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PhD thesis, 2007

Rikke Bjerring

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Abstract: Lake development is explored on a decadal to millennial scale on different lakes based on Cladocera subfossils analyses in lake sediment cores. Eutrophication was found to have occurred during centuries – or even millennia - in many Danish lakes. The effect of climate on lake ecosystems was investigated using a European latitudinal gradient as a climate proxy, showing a complex pattern of larger and occasionally acid tolerant species in northern cold, low nutrient and low conductivity lakes, whereas dominance of small and benthic-associated species prevailed in southern warm, nutrient rich and high conductivity lakes. Taxa richness was found to be highest at intermediate latitudes. Additionally, climate response was explored through changes in pollen and Cladocera subfossils during a cold event period 8200 years before present in a core from Lake Sarup, which indicated lake level to play a key role.

Keywords: Paleolimnology, Cladocera, eutrophication, reference state, climate change

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Content

Papers included

1. **R. Bjerring**, E. Bradshaw, S. L. Amsinck, L. S. Johansson, B. V. Odgaard, A. B. Nielsen and E. Jeppesen. Inferring recent changes in the ecological state of 21 Danish candidate reference lakes (EU Water Framework Directive) using palaeolimnology. *Revised version in review. (Printed with kind permission from the Journal of Applied Ecology)*

2. L. S. Johansson, S. L. Amsinck, **R. Bjerring** and E. Jeppesen, 2005. Mid- to late-Holocene land-use change and lake development at Dallund Sø, Denmark: trophic structure inferred from cladoceran subfossils. *Holocene 15 (8): 1143-1151. (Printed with kind permission from the Holocene).*

3. S. L. Amsinck, A. Strzelczak, **R. Bjerring**, F. Landkildehus, T. L. Lauridsen, M. Søndergaard and E. Jeppesen, 2006. Lake depth rather than fish planktivory determines cladoceran community structure in Faroese lakes - evidence from contemporary data and sediments. *Freshwater Biology 51: 2124-2142. (Printed with kind permission from Freshwater Biology).*

4. **Rikke Bjerring**, C. E. A. Simonsen, B. V. Odgaard, B. Buchardt, S. McGowan, P. Leavitt and E. Jeppesen. Climate-driven regime shift related to changes in water level: a decadal scale multiproxy study of the 8.2 kyr. cooling event in Lake Sarup (Denmark). *Draft manuscript*

5. **R. Bjerring**, E. Becares, S. Declerck, E. Gross, L. Hansson, T. Kairesalo, R. Kornijów, J. M. Conde-Porcuna, M. Seferlis, T. Nõges, B. Moss, S. L. Amsinck, B. V. Odgaard and E. Jeppesen. Using subfossils of cladocerans in surface sediments of 54 European shallow lowland lakes (latitude: 36-68 °N) to assess the impact of climate on cladoceran community structure. *Manuscript.*

6. **R. Bjerring**, M. Nykänen, K. Sarmaja-Korjonen, K. Jensen, L. Nevalainen, K. Szeroczyńska, A. Sinev and E. Zawisza. Description of the subfossil head shield of *Alona protzi* Hartwig 1900 (Anomopoda, Chydoridae) and the environmental characteristics of its finding sites. *In review. (Printed with kind permission from Studia Quaternaria).*

Preface

This thesis represents my Ph.D. studies during August 2003 - January 2004 and October 2004-August 2007, registered at University of Aarhus and undertaken at the Department of Freshwater Ecology, National Environmental Research Institute (NERI), Aarhus University. In addition, part of the work was carried out at the Department of Earth Sciences, Aarhus University. The project was funded by the International School of Aquatic Sciences, Aarhus University (SOAS) and NERI as well as ECOFRAME (EVK1–CT1999-00039), BIOMAN (EVK2-CT-1999-00046), EUROLIMPACS (GOCE-CT-2003-505540), the DK-funded CLEAR project (a Villum Kann Rasmussen Centre of Excellence project), the Danish Natural Science Research Council (research project “CONWOY” on the effects on climate changes on freshwater) and the Danish research project AGRAR 2000 (four Danish research councils). My research supervisors were Professor Erik Jeppesen (NERI), Professor Bent Vad Odgaard (AAU) and Associate professor Tom V. Madsen (AAU).

I am indebted to a number of people for their invaluable help and support. Most of all, I am grateful to my supervisor Professor Erik Jeppesen for his professional guidance, help and neverending constructive manuscript corrections, challenging my intellect as well as my expertise in reading hieroglyphs (Erik’s handwriting). Thanks also go to Professor Bent Vad Odgaard for his great help, valuable scientific discussions and advice as well as all those pieces of cake during coffee breaks.

I wish to thank all my colleagues in the Lake Group for a warm and friendly atmosphere with a touch of good humour, but also a constructive and inspiring working environment. Thanks to the paleo group for sharing practical experiences and to my “room mates” at NERI for good friendship and support during the weeks before my submission of this thesis.

Also special thanks to Susanne Amsinck for her support, friendship, inspiring discussions and input to – as well as critical review of – manuscripts, and to Karina Jensen for her excellent practical supervision in the lab. Thanks also to Emily Bradshaw, Kaarina Sarmaja-Korjonen and Mirva Nykänen for inspiring cooperation and friendship.

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Silkeborg, August 2007

Rikke Bjerring

1 Introduction

1.1 The role of nutrients in lake systems; contemporary and paleolimnological signals

Humans have had a major impact on lakes worldwide through alterations of the landscape, the hydrological cycle, contamination and waste disposal, and by altering species composition or promoting species invasion (Carpenter et al., 1992; Schindler, 1997; Wetzel, 2001). In particular eutrophication is regarded as one of the most severe stressors on fresh water ecosystems (Carpenter et al., 1992).

Increasing nutrient loading enhances the productivity at all trophic levels. However, major changes may occur that tip the balance in the lake ecosystem, leading to loss of submerged macrophytes, a shift towards dominance of plankti-benthivorous fish, high predation on zooplankton, noxious phytoplankton blooming and turbid water (Jeppesen et al., 2005; Schindler, 1977). Particularly in shallow lakes the shift from a clear water state of high ecological quality to a turbid water state may occur abruptly, depending on lake type and climate, when a certain nutrient threshold is reached (Irvine, Moss & Balls, 1989; Scheffer et al., 1993). This is because submerged macrophytes play a key role for maintaining lakes in a clear water stage in shallow lakes due to a number of positive feedback mechanisms: they take up nutrients, stabilise the sediment, increase sedimentation, potentially inhibit phytoplankton through allelopathy, and act as refuge for invertebrates, fish fry and piscivorous fish (Søndergaard & Moss, 1997). Fish predation by plankti-benthivorous fish on the zooplankton (top-down control) is also higher in shallow lakes and, therefore, changes in the fish community have more adverse effects in shallow than in the deeper lakes (Jeppesen et al., 2003a; Jeppesen et al., 1997).

As zooplankton constitute the link between primary production and predators, they respond to both food availability and predation, and they therefore have great potential as indicators of the ecological state of a lake. Zooplankton (in particular cladocerans) play a key role in controlling phytoplankton biomass and thus contribute significantly to maintain clear water phases (Jeppesen et al., 1999; Moss, 1998). The grazing capacity of cladocerans depends on size as the filtering rates increase with increasing body length (Brooks & Dodson, 1965). A positive relationship between body size and maximum particle size ingested is generally found for cladocerans (e.g. *Daphnia* spp. and *Bosmina longirostris*) (Burns, 1968, 1969) and, accordingly, large *Daphnia* can exploit a large size range of phytoplankton.

Several factors influence the size distribution of the cladocerans. Zooplanktivorous fish select for the larger-sized species (Langeland & Nost, 1995; Timms & Moss, 1984) and can effectively change the size distribution of cladocerans (Brooks & Dodson, 1965; Jeppesen et al., 2003a; Jeppesen et al., 1997). In temperate lakes macrophytes, in particular submerged taxa, provide a habitat rich refuge (Scheffer et al., 1993; Timms & Moss, 1984) that is exploited mainly by the larger pelagic and macrophyte-associated cladoceran species, as well as by predatory fish controlling the planktivorous fish stock (Jeppesen et al., 1997; Persson & Eklöv, 1995).

When studying the history of past environmental changes, i.e. eutrophication or climate change effects, long time series of monitoring data are highly valuable, but only rarely available for the time frame of interest (Anderson 1995). When available, the early data may be incomparable with modern methods of monitoring. Lake sediments, however, contain a tremendous library of information on past lake history and are a valuable alternative for studying long-term lake responses. Presently, there is no substitution for these sedimentary records until centuries of water quality data for each system of interest have been collected (Smol, 1992).

Most groups of aquatic organisms leave some sort of morphological or chemical record (Smol, 1992). This allows application of several indicators (proxies) in a study (multiproxy-study), such as algal pigments, diatoms, macrophytes, chironomids and cladocerans. Fragments of the proxies continuously accumulate in the sediment from the whole lake area, thereby integrating habitat availability and seasonal variation in the record and minimising the site-specific variability. This is an advantage which field studies rarely offer due to the labour-demanding and costly intensive sampling frequency.

The sedimentary record of algal pigment as well as diatom frustules can give valuable information on past algal communities as well as reflect the trophic state of lakes (Dressler et al., 2007; Fietz, Nicklisch & Oberhansli, 2007; McGowan et al., 2005). In particular diatoms are widely used for quantitative inference of the past epilimnion total phosphorous (TP) concentration (Bennion, Fluin & Simpson, 2004). Also chironomids have been used as a proxy for primary production through quantitatively inference of chlorophyll *a* and TP (Brodersen & Lindegaard, 1999; Lotter et al., 1998). In addition, in particular chironomids have been used for inference of hypolimnetic oxygen in eutrophication studies (Brodersen & Quinlan, 2006).

Historical changes in planktivorous fish abundance have been quantitatively or qualitatively inferred from lake sediment based on size differences in *Daphnia* resting eggs (ephippia) (Jeppesen et al., 2002a), *Bosmina* taxa (Gasiorowski, 2004; Sweetman & Finney, 2003) and from the ratio of large and small pelagic cladoceran ephippia (Amsinck, Jeppesen & Ryves, 2003; Jeppesen et al., 2003b). Planktivorous fish abundance has additionally been inferred in both freshwater lakes (Jeppesen et al., 2001b; Jeppesen et al., 1996; Johansson et al., 2005) and coastal brackish lakes (Amsinck, Jeppesen & Landkildehus, 2005a, b) based on cladoceran taxa.

Macrophyte subfossils directly reflect plant community structure and indicate, although usually qualitatively, the relative abundance of macrophytes (Hilgartner & Brush, 2006). Recently, the potential use of diatom subfossils for quantitative reconstruction of macrophyte cover has been evidenced (Vermaire, 2007). Also, macrophyte-associated cladocerans, especially chydorids, are considered useful indicators of past macrophyte cover in relation to eutrophication (Amsinck, Jeppesen & Ryves, 2003; Hann, 1989; Hofmann, 1986; Jeppesen, 1998; Whiteside & Swindoll, 1988). In addition Johansson et al. (2005) showed cladoceran inferred macrophyte cover for the last 7000 years to be related to eutrophication. Also the relative proportions of *Bosmina* and chydorid subfossils in sediment have been used to infer changes in macrophyte abundance following European settlement in billabongs in Australia (Thoms, Ogden & Reid, 1999). Likewise, the proportion of pelagic and benthic-associated subfossil cladoceran taxa has been used as an indicator of recent changes in trophic levels (reflecting habitat availability) (Hofmann, 1998). Chydorid subfossils have additionally been found to respond directly to nutrient concentrations (Brodersen et al., 1998; Lotter et al., 1998; Shumate et al., 2002); however, the responses most likely indirect reflect eutrophication-related changes in lake habitat and/or predation patterns as discussed above.

1.2 Climate effects on lake systems

While human induced changes in nutrient loads have had a marked effect on lakes, changes in climate also play a role. The key processes of climate variability are radiation (light, temperature regimes) and water balance (water level, retention time, stratification) and related factors (snow, wind) (Battarbee, 2000). Since lakes can be strongly influenced by changes in hydrology, they are particularly sensitive to climatic changes (Carpenter et al., 1992; Carpenter & Kitchell, 1992; Mason et al., 1994). Thus, indicators from lake sediment, ice cores, speleotherms (mineral deposits formed in caves) as well as tree rings have been used in climate studies. Several high-resolution studies of the early Holocene demonstrate abrupt climatic changes. The most prominent Holocene climate anomaly was the 8.2 kyr. cooling event (8200 years before the present) lasting 200-400 years (Alley et al., 1997; Dansgaard et al., 1993). Temperature reconstructions from Scandinavia during this period indicate an approximate drop of ca. 1-1.5 ºC based on pollen, diatoms and chironomids (Korhola et al., 2002; Korhola et al., 2000; Rosen et al., 2001; Seppa, Hammarlund & Antonsson, 2005). Other Holocene cooling events have been demonstrated – the latest cooling event usually referred to as the Little Ice Age took place 200-500 years ago. Warming also occurred (e.g. the medieval warm period ca. 850-1250 AD) and presently Europe is in a warming state (IPCC 2001).

Chironomid subfossils have been regarded as the most promising biological proxy for reconstructing temperature change due to a direct correlation between species assemblage and temperature (Korhola et al., 2002; Larocque & Hall, 2003; Lotter et al., 1999; Walker, 1991). However, this has been questioned by several authors (Brodersen & Anderson, 2002; Brodersen & Quinlan, 2006; Brooks, 2006), as the response is likely oxygen-driven and not a direct physiological temperature response. Also the proportion of cladoceran resting eggs (ephippia) relative to the sum of body shields and resting eggs has recently been related directly to temperature/length of growing season (Bennike, Sarmaja-Korjonen & Seppanen, 2004; Jeppesen et al., 2003b; Sarmaja-Korjonen, 2004; Sarmaja-Korjonen, Seppanen & Bennike, 2006).

However, in mid-latitude lowland systems, such as Denmark, which do not cover strong ecological border zones (e.g. tree line) hydrological changes rather than temperature probably have, and will probably be, the most important factor for lake ecosystems. Indeed, several studies (Hammarlund et al., 2002; Hammarlund et al., 2005; Nesje et al., 2006; Seppa, Hammarlund & Antonsson, 2005; Vassiljev, 1998) have demonstrated precipitation to be the most influential climatic change factor for lakes during the 8.2 kyr. event in northern Europe. Water level fluctuation may, depending on lake morphometry, have major effects on the relative proportion of the pelagic and littoral zone of lakes.

Several biological proxy assemblages reflect the relative proportion of littoral and non-littoral habitats. Thus, chironomids, encompassing littoral and profundal associated taxa, have been used to infer, quantitatively or qualitatively, water level changes related to climate changes (Ilyashuk et al., 2005), as have cladocerans (Alhonen, 1970; Koff et al., 2005; Korhola, 1992; Korhola, Tikkanen & Weckstrom, 2005; Sarmaja-Korjonen & Alhonen, 1999; Sarmaja-Korjonen et al., 2003; Sarmaja-Korjonen et al., 2006) and diatoms (Punning & Puusepp, 2007). Cladocerans and algae both have pelagic and littoral taxa.

Water level fluctuations may also result in changes in salinity/conductivity, particularly in arid regions or in lakes vulnerable to saltwater transgression. In paleo-studies cladocerans have been found to be related to salinity, showing alterations in community structure and decreasing species numbers with increasing salinity (Amsinck, Jeppesen & Ryves, 2003; Bos, Cumming & Smol, 1999; Sarmaja-Korjonen & Hyvarinen, 2002; Boronat, Miracle & Armengol, 2001; Hofmann & Winn, 2000; Verschuren et al., 2000). Also chironomids (Heinrichs & Walker, 2006), diatoms (Verschuren et al., 2000) and ostracods (Porter, Sauchyn & Delorme, 1999) have been used to infer salinity.

Community responses are seldom a direct response to a particular physical or chemical factor influenced by climate change, such as light, nutrients, salinity, oxygen availability or temperature, but rather a whole-ecosystem response (Battarbee, 2000). This fact complicates climate effect studies, especially in the latter part of the Holocene where anthropogenic factors including eutrophication strongly affected the lake ecosystems. Complexity makes it difficult to disentangle indirect climate responses to which communities react – for instance, are changes in nutrient concentration related to erosion processes from hydrological changes or derived from eutrophication. Thus, a major challenge is to disentangle climate and nutrient responses, not least now where many lakes are undergoing a re-oligotrophication process and coincident predictions of future climate in the Northern hemisphere (IPCC, 2001) will lead to increased precipitation and accordingly increased nutrient loading of lakes.

2 Aim

The overall aim of this thesis was to study lake responses to global change (cooling, warming and eutrophication), with special emphasis on Danish and other European shallow lakes.

Specific objectives were:

to elucidate recent (the last 150 years) changes in cladoceran communities in 21 potential Danish reference lakes and the long-term changes (the past 7000 years) in a eutrophic Danish lake (Lake Dallund), with focus on eutrophication related to land use changes (Papers 1 and 2).

to investigate lake ecosystem changes during a 200-year cooling event during the Holocene (the 8.2 cal. year BP event) with minimal human impact in a unique Danish annually laminated sediment core using cladocerans, pollen, pigments as well as stable isotopes as proxies (Paper 4).

to elucidate key variables determining the structure of cladoceran communities in 54 shallow freshwater lakes along a European climate gradient (36-68 °N) and in 29 shallow freshwater lakes distributed in a narrow geographical area (the Faroe Islands) by relating surface sediment samples to contemporary environmental data (Papers 3 and 5).

Table 1: Schematic overview of the studies conducted in this thesis

	Focus	Sediment samples			Proxies	Main influencing factor
		Core	Date	Surface		
Paper 1	Nutrients	x	1850-2000 AD	x	Diatoms Cladocerans	Nutrients
Paper 2	Nutrients	x	7000 BP		Cladocerans	Nutrients
Paper 3	Lake depth	x	6000 BP	x	Cladocerans	Lake depth
Paper 4	Climate	x	8700-8100 BP		Isotopes Organic content Pigments Cladocerans Pollen	Lake-level
Paper 5	Climate			x	Cladocerans	Conductivity – but see discussion
Paper 6	Taxonomy	x	-	x	-	-

3 Methodology

To study recent and long-term lake responses and lake structure an paleolimnological approach was used, with emphasis on cladoceran subfossils recovered from lake sediments (constituting the major part of preserved zooplankton remains) .

Two approaches were applied:

1) an investigation of historical changes in biological communities and lake ecosystem structure based upon analyses of subfossils of dated sediment cores (Paper 1-4).

2) a "space-for-time" approach for elucidating the changes in biological communities and ecosystem structure along an environmental gradient. This was based upon analyses of lake surface sediment samples related to contemporary environmental variables of the lakes in i) a narrow geographical area (Paper 3) and ii) at a wide European scale (Paper 5).

3.1 Core studies

Paper 1 and 2 focussed on lake response to historical eutrophication. Paper 3 focussed on historical changes in lake depth, whereas Paper 4 focussed on lake response to historical climate change.

In Paper 1 we intended to study the most recent (since 1850 AD) ecological development in 21 lakes selected to be relatively minimal human-impacted and thus representing potential reference sites according to the Water Framework Directive (WFD). The study lakes were distributed broadly throughout Denmark (Fig. 1) and were divided into Moderately to Highly Alkaline lakes (ALK, n=12), Low Alkaline Clear Water lakes (LACW, n=4) and Low Alkaline Coloured Lakes (LAC, n=5) based on proposed WFD thresholds (Søndergaard et al., 2005; Søndergaard, 2003). Subsamples, representing four different time periods (1850, 1900, 1950 and 2000 AD, the latter surface sediment) were investigated for cladoceran subfossils and diatom frustules in the 21 dated short sediment-cores. Total epilimnetic phosphorous was inferred based on diatoms (Bennion, 1996; Bradshaw et al., 2002), whereas macrophyte cover (Jeppesen, 1998) and fish abundance (Jeppesen et al., 1996) were inferred from cladocerans, using existing transfer functions. The reference condition was selected to be represented by 1850 AD, as in several other European studies (Andersen, Conley & Hedal, 2004; Bennion, Fluin & Simpson, 2004; Leira et al., 2006; Manca, 2002; Taylor et al., 2006).

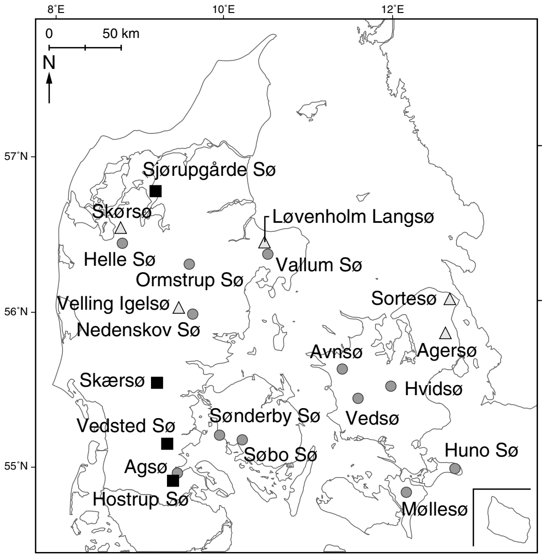

Figure 1. Location of 21 potential reference lakes in Denmark, investigated with respect to eutrophication during 1850-2000. Filled circles: Alkaline lakes (●), low alkaline coloured lakes (Δ), low alkaline clear water lakes (■). (From Paper 1)

In Paper 2 we studied recent and long-term changes, i.e. the last 7000 years, in lake trophic structure in a presently eutrophic shallow Danish lake (Lake Dallund). The analysis was based on changes in cladoceran subfossils and for the first time densities of planktivorous fish as well as submerged macrophyte cover were inferred quantitatively, also based on existing models (Jeppesen, 1998; Jeppesen et al., 1996), for a time period covering millennia.

In Paper 3 we investigated the historical change in water level during the last 6000 years in the Faroese Lake Heygsvatn, based on cladoceran subfossil assemblages.

Table 2

Parameter		Mean	Median	Min	Max	N
Latitude (°N)		51	53	36	68	54
Longitude	*	13	12	-6	27	54
Area (ha)	*	782	24	1	27000	54
Mean depth (m)		1.92	1.60	0.47	6.00	54
Total phosphorous (µg L^{-1})	*	107	71	6	470	54
Total nitrogen (µg L^{-1})	*	1936	1365	239	7710	54
Chl *a* (µg L^{-1})	*	47	24	1	331	54
Secchi depth (m)		1.5	1.1	0.2	5.6	54
Secchi/mean depth	*	0.9	0.6	0.1	4.6	54
Conductivity (µS cm^{-1})	*	775	313	9	7229	54
pH	*	8.0	8.1	5.1	9.5	54
Mean air temperature of the warmest month of the year (°C)	*	18.8	17	12	26.4	54
Mean annual temperature (1961-90) (°C)	*	8	8	-3	16	54
PVI submerged macrophytes (%)	*	15	5	0	87	44
Piscivorous fish biomass (kg net^{-1} night^{-1})		0.9	0.3	0	4.5	35
Planktivorous fish biomass (kg net^{-1} night^{-1})		2.3	0.9	0	11.1	35

* Included variables in multivariate statistics for elucidating influencing parameters for the subfossil cladoceran structure in 54 lakes along a European climate gradient. Plant filled volume of submerged macrophytes (PVI) were included in the analyses on a subset of 44 lakes (modified from Paper 5).

In Paper 4 we used varved sediment (sediment deposited in annual couplets) for the study of lake response to climatic change. In Lake Sarup (Paper 4), post-glacial varved sediment was found for the first time in Denmark (Rasmussen, 2002). Varves are typically formed in small, deep sheltered lakes, creating favourable limnological conditions for undisturbed surface-sediment in the deepest part of the lake. Such conditions include strong seasonal lake stratification and cycles in biological production as well as minimal bioturbation (O'Sullivan, 1983). The presence of varved sediment is relatively rare, but when present it yields outstanding properties for high-resolution studies.

Thus, a varved segment of the sediment core from Lake Sarup yielded a rare possibility of studying climate change during a period with minimal human impact in that it happened to cover the most abrupt Holocene climatic event (the 8.2 kyr. event). We selected the period 8700-8000 BP for analysis of climatic anomalies and used a multiproxy approach to study ecological changes in the lake (stable isotopes, varve thickness, organic content of sediment, pigments, cladoceran subfossils, pollen) and a time resolution of 10-40 year samples (Paper 4**)**.

3.2 Surface sediment studies

In Paper 3 we investigated contemporary data and sediment samples of 29 Faroese freshwater, mainly shallow oligotrophic lakes. Variables influencing the cladoceran subfossil structure were identified and transfer functions for the most important factor structuring the cladoceran community (maximum lake depth) were developed and applied to a long sediment core covering the last 6000 years.

In Paper 5 we elucidated the main structuring factors for the cladoceran subfossil assemblage in surface sediment samples by relating the taxa composition to 10 (11) contemporary physico-chemical and biological environmental variables (Table 2). The 54 shallow lowland freshwater lakes were distributed along a substantial climatic (36-68 °N) and trophic state (6-470 µg total phosphorous L^{-1}) gradient in Europe in order to study climate effects on lake structure. The lakes were located in Sweden (5), Finland (6), Estonia (6), Denmark (6), United Kingdom (5), Poland (6), Germany (6), Greece (4) and Spain (10) (Fig. 2).

3.3 Data analysis

We mainly applied multivariate statistical techniques, which generally are those most frequently used in paleolimnology due to the high degree of variation and complexity in the data, the occurrence of several possible explaining variables and species data expressed as proportional data when working with whole community assemblages. However, Paper 1 presents an alternative way of analysing simplified community variables using classical statistics on absolute species data.

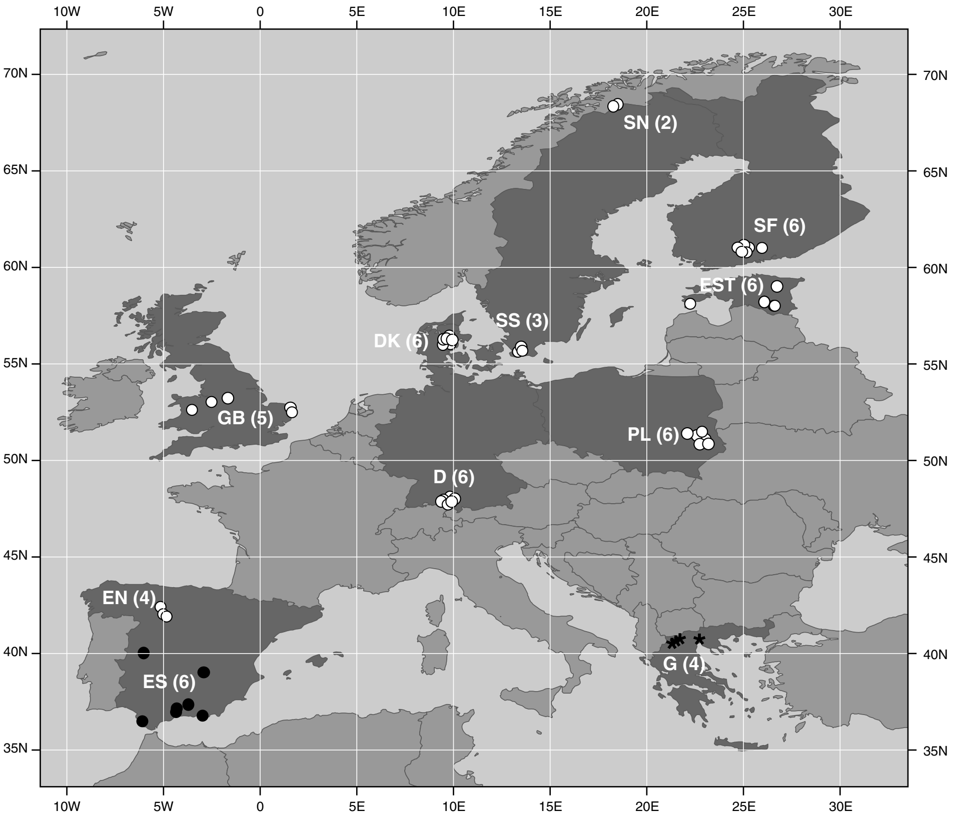

Figure 2 Geographical location of the 54 European lakes, in which cladoceran subfossils of surface -sediment samples were related to contemporary data. Capital letters denote country, subscript S= southern, N= northern. Numbers of study lakes are given in brackets. ○ ECOFRAME data set, ● BIOMAN data set, * Greek lakes. (From Paper 5).

A general problem of the multivariate methods is model validation, as statistical tests in real life generally are based on the same data used for model construction and not on independent test data sets (e.g. Birks, (1998); van Tongeren (1995) but see Hallgren, Palmer & Milberg (1999) ; Vermaire (2007)). Moreover, several multivariate methods (ordination, transfer function) assume linear or unimodal response curves to environmental variables for all species in the assemblage, an assumption that may not always hold. No such assumptions are, however, assumed in Multivariate Regression Tree Analysis (MRT), which in addition allows for high-order interactions between environmental variables (De'Ath, 2002). This approach was used in Papers 3 and 5.

3.4 Species identification

Most paleolimnological studies will be meaningless if species are misinterpreted. Photographs, detailed drawings and other descriptive material of described and undescribed species are important for identification to ensure the quality of the work (Cohen, 2003). Paper 6 provides photographs and a detailed drawing of *Alona protzi* head shield (Fig. 3) and is a contribution to the knowledge of species-specific identification of a small *Alona* head shield, which has not yet been described in full detail. The idea of this paper was developed during the Proceedings of the 8th Subfossil Cladocera Workshop 2006 and is a result of a co-operation between several international paleolimnologists, involving data from numerous studies. It is presented here, as it has status as background information for cladoceran subfossil analysis.

The special characteristics of the *A. protzi* head shield is a rounded and thick chitinous rostrum and a notched posterior margin of the head shield. *A. protzi* is a rare species with low abundance when present. Its geographical distribution seems rather wide in northern Europe. This paper documents its presence in lake sediments from five European countries (Sweden, Finland, Estonia, Denmark and Poland). The ecology of *A. protzi* is poorly known. The findings of our study suggest a wide tolerance of *A. protzi* with respect to trophic state, although most findings were in meso-eutrophic lakes with high to neutral pH and low macrophyte cover. However, the possibility that *A. protzi* mainly occurs in groundwater and occasionally is transported into lakes cannot be excluded.

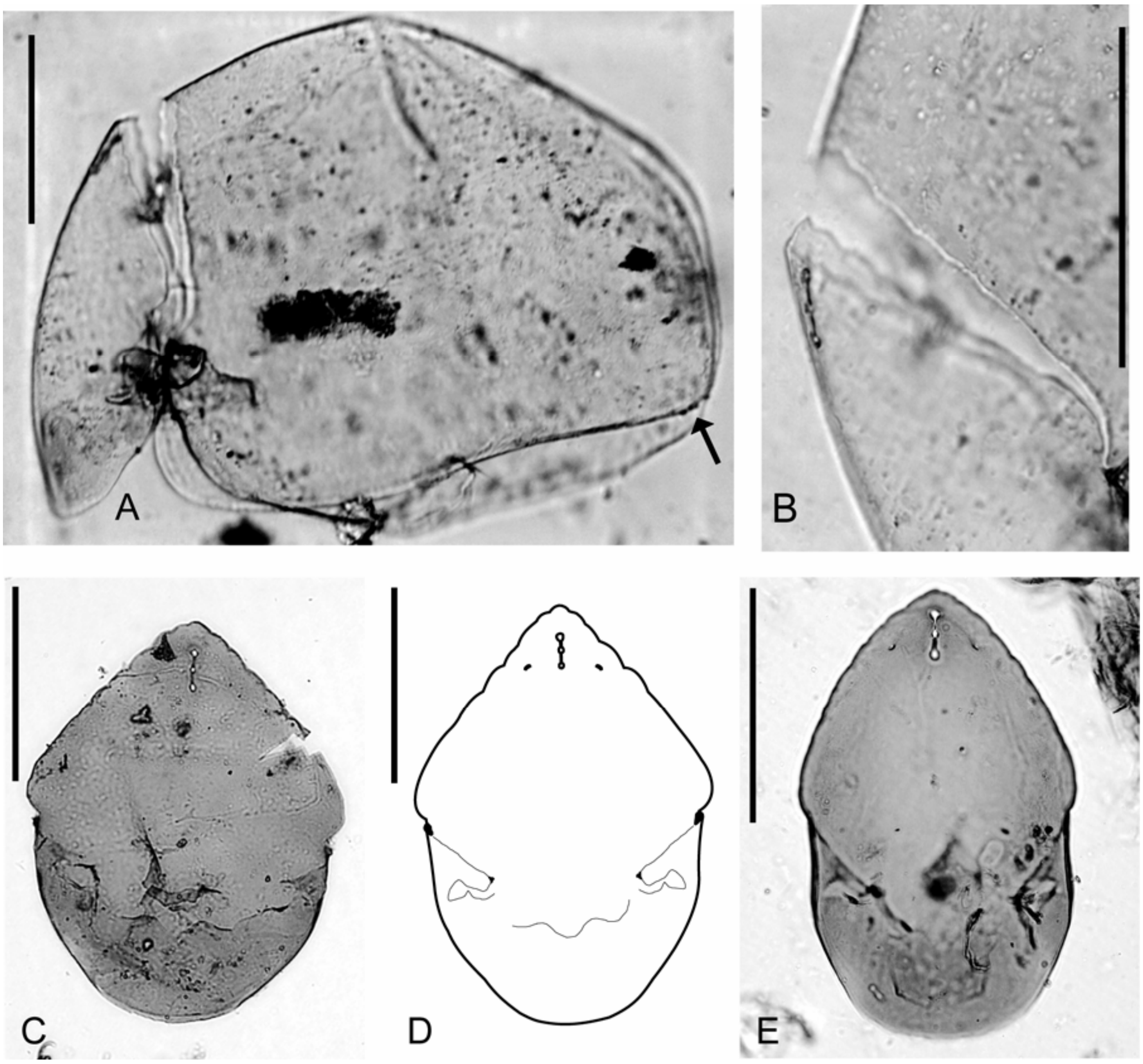

Figure 3. A) The subfossil head shield and carapace of *Alona protzi* from Lake Sarup, Denmark. An arrow indicates characteristic denticles on the posterior-ventral corner of the carapace. B) A detail of the opened molting seam between the head shield and carapace of *A. protzi*, showing the head pores and the c characteristic notched posterior margin of the head shield and the corresponding notched margin of the carapace. C) *A. protzi* head shield, Lake Jelonek, Poland. D) Drawing of *A. protzi* head shield, original from Lake Väike Juusa, Estonia. E) *A. protzi* head shield, Lake Krowie Bagno, Poland, the curvature of the head shield makes it look exceptionally narrow. Scale bar = 100 µm. (From Paper 6).

4 Summary of results and thesis papers

4.1 Recent and past lake development with emphasis on eutrophication

The most recent (since 1850 AD) ecological development was studied in 21 Danish lakes (Fig. 1) selected to be relatively minimal human-impacted, and thus potentially useful (at present or in the near past) as a reference site according to the definition in the Water Framework Directive (WFD) (Paper 1).

Contrary to our expectations, the majority of the 21 lakes were impacted by eutrophication already in 1850, as indicated by high accumulation rates of sediment and cladoceran subfossils, high abundance of pelagic cladoceran species, high diatom-inferred total phosphorous (particularly in moderately to highly alkaline lakes (ALK) and low alkaline clear water lakes (LACW)), high cladoceran inferred benthi-planktivorous fish abundance and low cladoceran inferred submerged macrophyte coverage (in ALK lakes). Supportingly, the percentage of land used for cultivation in the catchments was relatively high already in 1800, likely resulting in elevated nutrient input by leaching and soil erosion (Bradshaw, Nielsen & Anderson, 2006). Other paleolimnological studies of Danish lakes also indicate early eutrophication (Bradshaw, Rasmussen & Odgaard, 2005; Brodersen et al., 2001; Brodersen, Anderson & Odgaard, 2001; Jeppesen et al., 2001b; Odgaard & Rasmussen, 2000) (Paper 2). Since 1850 the study lakes developed towards more eutrophic conditions as evidenced by increasing accumulation rates of sediment and cladoceran subfossils and increasing proportions of pelagic diatom and cladoceran taxa (especially in ALK and LACW lakes).

In accordance with other Northern-European searches for potential reference lakes using the paleolimnological approach (Bennion, Fluin & Simpson, 2004; Leira et al., 2006), we found that only a small percentage of the study lakes exhibited minor diatom and cladoceran community changes for the time period investigated (Fig. 4).

Lakes with minimal changes since 1850 were found to be and remain oligotrophic in other Northern European studies (Bennion, Fluin & Simpson, 2004; Leira et al., 2006). In contrast, the Danish lakes showing minimal changes were eutrophic already since 1850. Moreover, based on diatom inferred TP-values, more than 70% of the Danish study lakes were in a WFD "moderate" to "poor" ecological state already in 1850. Our study clearly demonstrated the recent lake ecosystem development, showing the potential of using biological proxies for identifying reference conditions as well as identifying "true" reference sites, However, it also shows that it may be difficult to use 1850 to define the reference state for lakes situated in catchments with even moderate agricultural activities.

Certainly, the definition of 1850 as a period with minimal impact by humans does not fit to Lake Dallund either (Paper 2). This lake clearly illustrates early eutrophication in a Danish lake based on analysis of cladoceran subfossils representing the last approximately 7000 years. During the earliest period (ca. 4830-750 BC) cladoceran subfossil abundance and species richness were low and the community was dominated by the small-sized *Bosmina longirostris* (Paper 4). Presumably, during this period the lake was deep with a relatively small littoral zone inhabited by macrophytes and the fish predation pressure was high. The following period: late Bronze Age (ca. 650 BC – 1100 AD) was characterised by a marked increase in macrophyte-associated cladocerans (e.g. *Alonella nana, Eyrucercus lamellatus, Acroperus* spp.), indicating increased macrophyte production. Also, diminished fish predation pressure was indicated by the dominance of larger-sized ephippia (Jeppesen et al., 2002a; Jeppesen et al., 2001b). Supportingly, a marked decrease in pollen accumulation (ca. 700 BC) indicated forest clearance (Rasmussen, 2005) and, thus, enhanced leaching of nutrients through erosion.

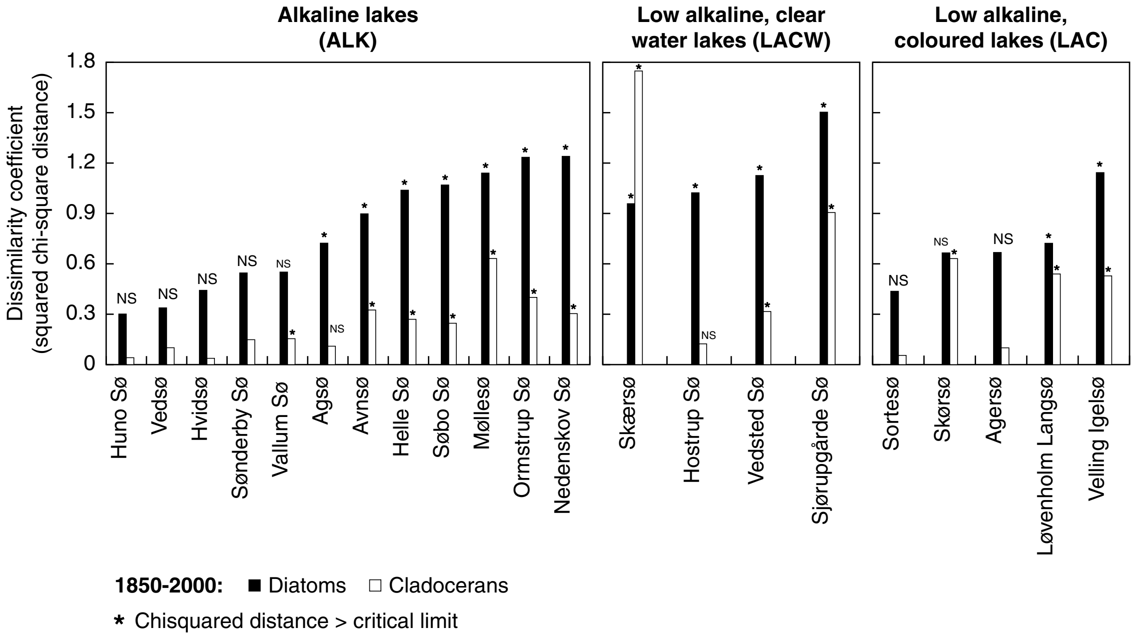

Figure 4. Lake-specific community changes (squared chi-square distance) between 1850 and 2000, sorted after increasing total diatom community change (left to right) within each lake type (ALK: Alkaline lakes, LACW: Low Alkaline Clear Water lakes, LAC: Low Alkaline Coloured lakes). * refers to squared chi-square distance higher than an estimated critical level, and are thus interpreted as lakes with changing communities, whereas NS refers to squared chi-square distance lower than the estimated critical level and thus are regarded as lakes of minimal community change (modified from Paper 1).

Coincident with the introduction of the mouldboard plough, intensifying agriculture, a marked increase in the accumulation of cladoceran subfossils occurred. In particular increases in pelagic species and *Chydorus sphaericus* can be traced around 1200 AD, suggesting higher nutrient input into the lake. Further development went towards increasing eutrophication beginning with the increase in the mud-dwelling *Leidigia leydigii* (ca. 1300-1700 AD) and eutrophic-indicating taxa (e.g. *Chydorus sphaericus*) (ca. 1700-1998 AD). The relative distribution between large and small-sized ephippia decreased again, indicating an increased fish predation pressure. The current ecological state of Lake Dallund has improved temporarily following fish manipulation in 1995-1997. However, this was not observable in the sediment core analysed, likely because of poor time resolution in the upper sediment. Our study indicated that a reference state with no or minimal human impact would refer to the late Bronze Age (ca. 750 BC) in Lake Dallund.

Based on the strong effect of fish predation on the zooplankton community structure both in Danish eutrophic lakes as well as in oligotrophic Greenland and Icelandic lakes (Antonsson, 1992; Jeppesen et al., 2001a; Lauridsen et al., 2001), we initially expected fish predation also to be the main structuring factor in Faroese lakes. This expectation also derived from a study of four Faroese lakes differing in trophic structure revealing differential fish predation pressure on zooplankton communities due to differential fish communities (Jeppesen et al., 2002b; Malmquist et al., 2002). Thus, lakes dominated by brown trout (*Salmo trutta*) exhibited low predation pressure, presence of brown trout and three-spined stickleback (*Gasterosteus aculeatus*) moderate predation pressure, and high predation pressure when arctic char (*Salvelinus alpinus*) was present in moderate numbers (Jeppesen et al., 2002b; Malmquist et al., 2002). However, as brown trout was the most abundant species and exclusively dominated the fish community in 12 out of 29 generally small and oligo-mesotrophic Faroese lakes, lake depth rather than fish planktivory was found to determine the community structure and body size distribution of the cladoceran subfossils in the Faroese lakes (Paper 3). The more omnivorous diet habits of brown trout than of arctic char (Malmquist et al., 2002) may imply a weaker predation pressure on the zooplankton, thus explaining the weak effect of fish predation on the cladoceran community recorded in the surface sediment. Instead, suitable habitat availability, reflected by lake depth, was recognised as the main structuring factor for the cladoceran community in agreement with the findings in 53 subarctic oligotrophic Fennoscandian lakes (Korhola, 1999; Korhola, Olander & Blom, 2000). Also O'Brien et al. (2004) showed the structure of zooplankton to be related to lake depth and area and to be the most important variables for zooplankton species richness, though they did not have data on fish. In the 29 investigated Faroese lakes, those with maximum depth larger than 5 m were dominated by pelagic species, whereas shallower lakes were dominated by benthic taxa, reflecting favourable conditions for benthic primary production in the shallower lakes (benthic cladoceran habitat). In contrast, lake chemistry seemed to have only limited impact on the cladoceran assemblage structure.

Based on the 29 Faroese surface sediment samples and contemporary data, predictive models of maximal lake depth were developed (Weighted Averaging procedures) and applied to subfossil cladoceran assemblages from a sediment core from the Faroese Lake Heygsvatn covering the period 5700 BP to the present. In contrast to inferences of lake depth in three continental sub-arctic lakes in Finnish Lapland (Korhola, Tikkanen & Weckstrom, 2005), no major changes in the lake depth of Lake Heygsvatn was observed during the last 5700 years. The inferred maximum lake depth corresponded well to the present-day depth, although a recent inferred increase in water level may instead reflect recent eutrophication, as nutrient poor species decreased (e.g. *Chydorus piger*) simultaneously with the increase in eutrophic species (e.g. *C. sphaericus*). Inference models of lake depth are driven by shifts in the relative distribution and importance of benthic and pelagic species. The study demonstrated that, inference of lake depth in long-core studies, based on cladocerans, should be interpreted with caution due to confounding factors, such as pH, eutrophication or changes in predator structure, in particular when covering the most recent decades (Hofmann, 1998), and even in relatively nutrient poor lakes such as Lake Heygsvatn (Paper 3).

4.2 Lake response in relation to climate change

4.2.1 Direct lake response to climate change

High accuracy of dating, clear isotopic anomalies and low human impact allowed studying of direct lake response to climate change in Lake Sarup. This enabled us to confidently interpret this period as the 8.2 cool event. The stable isotopic record indicated that hydrological induced changes were more important than the temperature shift, as the isotopic anomaly was too high to represent temperature only (Hammarlund et al., 2002; McDermott, Mattey & Hawkesworth, 2001).

In correspondence, changes in net precipitation, rather than temperature, have been suggested to be the driving force for lake level changes during the Holocene in Europe (Harrison, Prentice & Guiot, 1993), with an increase in humidity at latitudes north of 50 °N and south of 43° N based on different proxies (Magny & Begeot, 2004; Magny et al., 2003). The lake topography indicates a deep central basin surrounded by shallow areas (Fig. 5). Therefore, an increased lake level would result in an increased surface/volume ratio and, with it, an increase in the relative availability of benthic habitats and vice versa (Fig. 5 A, B). We interpret the changes in proxies 8359-8225 BP in Lake Sarup as a lake level increase (Fig. 6). Firstly, accumulation of inorganic as well as organic sediment accumulation increased coinciding with a decrease in the sediment organic content during this period. This indicated allochthonous inorganic and organic matter input from the surroundings, as expected from increased precipitation. Higher allochthonous input may have caused increased turbidity and a resultant decrease in primary producers, as indicated by the reduced accumulation of algal pigments, increases in the turbidity-tolerant bryozoans (*Plumatella fruticosa, P. casmiana*) (Bushnell, 1974; Økland & Økland, 2002) as well as increases in *Chaoborus* remains. The latter may be due to decreased fish predation as a result of lower water clarity (Wissel, Boeing & Ramcharan, 2003; Wissel, Yan & Ramcharan, 2003). Moreover, an increase in Nymphaeaceae trichosclereids (remains from floating-leaved macrophytes) and associated cladocerans as well as sediment associated cladocerans indicate increased water level, allowing colonisation of shallow areas. In addition, a sudden (20-40 years) increase in *Tilia* (lime) and *Ulmus* (elm) pollen during this period most likely reflected an increase in erosion of soils containing pollen of these trees, as expansion of these long-lived climax trees is ecologically unlikely.

