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Ecological preferences of freshwater *Ulva flexuosa* (Ulvales; Ulvophyceae): development of macroalgal mats in a Tulce fishpond (Wielkopolska Region, Poland)

by

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Abstract

Research on the diversity of thalli and mats built by freshwater *Ulva flexuosa*, as well as the impact of abiotic factors on the development and distribution of *Ulva* mats, was conducted from May to August 2013. The study describes the dynamics of "green tides" formation by *U. flexuosa*. The study assessed abiotic factors that may influence the colonization and the growth of *Ulva* populations in freshwater systems. The results are of major importance to the trade and industry sectors. In total, eleven environmental parameters were included in the analysis. The most important environmental parameters characterizing the *Ulva* sites are sodium chloride levels and conductivity. High values of pH inhibited the development of *Ulva*, leading to a lower percentage of mature thalli in the mats. The water depth was positively correlated with an increase in the density of thalli in the mats and the average thallus length. Significant differences were identified between the concentrations of nitrates under and outside the *Ulva* mats. Concentrations of nitrate under the *Ulva* thalli were always lower.

Key words: Chlorophyta, environmental conditions, macroalgal mats, *Ulva flexuosa*

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Introduction

The *Ulva* (Chlorophyta) genus belongs to the cosmopolitan group of macroalgae (Hayden et al. 2003) that grow primarily in seawater and estuaries (McAvoy & Klug 2005). With a very high rate of biomass increase in eutrophic waters, these algae form "green tides", which have been repeatedly found in European and Asian coastal waters (Blomster et al. 2002; Liu et al. 2010; Pang et al. 2010). Moreover, *Ulva* thalli have been observed at inland sites in Europe. In the 19th century, *Ulva* thalli were recorded in inland salt pans and waters located near mineral water source areas (Göppert & Cohn 1850; Hansgirg 1892). At several sites, the waters of which contained high levels of mineral compounds, *Ulva* has been recognized as one of the native elements of the halophilic flora in these areas (or *flora marina* in aquatic ecosystems of inland areas not affected by the Baltic Sea water). In the 20th century, *Ulva* populations were also recorded in freshwater ecosystems, such as lakes and lowland rivers and ponds, where the NaCl level did not exceed 20 mg l^{-1} (Messyasz & Rybak 2009, 2011; Rybak et al. 2014). The abundant occurrence of *Ulva* has been observed in European lakes, ponds and rivers with optimal conditions for its development, becoming an obvious dominant on the water surface (Kaštovský et al. 2011). The occurrence of ten *Ulva* species was reported in the Baltic waters along the littoral sites (Pliński & Jóźwiak 2004). In the inland ecosystems of Europe, which are not exposed to the impact of sea and ocean waters, the *Ulva* species have been recorded at more than 220 sites (Rybak et al. 2014; Rybak 2015).

Species of the *Ulva* genus demonstrate considerable morphological plasticity, the expression of which is influenced by environmental conditions, such as nutrient concentration and water salinity. Populations of a given species may produce either tube-like or leaf-like thalli at the bottom or top part of the thallus, which complicates the identification of taxa. It has been reported that a wide morphological plasticity is strongly expressed in the case of fresh water *Ulva* populations, the thalli of which grow to sizes not observed in bibliographical references (Messyasz & Rybak 2011). Currently, phylogenetic methods have become increasingly popular for the identification of *Ulva* species. Among the markers used, ITS1 – 5.8S rRNA – ITS2, nrDNA, *rbc*L are the most common; additionally, a special marker is used for SSU rRNA to create more informative sequence alignments and cladograms with better topological support (Mareš et al. 2011; Melton et al. 2015).

However, the research on freshwater populations of *Ulva* are focused on several aspects. The available studies focus on (i) the analysis of phylogenetic affinity with sea populations (Mareš et al. 2011; Chen 2015), (ii) the expression of *ULL* (***Ulva***-**L**ectin-**L**ike) genes, which are most likely responsible for *Ulva* resistance to the conditions of gradual mineralization of waters (Ichihara et al. 2011, 2013), and (iii) the bioaccumulation of heavy and light metals (Rybak et al. 2011; Rybak et al. 2012a,b; Rybak et al. 2013). On the other hand, there are no studies concerning the monitoring of the freshwater *Ulva* population development at inland sites, and such studies are essential given the increasingly frequent occurrence of macroalgae mats with *Ulva flexuosa* in freshwater ecosystems of Europe.

This objective of this study is to provide additional information for the previous ecological studies of freshwater *Ulva* populations in inland water ecosystems. Indeed, detailed ecological analyses of parameters that influence the occurrence of *Ulva* species in freshwaters are needed. Therefore, the objective of this work was to determine the influence of physio-chemical factors on the colonization and the growth of limnic *Ulva* populations in freshwater ecosystems using pond samples. An additional concern was to evaluate the impact of *Ulva* thalli on local disturbances in the concentration of nutrients.

