Characeae Biomass: Is the Subject Exhausted?

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1. Introduction

Popularly known as stoneworts, brittleworts, muskgrass, muskworts or bass-weeds, Characeae are among the largest and most complex green algae. All common names come from some characteristics these plants may exhibit, such as the brittle, limestone (calcium carbonate) exoskeleton that can form on the external surfaces of the plant (e.g. *Chara vulgaris* and *Chara globularis*) and, particularly, from the distinctive smell of stale garlic emitted by the plant when crushed. It is important to note, however, that most charophyte species do not accumulate lime in observable amounts. The widespread misinterpretation that *Chara* plants generally form lime is understandable, since the two very common species above thrive in shallow waters where they are readily collected, forming spectacular extensive growths, and may solidify directly into a marl layer or onto a curious tufa rock, a porous limestone formed by deposits from springs. Plants accumulating lime become gray or whitish and quite opaque, whereas the many species without evident lime are generally soft, nearly transparent, with a glassy brilliance and rich green color.

Charophytes forms a significant part of the submerged vegetation of both natural and artificial systems represented by lakes, ponds, ditches, streams, canals, bog-pools, concrete tanks, reservoirs and excavations such as gravel pits, and are found on all continents except Antarctica. They are common in the littoral region of oligotrophic to moderately eutrophic water bodies (Kufel & Kufel 2002), and some authors (e.g. Krause 1985) consider these macrophytes indicators of water quality. *Nitella* specimens predominate in mildly acid water as in igneous rock areas, whereas *Chara*'s predominate in hard waters, but this is not a rule. They are characteristic of a disturbed habitat where periodic drastic changes create less favorable conditions for the growth of other algal species. They are often the first plants to colonize newly dug or cleared ponds and ditches, and some species are characteristic of ephemeral water bodies which dry up completely in the summer. The fast maturing charophytes have an advantage over the slow-growing macrophytes in such habitats. Charophytes are usually at a competitive disadvantage in shallow, moderately productive

habitats, but tend to dominate in deeper water at low light intensities, particularly where the water has a high pH value. They are more often found in mesotrophic and eutrophic, hard water, calcium rich and low in phosphate waters. Charophytes may grow in silt, mud, peat or sand and they often form a dense carpet, known as a charophyte meadow, which restricts colonization by other macrophytes. The more common charophyte species do not die down during the winter. They have been recorded growing down to 60 m deep in clear water, but usually prefer depths between 1 and 10 m. In tropical countries such as Brazil, charophytes grow best in shallow water bodies, mostly in small reservoirs built for cattle disedentation, where they form dense carpets at the littoral zone of the reservoirs, usually at 20-40 cm depths. They may often grow intermingled with other macrophytes, mainly with water lilies (*Nymphaea* spp.), whose floating leaves they use to cut down the light intensity.

In size, they are generally moderately large, average shoots varying from 15 to 30 cm in height, but they may range from 5 mm to 2 m at extremes. Specimens of *Chara hornemannii* collected from the Rodrigo de Freitas Pond, in the city of Rio de Janeiro, ranged between 1.9-2 m tall.

The charophyte 'plant' or thallus is erect, central axis or 'stem' is branched and differentiated into a regular succession of nodes and internodes (Figure 1). Each node bears a whorl of branches of limited growth (the 'leaves' or branchlets), but branches capable of unlimited growth may arise axillary to the leaves. The axis consists of a chain of alternating
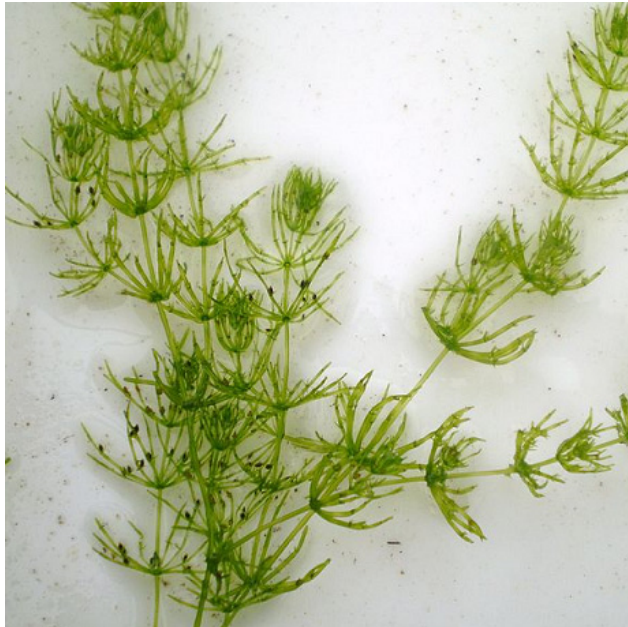

Figure 1. *Chara braunii* specimen showing the central axes branched and differentiated into a regular succession of nodes and internode, and oospores (black little rice-like structures at the verticelate branchlets) (source: Gutza Wikipedia)

long and short cells, the single long cells forming the internodes and the short, discoid cells forming the nodes. The single axial intermodal cell is commonly 1-4 cm long, but they may reach 50-60 cm in *Nitella cernua* and *Nitella translucens*. The intermodal cell is commonly 0.1-0.3 mm broad, but in the last two species, it may reach 2-3 mm broad. The plant is anchored by non-pigmented, single-celled processes, with or without a differentiation into nodes and internodes, the rhizoids, which penetrate the soil or substrate.

The importance of charophytes is indirect, as food for migratory waterfowl, protection of fish fry, and as a nuisance in shallow waters of reservoirs and recreational areas. They may also be used for sulfur baths, cattle food, fertilizers, scouring and filtering agents, and even for supposed control of mosquito larvae.

