

Niche specialization and functional traits regulate the rarity of charophytes in the Nordic countries

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ABSTRACT

1. Charophytes are benthic macroalgae that live in fresh and brackish waters. Given the historic deterioration of their habitats and their competitive inferiority relative to tall rooted plants under eutrophic conditions, it is hypothesized that charophytes are among the most threatened autotrophs. Also, it is expected that charophytes with generalist niches and functional traits, such as tolerance of a broad salinity range and large height, should thrive owing to available habitats and stronger competitive ability.

2. These hypotheses were tested comparing the rarity of charophytes in the Nordic countries with other autotrophs and analysing the relationship to species niche specialization and functional traits. A value on a rarity-commonness scale was assigned to each species based on their Red List status. Niche specialization was evaluated by the Outlying Mean Index using a large Danish dataset while traits were derived from the literature.

3. Supporting the hypotheses, 50–87% of charophyte species were Red Listed, which is much greater than for vascular aquatic (30–35%) and terrestrial plants (18–28%). Commonness of charophytes decreased significantly with niche specialization in separate analyses of brackish and freshwater sites. For brackish water species, the most influential parameter determining species distribution was salinity while for freshwater species, alkalinity, lake size and chlorophyll concentration played a major role. Four functional traits: shoot height, salinity tolerance, bulbil production and flexible life cycle duration were significantly positively related to commonness.

4. In conclusion, charophytes contain a larger fraction of threatened species compared with other groups of autotrophs. The few common charophytes are generalists tolerant of a wide range of conditions, while specialists often have short stature, restricted life cycle variability and are rare in the disturbed contemporary landscape. It is thus a conservation priority to delineate accurately the environmental conditions preferred by the threatened species and protect or restore proper habitats.

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INTRODUCTION

Charophytes belong to an ancient group of macroscopic, slow-growing green algae that live attached by hair-like rhizoids to soft sediments in lakes, ponds, streams and brackish waters (Kautsky, 1988; Krause, 1997; Matheson *et al.*, 2005). Some species are widely distributed from tropical to temperate and subarctic waters across the world, others are distributed in temperate or tropical regions and yet others are confined to narrower regions such as the Nordic countries (Langangen, 2007). Most charophytes grow in oligotrophic and mesotrophic waters, while in eutrophic waters they are often outcompeted by phytoplankton, blanketing filamentous algae and tall rooted angiosperms (Blindow, 1992; Sand-Jensen *et al.*, 2008). Charophytes should therefore include particularly high proportions of threatened species because widespread eutrophication has increased shading from all three groups of autotrophic competitors mentioned (Sand-Jensen *et al.*, 2008). However, some charophytes are able to colonize rapidly and establish temporary dominance in newly constructed or perturbed waters because of their high production of oospores that are efficiently dispersed by water birds (Crawford, 1977; Bonis and Grillas, 2002; Brochet *et al.*, 2010). This warrants an examination of both the conservation status of charophytes across a larger geographical scale, here the Nordic countries (excluding Iceland and Greenland where charophytes are not Red Listed), and a test of how variability in species niche specificity and functional traits (such as shoot height, reproductive investment and life cycle duration) influence their commonness.

Charophytes have experienced a dramatic decline during the last 150 years in all countries with dense human populations and intensive agriculture (Simons and Nat, 1996; Blindow, 2000; Auderset Joye *et al.*, 2002; Baastrup-Spoehr *et al.*, 2013b). Widespread cultural eutrophication has been the most important environmental factor causing deterioration of charophyte habitats and has led to restricted depth distribution or total extirpation of charophyte species in numerous water bodies (Blindow, 1992, 2000; Baastrup-Spoehr *et al.*,

2013b). Mechanical disturbance by dredging, construction, motor boat traffic and reed cutting can disturb sediment stability and increase turbulence and turbidity thereby reducing or excluding charophytes (Torn *et al.*, 2010). A third environmental factor, mainly operating from the 1950s to the 1980s, involved acidification of low-alkaline waters because of high sulphur deposition from the atmosphere that led to the replacement of charophytes by acid-tolerant mosses and filamentous green algae (Riis and Sand-Jensen, 1998). A final important cause of charophyte decline is the disappearance of numerous small water bodies and establishment of only a few new ones because of intensified agricultural exploitation of the landscape (Biggs *et al.*, 2005). As a consequence, many charophytes have become rare, are threatened by extinction and have been placed on the IUCN Red List in European countries, while the remaining common species have not declined to the same extent (Blazencic *et al.*, 2006; Gärdenfors, 2010; Auderset Joye and Schwarzer, 2012; Baastrup-Spoehr *et al.*, 2013b). Although charophytes have been subject to widespread and severe historical decline (Simons and Nat, 1996; Blindow, 2000; Baastrup-Spoehr *et al.*, 2013b) there has been no concerted effort to document their conservation status and to evaluate the possible determinants of species commonness on a larger geographical scale. In this study, conservation status is determined from the proportion of nationally Red Listed charophyte species. Also, the similarity in species composition and species commonness between the Nordic countries is examined. High similarity would support the notion that it is possible to identify mechanisms that determine whether species are common or rare.

