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Alkalinity and trophic state regulate aquatic plant distribution in Danish lakes

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Abstract

Main distribution patterns of submerged macrophytes in a large number of Danish lakes were determined and relationships to environmental variables evaluated by different multivariate analysis techniques. The lakes varied greatly in location, size, depth, alkalinity and trophic status. There were distinct differences in the distribution of species and growth forms among the lakes. The lakes separated into five groups of characteristic species compositions. Alkalinity was the main factor responsible for the species distribution. Lakes of high alkalinity were dominated by vascular plants of the elodeid growth form, lakes of intermediate alkalinity contained a variety of elodeids and vascular plants of the isoetid growth form, while lakes of low alkalinity and low pH had several isoetids and bryophytes, but very few elodeids. Alkalinity is a close descriptor of the bicarbonate concentration, which is an important source of inorganic carbon in the photosynthesis of many elodeids. The species distribution was related to their ability to use bicarbonate and extract inorganic carbon, implying that the observed distribution has an eco-physiological foundation, though a substantial variation suggests an influence of phenotypic plasticity and local environmental heterogeneity. Trophic state also influenced the distribution of species, with very eutrophic lakes having only a few robust elodeid species able to compensate for turbid conditions, while small elodeids and slow-growing isoetid species were absent. The distance separating the lakes did not influence similarity in species composition among them. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Species composition at a given site is determined by the multifactorial influence of historical, environmental and biological factors and is, therefore, a complex ecological question to evaluate (Johnson et al., 1993). In restricted regions with the same species pool, and

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for organisms with efficient means of dispersal among different sites, species composition is easier to study due to the main role of environmental and biological factors for the distribution.

Submerged macrophytes belong to a group of widely distributed organisms with efficient means of dispersal (Sculthorpe, 1967). They appear to show a distinct distribution among individual lakes, depending mainly on water chemistry and, in part, on transparency related to trophic level (Duarte and Kalff, 1990). Several early studies have correlated the distribution of lake macrophytes with water chemical parameters (reviewed by Hutchinson, 1975). However, focus on main factors responsible for the distribution pattern has shifted between several chemical variables such as pH (Iversen, 1929; Catling et al., 1986), al-kalinity (Moyle, 1945; Hellquist, 1980; Jackson and Charles, 1988), hardness, the sum of cations, calcium plus magnesium and conductivity (Spence, 1967; Seddon, 1972; Kunii, 1991). Close relationships exist between several of these variables, however, and it would be appropriate to use the most meaningful variable of importance to the physiology, growth and survival of the submerged species in comparative studies of plant distribution.

More recently, emphasis on environmental variables of possible importance to the distribution of lake macrophytes has been widened by including measurements of limiting nutrients such as nitrogen and phosphorus (Srivastava et al., 1995; Toivonen and Huttunen, 1995). In this study, we broaden the perspective further by including measurements of location, morphometry and the size of the lakes as well as their transparency and phytoplankton biomass.

We analyze species distribution and similarity among lakes by two different multivariate analysis techniques and, subsequently, determine how variation in species composition is correlated to the environmental factors, to ensure an objective analysis and avoid the initial subjective focus on certain environmental factors and plant traits. Different multivariate techniques are used to ensure that observed patterns are robust. These techniques, which have proved very useful in numerous terrestrial comparative studies (for references see Jongman et al., 1987; Kent and Coker, 1992), have been used in rather few comprehensive aquatic macrophyte studies (e.g. Jackson and Charles, 1988; Srivastava et al., 1995; Toivonen and Huttunen, 1995). Most of these studies evaluated a limited variation of the environmental variables of possible significance to the distribution of lake macrophytes, or a broader variation of the environmental variables but a rather limited number of localities, and thereby restricted the evaluation of variables of importance to plant performance and distribution. In contrast, we here include a broad range of relevant chemical and physical variables and a great number of lakes to ensure a comprehensive description of the environmental variability, in order to permit different possible patterns in plant distribution to be evaluated.

Environmental factors were selected for the following reasons. Lake surface area, mean and maximum water depth were included because they influence the vegetated area and habitat variability (Duarte et al., 1986) and thus the likelihood of species establishment and long-term survival (Rosenzweig, 1995). Lake location can account for the possibility that lakes in different geographical regions have a different species composition, independent of their water chemistry, and that closely neighbouring lakes have some species in common and generally support growth of more species because of mutual exchange of propagules (Møller and Rørdam, 1985; discussed by Weiher and Boylen, 1994). Water alkalinity is to a great extent determined by the bicarbonate content and is closely related to conductivity, the sum of cations, calcium plus magnesium and hardness in most freshwaters (Stumm and Morgan, 1981). Among these variables, bicarbonate (or alkalinity) is a preferable determinant because it is a specific measure and an important source of inorganic carbon for the photosynthesis and growth of many submerged macrophytes (Spence and Maberly, 1985; Madsen and Sand-Jensen, 1991). Measurement of pH is included as a possible controlling factor for the distribution of lake macrophytes because pH has a strong direct influence on membrane function, cell regulation and ion solubility (Larcher, 1995). While pH is closely related to the logarithm of bicarbonate concentrations in waters at approximate carbon dioxide equilibrium with the atmosphere over the pH-range from 5.5 to 8.5, the relationship vanishes under non-equilibrium conditions and in softwaters with a high content of humic acids and free mineral acids (Stumm and Morgan, 1981). As measures of lake trophic state, concentrations of total nitrogen and total phosphorus were used in accordance with most recommendations (e.g. Vollenweider, 1968). Finally, chlorophyll concentration was applied as a measure of phytoplankton biomass and Secchi-depth as a measure of light availability to submerged macrophytes. A close linear relationship was observed between maximum depth of macrophyte growth (zmax) and Secchi-depth (Sd) in a subset of 61 Danish lakes $(z_{\text{max}}=0.29+1.18 \text{ Sd}, r^2=0.85, p<0.001;$ Vestergaard, 1998), allowing the existence of different species in vertical depth zones of shifting light availability, sediment type and physical exposure.

The objective of the study was to find the main patterns in the distribution of submerged macrophytes in a large number of Danish lakes and evaluate the relationships between species distribution and environmental conditions by multivariate analysis techniques. Subsequently, we evaluated the morphological and eco-physiological background for the observed distribution patterns.

