as an important diagnostic feature in the taxonomic identification.

The floating large mats of freshwater *Ulva* were recorded in the Nielba river since 1993 [Messyasz 2009]. Alkaline pH is a characteristic feature for water of this small, lowland river, which could lead to intense precipitation of $CaCO_3$ on the surface of *Ulva* thalli. The size of the substrate surface is also important in the development of periphytic communities. The young thalli of freshwater *Ulva* are narrow and long, while mature are both wider and shorter. Therefore, calcification process and the rate of colonization by diatoms may depend on the stage of development of macroalgae. The main aim of the study was to compare the abundance and taxonomic composition of diatom communities on the surface of young and mature *Ulva* thalli. Followed, the relationship between the quantity of calcium carbonate and epiphytic diatoms, which are responsible for the roughness of the thalli were determined.

MATERIAL AND METHODS

Samples of the green algae from the *Ulva* genus were collected at 6 stations in the summer 2010 and in August 2011 from the Nielba river (Wągrowiec, Wielkopolska region) (Fig. 1) between Janowiecka street and the crossing of Nielba and Wełna rivers.

At the examined site physico-chemical parameters of water in Nielba river were study. Parameters such as: temperature (°C), pH, dissolved oxygen (%), electrolytic conductivity ($\mu S \cdot cm^{-1}$), TDS (total dissolve solids $mg \cdot l^{-1}$) and NaCl

($mg \cdot l^{-1}$) were measured using multiparameter probes *YSI*, model *Professional Plus*.

Content of Ca and Mg in the water and thalli were carried out at the Department of Supramolecular Chemistry, Faculty of Chemistry, Adam Mickiewicz University in Poznań. Quantitative analysis of thalli and water samples were studied using XRF spectroscopy and quantitative analysis were carried out using ICP-MS spectrometry. Prior to measurement the concentration of Ca and Mg samples were drying and mineralization. This process involves of a two stage of digestion by pressure in a closed MarsXpress oven.