Rare and declining species are often characterized by having specialized niches, which are outcompeted by species with more general preferences (Clavel *et al.*, 2011). If the rarity of charophyte species increases with niche specialization, it warrants a more focused conservation effort to protect or restore specific habitats. In this study, charophyte niche specialization was investigated to determine the relationship between niche and species rarity. Further, species traits are related to the overall

commonness of species (Sand-Jensen *et al.*, 2000) and these relationships can often give a functional explanation of the cause of species rarity and thus inform ways of reducing threats to particular species.

Therefore, the purpose of this study was to test how niche specialization and functional traits may account for the commonness of charophytes in the Nordic countries.

CHAROPHYTE NICHES AND TRAITS

High habitat specificity should, by definition, restrict species to a few special but very suitable habitats; they remain rare overall because the majority of habitats would be unsuitable for them (Brown, 1984). This concept of generalists and specialists is based on the trade-off between the capacity to exploit or tolerate a range of conditions (generalists) and performing better under specific conditions (Futuyma and Moreno, 1988). Furthermore, in disturbed contemporary landscapes a common pattern in the change of species composition has been observed for several species groups; i.e. a decline of specialist and an increase of generalist species (Clavel *et al.*, 2011). It can be predicted, therefore, that the degree of specialization of species with respect to physico-chemical and biological habitat conditions should be negatively related to the commonness of charophytes in the Nordic countries.

The response of species to environmental gradients is mediated through functional traits (Keddy, 1992). Conceptually, environmental conditions can be viewed as a set of filters only letting through those species with suitable traits for a given filter (Keddy, 1992). As species often face multiple abiotic and biotic gradients several traits can be involved in determining community composition (Shipley, 2010). For flowering aquatic plants, several traits have been shown to be significantly related to species occurrence and, in particular, plant height is positively related to occurrence both in lakes and streams (Sand-Jensen *et al.*, 2000; Willby *et al.*, 2000). It is thus hypothesized that taller charophytes should be more common than shorter species owing to the stronger competitive ability for light and space of the taller species.

Tolerance of salinity in charophyte species is related to the physiological ability of the species to regulate cell turgor pressure and experiments with several species have shown different salinity tolerances (Winter *et al.*, 1996; Blindow *et al.*, 2003). The observed tolerances correspond well with the distribution of the species within different salinity zones of the Baltic sea (Blindow, 2000). Because of the close proximity of brackish and freshwater habitats in the region investigated and the large number of brackish water habitats it is expected that increasing salinity tolerance will be positively related to regional commonness.

Charophyte oospores are dispersed by a wide variety of migratory waterfowl while bulbils (found in some species) are thought to be mainly important for local persistence (Van den Berg *et al.*, 2001; Bonis and Grillas, 2002; Brochet *et al.*, 2010). Therefore, production of oospores and vegetative propagules by charophyte species should be positively related to the likelihood of dispersal and local persistence, thereby increasing the regional commonness. Oospore formation requires the involvement both of male antheridia and female oogonia and these are present on the same individuals in most charophyte species (monoecious), while a few species have separate male and female (dioecious) individuals (Langangen, 2007). When species become rare, monoecy may maintain oospore formation better than dioecy, but dioecious species can better ensure outcrossing to maintain genetic variability and health, two factors that are important for small populations. Therefore, there is no a priori expectation as to whether common or rare species should have a larger proportion of monoecious or dioecious species.

Other biological traits may also influence charophyte growth and survival in their specific habitats and, thereby, their regional rarity or commonness. Certain species may have plasticity in their life cycle duration (annual vs. perennial), while other species are reported as being invariant (obligate annual or perennial). For example, summer-growing annuals should be selectively advantaged in shallow pond and lake sediments only disturbed by winter ice-scouring, while perennials should be advantaged in low-light and physically stable deep waters where a long time

period without disturbance can allow for population development. It is thus predicted that species capable of reproducing as both an annual and a perennial can occupy more habitats than species expressing only one of the two life cycle durations.