Material and methods

Study area

The study was located in Tulce village (Wielkopolska Region, Poland) in an open fishpond (52°20'35''N; 17°04'40''E). The pond (where freshwater *Ulva* thalli was found) is fed by waters carried by the Kopel River, one of the right-bank tributaries of the Warta River (the second longest river in Poland). *Ulva* thalli were sampled every week in the period from May through August 2013.

Analysis of the study material

Seasonal collections of *Ulva* thalli were carried out in the fish pond. They were identified to the species level using the ITS2 rDNA region and *rbc*L (Rubisco) gene sequences, which were previously described by Rybak et al. (2014). Identification of species was also based on the classic morphological and anatomical features presented by Bliding (1963, 1968), Koeman & van den Hoek (1982a,b; 1984) supplemented with measurements of the cell wall thickness, cell and pyrenoid circumferences, and diameter of pyrenoids, as proposed by Rybak et al. (2014). In addition, identification keys by Starmach (1972), Pliński (1988), Brodie et al. (2007), and Pliński & Hindák (2012) as well taxonomical studies by Hardy & Guiry (2008) were used. All specimens belonged to the subspecies *Ulva flexuosa* subsp. *pilifera* (Kützing) M.J. Wynne 2005: 107.

Both the percentage of water surface area covered by *Ulva* thalli and the density of *Ulva* thalli per m^2 of surface water were assessed in the field. The lengths and widths of thalli collected from each site were also measured. Macroalgal mats were analyzed to determine the number of furcated thalli and the presence of young and mature individuals. Laboratory analyses included measurements of the thalli structure (presence of proliferations) and the examination of cell morphology (the number of pyrenoids and the shape and arrangement of cells) was made using a light microscope Zeiss Axioskop 2 MOT and confocal microscope ZEISS LSM 700.

Chemical analysis

Physicochemical parameters of the water from the stand containing the limnic *Ulva* were analyzed when the macroalgae thalli were present, i.e. from May until August, 2013. Water temperature, pH, conductivity and oxygen levels were measured using YSI Professional Plus. Additionally, changes in the water depth were monitored. Water samples (500 ml) were collected for chemical analyses and preserved with 0.5 ml of chloroform. The samples were then stored in refrigerators at 4°C. Chemical analyses were performed in a laboratory using standard methods for a Hach DR 2800 spectrophotometer (APHA 1998). Concentrations were determined for the following parameters: ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), sodium chloride (NaCl), chloride (Cl^-) and iron (Fe_{total}).

Statistical analysis

STATISTICA 10.0 (StatSoft, Tulsa, OK, USA) and CANOCO 5.0. (ter Braak & Šmilauer 1998) software was used for the statistical analysis of the collected data. The correlation between the physicochemical parameters and morphometric features of thalli was defined using Pearson's linear correlation coefficient (r) including r^2 ratio. Differences in the average values of the physicochemical parameters at different sites were assessed using the Mann-Whitney *U*-test. The PCA (Principal Components Analysis) method was used to compare the diversity of physicochemical environmental parameters in stands of freshwater *U. flexuosa*.

Results

Physicochemical profile of the habitat

The average depth of water under the macroalga mats was 2.4 m. In the study period, water pH values fluctuated from 6 to 8, though the water in general was slightly alkaline. The water oxygenation rate (from 4.2 to 8.4 mg l^{-1}) was directly dependent on the amount of water supply from the Kopla River, which flows through the study site. The water contained high levels of nutrients, specifically N-NH_4^+, N-NO_3^-, and P-PO_4^{3-}. The water mineralization rate remained at a high level for the entire study period, with an average value of 1044.7 µS cm^{-1}, whereas the concentration of sodium chloride did not exceed 143.6 mg l^{-1} (Table 1).

Table 1

Average values of physicochemical factors of water for the examined site of *Ulva flexuosa* (n = 12)

Parameter	Units	Mean	Min.	Max	SD*
Temperature	°C	17.3	14.10	22.0	2.16
Depth of water	m	2.4	2.0	3.2	0.37
pH	-	7.2	6.03	8.4	1.37
Conductivity	µS cm^{-1}	1044.7	655.0	1421.0	196.38
O_2	mg O_2 l^{-1}	8.0	4.2	3.4	8.07
N-NH_4^+	mg N l^{-1}	1.2	0.18	4.0	1.26
N-NO_3^-	mg N l^{-1}	0.2	0.06	0.4	0.09
P-PO_4^{3-}	mg PO_4 l^{-1}	0.5	0.12	0.8	0.25
Total Fe	mg Fe l^{-1}	0.2	0.06	0.3	0.07
NaCl	mg NaCl l^{-1}	91.1	61.05	143.6	25.27
Cl^-	mg Cl l^{-1}	55.2	37.0	87.0	15.32

* – standard deviation

Out of physicochemical water parameters, reaction and reaction-related factors most clearly determined the observed variability. As far as some of the abiotic factors are concerned, the most important were: conductivity, chloride and oxygen.