2. Materials and methods

2.1. Regional setting and study sites

Denmark is a small lowland country in NW Europe. The mostly agricultural landscape is flat and open. About 15% is covered by forests or other natural types of vegetation. Soil types range from carbonate-rich moraine soils in the eastern part to carbonate-poor sandy soils in the western part of the country. Most lakes have medium to highly alkaline waters, but soft-water lakes are present as well. Some small soft-water lakes are acidic due to the influence of surrounding vegetation (i.e. conifer plantations or *Sphagnum*-bogs) and acid precipitation (Grahn, 1977; Nilssen, 1980; Riis and Sand-Jensen, 1998).

2.2. Vegetation data

Information on vegetation and environmental conditions was compiled from studies in 82 Danish lakes performed by universities and counties between 1983 and 1994. Aquatic vegetation was studied by wading, by boat using a rake or a water telescope or by Scuba diving. The abundance of macrophytes was studied in 31 of the lakes along several transects from the shore to the maximum depth of growth. In some lakes the littoral zone was divided

into about 10 sections along the periphery of the lake which were then studied seperately. In the remaining lakes, macrophyte species were searched throughout the littoral zone using a combination of direct observations by a water telescope and collection of plant samples from deep water with a rake. In order to include data on plant distribution from all 82 lakes, vegetation data was reduced to the presence or absence of species in the lakes in accordance with similar comparative macrophyte studies (Iversen, 1929; Lohammar, 1938; Spence, 1967; Seddon, 1972; Pip, 1979; Hellquist, 1980; Jackson and Charles, 1988; Kunii, 1991).

Macrophyte species were divided into six major plant groups based on differences in taxonomy and growth form: vascular plants (elodeids, isoetids, lemnids or helophytes), bryophytes and characeans (sensu Iversen, 1936). Only submerged species of elodeids, isoetids, bryophytes and characeans were included in the analysis because their distribution is likely to be strongly influenced by lake morphometry, transparency and chemical variables. Species of the elodeid growth form have leaves on erect stems of variable length (approx. 0.3–3 m), and they usually grow attached to the sediment by roots or rhizomes, though a few free-floating species (e.g. *Ceratophyllum* sp., *Lemna trisulca* and *Utricularia* sp.) were also included. Species of the isoetid growth form have leaves located in a rosette on a short stem, and most species are small with well-developed roots (e.g. *Lobelia dortmanna*). Bryophytes and characeans only included attached species. Species were identified according to Moeslund et al. (1990) which follows the nomenclature of Flora Europaea.

2.3. Environmental data

Data on environmental conditions in the lakes included location, surface area, mean and maximum water depth, alkalinity, pH, and the concentration of total phosphorus, total nitrogen and chlorophyll and Secchi-depth. Water sampling was carried out in accordance with Kristensen et al. (1990), and chemical analysis in accordance with Rebsdorf et al. (1988). In most of the lakes, limnological variables were measured monthly from early May to late September, but in some lakes measurements were restricted to 2–3 times between June and August. Measurements of water chemistry were done on integrated samples from the epilimnion taken in the middle of the lake. For each lake, a mean value was calculated based on all measurements. Unfortunately, the full set of environmental variables was not available in 31 of the lakes. Therefore, only 51 lakes could be included in the multivariate analysis of species distribution in relation to lake environment.

2.4. Data analysis

Relative occurrence of individual species was calculated based on their presence or absence in the 82 lakes. For each species, median values and mean values \pm S.E. of alkalinity and chlorophyll were calculated in the colonised lakes.

Multivariate analyses were used to describe patterns in species distribution among the lakes, and to determine how the overall species distribution was related to physical-chemical variables. Similarity in species composition among lakes was calculated as Bray-Curtis' similarity coefficients in cluster analyses (Clarke and Warwick, 1994), and indirect ordinations were carried out using detrended correspondence analysis (DCA-ordination; Hill and Gauch, 1980) based on the presence/absence of elodeids and isoetids in the 51 lakes.

A non-metric multidimensional scaling analysis (NMDS-ordination; Field et al., 1982; Clarke and Warwick, 1994) was also performed, although the NMDS-ordination is not presented or discussed any further because it yielded closely similar results to those of the DCA-analysis. With these procedures, patterns in species distribution were analysed independently of environmental factors. Subsequently, the variation in species composition, identified by the DCA-ordination, was correlated to the environmental variables to identify relationships between species distribution and the environment. Thus, Spearman rank correlation coefficients were calculated (i) between the environmental variables and the DCA-ordination-axes, and (ii) between subsets of environmental variables and ranked values of species similarity (performed by the BIOENV-procedure as described by Clarke and Ainsworth (1993)). By DCA-ordination, variation in species composition is expressed first along the single strongest gradient among the lakes and, subsequently, along the other gradients in decreasing order of importance.

With the DCA-technique the ecological interpretation of species distribution was performed indirectly, and the analysis was therefore objective in the sense that environmental factors did not direct the analyses towards specific well-known traits, but allowed patterns in species distribution to be fully expressed and evaluated. This procedure was favourable, since the purpose was to identify and evaluate all possible patterns in species distribution.

3. Results

3.1. Lake environment

The Danish lakes included in the analysis vary in surface area from 0.007 to 17.3 km^2 and in mean depth from 0.3 to 15.0 m among the lakes included in the multivariate analysis (Table 1). The lakes are dispersed throughout Denmark and include a wide variety of soil geology and water chemistry. Alkalinity ranged between 0.04 and 3.35 meq l⁻¹ and summer pH between 4.4 and 10.2. Concentration of total phosphorus and total nitrogen varied from low-medium in most lakes to high in some lakes (Table 1). Also the biomass of phyto-

Table 1

Median, minimum and maximum values of environmental variables for 51 Danish lakes included in the multivariate analysis^a

	Median	Minimum	Maximum
Area (km ²)	0.33	0.007	17.3
Maximum depth (m)	5.1	0.7	37.7
Mean depth (m)	1.9	0.3	15.0
Total phosphorus $(mg l^{-1})$	0.06	0.01	0.47
Total nitrogen (mg l^{-1})	0.96	0.08	4.61
Chlorophyll ($\mu g l^{-1}$)	8.03	1.20	200.00
Secchi-depth (m)	1.8	0.2	7.0
Alkalinity (meq l^{-1})	1.12	-0.04	3.35
pH	7.9	4.4	10.2

^a Mean values for each lake were calculated for total phosphorous, total nitrogen, chlorophyll, Secchi-depth, pH and alkalinity over the period 1 May–30 September.

plankton (1.2–200 μ g Chlorophyll 1⁻¹) and Secchi-depth (0.2–7.0 m) varied substantially (Table 1). Thus, the whole group of lakes represented a wide gradient from oligotrophic to eutrophic, from low alkaline to high alkaline and from low to high pH conditions.