2. Methods for biomass estimation

Papers dealing with charophytes biomass are not numerous worldwide, and methods to measure that attribute are more or less standard.

Two boat-based and one in-water sampling method were used by Rodusky et al. (2005) to collect submersed aquatic macrophytes (SAV) as part of a long term monitoring program in Lake Okeechobee, Florida, U.S.A. The boat-based methods consisted of a ponar dredge used only to collect *Chara*, and an oyster tongs-like apparatus to collect all other SAV. The in-water method involved use of a 0.5 m^2 PVC quadrat frame deployed by a diver. Comparison of the three methods above showed no consistent pattern to the significant differences found in sampling precision between the three sampling methods, regardless of the geographical location, sediment type, SAV species or density.

To estimate charophytes biomass, the quadrat method is the most used one. According to the method, first a quadrate shall be delimited in the field, e.g. a 25 cm^2 (Westlake 1965, 1971; Krebs 1989). Within this quadrat, a 5 cm diameter (area 19.7 cm^2) and 50 cm tall PVC tube is inserted. Tube wall must be perforated throughout the first basal 25 cm to allow water circulation and the gathering of the plants.

Once collected, material must be stored in glass vials (e.g. 50 ml volume) and taken to the laboratory. In the laboratory, charophytes must be gently washed and if necessary scrapped with a very soft brush to remove other algal material and sediments adhered to the plants. After washed and/or scrapped, the excess water must be dried with some paper towel and finally placed in a porcelain melter.

For the analytic procedure, the charophyte material must be calcinated at 550ºC during 1 hour, then cooled in a desiccator and weighted using an analytical scale to have P_0. Immediately after, plants must be taken to an aerated oven at 65-70ºC until no further weight change is observed for quantification of its dry weight (P_1), and after 1 hour calcination at 550ºC for determination of its ash dry weight (P_2) (Hunter 1976). Determination of the ash free dry mass (AFDM) (P_3) is done using the mathematics $P_3 = (P_1 - P_0) - (P_2 - P_0)$. If total phosphorus (TP) is required, Strickland & Parsons (1965) method is to be used, i.e. the calcinated material is washed with 25 ml of HCl 1N, crushed and heated in a

water-bath for 1 hour. After cooling, samples are diluted with 50-250 ml deionized water depending on the amount of calcinated material.

Palmer & Reid (2010) proposed a method they called 'invention' for the production of macroalgae to provide a sustained, economical source of biomass that may be used in various end-uses processes, including energy production. Their method provides specific combinations of macroalgae types, saltwater growth media compositions, and open pond water containers that resulted in biomass production beyond what may occur naturally without the required manipulation. Specifically, macroalgae that produce an exosqueleton in the presence of brackish water (e.g. stoneworths) have been found to provide excellent biomass production of at least 10 metric tons and up to 200 metric tons per acre per year under their method conditions.

Total phosphorus concentration is determined using a spectrophotometer. Another possibility for TP determination is by the molybdenum blue colorimetric method (Murphy & Riley 1962) after digestion with $K_2S_2O_8$ in an autoclave at 120ºC for 30 minutes (APHA 1995). Total nitrogen (TN) can be determined using spectrophotometry, based on the Koeofell colorimetric method. Calcium and magnesium can also be determined using a spectrophotometer, however, based on the Calmagite colorimetric method.

3. Charophytes biomass

The vast majority of papers published on charophytes worldwide, deals with their taxonomy and systematics. Comparatively, very few papers were published dealing with their biology, including citology, genetics, ecology and physiology.

Measure of biomass is one possibility to estimate the macrophyte's capacity to photosynthetize (Wetzel 1964) and the most used. Other possibilities include population density and biovolume. According to Wetzel (2001), the submersed macrophytes biomass is low if compared to that of other plants. The importance of the charophytes living at the littoral zone of lakes is directly related to the amount of submersed biomass, spatial structure and these plants association with other submersed and emerged macrophytes.

Literature regarding charophytes biomass is not rare neither profuse worldwide and dates mostly from 1980 on, when eutrophication was recognized to be one of the most important events of the century. While not profuse, literature available consents a pretty good overview on the subject.

3.1. Seasonal variation

In the temperate region of the World, aquatic macrophytes show very sharp annual variation, with a growth season of their aerial biomass during the spring and summer, and another season of the underground biomass and detritus accumulation during the fall and winter (Esteves & Camargo 1986). In the tropical region, however, deterministic of the aquatic macrophytes biomass seasonality are the rainy and dry periods (Esteves 2011). Very

little was done, however, up to now regarding the charophytes biomass seasonality in the tropics. Perhaps the only contribution in this regard is the work by Carneiro et al. (1994), who studied the extensive *Chara hornemanii* beds prospering in the Piratininga Lagoon, State of Rio de Janeiro, southeast Brazil at the depth from 0.30 cm to 1 m, and realized that N and P inputs, low water turnover and low water column depth favored growth of phytoplankton, macroalgae and aquatic macrophytes, including charophytes. The same authors also observed a very clear seasonal behavior of the charophyte population that stared during the winter and lasted until the beginning of summer, when the alga covered about 60% of the lagoon sediments. During the summer, the alga biomass reached 500 mg m^{-2} (Carneiro et al. 1994).

Using aerial photographs and field work in brackish water lagoons of Åland Island, Finland, Berglund et al. (2003) observed seasonal and interannual growth, distribution and biomass variation of some charophyte species. According to the last authors, filamentous green algae contributed with 45-70% of the total biomass studied, charophytes with 25-40% and vascular plants with 3-18%. The biomass peak was reached in July and August, and the average biomass was negatively correlated with the charophytes exposition to direct sun light, i.e. the charophyte coverage was greater when their exposition to solar radiation was low, being highly affected by the presence of filamentous algae.