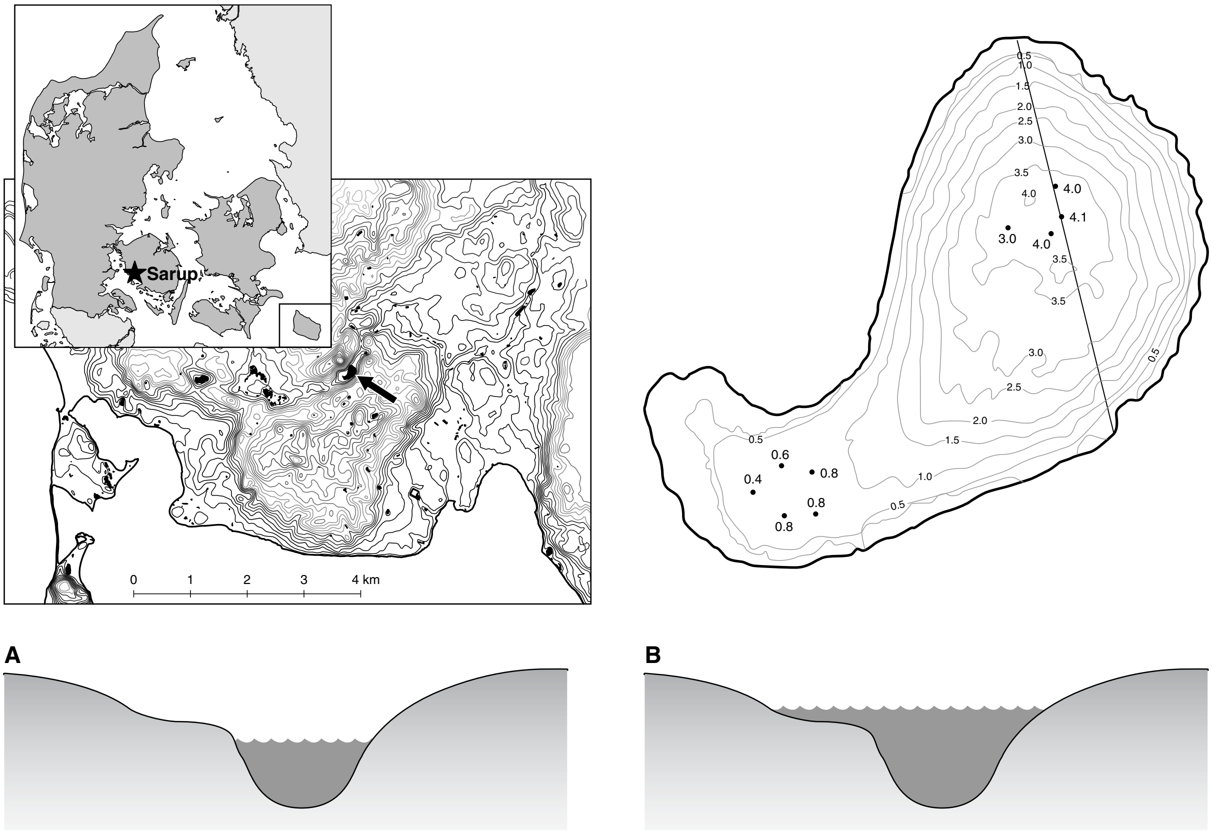

Figure 5. Location and hypsographic/typographic curves of Lake Sarup, Denmark, and its close surroundings. Schematic drawing of Lake Sarup at low-water level (**A**) and at high water level (**B)**.

Following 8225 BP the marked peak in *Betula* (birch), a pioneer readily invading new habitats, indicated an invasion of the former flooded areas. Withdrawal of the water table possibly led to improved water clarity followed by increased production, as indicated by enhanced accumulation of biological proxies and organic matter and a higher organic content in the sediment (Fig. 6). Thus, the climatic response in Lake Sarup is in accordance with the suggestion of drier conditions during the 8.2 kyr. event (Magny & Begeot, 2004; Magny et al., 2003), but contradicts interpretations from stable isotopic and pollen records in southern Sweden and Norway (Hammarlund et al., 2003; Hammarlund et al., 2005; Nesje et al., 2006; Seppa, Hammarlund & Antonsson, 2005). However the morphology of Lake Sarup and the surroundings complicate comparison with other kettle hole lakes.

In the recovery phase from climate anomaly (within the time span studied), Lake Sarup did not return to the initial state, but seemed more productive than before the climate anomaly. The evidence is a higher accumulation of sediment, higher accumulation of pigments (in particular cyanobacteria pigments and purple-sulphur bacteria pigments), higher relative abundance of cladoceran species related to meso-eutrophic conditions (e.g. *Leydigia ledigii, Alona quadrangularis*) and high abundance of Nymphaeaceae trichosclereids. The overall changes in the cladoceran community are relatively small during the studied period due to the predominance of *Bosmina longirostris* during the entire study (deep lake system). However, the decrease in this species, implicitly in the pelagic/benthic ratio, can most likely be attributed to increased relative abundance of littoral habitat (Alhonen, 1970; Hofmann, 1998; Korhola, Olander & Blom, 2000; Korhola, Tikkanen & Weckstrom, 2005).

Our study clearly shows the need for multi-proxy methods when interpreting abrupt changes in ecosystems, such as during the 8.2 kyr event. The conclusion of lake level changes would be difficult to reach solely by looking at cladoceran data.

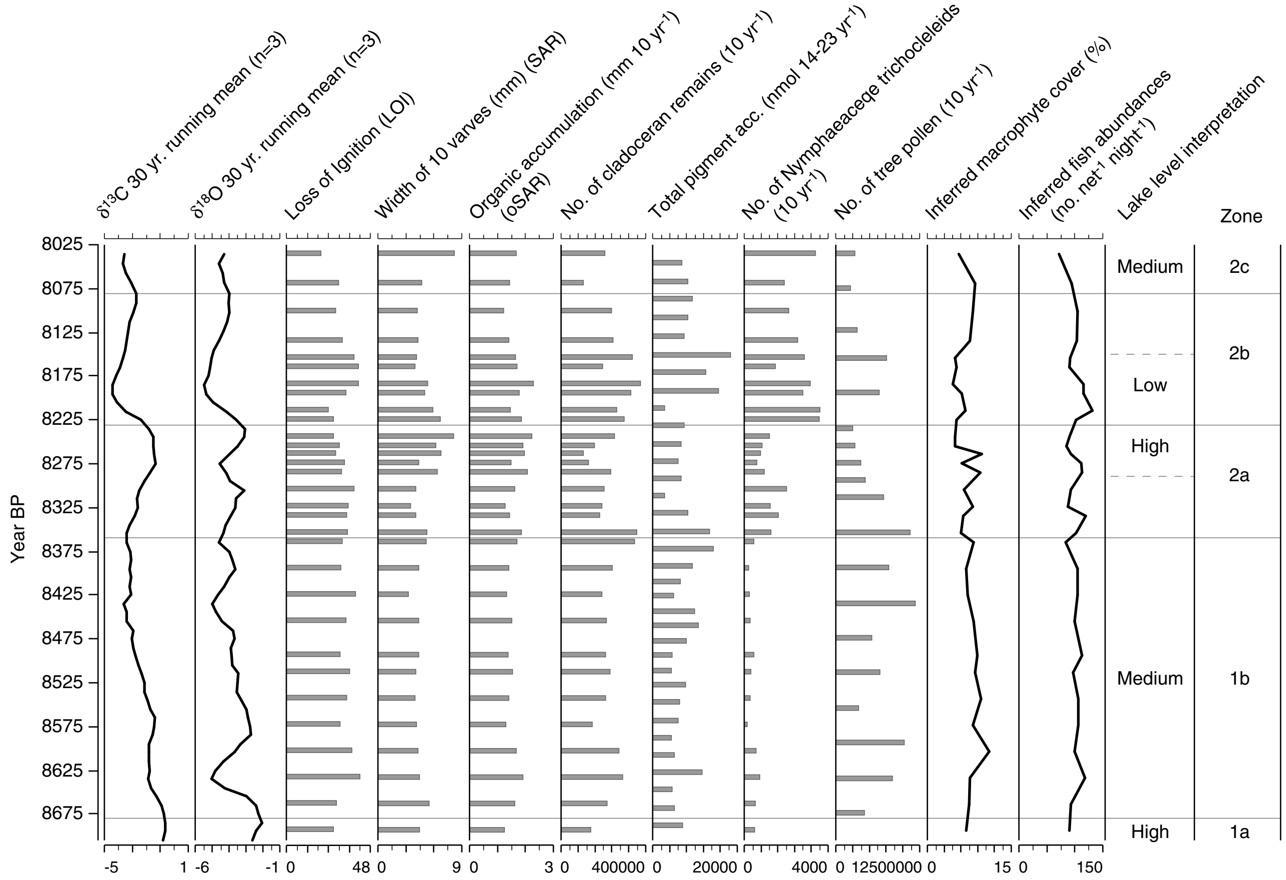

Figure 6. Stratiographical plot of stable isotopes ^{13}C and ^{18}O (‰) (30 yr. running mean, n=3), organic content (Loss of ignition) (%), thickness of 10 varves (mm), total accumulation of organic material (mm 10 yr.$^{-1}$), total accumulation of cladoceran remains (no. 10 yr.$^{-1}$), total accumulation of Nymphaeaceae trichosclereids (no. 10 yr.$^{-1}$), total accumulation of tree pollen (no 10 yr.$^{-1}$), total accumulation of algal pigments (nmol 14-23 yr.$^{-1}$), cladoceran inferred submerged macrophyte coverage (%) and fish abundance (no. net^{-1} night^{-1}) in Lake Sarup. Y-axis represent calender years before present (Paper 4).

4.2.2 Direct signal of climate

In contrast to most climate indicators, the production of ephippia (resistant resting eggs produced as a strategy for surviving environmental stresses) relative to the production of body shields, by members of the Cladocera group, can be directly related to climate or photo-period, although other factors such as intensive fish predation may also trigger the production (Carvalho & Hughes, 1983; Frey, 1982; Gyllström, 2004; Pijanowska & Stolpe, 1996; Stross & Kangas, 1969). An increased ephippia to body shield ratio has been related to colder temperature or increased length of ice-covered periods in several studies (Bennike, Sarmaja-Korjonen & Seppanen, 2004; Jeppesen et al., 2003b; Sarmaja-Korjonen, 2004; Sarmaja-Korjonen, Seppanen & Bennike, 2006). Accordingly, we found the ephippia to body shield ratio of both *Bosmina* spp. and Chydoridae to be highest in the most cold and northern lakes (Fig. 7), likely reflecting low temperature or shorter growing seasons in these lakes (Paper 5). However, food limitation could be a contributory factor, as resting egg abundance correlated negatively to chlorophyll *a* (a surrogate for phytoplankton biomass). However, using a larger gradient than in our study, Jeppesen et al. (2003b) showed that the effect of food and fish predation was of minor importance compared with changes in temperature. We expected similar results during the cold period studied in Paper 4; however, due to scarce abundance of ephippia during the whole study period (8700-8000 BP) no such relation could be identified. Also in Lake Dallund, *Bosmina* and *Daphnia* resting eggs were absent during 7000-5000 BP (Paper 2). This rarity, or absence, of ephippia could be due to a milder climate than today during the period studied (Vassiljev, Harrison & Guiot, 1998). Low sample size may also partly explain the low record in Lake Dallund (Paper 2).

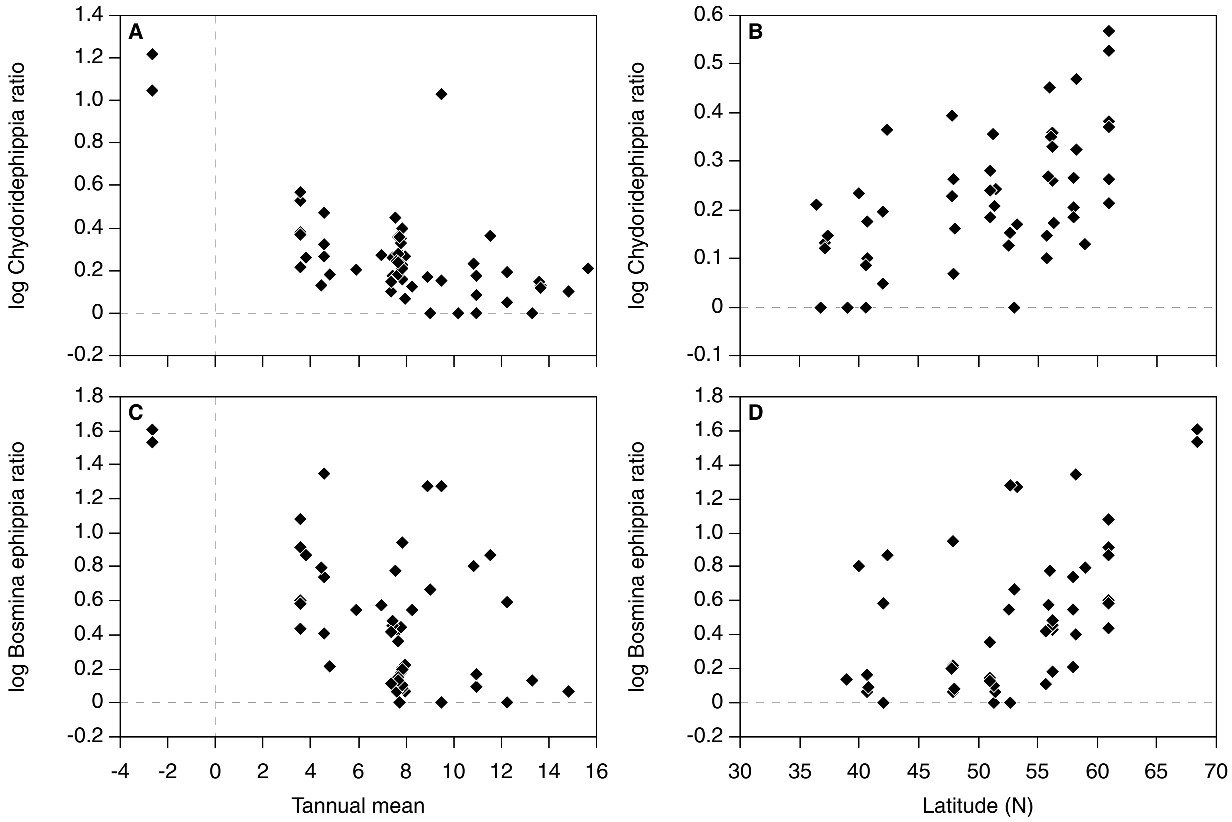

Figure 7. The ephippia to body-shield ratio of chydorids (A, B) and *Bosmina* (C, D) in relation to mean annual temperature (1961-1990) and latitude based on data from surface sediment from 54 shallow lakes covering a climate gradient from 36-68 ºN (Paper 5).

4.2.3 Indirect signals of climate

Although covering a large European climate gradient (representing mean annual temperature from -3 to +16 ºC) (Fig. 2) (Paper 5), we were not able fully to disentangle responses to climate-conductivity-trophy in the cladoceran community composition. Confounding factors were overriding a clear and direct climate effect. It is often more appropriate to regard the link between climate and the biological sedimentary record in sediments as an indirect response (Battarbee, 2000), even when encompassing large climate gradients (de Eyto et al., 2003; Gyllström et al., 2005; Jeppesen et al., 2003b; Korhola et al., 2000; Lotter et al., 1997; Sweetman & Smol, 2006) as those presented in Paper 5. Thus, in the European gradient study (Paper 5) conductivity was recognised as the main factor structuring the cladoceran assemblage based on two different multivariate analytical approaches (Redundancy Analysis (CCA) and Multivariate Regression Tree Analysis (MRT)). However, conductivity correlated closely with temperature and nutrients. Distinct cladoceran communities were present along the latitudinal gradient, separating particularly the most northern and the most southern lakes (Fig. 8), and they also differed in cladoceran size distribution. In mid-latitudinal lakes we found a somewhat weaker grouping among. These groups (Fig. 8 group 3-5) differed mainly with respect to conductivity.

The northern lakes were low-conductive acidic (pH 5-7) and showed a distinct cladoceran community composition with indicator species typical for acidic waters (e.g. *Alonella excisa, Alonopsis elongata, Alona rustica*) (Flössner, 2000; Røen, 1995). In correspondence pH and latitude were found to be the main factors influencing the chydorid fauna in a study of 54 European lakes, including 44 of the lakes included in Paper 5 (de Eyto et al., 2003). Moreover the low-conductive lakes were oligotrophic, with high light penetration, probably resulting in high benthic primary production (Liboriussen & Jeppesen, 2003; Vadeboncoeur et al., 2003) as macrophyte abundance was low. This also explains the relatively large distribution of benthic-associated cladocerans in these lakes.

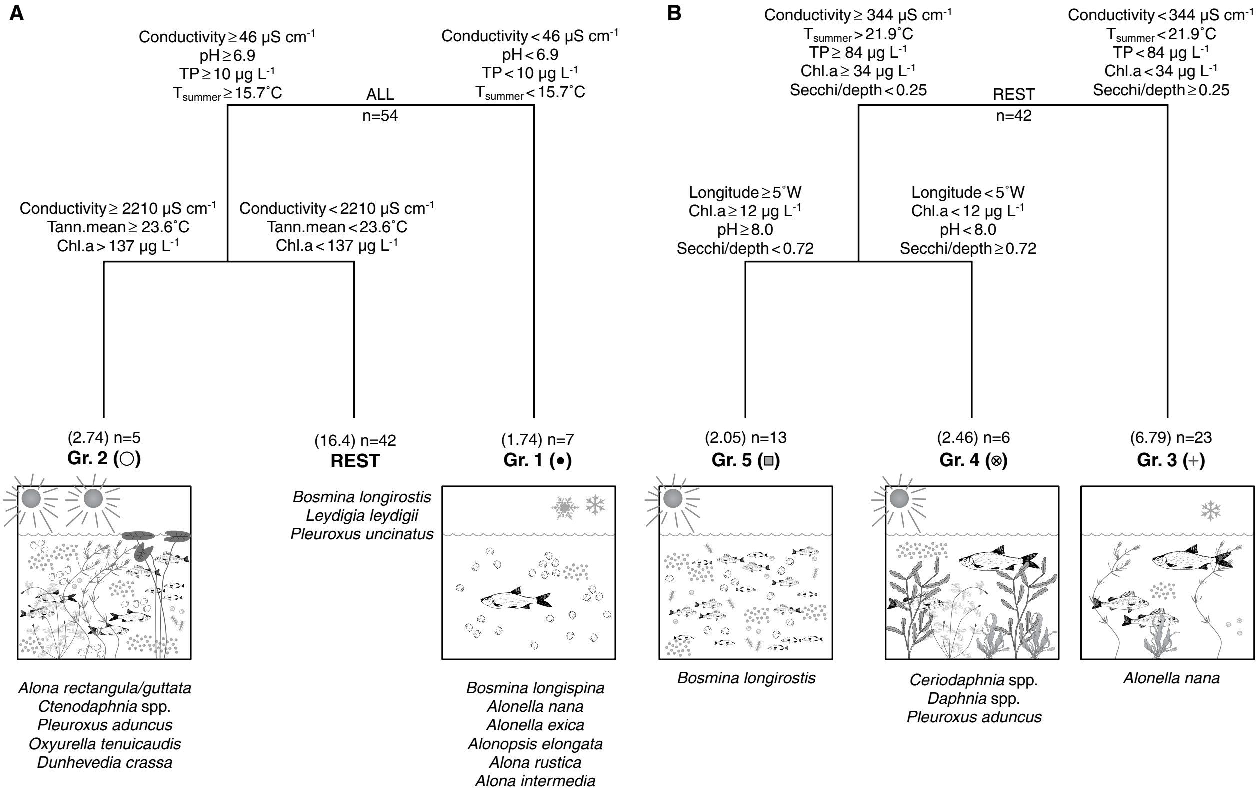

Figure 8. The resulting multivariate regression trees A: all 54 European lakes B: with the exclusion of low and high conductivity lakes. Group 1 is characterised by low-conductive, cool, northern oligotrophic lakes, dominated by the larger pelagic *Bosmina longispina*. The benthic species is probably supported by benthic production. Gr. 2 consists on high-conductive, warm, southern and eutrophic lakes with high plant cover. They are mainly dominated by small sediment-macrophyte associated cladoceran taxa. The division between group 3-5 was less strong. Group 3 is characterised by lower-conductive, colder and relatively nutrient-poor lakes with some macrophyte cover. The cladoceran community consist of both pelagic and littoral associated taxa. Group 4 resemble group 3 with respect to environmental conditions, although warmer and having higher conductivity as well as a tendency to higher macrophyte cover. Indicators are mainly taxa benefiting from macrophyte cover. Group 5 consists of higher-conductive, warmer and macrophyte-free eutrophic lakes, mainly dominated by the small pelagic *Bosmina longirostris.* Number of lakes per group (n) and indicator species are given for each group. (Modified from Paper 5).

The most southern lakes were high-conductive, saline, and were characterised by total absence of *Bosmina* and primary dominance of small benthic-macrophyte associated taxa (e.g. *Dunhevedia crassa, Oxyrella tennuicaudis, Pleuroxus aduncus*). Despite the eutrophic state of these lakes, a substantial submerged macrophyte cover was present (34-100%, except for one lake with 6%), explaining the presence of macrophyte associated species. However, the absence of larger pelagic and macrophyte associated cladoceran taxa, despite of high potential macrophyte refuge, is in contrast to findings in temperate lakes. Most likely, this absence is due to high fish predation pressure, even within the macrophyte beds, as found for Mediterranean (Castro, Marques & Goncalves, 2007) and subtropical and tropical lakes (Burks et al., 2002; Meerhoff, 2007). Thus, the differing cladoceran size distribution along the investigated gradient (north: large; south: small) probably reflected increased predation pressure towards the south. In contrast to the overall strong evidence of increasing species number towards the equator (Hillebrand, 2004; Mittelbach et al., 2007), we found a unimodal tendency along the investigated gradient. This is in correspondence with other European studies (de Eyto et al., 2003; Declerck et al., 2005) and likely reflects high conductivity and predation pressure in the southern lakes. We identified no marked species turnover, although we found some taxa only occurring in the southern lakes (e.g. *Dunhevedia crassa, Alona azorica, Trerocephala ambiqua, Moina* spp.) and some only in the northern-most lakes (*Polyphemus pediculus, Ofryoxus gracilis, Bythotrephes* spp.).

Although covering a large geographical scale, we were not able to fully distinguish between climate-conductivity and trophy related responses due to the correlative nature of the data (northern, cold, oligotrophic, low-buffered versus southern, warm, saline, eutrophic). Thus, our study highlights the complexity of disentangling a direct climate signal from indirect effects such as conductivity and predation when studying a climate gradient as proxy of future anthropogenic climate changes.

5 Concluding remarks and perspectives

Eutrophication is a widespread problem in densely populated areas such as Denmark. In 21 Danish lakes, selected as potential reference lakes according to the WFD, only 25 % showed minor changes in the communities of cladocerans and diatoms since 1850. In contrast to other Northern European studies, these lakes were already eutrophic in 1850. In fact, most of the 21 lakes had high nutrient levels and a considerable amount of their catchment was used for human activities already in 1850 and 1800, respectively. Thus, the WFD ecological state of the lakes in 1850 vas generally assessed as "moderate". Lake Dallund is an example of an early eutrophicated lake, which already showed signs of eutrophication in the early Medieval period, and eutrophication has been ongoing until lately. We therefore question the limit of 1850 as representing the reference state in the most typical Danish lake type (alkaline, eutrophic and shallow). Our study demonstrates the potential of applying a multi-proxy paleolimnological approach as a tool to define the "true" reference state in relation to the WFD.

Studies of Holocene historical abrupt climatic events, such as the 8.2 kyr cooling event, limit the confounding factors related to human impact. We found indication of lake level changes as a response to the 8.2 kyr. event in Lake Sarup. Comparisons with other Scandinavian studies of this event showed that lake responses to climatic changes may be site-specific. Due to the special morphology and catchment topography of Lake Sarup, a lake level increase was mirrored in the cladoceran community as a decrease in the relative distribution of pelagic taxa and an increase in macrophyte and sediment associated taxa. Overall, the changes in cladoceran community structure were not prominent and the application of other proxies is needed in such studies. We found that the ecological state of Lake Sarup (within the period studied) did not return to the state prior to the climate anomaly although the water level seemed to return to a level close to the initial one.

Applying cladoceran subfossils of surface sediment as a proxy for changing climate, implicitly using surface-sediment taken along a substantial climatic gradient in Europe (37-68 °N), clearly revealed differences in cladoceran structure. However, we were not able to fully disentangle the effects of temperature, conductivity and trophic level, as our study lakes were northern, cold, low-conductive and oligotrophic, while the southern lakes were warm, high-conductive and eutrophic. Thus, our study highlighted the difficulties in separating direct climate signals from anthropogenic impacts as well as the indirect effects of climate, such as conductivity, using a geographical gradient as climate-proxy.

The expected future climate change, which for Denmark is expected to appear as warmer and wetter winters, will presumably entail ecological changes as well. The wetter conditions will possibly increase the nutrient load in lakes with following cascading effects on the lake ecosystem. A warmer climate may increase the nutrient cycling and retention, enhance the growth potential for macrophytes and result in higher top-down control of grazing zooplankton (e.g. larger abundance of omnivorous and eutrophication resistant species, such as common carp (*Cyprinus carpio*)) (Jeppesen et al, 2007). As a result we expect a changed cladoceran community towards smaller size distribution and more eutrophic species, these being the main tendencies along the European climate gradient studied in this thesis. This may affect the resilience of shallow lakes and cause them to convert into a turbid state (Jeppesen et al., 2007; Mooij et al., 2005; Mooij et al., 2007). Under this predicted climate scenario, the "good" ecological state of the WFD may be difficult to obtain and the effects of ongoing lake restoration and re-oligotrophication may by counteracted. Thus, in the future lake managers should incorporate the potential effects of global climate change when setting targets for critical nutrient loading.

6 Future studies

The use of cladoceran subfossils as eutrophication indicators is fairly well established for shallow meso-eutrophic lakes. However, to quantitatively infer changes in fish abundance and macrophyte cover in less studied lake types (e.g. low alkaline or humic lakes, Paper 1) the calibration data set should be increased to include these types. Refining the models for quantitative inference of submerged macrophyte cover, based on macrophyte associated cladoceran taxa, is presently in progress (Davidson et al, submitted; S.L. Amsinck, personal communication). Also models inferring several mutual interacting variables are highly needed and some are underway (Davidson et al, submitted).

Distinguishing between natural variation and variation caused by human influence is essential when focussing on responses to anthropogenic driving forces such as global warming. Approaches that may be taken to improve our potential to distinguish between natural and anthropogenic variations could include studies of the rate of response and response rate comparisons among multiple proxies (e.g., Heegaard, Lotter & Birks, 2006). Development of analogues for defining response rates by selecting periods in fossil records exhibiting different rates of climatic changes (Anderson, 1995) is needed. High-resolution studies of long cores, preferably laminated, would in particular be beneficial when studying lake responses to historical Holocene climatic events, such as the 8.2 kyr cool event (8,200 years BP), the Medieval Warm Period (ca. 850-1250 AD), and the Little Ice Age (ca 1450-1900 AD). It may add to our understanding of lake responses and the rate of responses to differential climatic changes less confounded by eutrophication than is the case today. However, some sites may already early have responded to human impacts as is the case in Lake Dallund (Paper 2)

Application of stable isotope analysis (^{15}N, ^{13}C) of subfossil remains (e.g. cladoceran exuviae, fish scales) may provide information on the dominant sources of food intake and may potentially trace food web structure, which is related to the nutrient regime of the lake; a method widely used in contemporary studies (e.g. Vander Zanden & Rasmussen, 1999; Jeppesen, 2002c). In marine sediment ^{15}N in cladoceran exuviae (Struck et al., 1998) and fish scales (Struck et al., 2002) revealed a changed diet related to eutrophication and upwelling, respectively.

Hatching of sedimentary resting eggs (Barry et al., 2005; Courty & Vallverdu, 2001) may provide information on past adaptations to, for instance, predation pressure, salinity or temperature, thereby independently validating tendencies in other proxies. However, a major constraint is the longevity of resting eggs (decades to 300 years (Caceres, 1998; Hairston, 1996; Hairston et al., 1995, Michel et al, 2007)). Thus, the field of paleolimnology may benefit from innovative cross-use of traditional biological methods used in contemporary ecology today.

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7 References

Alhonen, P. (1970) On the significance of the planktonic/littoral ratio in the cladoceran stratigraphy of lake sediments. *Commentationes Biologicae*, **35**, 3-9.

Alley, R.B., Mayewski, P.A., Sowers, T., Stuiver, M., Taylor, K.C. & Clark, P.U. (1997) Holocene climatic instability: A prominent, widespread event 8200 yr ago. *Geology*, **25**(6), 483-86.

Amsinck, S.L., Jeppesen, E. & Landkildehus, F. (2005a) Inference of past changes in zooplankton community structure and planktivorous fish abundance from sedimentary subfossils - a study of a coastal lake subjected to major fish kill incidents during the past century. *Archiv Fur Hydrobiologie*, **162**(3), 363-82.

Amsinck, S.L., Jeppesen, E. & Landkildehus, F. (2005b) Relationships between environmental variables and zooplankton subfossils in the surface sediments of 36 shallow coastal brackish lakes with special emphasis on the role of fish. *Journal of Paleolimnology*, **33**(1), 39-51.

Amsinck, S.L., Jeppesen, E. & Ryves, D. (2003) Cladoceran stratigraphy in two shallow brackish lakes with special reference to changes in salinity, macrophyte abundance and fish predation. *Journal of Paleolimnology*, **29**(4), 495-507.

Andersen, J.H., Conley, D.J. & Hedal, S. (2004) Palaeoecology, reference conditions and classification of ecological status: the EU Water Framework Directive in practice. *Marine Pollution Bulletin*, **49**(4), 283-90.

Anderson, N.J. (1995) Using the Past to Predict the Future - Lake-Sediments and the Modeling of Limnological Disturbance. *Ecological Modelling*, **78**(1-2), 149-72.

Antonsson, U. (1992) The Structure and Function of Zooplankton in Thingvallavatn, Iceland. *Oikos*, **64**(1-2), 188-221.

Barry, M.J., Tibby, J., Tsitsilas, A., Mason, B., Kershaw, P. & Heijnis, H. (2005) A long term lake-salinity record and its relationships to Daphnia populations. *Archiv Fur Hydrobiologie*, **163**(1), 1-23.

Battarbee, R.W. (2000) Palaeolimnological approaches to climate change, with special regard to the biological record. *Quaternary Science Reviews*, **19**(1-5), 107-24.

Bennike, O., Sarmaja-Korjonen, K. & Seppanen, A. (2004) Reinvestigation of the classic late-glacial Bølling Sø sequence, Denmark: chronology, macrofossils, Cladocera and chydorid ephippia. *Journal of Quaternary Science*, **19**(5), 465-78.

Bennion, H., Juggins, S. & Anderson, N. J. (1996) Predicting epilimnetic phosphorous concentrations using an improved diatom-based transfer function and its application to lake eutrophication management. *Environmental Science & Technology*, **30**, 2004-07.

Bennion, H., Fluin, J. & Simpson, G.L. (2004) Assessing eutrophication and reference conditions for Scottish freshwater lochs using subfossil diatoms. *Journal of Applied Ecology*, **41**(1), 124-38.

Birks, H.J.B. (1998) D.G. Frey & E.S. Deevey review #1 - Numerical tools in palaeolimnology - Progress, potentialities, and problems. *Journal of Paleolimnology*, **20**(4), 307-32.

Boronat, L., Miracle, M.R. & Armengol, X. (2001) Cladoceran assemblages in a mineralization gradient. *Hydrobiologia*, **442**(1-3), 75-88.

Bos, D.G., Cumming, B.F. & Smol, J.P. (1999) Cladocera and Anostraca from the Interior Plateau of British Columbia, Canada, as paleolimnological indicators of salinity and lake level. *Hydrobiologia*, **392**(2), 129-41.

Bradshaw, E.G., Anderson, N.J., Jensen, J.P. & Jeppesen, E. (2002) Phosphorus dynamics in Danish lakes and the implications for diatom ecology and palaeoecology. *Freshwater Biology*, **47**(10), 1963-75.

Bradshaw, E.G., Rasmussen, P. & Odgaard, B.V. (2005) Mid- to late-Holocene land-use change and lake development at Dallund So, Denmark: synthesis of multiproxy data, linking land and lake. *Holocene*, **15**(8), 1152-62.

Bradshaw, E.G., Nielsen, A.B. & Anderson, N.J. (2006) Using diatoms to assess the impacts of prehistoric, pre-industrial and modern land-use on Danish lakes. *Regional Environmental Change*, **6**(1-2), 17-24.

Brodersen, K.P.W., Melburne C. and Lindegaard, Claus. (1998) Reconstruction of trophic state in Danish lakes using subfossil chydorid (Cladocera) assemblages. *Can. J. Fish. Aquat. Sci.*, **55**, 1093-103.

Brodersen, K.P. & Lindegaard, C. (1999) Classification, assessment and trophic reconstruction of Danish lakes using chironomids. *Freshwater Biology*, **42**(1), 143-57.

Brodersen, K.P., Odgaard, B.V., Vestergaard, O. & Anderson, N.J. (2001) Chironomid stratigraphy in the shallow and eutrophic Lake Sobygaard, Denmark: chironomid-macrophyte co-occurrence. *Freshwater Biology*, **46**(2), 253-67.

Brodersen, K.P., Anderson, N.J. & Odgaard, B.V. (2001) Long-term trends in the profundal chronomid-fauna in nitrogen-limited Lake Esrom, Denmark: a combined palaeolimnological/historical approach. *Archiv Fur Hydrobiologie*, **150**(3), 393-409.

Brodersen, K.P. & Anderson, N.J. (2002) Distribution of chironomids (Diptera) in low arctic West Greenland lakes: trophic conditions, temperature and environmental reconstruction. *Freshwater Biology*, **47**(6), 1137-57.

Brodersen, K.P. & Quinlan, R. (2006) Midges as palaeoindicators of lake productivity, eutrophication and hypolimnetic oxygen. *Quaternary Science Reviews*, **25**(15-16), 1995-2012.

Brooks, J.L. & Dodson, S.I. (1965) Predation Body Size and Composition of Plankton. *Science*, **150**(3692), 28-&.

Brooks, S.J. (2006) Fossil midges (Diptera : Chironomidae) as palaeoclimatic indicators for the Eurasian region. *Quaternary Science Reviews*, **25**(15-16), 1894-910.

Burks, R.L., Lodge, D.M., Jeppesen, E. & Lauridsen, T.L. (2002) Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. *Freshwater Biology*, **47**(3), 343-65.

Burns, C.W. (1968) Relationship between Body Size of Filter-Feeding Cladocera and Maximum Size of Particle Ingested. *Limnology and Oceanography*, **13**(4), 675-&.

Burns, C.W. (1969) Relation between Filtering Rate, Temperature, and Body Size in 4 Species of *Daphnia*. *Limnology and Oceanography*, **14**(5), 693-&.

Bushnell, J.H. (1974). Bryozoans (Ectoprocta). In *Pollution ecology of freshwater invertebrates* (ed C.W.a.F. Hart, S. L. H.), pp. 157-94. Academic Press, New York.

Caceres, C.E. (1998) Interspecific variation in the abundance, production, and emergence of Daphnia diapausing eggs. *Ecology*, **79**(5), 1699-710.

Carpenter, S.R., Fisher, S.G., Grimm, N.B. & Kitchell, J.F. (1992) Global Change and Fresh-Water Ecosystems. *Annual Review of Ecology and Systematics*, **23**, 119-39.

Carpenter, S.R. & Kitchell, J.F. (1992) Trophic Cascade and Biomanipulation - Interface of Research and Management - Reply. *Limnology and Oceanography*, **37**(1), 208-13.

Carvalho, G.R. & Hughes, R.N. (1983) The Effect of Food Availability, Female Culture-Density and Photoperiod on Ephippia Production in Daphnia-Magna Straus (Crustacea, Cladocera). *Freshwater Biology*, **13**(1), 37-46.

Castro, B.B., Marques, S.M. & Goncalves, F. (2007) Habitat selection and diel distribution of the crustacean zooplankton from a shallow Mediterranean lake during the turbid and clear water phases. *Freshwater Biology*, **52**(3), 421-33.

Cohen, A.S. (2003) *Paleolimnology. The Histrory and Evolution of Lake Systems* Oxford University Press, Inc., Oxford.

Courty, M.A. & Vallverdu, J. (2001) The microstratigraphic record of abrupt climate changes in cave sediments of the Western Mediterranean. *Geoarchaeology-an International Journal*, **16**(5), 467-500.

Dansgaard, W., Johnsen, S.J., Clausen, H.B., Dahl-jensen, D., Gundestrup, N.S., Hammer, C.U., Hvidberg, C.S., Steffensen, J.P., Sveinbjornsdottir, A.E., Jouzel, J. & Bond, G. (1993) Evidence for General Instability of Past Climate from a 250-Kyr Ice-Core Record. *Nature*, **364**(6434), 218-20.

Davidson TA, S.C., Perrow MR, Bramm M, Jeppesen, E. The simultaneous inference of zooplanktivorous fish and machrophyte density from subfossil cladoceran assemblages: A multiple regression tree approach. *Submitted.*

De'Ath, G. (2002) Multivariate regression trees: a new technique for modeling species-environment relationships. *Ecology*, **83**(4), 1105-17.

de Eyto, E., Irvine, K., Garcia-Criado, F., Gyllstrom, M., Jeppesen, E., Kornijow, R., Miracle, M.R., Nykanen, M., Bareiss, C., Cerbin, S., Salujoe, J., Franken, R., Stephens, D. & Moss, B. (2003) The distribution of chydorids (Branchiopoda, Anomopoda) in European shallow lakes and its application to ecological quality monitoring. *Archiv Fur Hydrobiologie*, **156**(2), 181-202.

Declerck, S., Vandekerkhove, J., Johansson, L., Muylaert, K., Conde-Porcuna, J.M., Van der Gucht, K., Perez-Martinez, C., Lauridsen, T., Schwenk, K., Zwart, G., Rommens, W., Lopez-Ramos, J., Jeppesen, E., Vyverman, W., Brendonck, L. & De Meester, L. (2005) Multi-group biodiversity in shallow lakes along gradients of phosphorus and water plant cover. *Ecology*, **86**(7), 1905-15.

Dressler, M., Hubener, T., Gors, S., Werner, P. & Selig, U. (2007) Multi-proxy reconstruction of trophic state, hypolimnetic anoxia and phototrophic sulphur bacteria abundance in a dimictic lake in northern Germany over the past 80 years. *Journal of Paleolimnology*, **37**(2), 205-19.

Fietz, S., Nicklisch, A. & Oberhansli, H. (2007) Phytoplankton response to climate changes in Lake Baikal during the Holocene and Kazantsevo Interglacials assessed from sedimentary pigments. *Journal of Paleolimnology*, **37**(2), 177-203.

Flössner, D. (2000) *Die Haplopoda und Cladocera Mitteleuropas* Backhuys Publishers, Leiden, The Netherlands.

Frey, D.G. (1982) Contrasting Strategies of Gamogenesis in Northern and Southern-Populations of Cladocera. *Ecology*, **63**(1), 223-41.

Gasiorowski, M., Szeroczynska, K. (2004) Abrupt changes in *Bosmina* (Cladocera, Crustacea) assemblages during the history of the Ostrowite Lake (northern Poland). *Hydrobiologia*, **526**, 137-44.

Gyllström, M. (2004) Induction and termination of diapause in a freshwater zooplankton community. *Archiv Fur Hydrobiologie*, **161**(1), 81-97.

Gyllström, M., Hansson, L.A., Jeppesen, E., Garcia-Criado, F., Gross, E., Irvine, K., Kairesalo, T., Kornijow, R., Miracle, M.R., Nykänen, M., Noges, T., Romo, S., Stephen, D., Van Donk, E. & Moss, B. (2005) The role of climate in shaping zooplankton communities of shallow lakes. *Limnology and Oceanography*, **50**(6), 2008-21.

Hairston, N.G., Vanbrunt, R.A., Kearns, C.M. & Engstrom, D.R. (1995) Age and Survivorship of Diapausing Eggs in a Sediment Egg Bank. *Ecology*, **76**(6), 1706-11.

Hairston, N.G. (1996) Zooplankton egg banks as biotic reservoirs in changing environments. *Limnology and Oceanography*, **41**(5), 1087-92.

Hallgren, E., Palmer, M.W. & Milberg, P. (1999) Data diving with cross-validation: an investigation of broad-scale gradients in Swedish weed communities. *Journal of Ecology*, **87**(6), 1037-51.

Hammarlund, D., Barnekow, L., Birks, H.J.B., Buchardt, B. & Edwards, T.W.D. (2002) Holocene changes in atmospheric circulation recorded in the oxygen-isotope stratigraphy of lacustrine carbonates from northern Sweden. *Holocene*, **12**(3), 339-51.

Hammarlund, D., Bjorck, S., Buchardt, B., Israelson, C. & Thomsen, C.T. (2003) Rapid hydrological changes during the Holocene revealed by stable isotope records of lacustrine carbonates from Lake Igelsjon, southern Sweden. *Quaternary Science Reviews*, **22**(2-4), 353-70.

Hammarlund, D., Bjorn, S., Buchardt, B. & Thomsen, C.T. (2005) Limnic responses to increased effective humidity during the 8200 cal. Yr BP cooling event in southern Sweden. *Journal of Paleolimnology*, **34**(4), 471-80.

Hann, B.J. (1989). Cladocera. In *Methods in Quaternary Ecology* (ed B.G. Warner), pp. 81-92. Geoscience Canada.

Harrison, S.P., Prentice, I.C. & Guiot, J. (1993) Climatic Controls on Holocene Lake-Level Changes in Europe. *Climate Dynamics*, **8**(4), 189-200.

Heegaard, E., Lotter, A.F. & Birks, H.J.B. (2006) Aquatic biota and the detection of climate change: Are there consistent aquatic ecotones? *Journal of Paleolimnology*, **35**(3), 507-18

Heinrichs, M.L. & Walker, I.R. (2006) Fossil midges and palaeosalinity: potential as indicators of hydrological balance and sea-level change. *Quaternary Science Reviews*, **25**(15-16), 1948-65.

Hilgartner, W.B. & Brush, G.S. (2006) Prehistoric habitat stability and post-settlement habitat change in a Chesapeake Bay freshwater tidal wetland, USA. *Holocene*, **16**(4), 479-94.

Hillebrand, H. (2004) On the generality of the latitudinal diversity gradient. *American Naturalist*, **163**(2), 192-211.

Hofmann, W. (1986) Developmental history of the Grosser Plöner See and Schöhsee (north Germany): cladoceran analysis, with special reference to eutrophication. *Archiv Fur Hydrobiologie*, **Supplement 74**(3), 259-87.

Hofmann, W. (1998) Cladocerans and chironomids as indicators of lake level changes in north temperate lakes. *Journal of Paleolimnology*, **19**(1), 55-62.

Hofmann, W. & Winn, K. (2000) The littorina transgression in the Western Baltic Sea as indicated by subfossil Chironomidae (Diptera) and Cladocera (Crustacea). *International Review of Hydrobiology*, **85**(2-3), 267-91.

Ilyashuk, E.A., Ilyashuk, B.P., Hammarlund, D. & Larocque, I. (2005) Holocene climatic and environmental changes inferred from midge records (Diptera : Chironomidae, Chaoboridae, Ceratopogonidae) at Lake Berkut, southern Kola Peninsula, Russia. *Holocene*, **15**(6), 897-914.

IPCC. (2001) *Climate Change 2001: The Scientific Basis. Contribution of Working Group 1 to the Third Assessment Report of the Intergovernmental Panel on Climate Change* Cambrigde University Press,, Cambrigde, United Kingdom and New York, NY, USA .

Irvine, K., Moss, B. & Balls, H. (1989) The Loss of Submerged Plants with Eutrophication .2. Relationships between Fish and Zooplankton in a Set of Experimental Ponds, and Conclusions. *Freshwater Biology*, **22**(1), 89-107.

Jeppesen, E., Madsen, E.A., Jensen, J.P. & Anderson, N.J. (1996) Reconstructing the past density of planktivorous fish and trophic structure from sedimentary zooplankton fossils: A surface sediment calibration data set from shallow lakes. *Freshwater Biology*, **36**(1), 115-27.

Jeppesen, E., Jensen, J.P., Søndergaard, M., Lauridsen, T., Pedersen, L.J. & Jensen, L. (1997) Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia*, **342**, 151-64.

Jeppesen, E. (1998) *The Ecology of Shallow Lakes - Trophic Interactions in the Pelagial.* Doctor's dissertation (DSc). National Environmental Research Institute. NERI Technical Report 247

Jeppesen, E., Jensen, J.P., Søndergaard, M. & Lauridsen, T. (1999) Trophic dynamics in turbid and clearwater lakes with special emphasis on the role of zooplankton for water clarity. *Hydrobiologia*, **409**, 217-31.

JJeppesen, E., Christoffersen, K., Landkildehus, F., Lauridsen, T., Amsinck, S.L., Riget, F. & Søndergaard, M. (2001a) Fish and crustaceans in northeast Greenland lakes with special emphasis on interactions between Arctic charr (Salvelinus alpinus), Lepidurus arcticus and benthic chydorids. *Hydrobiologia*, **442**(1-3), 329-37.

Jeppesen, E., Jensen, J.P., Skovgaard, H. & Hvidt, C.B. (2001b) Changes in the abundance of planktivorous fish in Lake Skanderborg during the past two centuries - a palaeoecological approach. *palaeogeography Palaeoclimatology Palaeoecology*, **172**(1-2), 143-52.

Jeppesen, E., Jensen, J.P., Amsinck, S., Landkildehus, F., Lauridsen, T. & Mitchell, S.F. (2002a) Reconstructing the historical changes in Daphnia mean size and planktivorous fish abundance in lakes from the size of Daphnia ephippia in the sediment. *Journal of Paleolimnology*, **27**(1), 133-43.

Jeppesen, E., Christoffersen, K., Malmquist, H.J., Faafeng, B., Hansson, L.-A. (2002b). Ecology of Five Faroese Lakes. Summary and Synthesis. In *Five Faroese Lakes. Physico-Chemical and Biological Aspects. Annales Societatis Scientiarum Færoensis.* (ed K. Christoffersen, Jeppesen, E., Enckell, P.H., Bloch, D.), Vol. Supplementum XXXVI, pp. 126-39. Føroya Fródskaparfelag.

Jeppesen, E., Landkildehus, F., Lauridsen, T.L., Jensen, J.P, Bjerring, R., Søndergaard, M. & Amsinck, S.L. (2002c). Food Web Interactions in Five Faroese. Lakes tracked by Stable Isotopes. In *Five Faroese Lakes. Physico-Chemical and Biological Aspects.* (ed K. Christoffersen, Jeppesen, E., Enckell, P.H. & Bloch, D.), Vol. Annales Societatis Scientiarum Færoensis. Supplementum XXXVI, pp. 114-25. Føroya Fródskaparfelag.

Jeppesen, E., Jensen, J.P., Jensen, C., Faafeng, B., Hessen, D.O., Søndergaard, M., Lauridsen, T., Brettum, P. & Christoffersen, K. (2003a) The impact of nutrient state and lake depth on top-down control in the pelagic zone of lakes: A study of 466 lakes from the temperate zone to the arctic. *Ecosystems*, **6**(4), 313-25.