3.2. Macrophyte species and their abundance

A total of 106 species of submerged macrophytes (including five varieties) was found in the 82 Danish lakes (Table 2). *Potamogeton pectinatus* was the most widespread species, *Elodea canadensis* was the second and *P. perfoliatus* the third most abundant species. Overall, species of the elodeid growth form were the most common. The abundance of elodeids and isoetids both followed an approximately linear course, when the logarithm of their relative abundance among all the lakes (y) were listed against their rank (x) (elodeids: $\log y=-0.06x-1.077$, $r^2=0.98$; isoetids: $\log y=-0.25x-0.58$, $r^2=0.96$). Many species were relatively rare as 53 grew in less than 5% of the lakes.

Mean number of species (\pm S.E.) within each lake was 7.0 \pm 0.6 elodeids, 1.8 \pm 0.3 isoetids, 1.3 \pm 0.2 characeans and 0.8 \pm 0.1 bryophytes. These figures, however, covered a substantial variation in the distribution of different growth form and species among the lakes. Some lakes included up to 25 species of elodeids and only a few isoetids, while other lakes included 5–6 species of isoetids and only a few elodeids.

3.3. Cluster analysis of species composition

Bray–Curtis' coefficients of similarity in the distribution of single species of elodeids and isoetids among the 51 lakes divided the species into two major groups (Fig. 1). The first group represented species of elodeids such as *Batrachium circinatum*, *E. canadensis*, *Myriophyllum spicatum*, *P. crispus*, *P. pectinatus* and *P. perfoliatus*. These specific species had a mutual Bray–Curtis similarity in their distribution of about 70%, implying that if *B. circinatum* was present in a given lake, it was very likely that the other five species were present as well. The other group of species represented elodeids such as *M. alterniflorum* and *P. gramineus* and isoetids such as *Littorella uniflora*, *Lobelia dortmanna* and *Juncus bulbosus*. The three isoetid species had a mutual similarity of about 90%, showing that they were almost always present in the same lakes.

Coefficients of similarity in the total composition of elodeids and isoetids among the lakes showed a similar pattern. At a similarity level on 46% it was possible to distinguish five groups of lakes, each with a very similar composition of species (Fig. 2). Three individual lakes, though, had a species composition that was very different from any other lakes, and were therefore not included in the final ordination in accordance with the recommendation of Kent and Coker (1992). Even the simple measure of the total number of elodeids, isoetids, characeans and bryophytes in each lake revealed distinct differences among the lakes (Fig. 3). Lakes within Group 1 only contained a few species and particularly elodeids. Lakes within Group 2 contained a higher number of elodeids and several characeans. Lakes within Group 3 were rich in elodeids and characeans, and a number of the lakes also included several isoetids. Within Group 4 the number of elodeids decreased, while several isoetids appeared. Lakes within Group 5 contained several isoetids and bryophytes, but very few elodeids.

Table 2
Species of elodeids, isoetids, characeans and bryophytes (submerged forms) listed in decreasing order of occurrence in 82 Danish lakes ^a

	Abundance	Alkalinity	Chl. a		Abundance	Alkalinity	Chl. a
	(%)	$(meq l^{-1})$	$(\mu g l^{-1})$		(%)	$(meq l^{-1})$	$(\mu g l^{-1})$
Elodeids							
Potamogeton pectinatus L.	50.0	1.50	21.70	Zannichellia pedunculata Rchb.	8.5	1.50	22.50
Elodea canadensis Michx.	39.0	1.30	10.20	Utricularia australis R. Br.	7.3	1.14	9.57
Potamogeton perfoliatus L.	39.0	1.30	16.85	Callitriche hermaphroditica L.	6.1	1.20	21.85
Batrachium circinatum Spach.	37.8	1.50	14.25	Myriophyllum verticillatum L.	6.1	1.50	10.20
Myriophyllum spicatum L.	37.8	1.50	19.85	Potamogeton alpinus Balb	6.1	0.83	8.85
Potamogeton crispus L.	37.8	1.50	17.00	Potamogeton zosterifolius Schum.	6.1	0.94	42.00
Potamogeton obtusifolius M. & K.	37.8	0.99	11.85	Batrachium trichophyllum Chaix	4.9	0.70	_
Potamogeton berchtoldii Fieb	34.1	0.99	13.35	Callittriche stagnalis Scop.	4.9	1.12	_
Ceratophyllum demersum L.	26.8	1.67	10.20	Zannichellia major Boenn.	4.9	1.85	30.50
Sparganium emersum Rehman	26.8	0.83	16.50	Batrachium hederaceum (L.) Gray	3.7	0.31	23.35
Potamogeton pusillus L.	22.0	1.12	19.85	Batrachium sp.	3.7	0.15	15.00
Callitriche platycarpa Kütz.	18.3	0.99	22.00	Ceratophyllum submersum L.	3.7	2.61	42.00
Lemna trisulca L.	18.3	1.01	11.40	Potamogeton x nitens Weber	3.7	1.01	12.00
Myriophyllum alterniflorum DC.	17.1	0.46	5.70	Najas flexilis (Willd.) R. & S.	2.4	0.85	3.95
Zannichellia repens Boenn.	15.9	1.50	22.00	Potamogeton acutifolius Link	2.4	1.49	_
Helosciadium inundatum (L.) Koch	14.6	0.40	7.20	Potamogeton rutilus Wolfg.	2.4	0.83	_
Callitriche hamulata Kütz	13.4	0.58	3.06	Ruppia maritima L.	2.4	1.01	22.00
Potamogeton gramineus L.	13.4	0.60	6.70	Sagittaria sagittifolia L.	2.4	1.77	15.79
Sparganium angustifolium Michx.	13.4	0.09	3.70	Scirpus fluitans L.	2.4	0.70	6.80
Hottonia palustris L.	11.0	0.99	_	Utricularia minor L.	2.4	0.38	4.35
Potamogeton lucens L.	11.0	2.09	42.00	Utricularia vulgaris L.	2.4	0.51	6.80
Batrachium aquatile L.	9.8	0.75	4.75	Callitriche pedunculata DC.	1.2	0.24	_
Hippuris vulgaris L.	9.8	1.12	16.50	Callitriche sp.	1.2	0.83	_
Potamogeton filiformis Pers	9.8	1.30	10.79	Luronium natans (L.) Raf.	1.2	1.01	22.00
Potamogeton praelongus Wulf.	9.8	0.83	4.00	Najas marina L.	1.2	2.53	9.57
Sparganium minimum Wallr.	9.8	1.01	19.25	Potamogeton x zizii Mert. & Koch	1.2	1.16	2.00
Batrachium baudotti Godr.	8.5	1.40	21.70	Ruppia cirrhosa (Petagna) Grande	1.2	1.60	43.00
Batrachium peltatum Schrank	8.5	0.62	6.80	Stratiotes aloides L.	1.2	1.77	-
Potamogeton friesii Rupr.	8.5	1.01	6.70	Utricularia ochroleuca R. Hartm.	1.2	0.83	-