Seasonal changes in the biomass of a monospecific community (*Chara globularis*) and of several communities with high charophyte coverage (*Chara globularis–Myriophyllum alterniflorum*, *Chara globularis–Potamogeton gramineus* and *Nitella translucens–Potamogeton natans*) were studied monthly, from May 1996 to June 1997, by Fernández-Aláez et al. (2002) in three shallow lakes in northwest Spain. Weather and hydrological regime strongly influenced the seasonal biomass patterns and the between-the-year differences in the biomass of the macrophytes. The *Chara globularis* community biomass showed a bimodal pattern, with maximum in mid-July (128 g DW m^{-2}) and late autumn (165 g DW m^{-2}). *Chara globularis* overwintered as a green plant and during the subsequent growth period characterized by high temperature and low rainfall reached a maximum of 305 g DW m^{-2} in June 1997. The highest biomass of *Chara globularis* in the *Chara–Myriophyllum* community was reached in July (Lake Sentiz 160 g m^{-2}, Lake Redos 204 g m^{-2}), while the minimum (Lake Sentiz 10 g m^{-2}; Lake Redos 3 g m^{-2}) was recorded in February or March. *Myriophyllum alterniflorum* (average biomass 95 g m^{-2}) was a better competitor than *Chara globularis* in Redos lake and appeared to be favored by the early beginning of the growing season in 1997 and by the later increase in the water level. *Nitella translucens* biomass (average 64 g m^{-2}) showed a high stability during the entire study period, but lacked a well-defined seasonal pattern. *Potamogeton natans* had a marked maximum biomass in August (426 g m^{-2}). Although the stability of the *Potamogeton natans* population was low, shading did not have a significant influence on the development of *Nitella translucens* biomass.

Torn et al. (2006) measured the seasonal dynamics of the biomass, elongation growth and primary production rate of *Chara tomentosa* in Rame Bay, NE Baltic Sea, a shallow and semi-enclosed sea inlet on the western coast of the Estonian mainland, during the vegetation

period of 2002. Their measurements showed extremely high plant heights (up to 1.42 m) and biomass values (5.2 kg (w.w.) m^{-2}) indicating the importance of the charophyte for the aquatic ecosystem. Torn et al. (2006) observed that the apical part of the plants grew more intensively from early spring to midsummer, whereas that of the subapical one was very low during the entire study period. The plant's net primary production rate peaked in July (43.4 mgO g(d.w.)$^{-1}$ 24 h^{-1}), remarkably lower rates being measured in May and September. The elongation growth and primary production were not correlated with the water nutrient concentrations and temperature. As the active growth of *Chara tomentosa* takes place during a relative short period at the beginning of summer, the amount of available solar radiation and the temperature levels during this sensitive time may have had a significant effect on the community in the same year (Figure 2).
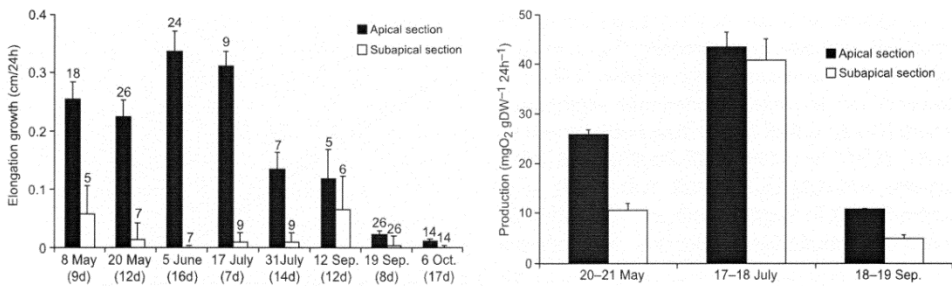

Figure 2. Left: seasonal variation in average elongation growth + S.E. of *Chara tomentosa*. Date of measurement and duration of the experimental period are indicated in between parenthesis. Number of replicates is indicated at the top of each bar. **Right**: Seasonal variation in average diurnal net primary production rate + S.E. of *Chara tomentosa*. In each period photosynthesis was measured in the period of 24 h replicated 3-fold (Torn et al. 2006).

Seasonal growth of *Chara globularis* var. *virgata* caused a regular summer depletion of Ca^{2+} and HCO$_3^-$ by associated CaCO$_3$ deposition, and a more extreme and unusual depletion of K$^+$ was followed over three years (1985-1987) by Talling & Parker (2002) in a shallow upland lake (Malham Tarn) in northern England. Chemical analysis of the *Chara globularis* var. *virgata* biomass and of the underlying sediments indicated a large benthic nutrient stock that far surpassed that represented by the phytoplankton. Growth in the *Chara globularis* var. *virgata* biomass and the magnitude of the water-borne inputs influenced removals of Ca^{2+}, K$^+$ and inorganic N. According to Talling & Parker (2002), several features of Malham Tarn are suggestive in relation to the general case of phytoplankton-phytobenthos interaction and possible long-term change. So, the low P concentrations in the open water are probably linked to the fairly low phytoplankton abundance and influenced by the dense benthic *Chara globularis* var. *virgata* with a major capacity for P uptake. Also, the additional *Chara globularis* var. *virgata* capacity for K$^+$ uptake led to a major seasonal reduction of concentration in the lake water and outflow, of a magnitude rarely if ever recorded elsewhere. The annual growth of *Chara globularis* var. *virgata* seemed to involve further translocation of N, P and K from stocks in the sediments.

