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Encrustations and element composition of charophytes from fresh or brackish water sites – habitat- or species-specific differences?

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ABSTRACT

Comparison of encrustation and analysis of element contents across species growing in freshwater (FW) and brackish water (BW) have never been done. Proportion of carbonate and element composition for Ca, K, Mg, Na, and P of plant DW were investigated in four charophyte species from ten freshwater and six brackish water sites in northern Europe. *Chara aspera* and *C. tomentosa* were found in both FW and BW sites, whereas *C. subspinosa* and *Nitellopsis obtusa* occurred in freshwater only. Significant differences in encrustation were found between FW and BW sites. Individuals from FW had a far higher carbonate content based on dry weight than individuals from BW. In BW, *C. tomentosa* was less encrusted than *C. aspera*. This might be explained by the osmoregulation capacities of the brackish water species. The K/Na ratio differed between FW and BW in a species-specific manner. Element composition was habitat-specific for Ca and Mg, species-specific for K, and habitat- and species-specific for Na. P contents showed no specific pattern.

1. Introduction

Charophytes are submerged macrophytes growing in fresh, brackish and saline waters (Krause, 1997; Blindow, 2000). Most species occur in freshwater and only a few are restricted to brackish or saline sites. Some species are able to thrive under freshwater and brackish conditions (Schubert and Blindow, 2003). Examples for these groups are: *C. subspinosa* Rupr. 1846 and *Nitellopsis obtusa* Groves 1919 as freshwater species, *C. canescens* Loisel. 1810, which is restricted to brackish water, *Lamprothamnium papulosum* Groves 1916 inhibiting brackish and saline waters (Wood and Imahori, 1965; Krause, 1997) and *C. aspera* Willd. 1809 and *C. tomentosa* L. 1753, which can be found under brackish as well as freshwater conditions (Hasslow, 1931; Wahlstedt, 1862). For the latter two species, heavy encrustations have been described for individuals growing in freshwater lakes (Schütte, 2003; Kufel et al., 2016; Pukacz et al., 2016), where both species prefer hard-water habitats (Teppke et al., 2016; Van de Weyer and Jordan et al., 2016). These are mostly alkaline- and hard-water lakes with bicarbonates as the main carbon source of the carbonate system (Wetzel, 1975). Van den Berg et al. (2002) showed that *C. aspera* assimilates bicarbonate more efficiently than *Stuckenia pectinata* Börner 1912. Charophytes of the genus *Chara* can therefore be assumed to have a competitive advantage over other macrophytes in hard-water lakes. The uptake of bicarbonate for photosynthesis can result in encrustations on the plant surface (McConnaughey and Falk, 1991; McConnaughey and Whelan, 1997; Pedersen et al., 2013). However, calcium carbonate can also be precipitated as a side-effect of active ion transport for nutrient uptake (McConnaughey and Whelan, 1997; Ullrich et al., 1998). In the process, H^+ are released forming acid regions along the plant. In ecorticated species a “banding pattern” of acid and alkaline regions was described, whereas corticated species exhibit strongly alkaline regions (Spear et al., 1969; McConnaughey and Falk, 1991; Ray et al., 2003; Kawahata et al., 2013). Together with carbonate phosphorus is co-precipitated (Otsuki and Wetzel, 1972; Murphy et al., 1983), which is considered to affect the P cycle of lakes by removal of total phosphorus from the water column (Kufel and Kufel, 2002; Siong and Asaeda, 2006).