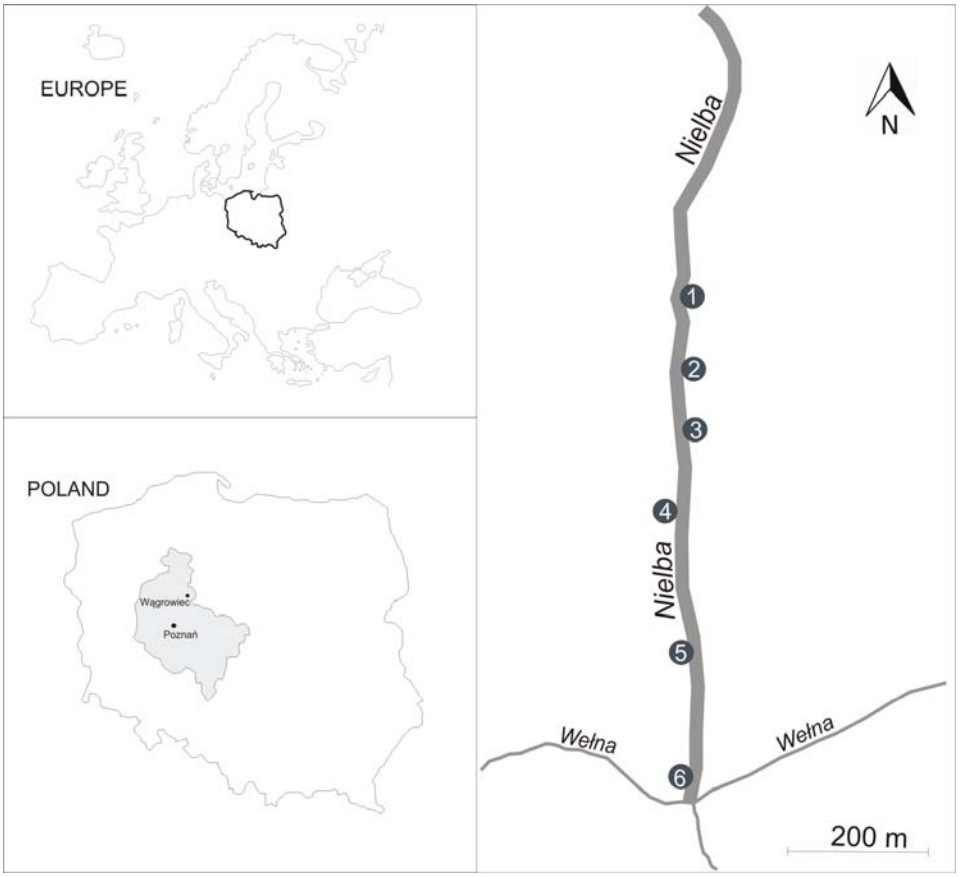

Fig. 1. The study area in the Wielkopolska Region (Nielba river), showing the locations of the sampling stations

Based on the length, width and color of thalli each of the collected macroalgae were separated into young, middle-mature and mature. Among the carefully grouped thalli wet weight of green algae were weighed (about 2 g) on a laboratory weight. Then weighted samples were dried at 105°C to weight stabilized. Method of determining participation of crystals and diatoms on the surface of *Ulva* thalli consisted of adding 10 ml of hydrogen peroxide (H_2O_2) into tubes

with dry mass of algae. The tubes were placed in a cylinders to half filled with water. To decomposition of organic matter were using H_2O_2 at 98°C for 8 hours. The remaining dry inorganic matter (diatom frustules and calcium carbonate crystals) was weighted and then to the separation them, $CaCO_3$ crystals were dissolved in 10 ml of dilute hydrochloric acid (HCl). Samples were re-weighted and based on the difference in the mass the contents of each fraction of the dry matter were determined.

Permanent preparations of purified diatoms were prepared using Naphrax. Then taxonomic analysis of diatoms were performed using light microscope Axioskop 2 MOT Zeiss. Terminology of recorded diatoms were adopted according to Krammer and Lange-Bertalot [1986–1991]. Photographic documentation was carried out in the Laboratory of Scanning Microscopy at Faculty of Biology, Adam Mickiewicz University.

RESULTS AND DISCUSION

Freshwater *Ulva* thalli have a wide range of tolerance to changes in salinity, temperature and light conditions. Mass development of *Ulva* in the Nielba river was observed for the first time in 1993 and then annually until 2012 from May to July, during which the thalli reach 12–17 cm long and 5–6 cm wide [Messyasz 2009, Messyasz and Rybak 2009]. In the collected samples period (2010–2011) the water level in the river was low and never exceed 1 m. The pH of water in the Nielba river was neutral, alkaline or slightly acidic (Tab. 1). Young *Ulva* thalli achieved an average 9.0 cm length and 0.3 cm width. In later phases of development algal thalli in the touch were distinctly rough and reached more than 50 cm long and 1.7 cm wide. However, the mature yellow-green thalli were widest, sometimes exceeded 6 cm (Tab. 2).

Table 1. Statement of average values of physical-chemical parameters in the Nielba river water and calcium and magnesium concentrations in thalli and habitat of green algae from the genus *Ulva*

Parameter	Unit	2010 (n = 22)	2011 (n = 3)
temperature	°C	20.8	19.8
O_2	%	95.8	96.76
conductivity	$\mu S \cdot cm^{-1}$	781.4	731.6
TDS[a]	$mg \cdot l^{-1}$	531.1	494.0
pH	-	8.15	8.09
NaCl	$mg \cdot l^{-1}$	585	855
Ca in water	$\mu g \cdot ml^{-1}$	106.8	103.4
Ca in thalli	$\mu g \cdot mg^{-1}$	227.9	210.1
Mg in water	$\mu g \cdot ml^{-1}$	18.3	17.6
Mg in thalli	$\mu g \cdot mg^{-1}$	6.6	5.7