METHODS

Study area

The Nordic countries in this context are Denmark, Sweden, Norway and Finland (Figure 1). Denmark (DK) is the southernmost and smallest country ($43\,095\text{ km}^2$) with the fewest freshwater bodies. Nonetheless, because of calcareous soils and high-alkalinity fresh waters suitable for a variety of *Chara* species, the country had a very rich charophyte vegetation 70 years ago (Olsen, 1944) before widespread eutrophication and drainage of

approximately two-thirds of all water bodies, which led to profound impoverishment of the flora (Baastrup-Spoerl *et al.*, 2013b). Sweden (SW) is the largest ($449\,964\text{ km}^2$) country and most variable with respect to geology, terrain and water types. Sweden had the greatest charophyte richness of the Nordic countries 70 years ago (Olsen, 1944). Eutrophication and acidification have reduced the number and quality of freshwater charophyte localities in Sweden, although not to the same extent as in Denmark except in the SE-region, Scania, where agriculture is most intense (Blindow, 1992, 2000). Norway (NO, $324\,220\text{ km}^2$) and Finland (FI, $337\,030\text{ km}^2$) are located furthest north and lack the charophyte species that have northern distribution limits in Denmark and southern Sweden, but they have a few species with a predominantly northern or eastern distribution (in the case of Finland, Langangen, 2007). Both

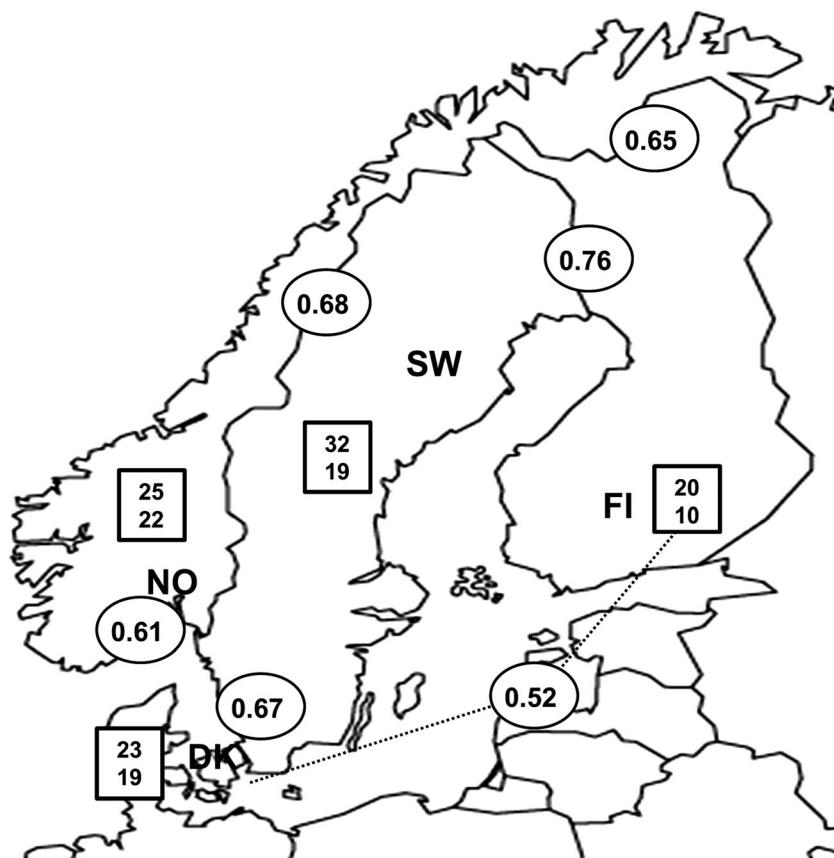


Figure 1. Number (in boxes) of all charophyte species (above) and Red Listed species (below) in the four Nordic countries. Bray–Curtis similarity (in circles) derived from Red List classifications of all species in pair-wise comparisons is shown at the national borders or along stippled lines connecting the countries.

countries have experienced eutrophication and acidification but, like Sweden, large proportions of the land area are forests or natural areas, although the calcareous regions most suitable for charophytes are cultivated, as is the case for all the Nordic countries.

Denmark, Sweden and Finland have coastlines facing the brackish waters of the Baltic Sea where several charophyte species are known to exist (Schubert and Blindow, 2003). These brackish populations have declined dramatically because of eutrophication of the southern Baltic Sea in Denmark, Germany and southern Sweden. The Swedish and Finnish coastlines in the central and northern parts of the Baltic Sea are less eutrophic and still support extensive, but diminishing, charophyte populations (Schubert and Blindow, 2003).

Measuring commonness, diversity and similarity

The Red List categories were used as a measure of the commonness of charophytes (from extremely rare to highly common) in all the countries investigated (Langangen, 2007; Gärdenfors, 2010; Koistinen, 2010; Sjøtun *et al.*, 2010). In the case of Denmark, a Red List proposal was used (Bastrup-Spohr *et al.*, 2013a) based on a recent (1989–2010) compilation of the presence of charophytes in 226 water bodies. In all countries the Red List was based on the IUCN guidelines (IUCN, 2001, 2003).

