Co-occurrence of Two Stoneworts of Reverse Ecological Spectra in the Same Lake Ecosystem. Habitat requirements of *Chara delicatula* Agardh and *Chara globularis* Thuillier in the Context of Bioindication

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Abstract

Regarding their distribution in lakes, *Chara delicatula* and *Chara globularis* are considered species of different or even opposite ecological requirements. *C. delicatula* is usually reported from oligotrophic lakes, but *C. globularis* from more fertile ones. Within Poland, both species rarely build extensive stands in the same ecosystem. The present study was carried out in a stratified, mid-forest lake in midwestern Poland where *C. delicatula* had not been found earlier. Based on the physical-chemical properties and analyses of phytoplankton, a transitional, meso-eutrophic status of the lake was stated. In the group of 15 more frequent macrophyte species, *C. delicatula* and *C. globularis* were among plants predominating the lake's macrovegetation. Both stoneworts built separate patches as well as contributed to other macrophyte assemblages. Growing under the same light and trophic conditions, the species revealed differences in ecological optima in relation to the depth of occurrence and bottom slope. The results are discussed in the context of the species identity: are *C. delicatula* and *C. globularis* separate species or forms within the same taxon?

Keywords: *Chara delicatula, Chara globularis,* ecological spectra, macrophytes, charophytes, habitat requirements

Introduction

Charophytes, a specific group of water macrophytes, have been extensively studied by many authors since the end of the nineteenth century. From the ecological point of view, however, this group of macroalgae has been treated since the 1930s [1 and references there] and used as indicators for good ecological states of water. Numerous environmental factors, including pH and hardness, calcium, chloride, organic matter, nutrient concentrations and light conditions, as well as biocoenotic interrelations have been taken under consideration [1-8]. Although the occurrence of charophytes is generally associated with clear waters of low fertility and good ecological status, the particular species distribution is affected by different environmental conditions [9-10].

Based on the literature, one may conclude that *Chara delicatula* Agardh and *Chara globularis* Thuillier are species with different or even opposite ecological requirements and indicator value in relation to trophical status. This is especially reflected in the species' distribution in lakes. *C. delicatula* localities have been reported from oligotrophic

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"Lobelia" lakes of low concentration of calcium, while *C. globularis* has been observed in lakes rich in calcium and phosphorus [10]. This concerns also assemblages built by both stoneworts [11]. Due to Blindow's [6] comparison of phosphorus concentrations and different species of charophytes in Swedish lakes, *C. delicatula* commonly developed at lower P concentrations than habitats containing *C. globularis*. Toivonnen and Huttunen [12] listed *C. globularis* among species indicating eutrophic waters, whereas Beauchamp et al. [13] documented large and dense *C. delicatula* meadows in an ultraoligotrophic lake.

Within Poland, C. delicatula has the status of a rare species and its localities were generally restricted to oligo- and mesotrophic "Lobelia" lakes and mountainous reservoirs [14-18]. This species mostly occupies well insolated mineral habitats from several centimeters up to over 10 meters in depth. By contrast, C. globularis might be characterized by a wide ecological amplitude and is frequently observed in different kinds of water bodies (with the optimum in meso-eutrophic and eutrophic waters), on the shallow, organic and organic-mineral habitats [14, 15, 19]. Both species are rarely observed in the same lake ecosystem, especially where they build monospecific stands and - in phytosociological terms - their own associations [15, 19, 20]. A lack of detailed data on the co-occurrence of both species in the same reservoir might result from morphological similarities of both stoneworts [15, 16, 21] which can lead to identification of individuals as one species.

The aim of this study was to characterize environmental conditions, including trophic ones, in a mid-forest lake where both species of interest occur abundantly. A comparative analysis of their habitat requirements was of the greatest interest. Since the species occurred in the same ecosystems, which means under similar light conditions and nutrient availability, such habitat factors as the depth and bottom slope were taken under consideration.