[a] TDS – Total Dissolved Solids

Table 2. The percentage share of calcium carbonate and periphytic diatoms on the surface of examined green algae thalli in various stages of growth

Parameter	*Ulva* sp.		
	young thalli	middle-mature thalli	mature thalli
Lenght, cm	3.20–24.50 (9.00)	0.80–56.50 (17.50)	1.60–72.20 (16.70)
Width, cm	0.10–0.90 (0.30)	0.40–4.50 (1.70)	0.70–7.00 (2.70)
$CaCO_3$, %	22.58–68.81 (48.83)	16.46–61.69 (44.12)	21.26–65.44 (48.59)
Diatoms, %	3.71–26.60 (12.22)	3.18–14.49 (8.96)	4.40–16.23 (9.20)

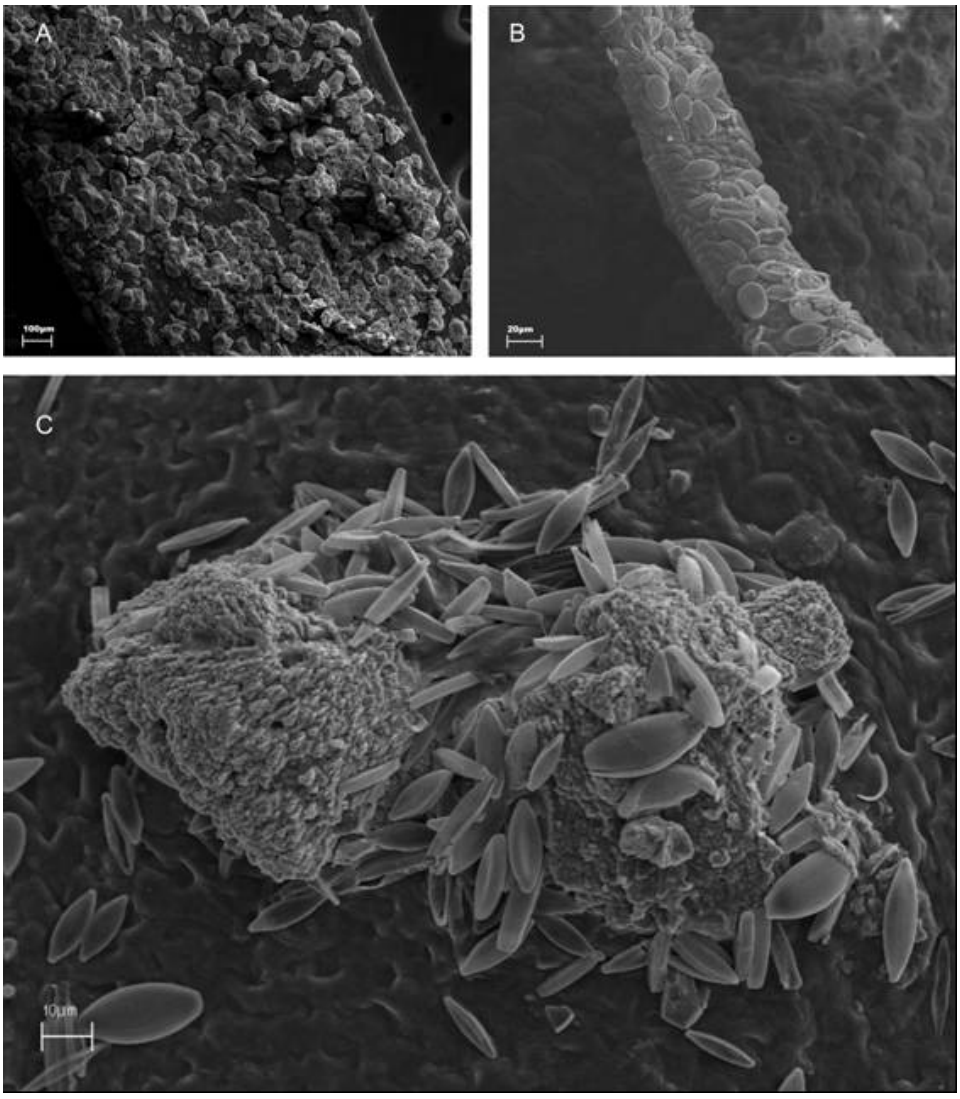

Fig. 2. Composition on the surface of freshwater *Ulva* thalli: A – crystals of calcium carbonate, B – periphytic diatoms, C – bands of crystals and periphytic diatoms

Ca ions are dominant in water environments and there is a balance in the system calcium – carbonic acid [McConnaughey and Falk 1991, McConnaughey 1997, Suzuki 1998]. Calcium ions associated with a carbonic acid in result formed soluble calcium bicarbonate. However, the biological activity disrupts this process, because plants used bicarbonate to photosynthesis and in the result on the surface precipitation poorly soluble calcium carbonate. Obtained results show that in dry weight of *Ulva* is about 33 times