3.2. Impact of climatic fluctuations on the biomass

Sender (2008) studied the long term changes of the macrophytes structure in the Lake Moszne located in the Poleski National Park in Poland. Lake Moszne is a relatively small (17.5 ha), distrophic and shallow (1 m) water body. The lake is not connected with the size, nor with the depth of the reservoir, thus depending on the climatic conditions as well as on the economic and recreational activities, and on the hydro-technical changes imposed to the lake (Sender 2008). As a result, a distinct decrease of the plant association variety was observed, as well as changes in their qualitative composition. In fact, changes in qualitative and quantitative structure of lake Moszne macrophytes were probably caused by both abiotic and biotic factors. The macrophytes structure was subject to fluctuation, the changes indicating notable growth of water trophy. The biomass of macrophytes also showed an increase tendency. Nowadays, the structure of vegetation of the lake does not show the typical features for distrophic lakes.

It is well known that algal populations are often present in considerable and varying densities within shallow lakes, as both planktonic and benthic components (Talling & Parker 2002), and that shallow lakes have become the archetypical example of ecosystems with alternative stable states (Scheffer & van Nes 2007). Moreover, that shallow lakes may switch from a state dominated by submersed macrophytes to a phytoplankton-dominated state when a critical nutrient is exceeded (Kosten et al. 2011). Last authors explored how climate change affected that critical nutrient concentration by linking a graphical model to data from 83 lakes along a large climate gradient in South America. Their data indicated that in warmer climates, submersed macrophytes may tolerate more underwater shade than in cooler lakes, although the relationship between phytoplankton biomass and nutrient concentrations did not change consistently along the climate gradient. According to Kosten et al. (2011), in several lakes in the warm and intermediate regions, submersed macrophytes were found until relatively greater depths than in the cool regions, taking the available light at the sediments surface into account.

Rip et al. (2007) is an excellent case-study of how temporal pattern of precipitation and flow from land to water, may give a coherent, quantitative explanation of the observed dynamics in P, phytoplankton, turbidity and charophytes. Studying the external P load to a wetland with two shallow lakes in the Botshol Nature Reserve, The Netherlands the above authors observed that P load reduction resulted in a rapid decrease of phytoplankton biomass and turbidity, and after four years in an explosive charophyte growth. Such a clear water state, however, was unstable and the ecosystem alternated between clear, high-vegetation and turbid, low-vegetation states. Rip et al. (2007) used a water quality processes' model in conjunction with a 14-year nutrient budget for Botshol to determine if fluctuations in precipitation and nutrient load effectively caused the ecosystem instability. Their results indicated that during wet winters when groundwater level rose above surface water level, P from runoff was stored in the lake sediments and banks (Figure 3). Stored P was released the following spring and summer under anaerobic sediments conditions, thus resulting in an increase of phytoplankton density and light attenuation in the water column. Also, in

years with high net precipitation, flow from land to surface water also transported humic acids, further increasing light attenuation. Conversely, in years with dry winters, P and humic acid loads to surface water were reduced, and growth of submersed macrophytes enhanced by clear water. Rip et al. (2007) concluded by stating that global warming caused winters in the Netherlands to become warmer and wetter during the last 50 years, consequently increasing flow from land to water of humic acids and P and, ultimately, enhancing instability of charophyte populations. Finally, in the first half of the 20th Century interannual variation in precipitation was not sufficient to cause large changes in the internal P flux in Botshol, and submersed macrophytes population were stable.
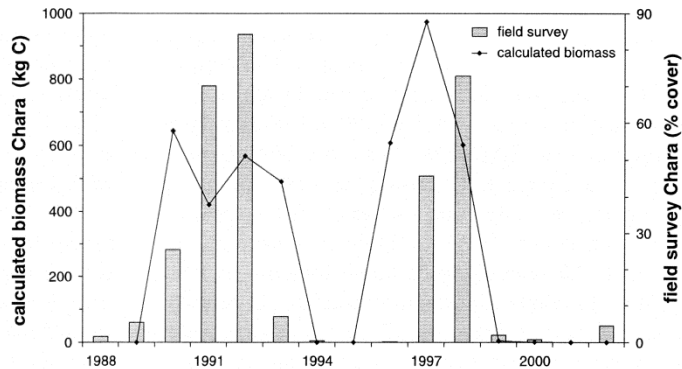

Figure 3. Calculated *Chara* biomass as model results and field surveys at subarea I of the Botshol Natural Reserve for 1989-2002 (Rip et al. 2007).

Recently, Salmaso et al. (2012) studied the combined effects of nutrient availability and temperature on phytoplankton in large and deep lakes of the Alps, lakes Garda, Iseo, Como, Lugano and Maggiore. A significant effect of temperature fluctuations and trophic status on the development of the main groups of cyanobacteria and eukaryotic phytoplankton was observed. However, high positive relationships of nutrient availability with temperature were found only in a few algal groups including charophytes, chlorophytes, dinophytes and, partly, cyanobacteria. Their results have implications in the evaluation of the impact of different climatic scenarios in lakes of different trophic status, suggesting a net increase of only selected eutrophic- or eurytrophic sensitive groups with increasing water temperature in more enriched systems.

3.3. Influence of depth and transparency

Once established, aquatic macrophytes have a positive effect on the transparency of water through several buffer mechanisms (Stephen et al. 1998). Furthermore, the presence of charophytes has been associated with the maintenance of clear water, and changes from a state of clear to turbid water have been associated with the eutrophication of the environment (e.g. Blindow et al. 1993, Kufel & Kufel 1997).

