CaCO$_3$ sedimentation by modern charophytes (Characeae): can calcified remains and carbonate $\delta^{13}$C and $\delta^{18}$O record the ecological state of lakes? – a review

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Abstract: Charophytes (Characeae), macroscopic green algae, are one of the macrophytes occurring mainly in the littoral zone of lakes. Their occurrence in a lake is considered as indicative of low trophy and high ecological status. Photosynthetic activity of charophytes leads to precipitation of autochthonous carbonates, that substantially contribute to lacustrine sedimentation. Calcified parts of their thalli can be preserved in sediments as thalli encrustations and calcified female fructifications called gyrogonites. The oldest charophyte fossils are Upper Silurian in age thus these macroalgae are a potential archive of palaeonvironmental information back to the middle Palaeozoic. Ability to recognize genera or even species of the fossil gyrogonites allows to use them as lacustrine biomarkers when contemporary ecological requirements of the taxa are known. Both gyrogonites and encrustations are frequently used in isotope investigations of lake sediments. However, in order to carry out correct, reliable palaeolimnological reconstructions using stable isotope record of the charophyte carbonates it is essential to know the relation between $\delta^{13}$C and $\delta^{18}$O of recent encrustations and gyrogonites and $\delta^{13}$C of DIC (dissolved inorganic carbon) and $\delta^{18}$O of ambient water, respectively. The main aim of the paper is to present the importance of charophytes in carbonate lacustrine sedimentation and to describe the possibilities to use their remains as biomarkers of current ecological state of lakes, potentially useful in palaeolimnological studies.

Keywords: Charophytes (Characeae), carbonates, encrustation, gyrogonites, stable isotopes, carbon, oxygen, ecological state, palaeolimnology, lakes
Charophytes as an important element of the lake ecology

The role of aquatic autotrophs in the lake functioning is commonly agreed upon. Depending on the lake morphometry and related mixing regime as well as intra-biocoenotic interrelationships either planktonic assemblages or communities of macroscopic plants (macrophytes) may be of prime importance (Alimov, 2003). Whereas in deep stratified lakes phytoplankton takes over the majority of photosynthetic productivity, macrophytes covering most of the lake basin are mainly responsible for the biomass production in shallow macrophyte water bodies. Among aquatic macrophytes, representatives of the family Characeae (Charales, Charophyceae, Chlorophyta), commonly referred to as ‘charophytes’ or ‘stoneworts’, can significantly affect the biotope – biocoenotic interdependencies and intra-biocoenotic interplay. As a consequence, charophytes can cause shifts in the water chemistry and clarity (van den Berg et al. 1998; van Donk & van de Bund, 2002; Pełechaty et al. 2006). These submerged macroscopic green algae are well distributed all over the world and occur in various types of aquatic environments (marine, brackish and freshwater, standing and floating, permanent and ephemeral), preferring freshwater lakes where they can occupy sites along a wide depth gradient (Wood & Imahori, 1965; Krause, 1997; Martin et al. 2003). Charophytes are today represented by ca. 400 species world-wide, gathered in six genera. The occurrence of most charophytes is limited to water bodies with clear, alkaline waters and low nutrient budget (Hutchinson, 1975; Murphy et al. 1983; McConnaughey, 1997; Krause, 1981, 1997). Hence, they become rare along with increasing trophy level and decreasing light availability. Thus, many species are applied as sensitive bioindicators of water quality and habitat conditions (Forsberg, 1964, 1965; Karczmarz, 1973; Ozimek & Kowalczewska, 1984; Krause, 1981; Hough & Putt, 1988; Blindow, 1988, 1992a, 1992b; Menendez & Sanchez, 1998; Schwarz et al. 1999; Pełechaty et al. 2004; Kłosowski et al. 2006). According to Rip et al. (2007) temporal pattern of precipitation and flow from land to water may explain the observed dynamics in phosphorus and related phytoplankton biomass, turbidity, and the occurrence or disappearance of Characeae. Global warming has caused winters to become warmer and wetter in the second half of the 20th century. As a result, increasing flows from land to water of phosphorus and humic substances enhanced instability of charophyte vegetation. Considering all the above, in studies of most recent lacustrine deposits shifts in the presence of charophyte remnants in the sediment record may reflect environmental changes, both on a local and a global scale (Rodrigo et al. 2009; Détriché et al. 2009 and literature quoted therein).