Jeppesen, E., Jensen, J.P., Lauridsen, T.L., Amsinck, S.L., Christoffersen, K., Søndergaard, M. & Mitchell, S.F. (2003b) Sub-fossils of cladocerans in the surface sediment of 135 lakes as proxies for community structure of zooplankton, fish abundance and lake temperature. *Hydrobiologia*, **491**(1-3), 321-30.

Jeppesen, E., Søndergaard, M., Jensen, J.P., Havens, K.E., Anneville, O., Carvalho, L., Coveney, M.F., Deneke, R., Dokulil, M.T., Foy, B., Gerdeaux, D., Hampton, S.E., Hilt, S., Kangur, K., Kohler, J., Lammens, E., Lauridsen, T.L., Manca, M., Miracle, M.R., Moss, B., Noges, P., Persson, G., Phillips, G., Portielje, R., Schelske, C.L., Straile, D., Tatrai, I., Willen, E. & Winder, M. (2005) Lake responses to reduced nutrient loading - an analysis of contemporary long-term data from 35 case studies. *Freshwater Biology*, **50**(10), 1747-71.

Jeppesen, E., Søndergaard, M., Meerhoff, M., Lauridsen, T.L. & Jensen, J.P. (2007) Shallow lake restoration by nutrient loading reduction - some recent findings and challenges ahead. *Hydrobiologia*, **584**, 239-52.

Johansson, L.S., Amsinck, S.L., Bjerring, R. & Jeppesen, E. (2005) Mid- to late-Holocene land-use change and lake development at Dallund So, Denmark: trophic structure inferred from cladoceran subfossils. *Holocene*, **15**(8), 1143-51.

Koff, T., Punning, J.M., Sarmaja-Korjonen, K. & Martma, T. (2005) Ecosystem response to early and late Holocene lake-level changes in Lake Juusa, Southern Estonia. *Polish Journal of Ecology*, **53**(4), 553-70.

Korhola, A. (1992) The Early Holocene hydrosere in a small acid hill-top basin studied using crustacean sedimentary remains. *Journal of Paleolimnology*, **7**, 1-22.

Korhola, A. (1999) Distribution patterns of Cladocera in subarctic Fennoscandian lakes and their potential in environmental reconstruction. *Ecography*, **22**(4), 357-73.

Korhola, A., Olander, H. & Blom, T. (2000) Cladoceran and chironomid assemblages as qualitative indicators of water depth in subarctic Fennoscandian lakes. *Journal of Paleolimnology*, **24**(1), 43-54.

Korhola, A., Weckstrom, J., Holmstrom, L. & Erasto, P. (2000) A quantitative Holocene climatic record from diatoms in northern Fennoscandia. *Quaternary Research*, **54**(2), 284-94.

Korhola, A., Vasko, K., Toivonen, H.T.T. & Olander, H. (2002) Holocene temperature changes in northern Fennoscandia reconstructed from chironomids using Bayesian modelling. *Quaternary Science Reviews*, **21**(16-17), 1841-60.

Korhola, A., Tikkanen, M. & Weckstrom, J. (2005) Quantification of Holocene lake-level changes in Finnish Lapland using a cladocera - lake depth transfer model. *Journal of Paleolimnology*, **34**(2), 175-90.

Langeland, A. & Nost, T. (1995) Gill Raker Structure and Selective Predation on Zooplankton by Particulate Feeding Fish. *Journal of Fish Biology*, **47**(4), 719-32.

Larocque, I. & Hall, R.I. (2003) Chironomids as quantitative indicators of mean July air temperature: validation by comparison with century-long meteorological records from northern Sweden. *Journal of Paleolimnology*, **29**(4), 475-93.

Lauridsen, T., Jeppesen, E., Landkildehus, F. & Søndergaard, M. (2001) Horizontal distribution of cladocerans in arctic Greenland lakes - impact of macrophytes and fish. *Hydrobiologia*, **442**(1-3), 107-16.

Leira, M., Jordan, P., Taylor, D., Dalton, C., Bennion, H., Rose, N. & Irvine, K. (2006) Assessing the ecological status of candidate reference lakes in Ireland using palaeolimnology. *Journal of Applied Ecology*, **43**(4), 816-27.

Liboriussen, L. & Jeppesen, E. (2003) Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and a turbid shallow lake. *Freshwater Biology*, **48**(3), 418-31.

Lotter, A.F., Birks, H.J.B., Hofmann, W. & Marchetto, A. (1997) Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps .1. Climate. *Journal of Paleolimnology*, **18**(4), 395-420.

Lotter, A.F., Birks, H.J.B., Hofmann, W. & Marchetto, A. (1998) Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. II. Nutrients. *Journal of Paleolimnology*, **19**(4), 443-63.

Lotter, A.F., Walker, I.R., Brooks, S.J. & Hofmann, W. (1999) An intercontinental comparison of chironomid palaeotemperature inference models: Europe vs North America. *Quaternary Science Reviews*, **18**(6), 717-35.

Magny, M., Begeot, C., Guiot, J. & Peyron, O. (2003) Contrasting patterns of hydrological changes in Europe in response to Holocene climate cooling phases. *Quaternary Science Reviews*, **22**(15-17), 1589-96.

Magny, M. & Begeot, C. (2004) Hydrological changes in the European midlatitudes associated with freshwater outbursts from Lake Agassiz during the Younger Dryas event and the early Holocene. *Quaternary Research*, **61**(2), 181-92.

Malmquist, H.J., Ingimasson, F., Jóhannsdóttir, E. E., Gíslason, D., Snorrason, S.S. (2002). Biology of Brown Trout (*Salmo trutta*) and Arctic Charr (*Salvelinus alpinus*) in Four Faroese Lakes. In *Annales Societatis Scientiarum Færoensis Supplementum XXXVI. Five Faroeses Lakes* (ed E.J. K. Christoffersen, P. H. Enckell, D. Bloch), pp. 94-113. Føroya Fróoskaparfelag, Torshaun.

Manca, M.A., M. (2002) Zooplankton of 15 lakes in the Southern Central Alps: comparison of recent and past (pre-*ca* 1850 AD) communities. *Journal of Limnology*, **61**(2), 225-31.

Mason, I.M., Guzkowska, M.A.J., Rapley, C.G. & Streetperrott, F.A. (1994) The Response of Lake Levels and Areas to Climate-Change. *Climatic Change*, **27**(2), 161-97.

McDermott, F., Mattey, D.P. & Hawkesworth, C. (2001) Centennial-scale holocene climate variability revealed by a high-resolution speleothem delta O-18 record from SW Ireland. *Science*, **294**(5545), 1328-31.

McGowan, S., Leavitt, P.R., Hall, R.I., Anderson, N.J., Jeppesen, E. & Odgaard, B.V. (2005) Controls of algal abundance and community composition during ecosystem state change. *Ecology*, **86**(8), 2200-11.

Meerhoff, M., Iglesias, C., Teixeira De Mello, F., Clemente, J.M., Jensen, E., Lauridsen, T.L. & Jeppesen, E. (2007) Effects of habitat complexity on community structure and predator avoidance behaviour of littoral zooplankton in temperate versus subtropical shallow lakes. *Freshwater Biology*, **52**, 1009-21.

Michels, H., Amsinck, S.L., Jeppesen, E. Meester, L.D. (2007) Interclonal variation in diel horizontal migration behavior of the water flea Daphnia magna - searching for a signature of adaptive evolution. . *Hydrobiologia*, in press.

Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., McCain, C.M., McCune, A.R., McDade, L.A., McPeek, M.A., Near, T.J., Price, T.D., Ricklefs, R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M. & Turelli, M. (2007) Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters*, **10**(4), 315-31.

Mooij, W.M., Hulsmann, S., Domis, L.N.D., Nolet, B.A., Bodelier, P.L.E., Boers, P.C.M., Pires, L.M.D., Gons, H.J., Ibelings, B.W., Noordhuis, R., Portielje, R., Wolfstein, K. & Lammens, E. (2005) The impact of climate change on lakes in the Netherlands: a review. *Aquatic Ecology*, **39**(4), 381-400.

Mooij, W.M., Janse, J.H., Domis, L., Hulsmann, S. & Ibelings, B.W. (2007) Predicting the effect of climate change on temperate shallow lakes with the ecosystem model PCLake. *Hydrobiologia*, **584**, 443-54.

Moss, B. (1998) *Ecology of Fresh Waters. Man and Medium, Past to Future*, Third edn. Blackwell Science Ltd., Oxford.

Nesje, A., Bjune, A.E., Bakke, J., Dahl, S.O., Lie, O. & Birks, H.J.B. (2006) Holocene palaeoclimate reconstructions at Vanndalsvatnet, western Norway, with particular reference to the 8200 cal. yr BP event. *Holocene*, **16**(5), 717-29.

O'Brien, W.J., Barfield, M., Bettez, N.D., Gettel, G.M., Hershey, A.E., McDonald, M.E., Miller, M.C., Mooers, H., Pastor, J., Richards, C. & Schuldt, J. (2004) Physical, chemical, and biotic effects on arctic zooplankton communities and diversity. *Limnology and Oceanography*, **49**(4), 1250-61.

O'Sullivan, P.E. (1983) Anually-laminated lake sediments and the study of quaternary environmental changes - A review. *Quaternary Science Reviews*, **1**, 245-313.

Odgaard, B.V. & Rasmussen, P. (2000) Origin and temporal development of macro-scale vegetation patterns in the cultural landscape of Denmark. *Journal of Ecology*, **88**(5), 733-48.

Økland, K.A. & Økland, J. (2002) Freshwater bryozoans (Bryozoa) of Norway III: distribution and ecology of Plumatella fruticosa. *Hydrobiologia*, **479**(1), 11-22.

Persson, L. & Eklov, P. (1995) Prey Refuges Affecting Interactions between Piscivorous Perch and Juvenile Perch and Roach. *Ecology*, **76**(1), 70-81.

Pijanowska, J. & Stolpe, G. (1996) Summer diapause in Daphnia as a reaction to the presence of fish. *Journal of Plankton Research*, **18**(8), 1407-12.

Porter, S.C., Sauchyn, D.J. & Delorme, L.D. (1999) The ostracode record from Harris Lake, southwestern Saskatchewan: 9200 years of local environmental change. *Journal of Paleolimnology*, **21**(1), 35-44.

Punning, J.M. & Puusepp, L. (2007) Diatom assemblages in sediments of Lake Juusa, Southern Estonia with an assessment of their habitat. *Hydrobiologia*, **586**, 27-41.

Rasmussen, P., Bradshaw, E. & Odgaard, B.V. (2002) Fortidens miljø arkiveret år for år. Fund af varvige sedimenter i Sarup Sø på Fyn. *Naturens Verden*, **5**, 34-40

Rasmussen, P. (2005) Mid- to late-Holocene land-use change and lake development at Dallund So, Denmark: vegetation and land-use history inferred from pollen data. *Holocene*, **15**(8), 1116-29.

Rosen, P., Segerstrom, U., Eriksson, L., Renberg, I. & Birks, H.J.B. (2001) Holocene climatic change reconstructed from diatoms, chironomids, pollen and near-infrared spectroscopy at an alpine lake (Sjuodjljaure) in northern Sweden. *Holocene*, **11**(5), 551-62.

Røen, U.I. (1995) *Gællefødder og Karpelus.* Dansk Naturhistorisk Forening, Vinderup Bogtrykkeri A/S, Vinderup, Denmark.

Sarmaja-Korjonen, K. & Alhonen, P. (1999) Cladoceran and diatom evidence of lake-level fluctuations from a Finnish lake and the effect of acquatic-moss layers on microfossil assemblages. *Journal of Paleolimnology*, **22**(3), 277-90.

Sarmaja-Korjonen, K. & Hyvarinen, H. (2002) Subfossil littoral Cladocera as indicators of brackish-water Littorina transgression of the Baltic Basin in a small lake in Finland. *Boreas*, **31**(4), 356-61.

Sarmaja-Korjonen, K., Kultti, S., Solovieva, N. & Valiranta, M. (2003) Mid-Holocene palaeoclimatic and palaeohydrological conditions in northeastern European Russia: a multi-proxy study of Lake Vankavad. *Journal of Paleolimnology*, **30**(4), 415-26.

Sarmaja-Korjonen, K. (2004) Chydorid ephippia as indicators of past environmental changes - a new method. *Hydrobiologia*, **526**(1), 129-36.

Sarmaja-Korjonen, K., Nyman, M., Kultti, S. & Valiranta, M. (2006) Palaeolimnological development of Lake Njargajavri, northern Finnish Lapland, in a changing Holocene climate and environment. *Journal of Paleolimnology*, **35**(1), 65-81.

Sarmaja-Korjonen, K., Seppanen, A. & Bennike, O. (2006) Pediastrum algae from the classic late glacial Bolling So site, Denmark: Response of aquatic biota to climate change. *Review of Palaeobotany and Palynology*, **138**(2), 95-107.

Scheffer, M., Hosper, S.H., Meijer, M.L., Moss, B. & Jeppesen, E. (1993) Alternative Equilibria in Shallow Lakes. *Trends in Ecology & Evolution*, **8**(8), 275-79.

Schindler, D.W. (1977) Evolution of Phosphorus Limitation in Lakes. *Science*, **195**(4275), 260-62.

Schindler, D.W. (1997) Widespread effects of climatic warming on freshwater ecosystems in North America. *Hydrological Processes*, **11**(8), 1043-67.

Seppa, H., Hammarlund, D. & Antonsson, K. (2005) Low-frequency and high-frequency changes in temperature and effective humidity during the Holocene in south-central Sweden: implications for atmospheric and oceanic forcings of climate. *Climate Dynamics*, **25**(2-3), 285-97.

Shumate, B.C., Schelske, C.L., Crisman, T.L. & Kenney, W.F. (2002) Response of the cladoceran community to trophic state change in Lake Apopka, Florida. *Journal of Paleolimnology*, **27**(1), 71-77.

Smol, J.P. (1992) Paleolimnology: an important tool for effective ecosystem management. *Journal of Aquatic Ecosystem Health: Rational, Challenges, and Strategies*, **1**, 49-58.

Søndergaard, M., Moss, B. (1997) *Impact of Submerged Macrophytes on Phytoplankton in Shallow Freshwater Lakes* K. Springer.

Søndergaard, M., Jeppesen, E., Jensen, J.P., Bradshaw, E., Skovgaard, H. & Grünfeld, S. (2003) *Vandrammedirektivet og danske søer. Del 1: Søtyper, referencetilstand og økologiske kvalitetsklasser* Danmarks Miljøundersøgelser.

Søndergaard, M., Jeppesen, E., Jensen, J.P. & Amsinck, S.L. (2005) Water framework directive: Ecological classification of danish lakes. *Journal of Applied Ecology*, **42**(4), 616-29.

Stross, R.G. & Kangas, D.A. (1969) Reproductive Cycle of Daphnia in an Arctic Pool. *Ecology*, **50**(3), 457-&.

Struck, U., Voss, M., von Bodungen, B. & Mumm, N. (1998) Stable isotopes of nitrogen in fossil cladoceran exoskeletons: Implications for nitrogen sources in the central Baltic Sea during the past century. *Naturwissenschaften*, **85**(12), 597-603.

Struck, U., Altenbach, A.V., Emeis, K.C., Alheit, J., Eichner, C. & Schneider, R. (2002) Changes of the upwelling rates of nitrate preserved in the delta N-15-signature of sediments and fish scales from the diatomaceous mud belt of Namibia. *Geobios*, **35**(1), 3-11.

Sweetman, J.N. & Finney, B.P. (2003) Differential responses of zooplankton populations (Bosmina longirostris) to fish predation and nutrient-loading in an introduced and a natural sockeye salmon nursery lake on Kodiak Island, Alaska, USA. *Journal of Paleolimnology*, **30**(2), 183-93.

Sweetman, J.N. & Smol, J.P. (2006) Patterns in the distribution of cladocerans (Crustacea : Branchiopoda) in lakes across a north-south transect in Alaska, USA. *Hydrobiologia*, **553**, 277-91.

Taylor, D., Dalton, C., Leira, M., Jordan, P., Chen, G., Leon-Vintro, L., Irvine, K., Bennion, H. & Nolan, T. (2006) Recent histories of six productive lakes in the Irish Ecoregion based on multiproxy palaeolimnological evidence. *Hydrobiologia*, **571**, 237-59.

Thoms, M.C., Ogden, R.W. & Reid, M.A. (1999) Establishing the condition of lowland floodplain rivers: a palaeo-ecological approach. *Freshwater Biology*, **41**(2), 407-23.

Timms, R.M. & Moss, B. (1984) Prevention of Growth of Potentially Dense Phytoplankton Populations by Zooplankton Grazing, in the Presence of Zooplanktivorous Fish, in a Shallow Wetland Ecosystem. *Limnology and Oceanography*, **29**(3), 472-86.

Vadeboncoeur, Y., Jeppesen, E., Vander Zanden, M.J., Schierup, H.H., Christoffersen, K. & Lodge, D.M. (2003) From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. *Limnology and Oceanography*, **48**(4), 1408-18.

van Tongeren, O.F.R. (1995). Cluster analysis. In *Data Analysis in Community and Landscape Ecology* (ed C.J.F.T.B. R. H. G. Jongman, O. F. R. van Tongeren), pp. 174-212. Cambrigde University Press, Cambridge.

Vander Zanden, M.J. & Rasmussen, J.B. (1999) Primary consumer delta C-13 and delta N-15 and the trophic position of aquatic consumers. *Ecology*, **80**(4), 1395-404.

Vassiljev, J. (1998) The simulated response of lakes to changes in annual and seasonal precipitation: implication for Holocene lake level changes in northern Europe. *Climate Dynamics*, **14**(11), 791-801.

Vassiljev, J., Harrison, S.P. & Guiot, J. (1998) Simulating the Holocene lake-level record of Lake Bysjon, southern Sweden. *Quaternary Research*, **49**(1), 62-71.

Vermaire, J.C.G.-E., I. (2007) Reconstructing changes in macrophyte cover in lakes across the northeastern United States based on sedimentary diatom assemblages. *Journal of Paleolimnology*, DOI 10.1007/s10933-007-9125-y. In press.

Verschuren, D., Tibby, J., Sabbe, K. & Roberts, N. (2000) Effects of depth, salinity, and substrate on the invertebrate community of a fluctuating tropical lake. *Ecology*, **81**(1), 164-82.

Walker, I.R., Mott, R. J., Smol, J. P. (1991) Allerød - Younger Dryas Lake Temperatures from Midge Fossils in Atlantic Canada *Science*, **253**(5023), 1010-12.

Wetzel, R.G. (2001) *Limnology, Lake and River Ecosystems*, 3rd edn. Academic Press, New York.

Whiteside, M.C. & Swindoll, M.R. (1988) Guidelines and Limitations to Cladoceran Paleoecological Interpretations. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **62**(1-4), 405-12.

Wissel, B., Boeing, W.J. & Ramcharan, C.W. (2003) Effects of water color on predation regimes and zooplankton assemblages in freshwater lakes. *Limnology and Oceanography*, **48**(5), 1965-76.

Wissel, B., Yan, N.D. & Ramcharan, C.W. (2003) Predation and refugia: implications for Chaoborus abundance and species composition. *Freshwater Biology*, **48**(8), 1421-31.

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Inferring recent changes in the ecological state of 21 Danish candidate reference lakes (EU Water Framework Directive) using palaeolimnology

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Summary

1. The European Water Framework Directive (WFD) requires that all European waterbodies obtain "good" ecological state by 2015 as judged primarily from biological indicators. So far, the five different ecological state categories of the WFD have only been vaguely defined. A suggested approach for defining the "high" status is to identify reference sites minimally impacted by human activities over time.

2. We selected the pre-industrial status at 1850 AD as reference state. Changes in ecological state during the last 150 years were analysed using a palaeolimnological approach in 21 Danish lakes assumed to be relatively low human impacted. Sediment samples representing the years 1850, 1900, 1950 and 2000 were analysed for diatoms and cladoceran subfossils. Existing transfer functions were used to infer key ecological variables for lake ecological state, i.e. total phosphorous concentrations from diatoms (DI-TP), submerged macrophyte coverage (SUB-COV) and benthi-planktivorous fish (BP-CPUE) abundance from subfossils of cladocerans.

3. Most lakes underwent major changes in diatom and cladoceran community structure during 1850-2000, especially during the most recent 50-year period. A higher accumulation rate of sediment and cladoceran subfossils and a higher ratio of pelagic to benthic taxa of diatoms and cladocerans indicated increasing eutrophication since 1850. Most lakes were characterised by high and stable DI-TP (median of 21 lakes =86 µg TP L^{-1}) and inferred BP-CPUE and low inferred SUB-COV since 1850.

4. *Synthesis and applications*. The study demonstrates that definition of the reference state (1850) may be questionable for lake types in a densely populated country such as Denmark. Less than 30% of the study lakes were in a "good" state in 1850 based on the proposed Danish WFD classification. Lakes with minimal change since 1850 were all nutrient-rich already in 1850, likely due to early eutrophication, and thus cannot be considered true reference sites by using 1850 as a target for the reference state. The study demonstrates the potential of applying a multi-proxy paleolimnological approach as a tool to define the reference state in relation to the WFD.

Introduction

Today, lakes are subject to intense public and political debate world-wide, mainly because their usage for recreational purposes has shown visible degradative changes. With the implementation of the EU Water Framework Directive (WFD), all natural water bodies are to show "good" status by 2015 (European Union 2000). In Denmark, excess nutrient loading from sewage and agricultural run-off has generated highly eutrophic conditions in many lakes. Contemporary monitoring data series are often too short to cover the reference state and typically only the largest and most abundant types of water bodies have been monitored (e.g. 38% of lakes >5 ha, 1.3% of lakes between 0.1-5 ha and

0.5% of lakes between 0.01-0.1 ha) (Lauridsen *et al.* 2005; Søndergaard *et al.* 2005b). Therefore, knowledge of smaller and rarer lake types is limited.

Palaeolimnological studies may serve as an alternative approach when time series are insufficient or absent (Anderson 1995). Such studies may provide important information on the onset and the rate of change in physico-chemical and biological processes within the water body assessed. Diatoms and cladoceran subfossils have been applied as ecological indicators (Battarbee 1986; Anderson 1995; Jeppesen *et al.* 2001) and for the quantitative reconstruction of variables of key importance to the ecological state of lakes; *i.e.* total phosphorous concentration (TP) (Bennion *et al.* 1996; Brodersen 1998), pH (Birks *et al.* 1990), submerged macrophyte cover (Jeppesen 1998) and fish abundance (BP-CPUE) (Jeppesen *et al.* 1996). Submerged macrophytes are vital to maintain a good state in shallow temperate lakes as they contribute to species diversity by providing microhabitats (Declerck *et al.* 2005), serve as a refuge for zooplankton against predation, possibly enhancing the grazing pressure on phytoplankton, and have a stabilising role in maintaining a clear water stage (Timms & Moss 1984; Søndergaard & Moss 1997). Also BP-CPUE may be indicative of ecological state, as high abundance signals high predation pressure on zooplankton and thus lower grazing of nuisance algae (Brooks & Dodson 1965; Jeppesen *et al.* 1997), leading to low water clarity. Furthermore, benthivorous fish may also increase sediment nutrient release and enhance lake turbidity by their predation on benthic invertebrates and through excretions (Jeppesen *et al.* 1997; Tarvainen *et al.* 2005).

For the purpose of defining a WFD reference state, palaeolimnological approaches have recently been applied in studies on British, Irish and Finnish lakes, involving comparisons of present and pre-industrial subfossil communities of diatoms and cladocerans (Bennion *et al.* 2004; Simpson *et al.* 2005; Leira *et al.* 2006; Räsänen 2006; Taylor *et al.* 2006). These studies found that only few lakes represented the WFD's reference state with respect to eutrophication (Finland, Scotland, Ireland) and acidification (UK, Ireland). We used a similar approach based on both diatom and cladoceran subfossils, but supplemented by inference of biological key variables (macrophyte, fish). We aimed at exploring lake changes since 1850 (time resolution of 50 years) in 21 Danish relatively low nutrient-impacted soft water and alkaline lakes with different land cover.

Materials and methods

Study sites

Well dated (^{210}Pb) sediment cores from 21 Danish lakes representing different lake types were obtained in a previous study (Nielsen 2003; 2004; Nielsen & Sugita 2005). These sites were selected (*i*) to be widely distributed (Fig. 1) and of relatively uniform size (all being small, between 3 and 30 ha with the exception of Lake Hostrup (210 ha)), (*ii*) to have no major inlets and a relatively long water retention time to obtain relatively low human and agricultural impact, and (*iii*) to be relatively deep for their size (Table 1), allowing reasonable dating.

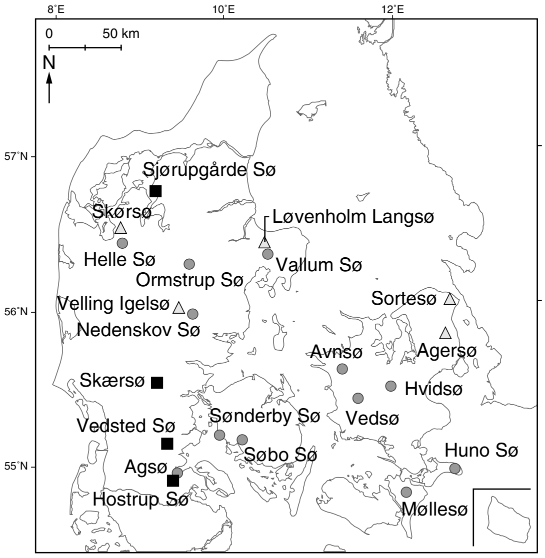


Fig. 1 Location of the 21 lakes in Denmark. Filled circles: Alkaline lakes (●), low alkaline coloured lakes (Δ), low alkaline clear water lakes (■).

Based on contemporary data from the last 5-10 years (Table 1) and the thresholds set for the Danish proposal regarding the WFD (Søndergaard *et al.* 2005b, Amsinck *et al.* 2003) we divided the lakes into three types: moderately to highly alkaline lakes: ALK (12 lakes), low alkaline clear water lakes: LACW (4 lakes) and low alkaline coloured lakes: LAC (5 lakes). As expected, their catchments were generally less impacted by humans compared to usual Danish conditions, with lower than average proportions of agricultural land and built-up areas (Table 1).

Table 1 Mean, median, minimum and maximum values of land cover variables (% of total lake catchment) and physico-chemical variables sampled between 1992 and 2002 in the 21 lakes divided into lake types. Aggregated variables MAN=agriculture+built-up area for year 2000 and year 1800, respectively. The percentage cover in 2000 of the total area of Denmark (DK) is given for each land cover variable, n denotes number of observations.

Variable	Lake type	Mean	Median	25% percentile	75% percentile	Min	Max	n
Area (ha)	ALK	11.2	9.8	6.2	12.4	5.0	26.7	12
	LACW	59.7	11.9	7.0	64.5	5.0	210.0	4
	LAC	9.3	8.8	3.7	9.5	3.5	20.8	5
Mean depth (m)	ALK	3.2	3.4	2.4	3.8	1.5	5.1	12
	LACW	2.8	2.1	1.4	5.0	1.4	5.0	3
	LAC	2.5	2.6	1.5	3.6	1.0	4.0	4
Secchi depth (m)	ALK	1.4	1.2	0.7	1.6	0.4	4.0	12
	LACW	1.5	1.5	0.8	2.2	0.6	2.4	4
	LAC	1.3	1.3	0.4	2.3	0.3	2.5	4
Total N (mg l^{-1})	ALK	1.44	1.19	1.10	2.00	0.52	3.03	10
	LACW	1.12	1.13	0.92	1.33	0.84	1.40	4
	LAC	0.88	0.77	0.61	1.20	0.45	1.37	5
Total P (mg l^{-1})	ALK	0.239	0.080	0.059	0.203	0.020	1.500	12
	LACW	0.063	0.060	0.050	0.075	0.050	0.080	4
	LAC	0.075	0.039	0.016	0.092	0.015	0.214	5
Chlorophyll *a* (µg l^{-1})	ALK	49	38	20	61	6	140	11
	LACW	31	29	17	46	13	53	4
	LAC	49	14	10	37	8	174	5
Total alkalinity (mmol l^{-1})	ALK	2.49	2.60	2.03	3.26	1.20	3.37	5
	LACW	0.44	0.41	0.26	0.62	0.20	0.74	4
	LAC	0.13	0.15	0.06	0.21	0.01	0.22	4
pH	ALK	8.4	8.4	8.3	8.7	7.9	8.8	9
	LACW	7.5	7.5	7.0	8.1	6.9	8.1	4
	LAC	6.4	6.2	5.9	7.5	4.3	7.9	5
Ecological classification (WFD) 1-5	ALK	3.9¤	4.0					12
	LACW	2.7#	2.8					4
	LAC	2.4*	2.0					5
Agricutural area (%) (DK: 68.3% of total area)	All lakes	35.8	41.6	6.4	61.1	0	80.2	18
Built-up area (%) (DK: 9.6%)	All lakes	5.2	2.7	1.1	6.7	0	21.3	18
Woodland and heathland area (%) (DK: 9.6%)	All lakes	32.6	28.3	10.8	55.5	0.0	89.0	18
Plantation & meadow area (%) (DK: 7.4%)	All lakes	8.2	3.5	0.2	8.0	0	46.1	18
MAN (%) (DK: 77.9%)	All lakes	41.0	44.5	8.0	71.4	0.0	82.6	18
	ALK	53.3	58.8	33.5	73.3	2.2	81.1	11
	LACW	67.5	-	-	-	82.6	52.4	2
	LAC	3.3	0.1	0.0	7.8	0	8.6	4
MAN (%) **year 1800**	ALK	52.9	48.3	36.7	73.3	23.2	77.7	11
	LACW	28.3	-	-	-	43.4	13.3	2
	LAC	13.4	4.1	0.7	5.3	0	57.0	4

* Classification based on total phosphor (TP) threshold only (1-5: high, good, moderate, poor, bad)

Classification based on thresholds of TP, total N, Chl *a*, Secchi (one lake only on TP)

¤ Classification based on thresholds of TP, total N, Chl *a*, Secchi, pH (6 lakes based on all thresholds, 3 lakes on 4 thresholds, 2 lakes on 2 thresholds). Thresholds were in accordance to Søndergaard *et al.* (2005b) and Amsinck *et al.* (2003).

Their location upstream in the watersheds also implies a relatively low nutrient impact compared to downstream lakes. Thus, they may potentially be as close to the reference state as can be found in Denmark, though the assessment of their ecological status (1-5, representing high-bad for one group of lakes (Table 1)) averaged 4 (ALK), 3 (LACW) and 2.4 (LAC) in the three lake groups based on the recent contemporary data.

Sampling and laboratory procedures

The sediment cores were taken from the centre of each lake between 1999 and 2001 using a combination of a HON Kajak corer (Renberg 1991) for the upper sediments and a Russian corer (Jowsey 1966) for longer cores. The cores were sliced at 2 cm intervals, and chronologies were established based on ^{210}Pb and ^{137}Cs dating of 5-9 samples per core. Errors on the earliest dates range from AD 1932 9 years to AD 1898 19 years (Nielsen & Sugita 2005). The ^{210}Pb chronologies were extrapolated back to AD 1850 by assuming a constant sediment accumulation rate below the base of the ^{210}Pb record. Sediment samples from four periods were selected: 1850, 1900, 1950 and the present (designated as year 2000) for analysis of diatom and cladoceran subfossils. The sediment accumulation rate was estimated by linear interpolation between dated samples. Further details on sediment sampling and dating can be found in Nielsen (2003).

Samples were prepared for diatom analysis following Renberg (1990) and slides were analysed under microscope (phase contrast, 1000x). Taxonomy followed several sources including Krammer & Lange-Bertalot (1986-1991) and pelagic diatom taxa were defined as taxa known to spend at least part of their life span in the pelagic (e.g. Bradshaw & Andersen 2003). Counts of at least 300 diatom valves were made and all taxa except unidentified valves were included in the data analysis.

For analysis of cladoceran subfossils (> 80 μm), approximately 5 g (wet weight) sediment was heated in 10% KOH for 20 minutes. Total counts of relatively rare fragments were performed on the 140 μm fraction to obtain reliable counts, while more common fragments were counted on subsamples (1-40% of total sample) from 80 and 140 μm fractions. Fragments were taxonomically identified in accordance with Frey (1959) and Flössner (2000) using a binocular microscope (100x) and an inverted light microscope (320x), and the most representative fragment of each taxa in all 21 lakes was used for the data analysis. The dry weight of each sample was measured to correct for water content, and accumulation of pelagic and benthic cladoceran taxa was expressed as number of fragments cm^{-2} year^{-1} (counts g^{-1} DW multiplied by accumulation rate). Cladocerans were separated into pelagic and benthic species according to Flössner (2000).

Data analysis

Between-year differences in the relative accumulation of pelagic and benthic cladoceran taxa (total number of cladoceran subfossils identified: 119,834, representing 49 taxa) were tested by paired t-tests of difference of means between two periods on ln-transformed counts for each lake type separately. The community change between the periods was calculated as squared chi-square dissimilarity (SCD) coefficients for diatoms and cladocerans (using the program ANALOG version 1.6 (H.J.B. Birks & J.M. Line, unpublished)). The SCD ranges from 0 (two identical species compositions) to 2 (two totally different species compositions). The critical limit to define sites with low community change was estimated based on the 5th percentile of the SCD distribution (see Bennion *et al.* 2004, Flower *et al.* 1997) between the 21 lakes within each year (2000, 1950, 1900 and 1850). In a comparative study of Irish lakes (Leira *et al.* 2006) the 2.5 percentile of SCD was chosen as the critical limit based on SCDs of a database of unimpacted lakes. Such independent information was not available for Danish lakes and the more conservative 5th percentile was therefore chosen, being SCD < 0.13 for cladocerans and SCD < 0.69 for diatoms.

Detrended correspondence analysis (DCA) was applied and showed gradient lengths > 3 SD units. The direction and magnitude of change in the community assemblage for each lake during the period 1850 to 2000 were determined by correspondence analysis (CA). Down-weighting of rare species was applied for diatoms due to high taxa richness (160 taxa), whereas for cladocerans (39 taxa) taxa present in at least three lakes were included. Univariate linear regression between CA-axis 1 scores (year 2000) and pH (n=18) and TP (n=21) was performed. Canonical correspondence analysis (CCA) was applied for year 2000 data with pH, TP and Chl *a* as environmental variables (all available for 17 lakes). TP and Chl *a* were log-transformed. SCD coefficients DCAs, CAs and CCAs were performed on percentage relative abundance for diatoms and cladoceran taxa to allow comparison of results. All ordinations were performed using CANOCO 4.5 (ter Braak & Smilauer 2002).

Table 2 Median values of sediment accumulation rate (g dw m^{-2} year^{-1}), accumulation rate of pelagic and benthic cladoceran fragments (cm^{-2} y^{-1}), relative abundance of pelagic cladoceran and diatom species (%), diatom-inferred total phosphorous (µg L^{-1}) and cladoceran-inferred benthi-planktivorous fish (BP-CPUE) abundance (number net^{-1} night^{-1}) and submerged macrophyte coverage (%) in year 1850. Range is given in brackets. No value available indicated by – .

1850	ALK	LACW	LAC
Sediment accumulation rate	359.5 (27.5-1632.6)	195.3 (149-680)	62.7 (50-460)
Accumulation of pelagic cladocerans	1339 (118-22715)	217 (129-364)	197 (8-922)
Accumulation of benthic cladocerans	1296 (41-11748)	267 (96-292)	133 (31-501)
Relative abundance of pelagic diatoms	87.2 (12.5-97.5)	14.9 (1.6-29.0)	49.7 (3.7-77.8)
Relative abundance of pelagic cladocerans	62.8 (42.9-92.5)	52.2 (33.2-64.0)	58.9 (9.0-67.1)
Diatom-inferred TP	94 (54-166)	61 (22-89)	- (11-17)
Cladoceran-inferred BP_CPUE	68 (37-133)	- (34)	- (73)
Cladoceran-inferred submerged macrophyte cover	4 (2-40)	- (5-20)	28 (11-63)

For inference of TP, WA models based on data sets including *i)* the total diatom assemblage (n=152 Northwest European lakes) (Bennion *et al.* 1996) and *ii)* the pelagic diatom assemblage (n= 29 Danish lakes) (Bradshaw *et al.* 1996), respectively, were used. For inference of SUB-COV and BP-CPUE, WA models based on data sets of *i)* macrophytes and macrophyte-sediment associated taxa (n= 13 taxa, n=19 Danish lakes) and *ii)* pelagic cladocerans (n=6 taxa, n= 31 lakes), respectively, were applied. Paired t-tests of difference of means were used to test for significant changes in ln-transformed inferred values between two periods. Estimation of the five EU ecological status classes of the lakes in 1850 was based on inferred values of TP and fish abundance according to thresholds for Danish lakes given in Søndergaard *et al.* (2005b) and Amsinck *et al.* (2003).

Historical data on land cover of catchments around 1800 AD for 18 (11 ALK, 5 LAC and 2 LACW lakes) of the 21 lakes was digitised from 1:20,000 scale parish maps (from 1770-1820) using the GIS software 'ArcInfo' (Nielsen 2003; Nielsen & Sugita 2005) and used as an approximation of the land cover concerning the 1850 samples. The percentages of land cover types were calculated on topographical catchment basis (Bradshaw *et al.* 2006). Modern land cover data of the lake catchments was derived from 1:25,000 digital map AIS (Aerial Information System) based on data collected during 1992-1999. Land cover was categorised into agricultural area (incl. dry grassland) (AGRI), heathland, built-up areas, other lakes in the catchment, woodland, plantations, meadows, bogs and unclassified for the total catchment and within an 1800 m radius from the centre of the lake (Bradshaw *et al.* 2006). Lake-specific percentages of change in heavily man-impacted areas (MAN: AGRI+ built-up areas; total catchment and 1800 m radius) between 1800 and 2000 were related to community changes in diatoms and cladocerans in the 18 lakes from 1850-2000.

Results

Accumulation of sediment and cladoceran subfossils

At the time of the selected reference state in 1850 the sediment accumulation rate (g m^{-2} year^{-1}) as well as the accumulation of pelagic (7 taxa) and benthic (32 taxa) cladoceran subfossils were highest in the ALK lakes, medium in the LACW lakes and lowest in the LAC lakes (Table 2). Paired t-test of difference of means of two periods showed that except for cladoceran pelagic taxa in LAC lakes the median of all accumulation rates increased significantly from 1850 to 2000 in all lake groups (Table 3). Additionally, the ALK lakes showed a significant increase in the sediment accumulation rate for each 50-year period as well as for pelagic cladoceran taxa from 1950-2000 (median 2535 and 7730 fragments g^{-1} cm^{-2}, respectively) (Fig. 2 A, Table 3).

The LACW lakes showed the most pronounced changes for both pelagic and benthic taxa: median pelagic taxa increased significantly from 1900 (median 238 fragments g^{-1} cm^{-2}) to 1950 (median 586 fragments g^{-1} cm^{-2}) (Table 3), whereas median benthic cladoceran accumulation increased significantly from 1950 (median 210 fragments g^{-1} cm^{-2}) to 2000 (median 1621 fragments g^{-1} cm^{-2}).

.

Table 3 Results of paired t-test on between-year differences in ln-transformed sediment accumulation rate (g dw m^{-2} $year^{-1}$) as well as ln-transformed number of fragments (cm^{-2} y^{-1}), pelagic and benthic cladoceran species, testing the relative change different from zero for each lake type separately (ALK: Alkaline lakes, LACW: Low Alkaline Clear Water lakes, LAC: Low Alkaline Coloured lakes). Only significant values are shown.

Lake type	Variable tested	Period	DF	t	P-value
	Sediment accumulation rate				
ALK		1850-1900	11	3.38	0.0062
		1900-1950	11	4.85	0.0005
		1950-2000	11	2.84	0.0161
LACW		1850-2000	3	4.33	0.0228
LAC		1900-1950	4	3.68	0.0212
		1850-2000	4	3.46	0.0258
	Cladoceran taxa accumulation				
ALK	Pelagic	1950-2000	11	2.14	0.0558
		1850-2000	11	3.15	0.0093
	Benthic	1850-2000	11	2.65	0.0225
LACW	Pelagic	1900-1950	3	3.62	0.0362
		1850-2000	3	4.47	0.0208
	Benthic	1950-2000	3	8.07	0.0040
		1850-2000	3	7.89	0.0042
LAC	Benthic	1850-2000	4	3.15	0.0345

The highest relative increase in cladoceran fragments occurred in ALK Lake Avn (40 times from 1850 to 2000).

Relative abundance of diatom and cladoceran subfossils

In 1850, pelagic taxa of diatoms (ALK lakes) and cladocerans (ALK, LAC lakes) dominated (Table 2, Fig. 2). Generally, the relative abundance of pelagic diatom and cladoceran taxa in ALK and LACW lakes increased during 1850-2000 (Fig 2 D & E), although this was only reflected in a marked increase in the 25th percentile for diatoms in the ALK lakes. In contrast, there are indications of a decrease in the median percentage of pelagic diatom taxa between 1850-1950 in the LAC lakes (median 50% and 33%, respectively) and between 1900-1950 for cladocerans (median 70% and 51%, respectively). In both types of low alkaline lakes, LAC and LACW, the distance between the 25th and 75th percentile in the relative abundance of pelagic diatom taxa increased towards recent time, whereas the opposite was seen for the ALK lakes.

Community change: dissimilarity analyses

There was a tendency for the median SCD coefficient of the diatom and cladoceran taxa assemblages to increase over time in the ALK lakes, reaching a critical limit during 1950-2000 (Fig. 2 F & G). Diatoms in the LAC and LACW lakes showed less difference in median SCD coefficient between the 50-year periods than the ALK lakes (Fig. 2 & 3) where only the cladoceran taxa assemblage showed an SCD median higher than the critical value between 50-year periods (Fig. 2G). Some lakes showed only negligible changes in taxa assemblage (ALK: Vedsø, Hvidsø, Huno Sø, LAC: Sorte Sø), whereas others displayed more significant changes (e.g. ALK: Ormstrup Sø, Møllesø, LACW: Vedsted Sø, Skærsø Sjørupgårde Sø, LAC: Velling Igelsø) (Fig. 4). For the majority of the study lakes SCD varied between proxies (Fig. 4). However, lakes exhibiting modest community changes showed similar changes in SCD. These lakes had high TP values already in 1850.

Community change: CA

In 1850 the LAC lakes were separated from the rest of the lakes on CA axis 1 in both diatom CA (n=160 taxa, n=21 lakes, $\lambda1= 0.736$) and cladoceran CA (n=36 taxa, n=20 lakes, $\lambda1=0.699$). Lake Sjørupgårde Sø was excluded from the cladoceran CA due to difficulties in identifying the abundant *Bosmina* (*Eubosmina*) to species level. The CA axis 1 scores of year 2000 correlated positively with summer mean pH for both diatoms and cladocerans (linear regression: F=65.65, P <0.0001, n=18 lakes and F=23.56, P =0.0002, n=17 lakes, respectively). In addition, CA axis 2 for diatoms (eigenvalue 0.625) correlated positively with contemporary TP (summer mean) (Linear regression: F=8.36, P <0.0094, n=21 lakes). No relation with TP was found for cladocerans, although the clear water species *Rhynchotalona falcata* as well as two macrophyte-associated taxa (*Acroperus, Graptoleberis testudinaris*) correlated positively with cladoceran CA axis 2. In the CCA (n=17 lakes) pH of year 2000 solely explained 16% and 28% of the total species variation of diatoms (total species variation = 33%) and cladocerans (total species variation = 30%), respectively, whereas TP solely explained 8% of the diatom species variation.

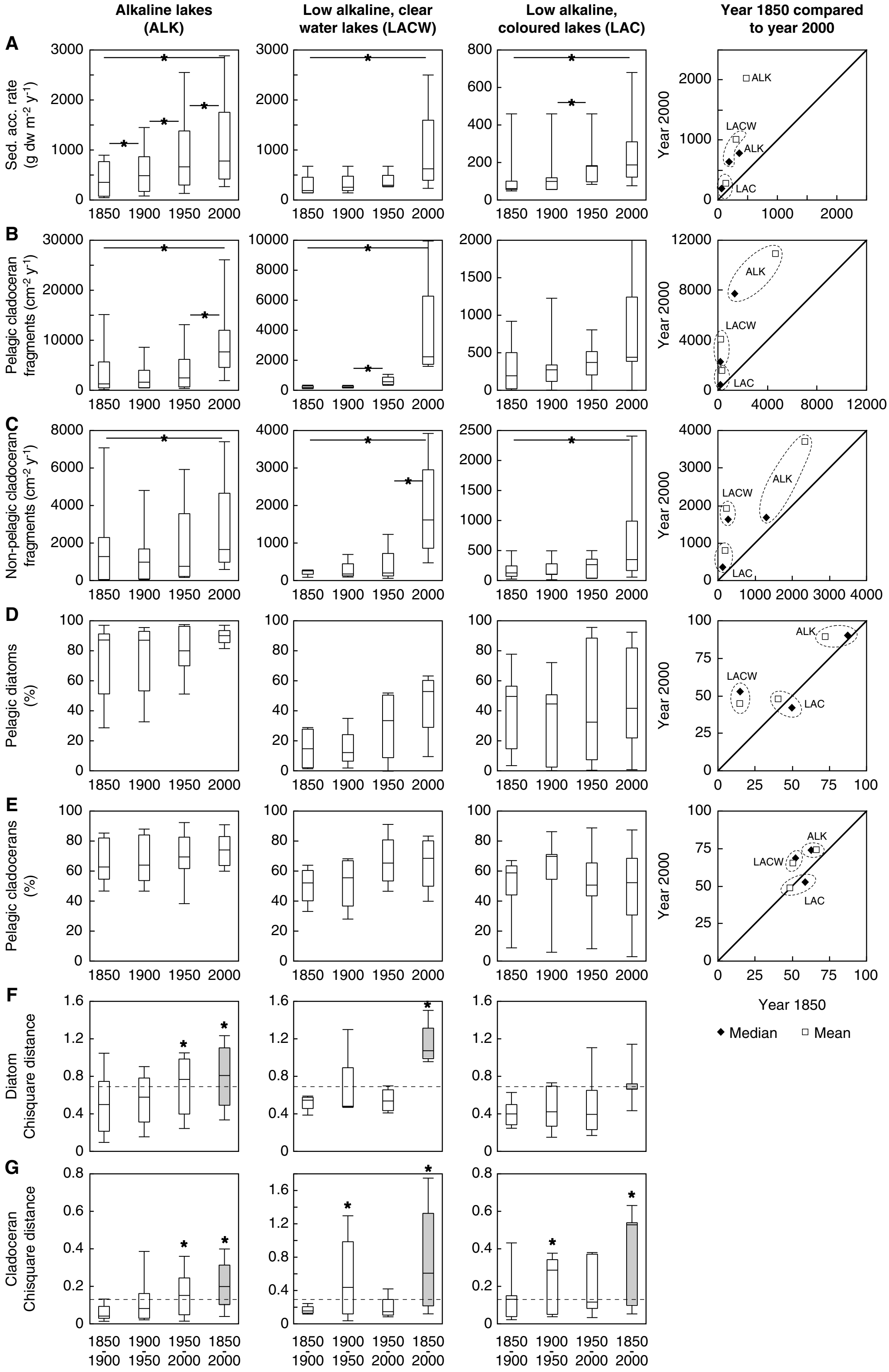


Fig. 2. Boxplots showing median, 25th and 75th quartiles; whiskers represent 10th and 90th percentiles for each year in 21 lakes and for each lake type (ALK: Alkaline lakes, LACW: Low Alkaline Clear Water lakes, LAC: Low Alkaline Coloured lakes). **A** Sediment accumulation (g dw m^{-2} $year^{-1}$), **B** Accumulation of pelagic cladoceran fragments (fragments cm^{-2} y^{-1}), **C** Accumulation of benthic cladoceran fragments (fragments cm^{-2} y^{-1}), **D** Percentage pelagic diatoms, **E** Percentage pelagic cladocerans, **F** Dissimilarity of diatoms (squared chi-square distance (SCD)) between 50-year intervals and 1850-2000 (grey) and **G** Dissimilarity of cladocerans (squared chi-square distance) between 50-year intervals and 1850-2000 (grey). ----*---- refers to significant difference at the 5% level. * refers to SCD higher than the critical level (dotted line in **F** and **G**). Comparison between 1850 and 2000 values of A-E for all lake types (mean (□) and median (♦)) is shown in the last figure column.