Table 2 (Continued)

	Abundance (%)	Alkalinity (meq 1 ⁻¹)	Chl. a (µg1 ⁻¹)		Abundance (%)	Alkalinity (meq 1 ⁻¹)	Chl. a (μ g1 ⁻¹)
Characeans			· ·	Isoetids			
Chara globularis Thuill.	20.7	1.33	9.57	Juncus bulbosus L.	37.8	0.15	5.45
Nitella flexilis (L.) Agardh	18.3	0.60	6.80	Littorella uniflora (L.) Aschers	37.8	0.16	5.45
Chara sp.	14.6	1.81	12.40	Lobelia dortmanna L.	28.0	0.10	5.20
Chara aspera Deth. Ex Willd.	13.4	1.70	16.40	Isoëtes lacustris L.	15.9	0.10	5.00
Chara globularis Thuillier	9.8	0.85	19.50	Eleocharis acicularis (L.) R. & S.	14.6	1.12	15.95
Chara vulgaris v. papillata Wallroth	4.9	0.70	_	Ranunculus flammula L.	13.4	0.15	5.00
Nitella sp.	4.9	0.49	10.00	Isoëtes echinospora Durieu	11.0	0.09	3.38
Nitellopsis obtusa (Desvaux) Groves	4.9	2.23	12.19	Elatine hexandra (Lapierre) DC.	8.5	0.13	4.70
Chara canescens Loisel	3.7	1.50	44.00	Ranunculus reptans L.	6.1	1.01	7.50
Chara tomentosa L.	3.7	1.92	9.57	Pilularia globulifera L.	4.9	0.35	5.45
Chara vulgaris (L.) Vaillant	3.7	2.06	21.70	Elatine hydropiber L.	3.7	1.12	_
Chara vulgaris v. contraria Wood	3.7	1.40	8.14	Scirpus parvulus Roemer & schult.	3.7	1.50	44.00
Tolypella nidifica Wood	3.7	1.50	32.50	Subularia aquatica L.	1.2	0.83	
Chara contraria A.Br. ex Kütz	2.4	2.20	12.13				
Chara globularis v. virgata Wood	2.4	0.90	14.40	Bryophytes submerged			
Chara hispida L.	2.4	2.46	16.40	Fontinalis antipyretica Hedw.	28.0	0.66	10.00
Chara vulgaris v. longibracteata Kütz	2.4	0.60	_	Sphagnum subsecundum Nees.	14.6	0.04	4.95
Nitella tenuissima (Desvaux) C. & G.	2.4	0.70	6.80	Drepanocladus sp.	13.4	0.06	5.25
Nitella translucens (Persoon) Agardh.	2.4	0.60	5.60	Sphagnum sp.	8.5	0.22	5.25
Tolypella glomerata (Desv.) v. Leonh.	2.4	0.81	_	Riccardia sinuata Dick	3.7	0.08	5.50
Chara connivens Salzm.	1.2	1.10	22.00	Chiloscyphus fragilis Roth.	2.4	0.06	5.05
Chara denudata A.Br.	1.2	2.30	3.13	Drepanocladus exannulatus Warnst	2.4	0.06	5.05
Chara fragilis Desvaux in Loisel.	1.2	1.92	18.00	Drepanocladus fluitans (Hedw.) War	. 2.4	0.01	5.67
Chara rudis v. Leonh.	1.2	1.92	18.00				
Chara vulgaris v. denudata A.Br	1.2	1.40	6.70				
Lamprothamnium papulosum Groves	1.2	1.70	43.00				
Nitella mucronata (A.Br.) Wood	1.2	0.88	-				

^a For each species median values of alkalinity and concentration of chlorophyll are calculated for the lakes in which they occur.



Fig. 1. Distribution of 33 species of elodeids and isoetids in 51 Danish lakes based on Bray–Curtis similarity in species composition among the lakes. Only species with an occurrence in at least five lakes are included.

In addition to changes in the abundance of different plant growth forms between the five groups of lakes, there was a pronounced shift in the distribution of individual species, particularly among elodeids (Table 3). Thus, *M. spicatum* and *P. pectinatus* dominated the lakes in Group 1. Within Group 2 also *C. demersum*, *E. canadensis* and *P. crispus* were abundant. Within Group 3 there was a more diverse range of species, and species such as *P. berchtoldii* and *P. filiformis* reached their highest abundance in these lakes. Many elodeid species were absent from Group 4, but *M. alterniflorum* and *P. obtusifolius* reached their highest abundance here. All species of isoetids were represented in Groups 4 and 5, whereas only a few widely distributed isoetids (e.g. *Littorella uniflora*) were present in Group 3. Consequently, there were major differences in species richness and the distribution of different growth forms and species among the lakes.