Although Characeae are multicellular organisms, they form neither tissues nor typical organs, such as leaves, roots or flowers. Their macroscopic, equisetum-like body is called ‘thallus’ (pl. ‘thalli’) and can grow from a few centimetres up to more than one meter high, attached to the bottom by delicate rhizomes (Groves & Bullock-Webster, 1924; Corillion, 1957; Wood & Imahori, 1965; Krause, 1997; Martin et al. 2003). The main axis (stem) of charophyte thalli is composed of a number of nodes and internodes (Fig. 1). The nodes are places where side-branches and whorls of branchlets are formed in numbers depending on the genus and species. Among all charophytes, the most diversified morphological structures are represented by the species of the genus Chara. Most of them have a complex morphology with an additional layer of cells on the stems and branchlets, forming the so called ‘cortex’ (Fig. 1). Chara species vary in the number of cortex cells. Three groups of corticate species can be distinguished: with haplostichous, diplostichous or tripllostichous cortex (the number of cells one, twice or three times higher than the numbers of branchlets, respectively). This specific morphological feature is frequently well preserved in carbonate remains after charophyte decay (Fig. 2), having potential to increase the environmental information gathered when using charophytes as biomarkers. Many corticate species form also additional spine cells, single or bunched with varied size. Similar cells, stipulodes, develop below stem nodes (Fig. 1).

With respect to the life cycle, charophytes can grow annually or perennially (Groves & Bullock-Webster, 1924; Krause, 1997; Martin et al. 2003). In some cases growth pattern can be related to local climatic conditions. Charophytes can grow sparsely or densely cover extensive areas, forming compact beds, the so called ‘charophyte meadows’ (Corillion, 1957; Krause, 1997; Martin et al. 2003), and, if so, play an important role in the water body functioning (van den Berg et al. 1998; Kłosowski et al. 2006). The increase in particle sedimentation from the water, reduction of sediment resuspension, competitive interactions with phytoplankton and periphyton, and the refugial role
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for zooplankton against predation are frequently cited mechanisms (as summarized by van den Berg et al. 1998; van Donk & van de Bund, 2002; Pelechaty et al. 2006). Many authors point to a potential role of charophyte vegetation as a nutrient (mainly phosphorus) sink in water bodies as a result of incorporation in biomass and carbonates or co-precipitation with calcite (Kufel & Kufel, 2002 and literature quoted therein; Siong & Asaeda, 2006, 2009a, 2009b).

Considering all the above, a new approach to the indicative value of charophytes has emerged along with the implementation of the Water Framework Directive 2000/60/EC (WFD, EU, 2000) in the European Union countries. According to the WFD, not trophy itself but the ecological state, emphasising the functional interdependencies within a lake, should be determined and monitored as a reflection of the quality of waters. In a result, the groups of organisms are nowadays applied instead of physical and chemical water properties, so far used as the main or even only reliable indicators. The significance of charophytes in the ecological state assessment of lakes has been well evidenced and pointed to this macroalgae as indicative of the so called ‘reference conditions’ (e.g. Pelechaty et al. 2006; Kolada, 2009). Consequently, the Polish macrophyte-based method of the determination of the lake ecological state attributes charophytes to an especially reliable indicative value (Ciecierska, 2008).

Fig. 1. Morphology of the charophyte thallus, an example of Chara rudis is given.
Carbonate precipitation by charophytes