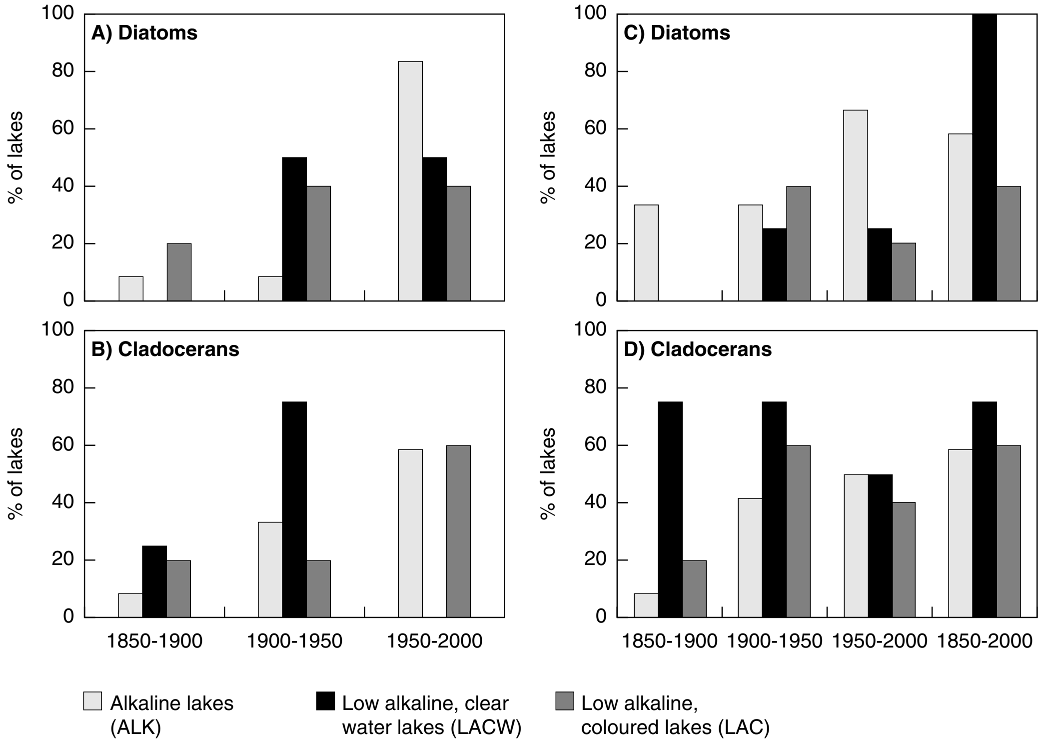


Fig. 3. Percentage of lakes within each lake type showing maximum lake specific community change (squared chi-square distance (SCD)) between 1850-1900, 1900-1950 and 1950-2000: **A** Diatoms, **B** Cladocerans. Percentage of lakes within each lake type with SCD coefficients > critical SCD values: **C** Diatoms, **D** Cladocerans.

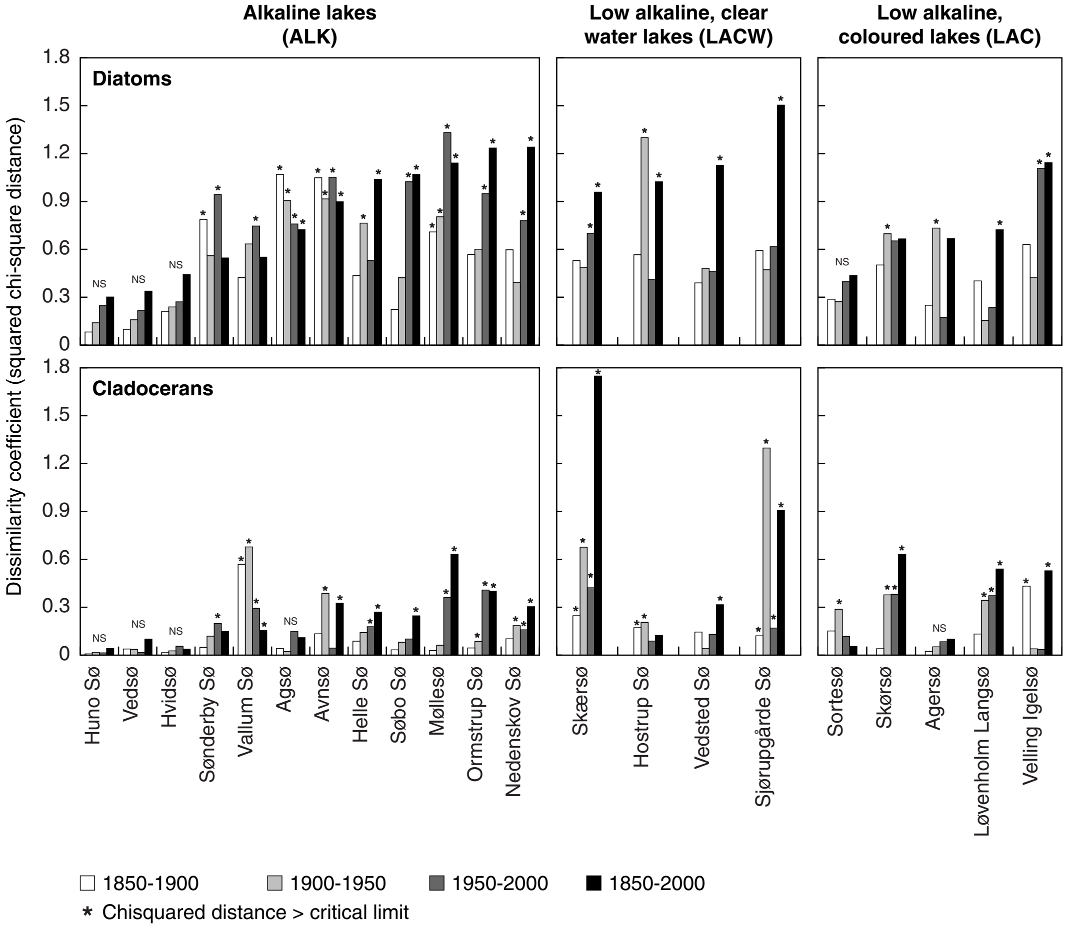
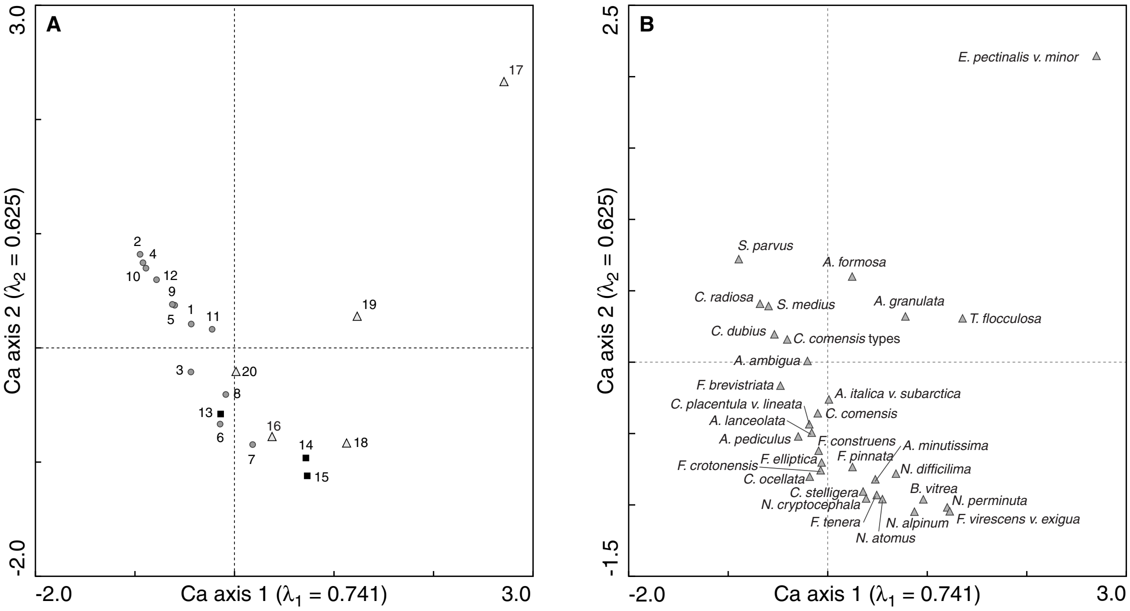
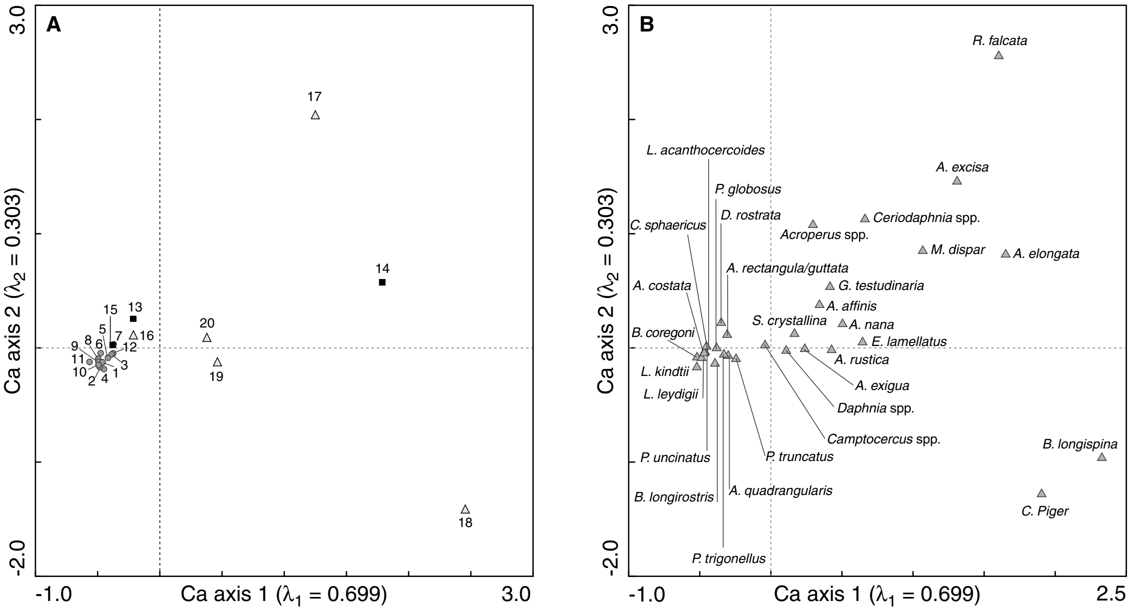


Fig. 4. Lake-specific community changes (squared chi-square distance) between 50-year periods and from 1850-2000 sorted after increasing total diatom community change (1850-2000) from left to right within each lake type (ALK: Alkaline lakes, LACW: Low Alkaline Clear Water lakes, LAC: Low Alkaline Coloured lakes). **A** Diatoms, **B** Cladocerans. * refers to SCD higher than the estimated critical level.

1 Diatoms



2 Cladocerans



● Alkaline lakes (ALK)

1. Agsø
2. Avnsø
3. Helle Sø
4. Huno Sø
5. Hvidsø
6. Møllesø
7. Nedenskov Sø
8. Ormstrup Sø
9. Søbo Sø
10. Sønderby Sø
11. Vallum Sø
12. Vedsø

■ Low alkaline, clear water lakes (LACW)

13. Hostrup Sø
14. Skærsø
15. Vedsted Sø

△ Low alkaline, coloured lakes (LAC)

16. Agersø
17. Løvenholm Langsø
18. Skørsø
19. Sortesø
20. Velling Igelsø

Fig. 5. CA ordination plots of sites (A) and taxa (B) in year 1850. 1 Diatoms, 2 Cladocerans.

Inferred TP, SUB-COV and BP-CPUE

DI-TP was inferred for 17 lakes only, as Nedenskov, Løvenholm Langsø, Skørsø and Sortesø were excluded due to poor analogue matching with both DI-TP calibration data sets. The inferred values based on pelagic taxa (n=29 sites) were significantly higher than those inferred on the total diatom assemblage (n=152 sites). No difference in means were found testing the H0: $\mu_{pelagic}$-(μ_{total} +20 μg L^{-1})=0 (paired t-test). The inferred DI-TP based on the total diatom assemblage was selected for further analysis due to the larger sample size of this calibration data set. Using DI-TP, only two lakes (Ager Sø, Skær Sø) could be classified as being in "good" state (Søndergaard *et al.* 2005b) in 1850. Generally, DI-TP values were high for both LACW and ALK lakes in 1850 (Table 2). Over time, no significant change in DI-TP was observed between lake types, although ALK lakes showed a marginally significant increase in the DI-TP median from 1900 (median 94 μg L^{-1}) to 1950 (median 129 μg L^{-1}) (t =2.16, *P* =0.06, DF=10, back transformed median relation$_{1950/1900}$=1.50) (Fig. 6 B). A separate test on LAC lakes was not performed as DI-TP was only estimated for two of the lakes within this group.

SUB-COV was inferred for only 13 of the study lakes: the remaining 8 lakes (mainly LAC and LACW lakes) contained communities poorly represented in the SUB-COV calibration data set. The inferred SUB-COV in 1850 was generally low for both LACW (n=4) and ALK lakes (n=9) (Table 2) and remained low until the present (Fig. 6D). However, the inter-period relative differences in median SUB-COV were significantly lower than 1 between 1850 and 2000 (median=5%, range: 2-40% and median=3%, range: 1-24%) (paired t-test: t =-4.99, *P* =0.001, DF=8, back transformed median relation$_{2000/1850}$=0.74), suggesting a significant decrease in SUB-COV in the ALK lakes (although the median difference was only 2%) (Fig. 6 D).

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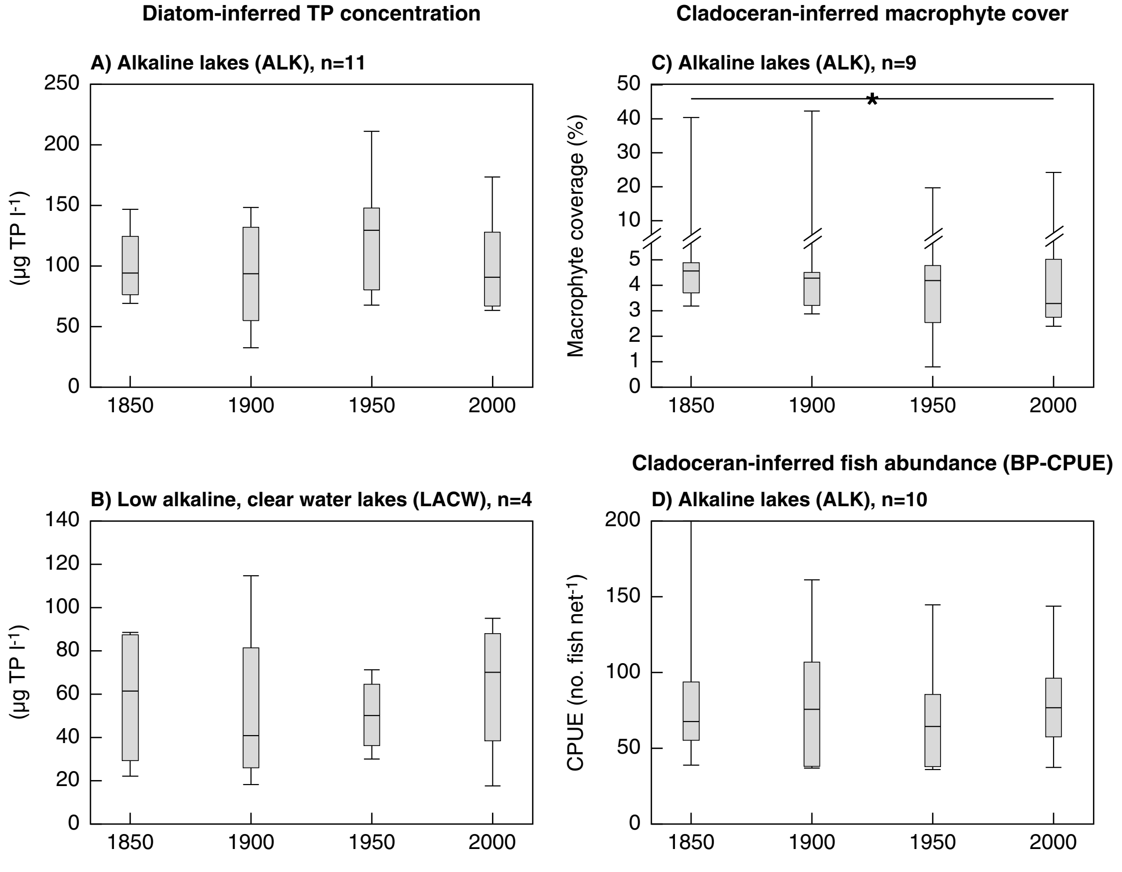

Fig. 6. Boxplots showing median, 25th and 75th quartiles; whiskers represent 10th and 90th percentiles for each year. **A**: Diatom-inferred total phosphorous (DI-TP) values of ALK lakes (Alkaline Lakes), **B**: DI-TP values of LACW lakes (Low Alkaline Clear Water lakes), **C**: Cladoceran-inferred submerged macrophyte cover in ALK lakes, **D**: Cladoceran-inferred benthi-planktivorous fish (BP-CPUE) abundance in ALK lakes. ----*---- refers to significant difference at the 5% level.

BP-CPUE was inferred for only 12 lakes (mainly ALK lakes) due to poor analogue matching between the surface sediments and the calibrations data set. Inference of BP-CPUE in the ALK lakes (n=10) showed high fish abundance already in 1850 (Table 2, Fig. 6 D) and revealed no significant inter-period changes.

Catchment changes since 1800

Despite the applied selection criteria for low-impacted lakes, the ALK lakes had a relatively large human-impacted area (MAN) already in 1800 (median 48%) and this increased slightly during 1800-2000 (Table1). The lowest MAN occurred in LAC lakes in both 1800 and 2000 when a mean increase of 5-7% was observed within an 1800 m radius catchment. The largest increases in MAN appeared in the two LACW lakes (40% for both lakes). No significant correlation was found between change in human-impacted area and diatom or cladoceran community changes (1850-2000) within lake types. However, for all 18 lakes with available land cover data, diatom and cladoceran SCD correlated positively with the change in MAN (1800 m radius) (Pearson correlation R=0.51 and 0.67, P = 0.03 and 0.002).

Discussion

The present study indicates that the majority of the 21 presumably low human-impacted Danish lakes were impacted by eutrophication already in 1850, as indicated by high accumulation rates of sediment and cladoceran subfossils, particularly in ALK and LACW lakes (constituting 57% and 19% of the studied lakes, respectively) (Fig. 2), high inferred values of both DI-TP (ALK, LACW lakes) and BP-CPUE (ALK lakes) and low inferred values of SUB-COV (ALK lakes). In addition, pelagic diatom and cladoceran species communities were abundant at most of the sites. Supportingly, the percentage of land used for cultivation purposes in the lake catchments (MAN) was high already in 1800 (ALK lakes), presumably leading to enhanced nutrient leaching by increased soil erosion and manuring (Bradshaw et al. 2006).

Most lakes developed towards higher nutrient loading and productivity during 1850-2000 BP as evidenced by the biological proxies. The ALK lakes seem to have responded later to enhanced eutrophication (1950-2000) than LACW and LAC lakes, which is indicated by both diatom and cladoceran SCDs, although 1-4 lakes (depending on proxy) did have significant SCD coefficients already in 1850-1900 or 1900-1950 (Fig. 4). Already in 1850 and throughout the study period most ALK lakes showed high DI-TP and inferred values > 50 fish net^{-1} night^{-1}. Typically, BP-CPUE is 50-200 fish net^{-1} night^{-1} in shallow Danish lakes with TP >50 µg P l^{-1} (Jeppesen et al. 2003a), which, for Danish shallow lakes, is the selected TP boundary for a shift from “good” to a “moderate” ecological state (Søndergaard et al. 2005b). Thus, 80% of the ALK lakes were WRD-classified “moderate-poor” in 1850. Early eutrophication in ALK lakes has been seen in several studies of Danish lakes; in some cases even centuries or millennia ago (e.g. Odgaard & Rasmussen 2000; Bradshaw *et al.* 2005, 2006).

Only five, mainly ALK lakes being characterised as productive already in 1850 (DI-TP: 76-124 µg L^{-1}), showed minor community changes since 1850. The proportion of lakes with minimal community changes since 1850 resembles the findings in Scottish and Irish studies of potential “reference sites”; however their sites with minimal change remained oligotrophic since 1850, (Bennion *et al.* 2004; Leira *et al.* 2006), whereas ours were eutrophic. Therefore, combined with the finding that more than 70% of the study lakes were in a WRD moderate-poor ecological state in 1850, the use of the year 1850 to define the reference state in Danish lakes is questionable.

Even though no overall change in DI-TP occurred in ALK lakes, a tendency to enhanced eutrophication during 1900-1950 followed by a decrease in 1950-2000 could be traced (Fig. 6). The decrease in DI-TP possibly reflects the declining nutrient loading to Danish lakes caused by the nutrient-reducing measures implemented in recent decades (Søndergaard *et al.* 2005a; Jeppesen *et al.* 2002). As the loads and eutrophication peaked during the 1980s in Danish lakes, the period 1950-2000 covers both an increase and a decrease in loads which may explain the weak change in DI-TP. A significant decrease was found in inferred SUB-COV during 1850-2000 in ALK lakes, which coincides well with contemporary data and other palaeoecological studies showing an overall decline in macrophyte cover over the past decade in Danish lakes (Anderson & Odgaard 1994; Sand-Jensen *et al.* 2000; Rasmussen & Anderson 2005). Recently (1994-2004), however, macrophyte cover has increased in several Danish lakes following external nutrient loading reduction (Lauridsen *et al.* 2005; Jeppesen *et al.* 2005).

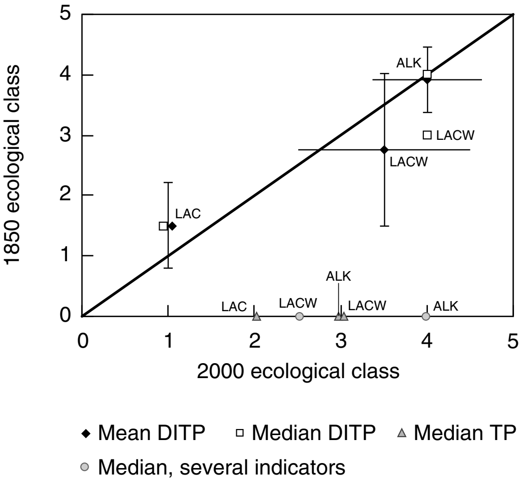

Fig. 7. Comparison of mean and median ecological band classification of lake groups based on diatom reconstructed total phosphorous (DI-TP) in 1850 and 2000. Classification (medians of lake types) based on TP contemporary measurements in 2000 (▲) and on several indicators (●) (TP, total nitrogen, Secchi depth, chlorophyll *a*, pH: contemporary data) (2-5 of these indicators available per lake).

LACW lakes showed the largest changes in SCD during the study period. LACW lakes also had the lowest median abundance of pelagic diatoms and cladocerans in 1850. Accordingly, the changes in the assessed WFD ecological state (Fig. 7) and MAN (Table 2) were larger in LACW lakes than in ALK lakes. The major changes in LACW lakes took place during 1900-1950, although earlier impacts may have occurred as cladoceran taxa composition changed already during 1850-1900 (Fig. 4).

The LAC lakes had the lowest accumulation rates during the period studied. However, indications of increased production over time could be traced, but for pelagic cladocerans these were not significant. Several of the cladoceran taxa found in relatively high abundances in the LAC lakes occur in low-nutrient and/or acidic lakes (Flössner 2000; Brodersen *et al.* 1998). The LAC lakes deviated somewhat from the ALK and LACW lakes by showing a decreasing trend in relative abundance of pelagic taxa. This occurred despite increasing nutrient loading and decreasing Secchi depth and macrophyte coverage (Frederiksborg Amt 2000; 2003; Århus Amt 2001; Ribe Amt 2006; Ringkøbing Amt 2006). However, the LAC lakes were inhabited or dominated by mosses (Frederiksborg Amt 2000; 2003; Århus Amt 2002; Ringkøbing Amt 2006; Ribe Amt 2006), with increasing moss coverage recently reported from two of the five LAC lakes (Frederiksborg Amt 2003; Ringkøbing Amt 2006). Thus, increased nutrient concentrations may have fuelled the development of epiphytes on plant and mosses, as stronger nutrient-induced stimulation of epiphytic to pelagic phytoplankton is common for shallow oligotrophic lakes (Sand-Jensen & Søndergaard 1981). This may explain the increased relative contribution of benthic taxa (Jeppesen *et al.* 2001), mimicking a situation of increased submerged plant coverage.

The changes in diatom and cladoceran community structure possibly reflect nutrient enrichment in that the number of species typically found in oligotrophic lakes decreased, whereas that of eutrophic lake species increased during the study period. However, the response patterns of diatoms and cladocerans differed, the earliest community changes appearing in ALK lakes for diatoms, but in LAC and LACW lakes for cladocerans (Fig. 3 C & D). In addition, the lake-specific trends in SCD coefficients as well as the lakes with highest SCD coefficients differed among proxies (Fig. 3, 4), and also the trend in the relative distribution of pelagic cladocerans and diatoms differed in half of the study lakes. Cladoceran community structure responds primarily to changes in trophic dynamics (e.g. fish predation) (Hofmann 1986; Hann *et al.* 1994; Jeppesen *et al.* 1996; 2002) rather than to altered nutrient levels to which phytoplankton may respond readily (Reynolds 1984; Zeeb *et al.* 1994). The response to shifting nutrient regimes may therefore differ for cladocerans and diatoms depending on the initial nutrient state, on habitat availability and fish community structure. The time resolution of this study was, however, too low to allow thorough analyses of possible time lags among proxies.

Despite major changes in community assemblage and sediment accumulation rates during the study period, DI-TP did not differ significantly. Surprisingly, many of the LACW and ALK lakes had relatively high TP-concentrations already in 1850. Even for the year 1800, high DI-TP values were inferred (mean DI-TP 112 µg TP L^{-1}) in 16 lakes included in the present study (Bradshaw *et al.* 2006). In our study the DI-TP values based on planktonic taxa only were generally higher than those based on the whole diatom community assemblage. Thus, the questioned applicability of DI-TP values based on whole diatom assemblages yielding too high values, due to a wide ecological tolerance of common non-planktonic taxa, especially in shallow productive lakes with high seasonal variation in TP concentrations (Bennion *et al.* 2005), would not change the conclusion that our study lakes were early productive.

In Denmark, precipitation has increased by 109 mm during the last 180 years and run-off by 56 mm during the last 75 years (Larsen *et al.* 2005), while the yearly mean temperature has increased 1.2 ºC since the instrumental recordings began in 1873 (Cappelen 2002). The low time resolution in our study prevents us from quantitatively evaluating such potentially climate induced effects. Thus, we cannot fully exclude that increases in temperature and higher precipitation mediated an increase in natural loading (Jeppesen *et al.* 2003b; McKee *et al.* 2003) and reinforced the enlarged eutrophication observed during the past century due to human activities in the catchments. However, the major changes in land-use and nutrient loading likely override the effect of changes in climate (Jeppesen et al. 2005).

Conclusions

Our study demonstrates that lakes presently being negligibly impacted by humans may be scarce, if not non-existing, in a densely populated and cultivated country such as Denmark. The large majority (75%) of our study lakes showed changed diatom and cladoceran community assemblages during the past 150 years. The 25% which did not show such changes were all eutrophic and likely impacted already before the onset of the industrial revolution in 1850. Our study additionally demonstrated the potential of applying a palaeolimnological approach to define reference conditions and identify "true" reference sites based on biological proxies.

Acknowledgements

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References

Amsinck, S. L., Johansson, L. S., Bjerring, R., Jeppesen, E., Søndergaard, M, Jensen, J. P., Jensen, K., Bradshaw, E. Anderson, N. J., Nielsen, A. B., Rasmussen, P., Ryves, D., Stavngaard, B., Brodersen, K., McGowan, S. Odgaard, B. V. & Wolin, J. (2003) *Vandrammedirektivet og danske søer. Del 2: Palæoøkologiske undersøgelser*. Danmarks Miljøundersøgelser. 120 s. – Faglig rapport fra DMU. nr. 476

Anderson, N. J. (1995) Using the past to predict the future: lake sediments and the modelling of limnological disturbance. *Ecological Modelling*, **78**, 149-172

Anderson, N. J. & Odgaard, B. V. (1994) Recent palaeolimnology of three shallow Danish lakes. *Hydrobiologia*, **275/276**, 411-422.

Battarbee, R. W. (1986) Diatom analysis. *Handbook of Holocene Palaeoecology and Palaeohydrology* (eds. Berglund, B. E), pp. 527-570. Wiley, Chichester.

Bennion, H., Johnes, P., Ferrier, R., Phillips, G. & Haworth, E. (2005) A comparison of diatom phosphorous transfer functions and export coefficient models as tools for reconstructing lake nutrient histories. *Freshwater Biology*, **50**, 1651-1670.

Bennion, H., Fluin, J. & Simpson, G. (2004) Assessing eutrophication and reference conditions for Scottish freshwater lochs using subfossil diatoms. *Journal of Applied Ecology*, **41,** 124-138.

Bennion, H., Juggins, S. & Anderson, N. J. (1996) Predicting epilimnetic phosphorous concentrations using an improved diatom-based transfer function and its application to lake eutrophication management. *Environmental Science & Technology*, **30**, 2004-2007.

Birks, H. J. B., Line J. M., Juggins S., Stevenson, A. C. & Ter Braak, C. J. F. (1990) Diatoms and pH reconstruction. *Philosophical Transactions of The Royal Society of London Series B-Biological Sciences*, **327**, 263-278.

Bradshaw, E. G., Nielsen, A. B. & Anderson, N. J. (2006) Using diatoms to assess the impacts of prehistoric, pre-industrial and modern land-use on Danish lakes. *Regional Environmental Change*, **6**, 17-24.

Bradshaw, E.G., Rasmussen, P. & Odgaard, B. V. (2005) Mid- to late-Holocene land-use change and lake development at Dallund Sø, Denmark: synthesis of multiproxy data linking land and lake. *Holocene*, **15**, 1152-1162.

Bradshaw, E.G. & Anderson, N.J. (2003) Environmental factors that control the abundance of Cyclostephanos dubius (Bacillariophyceae) in Danish lakes, from seasonal to century scale. *European Journal of Phycology*, **38**, 265-276.

Bradshaw, E. G., Anderson, N. J., Jensen, J. P. & Jeppesen, E. (2002) Phosphorous dynamics in Danish lakes and the implications for diatom ecology and paleoecology. *Freshwater Biology*, **47**, 1963-1975.

Brodersen, K. P., Whiteside, M. C. & Lindegaard, C. (1998) Reconstruction of trophic state in Danish lakes using subfossil chydorid (Cladocera) assemblages. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1093-1103.

Brooks J. L. and Dodson, S. I. (1965) Predation, body size and composition of plankton. *Science*, **105**, 28-35.

Cappelen, J. (2002) Yearly temperature, precipitation, hours of bright sunshine and cloud cover for Denmark; 1873-2001. *Technical Report 02-07*. Danish Meteorological Institute, 14 pp.

Declerck, S., Vandekerkhove, J., Johansson, L., Muylaert, K., Conde-Porcuna, J. M., Van der Gucht, K., Perez-Martinez, C., Lauridsen, T., Schwenk, K., Zwart, G., Rommens, W., Lopez-Ramos, J., Jeppesen, E., Vyverman, W., Brendonck, L. & De Meester, L. (2005) Multi-group biodiversity in shallow lakes along gradients of phosphorus and water plant cover. *Ecology*, **86**, 1905-1915.

European Union (2000) *Directive 2000/60/EC of the European Parliament and of the Council Establishing a Framework for the Community Action in the Field of Water Policy*. European Commission, off. J. Eur. Commun. L327 (2000) 1.

Flower, R. J., Juggins, S. & Battarbee, R. W. (1997) Matching diatom assemblages in lake sediment cores and modern surface sediment samples: the implications for lake conservation and restoration with special reference to acidified systems. *Hydrobiologia*, **344**, 27-40.

Flösner, D. (2000) *Die Haplopoda und Cladocera Mitteleuropas*. Backhuys Publishers, Leiden, The Netherlands

Frederiksborg Amt (2003) *Sortesø 2000*. Teknik og Miljø, Landskabsafdelingen, 26 pp. In Danish

Frederiksborg Amt (2000) *Agersø 1999*. Teknik og Miljø, Miljøafdelingen, 24 pp. In Danish

Frey, D. G. (1959) The taxonomic and phylogenetic significance of the head pores of the *Chydoridae* (*Cladocera*). *Internationale Revue der gesamten Hydrobiologie*, **44**, 27-50.

Hann, B. J., Leavitt, P. R. & Chang, P. S. S. (1994) Cladoceran Community Response to Experimental Eutrophication in Lake 227 as Recorded in Laminated Sediments. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 2312-2320.

Hofmann, W. (1986) Developmental history of the Grosser Plöner See and the Schöhsee (north Germany): cladoceran analysis, with special reference to eutrophication. *Archiv für Hydrobiologie*, **74**, 259-287.

Jeppesen, E., Jensen J. P., Lauridsen, T. L., Amsinck, S. L., Christoffersen, K., Søndergaard, M. & Mitchell, S. F. (2003a) Sub-fossils of cladocerans in the surface sediment of 135 lakes as proxies for community structure of zooplankton, fish abundance and lake temperature. *Hydrobiologia*, **491**, 321-330.

Jeppesen, E., Søndergaard, M. & Jensen J. P. (2003b) Climatic warming and regime shifts in lake food webs – some comments. *Limnology & Oceanography*, **48**, 1346-1349.

Jeppesen, E., Jensen, J. P. & Søndergaard, M. (2002) Response of phytoplankton, zooplankton, and fish to re-oligotrophication: An 11 year study of 23 Danish lakes. *Aquatic Ecosystem Health & Management*, **5**, 31-41.

Jeppesen, E., Leavitt, P., De Meester, L. & Jensen, J. P. (2001) Functional ecology and paleolimnology: using cladoceran subfossils to reconstruct anthropogenic impact. *Trends in Ecology & Evolution*, **16**, 191-198.

Jeppesen, E., Søndergaard, M., Jensen, J.P., Havens, K., Anneville, O., Carvalho, L., Coveney, M.F., Deneke, R., Dokulil, M.T., Foy, B., Gerdeaux, D., Hampton, S.E., Kangur, K., Köhler, J., Körner, S., Lammens, E., Lauridsen, T.L., Manea, M., Miracle, R., Moss, B., Nöges, P., Persson, G., Phillips, G., Portielje, R., Romo, S., Schelske, C.L., Straile, D., Tatrai, I., Willén, E., Winder, M., (2005) Lake responses to reduced nutrient loading – an analysis of contemporary long term data from 35 case studies. *Freshwater Biology*, **50**, 1747–1771.

Jeppesen E. (1998) *The Ecology of Shallow lakes. Trophic Interactions in the Pelagial*. NERI Technical Report No. 247.

Jeppesen, E., Jensen, J. P., Søndergaard, M., Lauridsen, T. L., Pedersen, L. J. & Jensen, L. (1997) Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia*, **342/343**, 151-164.

Jeppesen, E., Madsen, E. A. & Jensen, J. P. (1996) Reconstructing the past density of planktivorous fish and trophic structure from sedimentary zooplankton fossils: a surface sediment calibration data set from shallow lakes. *Freshwater Biology*, **36**, 115-127.

Jowsey P.C. (1966) An improved peat sampler. *New Phytologist*, **65**, 245-248.

Krammer, K & Lange-Bertalot, H. (1986-1991) *Susswasserflora von Mitteleuropa. Bacillariophyceae*. Verlag, Stuttgart

Larsen, S. E., Kronvang, B., Ovesen, N. B. & Christensen, O. B. (2005) Afstrømningens udvikling i Danmark. *Vand & Jord*, 12, 8-13. In Danish

Lauridsen, T.L., Jensen, J.P., Søndergaard, M., Jeppesen, E., Strzelczak, A. & Sortkjær, L. (2005) Søer 2004 NOVANA. 66 pp. NERI Technical Repport No. 553. In Danish http://fagligerapporter.dmu.dk

Leira, M., Jordan, P., Taylor, D., Dalton, C., Bennion, H., Rose, N. & Irvine, K. (2006) Assessing the ecological status of candidate reference lakes in Ireland using palaeolimnology. *Journal of Applied Ecology*, **43,** 816-827.

McKee, D., Atkinson, D., Collings, S. E., Eaton, J.W., Gill, A. B., Harvey. I., Hatton. K., Heyes. T., Wilson. D. & Moss, B. (2003) Response of freshwater microcosm communities to nutrients, fish, and elevated temperature during winter and summer. *Limnology and Oceanography*, **48**, 707-722.

Nielsen A. B. (2003) *Pollen based quantitative estimation of land cover. Relationships between pollen sedimentation in lakes and land cover as seen on historical maps in Denmark AD 1800.* GEUS Rapport 2003/57 Geological Survey of Denmark and Greenland.

Nielsen, A.B. (2004): Modelling pollen sedimentation in Danish lakes at ca. AD 1800 - an attempt to validate the POLLSCAPE model. *Journal of Biogeography*, **31**, 1693-1709.

Nielsen, A.B. and Sugita, S. (2005): Estimating relevant source area of pollen for small Danish lakes around AD 1800. *The Holocene*, **15**, 1006-1020.

Odgaard, B. V. & Rasmussen, P. (2000) Origin and temporal development of macro-scale vegetation patterns in the cultural landscape of Denmark. *Journal of Ecology*, **88**, 733-748.

Räsänen, J., Kauppila, T. & Salonen, V. (2006) Sediment-based investigation of naturally or historically eutrophic lakes – implications for lake management. *Journal of Environmental Management*, **79**, 253-265.

Rasmussen, P. & Anderson, N. J. (2005) Natural and anthropogenic forcing of aquatic macrophyte development in a shallow Danish lake during the last 7000 years. *Journal of Biogeography*, **32**, 1993-2005.

Renberg, I. (1991) The HON-Kajak sediment corer. *Journal of Paleolimnology*, **6**, 167-170.

Renberg, I. A. (1990) Procedure for preparing large sets of diatom slides from sediment cores. *Journal of Paleolimnology*, **4**, 87-90.

Reynolds, C. S. (1984) *The ecology of freshwater phytoplankton*. Cambridge University Press, 384 pp.

Ringkøbing Amt (2006) Miljøtilstanden i Skørsø 2004. Teknik og Miljø, 45 pp. In Danish

Ribe Amt (2006) *Skærsø har det fortsat dårligt.* http://www.ribeamt.dk/sw22765.asp. In Danish

Sand-Jensen, K., Riis, T., Vestergaard, O. & Larsen, S. E. (2000) Macrophyte decline in Danish Lakes and streams over the past 100 years. *Journal of Ecology*, **88**, 1030-1040.

Sand-Jensen, K. & Søndergaard, M. (1981) Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. *Internationale Revue der gesamten Hydrobiologie*, **66**, 529-552.

Simpson, G. L., Shilland, E. M., Winterbottom, J. M. & Keay, J. (2005) Defining reference conditions for acidified waters using a modern analogue approach. *Environmental Pollution*, **137**, 119-133.

Søndergaard, M., Jensen, J. P. & Jeppesen, E. (2005a) Seasonal response of nutrients to reduced phosphorous loading in 12 Danish lakes. *Freshwater Biology*. **50**, 1605-1615.

Søndergaard, M., Jeppesen, E., Jensen, J. P. & Amsinck L. S. (2005b) Water Framework Directive: ecological classification of Danish lakes. *Journal of Applied Ecology*, **42**, 616-629.

Søndergaard, M. & Moss, B. (1997) Impact of submerged macrophytes on phytoplankton in shallow freshwater lakes. In: *The structuring role of sub-*

merged macrophytes in lakes (eds E. Jeppesen, Ma. Søndergaard, Mo. Søndergaard & K. Christoffersen), pp. 115-132. Springer-Verlag, New York.

Tarvainen, M., Ventela, A.M., Helminen, H. & Sarvala, J. (2005) Nutrient release and resuspension generated by ruffe (*Gymnocephalus cernuus*) and chironomids. *Freshwater Biology*, **50**, 447-458.

Taylor, D., Dalton, C., Leira, M., Jordan, P., Chen, G., León-Vintró, L., Irvine, K., Bennion, H. & Nolan, T. (2006) Recent histories of six productive lakes in the Irish Ecoregion based on multiproxy palaeolimnological evidence. *Hydrobiologia*, **571**, 237-259.

ter Braak, C. J. F & Smilauer, P. (2002) *CANOCO Reference manual and CanoDraw for Windows. User's guide. Software for Canonical Community Ordination* (version 4.5). Microcomputer Power, Ithaca, New York, USA.

Timms, R. M. & Moss, B. (1984) Prevention of Growth of Potentially Dense Phytoplankton Populations by Zooplankton Grazing, in the Presence of Zooplanktivorous Fish, in a Shallow Wetland Ecosystem. *Limnology and Oceanography*, **29**, 472-486.

Zeeb, B. A., Christie, C. E., Smol, J. P., Findlay, D. L., Kling, H.J. & Birks, H. J. B. (1994) Responses to Diatom and Chrysophyte Assemblages in Lake 227 Sediments to Experimental Eutrophication. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 2300-2311.

Århus Amt (2002) *Natur og Miljø i Nord- og Midtdjursland (2000)*, Natur og Miljø, 52 pp. In Danish.

Århus Amt (2001) *Vandkvalitetsplan 2001. Søer*. Natur og Miljø, 168 pp. In Danish.

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Mid- to late-Holocene land-use change and lake development at Dallund Sø, Denmark: trophic structure inferred from cladoceran subfossils

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Abstract: Analyses of cladoceran remains were conducted on an 11-m sediment core from Dallund Sø, Denmark, covering approximately the last 7000 years. The densities of planktivorous fish and macrophyte coverage were inferred from previously established transfer functions for Danish lakes using pelagic and plant-associated cladocerans, respectively, as palaeoenvironmental indicators. This is the first reconstruction of the abundance of fish and macrophytes covering millennial timescales. The cladoceran assemblages indicated an early period (4830 BC to *c*. 750 BC) with low species diversity, being dominated mainly by small-sized pelagic taxa. An intervening period (750 BC–AD 1100) followed, dominated by macrophyte-associated taxa and large-sized pelagic species. A marked increase in the abundance of remains occurred at *c*. AD 1200 coincident with the introduction of the mouldboard plough to Denmark and major forest clearance in the lake catchment. Further upcore (AD 1300–1700) mud-dwelling taxa increased in importance. Finally (AD 1700–1998), a shift occurred towards taxa characterizing eutrophic conditions. Redundancy analyses and cladoceran-inferred submerged macrophyte coverage and planktivorous fish density indicated overall low levels of nutrients and chlorophyll *a*, moderate macrophyte coverage (10–24%) and moderate to high fish predation prior to the Roman Iron Age (AD 0–400) followed by higher levels of nutrients and chlorophyll *a* and lower macrophyte coverage ($< 10\%$) and moderate fish predation in recent times. The results suggest that the lake became increasingly eutrophic through time, not least after forest clearance and intensification of agriculture in Mediaeval times.

Key words: Zooplankton remains, fish, macrophytes, long-term changes, lake development, land use, Dallund Sø, Denmark, Holocene.

Introduction

Since the last glaciation, the Danish landscape has altered as a result of climatic changes and, not least, human activity and agricultural development since the Late Bronze Age (Rasmussen, 2005, this issue). The nutrient loading to lakes has increased significantly, particularly during the last century as a consequence of sewage input, fertilization and the use of phosphorous detergents. Consequently, the trophic structure of the lakes has changed. As judged from both historical (e.g., Baagøe and Kølpin Ravn, 1895; Boye Petersen, 1917) and palaeoecological data (Klein, 1993; Anderson and Odgaard, 1994; Odgaard and Rasmussen, 2001; Jeppesen *et al*., 2001a,b), many Danish shallow lakes have shifted from a clearwater state with high coverage of macrophytes to a turbid state dominated by phytoplankton, typically during the period 1850–1980 (Amsinck *et al*., 2003). The changes have also affected the fish stock, and a shift has occurred from percid dominance in the mesotrophic state to cyprinid prevalence in the present eutrophic state (Jeppesen *et al*., 2000). This shift has had major cascading effects on the food web and water quality. With increasing eutrophication, the piscivores lose control over the planktivores. This is partly because planktivores are superior competitors to potential piscivores at the juvenile stage, and partly because eutrophication leads to higher turbidity and loss of submerged macrophytes, factors that promote cyprinid

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(typically planktivores) dominance over piscivores (Persson *et al.*, 1988; Jeppesen *et al.*, 2000). Higher cyprinid abundance leads to more intensive predation on zooplankton and thus decreasing grazer control of phytoplankton. Together with the enhanced nutrient input, this has led to phytoplankton blooming, low water transparency and loss of submerged macrophytes. Analyses of biological remains retrieved from short cores have revealed that major changes occurred in many lakes during the 1940s to 1950s (Anderson and Odgaard, 1994; Odgaard and Rasmussen, 2001; Amsinck *et al.*, 2003). In other lakes the deterioration occurred before the turn of the twentieth century (Jeppesen *et al.*, 2001b; Søndergaard *et al.*, 2003), but little is known about the status of Danish lakes prior to the recent centuries.

Lake sediments host remains of many pelagic and benthic cladocerans and these can be used to quantify the past trophic structure of lakes. Thick-shelled forms, such as chydorids, are well preserved, whereas the remains of thin-shelled chitinous taxa, such as *Daphnia*, are represented by smaller fragments (e.g., postabdominal claws, caudal cerca and mandibles) and resting eggs (ephippia). The cladocerans include species that are functionally adapted to different microhabitats (i.e., pelagic, plant-associated, benthic), and changes in the relative abundance of key taxa may therefore yield information about both habitat alterations, changes in lake trophic structure and lake depth (Frey, 1986; Jeppesen *et al.*, 2000; Korhola *et al.*, 2000). To date, cladoceran remains have been used to evaluate qualitative changes in lake productivity and climate (Frey, 1986) and, more recently, to elucidate quantitative changes in the water table (Korhola *et al.*, 2000), salinity (Bos *et al.*, 1996, 1999), temperature (Lotter *et al.*, 1997), chlorophyll *a* and TP (Brodersen *et al.*, 1998), fish abundance, per cent piscivorous fish, zooplankton grazing and macrophyte coverage (Jeppesen *et al.*, 2001a,b; Amsinck *et al.*, 2005). The findings have greatly increased the possibility of determining not only physicochemical variables but also past trophic structure and dynamics (Jeppesen *et al.*, 2001a,b).