Study Area and Methods

The study was performed in a stratified, mid-forest Lake Grzybno (Fig. 1) located on the area of Lubuskie Lakeland (mid-western Poland), the region where in pub-



Fig. 1. Location of Lake Grzybno in mid-western Poland (Lubuskie Lakeland). * maximum depth [34].

lished and unpublished studies (herbarium materials) *C. delicatula* had not been found earlier [22-24]. The lake is centrally located among 9 lakes forming a postglacial tunnel-valley near the town of Ośno Lubuskie. It is a typical ribbon outlet-lake, with a maximum depth of 13.9 m, occupying an area of 41.2 ha, one of the largest lakes in the region [34]. The direct drainage basin is dominated by forests with highly inclined slopes. As a result of the geomorphological structure (especially the bottom slope), rushes develop sparsely and form a narrow belt in the littoral.

Field investigations were carried out in Lake Grzybno in the summer seasons of 2001 and 2002. To define the trophic state, basic physico-chemical parameters (transparency by Secchi disc, conductivity, pH, oxygen concentration, water temperature and dissolved forms of nutrients) as well as the qualitative and quantitative structure of phytoplankton were analyzed. The temperature, oxygen concentration, conductivity and pH were measured in the deepest, central basin of the lake using the portable apparatus Elmetron CX-742. Water samples for the rest of chemical analyses and phytoplankton were collected in the same central basin and preserved with chloroform and Lugol's solution, respectively. The concentrations of nutrients were determined in the laboratory with the aid of a Hach DR/2010 spectrophotometer.

Phytoplankton composition was determined with light microscopy, and taxa abundance using a Fuchs-Rosenthal chamber. Estimation of phytoplankton biomass was based on biovolume measurements.

In particular, macrophyte community species were listed and their abundance was estimated (as a percentage cover of a sampled area, in accordance with the mid-European Braun-Blanquet's phytosociological method). The representative parts of patches (with clearly defined species composition and structure; in practice in the middle of a patch) and not patch-to-patch transitional zones were studied. 209 macrophyte patches were investigated. Additionally, the mean depth of each patch as well as the bottom slope were measured. The composition of the substratum (as proportion between mineral and organic compounds) was also estimated. The significance of the habitat differences between the studied charophytes was tested statistically with the use of Mann-Whitney U-test and presented in the form of box-whisker diagrams. To present possible interrelations between charophyte cover and the depth and slope of habitats, schematic models were generated based on linear dependence.

Charophyte individuals were collected from each locality by diving and identified with the use of an Olympus SZX 9 stereo microscope. The species determination followed charophyte identification keys [10, 14].

Results

Physical-Chemical and Algological Analyses

Analyses performed in the deepest basin of Lake Grzybno revealed vertical stratification of temperature Table 1. Nutrient concentrations in the surface and above-bottom waters of Lake Grzybno.

Sample	Dissolved phosphates mg PO ₄ l ⁻¹	Nitrate nitrogen mg N l ⁻¹	Ammonia nitrogen mg N l ⁻¹
Surface	0.28	below detection	0.03
Above-bottom	0.29	0.2	0.73

and oxygen with fully developed thin epi-, and thick meta- and hypolimnion. However, oxygen depletion below 1 mg l⁻¹ was noted in the hypolimnetic waters. Transparency (SD) was 4.5 m and Carlson's trophic state index TSI(SD) accounted on the basis of transparency was 40. This value placed the lake within the oligo-mesotrophic category. Electrolytic conductivity reached 360 μ S cm⁻¹, and pH exceeded 8.6. Nutrient concentrations are given in Table 1.

