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Photosynthesis and calcification of charophytes

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ABSTRACT

Charophytes of the genus *Chara* can form a bottom-dwelling calcified vegetation of high density in nutrient-poor hardwater lakes and ponds, whereas they are rare compared to tall canopy-forming vascular plants (elodeids) in nutrient-rich waters. Based on the preferred habitat and appearance, we hypothesized that *Chara* species may use bicarbonate efficiently for photosynthesis accompanied by calcification. We measured photosynthesis and calcification in incubation experiments of 3–18 h duration with seven species of *Chara* by quantifying changes in dissolved inorganic carbon (DIC), oxygen, alkalinity and calcium. All Chara-species used bicarbonate efficiently according to: 1) an unaltered photosynthetic rate between pH 7.5 and 8.5 and a modest decline at pH 9.5, and 2) their ability to drive pH above 10 and deplete CO_2 below 0.1 μM. Calcification measured by the parallel loss of bicarbonate and dissolved calcium to precipitated calcium carbonate increased markedly with rising pH, resulting in additional consumption of DIC and a falling molar quotient of oxygen release to DIC consumption from 1.61 at pH 7.5 to 0.68 at pH 9.5. The stoichiometry of alkalinity and calcium loss relative to oxygen production at pH 9.5 was in accordance with simultaneous calcification and photosynthetic assimilation, which buffers pH rise. Our findings support that *Chara* species are efficient bicarbonate users and calcifying organisms. Through calcification, *Chara* species maintain photosynthesis at high pH and can form dense stands in oligotrophic hardwater lakes and ponds.

1. Introduction

Charophytes is a globally widespread group of macroalgae mainly growing in freshwater lakes and brackish coastal waters (Olsen, 1944; Schubert and Blindow, 2003). They can be early pioneer species in newly established lakes and ponds (Hutchinson, 1975) because of high survival ability of their oospores in soils and sediments and efficient dispersal by waterfowl among habitats (Brochet et al., 2009; Stobbe et al., 2014; Alderton et al., 2017). Long-term dominance of charophytes in the submerged vegetation is maintained in nutrient-poor habitats (Baastrup-Spohr et al., 2013), whereas they decline due to shading from excessive growth of phytoplankton, mat-forming filamentous algae and tall rooted plants in nutrient-rich habitats (Blindow, 1992; Hilt et al., 2013; Sand-Jensen et al., 2017). As a consequence of cultural eutrophication in numerous countries during the last 50–150 years, charophytes have undergone dramatic reductions of distribution and abundance and a high proportion of the species have become rare and red-listed today (Simons and Nat, 1996; Baastrup-Spohr et al., 2015; Schneider et al., 2015).

In comparison with tall rooted plants with scattered leaves along an erect stem (elodeid growth form), charophytes appear to grow more slowly and succumb in the direct competition under nutrient-rich conditions (Kautsky, 1988; Christensen et al., 2013; Murphy et al., 2017). In evaluations of nutrient requirements according to Ellenberg N (Ellenberg and Leuschner, 2010), Melzer Index values (Melzer, 1999) and Grime's ecological strategies (Grime, 2002) charophytes can be viewed as predominantly oligotrophic, stress-selected species in comparison with eutrophic, competition-selected tall elodeids. Interspecific variability exists within the two groups, however (Baastrup-Spohr et al., 2015). Despite the suggested low growth rates of charophytes, several species, particularly of the genus *Chara*, are capable of forming very dense canopies in oligotrophic, calcareous lakes and ponds presumably due to high persistence and slow decomposition of the tissue (Kufel and Kufel, 2002; van den Bergh et al., 2002; Hidding et al., 2010; Andersen et al., 2017). *Chara* species can attain the same or even higher biomass densities than most elodeids (Duarte et al., 1986; Pukacz et al., 2014). In such dense *Chara* canopies, extensive calcification takes place and photosynthesis can continue despite marked depletion of dissolved inorganic carbon (DIC: CO_2 + HCO_3^- + CO_3^{2-}; van den Bergh et al., 2002; Andersen et al., 2017).