In the present study, we sought to elucidate changes in fish abundance and submerged macrophyte coverage from the sediment remains of zooplankton in an 11-m core covering the past 7000 years. The study is part of a multidisciplinary palaeoecological investigation aimed to determine the natural (i.e., prior to major human disturbance) status of Dallund Sø and to trace the link between catchment land use, lake water quality and trophic structure through time. For an introduction to the project see Rasmussen and Bradshaw (2005, this issue).

Materials and methods

Study area

Dallund Sø is a relatively small (15 ha) and shallow (mean depth 1.9 m, maximum depth 2.6 m) lake situated in the northern part of the island of Funen, Denmark, in a landscape heavily exploited for agriculture. Today, the small catchment of the lake (153 ha) is largely used for agricultural purposes (50%), but comprises also built-up areas, woodland and wetlands. The lake has no major inflow and only one major outflow. The residence time of the lake is 270 days. The lake is nutrient-rich (annual mean concentration of total phosphorus (TP) measured in the 1990s ranged between 65 and 120 μg/L; Secchi depth: 57 and 125 cm). The lake is encircled by reeds and submerged vegetation is sparse ($<1\%$ coverage). Until 1970, the lake received sewage from a recreational home. In order to restore the lake, fish manipulation was conducted from November 1995 to October 1997. In total, 3.3 t of mainly bream (*Abramis brama*) and roach (*Rutilus rutilus*) were removed and 22 500 pike (*Esox lucius*) fry were stocked (Sandby Hansen, 1998). In consequence, the fish biomass declined from 8.1 t to 4.2 t and water clarity improved, increasing from a summer average of 0.4–0.8 m to 1.1–1.2 m. Scattered colonies of *Potamogeton crispus* and *Ceratophyllum demersum* appeared in 1996, but in summer 1997 macrophyte abundance again declined and was now mainly composed of a few *Potamogeton pectinatus* stands and filamentous algae (Sandby Hansen, 1998).

Coring and dating

In March 1998, the uppermost 570 cm of lake sediment was cored from, approximately, the centre of the lake. The top 29 cm of loose sediment was collected using an HON Kajak corer (Renberg, 1991) and the rest of this sequence was sampled in 100 cm long, overlapping sections using a Russian corer (Jowsey, 1966). In October 1998, sediments from 570 cm to 1120 cm were raised using a piston corer with 210 cm metal tubes that allow individual core sections up to *c.* 200 cm long to be collected. The upper and lower sediment sequences were correlated using ignition residue profiles with 2 cm intervals. The terrestrial plant macrofossil content of 20 samples from the Dallund Sø sediment was used to obtain accelerator mass spectrometry (AMS) ^{14}C dates. Calibrated ages were calculated using CALIB version 4.1.2 (Stuiver and Reimer, 1993). If the calibration resulted in more than one date, the centre of the calibrated age interval was used for the construction of an age–depth curve for the sediment core. The dating of the upper (post-1900) sediments was imprecise (Rasmussen and Bradshaw, 2005, this issue) and so interpretation of changes in the last century are made with caution (further details about coring and dating are given in Rasmussen and Bradshaw, 2005, this issue).

Zooplankton

The sediment cores (see Rasmussen and Bradshaw, 2005, this issue) were sectioned horizontally in the laboratory at 2 cm intervals. Bradshaw (2001) found only very small changes in diatom assemblages before *c.* 750 BC. Therefore, the cladoceran analyses were focused on the subsequent period. A total of 31 depth intervals (*c.* 1–7 g wet weight sediment per depth interval) were used for the analyses. Subsamples for each depth interval were boiled in 30 ml 10% KOH for 20 minutes and subsequently kept cold (4°C) for no longer than 2 weeks until taxonomical analyses was performed. The samples were filtered manually, and remains of cladocerans >80 μm were identified using a stereomicroscope (Olympus SZX12) and an inverted light microscope (320 ×, Leitz Labovert FS). To facilitate counting, the remains were divided into two size fractions: >140 μm and 80–140 μm. Counting typically covered 1000–2000 remains in the upper part (surface at 204–698 cm) of the core and 200–1000 in the lower part (750–1322 cm) of the core where fragments were less abundant. Subsampling of the most abundant taxa (e.g., *Chydorus sphaericus*, *Bosmina* spp.) was undertaken when necessary. As the different fragments were unequally preserved, only the most abundant and the most representative fragment of a species was used for data analyses. For identification, the keys of Frey (1959), Margaritora (1985), Hann (1990), Røen (1995) and Flössner (2000) were used.

The diagrams use the period name abbreviations as follows: MESO, Mesolithic; EN, Early Neolithic; MNA, Middle Neolithic A; MNB, Middle Neolithic B; LN, Late Neolithic; EBA, Early Bronze Age; LBA, Late Bronze Age; PRIA, Pre-Roman Iron age; RIA, Roman Iron Age; LIA, Late Iron Age; MED, Mediaeval; and MoT, Modern Time.

Statistical methods

Detrended correspondence analysis (DCA) was applied to determine whether linear or unimodal statistical techniques would be most appropriate to model the species responses of the sediment record. Values below 2 standard deviation (SD) of the gradient length of 1-axis indicate that most species respond monotonically along the gradient (Birks, 1995; ter Braak, 1995). Principal component analysis (PCA) was performed to identify possible patterns in the zooplankton species distribution and to track the direction of changes in the sediment record. The DCA and PCA were based on 19 taxa, rare taxa occurring in less than three depth intervals were excluded from the analyses.

Redundancy analyses (RDA) were performed to qualitatively estimate the historical changes of Dallund Sø in relation to environmental variables. Species abundances from the sediment core samples were compared with the abundances of zooplankton species of two different calibration data sets, used for quantitative inference of macrophyte coverage and planktivorous fish (PL-CPUE) abundances, respectively. The lakes included in the two calibration sets were not identical, which is why two different calibration sets were used. The species abundances of the calibration data sets were treated as active samples in the RDA ordinations, while species abundances of the Dallund Sø sediment record were made passive. Hereby, the sediment core samples are projected passively into the ordination space without influencing the positions of the environmental vectors and the calibration samples (species and sites), making it possible to evaluate past conditions and trends in Dallund Sø simply on the basis of the position of the core samples to the environmental vectors. All ordinations were performed using CANOCO version 4.5 (ter Braak and Smilauer, 2002). The DCA was performed by detrending by segments, while the PCA and RDAs were made by scaling on interspecies correlation, dividing species scores with standard deviation and centred by species with no downweighting of species data. The ordinations (DCA, PCA, RCAs) and reconstructions were based on zooplankton taxa expressed as log (number of remains per g dry weight sediment +1).

The calibration data set used for inference of macrophyte coverage was based on the relationships between remains of macrophyte and macrophyte-sediment associated cladocerans ($n = 14$ taxa) from surface sediments and corresponding contemporary data of 19 Danish freshwater lakes (Jeppesen *et al.*, unpublished data, 1998). The coverage of submerged macrophytes expressed as percentage coverage (COV%) was reconstructed using a weighted-average (WA) model with and without zooplankton species ecological tolerance downweighting (tol) and inverse deshrinking ($R^2_{\text{apparent}} = 0.56$, root mean squared error of prediction, $\text{RMSEP}_{\text{boot}} = 0.59$ log (COV% +1) for a WA model and $R^2_{\text{apparent}} = 0.44$ and $\text{RMSEP}_{\text{boot}} = 0.63$ log (COV% +1) for a WA(tol) model) (Jeppesen *et al.*, unpublished data, 1998). Models were developed using the program WACALIB version 3.3 (Line *et al.*, 1994). Excepting the three species (*Alona elongata, Ilyocryptus sordidus* and *Pleuroxus truncatus*) not found in the sediment record, the remaining nine taxa of the genera *Acroperus*, *Alona*, *Camptocercus*, *Eurycercus*, *Graptoleberis*, *Leydigia*, *Pleuroxus* and *Sida* were included in the calibration data set used for the RDA ordination and the macrophyte coverage inference.

The calibration data set used for inference of PL-CPUE abundance was based on relationships established between remains of pelagic zooplankton ($n = 6$ taxa) from surface sediment samples and corresponding contemporary data of 31 Danish freshwater lakes (Jeppesen *et al.*, 1996 with minor modifications). PL-CPUE values expressed as catch per unit effort in multiple mesh-sized gill nets (14 mesh sizes: 6.25–75 mm) were reconstructed based on similar WA models as for the inference of COV%. With the exception of two taxa (*Leptodora kindtii* and *Brachionus* spp.), the remaining four taxa (*Bosmina longirostris*, *Bosmina coregoni*, *Daphnia* spp., *Ceriodaphnia* spp.) in the Dallund Sø record were included in the calibration data set used for both the RDA ordination and the PL-CPUE reconstruction.

Results

Zooplankton stratigraphy

A total of 26 cladoceran taxa were identified in the 31 samples. The 19 most abundant species, defined as species occurring at more than three depth intervals, are shown in Figure 1. In the bottom section of the core covering the Mesolithic to the middle of the Late Bronze Age (4830 BC to *c.* 750 BC), only few cladocerans occurred, pelagic *B. longirostris* being the dominant species (Figure 1A,B). On a percentage basis, the abundances of plant-associated species such as *Sida*, *Acroperus*, *Eurycercus* and *Graptoleberis* were relatively high compared with modern time (Figure 1B).

From the middle of the Late Bronze Age (*c.* 650 BC) to the beginning of the Pre-Roman Iron Age (*c.* 470 BC) sediment- and plant-associated species dominated, while both the abundance and the proportion of pelagic *B. longirostris* reached relatively low levels. *Alona* spp. was particularly abundant. *Alona quadrangularis* and *A. guttata/rectangula* were the most dominant species, but also *A. costata* and *A. affinis* peaked periodically.

During the next 1700 years, until the beginning of the Mediaeval (*c.* AD 1200), concurrently with a reduction in the percentage of tree pollen (Rasmussen, 2005, this issue), the number of cladoceran remains increased, and a shift occurred to higher dominance of true pelagic species and the pelagic-littoral *Chydorus sphaericus* (Figures 1). Pelagic large-bodied *Daphnia* (ephippia) showed a temporary increase in abundance from 470 BC to 40 BC, accounting for 0.5–3.2% of the remains (Figure 1B). *Bosmina coregoni* increased in abundance from *c.* 360 BC, but the smaller *B. longirostris* tended also to be numerous. Yet, remains of macrophyte- and sediment-associated cladocerans (especially *Alona* spp., *Pleuroxus* spp., *Acroperus* spp. and to a lesser extent *Leydigia* spp. and *Alonella* spp.) still contributed significantly to total abundance. A temporary reduction in the abundance of remains was seen in the twelfth century (between AD 1101 and 1182).

Hereafter (from AD 1182 to 1250) a marked increase in the abundance of remains occurred, especially of pelagic species and *C. sphaericus*, while the contribution of true plant-associated species declined substantially. As judged from the ratio of *Daphnia* to *Bosmina* resting eggs, the contribution of large-bodied pelagic *Daphnia* declined to very low levels around AD 1200 (Figure 3). Around 1975, the share of plant-associated species (especially *Alonella nana*, *Acroperus* sp. and *Sida crystallina*) again showed a short temporary increase, while the contribution of *C. sphaericus* decreased. Thereafter, pelagic species and *C. sphaericus* again dominated in the upper part of the sediment (Figure 2).

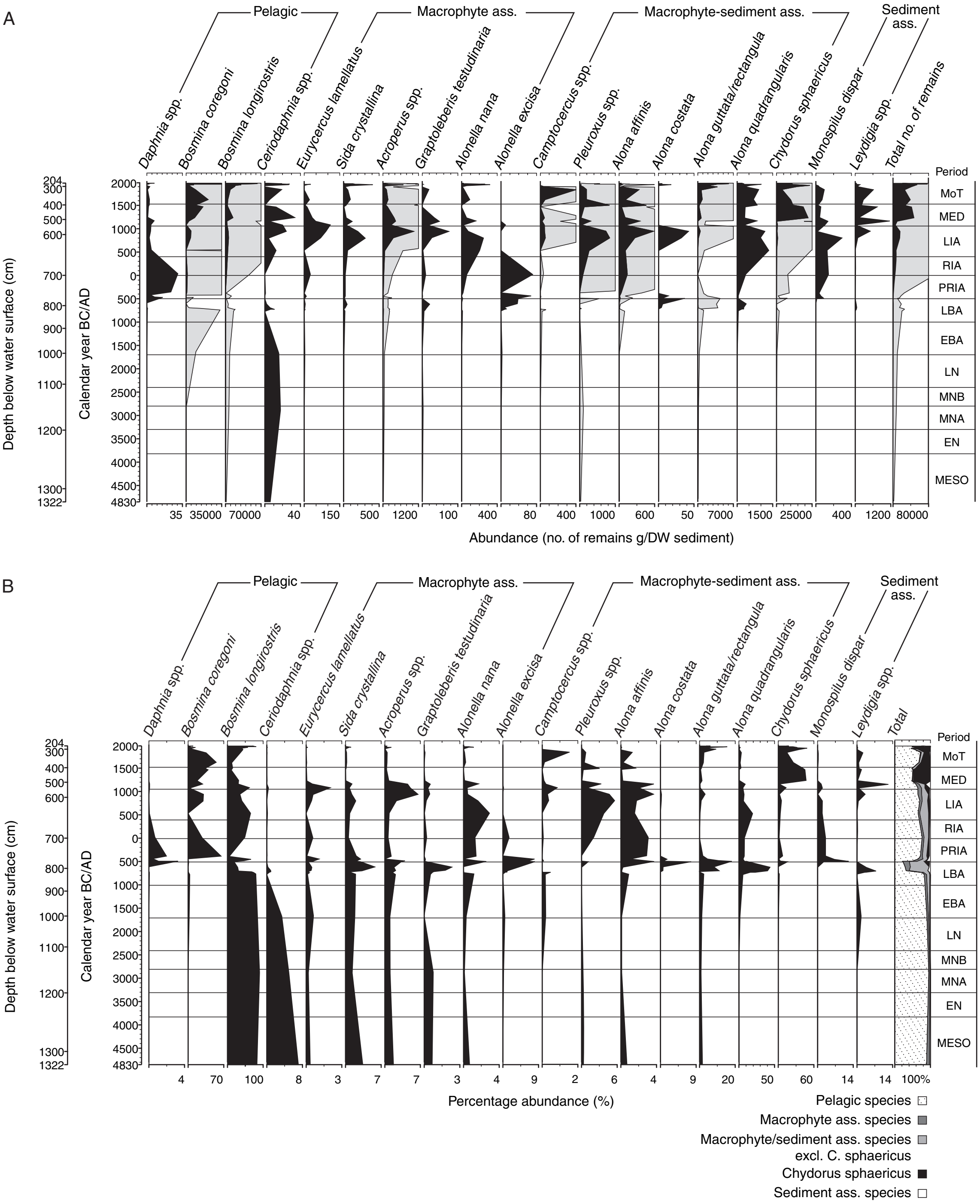

Figure 1 (A) Cladoceran stratigraphy of the Dallund Sø sediment core. The following exaggerations are shown in grey: *Bosmina coregoni*: 100×; *B. longirostris*: 20×; *Acroperus* spp.: 10×; *Camptocercus* spp.: 10×; *Pleuroxus* spp.: 100×; *Alona affinis* 10×; *A. guttata/rectangula*: 50×; *Chydorus sphaericus*: 100×; total number of remains: 20×. Note the different scales used for abundance data. Habitat classification according to Hann (1990) and Røen (1995). MESO, Mesolithic; EN, Early Neolithic; MNA, Middle Neolithic A; MNB, Middle Neolithic B; LN, Late Neolithic; EBA, Early Bronze Age; LBA, Late Bronze Age; PRIA, Pre-Roman Iron Age; RIA, Roman Iron Age; LIA, Late Iron Age; MED, Mediaeval; and MoT, Modern Time. (B) Percentage distributions of cladocerans calculated from the total number of remains for each depth of the Dallund Sø sediment core. For abbreviations of cultural period names see Figure 1A

Major changes occurred also in the relative size distribution of *Alona* and *Bosmina* (Figure 3). A remarkable shift occurred from dominance of small and medium-sized *A. guttata/rectangula* and *A. quadrangularis* together until the Pre-Roman Iron Age (*c*. 400 BC) to a higher proportion of the larger *A. affinis*, while the contribution of *A. guttata/rectangula*, in particular, declined. Yet around AD 1700, the pattern was reversed, and during the last 100 years, *Alona* was dominated by small-bodied *A. guttata/rectangula*. Likewise, among the small-bodied bosminids *B. longirostris* dominated totally until 400 BC. Then the proportion of the slightly larger *B. coregoni* increased, and it dominated periodically until the eighteenth century when a return to *B. longirostris* dominance took place, which has presently been sustained.

Ordinations

The gradient length of the first DCA axis (1.25 SD) suggested that the cladoceran species responses were largely monotonic when focusing on the sediment core data solely ($n = 19$ taxa). The eigenvalues of the first and the second DCA ordination

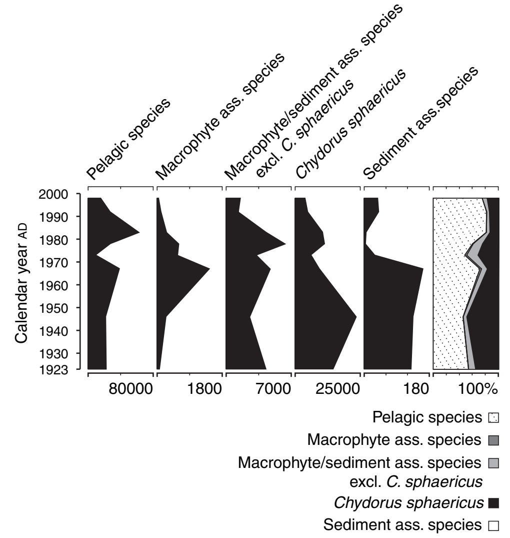

Figure 2 Cladoceran concentrations divided into habitat groups (number of remains per g DW sediment) for the period AD 1923–1998.

axes ($\lambda_1 = 0.108$, $\lambda_2 = 0.058$) explained 48% of the cumulative variation in species data. The PCA ordination ($\lambda_1 = 0.527$, $\lambda_2 = 0.164$) of Dallund Sø (Figure 4) indicated an early period (*c*. 1322–770 cm corresponding to 4830 BC to *c*. 500 BC) with low importance of the majority of taxa. This is presumably due to the overall low abundance of taxa found at the bottom section of the core (Figure 1), with the exception of *A. excisa*, which is the only taxon solely confined to depths below 554 cm (Figure 1). An intervening period followed (*c*. 750–520 cm, ~400 BC–AD 1100), which was dominated especially by macrophyte-associated taxa (e.g., *E. lamellatus*, *G. testudinaria*, *Camptocercus* spp.) as well as by the large bodied pelagic *Daphnia* spp. taxa. A shift occurred towards increasing importance of macrophyte-sediment associated taxa (e.g., *Pleuroxus* spp., *A. quadrangularis*) and the mud-dwelling taxon *Leydigia* spp. together with the macrophyte-associated taxa (*A. nana*, *S. crystallina*, *Acroperus* spp.) (*c*. 482–344 cm, ~AD 1300–1700). Finally, a more recent period (*c*. 346–204 cm, ~AD 1700–1998) with dominance of the small-bodied pelagic taxon *B. longirostris* and the macrophyte-sediment associated taxa *A. guttata*/*rectangula* and *C. sphaericus* appeared (Figure 4).

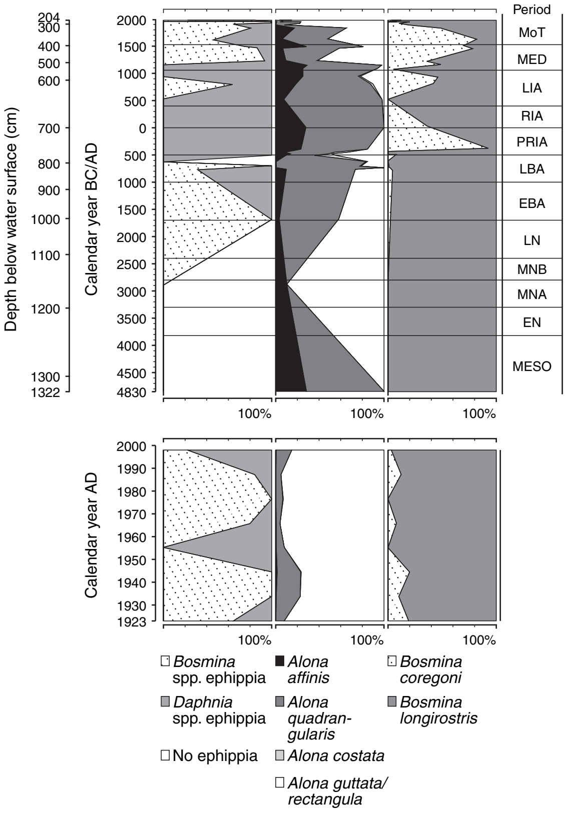

Figure 3 Percentage distributions of large-bodied and small-bodied cladocerans. Lower diagram shows details for the period AD 1923–1998. For abbreviations of cultural period names see Figure 1A

The distribution of the Dallund Sø core samples relative to the environmental vectors in the RDA ordination based on the calibration data set used for inference of COV% (Figure 5A) indicated overall low nutrient levels and low macrophyte coverage prior to the RIA (*c*. 1322–698 cm) with a intervening period with a minor increase in macrophyte coverage (*c*. 648–344 cm, ~AD 500–1700), followed by a more recent state with slightly higher levels of nutrients and chlorophyll *a* and lower macrophyte coverage (*c*. 344–204 cm, ~AD 1700–1900). The RDA ordination also indicates decreasing mean lake depth, which is supported by the fact that the sediment cores are long compared with the present low depth of the lake.

The RDA based on the calibration data set used for inference of PL-CPUE (Figure 5B) showed similar low overall levels of TP and chlorophyll *a* (*c*. 1322–750 cm) prior to the mid-PRIA. A minor increasing trend of PL-CPUE and decrease of Secchi depth were indicated post the mid-PRIA (*c*. 698–204 cm). The ordination suggested relatively high TN levels prior to the mid-PRIA followed by low TN levels post mid-PRIA. It must be emphasized, however, that only four of the six taxa used actively in the RDA were found in the Dallund Sø record. In addition, exclusively low abundances of these four taxa were found below the *c*. 750 cm depth. Thus, the distinct position of the core samples below 750 cm (in the upper left of the RDA plot) is therefore highly probable a consequence of taxa being few in numbers and low in abundances rather than high TN levels.

Inference of macrophyte coverage and fish abundance

As the two models WA and WA (tol) gave almost similar results for inference of macrophyte coverage and PL-CPUE abundances, only the results of the WA models are shown (Figure 6). The reconstructions of macrophyte coverage suggested overall low levels of macrophyte coverage (< 25%) during the study period (Figure 6). Prior to the RIA (1322–700 cm), macrophyte coverage appeared to be relatively high (*c*. 10–24%), while low levels (< 10%) seemingly have prevailed since RIA (above 700 cm) (Figure 6), with a minor temporary increase around AD 1100 followed by a decline to low levels since AD 1500.

The inference of PL-CPUE indicated generally high levels of PL-CPUE (> 61 fish per net per night) prior to mid-PRIA (1322–750 cm). Then a slightly decreasing trend appeared lasting until present day, however levels still being moderately high (> 37 fish per net per night) (Figure 6). Several periodic increases of PL-CPUE (at 224, 238, 648, 760–768, 794, 1166–1322 cm) are indicated (Figure 6). Yet, common for these abrupt peaks are the very low numbers of taxa shared between the Dallund Sø record and the PL-CPUE inference model (usually only two taxa) and the complete absence of *B. coregoni* (Figure 6, dashed lines), the latter occurring at all other depths. This increases the sensitivity of the PL-CPUE reconstruction and consequently reduces the reliability of the inference results.

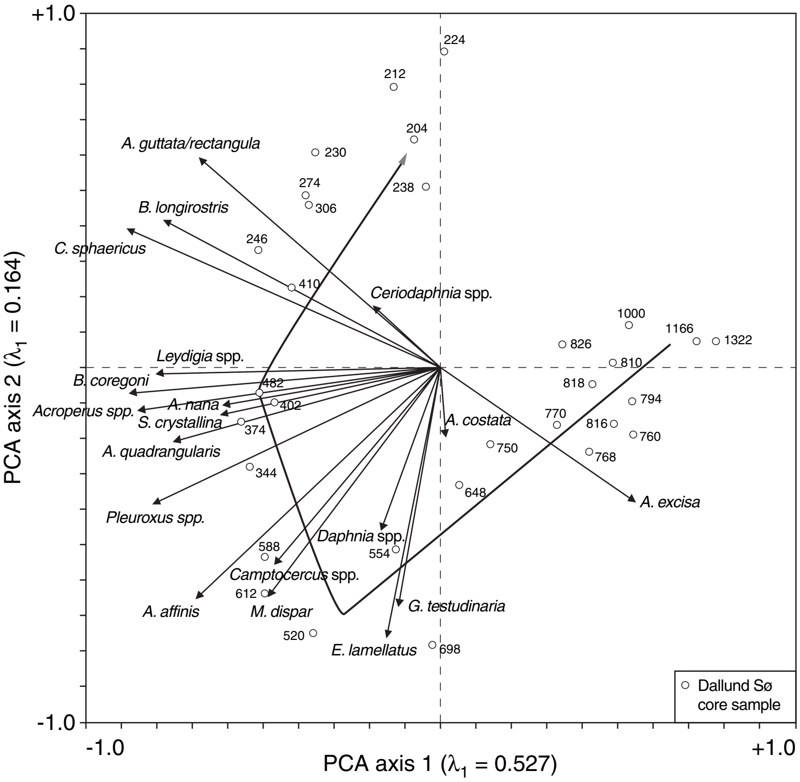

Figure 4 PCA biplot of zooplankton taxa ($n = 19$) and sediment core samples from Dallund Sø. Numbers refer to the specific sediment depth of the core sample. General trend arrow inserted from bottom (1322 cm) to the top (204 cm) of the core

Interpretation of the WA estimated PL-CPUE values must therefore be made with caution.

Discussion

Like the other inferred biological and physico-chemical variables (Bradshaw *et al.*, 2005, synthesis paper, this issue), the cladoceran data indicate stable conditions in Dallund Sø for the early part of the record (Late Mesolithic to Early Bronze Age; Figure 1), though based only on a few samples. Pelagic *B. longirostris* dominated exclusively followed by true macrophyte-associated species. The aquatic pollen record indicates the occurrence of *Myriophyllum verticillatum*, *Potamogeton* spp. and *Nymphaea* during this period (Bradshaw *et al.*, 2005, lake paper, this issue) and inferred macrophyte coverage was relatively high (10–24%). The RDA ordination, however, revealed low macrophyte coverage and a low nutrient level during this period. The diatom data also indicate a pelagic dominated system with low nutrient levels (inferred TP around 20 μg/L), and the combined proxy data suggest high transparency of the water (Bradshaw *et al.*, 2005, synthesis paper, this issue). It seems therefore reasonable to assume that a deep open water community was surrounded by a near-shore bed of floating-leaved plants and a shallow community of submerged plants in-between or outside these plant beds. Dominance of pelagic *B. longirostris* also indicates that a large volume of the lake was free of plants and that the predation risk was high in the open water. This may be explained by the fact that high clarity improves foraging conditions for visually hunting fish, and low food abundance for the zooplankton prolongs their generation time and therefore the period of exposure to predation before reproduction (Dahl-Hansen, 1995; Jeppesen *et al.*, 2003a). Accordingly, the inferred CPUE of planktivorous fish was relatively high during the period, indicating high predation risk for large-bodied zooplankton. No ephippia of *Daphnia* and *Bosmina* were found until 4830–2900 BC, which may in part reflect the overall low density of remains as seen in macrofossil analysis (Bradshaw *et al.*, 2005, lake paper, this issue), reducing the likelihood of finding the relatively scarce ephippia. Also, the relatively high temperatures during the Neolithic period (Sarmaja-Korjonen, 2003) may have reduced the need for resting egg production (Sarmaja-Korjonen, 2003; Jeppesen *et al.*, 2003b).

A major shift occurred in the last part of the Late Bronze Age (*c.* 750–600 BC). Both abundance and percentage contribution of pelagic species, most notably of *Bosmina* spp., decreased substantially, while the mud-dwelling *A. quadrangularis* and *Leydigia* spp. and true plant-associated species increased in abundance and not least in relative importance (Figure 1). This period is characterized by high input of minerogenic matter resulting from forest clearance (the percentage tree pollen decreased from 83 to 44; Rasmussen, 2005, this issue), leading to erosion and increased nutrient input (Rasmussen and Bradshaw, 2005, this issue). From around 480 BC the concentration of cladoceran remains increased substantially, indicating an increase in production. This correlates well with the increase in diatom-inferred TP and the raised concentrations of *Pediastrum* cells (Bradshaw *et al.*, 2005, lake paper, this issue) and with a major increase in loss-of-ignition in the sediment (Rasmussen and Bradshaw, 2005, this issue). Plant-associated cladoceran species were very abundant until *c.* AD 1200, coinciding with the period with high densities of *Chara* oospores in the sediment and the relatively high percentages of *Potamogeton* pollen and *Ceratophyllum* spines (Bradshaw *et al.*, 2005, lake paper, this issue). Probably plant density and height increased (despite lower coverage) with increased nutrient input, a well-known early stage of lakes undergoing eutrophication (Wetzel, 2001). Also the gradual change from a moderate deep to a shallow lake may have augmented this shift. During this period there are clear signs of reduced predation pressure. Thus, the high ratio between

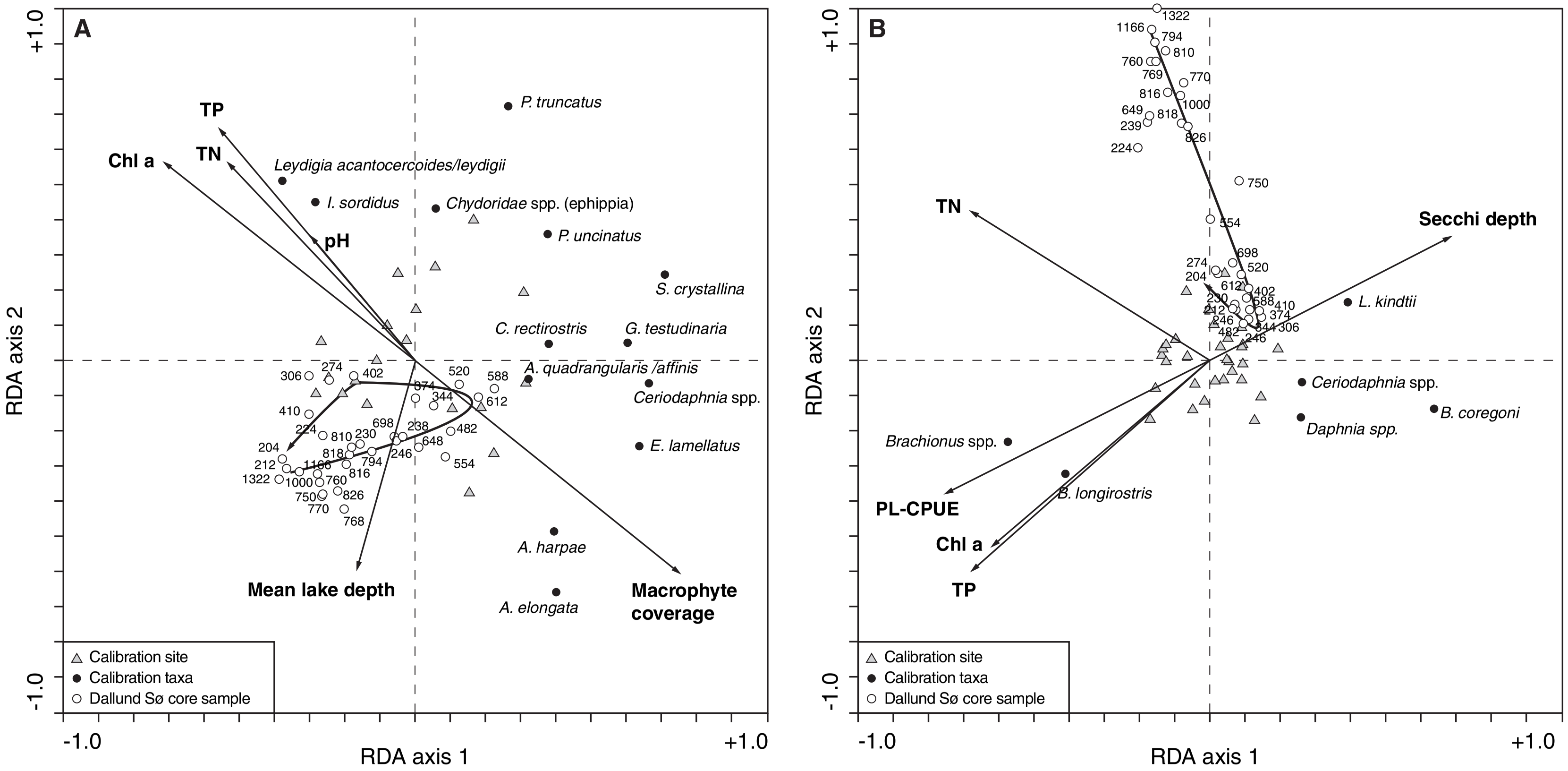

Figure 5 (A) RDA ordination biplot with sediment core samples of Dallund Sø plotted as passive samples including solely macrophyte and macrophyte-sediment associated taxa. Active samples are based upon the calibration samples used for inference of COV% ($n = 14$ taxa, $n = 19$ sites) (Jeppesen *et al.*, unpublished data, 1998). Numbers and trend arrow as in Figure 4. (B) RDA ordination biplot with sediment core samples of Dallund Sø plotted as passive samples including solely pelagic associated taxa. Active samples are based on the calibration samples used for inference of PL-CPUE ($n = 6$ taxa, $n = 31$ sites) (modified from Jeppesen *et al.*, 1996). Numbers and trend arrow as in Figure 4

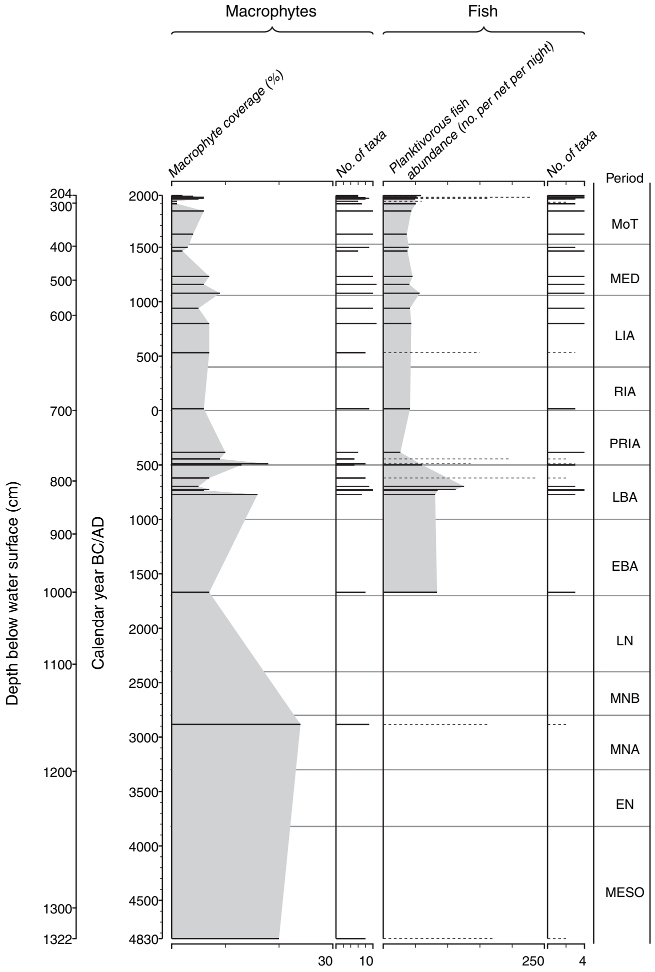

Figure 6 Zooplankton-inferred macrophyte coverage and planktivorous fish abundance based on WA models with inverse deshrinking. Number of taxa refers to taxa shared between the calibration data set and the Dallund Sø core and implicit taxa used for inferring macrophyte coverage and PL-CPUE. Note that coverage is not an estimate of surface plant coverage but of the size of the surface area covered by macrophytes, large as well as small. Dashed lines indicate less reliable estimates (see text for further explanation). Shaded area indicates overall trends. For abbreviations of cultural period names see Figure 1A

Daphnia:*Bosmina* ephippia suggests that predation sensitive large-bodied *Daphnia* dominated among the pelagic species during the period. Moreover, around 360 BC a shift occurred within the *Bosmina* community, from almost complete dominance of small *B. longirostris* to a more even distribution between *B. longirostris* and the larger and more predation sensitive *B. coregoni*. An increase in the size of dominant *Alona* species probably also reflect reduced predation owing to the circumstance that plants, when occurring in high densities, provide the large-bodied zooplankton with a daytime refuge against fish predation (Timms and Moss, 1984; Schriver *et al*., 1995; Burks *et al*., 2002). Accordingly, the inferred planktivorous fish density reached its minimum during this period.

Major changes occurred after AD 1200 when the nutrient input rose markedly (Bradshaw *et al*., 2005, lake paper, this issue) because of an intensification of agriculture, including extension of cultivated areas and use of deeper ploughing technology (Rasmussen, 2005, this issue). True macrophyte-associated zooplankton genera, such as *Sida, Eurycercus* and *Acroperus*, became scarce, while species indicative of a high-productivity lake (Frey, 1986; De Eyto *et al*., 2003) such as *C. sphaericus* and later *Alona rectangula*/*guttata*, occurred in high densities. A major decline in the *Daphnia*:*Bosmina* ephippia ratio and a later decrease in the proportion of *B. coregoni* among the bosminids (Figure 3) suggest a major increase in the fish predation pressure. This was, however, not fully supported by the inferred fish density showing only a slight increase.

Assessed from contemporary data, the environmental state of the lake improved temporarily after fish manipulation conducted during 1995–1997 as an attempt to restore the lake following a reduction in wastewater input. Water transparency (Secchi depth) increased, the in-lake TP concentration declined and submerged macrophyte abundance increased temporarily, but then declined in 1997 (see Materials and methods section). This recent improvement in the lake water quality is, however, not yet visible in the sediment record. The data presented suggest that Dallund Sø has changed from an oligo-mesotrophic to a eutrophic state through time, the deterioration accelerating after the forest clearance and intensification of agriculture that occurred in Mediaeval times (Rasmussen, 2005, this issue).

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References

Amsinck, S., Jeppesen, E. and **Landkildehus, F.** 2005: Relationships between environmental variables and zooplankton subfossils in the surface sediments of 36 shallow coastal brackish lakes with special emphasis on the role of fish. Journal of Paleolimnology 33, 39–51.

Amsinck, S.L., Johansson, L.S., Bjerring, R., Jeppesen, E., Søndergaard, M., Jensen, J.P., Jensen, K., Bradshaw, E., Anderson, N.J., Bennike, O., Nielsen, A.B., Rasmussen, P., Ryves, D., Stavngaard, B., Brodersen, K., McGowan, S., Odgaard, B.V. and **Wolin, J.** 2003: *Vandrammedirektivet og danske søer. Del 2: Palæoøkologiske undersøgelser*. Danmarks Miljøundersøgelser. Faglig rapport fra DMU nr. 476. Retrieved 25 July 2005 from http://www2.dmu.dk/1_viden/2_publikationer/3_fagrapporter/rapporter/FR476.pdf (in Danish).

Anderson, N.J. and **Odgaard, B.V.** 1994: Recent palaeolimnology of three shallow Danish lakes. *Hydrobiologia* 275/276, 411–22.

Baagøe, J. and **Kølpin Ravn, F.** 1895: Ekskursion til jydske søer og vandløb. *Botanisk Tidsskrift* 20, 288–326 (in Danish).

Birks, H.J.B. 1995: Quantitative palaeoenvironmental reconstructions. In Maddy, D. and Brew, J.S., editors, *Statistical modelling of Quaternary science data. Technical guide 5.* Cambridge: Quaternary Research Association, 161–254.

Bos, D.G., Cumming, B.F., Watters, E. and **Smol, J.P.** 1996: The relationship between zooplankton, conductivity and lake-water ionic composition in 111 lakes from the Interior Plateau of British Columbia, Canada. *International Journal of Salt Lake Research* 5, 1–15.

Bos, D.G., Cumming, B.F. and **Smol, J.P.** 1999: *Cladocera* and *Anostraca* from the Interior Plateau of British Columbia, Canada, as paleolimnological indicators of salinity and lake level. *Hydrobiologia* 392, 129–41.

Boye Petersen, J. 1917: Bemærkninger til plantekortene over Bastrup sø, Farum sø, Bagsværd sø og Lyngby Sø. In Wesenberg-Lund, C., editor, *Furesø studier*. Copenhagen: Det Kongelige Danske Videnskabernes Selskabs Skrifter (in Danish).

Bradshaw, E.G. 2001. Linking land and lake. The response of lake nutrient regimes and diatoms to long-term land-use change in Denmark. Ph.D. Thesis, University of Copenhagen, 118 pp.

Bradshaw, E.G., Rasmussen, P., Nielsen, H. and **Anderson, N.J.** 2005: Mid- to late-Holocene land-use change and lake development at Dallund Sø, Denmark: trends in lake primary production as reflected by algal and macrophyte remains, *The Holocene* 15, 1130–42.

Brodersen, K.P., Whiteside, M.C. and **Lindegaard, C.** 1998: Reconstruction of trophic state in Danish lakes using subfossil chydorid *Cladocera* assemblages. *Canadian Journal of Fisheries and Aquatic Sciences* 55, 1093–103.

Burks, R.L., Lodge, D.M., Jeppesen, E. and **Lauridsen, T.** 2002: Diel horizontal migration of zooplankton: costs and benefits of inhabiting littoral zones. *Freshwater Biology* 47, 343–65.

Dahl-Hansen, G.A.P. 1995: Long-term changes in crustacean zooplankton – the effects of a mass removal of Arctic charr, *Salvelinus alpinus* L., from an oligotrophic lake. *Journal of Plankton Research* 17, 1819–33.

De Eyto, E., Irvine, K., Bareiss, C., Gross, E., Cerbin, S., van den Bund, W., Criada, F.G., Gyllström, M., Jeppesen, E., Kornijow, R., Miracle, M.R., Nykanen, M., Salujõe, J. and **Stephens, D.** 2003: The distribution of chydorids Branchiopoda, Anomopoda in European shallow lakes. *Archiv für Hydrobiologie* 156, 181–202.

Flössner, D. 2000: Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas. Leiden: Backhuys Publishers.

Frey, D.G. 1959: The taxonomic and phylogenetic significance of the head pores of the *Chydoridae Cladocera*. *Internationale Revue der Gesamten Hydrobiologie* 44, 27–50

—— 1986: Cladoceran analysis. In Berglund, B.E., editor, *Handbook of Holocene palaeoecology and palaeohydrology*. Chichester: John Wiley, 667–92.

Hann, B.J. 1990: *Cladocera*. In Warner, B.G., editor, *Methods in Quaternary ecology*. Geoscience Canada Reprint Series 5. St Johns, Newfoundland: Geological Association of Canada, 81–91.

Jeppesen, E., Madsen, E.A., Jensen, J.P. and **Anderson, N.J.** 1996: Reconstructing the past density of planktivorous fish and trophic structure from sedimentary zooplankton fossils: a surface sediment calibration data set from shallow lakes. *Freshwater Biology* 36, 11–127.

Jeppesen, E., Jensen, J.P., Søndergaard, M., Lauridsen, T. and **Landkildehus, F.** 2000: Trophic structure, species richness and biodiversity in Danish lakes: changes along a nutrient gradient. *Freshwater Biology* 45, 201–18.

Jeppesen, E., Leavitt, P., De Meester, L. and **Jensen, J.P.** 2001a: Incorporating functional ecology in palaeolimnology: using pelagic and cladoceran remains to reconstruct anthropogenic impact. *Trends in Ecology and Evolution* 16, 191–98.

Jeppesen, E., Jensen, J.P., Skovgaard, H. and **Hvidt, C.B.** 2001b: Changes in the abundance of planktivorous fish in Lake Skanderborg during the past two centuries – a palaeoecological approach. *Palaeogeography, Palaeoclimatology, Palaeoecology* 172, 143–52.

Jeppesen, E., Jensen, J.P., Jensen, C., Faafeng, B., Brettum, P., Hessen, D., Søndergaard, M., Lauridsen, T. and **Christoffersen, K.** 2003a: The impact of nutrient state and lake depth on top-down control in the pelagic zone of lakes: study of 466 lakes from the temperate zone to the Arctic. *Ecosystems* 6, 313–25.

Jeppesen, E., Jensen, J.P., Lauridsen, T.L., Amsinck, S.L., Christoffersen, K. and **Mitchell, S.F.** 2003b: Sub-fossils of cladocerans in the surface sediment of 135 lakes as proxies for community structure of zooplankton, fish abundance and lake temperature. *Hydrobiologia* 491, 321–30.

Jowsey, P.C. 1966: An improved peat sampler. *New Phytology* 65, 245–48.

Klein, T. 1993: Impact on lake development of changed agricultural watershed exploitation during the last 3 centuries. *Hydrobiologia* 251, 297–308.

orhola, A., Olander, H. and **Blom, T.** 2000: Cladoceran and chironomid assemblages as quantitative indicators of water depth in subarctic Fennoscandian lakes. *Journal of Paleolimnology* 24, 43–54.

Line, J.M., ter Braak, C.J.F. and **Birks, H.J.B.** 1994: WACALIB version 3.3 – a computer program to reconstruct environmental variables from fossil assemblages by weighted averaging and to derive sample-specific errors of predication. *Journal of Paleolimnology* 10, 147–52.

Lotter, A.F., Birks, J.B.H., Hofmann, W. and **Marchetto, A.** 1997: Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. I. Climate. *Journal of Paleolimnology* 18, 395–420.

Margaritora, F.G. 1985: *Cladocera*. Fauna D'Italia Vol. XXIII. Bologna: Edizioni Calderini.

Odgaard, B.V. and **Rasmussen, P.** 2001: The occurrence of egg-cocoons of the leech *Piscicola geometra* L. in recent lake sediments and their relationship with remains of submerged macrophytes. *Archiv für Hydrobiologie* 152, 671–86.

Persson, L., Andersson, G., Hamrin, S.F. and **Johansson, L.** 1988: Predation regulation and primary production along the productivity gradient of temperate lake ecosystems. In Carpenter, S.R., editor, *Complex interactions in lake communities*. New York: Springer Verlag, 45–65.

Rasmussen, P. 2005: Mid- to late-Holocene land-use change and lake development at Dallund Sø, Denmark: vegetation and land-use history inferred from pollen data. *The Holocene* 15, 1116–29.

Rasmussen, P. and **Bradshaw, E.G.** 2005: Mid-to late-Holocene land-use change and lake development at Dallund Sø, Denmark: study aims, natural and cultural setting, chronology and soil erosion history. *The Holocene* 15, 1105–115.

Renberg, I. 1991: The HON-Kajak sediment corer. *Journal of Paleolimnology* 6, 167–70.

