ANALELE ŞTIINŢIFICE
ALE
UNIVERSITĂŢII „ALEXANDRU IOAN CUZA”
DIN IAŞI
(SERIE NOUĂ)

S E C Ţ I U N E A I

BIOLOGIE ANIMALĂ

Supliment

PROCEEDINGS OF THE INTERNATIONAL SCIENTIFIC CONFERENCE

THE ANTHROPOGENIC IMPACT UPON BLACK SEA REGION BIODIVERSITY

dedicated to celebrating 85 years since the establishment of „Prof. dr. Ioan Borcea” Marine Biology Research Unit – Agigea, „Alexandru Ioan Cuza” University of Iaşi

Agigea – Constanta, the 21st-22nd of October 2011

Editura Universităţii „Alexandru Ioan Cuza” Iaşi
2013

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SUMMARY

FERTILITY EFFECTS ON *ULVA* THALLI MASS DEVELOPMENT IN INLAND WATERS OF POLAND

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Abstract. The genus *Ulva* (Ulvophyceae, Chlorophyta) consists of some 140 species which are present mainly in marine waters. Nine species occur in the inland waters in Poland (central Europe), of which *U. flexuosa* subsp. *pilifera* (Kützing) Bliding is the most widespread in the Wielkopolska region. The occurrence of *Ulva* species in any particular locality seems to be governed mainly by the availability of nutrients (N, P) than by the high levels of anthropogenic salinity in freshwaters. Freshwater *Ulva* can grow in different types of ecosystems from reservoir (Malta) through ponds (Tulce) or rivers (Nielba) and ending on drainage ditches. The main question addressed by this study was does the quantity and size of *Ulva* thalli increase together with the amount of nutrients in water? In addition, the aim of the study was to determine whether the development and the persistence for the long time of the big and dense *Ulva* patches exert influence for the aqueous ecosystem. We used freshwater *Ulva* as a model study species.

Key words: macroalge, *Ulva* spp., freshwater ecosystems, thallus and cells size, morphology, Poland

Rezumat. Efectele fertilității asupra dezvoltării în masă a talului de *Ulva*, in apele interioare ale Poloniei. Genul *Ulva* (Ulvophyceae, Chlorophyta) este format din aproximativ 140 specii prezente în principal în apele marine. Nouă specii apar în apele interioare în Polonia (Europa Centrală), din care *U. flexuosa* subsp. *pilifera* (Kützing) este cea mai răspândită în regiunea Wielkopolska. Apariția speciilor de *Ulva*, pare a fi reglementată mai mult de disponibilitatea de elementelor nutritive (N, P), decât de un nivel ridicat al salinității din cauze antropice, în apele dulci. Speciile de *Ulva* de apă dulce pot să crească în diferite tipuri de ecosisteme, de la baraje (Malta), bălți (Tulce) sau râuri (Nielba) și până la șanțuri de drenaj. Scopul acestui studiu este de a vedea în ce măsură cresc cantitatea și dimensiunea talului la *Ulva* în funcție de aportul de nutrienți. În plus, scopul studiului a fost de a determina dacă dezvoltarea și persistența pentru mult timp a câmpurilor dense de *Ulva* exercită vreo influență pentru ecosistemele acvatice. Ca model de studiu am utilizat o specie de *Ulva* de apă dulce.

Cuvinte cheie: macroalge, *Ulva* sp., ecosisteme acvatice dulcicole, dimensiunea celulară și a talului, morfologie, Polonia

Introduction

The genus *Ulva* (Ulvophyceae, Chlorophyta) including species previously classified as the genus *Enteromorpha* (Hayden *et al.*, 2003) is frequent macroscopic green algae of the marine littoral zone and estuaries - widely distributed all over the world (Bäck *et al.*, 2000; Kirchhoff & Pflugmacher, 2002; Apeng *et al.*, 2008) and often shows a tendency to form blooms (Marěs *et al.*, 2011). Since the nineteenth century, occasionally, *Ulva* thalli been observed in the inland waters of Poland, mainly in northern and western parts of the country (Kozłowski, 1890; Sitkowska, 1999). However, in recent years their presence has significantly increased – particularly in Wielkopolska region (Messyasz & Rybak, 2009a). Currently recognize in Polish Baltic Sea thirteen species (Pliński & Jóźwiak, 2004) and nine species in inland waters (Rybak & Messyasz, 2009). The spread of marine *Ulva*

species into freshwater environment causes disturbances in structure and functioning of ecosystems.

In the Mondego estuary (west Portugal) growth rates of the opportunistic macroalgae – *Ulva intestinalis* L. (syn. *Enteromorpha intestinalis*) primarily depends on salinity (Martins *et al.*, 1999). However, the dominance of *Ulva* species in any particular locality in freshwaters seems to be mainly governed by the availability of nutrients, especially nitrogen and phosphorus than by the high levels of anthropogenic salinity (Messyasz & Rybak, 2010). Nutrient enrichment can cause change habitat of ecosystems and lead to excessive growth of primary producers (Zheng & Paul, 2012).