Wahlstedt (1875) already reported that brackish water charophytes (*C. aspera* and *C. tomentosa*) are far less encrusted than individuals from freshwater. Hasslow (1931) even distinguished between *C. aspera* “f. *incrustata*” from freshwater and “f. *munda*” from brackish water. In the brackish environment, charophytes have to cope with higher ion concentrations of the surrounding water, requiring osmoregulation as a countermeasure to prevent a loss of turgor (Bisson and Kirst, 1995). In the process of osmotic adjustment, concentrations of Cl^-, K^+ and Na^+

Abbreviations: AFDW, ash free dry weight; asp, *C. aspera*; BW, brackish water; DW, dry weight; FW, freshwater; LOI, loss of ignition; LSD, Least Significant Difference; NMDS, non-metric multidimensional scaling; SD, standard deviation; sub, *C. subspinosa*; TIC, total inorganic carbon; tom, *C. tomentosa*; TP, total phosphorus; obt, *N. obtusa*

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are actively regulated (Winter and Kirst, 1992). Especially the K^+/Na^+ ratio needs to be regulated, because too low ratios ($K^+/Na^+ < 1$) reduce the vitality of charophytes (Winter and Kirst, 1990; Winter et al., 1996). Both, ion contents and turgor regulation mechanisms have intensively been studied in the laboratory and found to differ widely among species (Winter and Kirst, 1990). However, differences among species in encrustation and internal ion contents with increasing salinity in the field are rather poorly investigated. Urbaniak (2010) compared different methods for estimation of carbonate and element content in five charophytes species occurring either in freshwater or brackish water and came to the conclusion, that carbonate must be analyzed separately. To our knowledge, ion content and encrustation patterns have never been compared between freshwater and brackish water specimens belonging to the same species. We therefore have chosen *C. aspera* and *C. tomentosa* from both freshwater (FW) and brackish water (BW) sites to investigate encrustation and ion content. For comparison, data were also collected for other charophyte species occurring in the same sites (*C. subspinosa* and *N. obtusa*). We assumed that the differences in encrustation already described (Wahlstedt, 1875; Hasslow, 1931) are reflected by a higher carbonate content in freshwater than in brackish water, and that the differences in turgor regulation among species are reflected by the ion contents of the plants. Our basic hypothesis was that the amount of precipitated carbonate encrustations are higher in freshwater than in brackish water also within the same species. The second hypothesis was that encrustations differ species-specific between FW and BW. We further aimed to investigate if internal element contents (Ca, K, Na, Mg, and P) are habitat-specific or species-specific.

2. Material and methods

2.1. Sampling sites

Ten freshwater lakes and six brackish water lagoons in northern Europe were sampled in June and July 2015 (Table 1 and Supplementary material). These water bodies were chosen by their charophyte flora, allowing for occurrence of the *Chara hispida*-species group *sensu* Wood (1962), selected for their ability to grow in freshwater as well as brackish water. Five of the freshwater lakes were localized in the biosphere reserve Schorfheide-Chorin in the north-eastern part of Brandenburg, Germany (Sabinensee, Gottsee, Kölpinsee, Parsteiner See, and Krüselinsee) and the other five in southern Sweden (Krankesjön, Börringesjön, Lyngsjön, Räpplinge, and Greby). All the brackish water lagoons were localized along the south-eastern coast of Sweden (Edenryd, Sibbaboda, Klumpudden, Loftahammar, Gällerskullaviken, and Hålviksfjärden).

2.2. Sample processing

Conductivity and pH of the surface water were measured directly at the sites with a HACH HQ40d (Hach-Lange, Germany). Surface water samples were collected in 50 ml tubes for the analysis of Ca^{2+}, Cl^-, K^+, Mg^{2+}, Na^+, SO_4^{2-}, and total inorganic carbon (TIC) at the Helmholtz Centre for Environmental Research (UFZ) – Central Laboratory for Water Analytics and Chemometrics. For quantitative analysis of main cation and anion concentrations, a Dionex ICS 3000 ion chromatograph with suppressor technique for both ion types was used. TIC was determined with a Dimatoc 2000. All measurements were conducted in accordance to EN ISO 14911:1999 and EN ISO 10304:2009-1. For total phosphorus (TP), surface water samples were collected in polypropylene tubes cleaned with 10% hydrochloric acid and ultrapure water. TP concentration was determined in duplicates after alkaline persulphate oxidation as described by Koroleff (1983) in the laboratory.