Røen, U.I. 1995: Danmarks Fauna Bd. 85, Krebsdyr V, Gællefødder *Branchiopoda* og Karpelus *Branchiura*. Copenhagen: Dansk Naturhistorisk Forening, Viderup Bogtrykkeri A/S (in Danish).

Sandby Hansen, K. 1998: Dallund Sø. In Søndergaard, M., Jeppesen, E. and Jensen, J.P., editors, *Sørestaurering i Danmark. Metoder, erfaringer og anbefalinger*. Miljønyt nr. 28. Copenhagen: Miljøstyrelsen 137–38 (in Danish).

Sarmaja-Korjonen, K. 2003: Chydorid ephippia as indicators of environmental change – biostratigraphical evidence from two lakes in southern Finland. *The Holocene* 13, 671–700.

Schriver, P., Bøgestrand, J., Jeppesen, E. and **Søndergaard, M.** 1995: Impact of submerged macrophytes on fish–zooplankton–phytoplankton interactions: large-scale enclosure experiments in a shallow eutrophic lake. *Freshwater Biology* 33, 255–70.

Stuiver, M. and **Reimer, P.J.** 1993: Extended ^{14}C data base and revised CALIB 3.0 ^{14}C age calibration program. *Radiocarbon* 35, 215–30.

Søndergaard. M., Jensen, J.P., Jeppesen, E. and **Bradshaw, E.,** editors 2003: *Vandrammedirektivets implementering i danske søer Del 1: Søtyper, referencetilstand og økologiske klasser*. Danmarks Miljøundersøgelser. Faglig rapport fra DMU nr. 475. Retrieved 14 October 2005 from http://www2.dmu.dk/1_viden/2_publikationer/3_fagrapporter/rapporter/FR475.pdf (in Danish)

ter Braak, C.J.F. 1995: Ordination. In Jongman, R.H.G., Ter Braak, C.J.F. and van Tongeren, O.F.R., editors, *Data analysis in community and landscape ecology*. Cambridge: Cambridge University Press, 91–173.

ter Braak, C.J.F. and **Smilauer, P.** 2002: *CANOCO reference manual and user's guide to CANOCO for Windows: software for canonical community ordination (version 4.5)*. New York: Microcomputer Power.

Timms, R.M. and **Moss, B.** 1984: Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. *Limnology and Oceanography* 29, 472–86.

Wetzel, R.G. 2001: *Limnology. Lake and river ecosystems*. San Diego CA: Academic Press.

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APPLIED ISSUES

Lake depth rather than fish planktivory determines cladoceran community structure in Faroese lakes – evidence from contemporary data and sediments

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SUMMARY

1. This study describes the environmental conditions and cladoceran community structure of 29 Faroese lakes with special focus on elucidating the impact of fish planktivory. In addition, long-term changes in biological structure of the Faroese Lake Heygsvatn are investigated.
2. Present-day species richness and community structure of cladocerans were identified from pelagial snapshot samples and from samples of surface sediment (0–1 cm). Multivariate statistical methods were applied to explore cladoceran species distribution relative to measured environmental variables. For Lake Heygsvatn, lake development was inferred by cladoceran-based paleolimnological investigations of a ^{14}C-dated sediment core covering the last ca 5700 years.
3. The 29 study lakes were overall shallow, small-sized, oligotrophic and dominated by brown trout (*Salmo trutta*). Cladoceran species richness was overall higher in the surface sediment samples than in the snapshot samples.
4. Fish abundance was found to be of only minor importance in shaping cladoceran community and body size structure, presumably because of predominance of the less efficient zooplanktivore brown trout.
5. Canonical correspondence analysis showed maximum lake depth (Z_{max}) to be the only significant variable in explaining the sedimentary cladoceran species (18 cladoceran taxa, two pelagic, 16 benthic) distribution. Multivariate regression trees revealed benthic taxa to dominate in lakes with $Z_{\mathrm{max}} < 4.8$ m and pelagic taxa to dominate when Z_{max} was > 4.8 m.
6. Predictive models to infer Z_{max} were developed using variance weighted-averaging procedures. These were subsequently applied to subfossil cladoceran assemblages identified from a ^{14}C-dated sediment core from Lake Heygsvatn and showed inferred Z_{max} to correspond well to the present-day lake depth. A recent increase in inferred Z_{max} may, however, be an artefact induced by, for instance, eutrophication.

Keywords: brown trout, cladoceran remains, Faroe Islands, fish planktivory, paleolimnology, regression tree analysis, transfer functions, water depth

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Introduction

In arctic and subarctic Greenland lakes (Jeppesen *et al.*, 2001a; Lauridsen *et al.*, 2001) and subarctic Icelandic lakes (Antonsson, 1992), fish have been shown to play a major role and exert a high predation pressure on the zooplankton, with a cascading impact on the remaining food web structure. In subarctic Fennoscandian lakes, however, Korhola (1999) and Korhola, Olander & Blom (2000) found lake depth to be the most important factor explaining cladoceran community structure. In addition, O'Brian *et al.* (2004) showed lake depth and area to be the single-most important factors influencing zooplankton and species richness in Alaskan arctic lakes. Yet, none of these studies included fish as an explanatory variable. A recent study of four subarctic Faroese lakes revealed major differences in trophic structure and fish predation pressures on zooplankton communities (Jeppesen *et al.*, 2002a). Analysis of fish diets (stomach content) (Malmquist *et al.*, 2002) and zooplankton biomass ratios (Jeppesen *et al.*, 2002a) thus indicated low predation pressure on cladocerans in the brown trout (*Salmo trutta*) only lake, moderate predation pressure in the two brown trout and three-spined stickleback (*Gasterosteus aculeatus*) lakes and high predation pressure on cladocerans in the brown trout and Arctic charr (*Salvelinus alpinus*) lake. A plausible explanation of the observed differences in predation pressure may be dominance of different fish species and implicitly then prey preferences. Thus, the zooplanktivorous predator Arctic charr dominated in the arctic and subarctic Greenland and Icelandic lakes (Antonsson, 1992; Riget *et al.*, 2000; Jeppesen *et al.*, 2001a), while the omnivorous brown trout was dominant in the few Faroese lakes expecting the one hosting Arctic charr (Malmquist *et al.*, 2002).

In the present study, we expanded the number of Faroese lakes to be investigated. We hypothesised that fish planktivory only plays a minor role in shaping the cladoceran community and body-size structure in brown trout dominated lakes. We related cladoceran assemblages to contemporary ecological variables of 29 predominantly shallow and oligotrophic lakes along a gradient of fish abundance. Cladocerans were collected as active individuals from pelagial snapshot samples. In addition, cladocerans were recovered as remains of surficial sediments, as recent paleoecological studies have demonstrated that such remains are useful indicators for elucidating both past and present-day fish predation intensity as well as changes in community structure in lake ecosystems (Jeppesen *et al.*, 2001b; Korhola & Rautio, 2001). Moreover, cladoceran assemblages of a ^{14}C-dated sediment core from Lake Heygsvatn were investigated with the purpose of describing lake development and past changes in fish predation pressure during the last ca 5700 years. Our study is the hitherto most comprehensive quantitative limnological investigation conducted in Faroese lakes.

Study site

The Faroe Islands are an archipelago situated in close proximity to the warm North Atlantic Current. The climate of the islands is therefore humid and cool in summer (average temperature in July 10.3 °C at Thorshavn) and mild in winter (average temperature in January 3.4 °C, Thorshavn; Danish Meteorological Institute). The low annual temperature regime along with the geographical remoteness of the islands (approximately 420 km south of Iceland, 600 km west of Norway, 300 km north of Scotland), their small size (1398 km^2 on 18 islands) and their relatively short colonisation period since the glacial retreat about 11 000 years ago presumably play an important determining role in shaping the community structure, species richness and ecosystem functioning of the lakes.

Methods

Study sites

Surface sediments and contemporary environmental variables were sampled during July and August 2000 in 29 Faroese lakes situated on the five islands of Suderoy, Sandoy, Vagar, Streymoy and Eysteroy (Fig. 1). In addition, sediment cores were recovered from Lake Heygsvatn [surface area 3.3 ha, maximum depth 4.3 m, catchment 23.2 ha (Dali, 1975)] located on the island of Suderoy (Fig. 1). The lakes cover a longitudinal gradient of 6.44–7.42°W, a latitudinal gradient of 61.29–62.17°N and an altitudinal range of 0–377 m above sea level.

Fish abundance

The composition and relative abundance of the pelagic fish stock in the lakes were determined with

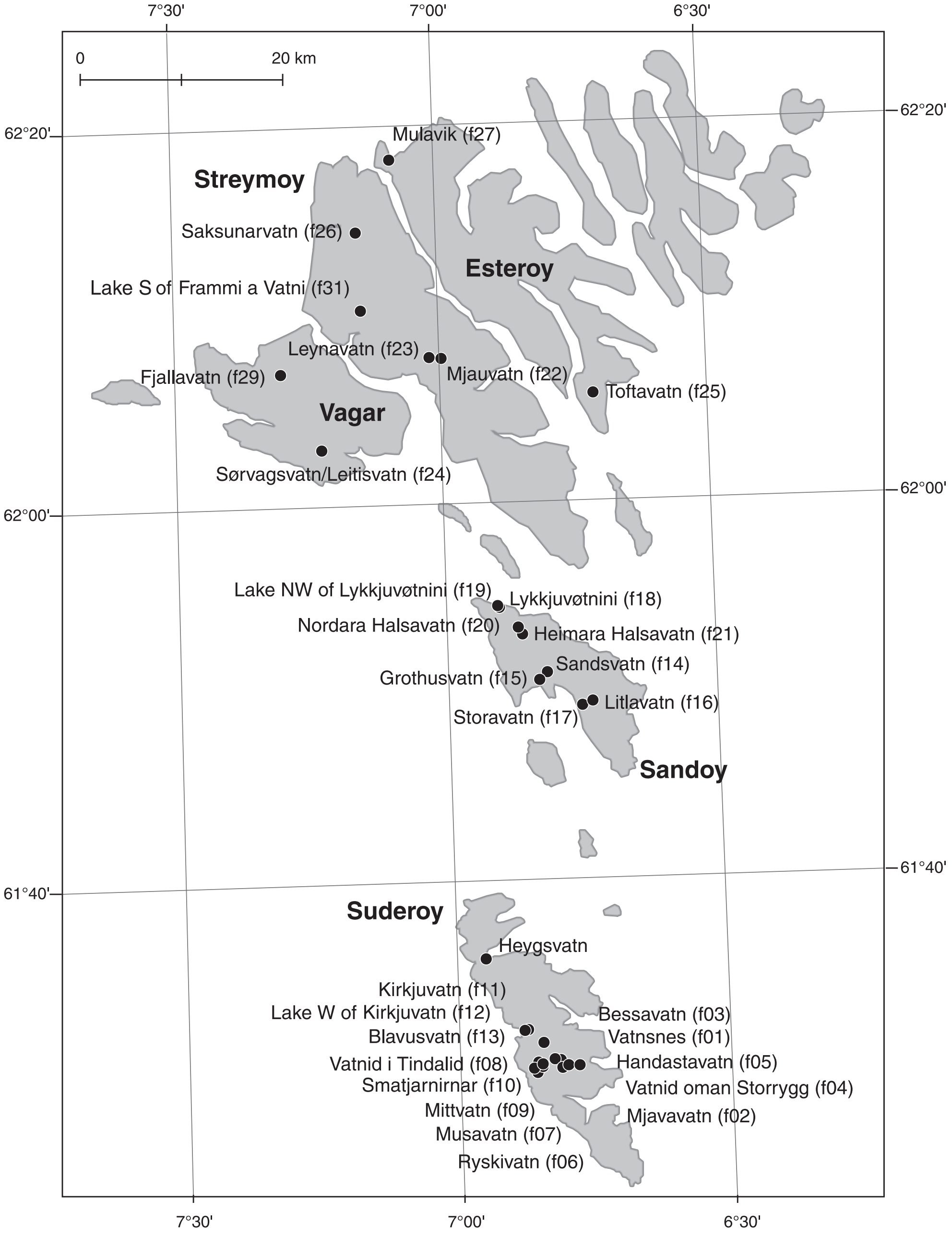

Fig. 1 Geographical location of the 29 Faroese study lakes. Abbreviations of lakes indicated in brackets and used in subsequent figures.

multiple mesh size gill nets (6.25, 8, 10, 12.5, 16.5, 22, 25, 30, 33, 38, 43, 50, 60 and 75 mm), the length and depth of each section being 3 and 1.5 m, respectively. Between two and 10 nets were used depending on lake size and depth. Nets were set in late afternoon and retrieved the following morning (approximately 18 h) in both the littoral zone and at the bottom in the pelagic zone and in deep lakes also in the open water of the pelagic zone. For each lake, catch per unit effort (CPUE) in terms of number of fish per net per night (approximately 18 h) was calculated.

Water chemistry

Water samples for determining total phosphorus (TP) and total nitrogen (TN; 200 mL unfiltered) and chlorophyll *a* (1 L) were collected from depth-integrated, mixed samples from the entire water column at mid-lake stations located in the pelagic (deepest part) using a Schindler sampler. Lake water TP concentrations were determined as molybdate reactive phosphorus (Murphy & Riley, 1972) following persulphate digestion (Koroleff, 1970), while TN concentrations were measured after oxidation as nitrite using a flow-injection analyser fitted with a copper-cadmium reductor column. Chlorophyll *a* was filtered on GF/C filters and concentrations determined spectrophotometrically after ethanol extraction (Jespersen & Christoffersen, 1987). Lake water conductivity (±1 μS cm^{-1}), salinity (±2 mg chloride L^{-1}), pH (±0.2) and maximum depth (±0.05 m) were determined *in situ* using a Mini-Sonde multiprobe (Hydrolab, Suite, Austin, U.S.A.).

Cladocerans sampled from the water

Cladocerans were collected in the central open water areas with a modified Patalas sampler (3.3 L). At each mid-lake station, a depth-integrated sample was taken by pooling samples from six to eight depths to represent the entire water column. Of this pooled sample, a 15–20 L subsample was filtered through a 20 μm mesh and preserved with acid Lugol's iodine (4%). The cladocerans were identified and quantified to genus or, when possible, to species level using a stereomicroscope (100×; Leica MZ12, Leica Microsystems Ltd, Heerbrugg, Switzerland) and the identification key of Røen (1995).

Cladocerans sampled in sediments

For each of the 29 lakes, five surface sediment (0–1 cm) samples were recovered using a Kajak surface corer (internal diameter: 5.2 cm) in the deepest part of the lake. The surface sediment samples were pooled for each lake and kept frozen (−18 °C) prior to analysis of cladoceran remains. In Lake Heygsvatn, 11 overlapping sediment cores were recovered using a Russian peat sampler and a Kajak corer in the middle of the lake (water depth: approximately 2 m). The cores were sectioned horizontally into 2 cm thick slices in the 20 cm overlap zones and into 4 cm thick slices in between. The core samples were kept frozen (−18 °C) until subfossil analysis. For taxonomical analysis approximately 5 g (wet weight) homogenised sediment was used. The subsamples were boiled in 50 mL 10% KOH for 15 min and subsequently kept cold (4 °C) for maximum 2 weeks until counting. Prior to the analyses, the samples were sieved manually. Remains >80 μm were all identified using a stereomicroscope (100×; Leica MZ12) and an inverted light microscope (320×; Leitz Labovert FS, Ernst Leitz Ltd, Midland Ontario, Canada). To facilitate counting, the remains were divided into two size fractions: >140 and 80–140 μm. Remains >140 μm were all counted, while remains in the 80–140 μm size fraction were subsampled and approximately 20–66% counted depending on the density of remains. A total of 27 189 remains were enumerated from the 29 surface samples, the median of remains counted per sample being 738 (minimum = 151, maximum = 2774). In addition, dorsal length of *Daphnia* spp. ephippia was measured. For taxonomical identification, the keys of Frey (1959); Margaritora (1985) and Røen (1995) were used. As the different fragments within the Cladocera suborder were unequally preserved, only the most abundant and the most representative fragment of a taxon or species was used for data analysis. Counting of remains was adjusted to represent individuals (e.g. number of carapace halves/2, number of headshields/1).

The sediment cores of Lake Heygsvatn were correlated using organic material profiles and to some extent magnetic susceptibility, the latter being conducted on the whole core (with 2 mm resolution) at Quaternary Department, University of Lund, Sweden. Loss-on–ignition (LOI) at 550 and 950 °C was used to determine the amount of organic material and limnic carbonate. Chronological control was based on nine ^{14}C accelerator mass spectrometry (AMS) dates conducted at the Institute of Physics and Astronomy, University of Aarhus, Denmark.

Statistical analyses

Prior to statistical analyses, environmental variables were screened to check for normality. Variables with skewed distribution were transformed using log or log $(x + 1)$ transformation (Table 1). Sedimentary cladoceran abundance was expressed as percentage relative abundance based on, respectively, number of remains per gram wet weight sediment per lake (surface sediment samples) and number of remains per gram dry weight sediment per depth (sediment core of Lake Heygsvatn). Similarly, cladoceran

Table 1 Survey of environmental variables measured in the 29 Faroese lakes

Variable	Unit	Median	Average	Minimum	Maximum	Transformation	Code
Area	ha	6	25	0.5	341	log	Area
Maximum lake depth	m	1.4	8.2	0.3	52	log	Z_{max}
Conductivity	μS cm^{-1} (20 °C)	216	374	110	4030	log	Cond
Salinity	‰	0	0.1	0	1.86	log(x + 1)	Sal
pH	−log[H$^+$]	6.9	7.2	5.5	9.2		pH
Total phosphorous	μg L^{-1}	26	37	3	225	log	TP
Total nitrogen	μg L^{-1}	250	300	100	780	log	TN
Chlorophyll *a*	μg L^{-1}	1.2	2.3	0.4	25.2	log	Chla
Total fish abundance	fish net^{-1} night^{-1}	8	11.5	0	30	log(x + 1)	CPUEtot
Brown trout abundance	fish net^{-1} night^{-1}	6.3	8.4	0	23.8	log(x + 1)	CPUEbt
Stickleback abundance	fish net^{-1} night^{-1}	0	1.75	0	25.5	log(x + 1)	CPUEst

Units of measurements, summary statistics, transformation applied in numerical analysis and abbreviated codes are given.

assemblages recovered from water samples were expressed as percentage relative abundance. Rare species, defined as taxa with a relative abundance <1% at <2 sites, were omitted from the data files before analysis to circumvent unreliability of species abundance because of low number of counts and the disproportionate impact of rare species on ordinations (Shi, 1993). Data analyses were conducted on the full data set including all 29 lakes and on subsets including lakes with maximum lake depth ≤4 m (20 lakes) and ≤10 m (23 lakes), respectively.

Ordinations

Relationship and redundancy (collinearity) among the environmental variables were explored by principal component analysis (PCA), based solely on the environmental data, and by the variance inflation factor (VIF) estimated using canonical correspondence analysis (CCA) (species and environmental data). Detrended correspondence analysis (DCA) of surface sediment cladoceran data was applied to determine the gradient length of axis 1 and values >2 SD units of species turnover, which are indicative of unimodal relationships (ter Braak, 1995). Biplots of the first two DCA axes were compared with correspondence analysis (CA) ordinations to examine if there was an arch in the data (ter Braak, 1995). CCA was applied to examine the relationships between the species and predictors and to identify suitable candidate parameters (predictors) for model development. Tests of significance of the ordination axes were performed by specifying, respectively, the first, second and third CCA axes as covariables. Suitable candidate parameters were evaluated on the basis of the regression coefficient's t-values with n-q-1 degrees of freedom (n = number of samples, q = number of environmental variables, significance level 5%), the inter-set correlation of the environmental variables with axis 1 and the significance of Bonferroni corrected type I error (α-corrected = 0.05 per q) of forward selected predictors within the CCA including all predictors. In addition, the significance of axis 1 and the ratio of the first constrained axis (λ_1) to the first unconstrained axis (λ_2), ratios > 0.5 for suitable candidate parameters (Kingston *et al.*, 1992), in single variable CCA's, were used for the evaluation (ter Braak & Smilauer, 2002). Partial CCA's with a single predictor specified as an active variable and the others as covariables were run to examine the contribution of explanatory power to the variance in species data by the single predictor. Single-variable detrended CCA's (DCCA) were performed to determine whether unimodal or linear based inference methods would be the most appropriate to apply, the latter being evaluated by the gradient length of axis 1 (Birks, 1998). All ordinations were performed using CANOCO version 4.5 (ter Braak & Smilauer, 2002). Detrending by segments was carried out in CA and DCA, and in all unimodal analyses down weighting of species was applied. Monte Carlo permutation significance tests were performed with 499 permutations.

Multivariate regression trees

Multivariate regression tree (MRT) analysis was used as an alternative tool to the ordination analyses and to determine the cut-off values of the environmental predictors most strongly separating the species data into clusters (habitat types). Contrary to the ordination analyses (DCA, PCA and CCA), MRT analysis makes no assumptions about the form of relationships (e.g. unimodal or linear) between species and their environmental predictors. Moreover, this method is applicable for complex ecological data with imbalance, non-linear relationships between variables and high-order interactions (De'ath & Fabricus, 2000). MRT models species-environmental relationships and forms clusters of the species assemblages and sites by repeated splitting of the data, with each split chosen to minimise the dissimilarity (sum of squared euclidian distances, SSD) of the species and sites within clusters (Breiman *et al.*, 1984; De'ath & Fabricus, 2000). The overall fit of a tree is specified as relative error (RE; SSD in clusters divided by SSD of undivided data), while the predictive accuracy is assessed by cross-validated relative error (CVRE; Breiman *et al.*, 1984; De'ath & Fabricus, 2000). In this study, the finally selected tree was the model with minimum CVRE, according to De'ath & Fabricus (2000), using 1000 multiple cross validations to stabilise the cross-validated error. Species distinctive for a given cluster were identified using an indicator species index (INDVAL) calculated by the product of the relative abundance and the relative frequency of occurrence within the cluster (Dufrene & Legendre, 1997). Significance of the species association to the particular cluster was accessed by permutation tests with 500 iterations. An INDVAL value of 1 indicates that the species is solely confined to a particular cluster, while an INDVAL of 0 indicates that the species are widely distributed among the different clusters. MRT analyses were carried out in R (The R Foundation for Statistical Computing Version 2.1.1) using the MVPART package (Multivariate), while INDVAL analyses were performed with the LABDSV package (Dynamic Synthetic Vegephenomenology).

Parametric statistical analysis

In cases where multivariate analysis appeared inappropriate because of too low species diversity and frequencies (e.g. zooplankton assemblages in water samples) Pearson correlation coefficients were applied to determine the trend and significance ($P < 0.05$) between the single taxon-predictor relationship. In addition, paired *t*-tests ($P < 0.05$) were conducted on Arcsine transformed percentage species data to elucidate single-taxon relationships in shallow (≤4 m) and deep (>4 m) lakes, respectively. The parametric statistical analyses were performed using SAS V8 (SAS Institute, 1999).

Model building

A variety of weighted averaging (WA) inference models, weighted averaging partial least squares regression (WA-PLS) models and partial least squares (PLS) were developed using C^2 version 1.4 (Juggins, 2004). Both tolerance down weighting and simple WA were used, with both classical and inverse deshrinking. The models were internally validated by the coefficient of determination (r^2) between the observed and predicted values of the predictor, the distribution of residuals (observed value – predicted value) and by the root mean square error of prediction (RMSEP). Predicted values and RMSEP were obtained by bootstrapping using 999 iterations. Bias (value dependent error) should be as low as possible. The optimal number of components to include in the WA-PLS and PLS model was assessed by leave-one-out-jack-knifing permutation tests (999 iterations). A higher component WA-PLS model was only accepted, if the improvement in RMSEP was >5% over the simpler (lower component) alternative (Birks, 1998).

Results

Present environmental state of the study lakes

The 29 lakes studied were generally small and oligo-mesotrophic with low chlorophyll *a* concentrations (Table 1). Maximum depth ranged from 0.3 to 52 m. The lakes were dilute (Table 1), excepting saline Lake Sandsvatn (conductivity > 4000 μS cm^{-1}). Eight lakes, all located on the island of Sandoy, were slightly brackish with a salinity range of 0.09–1.86‰. The majority of the lakes had pH values close to neutral (Table 1), while only one lake (Lake Vatnid Oman Storrygg) had pH < 6.5 and one lake (Lake Mulaik) had pH > 9.0. The total fish abundance covered a gradient of 0–30 fish net L^{-1} $night^{-1}$ (Table 1). Only one lake (Lake Handastavatn) was found to be fishless. Brown trout (*S. trutta*) was present in 26 lakes, while two lakes (Lake Musavatn, Lake Vatnid i Tindalid) were exclusively dominated by three-spined stickleback (*G. aculeatus*). Among the 26 lakes supporting brown trout populations, 12 were dominated exclusively by this species, while the remaining 14 lakes had additional populations of salmon (*Salmo salar*, Lake Vatnsnes), flounder (*Platichthys flesus*, Lake Sandsvatn), Arctic charr (*S. alpinus*, Lake Leynavatn, Lake Frammi a Vatni), rainbow trout (*Salmo irideus*, Lake Frammi a Vatni) and three-spined stickleback (12 lakes).

Statistical analyses

Exploratory analyses – environmental data. The salinity variable was omitted from our data analyses because of its strong correlation to conductivity ($r^2 = 0.88$, $P < 0.0001$) and its high VIF (12.5) compared with the VIF's of other predictors (VIF range 1.8–7.5). Initial CCA analysis including latitude, longitude and altitude in addition to the 10 other environmental predictors was performed to examine the impact of geographical location on cladoceran species community structure (e.g. isolation or dispersal hindrance between the five islands). The geographical predictors, however, did not contribute significantly to the species variation and did not markedly alter the CCA ordination. They were therefore excluded from further analyses.

Exploratory analyses – species data of water samples. Cladocerans were not recorded in the water samples from three lakes (Lake Mjavavatn, Lake Musavatn, Lake Frammi a Vatni) and only 11 cladoceran taxa (two pelagic taxa, nine benthic taxa) were recorded in the remaining 26 lakes (Fig. 2). The pelagic taxa (*Bosmina longispina* and *Daphnia hyalina/longispina*)

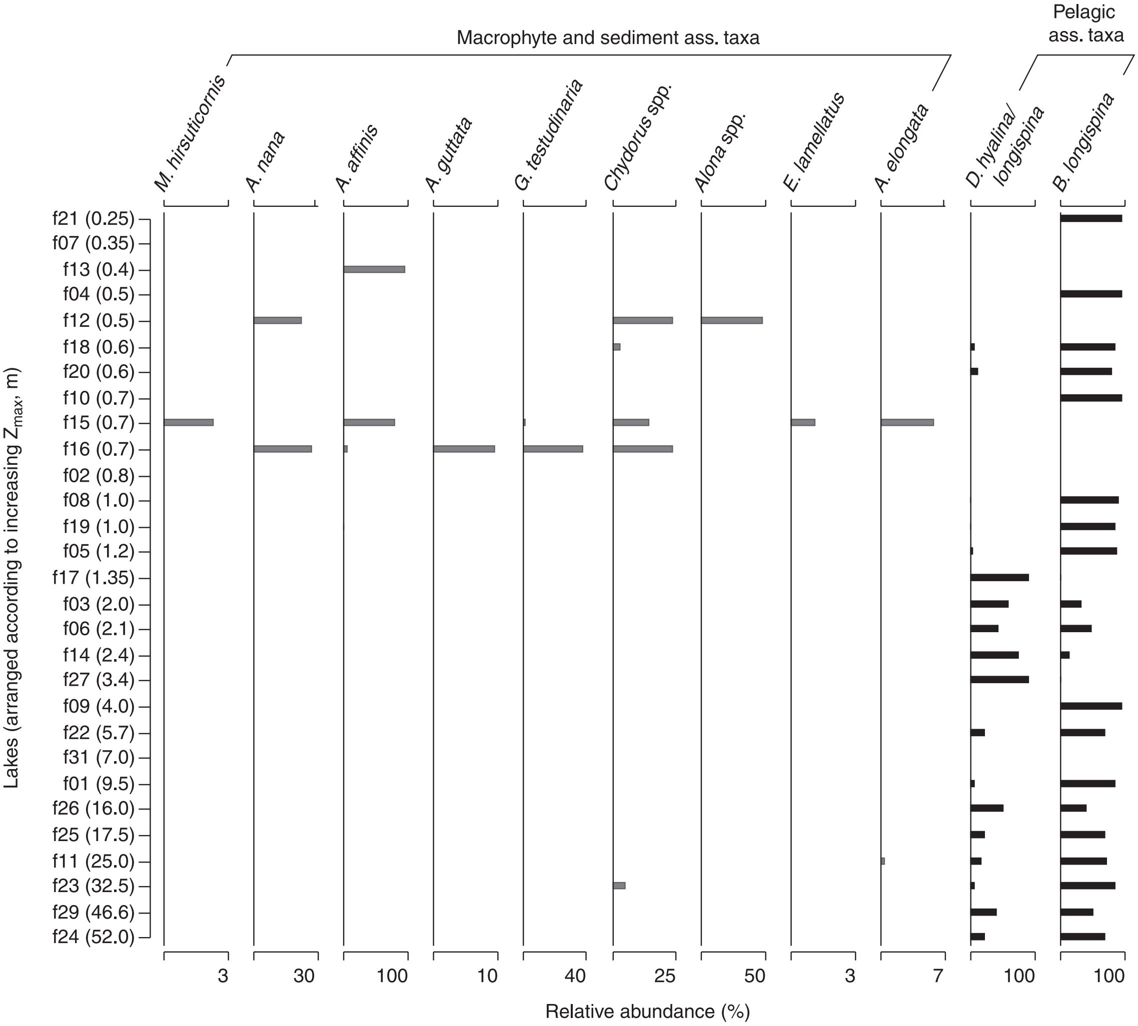

Fig. 2 Relative abundance of cladocerans recovered from water samples of the 29 study lakes. Lakes are arranged in order of increasing maximum lake depth (values given in brackets).

occurred exclusively in 14 lakes and dominated in the other lakes but 4 (Lake W. of Kirkjuvatn, Lake Blavusvatn, Lake Grothusvatn, Lake Litlavatn). Taxonomic species separation of *D. hyalina* and *D. longispina* could not be conducted; thus, the two taxa are termed *D. hyalina/longispina*. Benthic cladocerans generally occurred in low densities and only in a few lakes (Fig. 2), making them unsuitable for ordination analysis. The MRT analysis produced the lowest CVRE (1.076) for a one-leaf tree compared with larger sized trees (CVRE ≥ 1.644; Fig. 3a) and splitting the data into clusters was therefore pointless. Pearson correlation coefficients for the pelagic taxa showed only a significant relationship between $Z_{\max}$ and *D. hyalina/longispina* ($r^2 = 0.466$, $P < 0.0108$).

Exploratory analyses – species data of sediment samples. Cladoceran remains were recovered in all 29 surface sediments and a total of 18 taxa were identified, of which two were pelagic (*B. longispina*, *Daphnia* spp.) and 16 benthic chydorids (Fig. 4). *Alonella excisa* and *Monospillus dispar* only occurred in one though not the same lake and were therefore omitted from the data analyses. Taxonomic species separation of *Alona guttata* and *Alona rectangula*, and to some extent *Alona rustica* as well, could not be conducted for the surface samples as organic material adhered to the headshields and thus covered the headpores used for identification. In the following, these species are consequently referred to as *Alona* spp. Some of the carapaces and headshields of *Alona* spp. were dented and probably variants of tuberculata forms. A DCA with species samples produced a gradient length of axis 1 of 2.11 SD units, suggesting that application of unimodal methods could be useful (ter Braak, 1995). Ordinations of species and sites were almost similar for DCA and CA, and no arch was evident in the CA. Between 31.6% and 32.4% of the cumulative species variance was explained on axis 1, and a further 14.8% and 19.1% were explained on axis 2 in these ordinations.

Constrained ordinations of sedimentary species data. The eigenvalues ($\lambda_1 = 0.311$, $\lambda_2 = 0.088$) of the CCA based on the 29 lake data set were only slightly lower than those of the CA ($\lambda_1 = 0.329$, $\lambda_2 = 0.191$), which indicates that much of the variance from the CA was captured in the CCA, especially on axis 1. Only CCA axis 1 was significant ($P = 0.002$) using 499 Monte Carlo permutation tests. CCA axis 1 was most

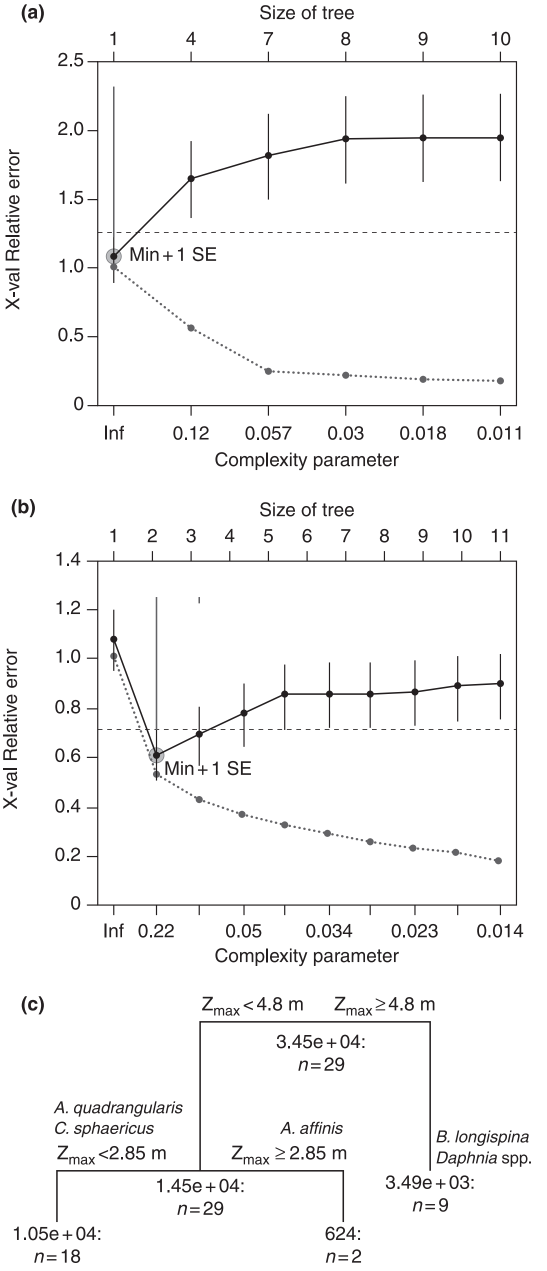

Fig. 3 (a) Cross-validation of the regression tree based on cladoceran water samples from the 29 study lakes. Shown are the explanatory power (lower line), the predictive power (upper line) and the distance of one standard error from the best model (solid horizontal line). The circled point is the model with the greatest cross-validated predictive accuracy. (b) Cross-validation of the regression tree based on cladocerans from surface sediment samples of the 29 study lakes (abbreviation as Fig. 3a). (c) Multivariate regression tree based on cladocerans from surface sediment samples of the 29 study lakes. The length of the vertical lines in the regression tree represents the deviance explained by each split. Cluster deviance (SSD) around the mean, number of lakes per cluster and indicator species are given at the tree leaves.

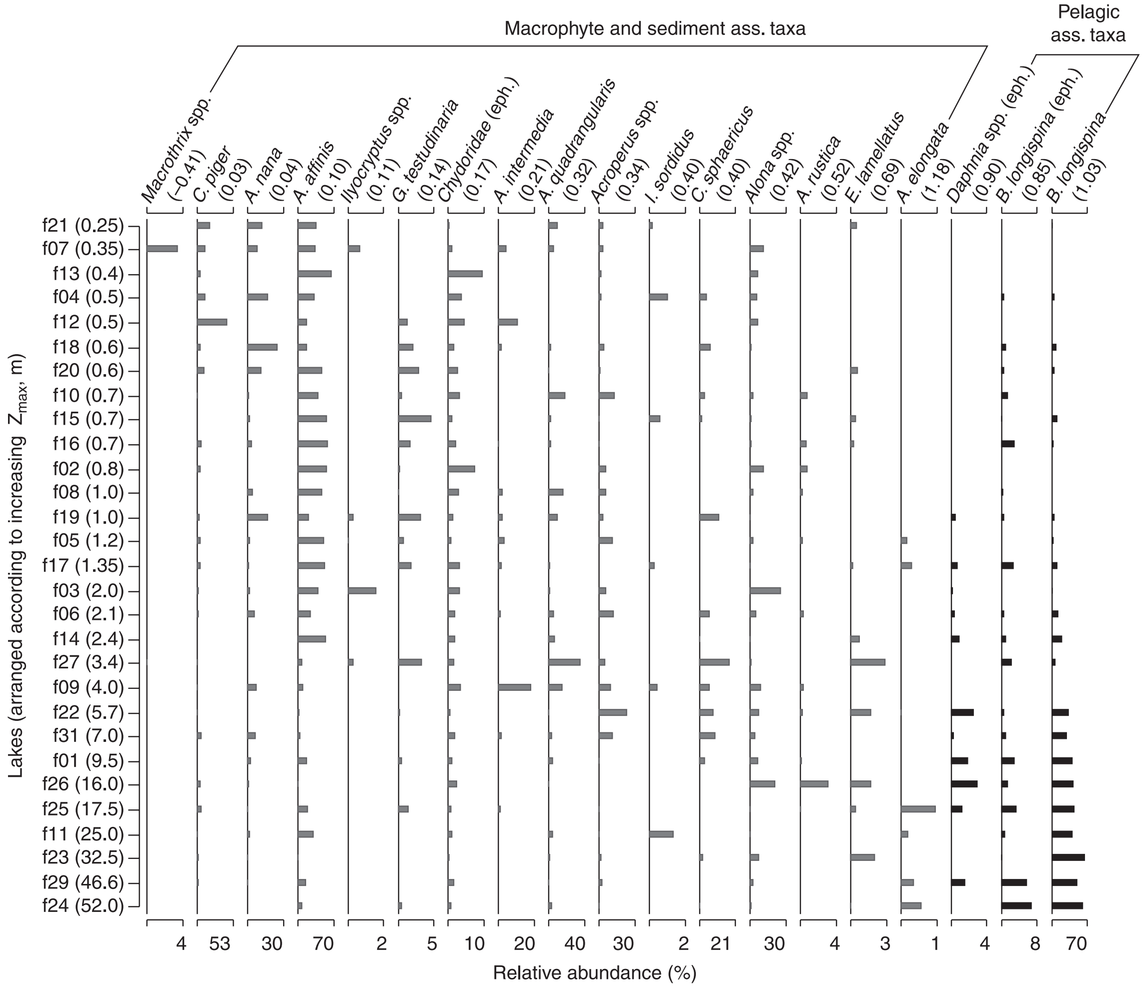

Fig. 4 Relative abundance of cladoceran remains recovered from surface sediments of the 29 study lakes. Lakes are arranged as in Fig. 2. Species are sorted by maximum lake depth weighted average optima (shown in brackets).

strongly influenced by Z_{max} (inter-set correlation = 0.95), area and TP (inter-set correlations = 0.72 and −0.66, respectively), while pH, total fish abundance (CPUEtot) and brown trout abundance (CPUEbr) contributed most strongly to axis 2 (inter-set correlations = 0.42, 0.38 and 0.34, respectively; Fig. 5). Yet, among these predictors only Z_{max} produced a significant *t*-value of the regression coefficients (Z_{max} *t*-value axis 1 = 6.88, critical value of Student's *t*-distribution with 18 degrees of freedom = 2.101). Z_{max} also appeared to be the most important predictor as it was persistently chosen as the only significant variable by Bonferroni-adjusted forward selection of CCA's based on the entire dataset (n = 29 lakes, n = 16 taxa) and on the two subsets based on lakes with $Z_{max} \leq 4$ m and ≤10 m, respectively. In addition, single variable CCA's showed Z_{max} to produce the highest λ_1/λ_2 value (1.5) compared with the other predictors (range $\lambda_1/\lambda_2 = 0.03–0.9$). Comparison of DCA axis 1 for sample scores with Z_{max} further confirmed that the major direction of variance within the cladoceran data was highly correlated with Z_{max} ($r^2 = 0.834$, Fig. 6). Z_{max} therefore seemed to be the most suitable candidate for the development of cladoceran inference models. The 10 predictors accounted for 53.4% (sum of all canonical λ's = 0.542, total inertia = 1.016) of the total species variation, of which Z_{max} uniquely accounted for 13.8% of the species variation.

MRT analyses of sedimentary species data. The MRT analysis produced the smallest estimated predictive error (CVRE = 0.612) for a two-leaf tree compared with those of the one-leaf tree (CVRE = 1.075) and

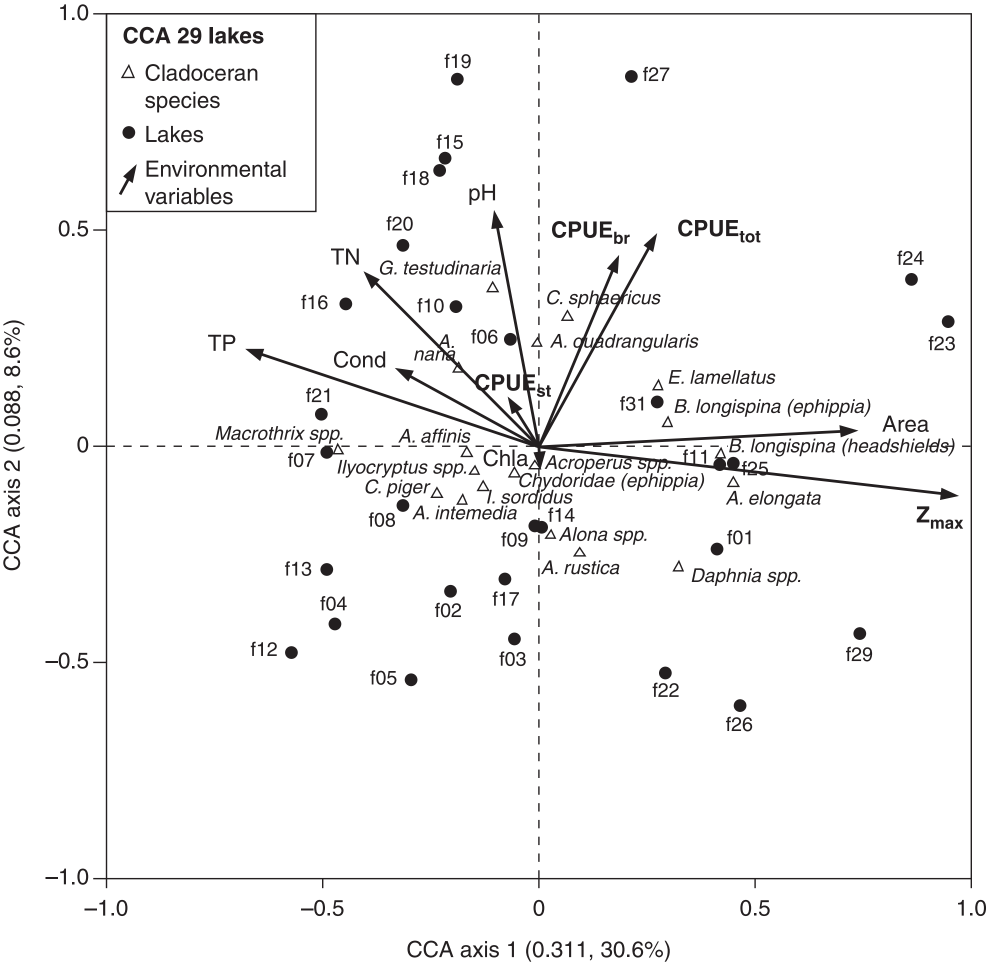

Fig. 5 CCA ordination plot of 18 cladoceran taxa identified in the 29 lake surface sediment samples. Solid arrow indicates significant variable determined by Bonferroni-adjusted forward selection ($P < 0.005$).

trees above two-leaf (CVRE ≥ 0.69; Fig. 3b). The primary split was defined by $Z_{max} < 4.8$ m (to the left; Fig. 3c), while the secondary split was based on $Z_{max} < 2.85$ m (to the left). For the primary split, surrogate variables for Z_{max} were given by TP (<12 μg L^{-1} to the right, $r^2 = 0.897$), conductivity (<167 μS cm^{-1} to the right, $r^2 = 0.862$) and TN (<155 μg L^{-1} to the right, $r^2 = 0.862$). For the cluster with $Z_{max} < 2.85$ m, *Alona quadrangularis* (INDVAL = 0.737, $P = 0.006$) and *Chydorus sphaericus* (INDVAL = 0.703, $P = 0.018$) were identified as indicator species, while only *Alona affinis* (INDVAL = 0.639, $P = 0.002$) was significantly associated with the cluster of 2.85 m ≤ $Z_{max} < 4.8$ m. Species significantly associated with the cluster of $Z_{max} \geq 4.8$ m were *B. longispina* (INDVAL = 0.7870, $P = 0.002$) and *Daphnia* spp. (INDVAL = 0.7452, $P = 0.014$; Fig. 3c).

Cladoceran distribution

A clear trend was observed in the distribution of sedimentary cladocerans regarding Z_{max} (Fig. 5). In the CCA the pelagic taxa *B. longispina* and *Daphnia* spp. had the greatest relative abundance in lakes with high Z_{max}, while truly sediment associated chydorids such as *Macrothrix* spp., *Ilyocryptus* spp. and *Chydorus piger* were more abundant in shallow waters (Fig. 5). This agrees well with the MRT analysis showing a significant association of pelagic species (*B. longispina*, *Daphnia* spp.) to the deep lakes ($Z_{max} \geq 4.8$ m) (to the right; Fig. 3c). In addition, light seemingly became attenuated in lakes with depths above approximately 5 m (Fig. 7a), concurrently with a clear shift from benthic to pelagic cladoceran dominance (Fig. 7b). Taxa with habitat preferences for either macrophytes

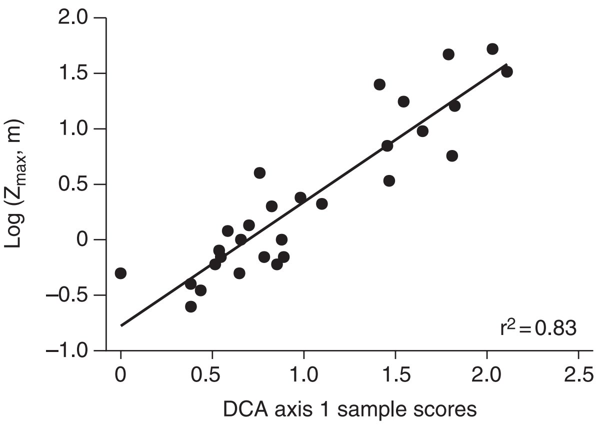

Fig. 6 Cladoceran DCA axis 1 scores against observed log (maximum lake depth) for the 29 study lakes.