Ulva can colonize lakes as well as ponds or rivers. It also occasionally grows in very slowly moving shallow water as for example drainage ditches (Sitkowska, 1999; Messyasz & Rybak, 2008; Rybak & Messyasz, 2009, 2010; Kowalski, 1975; Mareš, 2009). Shallow aquatic ecosystems are nutrient-rich and high-light environments (Sandgren *et al.*, 2004), on account it status of all of above places indicated high level of nutrient, which stimulate massive macroalgal growth. For the first time, great abundance in Poland of freshwater *Ulva prolifera* O. F. Müller arranged in dense mats (30 m^2) has been reported in small pond near Poznań in 2008 (Rybak & Messyasz, 2009; 2010). Then *Ulva* produced a very large amount of biomass for a short period of time. The reasons of the massive development of green algae from *Ulva* genus in some freshwater ecosystems are not fully investigated.

Expansion of *Ulva* species in inlands water is mainly inhibited by nutrient limitation and a variety of factors. The development of high biomass of *Ulva* thalli is under pressure of herbivores - specially snails, but as in the case of filamentous green algae in good nutrient conditions the grazer control was reduced (Power *et al.*, 2009).

Green algae *Ulva prolifera* O.F. Müller, *Ulva intestinalis* L. and *Ulva flexuosa* subsp. *pilifera* (Kütz.) Bliding occurs in different types of water bodies throughout Poland (Messyasz & Rybak, 2009b). The morphometric characteristic of thalli is acknowledged as a useful feature of *Ulva* when identificated to species. Nutrient contents is one of the factors which influence morphometric features of algae cells and thalli, and also individuality of species, stage of development or features of population.

The main aim of the study was to determine the relationship between uncontrolled presence of *Ulva* thalli mass development in Polish inland water and the content of nutrients in the water ecosystems.

Materials and Methods

The studies were carried out during summer in the years 2009-2011 on the different ecosystems, such as river (Nielba-N), pond (Tulce-T) and reservoir (Malta-M) (Table 1). Sites of the *Ulva* distribution were marked on the map of the Wielkopolska region (Fig. 1). Thalli of *Ulva prolifera* were found in the small pond in the Tulce (village near Poznań), *Ulva intestinalis* in lowland river Nielba (50 km from Poznań) and *Ulva flexuosa* subsp. *pilifera* from Malta reservoir (Poznań). The actual names of the species have been presented by Hayden *et al.* (2003).

The thalli samples of free-floating green algae (Fig. 2a) which were taken from surface by hand were collected into a plastic container and immediately transported to the laboratory. Next, the thalli were repeatedly rinsed with distilled water in order to remove any biotic and abiotic particles attached to them. During each research season about 500 thalli were measured (lengths, widths, presence of proliferations, Fig. 2c). From thalli samples were prepared microscopic slides to observe shape and composition of cells (Fig. 2b) and to measure length and width of cells, number of pyrenoids (staining Lugol's solution) with using a light microscope (40x) and the program ProCap.

The basic physico-chemical parameters of the water (temperature, conductivity, concentration of oxygen and Cl^- as well as the pH level) at the examined sites with *Ulva* were measured with the use of *YSI Professional Plus* handheld multiparameter meter. Water samples were also taken for detailed analyses at laboratory with a view to measuring the content of nitrogen and phosphorus by spectrophotometer HACH DR 2800.

Results and Discusion

Impact of biotic and abiotic factors on the freshwater *Ulva* development.

In general, the massive appearance of these green algae occurred mostly during summer from May or June, with maximum coverage in July or in the beginning of August. Thalli appeared in concentration tightly covering the column of water and were found in two forms - the submerged and free floating all over the surface water. Average concentrations of $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$, NaCl and total Fe took out 0.26 $mg.l^{-1}$, 0.86 $mg.l^{-1}$, 0.22 $mg.l^{-1}$, 284 $mg.l^{-1}$ and 0.21 $mg.l^{-1}$ respectively. The value of the electrolytic conductivity was fluctuated around 607 - 1421 $\mu S.cm^{-1}$ (Table 2). Among the studied water ecosystems, the highest values of nitrogen and phosphorus were reported in Tulce pond, where simultaneously were observed dense, monospecific free-floating mats on entire surface.

Table 1. Characteristics of the various *Ulva* patches on research sites in the Wielkopolska region.