Alkalinity also varied significantly among the five groups of lakes (Fig. 3, ANOVA, p < 0.001). Mean concentration of bicarbonate decreased from 2.1 meq1⁻¹ in Groups 1 and 2, to 1.6 meq1⁻¹ in Group 3, 0.4 meq1⁻¹ in Group 4, and to 0.08 meq1⁻¹ in Group 5. Mean summer pH declined along with the concentration of bicarbonate from pH>8.2



Fig. 2. Bray–Curtis similarity in the species composition of elodeids and isoetids in 49 Danish lakes. Based on a Bray–Curtis similarity of 46% (the dotted line), five main groups of lakes and three deviating individual lakes have been defined.

within Groups 1–3, pH 7.5 in Group 4 to pH 5.5 in Group 5. Concentrations of chlorophyll were very high in lakes within Group 1, but low or intermediate in the remaining lakes, while Secchi-depth varied inversely with the chlorophyll concentration. The composition of macrophyte species was consequently closely related to the concentration of bicarbonate in the lakes, while the relationship to the concentration of chlorophyll was less strong (Table 4).



Fig. 3. Number of species of elodeids, isoetids, bryophytes and characeans in 46 Danish lakes. The lakes are listed in five distinct lake groups based on Bray–Curtis similarity in species composition among the lakes (viz Fig. 2). Alkalinity, pH and concentration of chlorophyll are shown for each lake.

3.4. Ordination of species composition

DCA-ordination of the composition of species of elodeids and isoetids also expressed a significant change in species composition among the lakes. Thus, there was a characteristic distribution of the lakes along DCA-axis 1 (Fig. 4) with lakes dominated by elodeids at one end (e.g. Fure Sø and Esrum Sø, compare with Fig. 3), lakes dominated by mostly isoetids at the other end (e.g. Grane Langsø and Kalgård Sø), and lakes with representatives of both elodeids and isoetids in between (e.g. Almind Sø). The long gradient on DCA-axis 1 of 4.33 S.D. ('average standard deviation of species turnover', Gauch, 1982) reflected a complete shift in species composition between lakes located at opposite ends of the

			-		
	Group 1 (<i>n</i> =7)	Group 2 (<i>n</i> =7)	Group 3 (<i>n</i> =9)	Group 4 (<i>n</i> =7)	Group 5 (<i>n</i> =16)
Alkalinity (meq l ⁻¹)	2.00±0.3	2.22 ± 0.2	$1.65 {\pm} 0.2$	$0.44{\pm}0.1$	$0.08{\pm}0.0$
pH	$8.88 {\pm} 0.3$	$8.58 {\pm} 0.2$	8.22 ± 0.1	7.51 ± 0.3	5.46 ± 0.2
Secchi-depth (m)	$0.8{\pm}0.2$	$2.3{\pm}0.4$	$2.7{\pm}0.7$	$3.7{\pm}0.6$	$3.3{\pm}0.6$
Chlorophyll ($\mu g l^{-1}$)	93.53±24.2	25.21 ± 9.5	18.22 ± 6.4	$4.11 {\pm} 0.9$	$8.63 {\pm} 2.6$
Total-N (mg l^{-1})	$2.17{\pm}0.4$	$1.14{\pm}0.2$	$1.60{\pm}0.5$	0.37 ± 0.1	$0.68 {\pm} 0.1$
Total-P (mg l^{-1})	$0.16{\pm}0.1$	$0.11{\pm}0.0$	$0.06{\pm}0.0$	0.04 ± 0.0	$0.05{\pm}0.0$
Area (km ²)	$2.5{\pm}1.7$	$4.1{\pm}2.5$	$3.5{\pm}1.8$	0.5 ± 0.1	$0.2{\pm}0.1$
Mean depth (m)	$3.3{\pm}1.0$	$6.4{\pm}1.9$	$6.1{\pm}1.8$	$3.1{\pm}1.3$	$1.8{\pm}0.5$
Maximum depth (m)	7.3 ± 1.7	$14.1{\pm}4.9$	$12.5 {\pm} 4.0$	$7.2{\pm}2.7$	$4.2{\pm}1.0$
Relative occurrence	1.00 P. pectinatus	1.00 E. canadensis	1.00 B. circinatum	1.00 J. bulbosus	1.00 J. bulbosus
	0.86 M. spicatum	1.00 P. pectinatus	1.00 E. canadensis	1.00 L. uniflora	1.00 L. uniflora
	0.71 P. perfoliatus	0.86 B. circinatum	1.00 M. spicatum	1.00 L. dortmanna	0.94 L. dortmanna
	0.57 P. crispus	0.86 C. demersum	1.00 P. pectinatus	1.00 M. alterniflorum	0.69 S. subsecundum
	0.29 P. lucens	0.86 P. crispus	0.89 P. perfoliatus	0.86 P. gramineus	0.56 I. lacustri
	0.29 F. antipyretica	0.43 M. spicatum	0.78 P. crispus	0.86 P. obtusifolius	0.50 S. angustifolium

0.67 P. filiformis

0.67 P. berchtoldii

0.67 C. globularis

0.56 H. vulgaris

0.56 L. uniflora

0.56 P. pusillus

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0.86 P. perfoliatus

0.57 E. canadensis

0.57 F. antipyretica

0.57 B. aquatile

0.57 I. lacustris

0.57 N. flexilis

0.57 R. flammula

0.43 P. berchtoldii

0.43 P. perfoliatus

0.43 C. globularis

0.43 P. lucens

0.43 Z. repens

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0.14 B. circinatum

0.14 C. demersum

0.14 P. obtusifolius

0.14 Z. repens

0.14 Chara sp.

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Table 3 Mean values±S.E. of environmental variables and the most abundant species in decreasing order of occurrence in each of the five lake groups (compare with Fig. 2)

0.44 I. echinospora

0.19 M. alterniflorum

0.38 R. flammula

0.31 E. hexandra

0.19 R. sinuata

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able 4
pearman rank correlation coefficients between DCA-ordination axis 1, axis 2, axis 3 and axis 4 and the environmental variables for each of the lakes