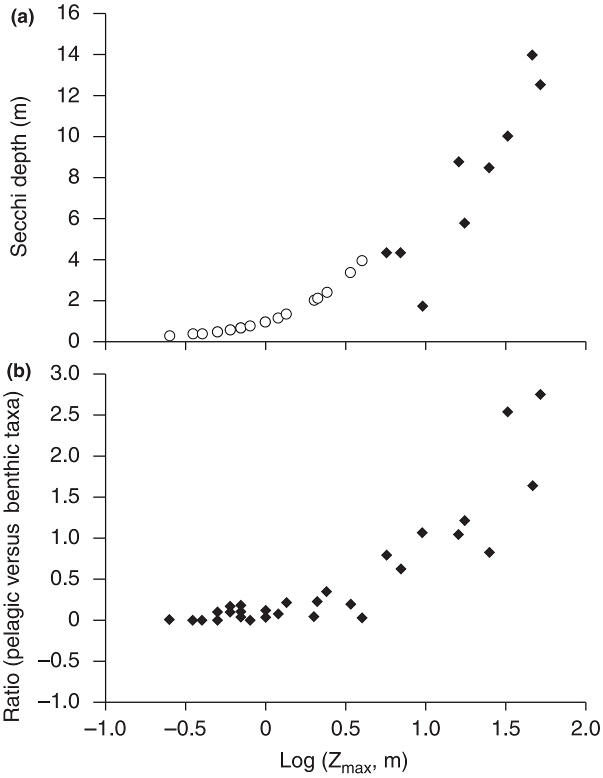

Fig. 7 (a) Relationship between Secchi depth and maximum lake depth for lakes with Z_{max}. Visibility to the lake bottom indicated by empty circles. (b) Relationship between relative abundance of benthic and pelagic cladoceran abundance and Z_{max} in the 29 study lakes.

or macrophytes and sediment showed less variation over the range of Z_{max}, with most species optima occurring near mean values, with the exception of the large bodied *Eurycercus lammelatus* and *Alonopsis elongata* that were more abundant in deeper waters (Fig. 5). However, paired *t*-tests conducted separately for each of these two species at shallow (<4 m) and deep lakes (>4 m) showed insignificant relationships between abundance and lake depth, respectively. Bonferroni-adjusted forward selection within the CCA's (based on the entire datasets, subsets of lakes ≤4 m and <10 m, respectively) suggested that the other variables additional to Z_{max} did not account for significantly more species variation than could be described by Z_{max} alone. Negligible importance of fish abundance in shaping the cladoceran community structure was further supported by insignificant relationships found between fish abundance (CPUEtot) and *Daphnia* spp. ephippial sizes and the ratio of *Daphnia* and *Bosmina* ephippia (*Daphnia*/(*Daphnia* + *Bosmina*); Fig. 8, left). Nor could the importance of Z_{max} in cladoceran community structure be explained by variations in fish abundance as CPUEbt and CPUEst did not differ significantly among shallow (<4 m) and deep (>4 m) lakes (paired *t*-tests, $P > 0.99$, $P > 0.68$, respectively). This was further supported by insignificant relationships between Z_{max} and *Daphnia* spp. ephippial sizes and the ratio of *Daphnia* and *Bosmina* ephippia (*Daphnia*/(*Daphnia* + *Bosmina*); Fig. 8, right). In addition, no difference in *Daphnia* spp. abundance was found in either the absence or presence of stickleback in shallow and deep lakes (paired *t*-tests, $P > 0.60$ and $P > 0.77$, respectively). However, it should be emphasised that because of distortion of the *Daphnia* spp. ephippia, size (dorsal length) could only be measured for half of the lakes (14 lakes), which adds to the uncertainty of these results.

Inference models

The DCCA with Z_{max} as the sole predictor produced a gradient length of axis 1 of 1.65 SD units, suggesting that both linear and unimodal based inference methods are appropriate for lake level inference. The second component WA-PLS and PLS did not contribute to a 5% improvement of RMSEP compared with the one-component alternative. As the one-component WA-PLS model is identical with the WA with inverse deshrinking, only the results of the WA and PLS models are described here. All inference models for inference of Z_{max} performed almost equally well with relatively high r^2, low RMSEP and low average bias (Table 2). Yet, no significant

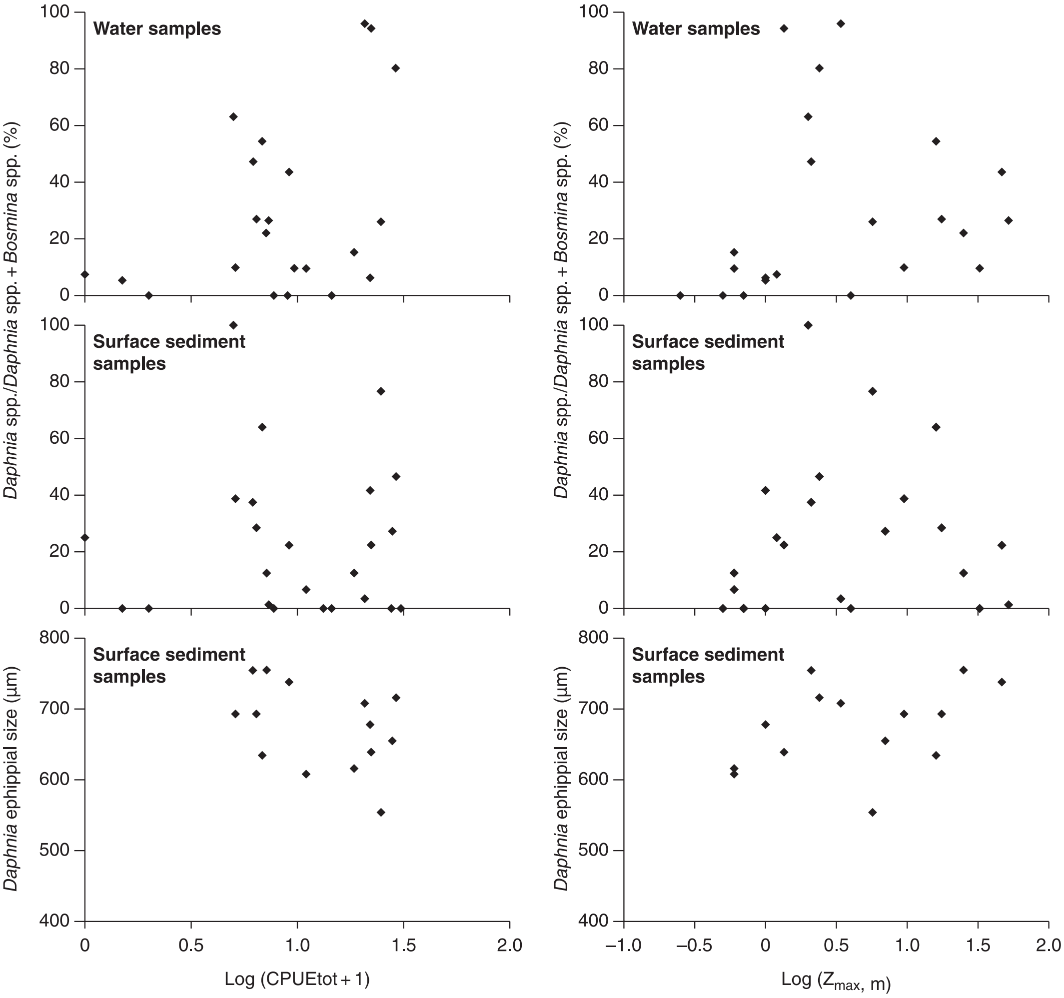

Fig. 8 The ratio of *Daphnia* spp. to the sum of *Daphnia* spp. and *Bosmina* spp. based on water and surficial sedimentary samples, respectively, and *Daphnia* ephippial size based on surficial sedimentary samples solely, in relation to CPUEtot and $Z_{\max}$, respectively.

Table 2 Summary statistics for $Z_{\max}$ inference models based on 16 cladoceran taxa and 29 lakes

	Inverse deshrinking WA	Classical deshrinking WA	Inverse deshrinking WA (tol)	Classical deshrinking WA (tol)	PLS component 1
Apparent					
r^2	0.907	0.907	0.900	0.900	0.851
RMSE	0.207	0.218	0.216	0.227	0.262
r^2 residuals	0.093	0	0.101	0	0.149
Bootstrapped					
r^2	0.876	0.877	0.838	0.839	0.819
RMSEP	0.263	0.260	0.317	0.310	0.303
r^2 residuals	0.272	0.068	0.411	0.180	0.198
Average bias	−0.006	−0.010	−0.006	−0.011	−0.009
Max bias	0.558	0.511	0.762	0.729	0.604

Units for bias, RMSE and RMSEP are $\log(Z_{\max})$.
WA, weighted averaging; PLS, partial least squares; tol, tolerance.

bias in residual structure was found in the simple WA models with classical deshrinking, making this model the most suitable.

Lake Heygsvatn

Chronological control based on the nine ^{14}C AMS dates showed that the Lake Heygsvatn sediment record covers the last ca 5700 years (Fig. 9). Measurements of magnetic susceptibility and organic content appeared to be relatively stable throughout the record, expect for a period starting ca 1714 ± 51 calendar years before present (BP) exhibiting a major increase in organic content. This rise was synchronous with a major change in the sedimentation rate. An age inversion (at 2235 ± 114 BP) just after the rapid increase in organic matter content supported the assumption of the occurrence of a period characterised by heavy soil erosion and consequent leaching of old carbon (for further details: see M. Grauert, S. McGowan and N.J. Anderson, unpubl. data).

In general the remains of cladocerans were well preserved and abundant throughout the core [median: 1904 remains (g DW sediment)$^{-1}$, range: 540–11 464 remains (g DW sediment)$^{-1}$]. A total of 16 taxa (two pelagic taxa, 14 benthic taxa) were identified in 23 depth core sections (Fig. 9). With the exception of *Ilyocryptus* spp. and *Macrothrix* spp., all taxa in the core were included in the calibration data set. Throughout the core the cladoceran stratigraphy was dominated by benthic taxa, mainly macrophyte associated *Eurycercus* spp., *Acroperus* spp., *Graptoleberis* spp. and *Alonella nana* and macrophyte and sediment associated taxa such as *A. affinis*, *A. quadrangularis*, *C. sphaericus* and *C. piger* (Fig. 9). The pelagic associated taxa *B. longispina* and *Daphnia* spp. maintained low abundances throughout the core, abundances being particularly low in the intermediate zone of approximately 800–500 cm below lake surface (Fig. 9). The median ephippial size (dorsal length) of *Daphnia* spp. ranged from 675 to 948 μm and the median ratio of *Daphnia* to *Daphnia* + *Bosmina* was low (median: 0.1) throughout the core. Yet, it must be emphasised that *Daphnia* spp. and *B. longispina* ephippia were absent at 12 and three depths, respectively (Fig. 9). In addition, when present, *Daphnia* ephippia numbers were low (Fig. 9), which adds to the uncertainty of the results, particularly as regards the estimation of past fish predation pressures. The inference of Z_{max} suggested overall low lake depth levels (range: 0.8–3.4 m ± 1.9 m, WA model with classical deshrinking) with only minor Z_{max} fluctuations to have persisted throughout the period covered by the core. Thus, around 840 cm below lake surface (around 1665 years BP) the inference (WA model) indicated an onset of a minor declining trend in Z_{max}. Shallowness (0–8–1.2 m) persisted until around 550 cm below lake surface (around 1420 years BP) where a slight increasing trend in Z_{max} emerged (Fig. 9). Almost coinciding (approximately 845–730 cm below lake surface) with the declining inferred Z_{max}, a pronounced temporary increase in organic content (LOI; Fig. 9) and sedimentation rate occurred, being indicative of catchment soil erosion and consequent lake shallowing (M. Grauert, S. McGowan and N.J. Anderson, unpubl. data).

Discussion

The present study demonstrated two major traits in regard to fish. First, brown trout was the most abundant species, being present in all except three and exclusively dominant in 12 of the 29 Faroese study lakes. Only two lakes supported populations of Arctic charr, while three-spined sticklebacks were present in 12 lakes. Second, fish abundance was apparently only of minor importance in shaping cladoceran community and body size structure (Figs 5 and 8, left). This contradicts the results of studies conducted in arctic and subarctic Greenland lakes (Jeppesen *et al.*, 2001a; Lauridsen *et al.*, 2001) and subarctic Icelandic lakes (Antonsson, 1992). In these lakes fish play a major role and exert a high predation pressure on the zooplankton, with a cascading impact on the remaining food web structure. A plausible explanation is that the zooplanktivorous predator Arctic charr dominates the fish population in lakes in Iceland and Greenland (Antonsson, 1992; Jónsson & Skúlason, 2000; Riget *et al.*, 2000; Jeppesen *et al.*, 2001a), whereas brown trout through its more omnivorous diet habits may exert a weaker predator effect on the zooplankton. Analysis of fish diets (stomach content; Malmquist *et al.*, 2002) and zooplankton biomass ratios (Jeppesen *et al.*, 2002a) in four of our study lakes thus suggest low predation pressure on cladocerans in the brown trout only lake, moderate predation pressure in brown trout and three-spined stickleback lakes, and high predation

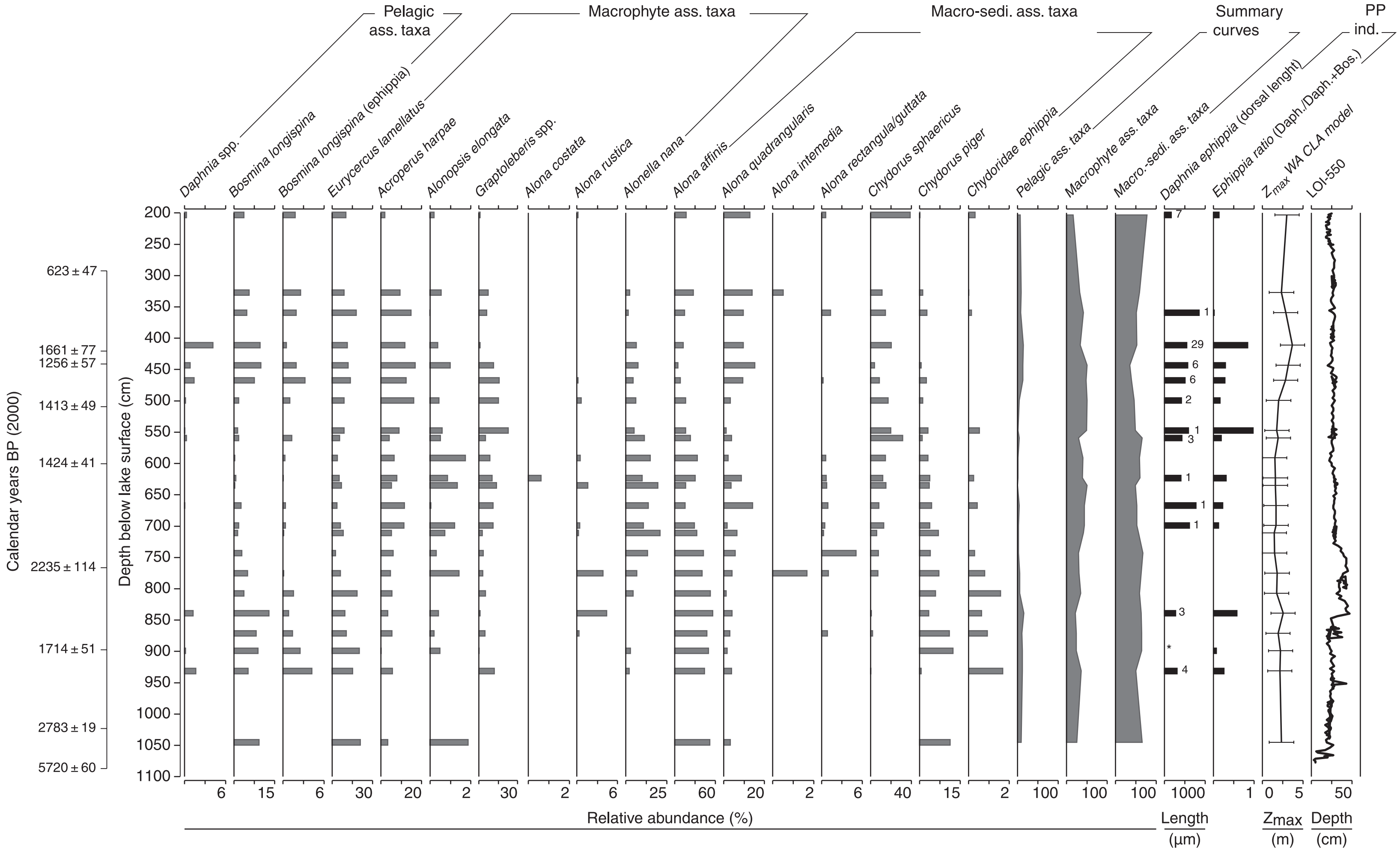

Fig. 9 Cladoceran stratigraphy, summary curves, cladoceran inferred $Z_{\max}$ and Loss-on-ignition (LOI-550) of the Lake Heygsvatn core. Classification into habitat preferences according to Hann (1990) and Røen (1995). Sediment age based on nine AMS ^{14}C- dating. Note: initiation of erosion (in-wash of old carbon from catchment) at approximately 1714 ± 51 and a subsequent age inversion of 2235 ± 114 and 1661 ± 77 (see M. Graunert, S. McGowan, J.N. Anderson, unpublished data, for further details). PP refers to predation pressure indicators. Numbers next to *Daphnia* ephippia refer to number of enumerated ephippia and asterisk refers to ephippia considered unsuitable for size measurement (partly torn).

pressure on cladocerans in the brown trout and Arctic charr lake. Moreover, stable isotope analyses of fish muscles in the four Faroese lakes show that brown trout forage indifferently in trout-only lakes, but forage to a higher degree in the pelagic zone when living in sympathy with stickleback and in the littoral zone when co-occurring with Arctic charr (Jeppesen *et al.*, 2002b). In addition, a recent 14 year monitoring study of the Norwegian Lake Atnsjøen shows zooplankton to contribute only negligibly to the diet of brown trout in general, while zooplankton was found to be the most important food item for Arctic charr (Saksgaard & Hesthagen, 2004). Moreover, Cavelli, Miquelis & Chappaz (2001) found the diet of brown trout to consist of mainly of chironomids and exogenous prey items, while Arctic charr additionally preyed upon cladocerans in a study of five high altitude lakes in the French Alps. The dominance of brown trout and its diverse foraging behaviour and diet may therefore explain why the impact of fish planktivory on cladocerans was markedly lower in the Faroese lakes when compared with other oligotrophic subarctic and arctic lakes. In addition, the diverse foraging behaviour and diet may serve as a plausible explanation to our finding of lake depth seemingly not altering fish predatory control of the pelagic cladocerans (Fig. 8, right), contrary to the findings in northern temperate lakes (Jeppesen *et al.*, 1997).

The larger success of brown trout compared with Arctic charr in Faroese lakes, both being native species (Malmquist *et al.*, 2002), may be climatically conditioned, as the optimum temperature for growth of brown trout is between 13 and 18 °C (Elliot, 1994; Klemetsen *et al.*, 2003), while the optimum of Arctic charr is around 10–12 °C (Jobling, 1983). In the 29 study lakes the average water temperature was measured to 13.8 °C (range: 11.4–17.4 °C, E. Jeppesen, unpubl. data) in August and thus exceeded the preferred temperature of Arctic charr. However, potential preference in stocking of brown trout in the lakes may have contributed as well.

The negligible impact of three-spined sticklebacks on cladoceran species composition and size structure contradicts the results of other studies (e.g. Pont, Crivelli & Guillot, 1991). However, the abundance of sticklebacks was relatively low (Table 1) in the 29 study lakes. A possible explanation is piscivory by brown trout on three-spined sticklebacks as found by Abée-Lund, Langeland & Sægrov (1992) in Norwegian lakes. In support of this, Jeppesen *et al.* (2002b) found the trophic position of brown trout in Faroese lakes with sticklebacks to be higher than in lakes without sticklebacks.

Our study demonstrates substantial differences in species frequency, richness and abundance of cladocerans derived from the water and surface sediment samples collected in 29 Faroese lakes. In the water samples, cladocerans were not found in three lakes and species richness was low (11 taxa). In contrast, surface sediment samples showed presence of cladocerans in all lakes and high species richness (18 taxa). The water samples were dominated by pelagic taxa, *B. longirostris* and *Daphnia* spp. being exclusively dominant in 50% of the lakes, whereas the sediment samples showed dominance of benthic taxa in 80% of the lakes. The results correspond well with those of recent studies (Brendonck & De Meester, 2003; Vanderkerkhove *et al.*, 2005). They all show that use of sedimentary cladoceran remains provides a more complete assessment of species richness and community structure than does conventional point-sampling in the pelagic zone. This is because the sedimentary samples include benthic communities and integrate spatial and seasonal species heterogeneity and year-to-year variations.

Compared with continental subarctic lakes (Korhola, 1999) and northern temperate lakes (Brodersen, Whiteside & Lindegaard, 1998), cladoceran species richness was lower in the subarctic Faroese lakes, which likely reflects the remoteness of the islands acting as a dispersal barrier and the relatively low temperature regimes of the Faroese lakes (Lauridsen & Hansson, 2002). Accordingly, cladoceran richness is higher in the Faroese lakes compared with the colder subarctic Icelandic lakes (Antonsson, 1992; Einarsson & Ornólfsdóttir, 2004), arctic north-eastern Greenland lakes (Jeppesen *et al.*, 2001a) and western Greenland lakes (Lauridsen *et al.*, 2001, Jeppesen et al., unpubl. data).

The multivariate ordination analyses and the MRT analysis based on the sedimentary cladoceran remains of the 29 study lakes unanimously indicated maximum depth to be the most important environmental variable influencing cladoceran community structure. A clear shift from benthic to pelagic cladoceran dominance was found around a maximum lake depth of 5 m (Fig. 7b), which agrees well with the primary split of 4.8 m and with the significant association of pelagic species (*B. longispina*, *Daphnia* spp.) to the deep lakes ($Z_{\max} \geq 4.8$ m; Fig. 3c). The boundary of 4.8 m seems reasonable as light penetrated to the bottom in lakes with depths below approximately 5 m, whereas lakes with depths above 5 m (Fig. 7a) exhibited less favourable conditions for benthic primary production. Lake chemistry, by contrast, seemed to have only limited impact on the cladoceran community structure, reflecting that the lakes were nutrient poor and dilute and had pH values close to neutral. Likewise, Korhola (1999) and Korhola *et al.* (2000) found maximum lake depth to be the most important factor explaining cladoceran distribution in 53 subarctic oligotrophic Fennoscandian lakes. In addition, in a survey based on contemporary spot sampling of 104 Alaskan arctic lakes O'Brian *et al.* (2004) showed lake depth and area to be the single-most important factors influencing zooplankton distribution and species richness. Yet, none of these studies included fish, which have been shown to be a major structuring factor in other studies (Jeppesen *et al.*, 2001c).

The weighted-averaging models for inference of maximum lake depth performed equally well with high r^2, low RMSEP and low average bias (Table 2), and they also compared well with similar models established for Fennoscandian (Korhola *et al.*, 2000) and Canadian lakes (Bos, Cumming & Smol, 1999). In addition, the cladoceran-inferred $Z_{\max}$ (approximately 2.6 m ± 1.9 m) in the upper part of the Lake Heygsvatn core corresponded well with contemporary measurements of $Z_{\max}$ (4.3 m; Dali, 1975) and average lake depth (1.5 m; Dali, 1975). However, interpretations must be made with caution. First, lack of documentary records (D. Bloch, pers. comm.) except that of Dali (1975) impedes any validation of the $Z_{\max}$ inference for Lake Heygsvatn. Second, the inference models are mainly driven by shifts in the relative importance of benthic and pelagic community structure. Therefore, any factor such as eutrophication (e.g. Hofmann, 1996), acidification (e.g. Nilssen & Sandøy, 1990) or changes in predation pressure (e.g. Jeppesen *et al.*, 2003), altering the relative importance of the two communities, will potentially influence the inference of lake depth and thereby introduce artefacts. For these reasons it cannot be clearly determined whether, for instance, the recent increase in inferred $Z_{\max}$ (around 1420 years BP, Fig. 9) is a fact (e.g. because of enhanced net precipitation or damming) or an artefact (e.g. because of eutrophication), the two latter events being likely as human settlement on the Faroe Islands happened almost simultaneously (Hannon, Jermanns-Audardottir & Wastegaard, 1998; Hannon & Bradshaw, 2000). However, the concurrent decrease in the abundances of *C. piger* and *A. affinis* (Fig. 9), characteristic of nutrient poor conditions (Whiteside, 1970), and the simultaneous increase in the abundances of *C. sphaericus* and *A. quadrangularis* (Fig. 9), characteristic of nutrient rich conditions (Whiteside, 1970), suggest that eutrophication is the driving factor behind the recent increase in inferred $Z_{\max}$. In addition, the diatom record, being the only proxy analysed besides cladocerans in the Lake Heygsvatn core, may serve as an indirect source of validation. Overall, the diatom record remained relatively unchanged up through the core and was dominated by benthic diatoms such as *Achnanthes* spp. (*A. minutissima* and *A. linearis*) and *Fragilaria* spp. (*F. exigua*, *F. pinnata* and *F. elliptica*; M. Grauert, S. McGowan and N.J. Anderson, unpubl. data), which agrees well with the benthic predominance of the cladoceran record. Around 1714 ± 51 years BP, a minor gradual change occurred in the diatom community (increasing *Fragilaria* sp. abundance), which coincided with an increase in organic content, factors that are both indicative of a continuous lake shallowing (M. Grauert, S. McGowan and N.J. Anderson, unpubl. data), which corresponds well with the onset of the cladoceran-inferred $Z_{\max}$ decline (Fig. 9). Further upcore, diatom data indicated an increase in nutrient concentrations or conductivity (M. Grauert, S. McGowan and N.J. Anderson, unpubl. data), which supports the eutrophication hypothesis.

In summary, unlike in arctic and subarctic Icelandic and Greenland lakes fish abundance was found to be less important in shaping cladoceran community and body size structures in our 29 Faroese study lakes, presumably because of predominance of the less efficient zooplanktivore brown trout. Lake depth, and thus implicitly light penetration, was found to be the single-most important determinant for the composition of the cladoceran community in the predominantly shallow, small-sized and oligotrophic study lakes. The long-core study, however, showed that inference of lake depth from cladocerans must be done with caution as confounding factors (like eutrophication) may be of importance.

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References

Abée-Lund J.H.L., Langeland A. & Sægrov H. (1992) Piscivory by brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.) in Norwegian lakes. *Journal of Fish Biology*, **41**, 91–101.

Antonsson U. (1992) The structure and function of zooplankton in Thingvallavatn, Iceland. *OIKOS*, **64**, 188–221.

Birks H.J.B. (1998) D.G. Frey & E.S. Deevey Review #1: Numerical tools in palaeolimnology – progress, potentials and problems. *Journal of Paleolimnology*, **20**, 307–332.

Bos D.G., Cumming B.F. & Smol J.P. (1999) Cladocera and Anostraca from the Interior Plateau of British Columbia, Canada, as paleolimnological indicators of salinity and lake level. *Hydrobiologia*, **392**, 129–141.

ter Braak C.J.F. (1995) Ordination. In: *Data Analysis in Community and Landscape Ecology* (Eds R.H.G. Jongman, C.J.F. ter Braak & O.F.R. van Tongeren), pp. 91–173. Cambridge University Press, Cambridge, England.

ter Braak C.J.F. & Smilauer P. (2002) *Reference Manual and User's Guide to for CANOCO for Windows (4.5)*. Microcomputer Power, New York.

Breiman L., Friedman J.H., Olshen R.A. & Stone C.G. (1984) *Classification and Regression Trees*. Wadsworth International Group, Belmont, California, U.S.A.

Brendonck L. & De Meester L. (2003) Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. *Hydrobiologia*, **491**, 65–84.

Brodersen K.P., Whiteside M.C. & Lindegaard C. (1998) Reconstruction of trophic state in Danish lakes using subfossil chydorid (Cladocera) assemblages. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1093–1103.

Cavelli L., Miquelis A. & Chappaz R. (2001) Combined effects of environmental factors and predator-prey interactions on zooplankton assemblages in five high alpine lakes. *Hydrobiologia*, **455**, 127–135.

Dali S. (1975) Uppmáting av vøtnum i Føroyum. *Fródskaparrit*, **23**, 63–135.

De'ath G. & Fabricus K.E. (2000) Classification and regression trees: a powerful and simple technique for ecological data analysis. *Ecology*, **81**, 3178–3192.

Dufrene M. & Legendre P. (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, **67**, 345–366.

Einarsson A. & Ornólfsdóttir E.B. (2004) Long-term changes in benthic Cladocera populations in Lake Myvatn, Iceland. *Aquatic Ecology*, **38**, 253–262.

Elliot J.M. (1994) *Quantitative Ecology and the Brown trout*. Oxford University Press, Oxford.

Frey D.G. (1959) The taxonomic and phylogenetic significance of the head pores of the *Chydoridae* (*Cladocera*). *Internationale Revue der gesamten Hydrobiologie*, **44**, 27–50.

Hann B.J. (1990) Cladocera. In: *Methods in Quaternary Ecology* (Ed. B.G. Warner), pp. 81–91. Geoscience Can. Rep. Ser. 5.

Hannon G.E. & Bradshaw R.H.W. (2000) Impacts and timing of the first human settlement on vegetation of the Faroe Islands. *Quaternary Research*, **54**, 404–413.

Hannon G.E., Jermanns-Audardottir M. & Wastegaard S. (1998) Human impact at Tjørnuvik in the Faroe Islands. *Fródskaparrit*, **46**, 215–228.

Hofmann W. (1996) Empirical relationships between cladoceran fauna and trophic state in thirteen northern German lakes: analysis of surficial sediments. *Hydrobiologia*, **318**, 195–201.

Jeppesen E., Jensen J.P., Søndergaard M., Lauridsen T., Pedersen L.J. & Jensen L. (1997) Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia*, **342/343**, 151–164.

Jeppesen E., Christoffersen K., Landkildehus F., Lauridsen T., Amsinck S.L., Riget F. & Søndergaard M. (2001a) Fish and crustaceans in northeast Greenland lakes with special emphasis on interactions between Arctic charr (*Salvelinus alpinus*), *Lepidurus arcticus* and benthic chydorids. *Hydrobiologia*, **442**, 329–337.

Jeppesen E., Leavitt P., De Meester L. & Jensen J.P. (2001b) Functional ecology and palaeolimnology: using cladoceran remains to reconstruct anthropogenic impact. *Trends in Ecology and Evolution*, **16**, 191–198.

Jeppesen E., Jensen J.P., Skovgaard H. & Hvidt C.B. (2001c) Changes in the abundance of planktivorous fish in Lake Skanderborg during the past two centuries – a palaeoecological approach. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **172**, 142–152.

Jeppesen E., Christoffersen K., Malmquist H.J., Faafeng B. & Hansson L. (2002a) Ecology of five Faroese Lakes: summary and synthesis. In: *Five Faroese Lakes, Editors: Annales Societatis Scientiarum Færoensis Supplementum XXXVI* (Eds K. Christoffersen, E. Jeppesen, P.H. Enckell & D. Bloch), pp. 126–139. Føroya Fróðskaparfelag Torshaun 2002, Five Faroese Lakes.

Jeppesen E., Landkildehus F., Lauridsen T.L., Jensen J.P., Bjerring R., Søndergaard M. & Amsinck S.L. (2002b) Food web interactions in five Faroese lakes tracked by stable isotopes. In: *Annales Societatis Scientiarum Færoensis Supplementum XXXVI* (Eds K. Christoffersen, E. Jeppesen, P.H. Enckell & D. Bloch), pp. 114–125. Føroya Fróðskaparfelag Torshaun 2002.

Jeppesen E., Jensen J.P., Jensen C., Faafeng B., Hessen D.O., Søndergaard M., Lauridsen T., Brettum P. & Christoffersen K. (2003) The impact of nutrient state and lake depth on top-down control in the pelagic zone of lakes: a study of 466 lakes from the temperate zone to the arctic. *Ecosystems*, **6**, 313–325.

Jespersen A.M. & Christoffersen K. (1987) Measurements of chlorophyll *a* from phytoplankton using ethanol as extraction solvent. *Archiv für Hydrobiologie*, **109**, 445–454.

Jobling M. (1983) Influence of body weight and temperature on growth rates of Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture*, **22**, 471–475.

Jónsson B. & Skúlason S. (2000) Polymorphic segregation in Arctic charr *Salvelinus alpinus* (L.) from Vatnshlidarvatn, a shallow Icelandic lake. *Biological Journal of the Linnean Society*, **69**, 55–74.

Juggins S. (2004) *Software for Ecological and Palaeoecological Data Analysis and Visualisation*. University of New Castle, England.

Kingston J.C., Birks H.J.B., Uutala A.J., Cumming B.F. & Smol J.P. (1992) Assessing trends in fishery resources and lake water aluminium from paleolimnological analyses of siliceous algae. *Canadian Journal of Fisheries and Aquatic Sciences*, **49**, 116–127.

Klemetsen A., Amundsen P.A., Dempson J.B., Jonsson B., Jonsson N., O'Connell M.F. & Mortensen E. (2003) Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish*, **12**, 1–59.

Korhola A. (1999) Distribution patterns of Cladocera in subarctic Fennoscandian lakes and their potential in environmental reconstruction. *Ecography*, **22**, 357–373.

Korhola A. & Rautio M. (2001) Cladocera and other branchiopod crustaceans. In: *Tracking Environmental Change Using Lake Sediments*, Vol. 4 (Eds J.P. Smol, H.J.B. Birks & W.M. Last), pp. 5–41. Kluwer Academic Publishers, Dordrecht.

Korhola A., Olander H. & Blom T. (2000) Cladoceran and chironomid assemblages as quantitative indicators of water depth in subarctic Fennoscandian lakes. *Journal of Paleolimnology*, **24**, 43–53.

Koroleff F. (1970) *Determination of Total Phosphorus in Natural Water by Means of Persulphate Oxidation*. An. Interlab. Rep. No. 3, Cons. Int. pour l'Explor. de la Mer., ICES, Hydrography COM., Copenhagen.

Lauridsen T.L. & Hansson L.A. (2002) The zooplankton community in five Faroese lakes. In: *Annales Societatis Scientiarum Færoensis Supplementum XXXVI* (Eds K. Christoffersen, E. Jeppesen, P.H. Enckell & D. Bloch), pp. 70–78. Føroya Fróðskaparfelag Torshaun 2002, Five Faroese Lakes.

Lauridsen T.L., Jeppesen E., Landkildehus F. & Søndergaard M. (2001) Horizontal distribution of cladocerans in arctic Greenland lakes – impact of macrophytes and fish. *Hydrobiologia*, **442**, 107–116.

Malmquist H., Ingimarsson F., Johannsdottir E.E., Gislason D. & Snorrason S.S. (2002) Biology of brown trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*) in four Faroese Lakes. In: *Annales Societatis Scientiarum Færoensis Supplementum XXXVI* (Eds K. Christoffersen, E. Jeppesen, P.H. Enckell & D. Bloch), pp. 94–113. Føroya Fróðskaparfelag Torshaun 2002, Five Faroese Lakes.

Margaritora F.G. (1985) *Cladocera*. Fauna D'Italia Vol. XXIII, pp. 1–399. Edizioni Calderini Bologna, Italy.

Murphy J. & Riley J.R. (1972) A modified single solution method for the determination of phosphate in natural waters. *Annales Chemica Acta*, **27**, 21–26.

Nilssen J.P. & Sandøy S. (1990) Recent lake acidification and cladoceran dynamics: surface sediment and core analyses from lakes in Norway, Scotland and Sweden. *Philosophical Transactions of the Royal Society of London*, **327**, 299–309.

O'Brian J.W., Barfield M., Bettez N.D. *et al.* (2004) Physical, chemical and biotic effects on arctic zooplankton communities and diversity. *Limnology & Oceanography*, **49**, 1250–1261.

Pont D., Crivelli A.J. & Guillot F. (1991) The impact of 3-spined sticklebacks on the zooplankton of a previously fish-free pool. *Freshwater Biology*, **26**, 149–163.

Røen U.I. (1995) *Danmarks Fauna Bd. 85, Krebsdyr V, Gællefødder (Branchiopoda)* og Karpelus (*Branchiura*), pp. 1–358. Dansk Naturhistorisk Forening, Viderup Bogtrykkeri A/S (in Danish).

Riget F., Jeppesen E., Landkildehus F., Lauridsen T.L., Geertz-Hansen P., Christoffersen K. & Sparholt H. (2000) Landlocked Arctic charr (*Salvelinus alpinus*) population structure and lake morphometry in Greenland – is there a connection. *Polar Biology*, **23**, 550–558.

Saksgaard R. & Hesthagen T. (2004) A 14-year study of habitat use and diet of brown trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*) in Lake Atnsjøen, a subalpine Norwegian lake. *Hydrobiologia*, **521**, 187–199.

SAS Institute Inc. (1999) *The SAS System for Windows V8*. Cary, NC, U.S.A.

Shi G.R. (1993) Multivariate data analysis in palaeoecology and palaeobiogeography – review. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **105**, 199–234.

R Development Core Team (2005) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, URL http://www.R-project.org.

Vanderkerkhove J., Declerck S., Brendonck L., Conde-Porcuna J.M., Jeppesen E., Johansson L.S. & De Meester L. (2005) Uncovering hidden species: hatching diapausing eggs for the analysis of cladoceran species richness. *Limnology & Oceanography Methods*, **3**, 399–407.

Whiteside M.C. (1970) Danish chydorid Cladocera: modern ecology and cores studies. *Ecological Monographs*, **40**, 79–188.

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Climate-driven regime shift related to changes in water level: a decadal scale multiproxy study of the 8.2 kyr. cooling event in Lake Sarup (Denmark)

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Abstract

We studied changes in trophic dynamics during the 8.2 kyr. cooling event in a wiggle-matched radiocarbon dated annually laminated sediment section (8700-8000 cal BP) from Lake Sarup (55 ºN) using a multiple proxy approach. Changes in $\delta^{13}C$ and $\delta^{18}O$ indicate that the lake hydrology is more driven by precipitation than by temperature-induced changes in evaporation. Sediment accumulation and multiple biological proxies indicated a lake level increase during 8359-8225 BP, followed by an abrupt lake level decrease during the 8.2 kyr event. Thus, the climate anomaly started some 100 years before the cooling event. A lake level increase during this period is supported by a higher load of inorganic and organic allochthonous sedimentation and coincidently lower accumulation of algae pigments, the latter possibly due to the enhanced turbidity-driven reduction in algae production. The lake level increase likely resulted in an extension of shallow areas, which may explain the higher accumulation of benthic associated cladocerans as well as Nymphaeaceae trichosclereids and bryozoans. Abrupt increases in *Tilia* and *Ulmus* pollen further indicate a lake level increase. In contrast, decreased accumulation of inorganic and organic matter during the 8.2 kyr event was observed followed, again, by an increase in algae pigment accumulation. Moreover, marked increases in *Betula* pollen suggest invasion of this species to the formerly flooded areas. Lake Sarup did not return to the initial stage, but stayed more productive after the climatic anomaly, as judged from the cladoceran, bryozoan and pigment assemblages and from their accumulation. Thus, the 8.2 kyr event apparently resulted in a regime shift in the lake. It is hypothesised that the expansion of *Alnus glutinosa* over the period studied induced more nutritious conditions in the terrestrial environment and that these may have affected the trophic level of the lake.

Introduction

Climate change effects on ecosystems have received considerable attention during the last decade, not least in consequence of the accelerating global warming (IPCC, 2001, 2007). Due to the long time scale of climatic change, contemporary data provide limited knowledge of climate effects on biological systems (Anderson, 1995). Paleolimnology offers tools to infer lake ecosystem responses to changes in climate related variables, such as temperature and lake level (Battarbee, 2000). Remote sites, preferably at a climatic borderline, are most suitable for studying recent (century to decadal scale) climate change effects (Battarbee, 2000; Quinlan, Douglas & Smol, 2005), as the signal in most other areas are confounded by human disturbance effects in the lake catchments (Battarbee, 2000). However, even at these disturbed locations previous responses to climate change can be elucidated using sediment from the early Holocene when human disturbance was low or absent. Remains of pollen, diatoms, cladocerans, chironomids (Anderson, 2000; Battarbee, 1986; Fritz, 1996; Korhola, 2001; Seppa, Hammarlund & Antonsson, 2005; Walker, 2001) as well as stable isotopes (Hammarlund et al., 2005; von Grafenstein et al., 1998) have been used to infer temperature and direct climate responses, such as changes in hydrology, lake depth, nutrients and lake stability.

The 8.2 kyr. event is identified as the most pronounced Holocene climatic event recorded in Greenland ice cores (Dansgaard et al., 1993; Grootes et al., 1993). It represents an estimated rapid cooling of 6±2°C over Greenland (Alley et al., 1997) and approximately 2 °C in Northern Europe during a 100-200 year period (Klitgaard-Kristensen et al., 1998; Veski, Seppa & Ojala, 2004; von Grafenstein et al., 1998). Although still a matter of debate, most researchers favour the hypothesis that the cooling during the 8.2 kyr. event derived from slowing of the ocean thermohaline circulation due to a freshwater pulse to the Hudson Bay from the proglacial Laurentide Lakes (Clarke et al., 2004; Muscheler, Beer & Vonmoos, 2004; Wiersma & Renssen, 2006). Evidence for a cooling in proxy records exists at an almost global scale (but see Nesje & Dahl, 2001; Thomas et al., 2007). Recently, Rohling & Palike (2005) and Alley & Agustsdottir (2005) have argued that most locations outside the North Atlantic show much longer responses (8500-8000 BP), starting earlier than the flood-related cold North Atlantic 8200-event, which seemed related to a larger climate deterioration caused by reduced solar activity (Muscheler, Beer & Vonmoos, 2004).

In mid-latitudes, changes in precipitation and evaporation as a result of temperature change may, however, be of higher importance for lake ecosystems than the temperature change itself. However, whether the lake level increased or decreased during the 8.2 kyr is debated. Using a simple water balance model Harrison, Prentice & Guiot (1993) argued that a change in precipitation was required to explain paleo-observations of lake level changes in European lakes during the Holocene as changes in insolation, temperature and cloudiness were not sufficient explanatory variables. Several paleolimnological studies (Scandinavia and USA) found winter precipitation important for the recharge of groundwater seepage lakes (e.g. Filby et al., 2002; Vassiljev, 1998; Vassiljev, Harrison & Guiot, 1998; Shuman & Donnelly, 2006). Especially lakes in forested regions - forest was the dominant vegetation in Central Europe until 6000 BP (Roberts, 1998) - are controlled primarily by winter precipitation (Carcaillet & Richard, 2000). A review of lake level anomalies in Europe around the 8.2 kyr. event indicates a more humid climate and lake level increases in mid-central Europe, but a drier climate north of ca. 50°N as well as south of ca. 43°N (Magny & Begeot, 2004; Magny et al., 2003). In contrast, increased lake level in a Swedish lake (58°N) during the 8.2 kyr. event was inferred from stable isotopes studies by Hammarlund et al. (2003, 2005) and their data indicate cold and dry winters and cold and wet summers for this event (Hammarlund et al., 2003; Hammarlund et al., 2005; Seppa, Hammarlund & Antonsson, 2005). Likewise, enhanced annual precipitation and sediment organic content as well as increased January temperatures and decreased July temperatures were inferred from the sediment pollen record in Lake Vanndalsvatnet, southern Norway (61°N), during the 8.2 kyr. event (Nesje et al., 2006). However, climatically induced water level changes depend on several lake-specific factors such as lake morphology, recharge source, topography and size of the catchment relative to lake size (Dearing, 1986; Vassiljev, 1998). Increased precipitation seems to have been the main factor affecting water level, especially during summer in Swedish Lake Igelsjön (Hammarlund et al., 2003, 2005), whereas decreased winter precipitation was the most important factor in Lake Bysjön (Sweden) and Lake Karujärv (Estonia) (Vassiljev, 1998; Vassiljev, Harrison & Guiot, 1998). Winter dryness may even have had a greater impact during the early Holocene than at present due to a generally warmer climate (less precipitation and snow than today) (Shuman & Donnelly, 2006). The resolution of the Lake Bysjön study was too low to catch the 8.2 kyr. event, but it did show a marked increase in water level at 9000-8000 ^{14}C yr BP (Vassiljev, 1998).

Studying the effects of abrupt past climate changes on lake ecology requires reliable dating. Annually laminated sediments provide an extremely precise absolute chronology of deposition, which can be identified and measured at an annual level (O'Sullivan, 1983; Zillen et al., 2003). Thus, annually laminated sediments provide a high potential to link specific changes in lake sediment to anomalies in ice core stable isotopes.

The aim of the present study was to explore the influence of climatic change around the 8.2 kyr. event on Lake Sarup, Denmark. We used a multiproxy approach (stable isotopes, varve thickness, organic content of sediment, pigments, cladoceran subfossils, pollen) on annually laminated sediment. We expected alterations in the aquatic biological community assemblages as well as in the rate of change to be most pronounced in the period during and immediately pursuing the climate event. By contrast, for pollen we would expect a

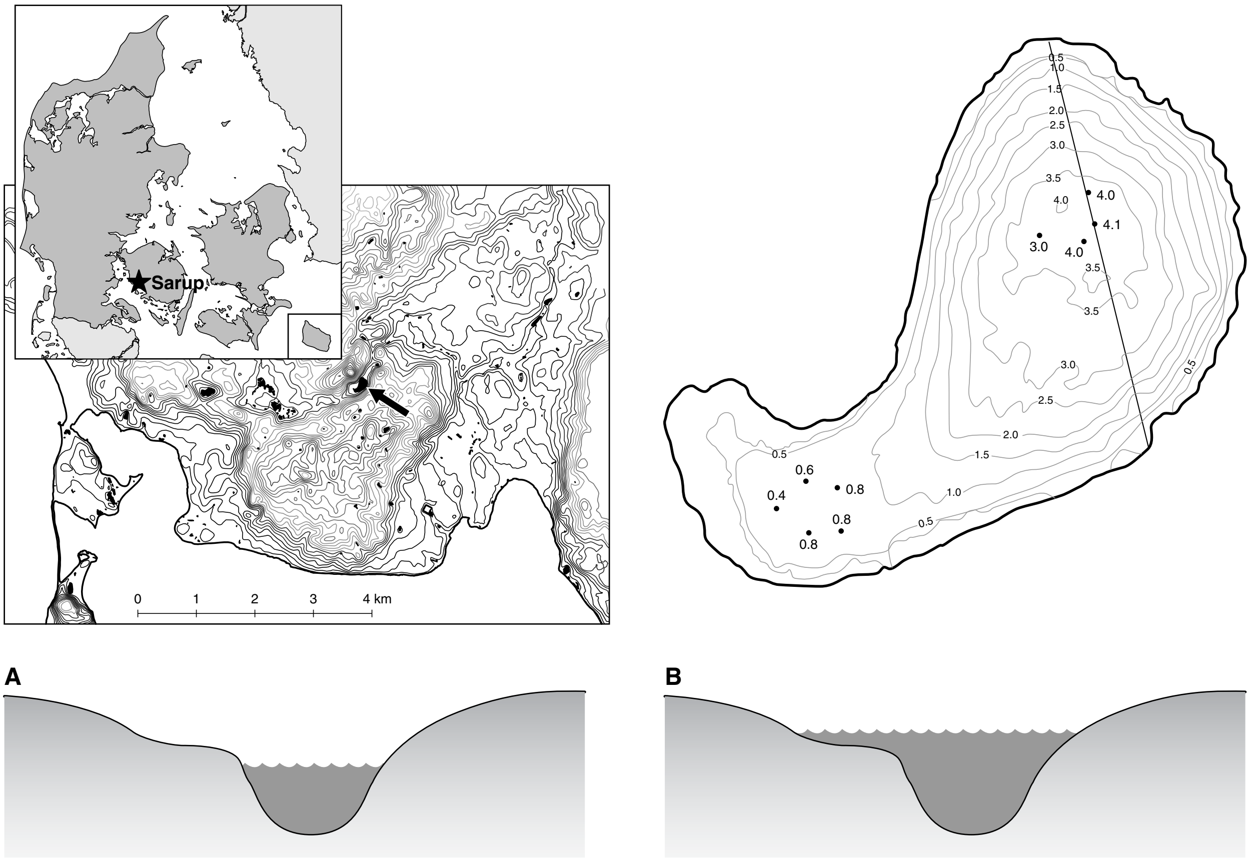

Fig. 1. Location and bottom morphology of Lake Sarup, Denmark, and its close surroundings. Schematic drawing of Lake Sarup at low water level (**A**) and at high water level (**B**).

time lag due to the longevity and resilience of forest ecosystems. Based on the assumption of cooler and drier conditions during the 8.2 kyr. event in northern Europe (ca. > 50°N) (Magny & Begeot, 2004), a lake level reduction in Lake Sarup (55°N) would be expected and, with it, a decreasing relative contribution of macrophyte associated cladocerans and increased relative abundance of pelagic to littoral species ratio (Fig. 1). Cooler and drier conditions are expected to reduce the frequency of plant species requiring high summer or winter temperatures such as *Viscum*, *Hedera* and *Tilia*. In areas with dominant brown earth soil types such as around Lake Sarup, reduced effective moisture would be expected to affect the local hydroseral vegetation more than the upland vegetation.