Qualitative analysis of phytoplankton assemblages showed 75 taxa representing 7 phytoplankton categories. In the surface and above-bottom water layers, similar numbers of phytoplankton taxa were identified. The diverse Chlorophyta-Cyanoprokaryota taxa were more common, with their representation considered typical for eutrophic waters. However, smaller contributions of Chlorophyta and larger contributions of Cyanoprokaryota taxa were observed in the abovebottom waters. This might be correlated with higher nitrogen concentration and oxygen deficit as compared to the surface layer (Table 1). As far as quantitative analysis is considered, phytoplankton abundance and biomass sharply indicated differences between surface and above-bottom water layers. Regarding taxa abundance, Chrysophyceae (43% of the total abundance, with the highest share of Erkenia subaequiciliata Skuja - 42%) and Bacillariophyceae (22%, including Fragilaria capucina Desmazieres - 20%) dominated the surface phytoplankton assemblage, while in the above-bottom one, Cyanoprokaryota - 72% (mostly Merismopedia tennuissima Lemmermann - 22% and Chroococcus minimus (Keissler) Lemmermann - 20%) decidedly dominated over the rest of phytoplankton taxa. In the case of biomass, in both surface and above-bottom phytoplankton assemblages, cyanoprokaryotic Aphanocapsa incerta (Lemmermann) Cronberg et Komárek had a significant share (17%, 16%, respectively). Aphanothece clathrata W. et G. S. West dominated the surface phytoplankton - 28% of the total biomass (2.1 mg l⁻¹). In the above-bottom sample, another prokaryotic species, Phormidium tenue (Ag. ex Gom.), and Ceratium hirundinella (O. F. Müller) Dujardin representing Dinophyta significantly contributed (22%, 33%, respectively) to total biomass $(3.5 \text{ mg } l^{-1}).$

These results provide evidence for transitional, mesoeutrophic conditions in the studied lake.

Macrophyte Characteristics

15 macrophyte species predominated the lake macrovegetation, forming complex assemblages as well as monospecific stands. Among emergent plants, *Phragmites australis* (Cav.) Trin. ex Steud. and *Typha angustifolia* L. were the most frequent species. Helophytes only locally formed a wide, compact reed belt. Large areas on the sloppy lake shores were sparsely inhabited by emergent vegetation or were vegetation-free.

Four species of submerged macrophytes formed extensive beds: *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L. among the angiosperms, and *C. globularis*, and *C. delicatula* among stoneworts. Besides *Chara* species, *Nitellopsis obtusa* (Desvaux) Groves also contributed to charophyte meadows. Although stoneworts commonly occupy the deepest phytolittoral regions in clear-water lakes, in Lake Grzybno *Ceratophyllum demersum* and *Myriophyllum spicatum* reached deeper (>6m) than charophyte species.

Floating-lived vegetation was poorly developed and predominated by *Nuphar lutea* (L.) Sibth. et Sm.

Al Comparison of *C. delicatula* and *C. globularis* Habitat Requirements

In terms of phytosociology, both stoneworts built their own associations, which in practice means they usually formed monospecific stands, sometimes with small or even negligible shares of other macrophytes. They also contributed to other macrophyte patches. In general, *C. globularis* appeared to be more frequent than *C. delicatula* (Figs 2, 3). Besides its own beds, it occurred frequently in *C. demersum* and *M*.



Fig. 2. The contribution of monospecific stands of *Chara delicatula* and *Chara globularis* to the vegetation of Lake Grzybno.



Fig. 3. *Chara delicatula* and *Chara globularis* frequencies in Lake Grzybno (in their own assemblages and in patches dominated by other macrophytes).

spicatum patches. The species was also found among helophytes. *C. delicatula*, by contrast, was more frequently noted within emergent vegetation (usually *P. australis* and *T. angustifolia*, where it occurred abundantly). Noteworthy was the fact that both studied charophytes only sporadically co-occurred in the same patches of vegetation.

Although the studied Chara species did not differ significantly in the substratum preferences (Fig. 4), the depth of occurrence (Fig. 5) and bottom slope (Fig. 6) appeared to differentiate both charophytes statistically significantly (Mann-Whitney U-test at p<0.05 and p<0.001, respectively). In order to find out whether these habitat factors might be of importance for the studied species' abundance, a schematic model of interrelation between charophytes cover, and the depth of patch and bottom slope was generated for monospecific stands of C. delicatula and C. globularis (Fig. 7). As might be concluded from Fig. 7, a difference in ecological optima of C. delicatula and C. globularis, co-occurring under similar trophic conditions, emerges with respect to the above-mentioned factors. However, it might be considered rather limited.



Fig. 4. Percentage contribution of different types of substratum in habitats occupied by *Chara delicatula* (N=24) and *Chara globularis* (N=66) in Lake Grzybno.