	DCA- axis 1	DCA- axis 2	DCA- axis 3	DCA- axis 4	Alkalinity	pН	Chl. a	Total-N	Total-P	Secchi- depth	Area	Mean depth	Maximum depth
Alkalinity	-0.91***	-0.24	-0.05	0.14									
pH	-0.80^{***}	-0.24	-0.08	0.19	0.81^{***}								
Chlorophyll	-0.64^{***}	0.03	-0.26	0.20	0.57***	0.67 ***							
Total-N	-0.46^{**}	0.04	-0.30^{*}	0.13	0.51***	0.49 ***	0.73***						
Total-P	-0.42^{**}	-0.10	-0.09	0.11	0.32*	0.44**	0.72***	0.49 ***					
Secchi-depth	0.30	0.09	0.31	-0.21	-0.18	-0.31	-0.73***	-0.60^{***}	-0.63***				
Area	-0.62^{***}	0.03	-0.09	0.36*	0.67 ***	0.66 ***	0.51 ***	0.29^{*}	0.31 *	-0.09			
Mean depth	-0.40^{**}	-0.07	-0.07	0.22	0.51***	0.44**	0.14	0.05	-0.20	0.65***	0.50***		
Maximum depth	-0.35^{*}	-0.06	-0.14	0.22	0.47**	0.39 **	0.16	0.07	-0.16	0.62***	0.49***	0.96***	
Longitude	-0.67^{***}	0.03	-0.01	-0.23	0.68***	0.52 ***	0.34*	0.20	0.08	0.28	0.36*	0.62***	0.57***
Latitude	0.59***	-0.17	0.11	0.14	-0.58^{***}	-0.43**	-0.42^{**}	-0.35^{*}	-0.10	0.01	-0.36^{*}	-0.46^{**}	-0.45^{**}

p*<0.05, *p*<0.01 and ****p*<0.001.



Fig. 4. DCA-ordination of 49 Danish lakes based on the occurrence of elodeids and isoetids in each lake (Eigenvalues: DCA-axis 1: 0.70, DCA-axis 2: 0.25, DCA-axis 3: 0.17, DCA-axis 4: 0.14, total: 4.26. Gradient length: DCA-axis 1: 4.33, DCA-axis 2: 2.71, DCA-axis 3: 2.28, DCA-axis 4: 2.13).

gradient, and a high eigenvalue of 0.70 indicated that a substantial part of the total variation in species composition among the lakes was expressed along axis 1 (Gauch, 1982). Most of the remaining variation was reflected by DCA-axis 2 with an eigenvalue of 0.25.

3.5. Species composition and lake environment

There was a very high Spearman rank correlation between alkalinity and DCA-axis 1 (r=-0.91, Table 4), indicating a strong relation between alkalinity and the species composition of the different lakes. Also, there was a high correlation between species composition and pH (r=-0.80, Table 4). Geographical location, lake surface area and the concentration of chlorophyll of the lakes explained a lesser part of the variation in species composition, and the concentration of total phosphorus and nitrogen, mean depth and maximum depth explained a relatively small part of the variation. A high intercorrelation existed between alkalinity and pH and between alkalinity and geographical location of the lakes (Table 4). These intercorrelations reflect that pH is largely controlled by the bicarbonate concentration, which varies among geographical regions of different soil type. That alkalinity and pH also had the strongest correlation with DCA-axis 2 of the environmental variables implies that most of the variation in species composition, which was not expressed along DCA-axis 1, was also explained by differences in alkalinity among the lakes.

The distribution of individual macrophyte species differed markedly among the lakes in relation to alkalinity indicated by differences of mean alkalinity for each species (Fig. 5).



Fig. 5. Mean, minimum and maximum values of alkalinity (meq l^{-1}) for 33 species of elodeids and isoetids for the lakes in which they occur. Only species with an occurrence in at least five lakes are included.

Among common species of elodeids *B. circinatum*, *C. demersum*, *M. spicatum*, *P. lucens* and *P. pectinatus* mainly grew in lakes with alkalinities above $1.0 \text{ meq } 1^{-1}$. *M. alterniflorum* was restricted mainly to lakes with low bicarbonate concentrations. Among the isoetids *Lobelia dortmanna* exclusively grew at low bicarbonate concentrations, while *Littorella uniflora* was also found in lakes of moderate concentrations.

The influence of light on species composition is indicated by the correlation coefficient between DCA-axis 1 and chlorophyll (r=-0.64, Table 4) and a decline in species number from several elodeids and some isoetids in lakes of Group 3 to fewer elodeids and no isoetids in Groups 1 and 2. The total number of species declined from 38 in Group 3, 19 in Group

2 and to 9 species in Group 1 along with a significant decline of mean Secchi-depth from 2.7 to 0.8 m among the three lake groups (Table 3, ANOVA, p < 0.05). Also, there was a change from a high relative occurrence of several species in Group 3 to only a few species with a high occurrence and mostly species of low relative occurrence in Group 1 (Table 3), and species composition changed from a diverse range of species (e.g. *B. circinatum*, *P. filiformis*, *P. berchtoldii*, *P. pusillus* and *Littorella uniflora*) to mainly *P. pectinatus*, *M. spicatum* and *P. perfoliatus* (Table 3).

3.6. Influence of distance between lakes

Similarity of species composition between pairs of lakes is shown as a function of their mutual geographical distance for three alkalinity groups (0–0.5, 0.5–2.0 and >2.0 meq 1^{-1} , Fig. 6). Low-alkaline lakes are restricted to the western part of Denmark, while lakes of intermediate and high alkalinity are distributed throughout the country. There was no relationship between Bray–Curtis similarity and the distance between lakes of low and intermediate alkalinity, but there was a very small, though significant negative



Fig. 6. Bray–Curtis similarity in species composition among Danish lakes as a function of the distance among the lakes. The lakes are divided into three levels of alkalinity: 0-0.5, 0.5-2.0 and >2.5meq 1^{-1} .

lakes. Some lakes with a mutual distance of 160 km between them have a Bray–Curtis similarity as high as 90%, while some very closely located lakes have no species in common.