One of the significant phenomena in the lake functioning is the carbonate precipitation from the water and its storage in the sediments, where, depending on the origin, carbonates can be chemical or biogenic compounds, serving as environmental archives and criteria of sediment classifications (Kelts & Hsü, 1978; Hammarlund et al. 1999; Schnurrenberger et al. 2003; Giguet-Covex, 2009). Apart from the physical and chemical mechanisms, photosynthetic activity of aquatic autotrophs seems to be of prime importance for the carbonate precipitation (Pomar & Halloch, 2008). Macrophytes are the main carbonate producers in the littoral zone (Wetzel, 1960) and phytoplankton, particularly autotrophic picoplankton, in the pelagial (Yates & Robbins, 1998; Dittrich & Obst, 2004; Dittrich et al. 2004). Carbonate precipitation is a consequence of CO₂ removal, that can be photosynthetically assimilated, from the soluble bicarbonates and their conversion into the insoluble calcium carbonate (McConnaughey, 1997), deposited directly onto the thalli surface as a mineral encrustation (Raven et al. 1986). Whereas the carbonate is usually dispersed from the vascular plants (Colletta et al. 2001), a specific carbonate zonation, related to the cell acidic and alkaic regions, or non-banded encrustation layer develop on the thalli surface of many charophyte species (Martin et al. 2003). Encrustation can be seen even with a naked eye (Fig. 3), particularly on dried specimens. Heavy encrustation is a feature typical of charophytes as their effectiveness to use bicarbonates over a wide range of concentrations in the water is higher as compared to vascular plants (van den Berg et al. 1999). Apart from the external encrustation on the cell walls, biologically induced internal incrustation and organic
matrix mediated calcification within the wall of female reproduction organ, the oogonium, participate in the total amount of carbonate precipitated (Borowitzka, 1987; Leitch, 1991; Anadón et al. 2002a). As a result of the latter mechanism, gyrogonites, the calcified female fructifications, are formed. This complexity in biomineralization makes estimation of the carbonate and element content in charophytes methodologically difficult (Urbaniak, 2010) and requires understanding of physiological and biochemical aspects of the organism functioning and the physical chemistry of carbonate precipitation (Borowitzka, 1987).

Depending on the thallus morphology (corticate or ecorticate) as well as growth conditions (mainly calcium and bicarbonate availability), charophytes can be slightly (e.g. Nitella species) or heavily (most of the Chara species) encrusted (Groves & Bullock-Webster, 1924; Krause, 1997; Martin et al. 2003). It is commonly accepted after Hutchinson (1975) that carbonate encrustation can reach up to 60% of the charophyte dry weight. Current studies, however, evidenced even higher contribution of carbonates. Urbaniak (2010) found over 80% content of ash and 77% of carbonates in the charophyte dry weight. Those values were species-related and visibly varied depending on the fresh- (higher values) or saline water (lower values) environment the species were collected from. Water chemistry is of prime importance for the precipitation itself and the type of carbonate deposited, as well. According to Müller et al. (1972), depending on the Mg/Ca ratio calcite or aragonite can precipitate, and the values below 2 or above 2, respectively, define the lake conditions under which either of them occurs. As an important environmental determinant, Mg/Ca ratio was also given by Anadón et al. (2002a). The authors, however, emphasized the possibility of polymineral
calcification which they evidenced for the first time for a single *Chara* oogonium. In accordance with their study, carbonate mineralogy and water salinity were not correlated and calcite, high-Mg calcite and aragonite were found in low salinity waters. Aragonitic composition of encrustations noted in some sediment layers of the 30 metres thick sequence of Pliocene Villarroya Lake in northern Spain was interpreted as a result of episodes of relatively high Mg/Ca ratio in waters (Anadón et al. 2002b).

Mg/Ca ratio may significantly affect not only the composition of encrustation but also the amount of precipitated material. Siong & Asaeda’s (2009a) study, performed in an Australian shallow coastal lake with low salinity, showed that high Mg concentration in the lake water reduced the calcification of charophytes. The CaCO$_3$ encrustation constituted less than 15% of the biomass dry weight.

Average percentage content of encrustation of up to 70% in dry charophyte mass was given by Blindow (1992b). In our unpublished study performed in a moderately productive lake with large areas of charophyte vegetation and significant contribution of lake marl in sediments (Lake Wigry, NE Poland, in prep.) the maximum of almost 80% encrustation in the dry plant mass was recorded.