The parameters correlated with the first PCA axis explained 70% of the variability; those correlated with the second PCA axis explained 20% of the variability (Fig. 1).

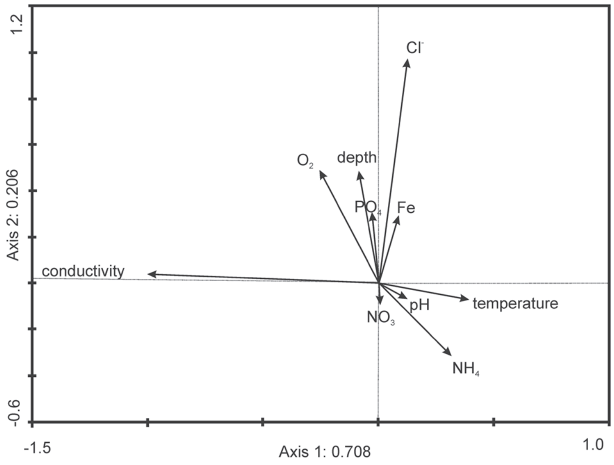

Figure 1

The PCA diagram for individual physicochemical factors of water from *Ulva flexuosa* site

Characteristics of *Ulva*

Freshwater *Ulva flexuosa* thalli developed at the above-mentioned site from May to August. The thalli occurred in two forms: submerged and recumbent thalli on the bottom and a compact thallus mat floating freely on the water surface. *Ulva flexuosa* thalli were not found to accrete with plant shoots or other substrates at the bottom. The submerged thalli were dark green, whereas the thalli from the water surface were light green in color (Fig. 2A and 2B). On average, the thalli were 4.2 to 66.40 cm long and 0.15 to 1.15 cm wide (Table 2). The submerged thalli featured strong morphological diversity, ranging from narrow and long thalli to very wide and short thalli (Fig. 2C). The occurrence of many long (0.5 to 3.5 cm) and thin (0.5 to 1.5 mm) thallus branches was the reason why the thalli tended to concentrate and form compact mats (Figs 2D and E). The submerged thalli were silky to the touch and flexible, whereas the thalli floating on the water surface had a rough surface and were highly fragile. Their cells had an average size of 11.15 μm × 8.08 μm; their shape was oval with jagged edges and a visible thick layer of the cell wall. Cells were arranged in irregular rows. Cells of the submerged thalli were nearly circular with visible pyrenoids (Fig. 2F). Cells from the floating thalli were smaller, cup-shaped with strongly wrinkled chromatophores on the light-incident side, and well-developed pyrenoids were rarely noticed (Fig. 2G). Cells contained no more than 4 pyrenoids per cell, and the average perimeter of a pyrenoid was 3.19 μm (Table 2).

The greatest biomass of young thalli was observed in that part of the pond where the river current was still noticeable. *Ulva* mats achieved their maximum extent in the second half of June (30 m^2); afterwards, the mats kept shrinking and became gradually dissipated in the following weeks to achieve the value of less than 1 m^2 in early August (Fig. 3, Table 2). Freshwater *Ulva* mats tended to settle along the

Table 2

Comparison of anatomical features of *Ulva flexuosa* (n =30) and average values of morphometric features of *Ulva* mats (n =12)

Features	Units	Mean	Min.	Max	SD*
Length of thalli	cm	20.00	4.2	66.40	0.53
Width of thalli		0.59	0.15	1.15	0.14
Length of cells	μm	11.15	8.10	15.5	1.99
Width of cells		8.09	4.96	10.78	1.39
Number of pyrenoids	-	2	1	3	0.75
Perimeter of pyrenoids	μm	3.19	2.40	3.90	0.38
Surface of mats	m^2	12.83	1.0	30.0	11.17
Density of thalli per m^2 of mats	m^{-2}	75.92	10.0	250.0	66.16
Percentage of branched thalli per m^2 of mats	%	49.58	10.0	90.0	26.67
Percentage of unbranched thalli per m^2 of mats		22.92	5.0	75.0	24.07
Percentage of young thalli per m^2 of mats		37.50	5.0	95.0	32.72
Percentage of mature thalli per m^2 of mats		55.92	1.0	95.0	31.74
Percentage of submerged thalli per m^2 of mats		35.25	0	95.0	38.78
Percentage of free-floating thalli per m^2 of mats		59.58	10.0	100	32.71