Steinman et al. (1997) studied the influence of water depth and transparency on the charophyte biomass distribution in the southern end of the subtropical Lake Okeechobee, U.S.A. Their first survey (August 1994) was conducted on 47 stations within the 3-Pole Bay. Subsequent surveys (November 1994-December 1996) were conducted on a monthly or bimonthly basis on 7 stations. According to the authors, the distribution and abundance of *Chara* population in the lake showed a marked seasonal phenology, although there were notable differences in biomass among the years and stations. *Chara* plants were observed only in August, September and October, and in 1996 also in November. Also, biomass never exceeded 20 g AFDM m^{-2} and declined significantly from 1994 to 1996. The charophyte biomass was inversely related to the water depth and positively related to the Secchi disc depth, suggesting that irradiance strongly influenced the charophyte distribution in the lake, a hypothesis that was confirmed by data they collected from photosynthetic measurements and phytosynthesis-irradiance curves (Steinman et al. 1997).

The role of charophytes in increasing the water transparency was also studied by Nõges et al. (2003). Under the frame of the EC project ECOFRAME, last authors worked out the water quality criteria for two shallow lakes of the Vooremaa landscape protection area, Central Estonia. Lake Prossa is a macrophyte-dominated system with an area of 33 ha and a mean depth of 2.2 m. Most of its bottom is covered by a thick mat of charophytes all year round. Lake Kaiavere is located 10 km far from Lake Prossa, is much larger (250 ha, mean depth 2.8 m) and is phytoplankton-dominated. Nevertheless, the nutrient dynamics was very similar in the two lakes (Figure 4). The first vernal phytoplankton peak was expressed in reduced Secchi depth in both lakes. After that peak, the water became clear in Lake Prossa, but remained turbid in Lake Kaiavere. Towards fall, the individual mean weight of zooplankton decreased in Lake Prossa, the *Chara*-lake, but remained smaller than in the plankton-dominated one (Lake Kaiavere) (Figure 5). Therefore, zooplankton grazing would initiate the clear-water phase in the *Chara*-lake, but other factors were needed for its maintenance. Another factor that showed a clear difference between the two lakes was the carbonate alkalinity that was rather stable or even increased during the spring in the phytoplankton-dominated lake, while it decreased by nearly 50% between April and July in the *Chara*-lake. The reduced sediment resuspension and the possible allellopathic influence of charophytes on phytoplankton remain the main explanations for the maintenance of the extensive clear-water period in the *Chara*-lake.

Blindow & Schütte (2007) worked with material from fresh and brackish water in Sweden and found out that both turbidity and salinity acted as stress factors on *Chara aspera*. According to the last authors, in clearwater lakes the species can occur in high densities and reach deep water, where the ability to hibernate as a green plant together with shoot elongation may further extend the lower depth limit. In turbid lakes, the plants can still form dense mats, but are restricted to shallow water due to the poor light availability, although shoot elongation may allow a certain extension of the depth range (Figure 6).
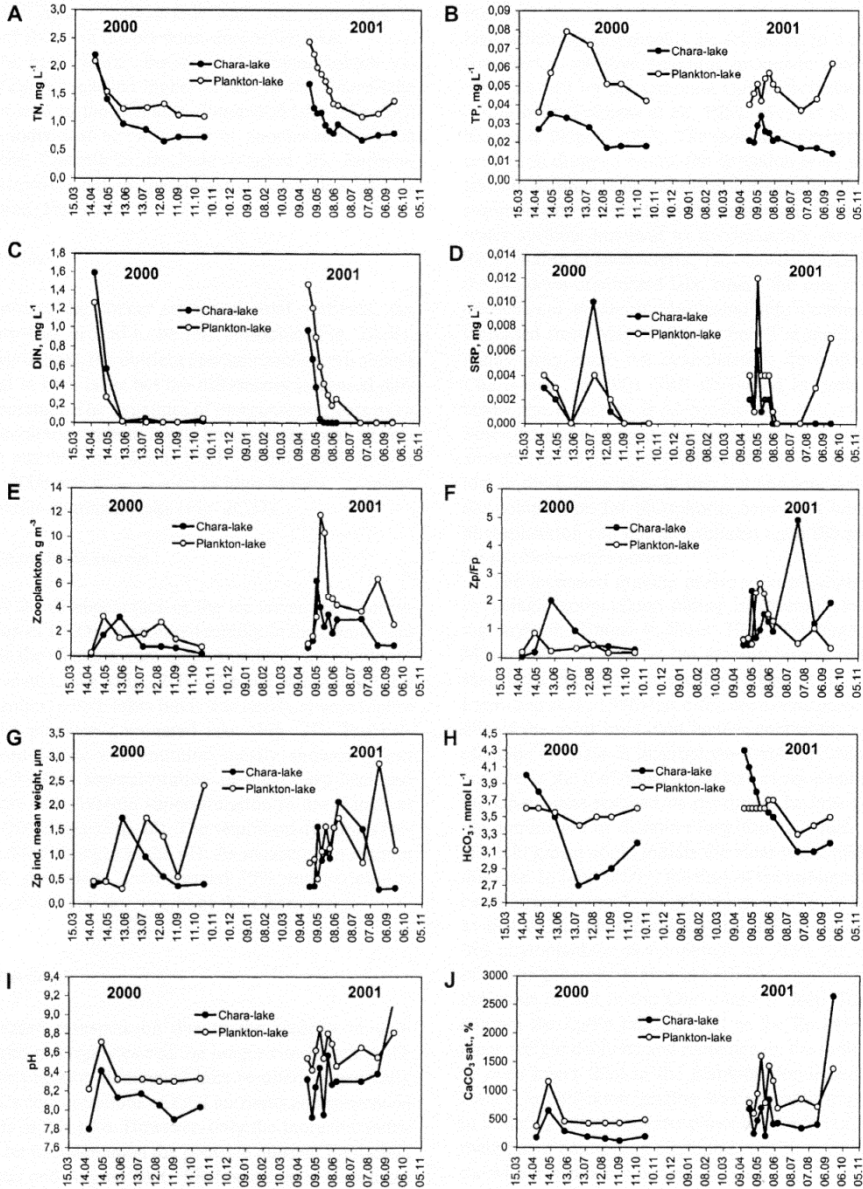
Figure 4. Seasonal dynamics of some chemical and biological features in lakes Prossa (*Chara*-lake) and Kaiavere (plankton-lake). A – total nitrogen, B – total phosphorus, C – dissolved organic nitrogen, D – soluble reactive phosphorus, E – zooplankton/phytoplankton biomass ratio, G – zooplankton mean individual weight, H – hydrocarbonate concentration, I – pH, and J – calcite saturation level (Nõges et al. 2003).
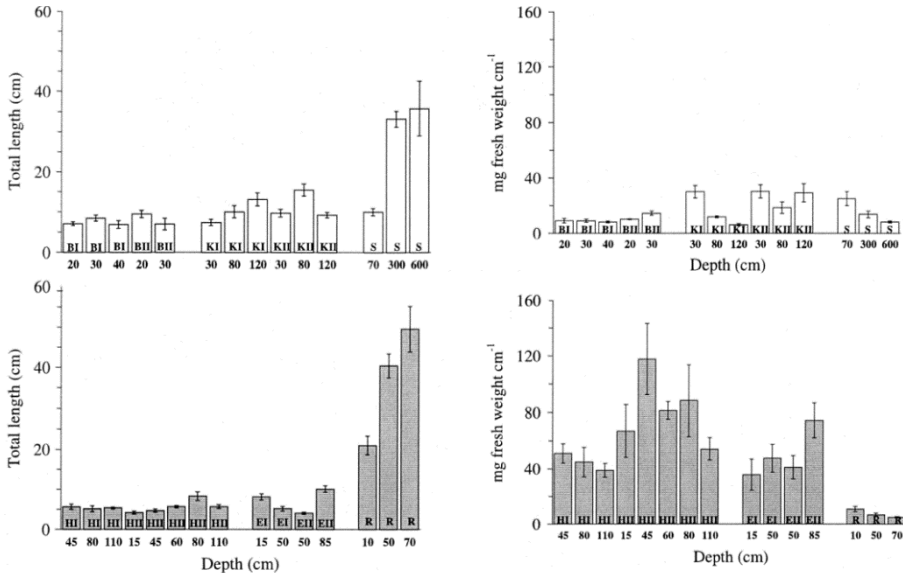
Figure 5. Left: total length of *Chara aspera* (mean values + S.E., n = 5), determined at all sites, depth ranges and sampling occasions. Above freshwater sites, bellow brackish water sites. K – Lake Krankesjön, B – Lake Börringesjön, S – Lake Storacksen, E – Edenryd bay, H – Höllviken bay, R – Redensee bay. I and II – first and second sampling occasions, respectively. Right: fresh weight:total length ratio of *Chara aspera* determined at all sites, depth ranges and sampling occasions (Blindow & Schütte 2007).

