Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 45, Issue 3, September 2016
pages (377-387)

ISSN 1730-413X
eISSN 1897-3191

Dry weight and calcium carbonate encrustation of two morphologically different *Chara* species: a comparative study from different lakes

by

Andrzej Pukacz[1,*], Mariusz Pełechaty[2], Marcin Frankowski[3], Eugeniusz Pronin[2]

DOI: **10.1515/ohs-2016-0034**
Category: **Original research paper**
Received: **October 27, 2015**
Accepted: **February 22, 2016**

[1]*Polish-German Research Institute, Collegium Polonicum, Adam Mickiewicz University in Poznań, ul. Kościuszki 1, 69-100 Słubice, Poland*

[2]*Department of Hydrobiology, Faculty of Biology, Adam Mickiewicz University in Poznań, ul. Umultowska 89, 61-614 Poznań, Poland*

[3]*Department of Water and Soil Analysis, Faculty of Chemistry, Adam Mickiewicz University in Poznań, ul. Umultowska 89b, 61-614 Poznań, Poland*

* Corresponding author: *pukacz@europa-uni.de*

Abstract

Two charophyte species (*Chara tomentosa* Thulli. 1799 and *Chara globularis* L. 1753) were studied to assess their biomass and $CaCO_3$ production in seven hard-water lakes in Western Poland. In each lake, samples of ten individuals from three study sites were collected for dry weight (DW) and calcium carbonate content (% $CaCO_3$) analyses. Additionally, physicochemical parameters of water collected from the above sampling sites were analyzed.

No significant differences were found between the study sites in each lake for any of the analyzed parameters. In all the lakes, DW of *C. tomentosa* (0.60 ± 0.23 g indiv.$^{-1}$) was significantly higher and more differentiated than DW of *C. globularis* (0.11 ± 0.08 g indiv.$^{-1}$), suggesting species-specificity. The $CaCO_3$ content in DW for *C. tomentosa* was higher (58.8-70.9%) than in *C. globularis* (50.1-68.3%), however, it did not reflect the DW differentiation, suggesting lake-specificity. The physicochemical properties of water revealed clear lake-to-lake differentiation. Different correlations between dry weight and calcium carbonate content and lake characteristics were found for each species. The results showed that DW and % $CaCO_3$ are closely related to habitat conditions and different factors may influence the individual biomass of each species.

Key words: charophytes, individual biomass, calcite encrustation, water properties, inter-lake variability

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Introduction

Charophytes are one of the main groups of macrophytes occurring in clean, hard-water lakes. They form large meadows and are therefore an important structural and functional element of the littoral zone. In the literature, there are more and more references to charophytes, not only in the context of their sensitivity as water quality indicators, but also as a significant habitat forming element (as summarized by Schneider et al. 2015). This is reflected e.g. in the alternative stable state theory for shallow lakes (Scheffer 1998). According to Van den Berg et al. (1998), charophytes play a special role in maintaining clean water quality, among others through: increasing the sedimentation from the ambient water, preventing the resuspension of bottom sediments or limiting the development of phytoplankton.

The key role is played by the quantitative contribution of charophytes (which form dense meadows in *Chara* lakes) in the littoral zone, and thus by the biomass they produce. In some lakes, charophyte meadows may cover more than 50% of the lake bottom surface (Pukacz, Pełechaty 2013) and are therefore an important environment-forming element influencing both the biocenosis and the water quality properties, as well as sediment formation processes (Pełechaty et al. 2013; Blindow et al. 2014). As evidenced by contemporary studies, the biomass of charophytes can be considerably high (over 2 kg dry weight m^{-2}), including more than 80% of carbonate encrustations (Pukacz et al. 2014a and literature quoted therein). Moreover, considerable amounts of nutrients are incorporated in charophyte biomass (Kufel, Kufel 2002; Rodrigo et al. 2007) and remain immobilized there for a long time. What is also important is that some charophytes (especially in clean lakes) can overwinter as green biomass (Blindow 1992a, Pukacz et al. 2014b). In addition, a considerable part of carbon and phosphorus is permanently immobilized in the form of carbonate encrustations, which get into the sediment and turn into lacustrine chalk (Otsuki, Wetzel 1972; Murphy et al. 1983; Pełechaty et al. 2013).

Despite the seemingly obvious impact of charophyte biomass on lake ecosystems (especially those with a considerable contribution in charophyte meadows), there is still little data concerning in situ variability of charophyte biomass production and factors affecting it.

Studies carried out so far suggest that charophyte biomass depends both on the species (Królikowska 1997), and on the lake (Kufel, Kufel 2002). There is also much evidence that the percentage contribution of precipitated carbonates is species-specific (Kufel et al. 2013, Pełechaty et al. 2013).