* – standard deviation

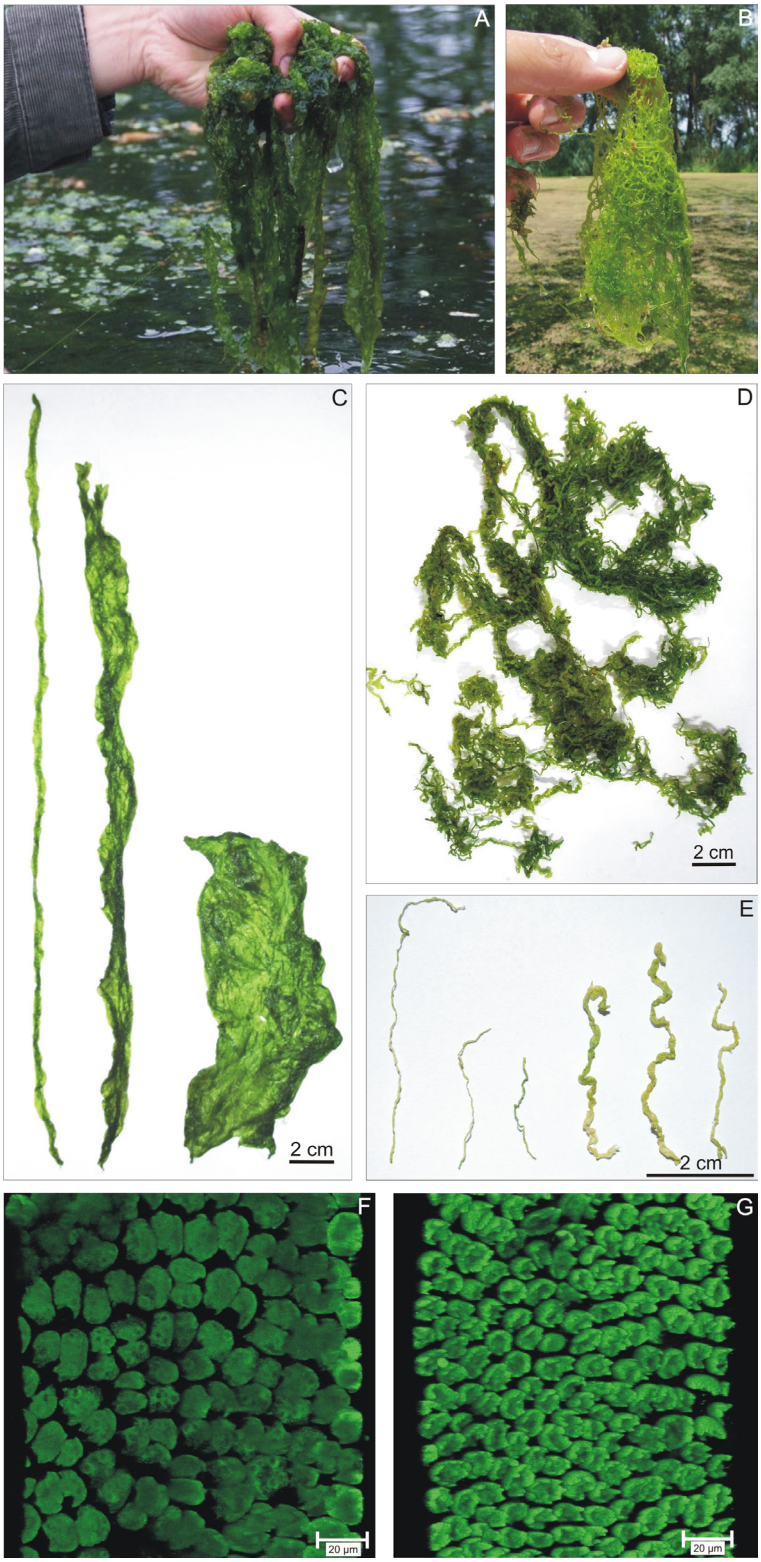

Figure 2

Thalli and cells of limnic *Ulva flexuosa*. A – submerged thalli; B – free-floating thalli; C, D and E – tube-like thalli from herbarium sheets; F – cells with 2-3 pyrenoids, rarely single, G – cells with cup-shaped chloroplasts (3D view from confocal microscope)