Assignment of individual species into the different Red List categories can be based on alternative criteria concerning rate of population decline (criterion A), geographic range size (criterion B) or population size (criterion C). For the majority of species in the Nordic countries criterion B has been used in the Red Listing process indicating that the categories should reflect the occurrence of species in each country. Red List categories were thus translated to a numerical scale appropriate for statistical analysis: 0 = regionally extinct, 1 = critically threatened, 2 = endangered, 3 = vulnerable and 4 = nearly threatened. Species present in other Nordic countries but absent from a particular country were also given the value 0. Common species not

present on the Red List were given a value of 6. It was assumed that these values approximate to a logarithmic series of species occurrence and this assumption was confirmed by finding a highly significant ($P < 0.001$) linear relationship between the logarithm of the number of localities (x) and the numerical Red List categories for both the largest (Sweden) and smallest (Denmark) country in the investigation (data extracted from Langangen, 2007; Gärdenfors, 2010; Bastrup-Spohr *et al.*, 2013a) (Supplementary material, Figure S1). Because of the strong association between the numerical Red List categories and occurrence data for these two countries (Figure S1) and identical criteria in the Red List regarding number of localities, the Red List values applied are arguably a reliable reflection both of national and overall occurrence. Therefore, the commonness of individual species in the whole of the Nordic countries was calculated as the sum of numerical Red List values in each of the four countries, making the maximum possible overall commonness 24 (=4 × 6).

Diversity indices were calculated for the four Nordic countries by applying species number and commonness as the numerical values for species categories on and off the Red List as presented above. Richness was expressed as species number (S), evenness as the Pielou index ($J' = H'/\ln S$) and the combination of richness and evenness as the Shannon-Wiener index ($H' = \sum p_i \ln p_i$, where p_i is the relative commonness of species according to numerical Red List categories). The similarity of species commonness among the four countries was calculated by the Bray–Curtis (BC) index (Bray and Curtis, 1957).

Niche parameters

There are many ways of measuring niche specialization each with its own advantages and drawbacks (Devictor *et al.*, 2010). In the present study, Outlying Mean Index analysis (OMI) was used (Doledec *et al.*, 2000) providing a measure of niche position along multiple environmental gradients while searching for the most influential parameters. The advantage of OMI is that it makes no assumption concerning the shape of the

species response curve to the environment, and it gives equal weight to species-rich and species-poor sites (Doledec *et al.*, 2000).

The analysis results in a description (the OMI index) of the mean niche position of each species, the species marginality, representing a measure of the distance between the mean conditions used by the species and the mean conditions of the study area. The OMI values presented are given as a percentage of inertia (Doledec *et al.*, 2000). In this case, the resulting OMI values describe the distance of the niche for each charophyte species from the mean niche of a hypothetical charophyte occupying all investigated sites. The method also yields a set of ordination axes and the loadings of the environmental variables included along these axes indicating the degree that individual variables affect the distribution of species among sites. OMI analysis has been applied successfully to terrestrial plants (Thuiller *et al.*, 2005) and freshwater environments (Doledec *et al.*, 2000; Dole-Olivier *et al.*, 2009). OMI analysis uses two matrices: sampling unit vs species matrix and sampling unit vs niche variables.

Environmental variables were used to delineate the species niche based on data collected by the Danish Environmental Agencies (Anonymous, 2012) from 1980 to 2011 consisting of coupled records of species occurrence and environmental parameters (Table 1). In the case of closed freshwater and brackish water bodies with distinct inlets and outlets, sites with both vegetation and

environmental records were considered the sampling unit. In more open brackish sites, the sampling units were marine environmental monitoring stations coupled with vegetation records. The monitoring stations are placed to best represent local conditions while avoiding barriers (islands, isthmuses, etc.). The generally well-mixed conditions of the surface layers ensured a good correspondence between measured environmental values and values experienced at the sites of vegetation recording (up to 5 km away). A few brackish sites were sampled twice with more than 5 years in between if they showed signs of major change in species composition over the period.

All records of species and associated environmental parameters were collected in the same year, except for a few instances where environmental data from the year immediately before or after species records were used. Environmental variables were calculated as time-weighted averages based on more than two measurements within the year.

Three niche positions were determined, one based on measurements from all sites investigated and the other two based on the freshwater and brackish ($>0.5 \text{ g NaCl L}^{-1}$) sites analysed separately (Table 1).

Species traits

A series of species-specific traits that potentially affect the species occurrence, and thus their commonness, were obtained from the literature on

Table 1. Number of sites and range (min–max) of the variables included in the Outlying Mean Index (OMI) analysis performed at all sites, brackish sites ($>0.5 \text{ g NaCl L}^{-1}$) and freshwater sites. Physico-chemical parameters were measured very close to (in the case of freshwater sites) or within 5 km of (in the case of brackish sites) the vegetation record. Data source is the Danish Environmental Monitoring Program. N/A indicates variable was not included in the OMI calculations for a given dataset

Variables	Full dataset	Brackish sites	Freshwater sites
Number of sites	226	56	168
Salinity (g NaCl L ⁻¹)	0-26.7*	0.6-26.7	N/A
Chlorophyll <i>a</i> (μg L ⁻¹)	0.9-295	0.9-295	1.1-128
Total phosphorus (μg L ⁻¹)	7.6-864	18.8-408	7.6-864
Total nitrogen (μg L ⁻¹)	245-5729	245-5729	286.4-4982
Alkalinity (meq L ⁻¹)			0.025-5.6
Secchi depth (m)			0.26-7.8
Lake depth maximum (m)			0.2-32.7
Lake area (ha)			0.17-1730**

*In all freshwater sites salinity was very low and measured infrequently, therefore all freshwater sites were given the value 0 in the full dataset and the variable was not included in the freshwater OMI analysis.