A field study conducted from July 2003 to May 2005 in the Myall Lake, a brackish shallow lake in New South Wales, Australia, revealed that *Chara fibrosa* var. *fibrosa* and *Nitella hyalina* occurred in areas of the entire lake that were deeper than 50 cm. Also, more fresh shoots were obtained during the winter (water temperature 13-16ºC), thus suggesting that winter may be their preferred growing season. Their biomass varied from 0 to 321 g DW m⁻², their maximum biomass being displayed between 1 and 2.5 m depth (Asaeda et al. 2007). These authors also observed that charophyte's shoots were longer in deeper waters, varying from c. 30 cm at 1 m depth to 60-90 cm between 2 and 4 m depth. Plants growing in shallow depths had shorter internodes implying a shorter life cycle of shoots. Also, nodal spacing was relatively regular in contrast to its deeper water counterparts although spacing tended to increase at locations farther from the apex (Figure 7). Finally, numbers of oospore and antheridia were higher in shallower water reaching their maximum at around 80 cm.

Chambers & Kalff (1985) used original data from eight lakes in southern Quebec, Canada and literature data from other lakes throughout the World to predict the maximum depth of charophytes colonization and the irradiance over the growing season at the maximum depth of colonization, concluding that the depth distribution of the aquatic macrophyte communities is quantitatively related to Secchi depth. According to regression models proposed in Chambers & Kalff (1985), natural distribution of aquatic macrophytes is restricted to depths of less than 12 m, whereas charophytes can colonize to great depths and up to a predicted 42 m in the very clearest lakes (Secchi depth 28 m).
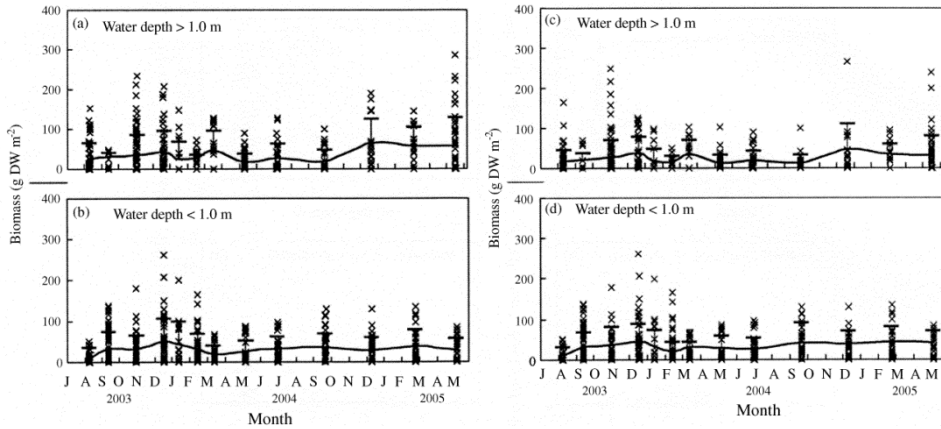
Figure 6. Seasonal variation of (a and b) *Chara fibrosa* and (c and d) *Nitella hyalina* biomass at location deeper than and shallower than 1 m. "X" markers denote individual measurements, the thick solid line represents monthly means, and short flat bars indicate standard deviations (mean + 1 S.D.) (Asaeda et al. 2007).