Besides, little is also known about factors affecting the charophyte biomass production and the formation of encrustations. Most works on the subject written so far are based on experimental studies investigating single factors which affect charophyte biomass (e.g. Ray et al. 2003). We know that the content of nutrients (Kufel, Rymuza 2014) and the amounts of calcium and magnesium ions in the water (Asaeda et al. 2014) are among the main environmental factors affecting the amount of biomass and the contribution of carbonates. There is much evidence that similar factors operate in the lacustrine environment, and the amount and precipitation rate of carbonates is site-specific (Pełechaty et al. 2015). This is suggested e.g. by the results obtained by Pukacz et al. (2014a), showing that both the dry weight of charophytes and the amount of carbonate encrustations they produce change with depth.

Learning exactly how the quantitative and qualitative structure of biomass changes in relation to environmental characteristics may be very important both for the current studies concerning the functioning of lake ecosystems, and – as Dittrich & Obst (2004) suggested – also in paleoecological and paleoclimatic research.

The aim of the presented study was to: a) quantify the dry weight and $CaCO_3$ precipitation of two charophyte species in different lake ecosystems, b) determine possible relationships between the environmental characteristics of the investigated lakes and the dry weight and $CaCO_3$ content in DW of the two species. We hypothesized that the amount of dry weight and $CaCO_3$ encrustation is species-specific but the $CaCO_3$ content in DW is lake-specific.

Methods

Study sites and sampling

The study was carried out between 20 and 26 July 2012, in seven lakes located in Western Poland. The same high-pressure weather (cloudless or slightly cloudy, high temperatures and light wind) prevailed during the whole study period. The lakes differed in terms of morphology, flow type and the nature of the drainage basin (Table 1). On the one hand, the group of lakes included a shallow, small polymictic lake (Lake Jasne) and on the other, a deep, large lake (Lake Niesłysz). The lakes had different hardness levels (from 3.62°dH in Lake Pierwsze to 12.79°dH in Lake Karskie) and the parameters related to hardness varied in a similar manner. Despite this, as well as a considerable

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Table 1

Characteristics of the studied lakes (based on: Jańczak 1996; Pełechaty, Pukacz 2006; Pełechaty et al. 2007)

	Unit	Jasne	Karskie	Malcz	Męckie	Niesłysz	Pierwsze	Złoty Potok
Geographical coordinates	-	52°17'7"N 15°03'06"E	52°55'4"N 15°04'8"E	52°21'1"N 15°13'3"E	52°22'0"N 15°11'2"E	52°13'9"N 15°23'8"E	52°23'11"N 15°09'18"E	52°13'0"N 15°22'5"E
Surface area	ha	15.1	150	36.2	40.9	486.2	19.3	32.8
Mean depth	m	4.3	6.2	3.4	9.2	7.8	4.7	5.9
Max depth	m	9.5	17.6	7.3	22.7	34.7	10.7	13.7
Lake type	-	outflow	flow	no flows	flow	no flows	no flows	no flows
Stratification	-	incomplete	complete	incomplete	complete	complete	incomplete	complete
SD visibility	m	3	3.1	3.8	6.5	4.2	5.4	4.3
C. tomentosa sampling depth	m	3	3		3	2		2
C. globularis sampling depth	m	4	3	3	4		4	

morphometric variety, all the lakes are classified as mesotrophic. This is evidenced by relatively low TP concentrations (from 0.010 to 0.033 mg l^{-1} in Lake Jasne and Lake Niesłysz, respectively) and TN concentrations (from 1.23 to 1.87 mg l^{-1} in Lake Złoty Potok and Lake Karskie, respectively). All the lakes had high water transparency (Secchi Depth visibility > 3 m), with Lake Męckie having the highest water clarity (SD = 6.5 m). What these lakes had in common was also well-developed aquatic vegetation, dominated by charophyte meadows. Both the structure of the vegetation and the habitat conditions found in most lakes during the study correspond to the data from previous years (e.g. Pełechaty, Pukacz 2006; Pełechaty et al. 2007).

The study concerns two morphologically different *Chara* species: *Chara globularis* and *Chara tomentosa*. *Chara globularis* is a small, green charophyte, with a thin axis, branchlets and few branches (Krause 1997; Urbaniak, Gąbka 2014). It usually occurs in meso- and eutrophic waters, where it forms dense meadows just above the bottom (Pukacz et al. 2011). *Chara tomentosa* belongs to large charophytes; it is extensively branched and has a thick axis and branchlets with characteristic reddish color (Krause 1997; Urbaniak, Gąbka 2014). The species mostly lives in mesotrophic, meso-eutrophic and weakly eutrophic lakes, where it can form large surface meadows (Pełechaty et al. 2015).