more calcium than magnesium (Tab. 1). The high degree of extracellular calcium carbonate precipitation by *Ulva* may contribute to biogenic decalcification in the Nielba river, resulting in the reduction the level of this element in the surrounding water. In our study, there was a big difference in the concentration of Ca in water (about 100 $\mu g \cdot ml^{-1}$) and in *Ulva* thalli (about 200 $\mu g \cdot mg^{-1}$ DW). Green algae take calcium ions from the water, there is precipitation no soluble in water $CaCO_3$ and calcium becomes biologically unavailable for a longer period of time. The average concentration of calcium in the green algae was almost two thousand times higher than in water, in which the examined thalli developed.

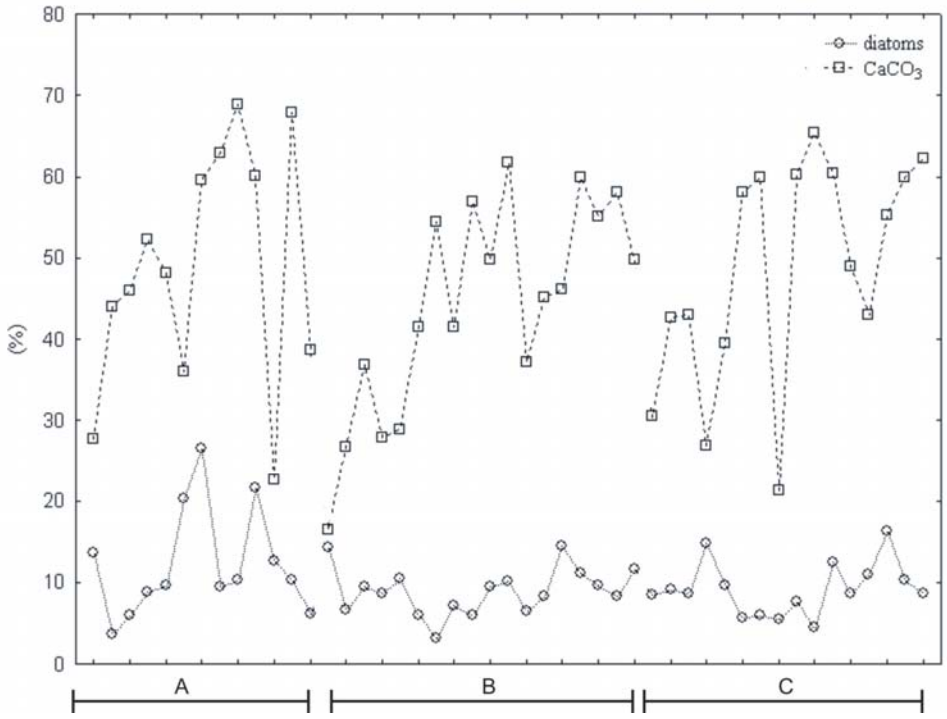

Fig. 3. The proportion between the percentage of calcium carbonate crystals and epiphytic diatoms in the dry weight of alga *Ulva* in its various stages of thalli growth: A – young thalli, B – matured thalli, C – aging thalli

SEM's analyzing show that both crystals and diatoms occupy a significant part of thalli surface (Fig. 2A, 2B). For the diatoms substrate may be not only *Ulva* cells, but also the crystals precipitated on the algal surface (Fig. 2C).