Plant material was collected by snorkeling or by means of a fork directly from the shore or boat in shallow water (0.1–1.5 m). For *C. subspinosa*, *C. tomentosa* and *N. obtusa* five undamaged individuals were picked from the gathered material for further analysis. In case of *C. aspera*, biomass of individuals was insufficient for analysis, so each of the five samples consisted of several plants. All sampled *Chara* species are corticated whereas *Nitellopsis* is an ecorticated species. After gentle removal of epibionts by means of a brush the plants were air-dried (around 24 h) and stored at room temperature until further processing. Four out of the five samples taken at each site were used for the determination of carbonate content, the fifth sample for the analysis of element contents of the biomass. Carbonate content was determined by the "loss of ignition" (LOI) method as described by Heiri et al. (2001). The samples were dried at 105 °C overnight followed by a two-step combustion process at 550 °C and 925 °C for two hours, respectively. The weight loss at 550 °C represents the ash free dry weight (AFDW), while at 925 °C CO_2 evolved. Carbonate content was calculated after Pełechaty et al. (2013) by multiplying CO_2 loss by 1.364 to obtain CO_3^{2-}, and then multiplied by 1.668 to achieve calcium carbonate. Mineral remains (= total minerals – carbonates) resulted from the subtraction of ash at 925 °C from calcium oxide, which was calculated from the CO_2 loss multiplied by 1.274 (fraction of CO_2 in CaO). Ca, K, Mg, Na and P contents in the plant were analyzed by inductively coupled plasma-optic emission spectroscopy (ICP-OES). For this, a sample of 0.1 g powdered dry weight (DW) was digested with 5 ml HNO_3 (65%) and 3 ml H_2O_2 (30%) for 1.5 h by microwave extraction (CEM MARS 6). The samples were filled up to 25 ml with ultrapure water and were measured for Ca (317.9 nm), K (766.5 nm), Na (589.6 nm), Mg (285.2 nm) and P (214.9 nm) with an Optima 8300 spectrometer from Perkin Elmer. Ca and Mg contents are presented as g kg^{-1} DW and K, Na, and P were calculated as g kg^{-1} AFDW. It is assumed that K, Na, and P contents are mainly stored in the organic part of the plants. Mg/Ca and K/Na mass ratios are calculated from individual sample weight.

Table 1
Sampling sites in Germany and Sweden investigated with coordinates and species sampled. Sites and species were abbreviated as first three letters.

site	abbreviation	coordinates	species
freshwater			
Sabinensee	Sab	53°06'44.0" N 13°45'59.4" E	sub, tom
Gottssee	Got	53°05'15.6" N 13°40'45.1" E	tom
Kölpinsee	Köl	53°06'42.1" N 13°39'35.6" E	tom, obt
Parsteiner See	Par	52°55'43.9" N 13°59'06.1" E	tom
Krüselinsee	Krü	53°16'02.3" N 13°24'59.7" E	sub, tom, obt
Krankesjön	Kra	55°42'22.8" N 13°28'38.3" E	asp, sub, tom
Börringesjön	Bör	55°29'22.1" N 13°19'17.5" E	asp
Lyngsjön	Lyn	55°55'54.3" N 14°04'05.0" E	tom
Räpplinge	Räp	56°48'54.9" N 16°36'29.0" E	asp
Greby	Gre	56°48'57.3" N 16°36'11.1" E	asp
brackish water			
Gällerskullaviken	Gäl	57°39'52.4" N 16°36'02.3" E	tom
Hålviksfjärden	Hål	58°30'08.2" N 16°54'13.9" E	asp, tom
Loftahammar	Lof	57°53'51.8" N 16°41'13.4" E	asp
Klumpudden	Klu	56°48'51.1" N 16°24'56.1" E	tom
Edenryd	Ede	56°02'30.7" N 14°31'49.8" E	asp
Sibbaboda	Sib	56°07'53.4" N 15°53'44.4" E	asp