At five out of 15 study sites of *C. tomentosa*, the cover of this species reached 100%, whereas at seven sites it was equal to or exceeded 70%. In three stands, the species cover was 50%. Regardless of the exact cover of *C. tomentosa* in all the studied sites, the species was always a dominant taxon with minor or negligible importance of vascular plants and mosses. The cover of *C. globularis* was in many cases greater. In nine out of 15 studied stands, this species covered 100% of the studied stand and in five stands the cover reached or even exceeded 70%. At one site, the cover was only 40%. Similarly to *C. tomentosa*, *C. globularis* was always the dominant species compared to much less abundant accompanying single vascular plants and mosses.

Each species was sampled in five lakes from three different sites (Table 1). In three of the lakes (Jasne, Karskie, Męckie), both species co-occurred. In the four other lakes, only one of these species was found, each of them in two lakes. All the sampling sites for each species within a particular lake were located at the same depth. The sites and depths were determined in the preliminary research on the basis of the community formation: the sites where each species formed dense patches, in good condition and with structure typical of a given lake were selected. The samples were manually harvested from the bottom by diving from the central part of a patch. In addition, before sample harvesting, the percentage cover of the bottom by the dominant species (*C. globularis* or *C. tomentosa*) and the number of species in the community were determined.

From each of the plant samples, 10 entire individuals (not damaged) were selected for further measurements. The selected individuals were separately transported to a laboratory in plastic bags.

Prior to charophyte sampling, basic physicochemical parameters of water from above each sampling site (a few cm above charophyte meadow) were measured. The following parameters were measured:

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visibility (in each lake at one pelagial site, using a Secchi disc), dissolved oxygen concentration and temperature (using an Elmetron CX-401 portable meter), electrolytic conductivity and pH (with a Cyber-Scan 200). Similarly, water samples for laboratory analyses were collected directly from above the sampling sites using an electric pump. The samples were collected in one-liter plastic bottles and kept in a portable refrigerator. First, alkalinity analysis was performed (in a laboratory within 6 hours after the sampling). Then, the samples were kept in a refrigerator (at 4°C) until the remaining chemical analyses were performed.

Laboratory analyses

Immediately after their collection at the lake, the individuals were air-dried for 24 hours using a laboratory ventilation system to avoid decomposition. After that, the plants were dried at 105°C for three hours in an electric drier to determine the dry plant weight (DW). The calcium carbonate content (% $CaCO_3$) was determined by the two-step weight loss on ignition method (Heiri et al. 2001). Powdered samples were first combusted at 550°C for 4 hours and subsequently at 950°C for 2 hours. Carbonate content was calculated by multiplying the mass of CO_2 evolved in the second step of the analysis by 1.36. Finally, the $CaCO_3$ content was calculated by multiplying the CO_3^{2-} content by 1.66. The loss on ignition at 550°C is presumed to represent an organic matter percentage. The whole procedure was performed separately for each of the 10 individuals taken from a single sampling site.

Alkalinity was determined by the titration method with an indicator and color using the visual method against the platinum scale. The total water hardness was determined by the versenate method. In order to determine Ca^{2+} and Mg^{2+}, a Metrohm ion chromatograph, the 881 Compact IC Pro model (Metrohm, Switzerland) was applied, using columns Metrosep C4 Guard (the guard column) and Metrosep C4 150 (the separating column). Total nitrogen was determined by a TOC-L Shimadzu analyzer with a TNM-L unit using catalytic thermal decomposition and chemiluminescence methods (Shimadzu, Japan). Total phosphorus was determined by the molybdate method with ascorbic acid as a reducer using a Merck Spectroquant® Pharo 100 apparatus (Merck KGaA, Darmstadt, Germany). The same analyses were performed separately for each of the samples.

Data analyses

Statistical analyses were performed using STATISTICA 10.1 (StatSoft Inc., Tusla, OK, USA) software. The normality of distributions of the analyzed variables and the homoscedasticity of the samples were tested with the Shapiro-Wilk and Levene tests, respectively. The conditions were satisfied in both cases, thus one-way ANOVA and the post-hoc Scheffe test were used to compare the means of the variables. $P < 0.05$ was accepted as being statistically statistically significant. Principal Component Analysis (PCA) was performed for the statistical analysis of the data set of physicochemical parameters. Prior to this analysis, the data were subjected to a logarithmic transformation. Since the number of samples was limited, Spearman rank correlation was applied to test the relationships between the charophyte dry weight, the calcium carbonate content and physicochemical properties of water.