Site	Malta Reservoir (M)	Tulce Pond (T)	Nielba River (N)
Location	N 52°24'9.19" E 16°58'13.7"	N 52°20'34.5" E 17°4'39.16"	N 52°48'39.32" E 17°13'18.46"
mean depth (m)	3.1	2.4	0.75
structure of mats	loose	dense	dense
Taxon	*Ulva flexuosa* subsp. *pilifera* (Kützing) M.J.Wynne	*Ulva prolifera* O.F.Müller	*Ulva intestinalis* L.
accompanying	filamentous green algae	macrophytes	filamentous green algae
mats area (m^2)	1-5	30	10-15
Forms	free-floating, submerged	free-floating, submerged	free-floating, submerged
Occurrence	V-VII	V-VIII	V-VII

Table 2. Values (min-max; average) of water main physicochemical factors at the examined sites.

factors / locations	pH	conductivity ($\mu S.cm^{-1}$)	$N\text{-}NO_3$ ($mg.l^{-1}$)	$N\text{-}NH_4$ ($mg.l^{-1}$)	$P\text{-}PO_4$ ($mg.l^{-1}$)	NaCl ($mg.l^{-1}$)	$Fe_{tot.}$ ($mg.l^{-1}$)
Tulce pond	5.03-9.83 (8.3)	655-1421 (978)	0.06-1.11 (0.28)	0.19-4.27 (1.20)	0.02-0.80 (0.44)	61-597 (154)	0.02-0.31 (0.15)
Maltański Reservoir	7.40-8.99 (8.1)	607-805 (714)	0.02-0.14 (0.06)	0.03-1.73 (0.61)	0.01-0.14 (0.05)	98-120 (102)	0.01-0.05 (0.02)
Nielba river	7.40-9.97 (8.6)	719-860 (806)	0.09-0.43 (0.25)	0.22-1.81 (0.54)	0.00-0.23 (0.05)	92-181 (107)	0.01-0.29 (0.05)

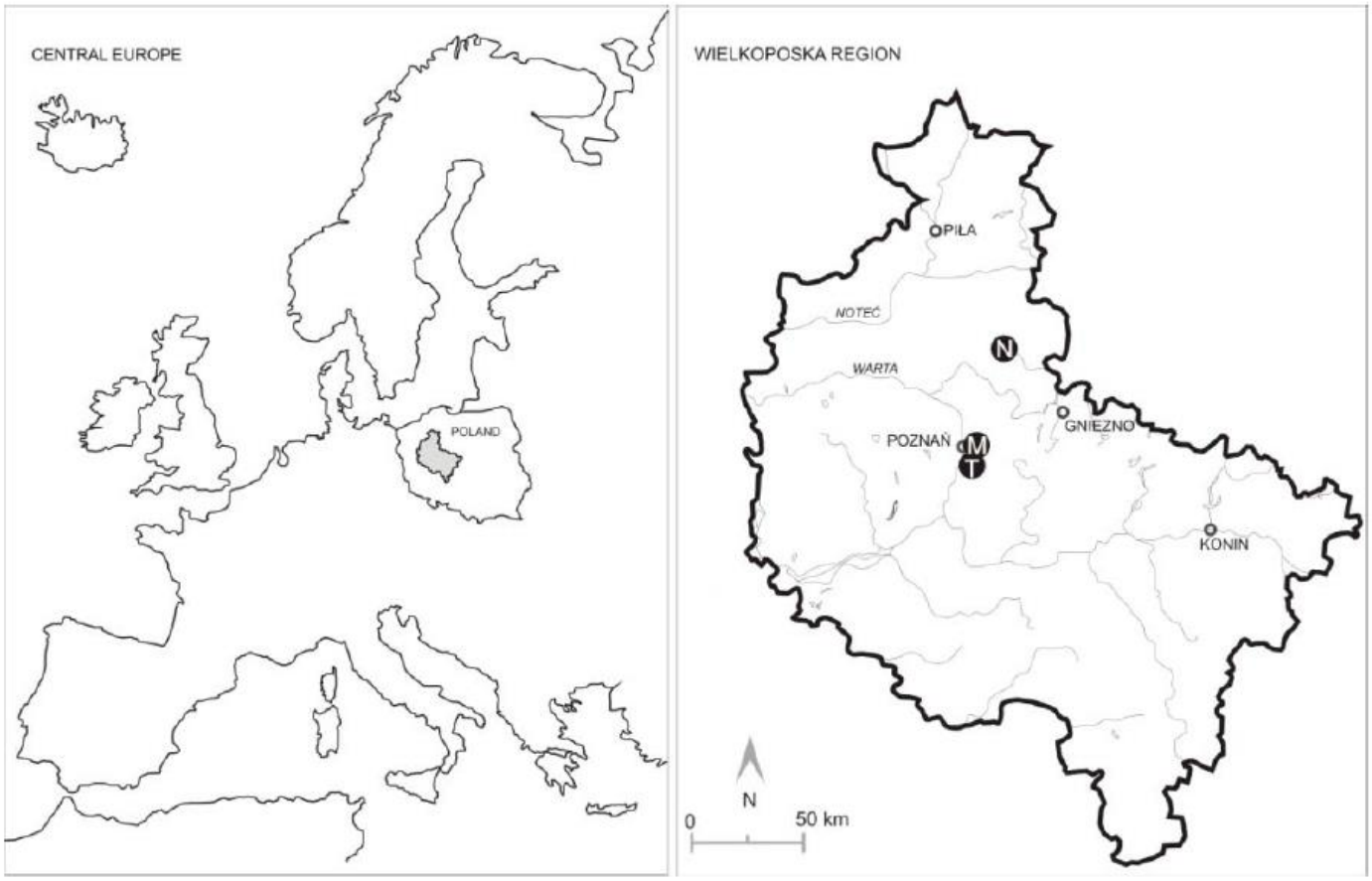

Figure 1. Location of sampling sites for the freshwater *Ulva* in the Wielkopolska region of Poland.
N – the Nielba river; M – the Maltański Reservoir; T – the Tulce pond.