**Lake area was log transformed in the OMI analysis.

Nordic charophytes (Langangen, 2007) and supplemented by a Baltic and Polish investigation in the few cases where Nordic data were missing (Schubert and Blindow, 2003; Gabka, 2009). The traits included were shoot height, life cycle duration, monoecy/dioecy and production of oospores and bulbils. The maximum shoot height (cm) was converted to a categorical scale: (0) 0–20 cm, (1) 21–40 cm and (2) >41 cm to account for the variation within and among species. These size groups reflect the different size ranges that charophytes exhibit while ensuring a similar number of species in each group. Life cycle duration data were included as (0) restricted life-cycle (obligate annual or perennial) or (1) flexible life cycle (either annual or perennial). In a more detailed analysis of the effect of duration on commonness, the following categorical variables were used: obligate annual, obligate perennial and both annual and perennial. Some species might on special occasions deviate from the recorded life cycle duration but the recorded durations are those that are most commonly observed and thus the most ecologically relevant. The same two data sources were used to determine whether species are monoecious (0) or dioecious (1). *Chara canescens*, a dioecious species, only reproduces parthenogenetically in the focal area. It thus shares the advantages and disadvantages of a monoecious species in this region and is classified as such in the analyses (Schubert and Blindow, 2003). The intensity of oospore production was provided by Langangen (2007) for species with low (0) and high (2) production while species lacking detailed information were considered intermediate (1). Some species produce bulbils as a mean of vegetative reproduction and these species were given the value 1, while the rest were assigned the value 0. Species salinity tolerance was determined from Schubert and Blindow (2003), and was included as the range in salinity (max–min) a given species inhabits. The brackish water species, *Tolyella normaniana*, was not included in the analysis because tolerance values could not be obtained from the literature. A summary of the investigated traits and the commonness in the Nordic countries is provided in the Supplementary material (Table S1).

RESULTS

Species richness and similarity

Thirty-six species have been found in the Nordic countries within the last 50 years. Historically, a few more species such as *Chara baueri* and *Nitella tenuissima* have been recorded but are now considered regionally extinct. Species richness of charophytes was highest in Sweden (32) and lower in the other countries (20–25, Figure 1). A large number of species were on the Red List in all countries (10–22, Figure 1) corresponding to 50–87% of all species. The Shannon–Wiener biodiversity index, combining richness and evenness, was highest in Sweden (3.38) with lower values in Norway (3.07), Denmark (3.00) and Finland (2.93), mainly owing to changes in species richness. The Pilou evenness index was about the same in all four countries (0.958–0.977).

Similarity in relative commonness of charophytes between the countries reflected their proximity (Figure 1). Similarity was high between neighbouring countries and declined with distance between countries.

Niche and commonness

The OMI analysis used records of charophytes at 226 Danish sites with a total of 19 species (Table 1). Omitting one species that only occurred once (*Chara rufis*) and using the restricted number of variables sampled across all brackish and freshwater sites yielded an OMI first axis that explained 78% of the observed variability, with high scores for salinity in particular (Table 2). Several environmental variables were measured only at the freshwater sites, therefore the dataset was separated into brackish and freshwater partitions to allow for a more detailed description of the relative niche position of the freshwater species. In the freshwater and brackish sites investigated, 13 and 12 species were recorded, respectively. In total, seven species were found in both freshwater and brackish sites.

At both the freshwater and brackish sites the first OMI axis explained a high percentage of the variability (Table 2). In the brackish dataset, the niches separated primarily along a main gradient

Table 2. Results of the Outlying Mean Index (OMI) analyses performed on the full dataset, brackish ($>0.5 \text{ g NaCl L}^{-1}$) and freshwater sites. Scores of the variables and the amount of variability explained are given for the first two axes