For the interpretation of the obtained results, the Ca/Mg ratio and the saturation index (SI) were calculated. For SI, the formula by Kelts and Hsü (1978) was applied:

$$SI = \frac{\log(IAP)}{K_c}$$

where *IAP* is the ion activity product of Ca^{+2} and CO_3^{-2} and K_c is an equilibrium constant for the reaction:

$$CaCO_3 <=> Ca^{2+}(aq) + CO_3^{2-}(aq).$$

Results

Dry weight and $CaCO_3$ percentage differentiation

Dry weight of *Chara tomentosa* individuals (mean DW = 0.60 ± 0.23 g indiv.$^{-1}$) was five times higher (ANOVA, $P < 0.0001$) than that of *Chara globularis* individuals (mean DW = 0.11 ± 0.08 g indiv.$^{-1}$) in all lakes (Fig. 1). Differentiation of the carbonate weight (in g indiv.$^{-1}$) directly reflected the differentiation of individuals' dry weight presented in Fig. 1. No significant differences were found in any of the lakes for dry weight between the sites of a given species (ANOVA, $P > 0.05$).

Values of *C. tomentosa* dry weight varied significantly between most of the lakes (Fig. 1). In the case of *C. globularis*, dry weight was significantly higher in Lake Jasne, while no significant differences were found among the other lakes.

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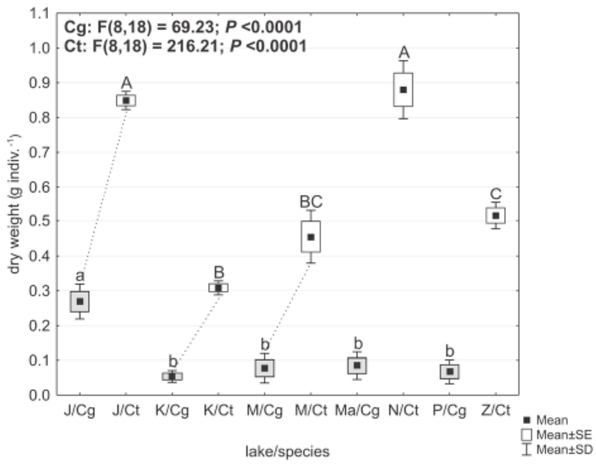

Figure 1

Lake-to-lake variability of charophyte dry weight. The dashed line points at the lakes in which both species occurred. Each box-whiskers plot n = 30 (10 individuals × 3 sites). Abbreviations on the horizontal scale indicate the name of the lake (J – Jasne, K – Karskie, M – Męckie, Ma –Malcz, N – Niesłysz, P – Pierwsze, Z – Złoty Potok) slashed with the species name (Cg – *Chara globularis*, Ct – *Chara tomentosa*). The colors indicate species (gray is for *C. globularis*, white is for *C. tomentosa*). Different letters (lower case – *C. globularis*, uppercase – *C. tomentosa*) indicate significant (P < 0.05) lake-to-lake differences.

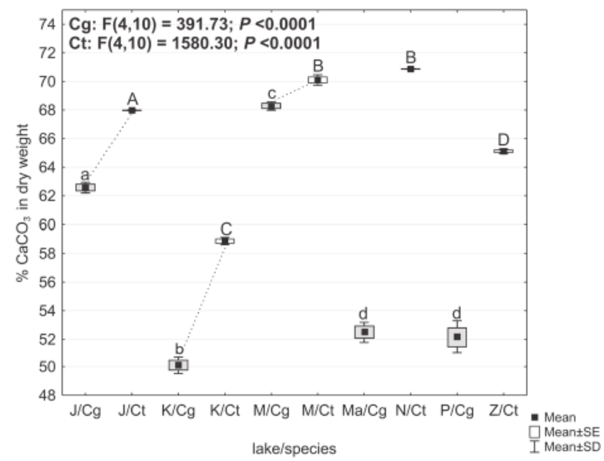

Figure 2

Lake-to-lake variability of calcium carbonate content in charophyte dry weight. The dashed line points at the lakes in which both species occurred. Each box-whiskers plot n = 30 (10 individuals × 3 sites). Abbreviations on the horizontal scale indicate the name of the lake (J – Jasne, K – Karskie, M – Męckie, Ma –Malcz, N – Niesłysz, P – Pierwsze, Z – Złoty Potok) slashed with the species name (Cg – *Chara globularis*, Ct – *Chara tomentosa*). The colors indicate species (gray is for *C. globularis*, white is for *C. tomentosa*). Different letters (lower case – *C. globularis*, uppercase – *C. tomentosa*) indicate significant (P < 0.05) lake-to-lake differences.