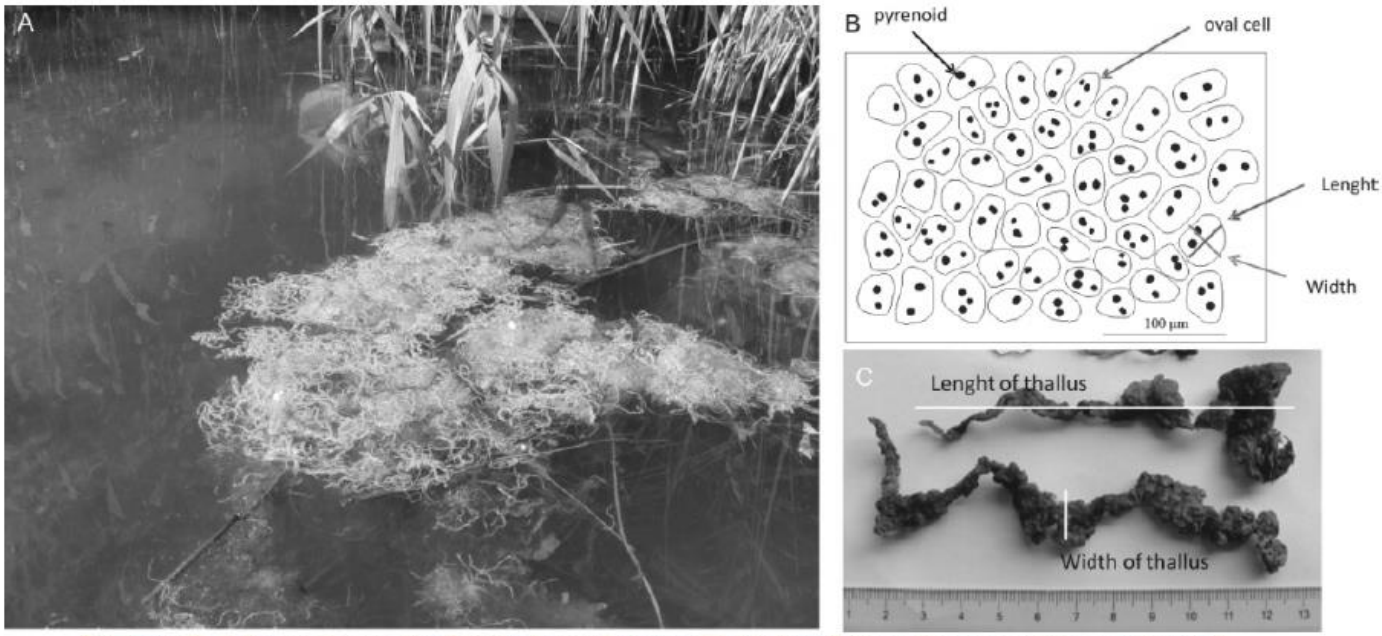

Figure 2. *Ulva* halli in the Maltański Reservoir (A) and the scheme of the measured morphometric features of macroalgal cells (B) and thalli (C).

In addition, thalli achieved average length 20 cm and reached 45 cm maximum and the width from 0.1 up to 4.7 cm (Fig. 3), where thalli collected in May from a pond achieved a much larger size – more than 2 m (Messyasz & Rybak, 2009). The cells had the length from 2.0 up to 24.2 cm and the width in the range from 3.8 up to 17.6 cm (Fig. 4). Length of the thalli suggests that in running waters *Ulva* can reach higher values of these characteristics than in stagnant waters as a result of water flow and habitat conditions. Common situation is with width of the thalli, where in river it is higher than in the reservoir or pond. Therefore, water flow has a significant influence on mats development. For each species (*Ulva intestinali, U. prolifera* and *Ulva flexuosa* subsp. *pilifera*) were observed common results of length and width of thalli and cells, number of pyrenoids (1-3) and shape of cells (oval or square). There were also many differences. Causes of this situation are habitat conditions, e.g. water temperature, nitrogen, phosphorus and, in a lower degree, chloride concentrations. The obtained results demonstrated that even though all water bodies habitats were enriched with N and P they differed from each other in *Ulva* morphology features development of the same species in many respects (size of cells and thalli, young and old thalli participation, presence of branches). Usually, there is a constant number of pirenoids in cells of particular taxon. This phenomenon appeared in *Ulva prolifera* most often. Results of this study highlight the importance of affluence of water in nutrients which allows *Ulva* thalli to obtain good conditions for development shown in its morphometric features.