The amounts of carbonate deposited by charophytes in the sediments depend on the biomass production by plants and are regulated by environmental conditions (depth, light and bicarbonate availability, weather fluctuations, human impact). If we assume after Kufel & Kufel (2002, based on the literature data reviewed therein) the average production of charophyte biomass of 279 gm$^{-2}$ and, after Hutchinson (1975), 60% content of encrustation in their dry mass, it can be estimated that charophytes precipitate 167 g CaCO$_3$ m$^{-2}$ on an average. Our above-mentioned study in a dense charophyte meadow in Lake Wigry allowed to assess higher mean carbonate precipitation of almost 430 gm$^{-2}$ in the middle of growing season, and the contribution of other than carbonate mineral substances was < 6%.

Keeping in mind that the above-presented data are average values and the maximum biomass production can be higher, one may figure out the significance of charophytes in the deposition of carbonates in sediments, being an important record of environmental conditions (Dittrich & Obst, 2004).

Charophytes in a fossil record

The oldest charophyte fossils are Late Silurian in age (Croft, 1952), thus these macroalgae are a potential archive of palaeoenvironmental information back to the middle Palaeozoic. Abundant charophytic carbonates have been documented in lacustrine sediments of different age, e.g. Cretaceous (Glass & Wilkinson, 1980), Paleogene and Neogene (Becker et al. 2002), Neogene (Anadón et al. 2000) and Recent (Rutkowski et al. 2007; Rutkowski et al. 2009).

Charophyte fossils preserved in lacustrine sediments occur in two forms, as encrustations and gyrogonites (Figs. 2, 4). The later have larger preservation potential. Encrustations occurring at charophyte stems often fall apart after algae death and constitute a fine-grained carbonate fraction of the lacustrine sediment. This is observed especially in species with delicate, thin thalli. In the case of corticate charophytes fragments of carbonate encrustation can remain in sediments (Figs. 2, 4). If not destroyed by post-sedimentary processes, charophyte fossils may become lithified, as in the Upper Jurassic charophyte limestone from Saxony in Germany (Fig. 5). Charophyte encrustations preserved in sediments allow to conclude about the possible post-sedimentary, diagenetic processes such as compaction of the sediment (Anadón et al. 2000).

The presence of charophyte fossils in sediments is an important environmental indicator (Soulié-Märsche, 1991; Martín-Closas et al. 2006). As mentioned in the previous chapter preferential occurrence of the algae in fresh, less fertile, alkaline, unpolluted waters between about 1 m to 10 m of depth (Garcia, 1994) can be assumed. However, remains of some saline water preferring species may indicate low salinity phases of the basin in the past (Soulié-Märsche, 2008). Presence of fossil gyrogonites and encrustations indicate that the water basin existed at least for three months because this is the time required for the plant to complete a full cycle of growth (Soulié-Märsche, 1991). Charophyte deposits can therefore not be attributed to ephemeral environments created by stagnant water, e.g. after flooding.

Morphology of gyrogonites allows determination of genera and in many cases also species of the Characeae (Horn af Rantzien, 1956; Soulié-Märsche, 1991; Haas, 1994; Hutorowicz, 2008; Rodrigo et al. 2009). Knowledge about the contemporary ecological requirements of charophyte species can be helpful in environmental interpretation when gyrogonites
Fig. 4. Charophyte remains: thalli encrustations and a gyrogonite (in the centre), picture taken from surface sediments of Lake Wigry, north-eastern Poland (a sediment sample provided by Prof. Dr J. Rutkowski).

Fig. 5. Upper Jurassic (Kimmeridgian) charophyte limestone composed of stem encrustation debris and peloids viewed in the thin section. Süntel Formation, Wolfsburg-Nordsteimke, Lower Saxony, Germany (photo provided by Prof. Dr Michael Schudack).
are applied as lacustrine biomarkers, reflecting the ambient conditions of the water body where charophytes grew (Martín-Closas et al. 2006; Wasylikowa et al. 2006). Gyrogonites preserved in Quaternary charophyte deposits located in North Africa allowed to reconstruct local environment of a number of sites where human settlements were located (Soulié-Märscche, 1991). Not only the occurrence itself, but also the abundance of gyrogonites preserved in sediments may provide information about past environment and sedimentary conditions. Sediment layers with high gyrogonite frequencies (more than 100 specimens) are interpreted as corresponding to in situ growth of the submerged vegetation and allow conclusions about the environmental characteristics according to the ecological requirements of extant representatives of the taxa. Samples, that contain few gyrogonites are treated as indicating intra-lacustrine or synsedimentary transportation (Ghetti et al. 2002). Special attention is paid not only to morphological differences between gyrogonites but also to preservation state of the specimens, i.e. gyrogonites filled and empty, diagenesis, recrystalization (Becker et al. 2002). The species distribution of charophytes and degree of preservation of charophyte remains (encrusted stems and, particularly, gyrogonites) may register changes in lake level, e.g. a series of lowstands with subsequent proximal palaeosol formation recognized by Détriché et al. (2009). Charophyte remains may be successfully used for the reconstructions of leading environmental factors and vegetation composition and structure (e.g. Rodrigo et al. 2009).