The $CaCO_3$ content in DW varied unlike the pattern of dry weight (Fig. 2). In all three lakes where both analyzed species occurred, the $CaCO_3$ content in DW of *C. tomentosa* was higher than in DW of *C. globularis*. The smallest differences occurred in Lake Męckie (less than 2%), and the biggest ones – in Lake Karskie (more than 8%).

The range of $CaCO_3$ content in DW was 58.8-70.9% for *C. tomentosa* and 50.1-68.3% for *C. globularis*.

Values of $CaCO_3^-$ in DW of *C. tomentosa* in Lake Męckie were similar to those in Lake Jasne and much higher compared to lakes Złoty Potok and Karskie.

Mean values of $CaCO_3^-$ content in DW varied considerably between most lakes, both for *C. tomentosa* and for *C. globularis*. No statistically significant differences (ANOVA, $P > 0.05$) for *C. tomentosa* were found only between lakes Męckie and Niesłysz and for *C. globularis* – between lakes Malcz and Pierwsze.

Habitat-based differentiation

The Principal Component Analysis (PCA) showed clear differences between the investigated lakes in relation to physicochemical parameters (Fig. 3), mainly due to conductivity, alkalinity, total hardness, Ca^{2+} and Mg^{2+} (correlated with the first principal component) and oxygen (correlated with the second principal component). These parameters explained over 70% of the observed variance, with r > 0.8. This was also confirmed by additional tests, which indicated statistically significant lake-to-lake differences (ANOVA, $P < 0.05$) for all the above-mentioned parameters (Table 2). In the lakes where both species occurred, no significant differences (ANOVA, $P > 0.05$) were found between the sites of *C. tomentosa* and *C. globularis*.

Lake Karskie was most different from the other lakes. The waters of this lake were characterized by the highest values of conductivity, alkalinity, hardness and total nitrogen, whereas the lowest values of these parameters were determined in Lake Pierwsze. Lakes Niesłysz and Złoty Potok also clearly stood out in PCA. Both of them had similar water characteristics, which indicate intermediate conditions between those of Lake Karskie and Lake Pierwsze. The other lakes (Malcz, Jasne and Męckie) were clustered together as a group with similar water characteristics. Their differentiation

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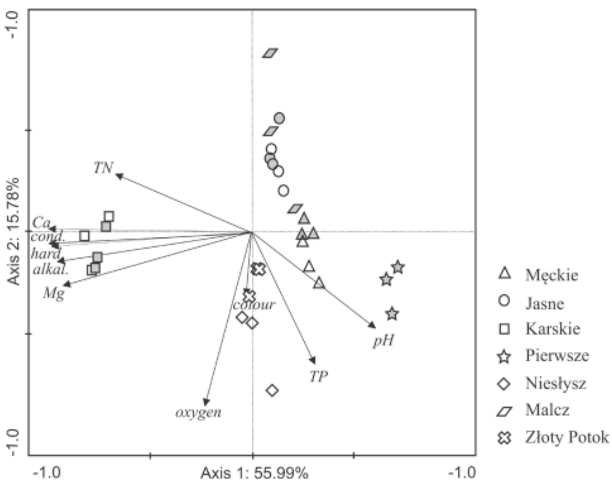

Figure 3

PCA output for physicochemical water properties at all the study sites. The symbols indicate particular lakes. The colors indicate species (gray is for *C. globularis*, white is for *C. tomentosa*).

for the second principal component was mainly related to the values of oxygen and, to a lesser extent, TP concentration.

Relationships between physicochemical properties and DW and carbonate content

Significant correlations were found for most of the physicochemical parameters of ambient waters and additional characteristics of the sampling sites with DW and the percentage of $CaCO_3$ (Table 3). No statistically significant correlation ($P > 0.05$) was found only for the color. The correlations were different for *C. globularis* and *C. tomentosa*. In the case of *C. globularis*, DW was significantly correlated with oxygen concentration only, and the percentage of $CaCO_3$ was correlated with sampling depth, percentage of bottom cover and the number of species at a sampling site. For *C. tomentosa*, DW was significantly correlated with the temperature, alkalinity and total phosphorus concentration, and the percentage of $CaCO_3$ in DW was correlated with conductivity, alkalinity, Ca^{2+}, Mg^{2+} and total hardness.