Axes	Full dataset		Brackish sites		Freshwater sites	
	1	2	1	2	1	2
% of variability	78	17	86	10	40	33
Salinity (g NaCl L ⁻¹)	0.78	-0.11	0.61	-0.31		
Chlorophyll-a (μg L ⁻¹)	-0.10	-0.25	-0.54	-0.17	-0.18	-0.05
Total phosphorus (μg L ⁻¹)	-0.10	-0.17	-0.51	-0.13	-0.04	-0.17
Total nitrogen (μg L ⁻¹)	-0.19	-0.22	-0.56	-0.06	-0.10	-0.16
Alkalinity (meq L ⁻¹)					-0.37	-0.07
Secchi depth (m)					0.07	0.07
Lake depth maximum (m)					-0.03	0.06
Lake area (ha)					-0.17	0.36

of salinity and were negatively associated with nutrients and chlorophyll-*a* (Table 2). In the analysis of the freshwater sites the separation of species niches was linked to two gradients: alkalinity and lake area. This indicates that niches of freshwater species separate along gradients (e.g. alkalinity) that are not relevant in the permanently alkaline brackish waters. Variables linked to eutrophication, such as concentration of chlorophyll-*a*, total phosphorus and total nitrogen had intermediate loadings along the first and second axes indicating smaller impact on the distribution of species among sites (Table 2).

A high OMI index value of a species indicates that the environmental conditions of its growth sites deviate strongly from average conditions in the whole dataset and thus show a high degree of specialization. To test if the commonness of charophytes in the Nordic countries was related to niche specialization, the commonness and the OMI index values of each species were investigated by correlation analysis. Using the niche specialization from the full dataset, a tendency towards a negative relationship between niche specialization and commonness was found ($r = -0.41$, $P = 0.09$) (Figure 2). Separating freshwater and brackish sites to allow inclusion of variables important to niche separation in fresh waters, however, yielded a significant ($P < 0.05$) relationship between commonness in the Nordic countries and niche specialization (Figure 2). It should also be noted that species occurring in the brackish sites generally had a higher OMI index ($49 \pm 30\%$) compared with species occurring in

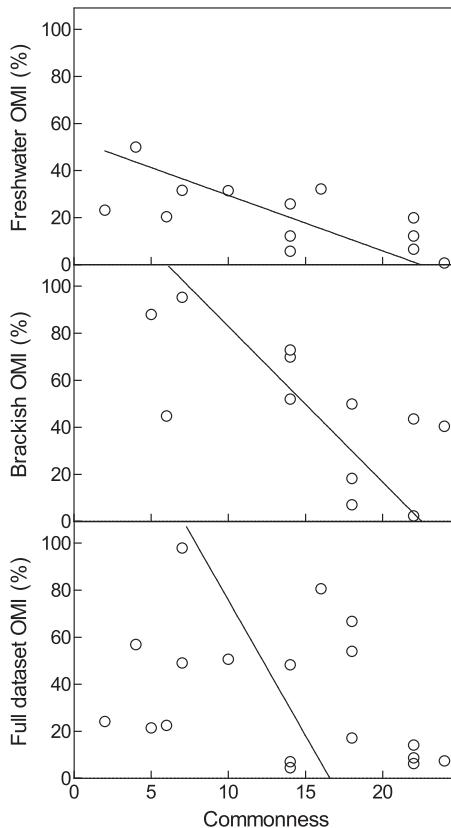


Figure 2. Relationship between niche specialization and commonness in the Nordic countries. Niche specialization was calculated as the Outlying Mean Index (OMI) using specific environmental conditions for Danish sites. Niche specialization was calculated based on three datasets: (a) using all sites (includes 18 species); (b) brackish ($>0.5 \text{ g NaCl L}^{-1}$) sites (11 species); and (c) freshwater sites only (13 species). OMI values are shown as % of inertia. The lines shown are model II regressions. The slopes of the relationship between commonness and OMI are significant for the separate freshwater and brackish datasets (Spearman, $P < 0.05$).

freshwater sites ($21 \pm 14\%$) stressing the large effect of salinity on species occurrence. In general, there is robust evidence supporting the hypothesis that common charophytes are less specialized than rare species and that variables separating the niches are fundamentally different in the brackish and freshwater environments.

Traits and commonness

The commonness of charophytes in the Nordic countries was significantly related to several of the species traits investigated. Taller species tended to be more common than small species, which was also the case for species with wide salinity tolerance (Table 3). Annual species were significantly less common than species capable of expressing both an annual and perennial reproductive cycle, while obligatory perennials were between these groups (Kruskall-Wallis, $P = 0.03$). However, merging the two groups of restricted life cycles (obligate annual and perennial) and comparing them with species with flexible life cycles showed that flexible species were generally more common (Table 3). While the intensity of spore production did not affect the commonness, the presence of bulbils increased it. Species bearing bulbils also tended to have wider salinity tolerance, lower sexual reproductive investment and to be dioecious (Table 3).

DISCUSSION

Diversity and similarity

The most species-rich of the Nordic countries was Sweden and, owing to the generally high evenness in all countries, also exhibited the highest Shannon diversity. The most likely explanation for the high

species richness is that Sweden is the largest of the countries in the investigation and also the country with the most diverse aquatic habitats suited for charophytes. None of the other Nordic countries investigated have Sweden's environmental gradients of a temperate zone underlain with chalk-rich parent material in the south, grading to a subarctic zone with low alkaline substrate in the north, coupled with a long coastline having a strong salinity gradient.