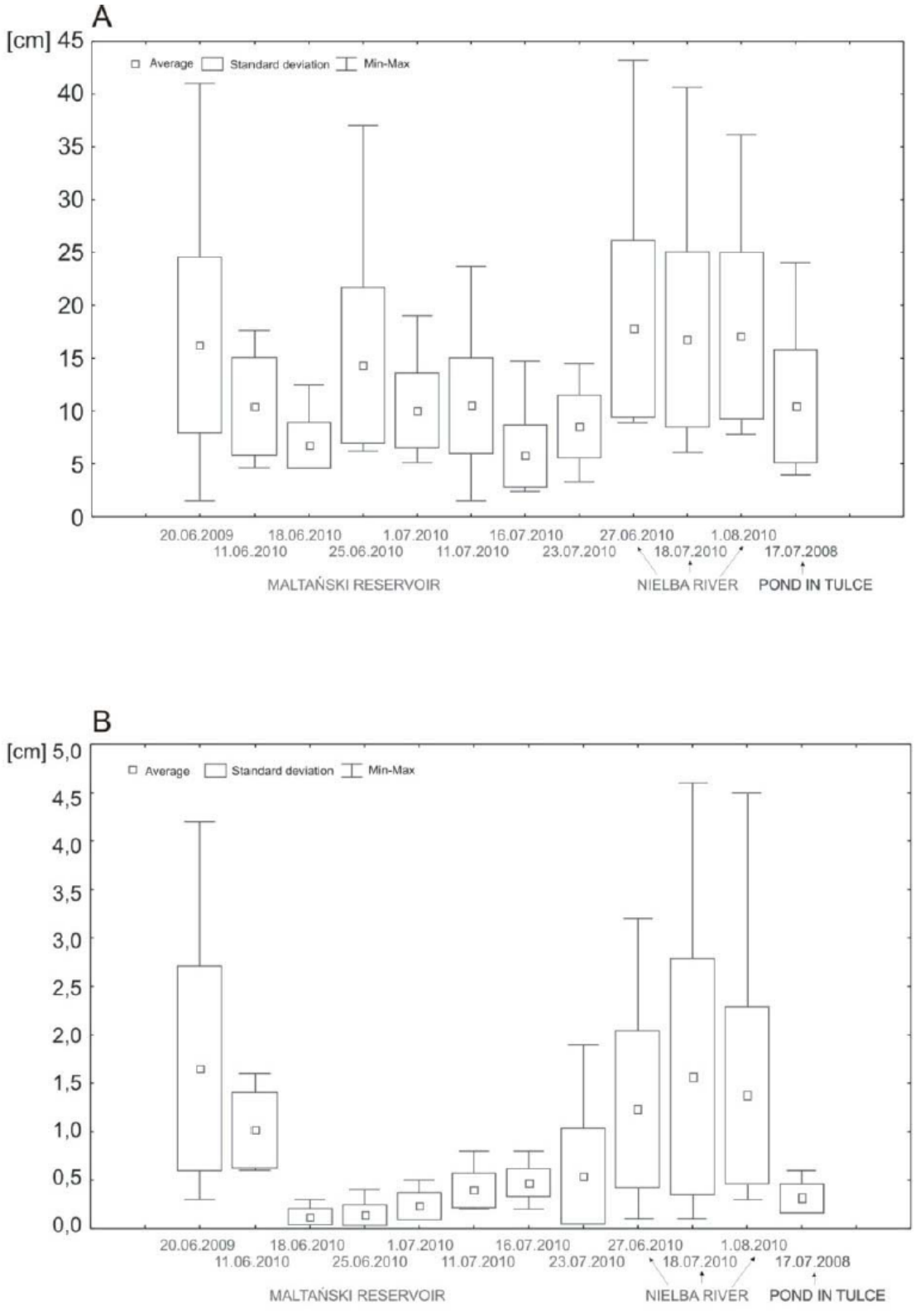

Figure 3. Length (A) and width (B) of *Ulva* thalli in particular types of water body.

The fast growth of thalli in littoral waters, rich in nutrients, was a frequent reason of using *Ulva* biomass in cleaning off waters originating from the fish and shrimp farming (Cohen and Neori, 1991; Neori *et al.*, 1991; Sato, 2006). *Ulva reticulata* thalli implemented into the system of fish breeding ponds grew 4% per