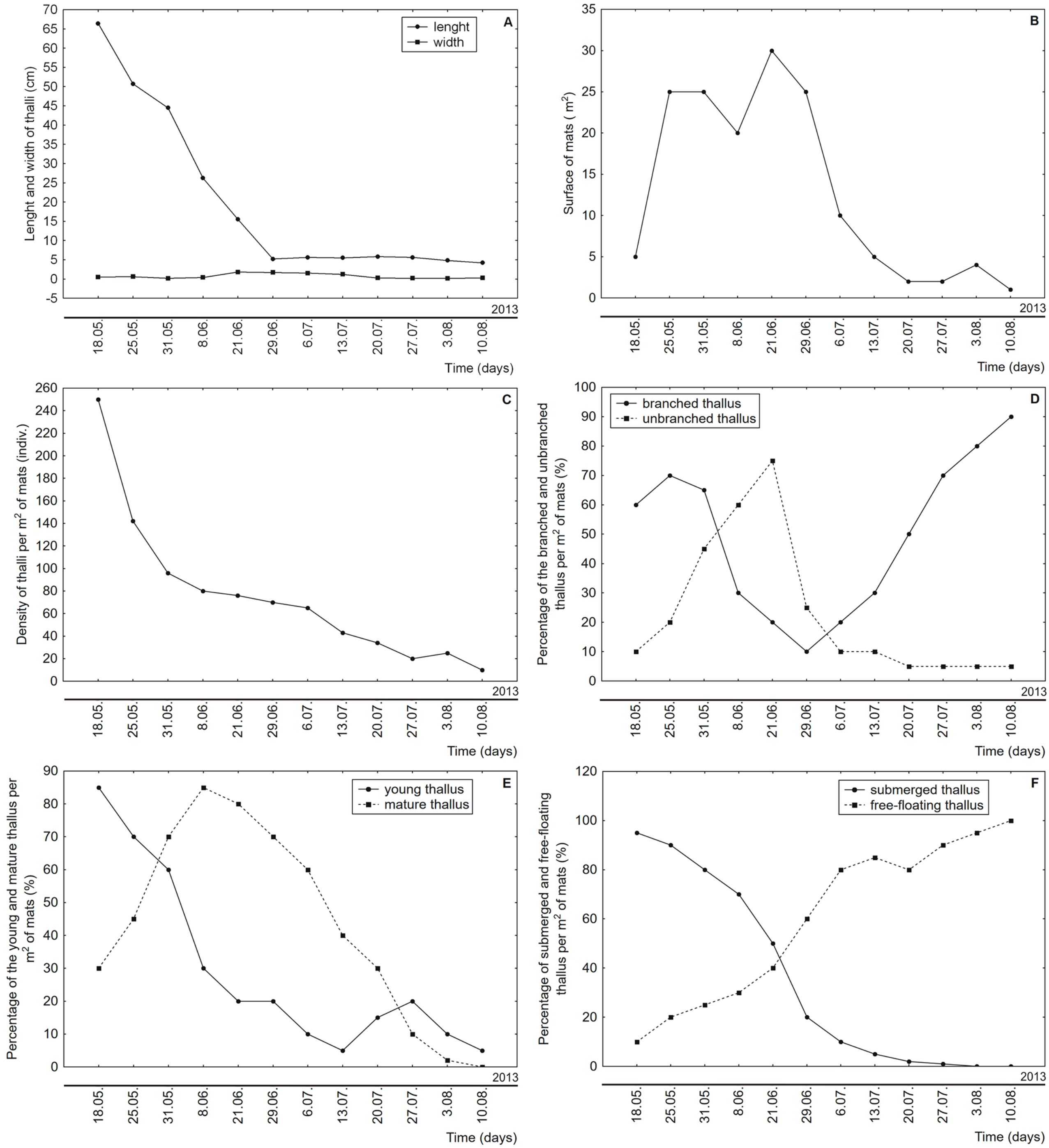

Figure 3

The succession dynamics of a macroalgal community with limnic *Ulva flexuosa*

flow of the Kopla River through the pond, which was observed in June (Fig. 3). The longest thalli were noticed in May and in early June, whereas the widest specimens occurred at the turn of June and July (Fig. 3A). In early June, a sudden decrease in the *Ulva* mat area was noticed, from 25 down to 20 m^2, due to violent rain flows which flooded the thalli (Fig. 3B). The density of thalli in the mats systematically decreased from May (the highest value, on average 250 thalli per m^2) until August when only several thalli were noticed inside the structure of the macroalgae mat (Fig. 3 C, Table 2). The contribution of mat-building branched and non-branched thalli changed. In May, July, and August, the mat-building branched thalli dominate (from 60 to 90%) while in June, non-branched thalli dominated (75%) (Fig. 3D, Table 2). Similarly, the contribution of young and mature individuals changed in the period of mat building. The highest contribution of young individuals occurred in May (85%) to decrease in the following weeks to 5% in the last week of the study period (Fig. 3 E). The highest contribution of adult individuals occurred in early June (85%) to decrease in May (30%) and in the last weeks of mat occurrence in the pond (1 to 2%) (Table 2). The contribution of submerged and floating thalli changed at the end of June (Fig. 3 F). Until that time, the mats had been mostly composed of submerged thalli (as many as 97%) whereas the contribution of free-floating thalli increased as early as in June and August up to 98-100% (Table 2). In August, neither submerged thalli nor thalli deposits at the bottom of the pond were noticed.