Discussion

Species- and lake-based comparison of DW and $CaCO_3$ content

The individuals of *C. tomentosa* had higher DW in all the lakes, which reflects the morphological differentiation between the investigated species (e.g. Wood, Imahori 1865; Krause 1997). The significant differences in *C. tomentosa* and *C. globularis* DW, particularly for lakes in which both species occurred, indicate that charophyte biomass is species-specific. This finding is consistent with interspecific differences in charophyte biomass, as reported by Pukacz et al. (2014) for a single lake.

Much greater lake-to-lake differentiation for *C. tomentosa* (especially in the lakes where both species occurred) suggests that the production of charophyte biomass is not only species-specific. *C. globularis* has a wide ecological amplitude and usually occurs in small lakes with a higher trophic status (Pukacz et al. 2011). *C. tomentosa*, on the other hand, is a much rarer species and usually occurs in clean, hard-water and mesotrophic lakes

Table 2

Means and standard deviations of the physicochemical water properties significantly differentiating the investigated lakes. Results of ANOVA tests (F and p-values are indicated; degrees of freedom: 6 and 23). Results of post hoc analyses are indicated by means of capital letters.

	ANOVA	Jasne (n = 6)	Karskie (n = 6)	Malcz (n = 3)	Męckie (n = 6)	Niesłysz (n = 3)	Pierwsze (n = 3)	Złoty Potok (n = 3)
O_2 (mg l^{-1})	F = 31.4 $P < 0.001$	A 6.0 ± 0.1	BCD 7.4 ± 0.3	A 5.3 ± 0.1	BCD 7.4 ± 0.2	C 8.2 ± 0.6	D 7.0 ± 0.2	D 7.0 ± 0.2
Alkalinity (mval l^{-1})	F= 345.8 $P < 0.001$	A 1.6 ± 0.1	B 2.8 ± 0.0	CD 1.9 ± 0.06	C 1.7 ± 0.1	D 1.9 ± 0.0	E 1.2 ± 0.1	F 2.2 ± 0.6
Conductivity (µS cm^{-1})	F = 803.3 $P < 0.001$	A 304 ± 13.4	B 590 ± 11.9	C 266 ± 6.6	C 263 ± 3.7	A 318 ± 18.5	D 158 ± 5.8	A 332 ± 5.6
Ca^{2+} (mg l^{-1})	F = 1512.1 $P < 0.001$	A 47.4 ± 1.4	B 77.8 ± 1.2	C 38.3 ± 0.3	C 40.4 ± 0.5	D 44.0 ± 0.2	E 22.6 ± 0.2	A 49.7 ± 1.0
Mg^{2+} (mg l^{-1})	F = 61.3 $P < 0.001$	A 2.9 ± 0.6	B 9.6 ± 1.4	A 2.7 ± 0.3	A 2.2 ± 0.4	C 5.3 ± 1.0	A 2.1 ± 0.5	C 5.9 ± 0.2
Hardness (°dH)	F = 1433.5 $P < 0.001$	A 7.3 ± 0.2	C 13.1 ± 0.2	B 6.0 ± 0.1	B 6.2 ± 0.1	A 7.4 ± 0.2	D 3.7 ± 0.1	E 8.3 ± 0.1

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Table 3

Significant correlations between the dry weight and calcium carbonate content of each charophyte species and the habitat characteristics of the study sites. For each characteristic n = 15 (DW and % $CaCO_3$ were represented by mean of 10). The asterisks (*) indicate the significance level (< 0.05, < 0.01, < 0.001, respectively)

	Chara globularis		*Chara tomentosa*	
	Dry weight	% $CaCO_3$	Dry weight	% $CaCO_3$
Temperature			0.61*	
O_2	-0.53*			
Conductivity				-0.63**
Alkalinity			-0.59*	-0.59*
Ca^{2+}				-0.88***
Mg^{2+}				-0.55*
Hardness				-0.75***
TP			0.73**	
Sampling depth		0.69**		
% bottom cover		0.72**		
No. of species		-0.58*		

(e.g. Pełechaty, Pukacz 2008; Urbaniak, Gąbka 2014). In this context, it can be concluded that the differences in *C. tomentosa* individual biomass reflect greater sensitivity of this species to habitat conditions.

The fact that DW at all 3 sites in each lake was similar for a given species indicates that the sampling sites did not differ significantly in terms of habitat conditions. The main factor responsible for this situation was certainly the same sampling depth, and thus the availability of light, which is the main limiting factor for charophytes (Blindow et al. 2002; Pukacz et al. 2014a).