Species composition was generally similar, but reflected the distance between countries. The decrease in similarity with distance is probably the combined effect of multiple factors correlated with distance but with aquatic plants being well dispersed (Santamaría, 2002) it is likely that similarity in species composition mainly reflects the similarity of habitat types available between neighbouring countries.

Despite the differences in species richness and similarity, the compiled Red List data confirmed the hypothesis that the proportion of threatened species is high throughout the Nordic countries (50–87%). This is also seen in Germany, Switzerland, Great Britain and countries on the Balkan Peninsula (Stewart and Church, 1992; Schmidt *et al.*, 1996; Blazencic *et al.*, 2006; Auderset Joye and Schwarzer, 2012) emphasizing the critical status of charophytes here and elsewhere in Europe (Simons and Nat, 1996).

These high proportions of Red Listed species are unprecedented among aquatic submerged plants in Denmark and Norway (30–35%) and terrestrial plants in all four Nordic countries (18–28%) (Gärdenfors, 2010; Kålås *et al.*, 2010; Koistinen, 2010; Wind and Pihl, 2010). The imperilled status of charophytes can be explained by the fact that habitats suitable for charophytes are relatively small in size and limited in number in the Nordic

Table 3. Relationships between charophyte commonness in the Nordic countries and species traits investigated. Within-species correlations of functional traits are also shown. Values are Spearman rank correlations with P -values in parentheses

Trait	Commonness	Height	Salinity tolerance	Oospore production	Bulbil production	Life-cycle duration
Height	0.46 (<0.01)					
Salinity tolerance	0.59 (<0.01)	0.16 (0.34)				
Oospore production	0.05 (0.74)	-0.27 (0.11)	0.11 (0.54)			
Bulbil production	0.35 (0.03)	0.36 (0.03)	0.48 (<0.01)	-0.39 (0.02)		
Life cycle duration	0.40 (0.02)	0.40 (0.02)	0.28 (0.12)	-0.11 (0.54)	0.16 (0.37)	
Monoecious/dioecious	0.05 (0.78)	0.05 (0.77)	0.29 (0.09)	-0.20 (0.24)	0.36 (0.03)	-0.04 (0.82)

countries because of extensive habitat loss and deterioration by eutrophication, acidification, and mechanical perturbation (Baastrup-Spoehr *et al.*, 2013b). Moreover, charophytes are more sensitive to eutrophication than other aquatic plants because they are inferior competitors under nutrient-rich conditions and often occupy light-limited habitats (i.e. below the canopy of taller plants and in the deepest depths recorded for submerged vegetation), thus they are highly susceptible to local extinction (Blindow, 1992; Van den Berg *et al.*, 1998). For example, as Lake Fure, Denmark, became eutrophied and turbid from 1940 to 1985, all 11 charophyte species were lost, whereas only eight of the original 21 vascular plant species disappeared (Sand-Jensen *et al.*, 2008).

Commonness, specialization and traits

As predicted, charophytes with low niche specialization were more common in the Nordic countries while specialist species were more rare. This relationship will exist if the species that have broad environmental tolerances and are able to use a wide variety of resources will, in so doing, achieve high local densities and be able to survive in more places over larger areas (Gaston and Spicer, 2001).

Some investigators argue that one of the main analytical problems in studies of commonness versus niche specialization is that species are evaluated based on their realized niche, while the fundamental niche is best determined experimentally. Experimental determinations of the fundamental niche breadth with respect to several biological features are few because of tradition and insufficient experimental expertise among investigators. However, even if experimental studies were available, they may not provide the necessary answers because they may be restricted in time and space, include a limited number of uninformative traits, and lack the variety of genotypes often present in widely distributed species. Among charophytes, for example, the common species *Chara vulgaris* is highly polymorphic with at least five noticeable varieties in the Nordic countries. These varieties may ensure greater ecophysiological variability

and permit the species to thrive in varying conditions (Langangen, 2007). It is highly unlikely that all five varieties are ever accounted for in the description of the fundamental niche. Therefore, there is no guarantee that experiments will determine the relevant fundamental niche for this species.

Historical changes in landscape development, particularly in Denmark and southern Sweden, have been characterized by widespread eutrophication. Such changes can lead to biotic homogenization with a decrease in specialists and an increase in generalists (McKinney and Lockwood, 1999). Charophytes such as *Chara globularis* and *C. vulgaris* that have been shown to increase or retain high occurrence in Denmark, The Netherlands and Switzerland within the last century, all have low values of specialization (data not shown) (Simons and Nat, 1996; Auderset Joye *et al.*, 2002; Baastrup-Spoehr *et al.*, 2013b). In contrast, highly specialized species such as *Laprothamnium papulosum* have declined dramatically (data not shown) (Schubert and Blindow, 2003). The knowledge that rare species tend to be specialists has implications for the conservation measures that could be used to improve the status of rare charophytes. Probably, it is not simply more area of aquatic habitat that is needed, but particular types of habitat suited to specialist taxa. Further research effort is needed to delineate the conditions suitable for particular species enabling targeted conservation measures.