Fig. 5. Statistically significant difference in depth of occurrence of *Chara delicatula* (N=24) and *Chara globularis* (N=66) in Lake Grzybno.

Discussion

Although C. delicatula is considered a sub-cosmopolitan species with the distribution well documented in the international literature [6, 10, 13, 16, 25, 26], its occurrence, in contrast to C. globularis, has rarely been noted in Poland. The localities in Lake Grzybno and in nearby lakes are the first records in mid-western Poland [22-24]. Earlier, the species was found in poorly fertilized "Lobelia" lakes of northern Polish lakelands [14-16, 27-29]. Although the distribution of C. delicatula is also wide in lakes of mid-eastern Poland, its occurrence is generally limited to the group of lakes in which Myriophyllum alterniflorum DC, one of the species characteristic of ageing "Lobelia" lakes, is present [16-18]. So far, the co-occurrence of both Chara species in the same lake has rarely been reported from Polish lakes [15, 16, 19, 20]. Different habitat requirements may be of fundamental importance. In fact, morphological similarities can possibly lead to incorrect identification of both species as only one species, C. globularis. According to Karczmarz [16 and references there] C. delicatula and C. globularis undoubtedly are separate species and distinctly differ in both morphology and genetics. Thus, even atypically developed forms of one species should not be taken for the other. This point of view, however, is not generally agreed upon [21,30] and individuals are treated as two varieties of one species complex of C. globularis, with a set of transition forms. Such phenomenon was observed in a eutrophicated shallow lake in Wielkopolski National Park [31]. It is noteworthy, that individuals collected in the present study were morphologically distinguishable in each case and no transitions between both species were found. Moreover, both stoneworts only occasionally cooccurred in the same patches. Even in such a case, both or one of them occurred in small numbers.

Interestingly, the studied charophytes not only occurred within emergent and submerged vascular plants but



Fig. 6. Statistically significant difference in bottom slope of habitats occupied by *Chara delicatula* (N=24) and *Chara globularis* (N=66) in Lake Grzybno.



Fig. 7. Dependence of *Chara delicatula* (N=12) and *Chara globularis* (N=33) cover on the depth and bottom slope of habitats in phytocoenoses dominated by the studied charophytes. A schematic comparison of monospecific stands.

also formed monospecific stands in the lake. Occurring in the same reservoir, which means under similar environmental conditions (trophy, light climate and competition with other micro- and macrophytes), both charophytes inhabited habitats of different depth and bottom slope (as evidenced by the use of statistical tests). Therefore, different requirements in relation to these factors may have substantial consequences for the distribution of the studied species.

Physical-chemical and algological analyses as well as the composition of macrovegetation suggest a transitional, meso-eutrophic status of Lake Grzybno. Carlson's trophic state index points to oligo-mesotrophic conditions in the lake [32], whereas the floristic composition and quantities of phytoplankton suggest ongoing eutrophication. Noteworthy is the fact that C. delicatula did not form organs of sexual reproduction, likely a result of unfavorable conditions and increasing trophy where C. globularis might replace C. delicatula [16]. On the other hand, in Blindow's [6] opinion, small charophyte species, including both C. delicatula and C. globularis, might occur in turbid lakes where they are often found on wind-exposed shores, whereas large charophyte species are among the first submerged macrophytes to disappear during eutrophication. In fact, small species dominated charophyte meadows in Lake Grzybno (although Nitellopsis obtusa was locally abundant) and often occupied shallow waters, where angiosperms were sparse or absent. A lack of strong competitors, which developed in deeper habitats, in combination with the depth, bottom slope and water trophy, might be factors governing the charophytes' composition and distribution in the studied lake. Due to Pełechaty and Antonowicz [33], C. delicatula has expanded in a eutrophic shallow lake and spread over shallow areas of the phytolittoral where macrophytes had earlier been removed by anglers. Further research on the habitat requirements and scope of charophytes are desirable, especially in the case of C. delicatula, whose ecological spectrum seems to be

broader than considered so far. Such results as presented here might have important consequences for the evaluation of a lake status based on charophyte species, often applied as sensitive bioindicators of good ecological conditions and low trophy.

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