By comparing the data of crystals and diatoms participating in the dry matter was clearly indicated a trend that together with decreases in the crystal quantity at the same time increasing number of diatoms, and inversely (Fig. 3). The largest increase of crystal numbers was in young thalli. Precipitation of calcium carbonate was associated with an intense process of photosynthesis, designed to preserve carbonate equilibrium. In the case of diatoms, the situation was similar, the largest participation was recorded in young thalli (12.2%) and less than 2–3% in mature (9.2%). This may be due to the fact that on the young (light green) thalli is more free space to colonize. In the case of middle-mature (live green) and mature (yellow green) green algae amount of both diatoms and crystals was more or less uniform (Tab. 2). The results are in accordance with earlier studies of Messyasz *et al*. [2010] conducted for *Ulva prolifera* (O.F. Müller), which showed that diatoms accounted for approximately 13% and calcium carbonate 48% dry weight of algae.

Surface structure of the substrate (stability, hardness) may be a favoring factor for colonization by diatoms. The entire structure of *Ulva* thalli were covered by calcium carbonate and epiphytic species. This study indicated, that the thalli of freshwater *Ulva* were inhibited by 62 taxa of diatoms. Literature data show that the *Ulva* thalli were primarily overgrown by species such as: *Cocconeis placentula* Ehr., *Achnanthidium minutissimum* (Kütz.) Czarnecki and *Fragilaria* spp. [Messyasz *et al*. 2009, Wetherbee *et al*. 1998]. Other species of diatoms appear later with the increase of periphyton layer in thickness and were represented by: *Asterionella, Cyclotella, Cymbella, Diatoma, Melosira, Navicula, Nitzschia* and *Gomphonema* [Kawecka and Eloranta 1994]. During analysis it was observed that *Achnanthidium minutissimum* (Kütz.) Czarnecki was recorded only on the surface of narrow and young thalli. It was the same with taxa from *Fragilaria* genera, which were both on the young and mature thalli, but in small numbers. *Cocconeis placentula* (Fig. 2B, Tab. 3) is a diatom, which colonized as the first the substrate, occurred both on the young and mature thalli, suggests that this species has a good conditions for overgrowing on all of macroalgae surfaces. It was also noted, that *Ulva* was rapidly colonized, because the number of diatom taxa was higher at the mature (55.17% of the total number of cells), than at the young thalli (43.81%). Other diatom species were accompanying *Cocconeis placentula*, but their number was definitely smaller. This species probably choose plant as substrate, what allow unlimited access to different organic substances secreted by plant [Gumiński 1990, Lee 1999]. The rate of colonization by diatoms in the Nielba river was higher on the macrophytes (*Callitriche* sp., *Potamogeton Crispis)*, than on the free-floating *Ulva* thalli, probably it was caused by strong current, which detached diatoms [Łepkowska 2012].

Table 3. The structure of the dominant diatom species and numerously represented in the epiphytic communities on freshwater *Ulva* thalli in various stages of growth in the Nielba river (values refer to the percentage of the total number of cells; bold highest values within the examined algae thalli)