Recent studies on terrestrial plant species suggest that their success is intimately linked to their functional traits (Mokany and Roxburgh, 2010). Charophytes of the Nordic countries support this pattern because species commonness was significantly linked to several of the investigated traits. Shoot height of charophytes, a proxy for competitive ability for light and space in dense stands, was positively linked to commonness. This finding is similar to that for vascular *Potamogeton* species (Sand-Jensen *et al.*, 2000) and indicates that the ability to compete for light plays an important role in shaping the charophyte community.

The ability to tolerate physiologically a large range of salinities should be of great advantage to

aquatic plants in the Nordic countries because of the proximity and close linkage between brackish and freshwater habitats and the increase in the number of potential growth sites. This was verified by the relationship between commonness and salinity tolerance, implying that species with wide tolerances were generally more abundant than species with narrower tolerance and smaller ranges. For rare species with relatively narrow salt tolerances, such as *Laprothamnium papulosum*, it is important to protect the remaining sites within their salinity range from other stressors such as increased eutrophication. Interestingly, the salinity tolerance seems to co-vary with the formation of bulbils, suggesting that this mode of reproduction is more essential for dispersal and survival of species in brackish waters than in fresh waters. Indeed, apomictic reproduction has been shown to be more abundant in harsh environments (Richards, 2003). Brackish environments with changing salinity and strong physical disturbance (Idestam-Almquist, 2000) could be regarded as harsher than fresh waters giving rise to more apomictic species.

Commonness of charophyte species in the Nordic countries was also positively correlated with the ability of a species to express either annual or perennial life-cycles, as opposed to expressing one or the other. This effect could be straightforward because variety in life-cycle duration increases the ability of a species to thrive in both perturbed habitats as an annual and in stable habitats as a perennial. Charophytes develop dense, monospecific stands of winter annuals in ephemeral ponds that dry out during summer. This is attributable to efficient survival and sprouting of numerous oospores during rewetting in autumn. Vascular aquatic plants are impeded more than charophytes by summer desiccation, as their abundance increases gradually from temporary to permanent calcareous ponds (Sand-Jensen *et al.*, 2010). In permanent ponds and lakes, annual forms may thrive during summer in shallow waters and survive heavy physical disturbance and thick ice cover during the winter as oospores and vegetative propagules. A conservation measure that could improve the status of rare winter annual charophyte species is

to allow more ponds to dry out in summer or reset the pond succession through management, such as sediment removal, which has been shown to benefit charophytes (Sayer *et al.*, 2012). Perennial forms may grow year-round in the more physically stable deeper waters where longer time is needed for population growth because of low light availability. Certain charophytes have an exceptional capacity for growing at lower light and temperature than vascular plants in the cold bottom waters of temperate lakes and are thus dependent on water clarity for survival (Nygaard and Sand-Jensen, 1981).

A diagnosis of being common or rare

The data confirmed that charophyte traits and degree of niche specialization are important determinants of whether species are common or rare. In common species, the prevalent traits are related to being large, having flexible life-cycle duration and a high salinity tolerance. In rare species, traits are associated with having more specialized niches.

Some rare charophyte species are derived from common species, which they still resemble. For instance, *Chara filiformis* resembles the common *Chara contraria*, and *Nitella capillaris* resembles the common *Nitella opaca* (Langangen, 2007). In reference to the rich, terrestrial South African Cape flora with many endemics, Davies *et al.* (2011) proposed that if plants speciate via small isolated populations at the edge of larger species ranges (peripatric), then lineages that are diversifying rapidly will have many threatened species. Darwin (1859) noted the pattern of widespread, variable plant species acting as species pumps for peripheral sister species of restricted distribution. If this mechanism also operates in charophytes, then formation of peripheral species in cold regions of the Nordic countries could be driven by widespread, common species. If the ranges of these common species are fragmented they will lose their ability to operate as species pumps in the future (Knapp, 2011).

In conclusion, most charophytes are rare in the Nordic countries because of too few suitable habitats and high niche specificity. Contrary to

previous belief, charophytes are not solely restricted to stable, resource-poor habitats (ultra-oligotrophy or deep waters of low irradiance). Many charophytes flourish in recently constructed ponds and restored lakes at relatively high nutrient levels before phytoplankton or tall vascular plants overtake and outcompete them (Crawford, 1977; Wade, 1990; Bastrup-Spoerl *et al.*, 2013b). The best way to set long-term priorities for the conservation of both common and rare charophyte species is to create or encourage new pond habitats, ensure diverse habitat conditions with clear water, and avoid physical deterioration of existing populations.

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