4. Discussion

4.1. Distribution patterns relative to environmental variables

Submerged vegetation in Danish lakes includes many species of elodeids and fewer species of isoetids, characeans and bryophytes as is the situation in most other temperate countries and regions (Hutchinson, 1975). Several previous comparative studies have shown a strong influence of water chemistry on the distribution of these submerged species and growth forms (Spence, 1967; Seddon, 1972; Hutchinson, 1975; Catling et al., 1986; Jackson and Charles, 1988). Duarte and Kalff (1990) demonstrated that water chemical parameters control the presence or absence of species among lakes, while physical variables such as water depth, littoral slope and sediment composition are important for the site-specific distribution of species within each lake.

This study confirmed the strong correlation between species composition, alkalinity and pH, while the correlation was weaker to other environmental variables such as nutrients, water transparency and phytoplankton biomass. Also lake size, depth, geographical location and distance between lakes have little influence on species composition when differences in alkalinity are accounted for. Therefore, the result reflects a close relationship between species distribution and the concentration of bicarbonate, as bicarbonate (plus carbonate in a few lakes of high pH) is responsible for more than 95% of the alkalinity in most Danish lakes as well as other lakes worldwide (Stumm and Morgan, 1981). Species of different growth forms have a relatively distinct distribution relative to alkalinity in the lake waters (Figs. 3 and 5). Elodeids dominate the vegetation at high alkalinity and isoetids at low alkalinity, while Danish lakes of intermediate alkalinity have several representatives of both elodeids and isoetids (Fig. 3).

The results support previous studies showing clear relationships between species distribution of macrophytes and either pH (Iversen, 1929; Catling et al., 1986; Arts et al., 1990a), alkalinity (Moyle, 1945; Hellquist, 1980; Jackson and Charles, 1988), conductivity, hardness ratio or the sum of cations (Spence, 1967; Seddon, 1972; Kunii, 1991; Srivastava et al., 1995; Toivonen and Huttunen, 1995). Among these variables, alkalinity, and thus bicarbonate, should be considered the main determinant because bicarbonate concentrations are closely and linearly related to conductivity in most types of freshwaters, and pH is likewise closely regulated by the concentration of bicarbonate across the range from low-alkaline to high-alkaline lakes (Leuven et al., 1992). The present study has, however, some advantages relative to previous studies in that, (i) the dataset was rather comprehensive in terms of the number of lakes and the range of environmental variables, (ii) patterns in species distribution and relations to environmental variables were evaluated with an objective method, and (iii) distinct distribution patterns could be established for a large number of common macrophyte species as a function of alkalinity/bicarbonate concentration (Fig. 5).

4.2. Why and how is bicarbonate important for elodeids

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The strong relationship between species distribution and bicarbonate concentration suggests a direct influence of bicarbonate on photosynthesis, growth and long-term survival of aquatic plants. Many experiments have documented the ability of most characeans and elodeid species to utilise bicarbonate as a carbon source for photosynthesis, while most mosses and isoetids are unable to utilise bicarbonate, thereby restricting their carbon source to free carbon dioxide (Steemann-Nielsen, 1947, 1960; Allen and Spence, 1981; Maberly and Spence, 1983; Sand-Jensen and Gordon, 1984; Prins and Elzenga, 1989; Madsen and Sand-Jensen, 1991). A long-term stimulation of growth rates of elodeids by bicarbonate and elevated inorganic carbon concentrations (DIC) in the water has been shown in laboratory cultures and field experiments (Madsen and Sand-Jensen, 1987, 1994; Vadstrup and Madsen, 1995). Thus, the different modes of carbon use between different growth forms and taxonomic groups have clear implications for their distribution in lakes of different bicarbonate concentrations (e.g. Hutchinson, 1975; Spence and Maberly, 1985). When it comes to the distribution of individual species, however, relationships between performance in short physiological experiments and natural distribution are much weaker. The ability to use bicarbonate and the affinity for bicarbonate vary among species with the same growth form, and within each species physiological acclimation is closely influenced by carbon availability, light, nutrients and temperature conditions in the natural growth environment (Maberly and Spence, 1983; Sand-Jensen and Gordon, 1984, 1986; Madsen and Sand-Jensen, 1991).

The extraction capacity for inorganic carbon, measured as the ratio of final total inorganic carbon to initial alkalinity (DIC/Alk) in pH-drift experiments, can be used as an index of bicarbonate affinity (Maberly and Spence, 1983). Thus, efficient bicarbonate users have ratios below 0.5 and non-users have ratios close to 1.0 (Maberly and Spence, 1983). Comparison of measurements of DIC/Alk ratios from the literature (Maberly and Spence, 1983; Madsen and Sand-Jensen, 1991) and the median bicarbonate concentration derived from the frequency distribution of the species in the Danish lakes studied here show a negative relationship with a relatively wide data scatter. The pattern mainly emerges, because isoetid species (DIC/Alk ratios: ~ 1.0) predominantly grow in lakes of low bicarbonate concentrations, whereas elodeid species with variable bicarbonate affinity are widely distributed in lakes ranging from medium to high bicarbonate concentrations. Nonetheless, some differences exist between efficient bicarbonate-users such as E. canadensis, M. spicatum, P. pectinatus, P. perfoliatus and Zannichellia palustris (DIC/Alk ratios: 0.24-0.50) having a wide distribution in alkaline lakes, and less efficient bicarbonate-users such as *B. aquatile*, M. alterniflorum and P. praelongus (DIC/Alk: 0.62–0.86) growing predominantly in lakes of low to moderate alkalinity (Fig. 5).

Bicarbonate can be used in photosynthesis as a supplement to free carbon dioxide which is used more readily for the same external concentration (Madsen et al., 1996). The importance of bicarbonate for photosynthesis and plant growth, therefore, depends on the absolute concentrations and concentration ratios of bicarbonate to carbon dioxide in the water as well as on carbon requirements of the plants (Sand-Jensen and Gordon, 1986). A substantial intraspecific variability of the carbon extraction capacity has been observed for *E. canadensis* and *M. alterniflorum* retrieved from different sites in lakes and streams (Sand-Jensen and Gordon, 1986; Madsen et al., 1996). These findings can account for the relatively weak relationship between mean carbon extraction capacity of the species observed in photosynthesis experiments and the median alkalinity derived from their frequency distribution among lakes.