Taxon	*Ulva* thalli from Nielba river		
	Young, %	Middle-mature, %	Mature, %
Cocconeis placentula Ehr.	**43.81**	**55.17**	**44.91**
Melosira varians Ag.	**12.78**	**9.16**	5.36
Navicula cryptocephala Kütz.	**6.68**	**9.66**	**11.38**
Gomphonema olivaceum (Horn.) Brėb.	5.93	**8.49**	2.75
Gomphonema parvulum (Kütz.) Kütz.	5.10	5.66	**5.19**
Diatoma tenuis Agardh	4.51	2.43	1.45
Gomphonema olivaceum (Horn.) Brėb.	3.82	**8.49**	2.55
Fragilaria biceps (Kütz.) Lange-Betalot	3.82	-	-
Cyclotella menighiniana Kütz.	2.05	**12.26**	**6.48**
Navicula cincta (Ehr.) Ralfs)	1.10	1.48	**5.82**
Achnanthes linearis (W. Sm.) Grunow	-	3.30	1.85
Fragilaria ulna (Nitzsch) Lange-Bertalot	-	2.83	**6.02**
Gomphonema acuminatum Ehr.	-	0.55	**4.73**

Except *Cocconeis placentula* in dominant diatom communities on the surface of *Ulva* thalli were also *Melosira varians* Ag. (young thalli), *Navicula cryptocephala* Kütz. (middle-mature and mature thalli), *Cyclotella menighiniana* Kütz. (middle-mature and mature thalli) and *Gomphonema olivaceum* (Horn.) Bréb. (mature thalli) (Tab. 3). Periphytic diatoms were dominated by species such as: *Amphora ovalis* (Kütz.) Kütz., *Cocconeis placentula* Ehr., *Gomphonema olivaceum* (Hor.) Bréb. and *Fragilara ulna* (Nitzsch) Lange-Bertalot, which were also identified on the surface of freshwater *Ulva prolifera* [Messyasz *et al*. 2009]. In the examined forms of *Ulva* the structure of epiphytic communities developed similarly like on the surface of *Ulva prolifera*, but in contrast *Diatoma vulgaris* Bory*, Fragilaria capucina* Desm. or *Navicula cryptocephala* Kütz. on the young thalli of *U. prolifera* were occurred more frequently. On the other hand, the arrangement of dominant diatoms on mature thalli was similar in both cases and included: *Cyclotella meneghiniana* Kütz. and *Gomphonema acuminatum* Ehr.

It was also observed the rapid rate of diatoms succession, because on the surface of young *Ulva* thalli occurred significantly less Bacillariophyceae species (20–25), while on the mature forms were more than 40 taxa stated. Analyzing the taxonomic structure of periphyton some characteristic species for particular development phase of thalli has been identified. Only on the young *Ulva* thalli were: *Cyclotella cyclopuncta* Hak. & Carter, *C. radiosa* (Grun.) Lemm., *Cymbella turgidula* Grun., *Fragilaria biceps* (Kütz.) Lange-Bertalot and *Navicula lanceolata* (Ag.) Ehr. On the mature thalli much more frequently grew species of the *Gomphonema* genus*,* which settled by using long and branched stalks. The big number of epiphytic diatoms confirms that *Ulva* is microhabitat for them, because the algal surface is enough stable environment and the competition for light could occur there [Messyasz *et al*. 2009]. Solely on the mature green algal thalli were observed: *Planothidium oesterupii* (A. Cl.) Round & Bukht., *Nitzschia*

incospicua Grun. and *Placoneis gastrum* (Ehr.) Meresch. However, on the middle-mature and mature thalli occurred 12 taxa, which were not reported on the younger forms of *Ulva*: *Cyclotella bodanica* Grun., *Cymatopleura elliptica* var. *nobilis* (Hant.) Hust., *Cymatopleura solea* (Bréb.) W. Smith, *Cymbella lanceolata* (Ehr.) Kirch., *C. prostrata* (Berk.) Cleve, *Fragilaria construens* (Ehr.) Grun., *F. crotonensis* Kitton, *F. dilatata* (Bréb.) Lange-Bertalot, *Gomphonema affine* Kütz., *Hantzschia amphioxys* (Ehr.) Grun., *Meridion circulare* Ag. and also *Nitzschia amphibia* Grun.