day, removing in this process 6.5 g N m^{-2} day^{-1} (TAN) total ammonia nitrogen from water (Msuya, 2006). It was also made an attempt at composting the biomass of sea species from the *Ulva* genera in order to use it as fertilizer (Wosnitza & Barrantes, 2006). Freshwater *Ulva* which the mass development was observed in the Tulce pond in the period since May by August also influenced the concentration of nitrogen ranked in the water under its thalli. Directly under thalli the concentration of nitrates amounted about 0.20 $mg.l^{-1}$ while, in sites where *Ulva* thalli didn't appear it was higher and gained on average – 0.37 $mg.l^{-1}$ (Fig. 5). We also observed that in individual phases of the freshwater *Ulva* development the ability to remove nitrogen from water was changeable. During appearence of young *Ulva* thalli in the water, under freshwater macroalgal mats concentrations of nitrates amounted from 0.18 to 0.22 $mg.l^{-1}$ at the beginning of the vegetative period. Next, in the course of the thalli development, a decrease in the concentration of nitrates was registered to 0.17 $mg.l^{-1}$ and in the phase of thalli decay repeated increase 0.21 $mg.l^{-1}$ $N\text{-}NO_3$ was recorded. In the end of July an appearance of the second generation of thalli was noted. Young thalli of *Ulva*, which in the sequence of two weeks covered almost entire surfaces of the pond, contributed to the fall in the concentration of nitrates directly under mats to 0.14 $mg.l^{-1}$ (Fig. 6). Under mats with *Ulva*, concentrations of $N\text{-}NO_3$ always were lower than outside them. Observed differences in the $N\text{-}NO_3$ concentration which were noted under and outside mats formed by freshwater *Ulva* were statistically significant (Kruskal-Wallis test H: 5.25; $p< 0.05$). However, in the case of remaining nutrients differences in their concentrations under and outside mats built by freshwater *Ulva* were not statistically significant.

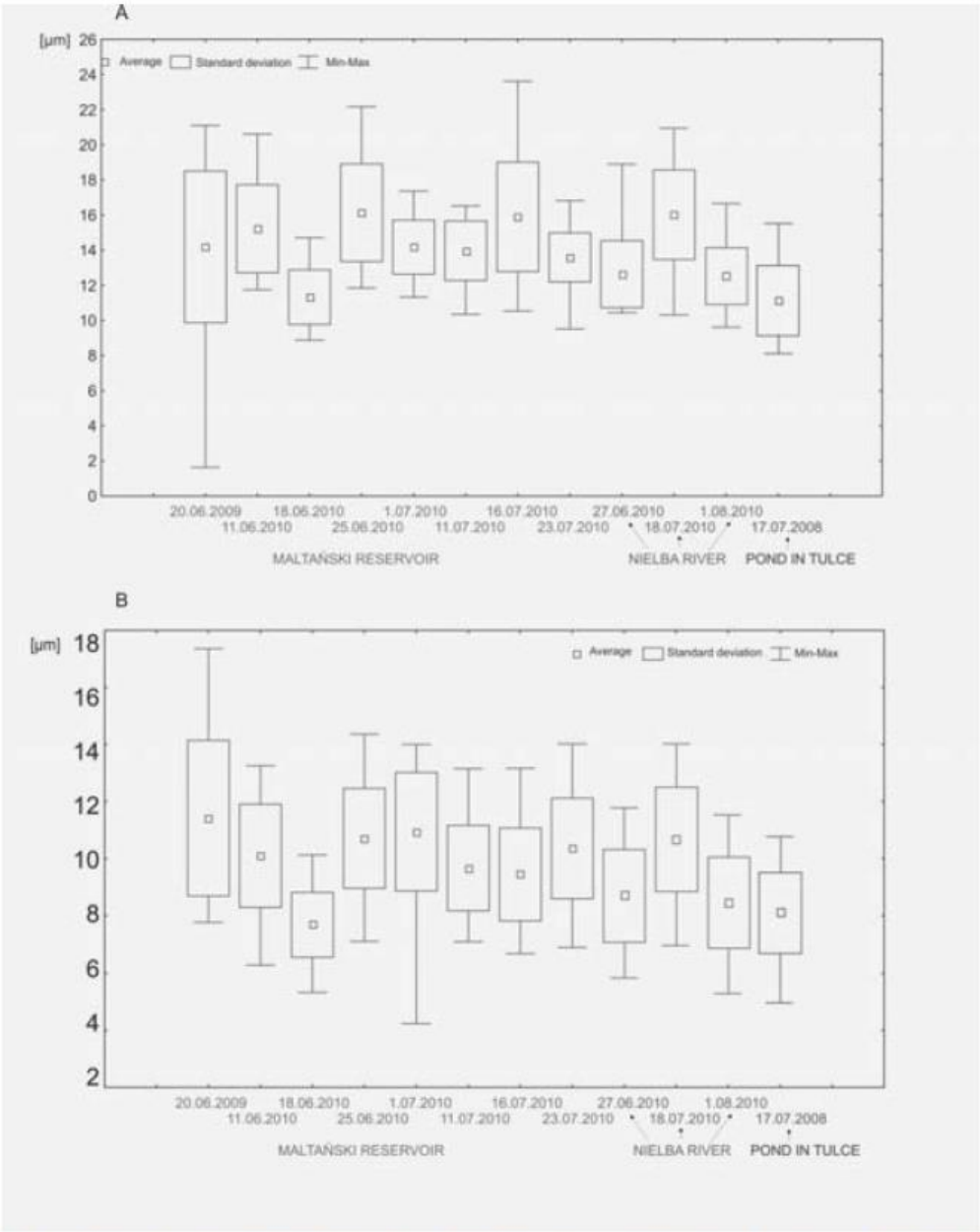

Figure 4. Length (A) and width (B) of *Ulva* cells in particular types of water body.