Effect of abiotic factors on *Ulva* development

Of all the parameters studied in the present work, the highest impact on the thalli development and mat arrangement was attributed to: pH, concentration of $N\text{-}NH_4$ and $P\text{-}PO_4$, water depth, oxygenation rate, and temperature (Table 3). Many of the correlations between thalli and mat morphometric features and physical and chemical conditions were observed for pH, water depth and ammonium. The depth was most strongly correlated with the average *Ulva* thalli length ($r = 0.90$; $r^2 = 0.82$; $P < 0.001$) and thalli density inside the mat ($r = 0.89$; $r^2 = 0.80$; $P < 0.001$). The strongest negative correlation was determined between the contribution of adult thalli inside the mat and the concentration of $N\text{-}NH_4$ ($r = -0.84$; $r^2 = 0.71$; $P < 0.001$) and pH values ($r = -0.82$; $r^2 = 0.68$; $P < 0.001$ (Table 3).

Statistically significant differences between the values of conductivity and $N\text{-}NO_3$ levels were determined; measurements were taken under and outside the mats built by fresh water *Ulva* (Table 4). $N\text{-}NO_3$ levels were always lower under the *Ulva* mats than outside the mats. In the case of conductivity, the levels of this parameter were lower outside the *Ulva* mats than under the mats (Table 4). Although the remaining abiotic factors were also studied under and outside the mats, no statistically significant differences were found between the concentration of pH, chloride or other parameters (Table 4).

Table 3

Values of Pearson's linear correlation coefficient and the coefficient of determination (r^2) for morphometric parameters of *U. flexuosa* thalli and macroalgal mats with physicochemical factors

	pH	Depth	$N\text{-}NH_4^+$	$P\text{-}PO_4^-$	O_2	Temperature
Average length of a thallus	-0.45 ns -	0.90*** (0.82)	-0.45 ns -	0.10 ns -	0.68* (0.45)	-0.38 ns -
Average width of a thallus	-0.18 ns -	-0.03 ns -	-0.23 ns -	-0.40 ns -	-0.08 ns -	-0.63* (0.39)
Surface of mats	-0.73** (0.54)	0.43 ns -	-0.62* (0.39)	-0.75** (0.56)	-0.20 ns -	-0.47 ns -
Density of thalli per m^{-2} of mats	-0.48 ns -	0.89*** (0.80)	-0.53 ns -	0.06 ns -	0.82** (0.67)	-0.42 ns -
Percentage of branched thalli per m^2 of mats	0.54 ns -	0.10 ns -	0.59* (0.35)	0.59* (0.35)	0.14 ns -	0.16 ns -
Percentage of unbranched thalli per m^2 of mats	-0.76** (0.58)	0.31 ns -	-0.46 ns -	-0.67* (0.45)	-0.15 ns -	-0.31 ns -
Percentage of young thalli per m^2 of mats	0.60* (0.36)	-0.48 ns -	0.70** (0.50)	0.30 ns -	-0.31 ns -	0.52 ns -
Percentage of mature thalli per m^2 of mats	-0.82*** (0.68)	0.43 ns -	-0.84*** (0.71)	-0.59* (0.35)	0.05 ns -	-0.34 ns -
Percentage of submerged thalli per m^2 of mats	-0.66* (0.44)	0.89*** (0.80)	-0.58* (0.33)	-0.14 ns -	0.50 ns -	-0.43 ns -
Percentage of free-floating thalli per m^2 of mats	0.73** (0.54)	-0.86*** (0.74)	0.70* (0.49)	0.22 ns -	-0.48 ns -	0.40 ns -

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns – not significant, coefficient of determination – r^2 in brackets

Table 4

Results of the Mann-Whitney *U* test of the difference in mean values of physicochemical factors under and outside of *Ulva* mats (n = 12). Results where *P* < 0.05 are statistically significant and given in bold.