Dense precipitates of calcium carbonate ($CaCO_3$) on the outer surface are conspicuous among most *Chara* species accompanying their

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ability to use bicarbonate (HCO_3^-) in photosynthesis (McConnaughey, 1991; Herbst et al., 2018). Bicarbonate use enables charophytes to maintain photosynthesis in alkaline waters of relatively high pH (i.e. > 9.0) where bicarbonate is the predominant inorganic carbon species and low CO_2 concentrations are unable to sustain appreciable photosynthesis (Lucas, 1979). Use of bicarbonate in assimilation of organic matter (CH_2O) without calcification is accompanied by the release of hydroxyl ions and rising pH (eq. 1: $HCO_3^- + H_2O \rightarrow CH_2O + OH^- + O_2$) that will ultimately reduce photosynthesis as pH approaches 10 and bicarbonate is converted to carbonate (Christensen et al., 2013). High external pH may also directly inhibit proper cell functioning and photosynthesis (Sand-Jensen, 1983). Calcification is a way to prevent the pH rise and better maintain photosynthesis when accompanied by bicarbonate use (Eq. 2: $Ca^{2+} + 2\ HCO_3^- \rightarrow CaCO_3 + CH_2O + O_2$).

Carbon assimilation without or with associated calcification will markedly affect the stoichiometry of DIC use, alkalinity decline and oxygen production. Photosynthesis without calcification (eq. 1) has a 1:1 molar relationship between DIC decline, carbon assimilation and oxygen production. Photosynthesis with calcification (Eq. 2) has a 1:1 quotient between DIC and alkalinity (ANC: acid neutralizing capacity) decline, but a 2:1 molar quotient between DIC decline relative to calcification, carbon assimilation and oxygen production. The extent of calcification is likely to depend on pH and the concentrations of bicarbonate and calcium (McConnaughey and Whelan, 1997) and perhaps on individual characteristics of the charophyte species as well. At slightly acidic to neutral pH, proportions of CO_2 of DIC are high and calcification rates may be low, while calcification may gradually increase at higher pH as proportions of bicarbonate and carbonate increase (McConnaughey, 1991). Therefore, we should not expect that photosynthesis and calcification strictly follows either equation 1 or 2, but rather that a mixed stoichiometry and pH dependent rates of photosynthesis and calcification exist (viz. McConnaughey and Whelan, 1997).

In order to test those relationships, we measured DIC consumption, alkalinity change, calcification and oxygen production in photosynthesis experiments at three different initial pH levels (7.5, 8.5 and 9.5) with seven corticated *Chara* species: *C. aculeolata*, *C. aspera*, *C. globularis*, *C. hispida*, *C. tomentosa*, *C. vulgaris* and *C. subspinosa.* The entire exterior of the cortex was calcified in all *Chara* species and no banding pattern was observed like in *Nitella* species and ecorticated *Chara corallina* (Lucas, 1979; McConnaughey, 1991; Kawahata et al., 2013). Our three main hypotheses were that: (i) *Chara* species would be able to use bicarbonate efficiently and, thereby, maintain photosynthesis up to high pH, (ii) species would accompany photosynthesis with calcification and show proportionally increasing calcification as pH rises, and (iii) rates of DIC use, alkalinity decline and calcification would increase relative to oxygen production with rising pH.

2. Materials and methods

Seven *Chara* species were collected for experiments in May-June 2017 from transparent alkaline waters in ponds and lakes located on Zealand, Denmark and Öland, South-Sweden (Sand-Jensen et al., 2010). Species identification and taxonomy followed Schou et al. (2017). One of the species, *Chara aspera* also sprouted and grew in natural sediments collected from a pond on Öland (Christensen et al., 2013) under a natural light regime during April in a greenhouse facility.