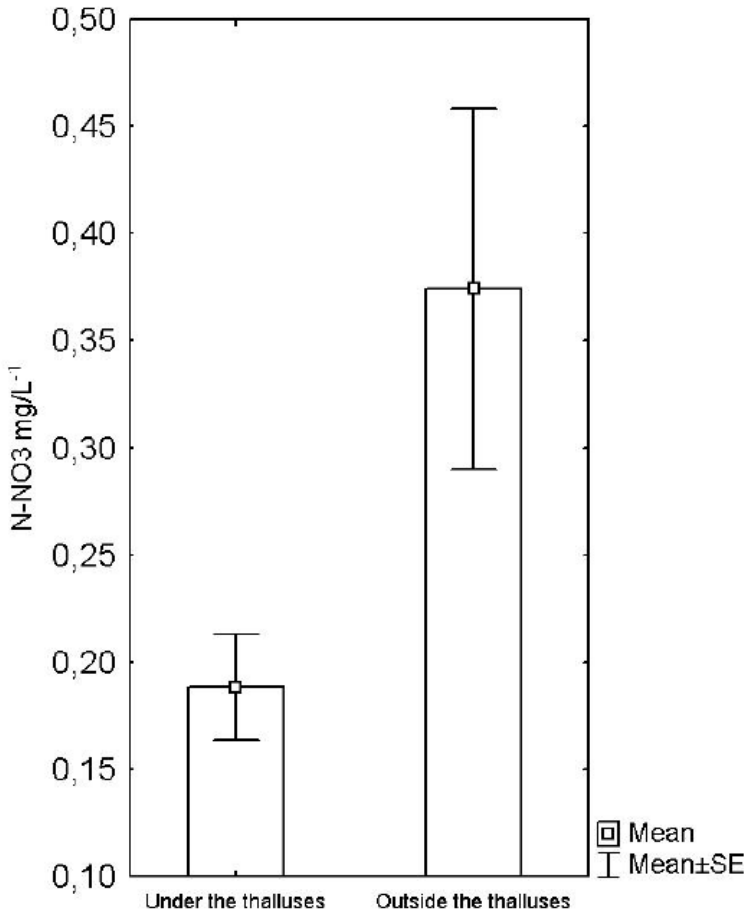

Figure 5. The concentration of nitrate in particular habitats of the pond Tulce.

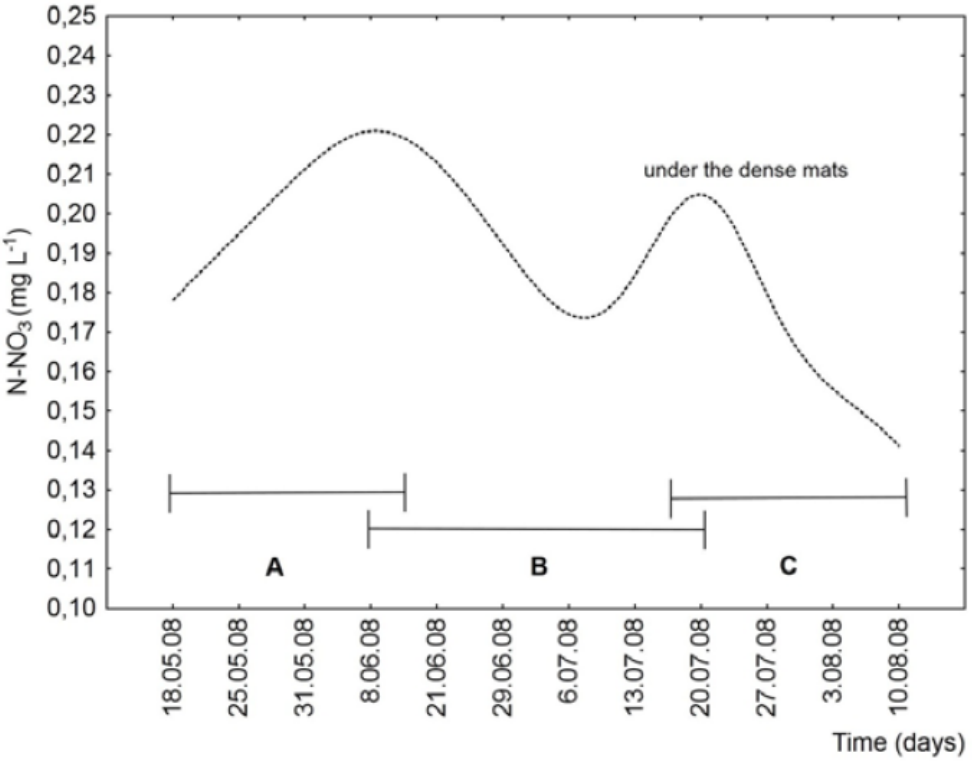

Figure 6. Changes of the concentration of N-NO_3 under the dense mats of *Ulva prolifera* (A – young thallus; B – ripening and decaying thallus; C – young thallus).