Both species produced considerable amounts of carbonate encrustations, which shows they are an important sediment-forming element in the littoral zone (e.g. Wetzel 1960; Hutchinson 1975). The mean values of $CaCO_3$ content in DW of *C. tomentosa* were similar to literature data quoted previously for hard-water lakes by Blindow (1992b) and Pełechaty et al. (2013). In the case of *C. globularis*, the values were much lower in most lakes (Karskie, Malcz and Pierwsze) than previously reported by Pentecost (1984) and Kufel et al. (2013). The generally higher values of % $CaCO_3$ in *C. tomentosa* DW suggest that this species is more efficient in carbonate precipitation than *C. globularis*. It must be stressed, however, that sampling sites of *C. tomentosa* were shallower than those of *C. globularis*, and hence they received larger amounts of lights. In addition, much more complex individual morphological features of *C. tomentosa* (big, branchy and very spiny) favor deposition of insoluble calcium carbonate directly onto the surface of a charophyte thalli (Pukacz et al. 2014).

Apart from the above-mentioned higher mean values of % $CaCO_3$ in *C. tomentosa* DW, the carbonate precipitation seems to be lake-specific. This finding is not in line with the results obtained by Kufel et al. (2013) who have investigated the $CaCO_3$ content in DW of five species in three lakes. In our study, the lake-specificity of $CaCO_3$ content in DW was evidenced by great lake-to-lake differences of this property for both species. Moreover, despite a constant trend regarding the differences in $CaCO_3$ content in DW (in the lakes where both species occurred, the values of % $CaCO_3$ in *C. tomentosa* were higher), the content of $CaCO_3$ in *C. globularis* DW may be higher than in *C. tomentosa*. This is exemplified by Lake Męckie where the $CaCO_3$ content in *C. globularis* DW was higher than in *C. tomentosa* in Lake Karskie. What is also noteworthy is that the two lakes differed greatly in terms of habitat characteristics.

The obtained results show clearly that the percentage of $CaCO_3$ does not directly reflect the growth (DW) of charophytes. This was indicated by different variability patterns of DW and % $CaCO_3$. It is particularly evident in Lake Męckie where values of % $CaCO_3$ were among the highest for both species, while DW values were relatively low. Therefore, such results suggest that the factors affecting the charophyte growth are different from those affecting the formation of carbonate encrustations. These observations are in line with the results obtained by Pukacz et al. 2015 (unpublished data) who found that the percentage of $CaCO_3$ in DW of *C. polyacantha* may change in time regardless of the biomass and mostly depends on habitat characteristics. Therefore, we may conclude that direct interpretation of the $CaCO_3$ amount as a measure of primary production is not possible in every case, as it was suggested by McConnaughey & Falk (1991).

Habitat-based differentiation

The analyses of water characteristics showed that lake-based habitat differences are much bigger than the site-based ones. The lack of significant differences between sites within the same lake is mostly the

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consequence of a) intensive water mixing in the littoral zone (Wetzel 2001) and b) similar depth of sampling sites (Pukacz et al. 2014). The high contribution of charophyte vegetation may also be an important factor, which stabilizes the habitat conditions within phytolittoral (e.g. van den Berg et al. 1998; Kufel, Kufel 2002).

The significant lake-to-lake differences based on physicochemical water properties showed a great diversity of typical *Chara*-lakes and a wide spectrum of habitat conditions in which *C. tomentosa* and *C. globularis* may occur. Our study revealed that the parameters related to hardness were the most significant ones for the lake-to-lake differences and, as a consequence, for the differentiation of individual charophyte biomass. This applies in particular to calcium carbonate encrustation, which depends to a large extent on water chemistry – temperature, and pH-dependent proportion between CO_2, HCO_{3-} and CO_{3-}^{2-} (Smith, Walker 1980). The pH values of waters, almost equal in all the studied lakes, exceeded 8 at each study site, which allowed the assumption that HCO_{3-} ions were the main form of inorganic carbon in waters sampled above all charophyte beds. The availability of HCO_{3-} ions for autotrophs in the studied lakes was also reflected in the abundant encrustation present on the charophyte thalli and in the calculated values of the calcite saturation index (SI). The SI values indicate supersaturation with respect to HCO_{3-} ions, which means favorable conditions for calcite precipitation in all the studied lakes (Baumgartner et al. 2006). The additionally calculated Mg/Ca ratio varied from 0.04 in Lake Jasne to 0.14 in Lake Złoty Potok. Referring to the above-mentioned SI values, this indicates, according to Müller et al. (1972), that the conditions for low-Mg carbonate precipitation occurred in all the studied lakes.