Isotope studies of Chara carbonates

Fossil charophytes

Carbon and oxygen stable isotope analyses of charophyte carbonates are often carried out in order to reconstruct the past climate and environment (Anadón et al. 2000; Becker et al. 2002; Apolinarska, 2009). In the studies of postglacial lacustrine deposits parallel analyses of encrustations preserved in the sediment and lake marl are carried out, often supplemented by analyses of other biogenic carbonates, e.g., mollusc shells (Hammarlund et al. 1997; Apolinarska & Hammarlund, 2009). Usually, the isotope signatures measured for encrustations and lake marl closely resemble each other proving that the marl is in fact composed of disaggregated calcitic encrustations (Fritz et al. 1987; Hammarlund et al. 1999; 2003; von Grafenstein et al. 2000). The similarity of the carbon and oxygen isotopic composition of sedimentary carbonate to that of co-existing Chara encrustations may be used as an indicator of minimum contributions of allochthonous carbonates to a lake basin (Hammarlund et al. 2003).

Recent charophytes

In order to carry out correct, reliable palaeolimnological reconstructions using the stable isotope record of encrustations and gyrogonites it is essential to know the relation between δ13C and δ18O of charophyte carbonates and δ13C of DIC (dissolved inorganic carbon in water) and δ18O of water, respectively, and ecological variables of a water body. There are only a few isotope studies of contemporary Chara and ambient conditions of their growth.

Chara encrustations

Isotope analyses of recent charophytes involved Chara hispida L. (Coletta et al. 2001; Pentecost et al. 2006), Chara globularis Thuill. (Coletta et al. 2001; reviewed by Andrews et al. 2004), Chara vulgaris L. (Coletta et al. 2001) and Chara rudis A. Br. (Pełechaty et al. 2010). δ13C and δ18O of encrustations were compared with isotope composition of water sampled twice during vegetative season (Coletta et al. 2001), in two weeks intervals (Pentecost et al. 2006) and monthly (Pełechaty et al. 2010). In the investigations of Chara hispida (Coletta et al. 2001), Chara globularis Thuill. (Andrews et al. 2004) and Chara rudis (Pełechaty et al. 2010), carbonates were found to be precipitated in isotopic disequilibrium with the ambient waters. Encrustation was enriched in 13C due to photosynthesis, and depleted in 18O relative to δ13C DIC and δ18O of water, respectively, possibly because of kinetic effects during rapid calcification. Strong isotopic disequilibrium between δ18O of carbonates and δ18O of water and also between δ13C of carbonates and δ13C of DIC was observed when charophyte growth was subject to extreme conditions, e.g. gradual fall of the water in the pond during vegetative season, until an eventual drying out of the basin during summer (Pentecost et al. 2006). Despite the disequilibrium observed, the results obtained by Pełechaty et al. (2010) indicate the presence of correlation between δ13C and δ18O of encrustations and δ13C DIC and δ18O of water, respectively. It was suggested that oxygen isotope composition is more reliable indicator of environmental changes since it seems to be less susceptible to the vegetation...
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composition and structure as compared to the carbon one, highly influenced by the photosynthetic activity and reflecting in such a case a state of isotopic disequilibrium with water DIC (Pełechaty et al. 2010). On the other hand, δ¹⁸O of charophyte encrustation is sensitive to water temperature changes: a negative relationship was noted irrespective of other environmental factors.