Parameters	Unit	Mann-Whitney *U* test	Statistical significance		Sign	
pH	-	66.0	$P = 0.75$	UMM	>	OMM
Conductivity	μS cm^{-1}	**31.0**	$\mathbf{P = 0.01}$		>	
$N\text{-}NH_4^+$	mg l^{-1}	64.0	$P = 0.66$		>	
$N\text{-}NO_3^-$		**32.5**	$\mathbf{P = 0.02}$		<	
$P\text{-}PO_4^{3-}$		49.0	$P = 0.19$		>	
Fe_{total}		63.0	$P = 0.62$		>	
NaCl		67.5	$P = 0.85$		>	
Cl^-		67.5	$P = 0.81$		>	

UMM - under macroalgae mats; OMM - outside macroalgae mats

Discussion

The abundant occurrence of *Ulva flexuosa* in freshwater ecosystems in the form of dense mats which cover considerable areas of water surface is not a common phenomenon. In Europe, several to dozens freshwater populations of *U. flexuosa* subsp. *pilifera* were observed creeping along the basin floor or floating freely on the water surface (Messyasz & Rybak 2009; Mareš et al. 2011). In 2007-2008, the freshwater population of *U. flexuosa* was observed forming the mats of just 4 m^2 (Messyasz & Rybak 2011). In that case, high levels of ammonia nitrogen and the increasing water depth were the main factors determining the process of mat formation. Under such conditions, the records evidenced the highest concentration of thalli in 1 dm^3 of water (Messyasz & Rybak 2009; Messyasz & Rybak 2011). In the studies of the freshwater population of *U. flexuosa* originating from the fish pond, the water depth and concentration of ammonia nitrogen were not as important as the level of conductivity and chloride concentration. These two parameters determined the chemical specificity of the studied stand. García & Aboal (2014) have also indicated the conductivity as the main determinant of *U. flexuosa* occurrence in their field research on the dynamics of the macroalgae population development in the area of the Mediterranean marshes. However, in the fish pond where the *U. flexuosa* "green tide" was monitored, the concentration of nutrients, and in particular ammonia (> 4 mg l^{-1}), was high.

The abundant occurrence of *Ulva* in saltwater ecosystems is connected with higher concentrations of nutrients in water, specifically nitrogen and phosphorus (Valiela et al. 1997). *Ulva* species which form the "green tide" are mainly: *U. intestinalis*, *U. lactuca* (Blomster et al. 2002), *U. ohnoi* (Hiraoka et al. 2003), *U. pertusa* (Ishii et al. 2001), and *U. prolifera* (Liu et al. 2010). The correlation between quick biomass growth of *Ulva* species thalli and high concentrations of nutrients in water often made thalli of such species as *U. rotundata*, *U. reticulata*, and *U. lactuca* useful as nutrient biological filters, e.g. in breeding ponds with shrimps and saltwater fish (Neori et al. 1991; Jimenez del Rio et al. 1996; Hernández et al. 1997; Schroeder et al. 2013). Some species like *U. lactuca* or *U. intestinalis* are used for monitoring of over-fertilized waters (Reed & Moffat 2003). For this reason, *Ulva* opportunistic macroalgae have been included on the list of water quality indicators, which was developed for the purposes of the European Water Framework Directive (WFD 2000).

Brault & Quéquiner (1989) demonstrated that *Ulva gigantea* biomass would keep growing under the influence of increasing concentration of ammonia nitrogen. In the laboratory studies of the freshwater population of *U. flexuosa* (Rybak et al. 2012c), the contribution of organic nitrogen was measured at the level of 1.79-2.29% dry mass (KNO_3 being the source of nitrogen). On the other hand, the thalli incubated on NH_4Cl culture medium featured the concentration of organic nitrogen from 1.89 to 2.48% dry mass. The nitrogen level in *U. flexuosa* thalli was very similar to the content of nitrogen in a saltwater tubular thallus (the assay up to *Enteromorpha* sp. genus only) where the value of organic nitrogen in the thallus amounted to 0.7 through 3.5% dry mass (Fong et al. 1994). In their studies of *U. clathrata*, Copertino et al. (2009) demonstrated the highest accumulation of nitrogen in the ammonium form compared with other available nitrogen forms. Furthermore, these studies proved the lowest possible efficiency of phosphate biofiltration from the medium. A similar relationship was found by Taboada (2009) in his studies using *U. reticulata* and *U. lactuca*. In this case, the uptake of nutrients (in the form of NH_4^+ and PO_4^{3}) was consistent with Michaelis-Menten kinetics, which allows to conclude that the rate of nutrients' consumption from the medium depends on the concentration of such elements in the water, but it will grow up to a certain level of nutrient concentration and a certain value of macroalga biomass. The efficiency of consumption of nitrogen in NH_4^+ from the water contaminated by *U. reticulata* and *U. lactuca* thalli amounted to 95.8% and 93.5%, respectively. For the purpose of comparison, the efficiency of consumption of phosphorus in the PO_4^{3-} form by the two species was 78.6% and 78.3%, respectively (Taboada 2009). In the case of the marine species *U. rotundata*, the rate of ammonia nitrogen consumption reaches the level of 98% in just 30 minutes of incubation. On the other hand, the nitrate removal efficiency was at the level of 84% in the same period of incubation (Anibal et al. 2013). Although the rate of ammonia nitrogen consumption is definitely higher than in the case of nitrates following the beginning of incubation, the levels of the two nutrients in the medium will drop to zero in one hour (Anibal et al. 2013).