Considerable variation in the characteristic species structure for young and mature thalli may be associated with the colonization ability by diatom species and indirectly may influenced on degree of *Ulva* roughness in the touch. Diatom *Cocconeis placentula* is sedentary and practically no moving species, which means that entire surface of cells attached to substrate, thus is more difficult to break away. Frustules form a tape system and quickly dominate on the surface of substrate. This coverage substrate surface like „a carpet" does not significantly increasing the rate of roughness of the young thalli, especially that the increase of the number of diatoms was associated with *Cocconeis placentula* and less with members of other taxa. Together with the increase of the surface of *Ulva* thalli increases its colonization, mainly by *Cocconeis placentula,* but simultaneously gradually increase layer thickness of epiphytic communities. Therefore, diatoms show various types of methods of attaching to a substrate, for example: by mucilage stalks (*Gomphonema*), by one end of the valve (*Fragilaria*, *Nitzschia*), by mucilage tube (*Cymbella*) or by cover the entire valve surface (*Cyclotella*, *Navicula*, *Cymatopleura*).

To sum up, the roughness structure of the *Ulva* thalli surface depends mainly from calcium carbonate crystals and to a lesser extent on its growing periphyton, which amount is maintained constant at about 10%. The changes in the species structure of periphyton assemblages were connected with increasing macrogreen algae thalli. The process of calcification is most intense for young thalli and later, with their maturing persists at a high level. However, small variations in the mass of crystals probably result from the increase of their thickness. Small crystals can not express feeling of roughness on young thalli. While the mature forms of *Ulva* become more wrinkled or even curly-bubbled and may be flattened and overgrown by filamentous green algae [Starmach 1972, Pliński 1976, Pliński and Hindák 2012]. Strongly corrugated thalli and changes in the surface structure from a smooth in the direction of bubble of the middle – mature and mature thalli may increase the roughness.

CONCLUSION

Thalli of freshwater *Ulva* provide a good substrate for development of peryphytic diatoms, mainly *Cocconeis placentula,* while photosynthesis process is responsible for the precipitation of calcium carbonate in the form of calcite

crystals. The level of thalli calcification ranges from 16.46 to 48.83% and the degree of colonization surface by epiphytic diatoms is approximately 10%. Research have shown that changes in the structure of epiphytic diatom communities are mainly qualitative less quantitative. Roughness of the surface increased with development of the *Ulva* due to the folding of the thalli during aging. The succession of individual diatoms on freshwater *Ulva* thalli requires further and more detailed research.

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CHARAKTERYSTYKA ZBIOROWISKA EPIFITYCZNYCH OKRZEMEK I KRYSZTAŁÓW WĘGLANU WAPNIA NA POWIERZCHNI PLECH SŁODKOWODNYCH FORM *Ulva*

Streszczenie. Na powierzchni plech słodkowodnych form zielenic z rodzaju *Ulva* zachodzi proces inkrustacji, w wyniku którego wytrącają się kryształy węglanu wapnia ($CaCO_3$). Równocześnie powierzchnia ta jest licznie zasiedlana przez zbiorowiska organizmów peryfitonowych, głównie przez okrzemki. Krzemionkowe pancerzyki i kryształy kalcytu są odpowiedzialne za szorstkość plech we wszystkich okresach ich wzrostu. Zbiorowiska okrzemek zasiedlających młode, cienkie plechy *Ulva* zdominowane były przez *Cocconeis placentula* i *Melosira varians*, natomiast dojrzałe przez *Cocconeis placentula* i *Navicula cryptocephala*. Z uzyskanych danych wynika, że szorstkość plech zależy głównie od zawartości węglanu wapnia w suchej masie glonu, a udział okrzemek zmienia się w niewielkim stopniu wraz z wiekiem plech *Ulva*.

Słowa kluczowe: powierzchnia plech, makroglony, *Ulva*, peryfiton, węglan wapnia, szorstkość