The fact that Lake Karskie was most different from the other lakes was probably due to the high fluctuations of the water level (rising and then falling within a few months) observed in the year of the study and the preceding year (according to various sources, from 0.5 to 0.8 m). This caused the leaching of mineral and organic substances from the lake margins, and thus considerable changes in water chemistry. This may also be reflected in the brownish color of the water observed during the study. Changes in Lake Karskie probably influenced the development of charophytes, as indicated by the lowest DW values. During the study, we also observed a considerable decrease of the number of species and the area covered by charophytes in Lake Karskie, as compared to 2009 (Cyrwus 2009). It shows that changes of water chemistry may result in changes of quantitative and qualitative structure of charophyte biomass, which was evidenced, among others, by the results of experimental research by Kufel & Rymuza (2014).

Even though lakes Złoty Potok and Niesłysz differ significantly in terms of their morphometry (Lake Niesłysz is more than 10 times larger and much deeper than Lake Złoty Potok), they had very similar water properties. This is also confirmed by previous studies (Pełechaty et al. 2015). This probably results from the fact that the lakes are adjacent and have almost identical drainage basins, both in terms of hydro-geological properties and the use by man. However, both DW and % $CaCO_3$ of *C. tomentosa* were significantly different in these two lakes, which suggests that there are other factors affecting the values of these qualities (e.g. strong pressure for recreation in Lake Niesłysz). An opposite situation was observed for lakes Pierwsze and Karskie, which were different in terms of water chemistry but similar in values of DW and % $CaCO_3$ as *C. globularis*. In our opinion, this shows that DW and % $CaCO_3$ are also affected by indirect factors such as competitive relationships (mainly competition for light) with phytoplankton, which may significantly reduce the growth of charophytes (Blindow 1992b; Pełechaty et al. 2013). This finding, however, needs to be verified, which will be the subject of our further considerations.

Relationships between physicochemical properties and DW and $CaCO_3$ content.

The significant correlations for most physicochemical parameters of ambient waters and additional characteristics showed that both DW and % $CaCO_3$ are closely related to the habitat conditions. However, the differences in correlation coefficients for *C. globularis* and *C. tomentosa* indicate that their biomass is affected by different factors, thus these relationships should be considered separately. This can be explained by different habitat requirements and ecophysiological characteristics of *C. globularis* and *C. tomentosa* (Krause 1997; Urbaniak, Gąbka 2014). As exemplified by Blindow (1992b), charophytes tend to disappear as the quality of water deteriorates (especially when visibility decreases). In such conditions, large charophyte species, including *C. tomentosa*, withdraw first, while small species such as *C. globularis* "retreat" to shallower sites with a sufficient amount of light.

The larger number of correlations found for *C. tomentosa* suggests that this species is more habitat--dependent than *C. globularis*. This applies in particular to parameters related to hardness, as confirmed by positive correlations for % $CaCO_3$ in *C. tomentosa* DW. On the other hand, the positive correlation between

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DW of *C. tomentosa* and TP concentration seems to be contradictory to the above-mentioned information on the habitat preferences of the species, as well as other charophytes. It must be emphasized, however, that relatively low TP concentrations were found in all the lakes where this species occurred (from 0.013 mg l^{-1} in Lake Męckie to 0.025 mg l^{-1} in Lake Niesłysz). This suggests that with a relatively low trophic status and good light conditions (as evidenced by high SD visibility), an increase in phosphorus content may lead to the intensification of photosynthesis. This is consistent with the contemporary literature data on other species (e.g. Dittrich, Koschel 2002; Kufel, Kufel 2002).

The positive correlations found for $CaCO_3$ content in *C. globularis* DW proved that calcite encrustation in this species is determined rather by the structure of community and light conditions than the water chemistry. Although the depth of the sampling sites in each lake was the same, they varied between the lakes. It was similar in the case of bottom vegetation cover. Since there were no significant correlations found for physicochemical parameters, we believe that these correlations may show that the efficiency of $CaCO_3$ precipitation (as a consequence of photosynthesis) increases as a result of elongation forced by lower availability of light at greater depths and self-competition. The phenomenon was described, among others, by Pukacz et al. (2014). Whereas the negative correlation of $CaCO_3$ content in DW of *C. globularis* with the number of species may indicate that species compete with each other in more complex communities.

Most of the results discussed above confirm the significance of charophytes as an important element of lake ecosystems and their functioning. However, due to considerable limitations of the in situ study (e.g. difficulties with sampling and a number of potential external factors affecting the results of the analyses), the results presented herein must be considered with caution. A detailed study, including both a wider range of analyses and elements of experimental research (mesocosm studies), would be desirable.

Acknowledgements

This paper presents results obtained during research project N N304 0425 39, which was financially supported by the National Science Centre (Poland) in 2010-2013.

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