Furthermore, under *ex situ* conditions, the rates of nutrients' consumption by the species with tubular thalli (such as *U. prolifera*) were much higher than in species with leaf-shaped thalli (e.g. *U. rigida*) (Kim et al. 2007). This phenomenon may be explained by the fact that the area of cell-water contact is larger in tubular thalli compared with leaf-shaped thalli (Luo et al. 2012). Many field studies have indicated that the growth of biomass in *Ulva* marine species from coastal waters ranges from 4 to 33 g m^{-2} d^{-1} (Sfriso et al. 1987, 1988; Hernández et al. 1997). The growth of *Ulva* biomass in basins designed for saltwater pisciculture was on average 14 g m^{-2} d^{-1} (Israel et al. 1995).

Advanced numerical modelling of "green tides" of macroalgae demonstrated that the main drivers in the formation of large *Ulva* mats in the spring season at the coasts of France were high concentrations of nitrates (Perrot et al. 2014). Hence, the only method which may reduce the frequent blooming of macroalgae in coastal waters consists in the reduction of water discharge contaminated with nutrients (Beaujouan et al. 2002; Ruiz et al. 2002; Guidone & Thornber 2013). The occurrence of considerable *Ulva* biomass in coastal waters and on the beaches may considerably reduce the tourist attractiveness of such regions as well as produce the potential risk to human health because of the gases generated by putrefaction of thalli heaps (CEVA 2011). On the coasts of France, a horse and several dozens of wild boars died from intoxication with hydrogen sulfide which was released from under the decaying *Ulva* mats (Smetacek & Zingone 2013; Charlier et al. 2008). *Green tides* of *U. flexuosa* are more and more frequently observed in European lakes and rivers (Rybak et al. 2014). They may become potentially hazardous to native aquatic life forms as well as generate many problems for holiday and health resorts.

The use of *Ulva* marine species as biological filters (macroalgal "nutrient traps") in fish and shrimp breeding ponds is limited to brackish or salty basins only, i.e. the sites located directly on the sea or ocean coasts. For the purpose of saltwater fish breeding (e.g. gilt-head (sea) bream – *Sparus aurata*), the optimum salinity for many *Ulva* species (e.g. *U. lactuca, U. intestinalis* or *U. compressa*) ranges from 15 to 37 PSU (de Paula Silva et al. 2008). Thus, *Ulva* marine species cannot be used as biological filters of nutrients in ponds designed for freshwater pisciculture, e.g. carp breeding. In this case, one of the alternative solutions is the use of *U. flexuosa* freshwater population thalli since *U. flexuosa* occurs in waters with salinity of up to <0.5 PSU, and the thalli will tolerate the increased salinity of up to 6 PSU (Rybak & Messyasz 2011; Rybak et al. 2014). Another thalli may come from *U. limnetica*, which features the largest spectrum of tolerance towards the salinity (0-30 PSU) among the identified freshwater *Ulva* taxa (Ichihara et al. 2013).

In the case of limnetic *U. flexuosa* from the studied fish pond, statistically significant differences in nitrate concentrations directly under and outside the macroalga mats were determined – up to 0.1 mg l^{-1}. Nevertheless, the water flow system in this fish pond features the permanent renewal and the measurements produce considerably high values.

Conclusions

The study presents the results of macroalgal monitoring connected with the formation of the *Ulva flexuosa* "green tide" in the freshwater ecosystem. The case was the first bibliographical reference describing the example of "green tides" with *Ulva flexuosa* formation outside the coastal ecosystems. In connection with a real risk of occurrence of "green tides" in lakes and ponds (also those used in the economy), which feature high nutrient values, a detailed analysis will be needed to study the abundant occurrence of freshwater *Ulva*, also on a smaller scale. Furthermore, such studies should account for the impact of *Ulva* blooming on aquatic biocoenosis (phytoplankton or zooplankton), since the effects of long-term impact of *Ulva*-containing macroalgal mats on the native freshwater hydrocenosis, e.g. lakes, remain unknown. These issues should be addressed by more detailed hydrobiological studies scheduled for many independent study sites and aquatic ecosystem types featuring various types of development.

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