Changes in the isotope composition along the stem, the so called “age gradients”, were also noted, what indicates that seasonal shifts in δ¹³C of DIC and δ¹⁸O of water were recorded in the isotope composition of encrustations (Coletta et al. 2001). However, the presence of the “age gradients” is strongly dependent on environmental characteristics. Those were absent in encrustations of Chara vulgaris sampled from a stream where continuous exchange of water prevented shifts in isotope composition to occur (Coletta et al. 2001).

Chara gyrogonites

Isotope studies of recent gyrogonites point to δ¹⁸O of the carbonates to be in equilibrium with δ¹⁸O of the water while disequilibrium was noted for δ¹³C (Jones et al. 1996). However, disequilibrium between the isotopic composition (both δ¹³C and δ¹⁸O) of the gyrogonites and the lake’s water was also observed (Huon & Mojon 1994).

Isotope investigations of sub-recent samples involved comparison between encrustations and gyrogonites. Sub-recent Chara globularis encrustations were more ¹³C-enriched and less ¹⁸O-enriched than the associated gyrogonites and suggested that this resulted from different calcification processes, described earlier in the text. However, as previously suggested by Jones et al. (1996), the process of oogonium calcification needs a short time period and thus isotope composition of gyrogonite carbonates represent a ‘time window’. The problem of rapid precipitation of CaCO₃ of gyrogonites was also discussed by Andrews et al. (2004). Authors suggested that sub-recent and recent stem encrustations were further from oxygen isotopic equilibrium than gyrogonite calcites due to kinetic effects during rapid precipitation of CaCO₃, suggesting that stem calcification is rapid relative to oogonium calcification.

The need for more experimental work on the precise mechanisms of calcification in charophytes and more empirical data on the relationship between δ¹³C and δ¹⁸O values in Chara stem encrustations and gyrogonites is stressed (Andrews et al. 2004).

Herbarium collections

Isotope studies of Chara encrustations also involved specimens from herbarium collections, Coletta et al. (2001) investigated stable isotope composition of encrustations of 12 charophyte species, e.g. Chara globularis, Chara hispida, Chara tomentosa, Chara vulgaris, collected at numerous locations in Britain and Ireland. Despite general information about a collecting site was given (flowing or stagnant water, small or a big lake) still the exact isotopic characteristics of water were not known. Isotope composition of water may differ significantly between lakes and only observations of recent sites allow to make reliable conclusions on the relation between stable isotope composition of charophyte carbonates and ambient water. Still, isotope studies of herbarium specimens, especially different species collected at one site, may provide information about interspecific differences in isotope composition of Chara carbonates. Five environmental factors influencing the stable C and O composition of charophyte carbonate were identified: evaporation, temperature, photosynthesis, groundwater composition, water flow (Coletta et al. 2001).

The above review emphasizes the complexity of phenomena, which can be of importance when interpreting the information provided by charophyte fossils and isotope composition of carbonates produced by modern charophytes. Considering the latter one, further study on conditions and mechanisms leading to the state of isotopic equilibrium/disequilibrium as well as interspecific and intraspecific (site-to-site and lake-to-lake) variability is required.

Summary

Charophytes play an important role in lacustrine ecosystem and can be applied in studies of both recent and fossil lakes:

• depending on their abundance and the type of ecosystem charophytes can cause shifts in the water chemistry and clarity, creating preconditions of a good ecological state;
• charophytes significantly contribute to lacustrine sedimentation by precipitation of carbonates on stems and oospores;
• the presence of charophyte fossils in sediments is an important environmental indicator due to the specific demands of these macroalgae. Knowledge about the contemporary ecological requirements of charophyte species allows to apply gyrogonites
as lacustrine biomarkers reflecting the ambient conditions of the water body where charophytes grew;
• despite the differences in isotope signatures noted between *Chara* carbonates and ambient waters, $\delta^{13}C$ and $\delta^{18}O$ of charophytes reflect environmental conditions and thus are used in palaeolimnological studies. However, the relation between carbon and oxygen isotope composition in recent *Chara* encrustations and $\delta^{13}C$ in DIC and $\delta^{18}O$ in water still needs to be explored.

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