3.4. Nutrients

The concentrations of N, P and C in the above-ground biomass of 14 dominant macrophyte species (including *Chara globularis* and *Nitella translucens*) in seven shallow lakes of NW Spain were measured by Fernández-Aláez et al. (1999) that found significant differences for the three nutrients among the species and among the groups of macrophytes. The charophytes showed the lowest P (0.053% dry weight) and C (35.24% dry weight) content. Also, only the charophytes exhibited a strong association between N and P ($r = 0.734$, $p < 0.0001$), reflecting an important biochemical connection in these species.

Phosphorus was established as a limiting factor of all the macrophytes (N:P = 35:1), especially charophytes, in which it was below the critical minimum. Siong et al. (2006) used sequential P fractionation to study the nutrient speciation in three submersed macrophytes species, *Chara fibrosa*, *Najas marina* and *Vallisneria gigantea*, and the implications for P nutrient cycling in the Myall Lake, New South Wales, Australia. The mean TP of both *Najas marina* and *Vallisneria gigantea* was significantly higher than that of *Chara fibrosa*, even when the comparison made was based on the ash-free dry weight (AFDW). However, P co-precipitation with calcite ($CaCO_3$) induced during intense periods of photosynthesis occurs in hard water lakes, and this indirect mechanism of reducing P bioavailability in the water column may have been underestimated in assessing *Chara* beds acting as nutrient sink in shallow lakes. According to their results, besides the indirect mechanism above, P in the water column was also directly co-precipitated with encrusted calcite along the charophyte intermodal cell, and such a calcification should be regarded as a positive feedback in stabilizing *Chara* dominance in lakes. Siong & Asaeda (2009) studied the effect of Mg on the charophyte calcite encrustation, and assessed whether charophytes growing on the non-calcareous sediments of the Myall Lake could function as an effective nutrient sink for P in a

similar manner to charophytes growing on the calcareous sediments of freshwater calcium-rich hard water systems. According to the last authors, calcification of *Chara fibrosa* was significantly inhibited by Mg in the water column and, consequently, reduced the formation of Ca-bound P that has a potential sink for P. However, a large percentage of non-bioavailable forms of P in the lake sediments suggested that P sink was through burial of dead organic matter and subsequent mineralization process.

The inorganic phosphorus concentration was not yet significantly related to the charophyte biomass. Palma-Silva et al. (2002) observed that the charophyte community (*Chara angolensis* and *Chara fibrosa*) sometimes occupied the entire benthic region in the Imboacica coastal lagoon in Brazil, and presented a large variation in C:N:P ratio. Results of their investigation (samples taken in March, April, May, July and October 1997) indicated that the charophytes fast growth may have absorbed a great amount of the nutrients entering the lagoon. Values of nutrient concentrations in the charophytes biomass were, according to those authors, within the expected range for the group, with the most eutrophic sampling station in the lake showing the highest N and P values. C:N:P ratios presented high values, and the biomass values were higher in the less eutrophic areas. The biomass reached maximum values of between 400 and 600 g DW m^{-2}, and the C:N:P ratio varied from 51:7:1 to 1603:87:1, indicating that the two *Chara* species may grow in a wide range of nutrient concentration. The same authors concluded that the charophyte community would be responsible by the nutrient decrease in the water column and keeping the water clear after drawdowns (Palma-Silva et al. 2002).

Several authors concluded that the nutrient kinetics favor the phytoplankton growth over *Chara*, thus assuming a P-limited condition. Therefore, although nutrient concentration may influence the charophyte phenology and abundance, light appeared to be a stronger regulator in the Okeechobee Lake. Schwarz & Hawes (1997) also observed the influence of the water transparency on the variation of the charophyte biomass in the Coleridge Lake, New Zealand. In the latter lake, total algal biomass did not surpass 180 g DW m^{-2} between 5 and 10 m depth. Pereyra-Ramos (1981) worked with seven charophyte species collected from Polish lakes and observed an increase of their fresh dry weight during the summer (July): *Chara rudis* 2.07 kg m^{-2}, *Chara vulgaris* 1.61 kg m^{-2}, *Chara contraria* 0.54 kg m^{-2}, *Chara fragilis* 0.39 kg m^{-2}, *Chara jubata* 0.37 kg m^{-2}, *Chara tomentosa* 0.28 kg m^{-2} and *Nitellopsis obtusa* 0.24 kg m^{-2}. Together, the charophytes represented 53% of the total submersed macrophytes biomass, 28% of *Elodea* sp. and 8% of *Ceratophyllum demersum*, two submersed macrophytes. According to Howard-Williams et al. (1995), *Chara corallina* biomass in deep (average 90 m depth) New Zealand lakes ranged around 300 g DW m^{-2}. Bakker et al. (2010) registered a strong decline of the *Chara* sp. biomass under the nutrient enriched condition of Lake Loenderveen, Norway. Similar situation was already detected by Blindow et al. (1993) and van de Bund & van Donk (2004) for other water bodies.