Most macrophytes are densely overgrown by periphytic communities (Messyasz & Kuczyńska-Kippen, 2006). Filamentous algae or thalli of macroalgae also constitute an available to the development of attached diatoms. Representatives of the freshwater form of *Ulva* may have two types of thalli - smooth or rough (Messyasz & Rybak, 2010). The first type of *Ulva prolifera* thalli with intestinally-undulating structure was characteristic for young plants while the second type occurs in mature and withering specimens and has a characteristic, curly-bubbled structure. Furthermore, scanning electron microscope observations of *Ulva* thallus revealed that its surface is covered by crystals and on them large numbers of epiphytic diatoms were also noted. During the study period, 89 taxa of diatoms were identified in total. The richest taxonomical structure was recorded for the pond Tulce (63 taxa), the Nielba river (45), while the poorest for the lake Malta (31). The majority of the dominant peryphytic species found on the *Ulva* are cosmopolitan, alkaliphilous, eutrophic and preferring well oxygenated waters. It is interesting to note that species composition did not show much variation in response to thallus growth form. The species *Nitzschia palea* (Kützing) W. Smith, *Cocconeis placentula* Ehr. and *Navicula halophila* (Grunow) Cleve were dominant during all sampling periods. The obtained results revealed that water body type and physical-chemical features of water also seem to play a role in the development of individual differences in epiphytic diatoms assemblages.

Ulva influence for the aqueous ecosystem.

Due to possibility of *Ulva* influence for the aqueous ecosystem, the freshwater thalli consumption was only observed by pond snails (*Lymnaea stagnalis* L.). The freshwater snails observed during natural and experimental conditions were mainly interested in young thalli with a smooth surface and did not generally consume the mature and strongly deformed algae. Freshwater full-grown *Ulva* thalli were characterized by a strongly creased and rough surface. Such structure of a thallus was a result of the mass prevalence of calcium carbonate ($CaCO_3$) crystals and diatom microflora (Messyasz *et al.*, 2010). Consequently, it may be assumed that the occurrence of too developed $CaCO_3$ incrustation may reduce the attractiveness of *Ulva* thalli as a source of food for herbivorous snails. The marine shellfish feeding on periphiton contribute to the increase in light and nutrient accessibility, which enhances the growth of the algal biomass. This type of predation may also control or influence the growth of thalli biomass by other invertebrates eating epiphytic microorganisms (Kamermans *et al.*, 2000). Consequently, it is possible that freshwater species of snails on *Ulva* thalli at freshwater sites (e.g. lakes, ponds and rivers) can consume a large number of periphiton which would stimulate thalli growth.

Moreover, it was found that *Ulva* mats were a very important factor in shaping the phytoseston structure because not all species of algae are tolerating the strongly limited availability of light in their habitats. Only diatoms, cryptophytes and euglenophytes found favourable conditions for their development within *Ulva* mats, irrespective of the site, reaching very high biomass values (on average 4.960 $mg.l^{-1}$ in the pond Tulce; 0.402 $mg.l^{-1}$ in Lake Malta; 0.217 $mg.l^{-1}$ in the Nielba

river). Similar differences were also observed at the level of dominating taxa. The most abundant species in all sites under *Ulva* mats were: *Cryptomonas rostrata* Troitzkaja emend. I. Kiselev (on the average - 18% of the total numbers of alls), *Gomphonema olivaceum* (Horn.) Breb. (15%), *Gomphonema ventricosum* Gregory (12%), *Cocconeis placentula* Ehr. (28-42%), *Gomphonema acuminatum* Ehr. (15%), *Meridion circulare* Ag. (11%), *Cymbella tumidula* Grunow (11%). Microscopic green algae (average biomass 0.098 $mg.l^{-1}$) development in phytoseston communities was strongly restrained by *Ulva* thalli overshadowing. Only in the case of the pond Tulce, green algae achieved much higher biomass than in streams, what was connected with the eutrophic phytoplankton character in all parts of this water body with occurrence of *Scenedesmus*, *Desmodesmus*, *Monoraphidium* and *Tetraëdron*.

Conclusions

The obtained results demonstrated increase in the number of *Ulva* thalli and their size along with the growth of the N and P concentrations in the water. However, the decrease of nutrients concentrations during the *Ulva* development was rather small on account of the permanent supply from the drainage area. The growth of thalli is also affected by a large numbers of epiphytic diatoms (primarily *Cocconeis*) and crystals bands, which excessive contribution may lead to dying of algae.

On the other hand, mass development of these macroscopic algae can contribute to considerable limiting of the light availability in the water, and in consequence reduced the rise in the microscopic algae densities. The fact that *Ulva* thalli are actively consumed by *L. stagnalis*, which used the free floating algal mats as a source of food and shelter, indicates natural character of the *Ulva* phytocoenoses in examined water bodies.

Acknowledgements

The study was supported by the Polish Ministry of Education Science grant No. N N304 0134 37 and partially from projects GDWB-07/2011

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