2.1. Short-time photosynthetic experiments as a function of pH

Five-cm long apical shoots of six *Chara* species were used for photosynthesis experiments. *Chara aculeolata* being only available on Öland was not included in these experiments. Apical shoots had a sparse cover of epiphytes and it is clear that the measured processes are predominantly due to the charophytes. The medium was tap water - high quality groundwater from calcareous aquifers. It was diluted with ion-free milliQ water to yield DIC concentrations close to 1.25 mM and Ca^{2+} close to 0.5 mM in all experiments to mimic the chemical conditions in sampling ponds on Öland (Sand-Jensen et al., 2010). Experimental water was bubbled with atmospheric air for at least 45 min to ensure 100% oxygen saturation. It was distributed in 1 L glass flasks and titrated to pH 7.50, 8.50 and 9.50, respectively with either HCl or freshly prepared NaOH (Radiometer pH equipment, Copenhagen). Inorganic carbon concentrations in the media for experiments at 15 °C were 1.15 mM HCO_3^- and 96 µM CO_2 at pH 7.5, 1.23 mM HCO_3^- and 11 µM CO_2 at pH 8.5 and, finally, 1.11 mM HCO_3^- and 0.9 µM CO_2 at pH 9.5. A large batch was prepared to be used in several experiments. The glass bottles were closed to avoid air contact and exchange of CO_2 before the experiment. Before use water was kept cold at 5 °C.

Experiments were performed with four replicates of each *Chara* species and four blanks at each pH in 25 ml or 50 ml closed glass bottles. Water was siphoned into the incubation bottles with overflow to prevent air contact. Bottles were mounted on a rotating wheel (12 rpm) in a temperature constant incubator set at 15 °C in experiments in April-May and 20 °C in June. Bottles were illuminated by fluorescent light at 590 µmol photons m^{-2} s^{-1} (PAR, 400–700 nm) which should saturate photosynthesis according to earlier studies on charophytes (e.g. Blindow et al., 2003; Christensen et al., 2013). Experiments lasted 2.5–3 h and dissolved oxygen was measured every 0.5 h using the Presense precision optode system and the programme Oxiview PST3-V6.O2 (Firesting PyroScience, Aachen, Germany) to ensure that photosynthesis had attained constant rates. After incubation, pH, alkalinity, calcium and DIC were measured in replicate samples and blanks in all experiments. Charophytes were dried overnight at 105 °C. Rates of DIC loss, alkalinity change, calcium change and oxygen production were calculated as the differences in concentrations between incubation bottles and blanks and expressed relative to incubation time and dry mass.

Alkalinity (ANC) was measured by acidimetric Gran titration (Gran, 1952) on a 20 ml water volume by repeated addition of 10 µL 0.1 N HCl with a high precision repetition pipette. Calcium was measured on 10 ml water samples by titration with EDTA according to Standard Methods (1971). DIC was either measured directly on an infrared gas analyser (IRGA, Vermaat and Sand-Jensen, 1987) or calculated from alkalinity, pH and temperature according to Lewis and Wallace (1998). DIC was measured directly by injecting 100 µL water through a serum stopper into 5% HNO_3 in a bubble chamber purged with N_2 gas carrying the evolved CO_2 into an IRGA system (ADC, Hoddesdon, England). For every three samples a known standard was measured. The CO_2 response curve from the IRGA was transferred to a laptop computer using Picolog and the response, representing the DIC pool, was calculated in the program OriginPro 2017 ®. The relative contribution of CO_2, HCO_3^- and CO_3^{2-} to DIC was calculated from Lewis and Wallace (1998).

2.2. DIC consumption and calcification in long-time drift experiments

DIC consumption and calcification were measured on four *Chara* species in 18-hours long *incubations* at 20 °C using the same setup and replication as described above. The four species available for these experiments on Öland were: *Chara aculeolata*, *C. globularis*, *C. vulgaris* and *C. hispida.* The initial pH was adjusted to 8.85 in all bottles and a headspace of air was left to reduce accumulation of dissolved oxygen and photorespiration during incubation. After incubation, pH, alkalinity and calcium were measured and DIC calculated as already described.

2.3. Data analysis

Statistical analyses were carried out with the GraphPad Prism 6 software package (GraphPad Software, San Diego, CA, USA). Multiple comparisons were conducted using one- or two-way ANOVA followed