3.5. Trace contaminants

The Anthropocene period is characteristic by rapid urbanization, industrialization, mining activities, metal ore refining, agricultural chemicals, liquid and solid wastes, resulting in

heavy metal pollution of water and land resources. There has been an increasing load of heavy metals (Cu, Zn, Cd, Cr, Hg and Ni) in the aquatic ecosystems, which in turn are being assimilated and transferred within food chains by the process of biomagnification. The problem with the heavy metals is their non-biodegradable nature. The conventional methods used to remove metal ions include chemical precipitation, lime coagulation, ion exchange, reverse osmosis solvent extraction, aeration, chemical oxidation, electrodialysis, ultra filtration, and chlorination (Rich & Cherry 1987).

Research was carried out recently to evaluate the metal accumulation in charophytes. Hence, Bibi et al. (2010) investigated the effects of Cd, Cr and Zn on the growth of *Nitella graciliformis* and their bioaccumulation in the plant under laboratory conditions. Charophyte specimens were exposed to different Cd, Cr and Zn concentrations, and it was observed that the heavy metals concentrations in the plant increased with the increasing metals concentrations in the mediums. As a result, negative growth occurred and the internode elongation was reduced when exposed to these metals at any concentration, however, intracellular *Nitella gracilliformis* has a potential for accumulating Cd, Cr and Zn. Bibi et al. (2010) concluded their investigation by stating that their study should be an integral part of the sustainable development of ecosystems and pollution assessment programs.

Absorption processes are being widely used for the removal of heavy metals from aqueous solutions. According to Shaikh Parveen & Bhosle Arjun (2011), use of various products has been widely investigated in the recent years as an alternative for the currently expensive methods of water treatment, and some natural products can be effectively used as a low cost absorbent. The above mentioned authors conducted batch studies of *Hydrilla* sp. and *Chara* sp. to evaluate the uptake of Cr from aqueous solutions. They found out that about 91.7% removal was obtained with 2 mg L^{-1} of *Chara* sp. at 2 mg L^{-1} Cr concentration after a period of seven days at pH 4. Their results also indicated that the metal removal increased as the days were extended, however, with the increasing contact time *Hydrilla* sp. proved to be better than *Chara* sp. in the Cr removal.

4. Final remarks

As it was mentioned before, literature on charophytes biomass is not rare neither profuse worldwide and dates mostly from 1980 on, when eutrophication was recognized to be one of the most important events of the century. Despite of not being profuse, literature available consents a pretty good overview on the subject.

In the temperate region of the World, aquatic macrophytes show very sharp annual variation, with a growth season of their aerial biomass during the spring and summer, and another season of the underground biomass and detritus accumulation during the fall and winter. Very little, however, was done up to now regarding the charophytes biomass seasonality in the tropics. The single paper published based on charophytes from the tropical region defined, however, deterministic of the aquatic macrophytes biomass seasonality the rainy and dry periods. Water temperature and rain precipitation are, nevertheless, somewhat connected to each other, since the rainy season in the tropics somewhat coincides with the high temperature season.

A climate gradient in South America was studied, indicating that in warmer climates, submersed macrophytes may tolerate more underwater shade than in cooler lakes. Moreover, in several lakes in the warm and intermediate regions, submersed macrophytes were met until relatively greater depths than in the cool regions, taking the available light at the sediments surface into account. According to a very detailed long term study, global warming has been causing winters in the Netherlands to become warmer and wetter during the last 50 years, consequently increasing flow of humic acids and P from land to water that, ultimately, has been enhancing instability of charophyte populations. Such studies conclusion is that in the first half of the 20th Century interannual variation in precipitation was not sufficient to cause large changes in the internal P flux, and submersed macrophytes population was stable.

The presence of charophytes has been associated with the maintenance of clear water, and changes from a state of clear to turbid water have been associated with the eutrophication of the environment. Original data from eight lakes in southern Quebec, Canada and literature data from other lakes throughout the World were used to predict the maximum depth of charophytes colonization and the irradiance over the growing season at the maximum colonization depth, concluding that the depth distribution of the aquatic macrophyte communities is quantitatively related to the Secchi depth. Regression models using the same information above, defined that natural distribution of aquatic macrophytes is restricted to depths of less than 12 m, whereas charophytes can colonize to great depths and up to a predicted 42 m in the very clearest lakes.

The inorganic phosphorus concentration was not yet significantly related to the charophyte biomass. Concentrations of N, P and C in the above-ground biomass of 14 dominant macrophyte species (*Chara globularis* and *Nitella translucens* included) in seven shallow lakes of NW Spain pointed to significant differences for the three nutrients among the species and among the macrophytes groups, the charophytes showing the lowest P and C content. Also, only the charophytes showed a strong association between N and P.

Only recently some research has been carried out to evaluate the metal accumulation in charophytes. Therefore, charophyte specimens were exposed in laboratory experiments to different Cd, Cr and Zn concentrations, showing that the heavy metals concentrations in the plant increased with the increasing metals concentrations in the cultivation mediums used. As a result, negative growth occurred and the internode elongation was reduced when exposed to these metals at any concentration, however, intracellular *Nitella gracilliformis* revealed a potential for accumulating Cd, Cr and Zn.

Summarizing, all research done up to now on the charophytes biomass is still very punctual, i.e. they most often focused one special environment under very specific conditions. There are very few studies focusing a larger time scale and comparing several localities. In the last cases, results are much more consistent. The scientific community needs much more studies, to be able to formulate generalizations. In other words, despite of producing some important information, study of charophytes biomass is far from being exhausted, on the contrary they have just started.

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