4.3. Why are isoetids common in low-alkaline lakes

Isoetid species such as *Isoetes lacustris*, *Littorella uniflora* and *Lobelia dortmanna* are widespread and dominant in low-alkaline Danish lakes. The same pattern has been reported from other countries in northern Europe (Lohammer, 1938; Seddon, 1965; Jensén, 1979; Farmer and Spence, 1986). Isoetid species are unable to use bicarbonate, but the three mentioned species, and probably several others with the same growth form and anatomy, are capable of using sediment carbon dioxide as a main carbon source for photosynthesis (Wium-Andersen, 1971; Søndergaard and Sand-Jensen, 1979; Richardson et al., 1984; Sand-Jensen, 1987), which makes them photosynthetically independent of the bicarbonate concentration in the water.

The limited occurrence of isoetids in alkaline lakes is likely to be due to intensive competition for light and space from elodeids benefiting from the supply of bicarbonate for photosynthesis (Seddon, 1965; Farmer and Spence, 1986). Elodeids are capable of developing a leaf canopy in the water column (Adams et al., 1974; Bijl et al., 1989) and thereby tolerate greater light attenuation by phytoplankton and reduce the light availability for isoetids with leaves located close to the sediment. When isoetids occur in alkaline lakes, they are often restricted to shallow, wave-exposed sites with coarse sediments, which several isoetid species can tolerate better than elodeids and emergent helophytes (Seddon, 1965; Farmer and Spence, 1986; Sand-Jensen and Søndergaard, 1997). Among the isoetids, *Littorella uniflora* is apparently able to grow in Danish and Scottish lakes of higher alkalinity than *Lobelia dortmanna* or *I. lacustris* (Fig. 4; Farmer and Spence, 1986).

4.4. Acid lakes

Acid lakes of low alkalinity (lakes with pH<5.0 in Group 5, Fig. 2) are generally poor in submerged species, as the species-rich group of elodeids has only one representative as an average and a total of only five species in these lakes. In contrast, isoetid species are common in the acid lakes with *J. bulbosus* and *Littorella uniflora* being the most abundant species. Among the vascular plants, *J. bulbosus* is particularly known for its ability to grow in very acidic waters in pits and streams from lignite mining areas with pH levels of only 2–3 (Ohle, 1936; Sand-Jensen and Rasmussen, 1978; Roelofs, 1983). Bryophytes of the genera *Drepanocladus* and *Spaghnum* are also common, and their abundance often increases relative to isoetid species under very acidic conditions, apparently because bryophytes are more tolerant than isoetids to low pH (Grahn et al., 1974; Wetzel, 1983). Overall, the observed patterns agree with other studies elsewhere in northern temperate regions (Jackson and Charles, 1988; Farmer, 1990; Arts et al., 1990a) and with observations of progressive changes in the vegetation accompanying anthropogenic acidification of the lakes (Grahn, 1977; Nilssen, 1980; Roelofs, 1983; Roberts et al., 1985; Riis and Sand-Jensen, 1998).

4.5. Influence of nutrients and light on species composition

Species composition is also influenced by lake trophy and transparency. The variety of elodeids and isoetids in lakes of Group 3 is reduced to much fewer elodeids and no isoetids in Groups 1 and 2, as nutrient concentration increases and Secchi-depth declines (Table 3). The same tendencies have been found in other studies (Chambers, 1987; Arts et al., 1990b; Rørslett, 1991; Srivastava et al., 1995). With increasing lake trophy, species composition changes due to different nutrient demands and light requirements of the species. Moreover, areal cover and biomass increase among the remaining species in the vegetated zone, enhancing the likelihood of competitive exclusion (Sand-Jensen, 1997) similar to the response observed in terrestrial vegetation in nutrient-rich habitats of high biomass (Tilman, 1986). In lakes, isoetids with their inherently slow growth rate and short shoots well adapted to oligotrophy are, therefore, susceptible to competition from elodeids. Presently, *P. pectinatus* is the most abundant submerged species in Danish lakes, probably benefiting from the ability to grow rapidly from starch-filled tubers in the sediment and develop a dense canopy close to the water surface (Bijl et al., 1989). The same prolific growth in eutrophic lakes has been reported for M. spicatum (Adams and McCracken, 1974). Together with P. crispus these two species dominate the species-poor elodeid vegetation in the eutrophic Danish lakes of Group 1 (Fig. 2, Table 3).

Several studies of the historical development of the submerged vegetation with increasing eutrophication of lakes document that isoetids such as *I. lacustris*, *Littorella uniflora* and *Lobelia dortmanna* are replaced by elodeids, and among elodeids small slow-growing species are replaced by large fast-growing species (Baagøe and Ravn, 1896; Wesenberg-Lund et al., 1917; Christensen and Andersen, 1958; Sand-Jensen and Søndergaard, 1981, 1997). For example, the species richness of submerged macrophytes in Lake Fure Sø in Denmark has declined from 33 to 10 during the last 100 years along with a 30-fold increase of external phosphorus loading, and while *Littorella uniflora* was out competed by helophytes in shallow water, and all deep-growing mosses and characeans disappeared, robust and fast-growing elodeids have survived with *P. pectinatus* increasing in abundance-rank among submerged species from number fifteen 100 years ago to number one today (Sand-Jensen, 1997). Also, in a comparison of the *Potamogeton*-vegetation in 17 Danish lakes studied between 1890 and 1920 with the present status in 82 lakes (Sand-Jensen et al., unpublished) *P. crispus*, *P. pectinatus* and *P. perfoliatus* have maintained the same percentage occurrence in the lakes today as before, while all other species have declined.

The present study demonstrates statistically the characteristic changes in species composition of aquatic macrophytes along a wide gradient of bicarbonate availability in Danish lakes, emphasising the importance of modes of inorganic carbon use for species distribution. The species compositions of Danish lakes are only to a small extent influenced by the distance to neighbouring lakes (Fig. 6). Apparently there is an effective dispersal and establishment of plants throughout the country, so the actual species composition of individual lakes is mainly regulated by the environmental conditions.

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