Materials and methods

Field and laboratory methods

Lake Sarup is a small (3.6 ha), alkaline, shallow (mean depth = 1.7 m, maximum depth = 4.1 m) wind-sheltered kettle-hole lake (Fig. 1). A dead ice remnant from the Weichselian glaciation melted out during the earliest part of the Holocene, resulting in the formation of the lake basin, at that time with a maximum depth of around 19 m. Today, Lake Sarup has one outlet, but no major inlets, and is mainly groundwater fed with a hydraulic retention time of 152 days and has a relatively small catchment area of 35 ha. (Fyns Amt, 1995). In this lake annually laminated sediments were found for the first time in Denmark in 2001 (Rasmussen, 2002). Re-sampling was performed in the middle of the lake (water depth 3.5 m) in July 2003 using a Usinger piston corer (Mingram et al., 2007) from a fixed platform. Approximately 1.8 m of the core was clearly laminated (18.10-16.30 m below lake surface) and constituted an early part of a 15 m long Holocene sediment core. To facilitate sampling the laminated part of the core was marked for each 0.5 centimetre and photographed.

The bottom sample (no. 191) of the most clearly laminated series of the core was dated to 8055-8000 BP (68 % probability, BP = before year AD 2000) using a series of fifteen ^{14}C-dates conducted within an interval of about 1400 years and wiggle-matched to the IntCal04 calibration curve (Björck, 2001). The date of sample 191 was accordingly set to 8025 BP as the midpoint of this interval. Beneath sample 191 it was not possible to identify varves unambiguously by eye, but in thin sections of sediment embedded in epoxy varves were clear and countable. Each varve consists of a light $CaCO_3$-rich layer and a dark organic-rich layer. Microfossil analysis of these sub-layers has documented that light layers were precipitated between May and mid-August, while dark layers were deposited during the rest of the year (Rasmussen, 2002). In this case the term varve refers to a couple/combination of a light and a dark layer representing the sedimentation of one full year. Varves were counted on digital photographs of the thin sections, the cumulative deviation of three independent counts being 1-3% of the mean of total counted varves. Photographs and epoxy blocks were used to locate sampling intervals on the core, aiming at a resolution of 10 varves per sample. This resulted in 67 samples, although the 10 first samples were misinterpreted and comprised 11 years each. Thus, the study period spanned 680 years from 8705-8025 BP. All dates are presented graphically by the earliest date, for instance 8725 BP representing 8725-8715 BP.

Carbon and oxygen stable isotope measurements were made on the carbonate fraction (bulk carbonate) of 67 freeze-dried and homogenized sediment samples in a continuous flow IsoPrime mass spectrometer equipped with a MultiFlow automized preparation system. The sample size corresponded to a carbonate content of 0.5 mg. Samples were placed in septum-capped vials in the MultiFlow system and flushed with He. Phosphoric acid (100 *per cent*) was added manually from a syringe and the samples were left to react for more than 1 hour at 70 ºC. CO_2 was extracted from the vials by a Gilson autosampler, passed through a chromatographic column, cleaned for water and carried to the mass spectrometer by a flow of He. Each batch of analyses included 50 samples and 10 internal standards (Carrara marble LEO). After correction for linearity slope, reproducibility for $\delta\ ^{13}C$ is better than 0.1‰ and for $\delta^{18}O$ better than 0.2‰ as measured on 10 identical standards. All numbers are given in delta-values and have been recalculated to the international V-PDB values using the NBS-19 international standard for calibration. All numbers are given as averages of at least two individual determinations.

Dry matter, organic content and the $CaCO_3$-content for each sample were determined by weight loss after ignition at 105 ºC, 550 ºC and 950 ºC for 20, 4 and 2 hours, respectively. Measurement of sample thickness (accumulation rate in mm per 10 years) was performed on the digital photographs of polished sediment blocks of the core.

Approximately 3 g (wet weight) of sediment per sample was prepared for cladoceran analysis according to Korhola & Rautio (2001). In order to facilitate counting the samples were filtered on a >140 µm sieve for total count on this fraction. Abundant and small fragments were counted on sub-samples of the >80<140 µm fraction (7.5-10% of the total sample), whereas the very abundant *Bosmina* as well as some Chydoridae carapaces were subsampled on both fractions (2-15% counted on the >140 µm fraction, 0.5-2.5% counted on the >80<140 µm fraction). Cladoceran remains were identified using Frey (1959), Røen (1995) and Flössner (2000). The most abundant fragment of each cladoceran taxon was selected to represent one individual. For *Chydorus* spp. (excluding *Chydorus piger*, which was counted separately) there was no clear relationship between head shield and carapace abundance, and *Chydorus* spp. was therefore represented by the average of head shields and carapaces for each sample. Three distinctive morphotypes of *Bosmina longirostis* occurred: a cornuta type with (i) very curved antennae, ii) a very short and less curved antennae, and iii) with a longer slightly curved antennae (e.g. Kerfoot, 1981; Sanford, 1993), and were counted separately. In addition to cladoceran remains, resting eggs of rotifers, *Chaoborus* mandibles, Nymphaeaceae trichosclereids and bryozoan statoblasts were counted, identification of the latter to species level based on Ricciardi & Reiswig (1994).

Pollen samples were treated according to standard procedures (Fægri, 1989) including HF to dissolve small inorganic particles. Tables with pre-acetolyzed *Lycopodium*-spores were added at the beginning of the chemical treatment to allow estimation of the pollen concentration (Stockmarr, 1971). A ratio of 1:2 between *Lycopodium* spores and the terrestrial pollen sum was aimed at (Maher, 1981). Counting of pollen, spores and other palynomorphs was continued for each sample until at least 500 pollen grains of trees and terrestrial herbs were tallied.

Pigments were analysed on samples previously taken from the same core as Cladocera and pollen, at 1 cm intervals, thus including 14-23 years per sample. Pigments of various chlorophylls (chls), carotenoids and their derivatives were analysed using HPLC (High Performance Liquid Chromatography) according to Leavitt & Findlay (1994). The analysed pigments included pigments from all algae and plants (β-carotene, chl *a*, pheophytin *a*), chlorophytes (chl *b*, pheophytin *b*, lutein), total cyanobacteria (echinenone, zeaxanthin), colonial cyanobacteria (myxoxanthophyll, canthaxanthin), diatoms (diatoxanthin), cryptophytes (alloxanthin) and photosynthetic sulphur bacteria (okenone). Pigments are presented as total accumulation per sample (14-23 years).

Data analysis

Accumulation rate, pigment preservation and data transformation

For calculation of accumulation per sample of biological proxies, a constant conversion factor of 0.75 between g wet weight and volume wet sediment was used. This constant was the mean of 21 measurements on evenly scattered sediment samples between 8385-8045 BP (mean=0.75, std=0.037) and assumed applicable due to the relatively constant dry matter content of the samples (24-37%, mean = 31% std = 2.1, n = 31). For pigment samples (1 cm sediment) values of g wet weight measured on overlapping cladoceran samples were used. Whenever the pigment sample covered a longer time span than the date-corresponding cladoceran sample time span, the mean of the g wet weight values from the cladoceran samples covering the time span of pigment sample was used.

Preservation of pigments varies and was estimated as the ratio of the labile chl *a* to the sum of chl *a* and the more degradation resistant chl *a* degradation products (pheophytin *a*, Chl *ap*) (Buchaca, 2007; Steenbergen, Korthals & Dobrynin, 1994).

Non-cladoceran fragments are shown as percentage of total cladoceran fragments (each Cladocera individual being represented by the most frequent and/or the most characteristic fragment) to relate abundance to the cladoceran community pattern. Before statistical analyses, cladoceran as well as terrestrial pollen percentage data were arcsin-transformed in order to normalise data (Legendre & Legendre, 1998).

Changes in assemblage compositions

Identification of differential cladoceran and terrestrial pollen assemblage zones was performed by optimal splitting based on information content dissimilarity (taxa with values larger than 0.01% (Cladocera) and 3% (pollen) were included) using PSIMPOLL version 4.25 (Bennett, 2005). Splitting was continued until the reduction in variation when adding a new zone was smaller than expected when comparing to a Broken Stick model (Legendre & Legendre 1998) as implemented in PSIMPOLL (Bennett, 1996).

We also conducted ordination analysis. Detrended Correspondence Analysis (DCA) was carried out (down-weighting of rare species) to help deciding whether linear or unimodal ordination methods were the most appropriate. As gradient lengths for this short time interval studied were <1 for all DCA's (pollen, pigments (log-transformed accumulation), benthic, pelagic and total cladoceran assemblage) a linear method, Principal Correspondence Analysis (PCA), was chosen (ter Braak, 2002). Taxa found in less than three samples were excluded. Redundancy analysis (RDA) was performed on biological assemblages in order to investigate responses to changes in the isotopic record, thus using $\delta^{13}C$ as single explanatory variable.

In order to investigate whether changes in pollen assemblages (as a proxy of terrestrial plant communities) had an isolated effect on the in-lake system, we used PCA axis 1 sample scores of the pollen assemblages as single explanatory variable in a partial RDA (pRDA) on the cladoceran assemblage – attempting to partial out the variance explained by climate change by using $\delta^{18}O$ and $\delta^{13}C$ as co-variables. Due to the longevity of trees and the resilience of forest ecosystems, a delayed response to environmental changes might be expected. Thus, pRDA's on sequential steps moving the pollen record 40 years ahead, while holding the cladoceran time record constant, were applied to investigate terrestrial community change effect on the lake system. As sediment samples analysed for pollen and cladocerans were not always identical, cladoceran percentage data were linearly interpolated for this time series analysis to the lowest time resolution, 40 years, between samples.

Possible time lags between the isotopic record and important cladoceran taxa or groups of taxa as well as cladoceran community assemblage change (PCA axes) were investigated by cross-correlation using the program PAST (Hammer, 2006). All variables were detrended (least squares linear regression). We applied all possible samples for the detrending, as detrending using a lower resolution of 30-year samples yielded only minor deviations from detrending including all samples. For cross-correlation analysis 30-year time steps were applied, this being the highest resolution of counted samples for the whole period investigated.

Cladoceran inference of macrophyte cover and fish abundance

Cladoceran inferred macrophyte cover (%) as well as cladoceran inferred planktivorous fish abundance (CPUE, no net $^{-1}$night^{-1}) were estimated using weighted-averaging based on a model developed for 19 and 31 Danish shallow lakes ($RMSE_{macrophyte}$=0.41 log_{10} % cover; $RMSE_{CPUE}$=0.33 log_{10} CPUE) (Jeppesen, 1998; Jeppesen et al., 1996), respectively.

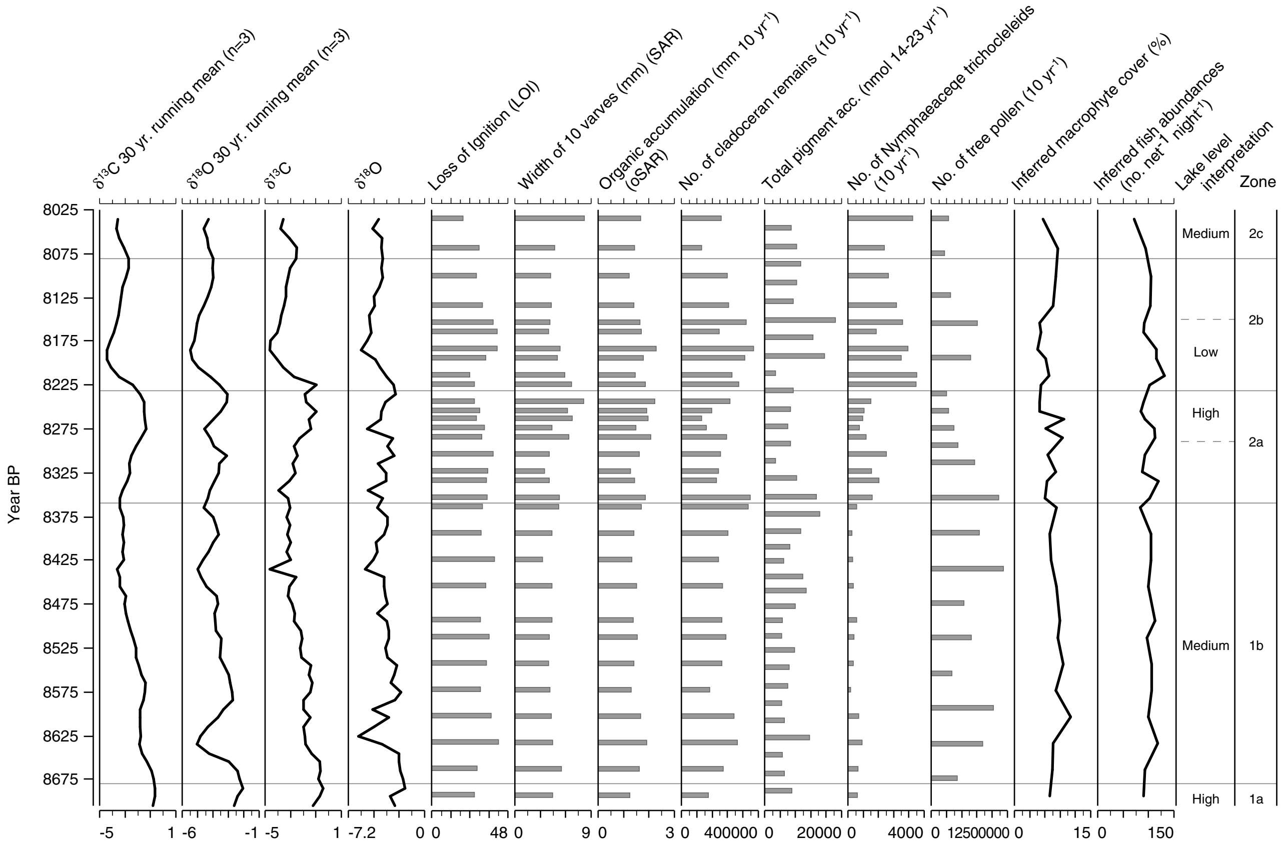

Fig. 2. Stratigraphical plot of stable isotopes $\delta^{13}C$ and $\delta^{18}O$ (‰): mean of at least two measurements, running mean (n=3), organic content (Loss of ignition- LOI) (%), Width of 10 varves (mm) (SAR), total accumulation of organic material (mm 10 $yr.^{-1}$) (oSAR), total accumulation of cladoceran remains (no. 10 $yr.^{-1}$), total accumulation of pigment concentration of sediment (14-23 $yr.^{-1}$), total accumulation of Nymphaeaceae trichosclereids (no. 10 $yr.^{-1}$), total accumulation of tree pollen (no 10 $yr.^{-1}$), cladoceran inferred submerged macrophyte coverage (%) and fish abundance (no. net^{-1} $night^{-1}$). Lines represent zonation by optimal splitting based on the cladoceran assemblage.

Results

Core chemistry

Organic content, sediment accumulation rates and stable isotope records of carbonate

The isotopic records of $\delta^{13}C$ and $\delta^{18}O$ generally showed similar trends and were significantly linearly related (F=59.94, P<0.0001) although the $\delta^{18}O$ record was more scattered and exhibited large deviations (Fig. 2, 3). This variability is most likely due to different origins of the measured carbon. The correlation among the isotopic records, as well as the major changes in $\delta^{18}O$ (3.3 ‰ from 8225-8175 BP, and up to 4.1‰ during the whole period and SD<0.6‰), suggest that the isotopic composition of carbonates is mainly controlled by hydrology rather than by lake water temperature (Talbot, 1990) or by production. Overall, $\delta^{13}C$ decreased during the study period. However, a temporarily higher level occurred during 8355-8225 BP and a minor peak occurred again in ca. 8075. Moreover, a rapid and abrupt decrease occurred at 8225, spanning a 40-year period.

The organic content of the sediment (LOI) was relatively high and tended to correlate negatively, though insignificantly, with stable isotope values ($\delta^{13}C$: r= -0.31, p=0.08, n=31; $\delta^{18}O$. r= -0.34, p=0.07, n=31). The measured thickness of 10 varves, referred to as the sediment accumulation rate (SAR), correlated closely and inversely with LOI (Pearson r= -0.65, p<0.0001, n=31), whereas the organic accumulation rate per 10 years (oSAR) showed no correlation with LOI. Neither SAR nor oSAR correlated significantly with stable isotopes, the latter supporting the conclusion that $\delta^{13}C$ does not generally reflect productivity in Lake Sarup.

Along with the increase in $\delta^{13}C$ and during the most positive isotopic values of $\delta^{13}C$ (and $\delta^{18}O$) 8305-8225 BP, SAR and less strongly oSAR increased, whereas LOI decreased (Fig. 2). The opposite trend was observed for SAR during the major decrease in $\delta^{18}O$ and $\delta^{13}C$ (3.0‰ and 3.7‰, respectively) at 8225-8175. This is indicative of a major shift in lake hydrology, mainly reflected in a major increase in the organic content (8215-8175 BP) and a decrease in SAR (8235-8175 BP), whereas the organic accumulation rate stayed high (Fig. 2). After 8175 BP LOI continued to decrease, whereas SAR and oSAR remained low until the last sample(s) (Fig. 2).

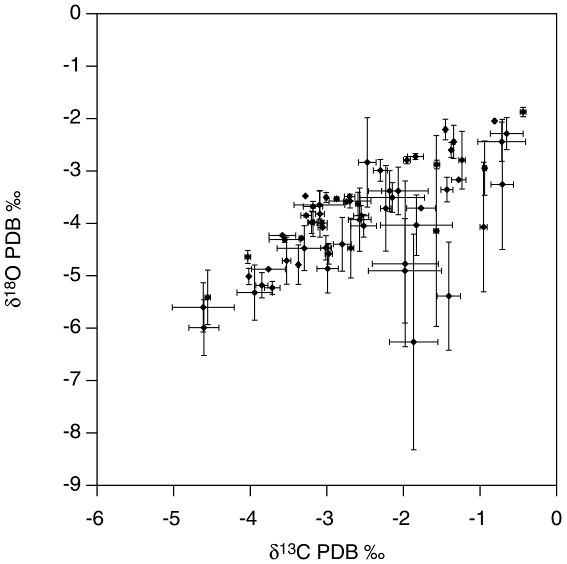

Fig. 3. Correlation between $\delta^{18}O$ and $\delta^{13}C$ ± standard deviation.

Concentration and accumulation of biological proxies

The total cladoceran concentration showed a similar trend as LOI (Pearson correlation $r = 0.72$, $p<0.0001$, $n=31$), except during 8335-8305 BP, coinciding with a sudden increase in the density of the floating-leaved macrophyte Nymphaeaceae trichosclereids (Fig. 2). The total accumulation rate of cladocerans, pollen (number per 10-11 years) and pigments (nmol per 14-23 years) did not show any significant correlation with LOI or SAR. However, the cladoceran accumulation rate correlated positively with oSAR ($r_{cladoceran}= 0.43$, $p=0.01$, $n=31$), whereas pigment accumulation correlated only marginally with oSAR ($r_{pigment}= 0.31$, $p=0.06$, $n=36$). Tree pollen accumulation rates were uncorrelated with oSAR. Moreover, both cladoceran and pigment accumulation rates correlated negatively with the two stable isotopes ($\delta^{13}C$: $r_{cladoceran}= -0.47$, $p=0.01$, $n=31$; $\delta^{18}O$: $r_{cladoceran}= -0.34$, $p=0.07$, $n=31$; $\delta^{13}C$: $r_{pigment}= -0.61$, $p<0.0001$, $n=36$; $\delta^{18}O$: $r_{pigment} = -0.62$, $p<0.001$, $n=36$), whereas the total accumulation of tree pollen was marginally significantly related to $\delta^{13}C$ ($r= -0.42$, $p=0.06$, $n=20$).

The accumulation rates of cladocerans and Nymphaeaceae remains showed similar responses from 8305 and onwards, whereas total pigment accumulation showed a later increase in the accumulation rate coinciding with the abrupt decrease in stable isotopes (Fig. 2).

Biological assemblages, zonation, rate of change profile

The cladoceran assemblages were represented by 27 benthic and 4 pelagic cladoceran taxa, in total varying from 19-28 (median=23) taxa over time. The cladoceran assemblages were dominated by the pelagic *Bosmina longirostris*, constituting 93-97% of the assemblages throughout the core. Accordingly, assemblage changes were mainly found in the benthic cladocerans. The taxon diversity of the benthic forms showed a slight increase during the period with marked changes in stable isotopes (8355-8155 BP) (evenness ranging from 0.58-0.78) (Fig. 2).

Optimal splitting guided by a Broken Stick model of the 31 cladoceran samples (27 taxa included) and the 20 pollen samples (21 taxa included) both resulted in one split, dividing the core into two zones: 8695-8360 (*Zone 1*) and 8360-8025 (*Zone 2*) yr BP for cladocerans and 8695-8215 BP and 8215-8025 for pollen. The split in cladocerans corresponded to a major decrease in all algal pigment accumulation rates (Fig. 2). Pigment preservation was relatively stable (mean: 0.13, range: 0.08-0.31) and, in general, pigment accumulation rates showed no correlation with preservation (Pearson correlation, p-value>0.05) except for echinenone, beta-carotene and pheophytin *a* (Pearson correlation, p-values<0.03). Thus, the changes in pigment accumulation rates were not a simple function of preservation.

Optimal splitting separately on cladoceran benthic taxa (n=27) yielded an identical split as for the whole cladoceran assemblage, whereas split based on cladoceran pelagic taxa (n=4) resulted in one split at 8085 BP. Instances of sub-zone splitting were found (although with lower variance reduction than expected from a Broken Stick Model): 8695-8680 (*Zone 1a*), 8680-8360 (*Zone 1b*), 8360-8220 (*Zone 2a*), 8220-8085 (*Zone 2b*) and 8085-8025 (*Zone 2c*) BP (Fig. 4). *Zone 1* is represented by relatively stable isotopic values, high LOI, relatively low oSAR and SAR. Accumulations of cladocerans were relatively stable and of median values, whereas the accumulation of most pigments was low, generally increasing towards the beginning of *zone 2*. Total accumulation of tree pollen was relatively high, but less stable (Fig. 2). Nymphaeaceae trichosclereid accumulation and inferred submerged macrophyte cover were low and stable. Inferred fish CPUE was high and constant. Isotopic $\delta^{13}C$ and

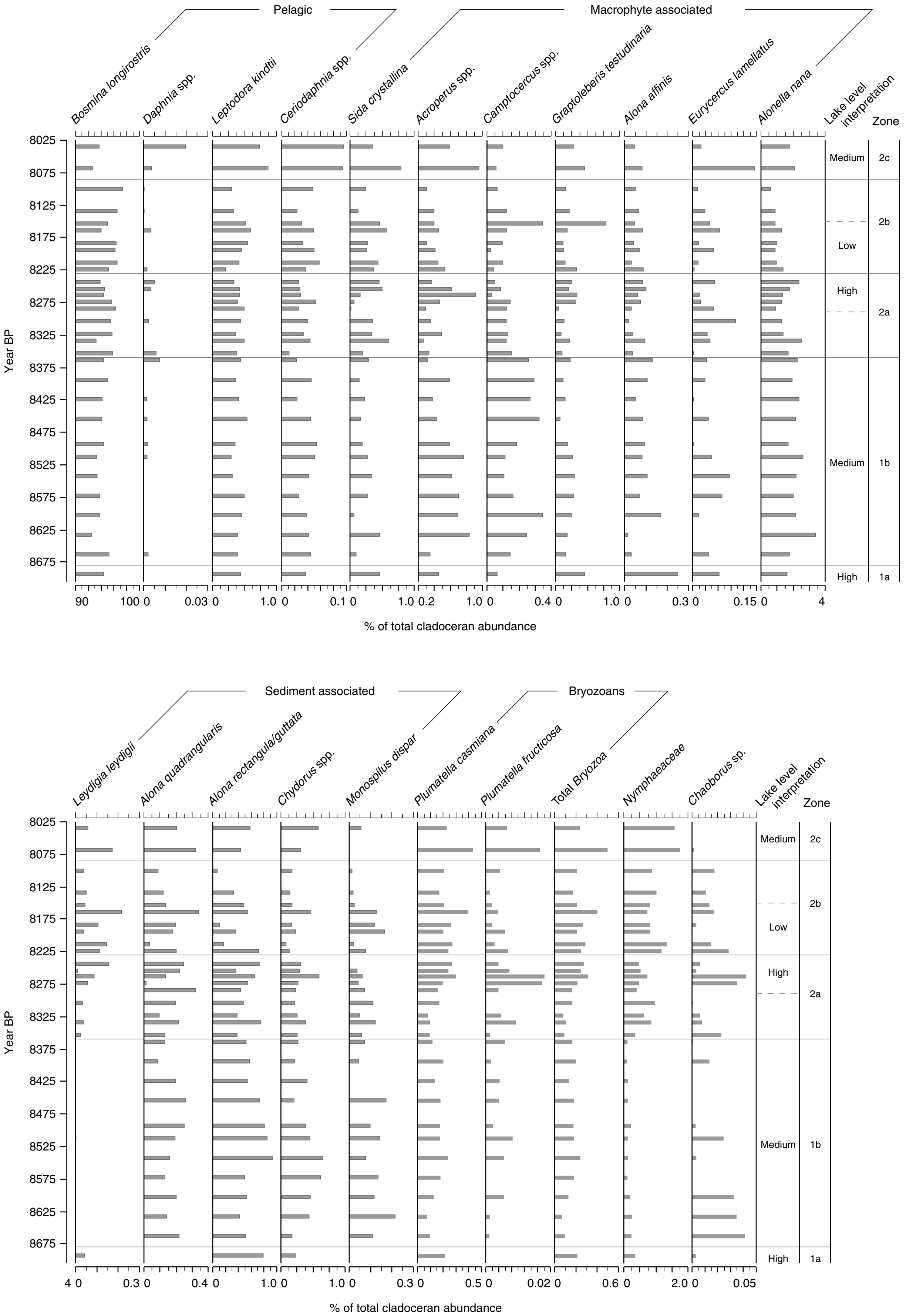

Fig. 4. Stratigraphical plot of percentage distribution of selected cladoceran taxa grouped into pelagic, macrophyte and sediment associated taxa. Bryozoans, Nymphaeaceae trichosclereids and *Chaoborus* remains plotted as percentage of cladoceran remains. Lines represent zonation by optimal splitting based on the cladoceran assemblage.

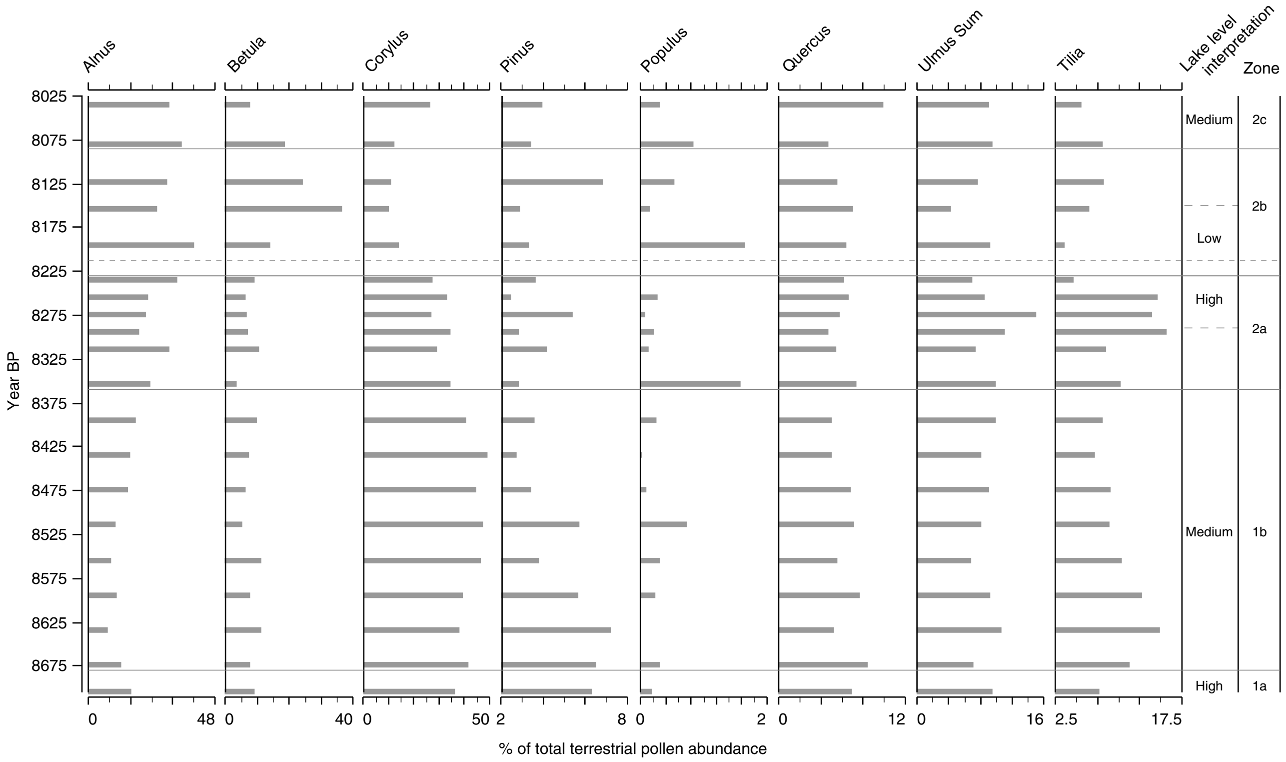

Fig. 5. Stratigraphical plot of percentage distribution of tree pollen taxa. Solid lines represent zonation by optimal splitting based on the cladoceran assemblage, dashed line show the pollen zonation.

$\delta^{18}O$ decreased gradually during the period, although $\delta^{18}O$ showed some variation.

Zone 1a consists of a single sample and is only reflected in the cladoceran record. It is characterised by the presence of *Leydigia leydigii* and a relatively high abundance of macrophyte associated taxa (*Graptoleberis testudinaris, Sida crystallina, Alona affinis*) as well as *Alona retangula/guttata*. The relative abundance of bryozoans is median for the core (*P. fructosa* is absent). The accumulation rates of cyanobacteria-related pigments seem relatively high (Fig. 6). During *zone 1b*, representing 320 yr, higher relative abundances of several macrophyte associated cladoceran species (primarily *Acroperus* spp., *Camptocercus* spp.) appear around 8625 BP, coinciding with an increase in inferred submerged macrophytes as well as in *Tilia* and *Pinus* (Fig. 2, 4, 5). By contrast, the contribution of sediment associated taxa *Chydorus* spp. and *Alona rectangula/guttata* declines (Fig. 4). *Leydigia leydigii* is absent during *zone 1b*.

Zone 2 covers the period with major changes in all proxies. In general, cladocerans, Nymphaeaceae, pigments, SAR and oSAR peaked during this period (8275-8125 BP). In contrast, total tree pollen accumulation as well as LOI and submerged macrophyte cover reached their minimum during the same period. A shift in the dominant pollen taxa from *Corylus* to *Alnus* appeared and all accumulation rates of pigments generally showed an increasing trend (Fig. 5). In *zone 2a Leydigia leydigii* reappeared and increased in abundance. Additionally, Nymphaeaceae accumulation rates increased markedly. In contrast, all algal pigment accumulations were low during the entire period, thus diverging from the trend in oSAR. In the pollen record *Corylus* decreased, whereas *Alnus* increased. *Tilia* and *Ulmus* showed a marked peak in the middle of the period. Towards the end of this zone a general increase occurred in both macrophyte and sediment associated cladoceran taxa as well as in the abundance of bryozoans. However, *P. fructosa* showed a marked peak around 8275 BP, thus responding differently than *P. casmiana* (Fig. 4). In contrast, inferred submerged macrophyte cover decreased towards the end of the zone. These changes coincided with the maximum values of stable isotopes, a decrease in LOI, an increase in cladocerans, pigments, SAR and oSAR (Fig. 2).

During the transition from *zone 2a* to *2b* most cladoceran taxa showed a decrease, except for the

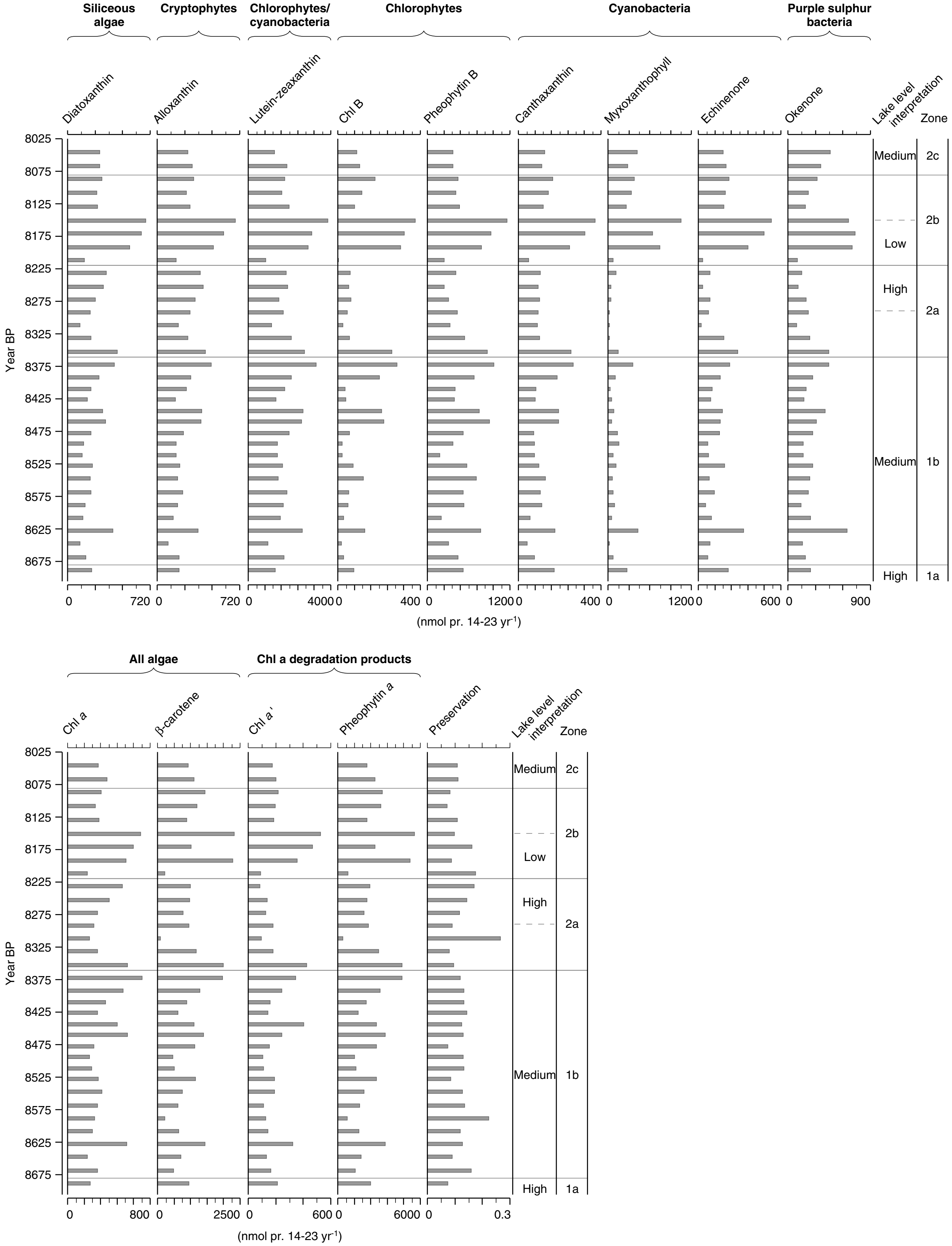

Fig. 6. Stratigraphical plot of absolute pigment accumulation (nmol 14-23 yr^{-1}). Lines represent zonation by optimal splitting based on the cladoceran assemblage.

pelagic taxa. Correspondingly, inferred planktivorous fish CPUE increased. Interestingly, most cladoceran taxa generally stayed relatively stable during *zone 2b*. However, a peak in relative abundance in 8155 or 8165 BP could be observed for several taxa (*E. lamellatus*, *G. testudinaris, S. crys-*

tallina, Chydorus spp., *A. quadrangularis*, *Alona rectangula/guttata*, *L. leydigii* and *P. camiana*). This was also the case for *Betula* as well as for all algal pigments, which generally all increased markedly during the first part of *zone 2b* (Fig. 5, 6). At the same time LOI peaked, whereas oSAR decreased. In general, the accumulation rate of the biological proxies, except pollen and fish CPUE, followed the trend of the oSAR (Fig. 2). These changes coincided with the rapid shift towards the most negative isotope values recorded (Fig. 2). The accumulation rate of Nymphaeaceae was at its maximum but decreased during the entire zone, whereas their relative abundance to cladocerans was high but stable (Fig. 2, 4).

Among cladocerans, *zone 2c* was characterised by a decrease in *B. longirostris* and an increase in the vast majority of the remaining cladoceran taxa. Also the cladoceran accumulation rate increased as did that of Nymphaeaceae and SAR (Fig. 2, 4), whereas *Betula* continued a decreasing trend starting in zone 2b. In contrast, *Corylus* and *Quercus* increased (Fig. 5). Algal pigments were stable but higher than prior to the isotopic anomaly, in particular cyanobacteria related pigments (Fig. 6).

Ordination and rate of change

Most of the variation in cladoceran assemblages was explained by PCA axis 1 (λ_1=0.43, λ_2=0.14). PCA axis 1 was strongly positively related to the occurrence of *B. longirostris* and negatively to *A. nana*, whereas macrophyte associated species (especially *S. crystallina* and *G. testudinaris*) were related to PCA axis 2. The trend seen in the ordination diagram over time (not shown) resembled that elucidated by the optimal splitting analysis: a distinct group of samples from 8355-8275 yr PB (*zone 2a*). The proximity of the oldest sample (8695 yr BP, *zone 1a*) to the earliest sample (8036 yr BP, *zone 2c*) is noteworthy. The distribution of the remaining samples along PCA axis 1 and 2 was relatively scattered. However, the largest distance between consecutive samples occurred between 8102-8069 BP, representing the most pronounced changes in the pelagic species assemblages. This is also evidenced from the PCA axis 1 of the ordination plot of pelagic taxa (n=4) ($\lambda_{1pelagic}$=1). In the PCA plot (Fig. 7) of benthic taxon scores (n=27) ($\lambda_{1benthic}$=0.31, $\lambda_{2benthic}$=0.16), axis 1 was closely positively related to *L. leydigii* and *G. testudinaris* and *Acroperus* spp. PCA axis 2 was generally related to sediment associated taxa. Again the pattern in the ordination diagram resembled the zonation, the earliest part of the core represented to the left and the latest part to the right in the ordination plot – transition state around the origin (Fig. 7). The oldest sample (8695 yr BP, *zone 1a*) was relatively close to the earliest sample (8036 yr BP, *zone 2c*) (Fig. 7). Large assemblage changes during time, expressed as PCA axis 1 sample scores, occurred increasingly with the onset of the changes in stable isotopes around 8375 BP (Fig. 8). A comparatively large change appeared in the beginning of the core (*zone 1a*) followed by a 330-year long relatively stable period (*zone 1b*). These findings were in agreement with cladoceran RDA's (Table 1).

The pollen assemblages were totally dominated by tree pollen (95-99%) and in contrast to the cladoceran assemblage profile, the main change in the pollen assemblage involved a shift in the dominant taxa (from *Corylus* to *Alnus*) mainly at the transition state between *zone 2a* and *2b* (ca. 8225 BP) (Fig. 5 and 8). The vast majority of the variation in PCA performed on pollen and algal pigment (the latter $\log_{10}$ transformed accumulation rate) was captured by PCA axis 1 ($\lambda_{1pollen}$=0.61, $\lambda_{2pollen}$=0.14; $\lambda_{1pigment}$=0.92, $\lambda_{2pigment}$ =0.05, respectively) and large assemblage changes occurring during and after the abrupt isotopic changes (Fig. 8).A large part of the variation in the algal pigment variation (27%) was explained by variation in δ^{13}C, whereas the total pollen assemblage variation could only marginally be explained by δ^{13}C changes (Table 1). Pollen PCA axis 1 sample scores explained a significant proportion of the

Table 1 Summary results from RDAs performed on the biological assemblages. Bold numbers indicate significance.

RDA	λ_1	F-ratio (1st RDA axis)	P-value	Explaining variable/s	% explained
Algal pigment assemblage	0.272	13.347	**0.001**	δ^{13}C	27.2
Pollen assemblage	0.131	2.707	**0.050**	δ^{13}C	13.1
Cladoceran assemblage, all	0.078	2.450	**0.044**	δ^{13}C	7.8
Cladoceran assemblage, pelagic	0.10	3.307	0.069	δ^{13}C	NS
Cladoceran assemblage, benthic	0.064	1.985	**0.029**	δ^{13}C	6.4

variation in the cladoceran assemblage with no lag (significance of pRDA axis 1: F=3.483, P=0.0100), a 40-year time lag (significance of pRDA axis 1: F=3.531, P=0.0120) and a 160 year time lag (significance of pRDA axis 1: F=4.343, P=0.0080).

Time lags between isotope and Cladocera responses

There was no time lag between changes in isotopes and SAR (resolution: 10 years, n=67 samples) or LOI (resolution: 30 years, n=31 samples). Relating the taxa responses to the isotopic signals by cross-correlation resulted in less consistent results. The $\delta^{13}C$ signal was chosen for cross correlation analysis as it showed lower scatter than $\delta^{18}O$ results. *Leydigia leydigii*, which appeared only in the upper part of the core, showed a 1-2 step time lag (30-60 years). In contrast *L. leydigii* plus strictly plant associated species (*Sida crystallina, Eurycercus lamellatus* and *Graptoleberis testudinaris*) showed no time lag (implicit response within 30 years), whereas aggregating the most abundant taxa of *Zone 1* (*Alonella nana, A. exigua, Camptocercus* spp., *Acroperus* spp. and *Chydorus* spp.) showed no relation to $\delta^{13}C$. Also at the assemblage level, benthic taxa, pelagic taxa and the entire cladoceran assemblage showed no relation to the isotopic signals along PCA axis 1, whereas PCA axis 2 of benthic taxa as well as the whole community assemblage showed a positive response and no time lag relative to $\delta^{13}C$.

Bosmina, morphology and predation indices

The relative contribution of *Bosmina longirostris* morphotypes showed no clear shifts in the series. The long antennae form has a median contribution of 56 % of the *Bosmina* head shields, the *cornuta* type contributes 16% and the short antennae type 28%. Also, there seemed to be no relation between the *cornuta* type percentage and the short antennae type. Neither the variation in the rare invertebrate predator *Chaoborus* (0.5-4.5 encountered individuals) nor in the more abundant *Leptodora* (4-41.5 individuals) was correlated with the distribution of *Bosmina* head shield morphotypes. Fish were probably the most important predators, as inferred values indicate a relatively constant and high planktivorous fish abundance (71-132 fish net^{-1}, although based on an inference model for shallow lakes) (Fig. 2).

Inferred macrophyte cover

Inferred coverage of submerged macrophytes was low (4-10 %) and stable, although a local minimum was present at the time with major changes in the isotopic records (8255-8155 yr BP) (Fig. 2). The macrophyte cover data must be interpreted with caution, as the estimates are derived from a model developed for shallow lakes, in which macrophytes have a relatively larger role than in deep lakes.

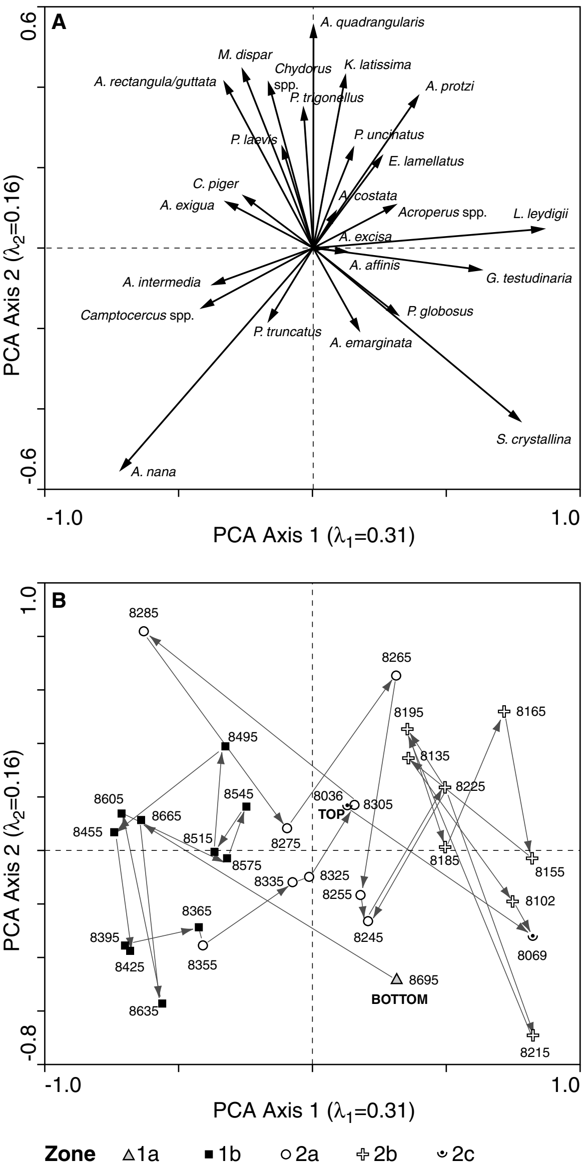

Fig. 7. PCA of arcsin transformed percentage data for the benthic cladoceran community assemblage. A. Species plot on axes 1 and 2. B Plot of sample scores on axes 1 and 2, sample symbols refer to the cladoceran assemblage zonation.

Discussion

A regime shift towards a more productive system occurred during the selected study period as judged from the isotopic record and several biological proxies (Fig. 2, 4 and 5-7). All biological assemblages responded to the climatic change, as evidenced by significant proportion of the taxon variation being explained by $\delta^{13}C$ with no overall time lag (response within 30 yr), although different lags appeared when

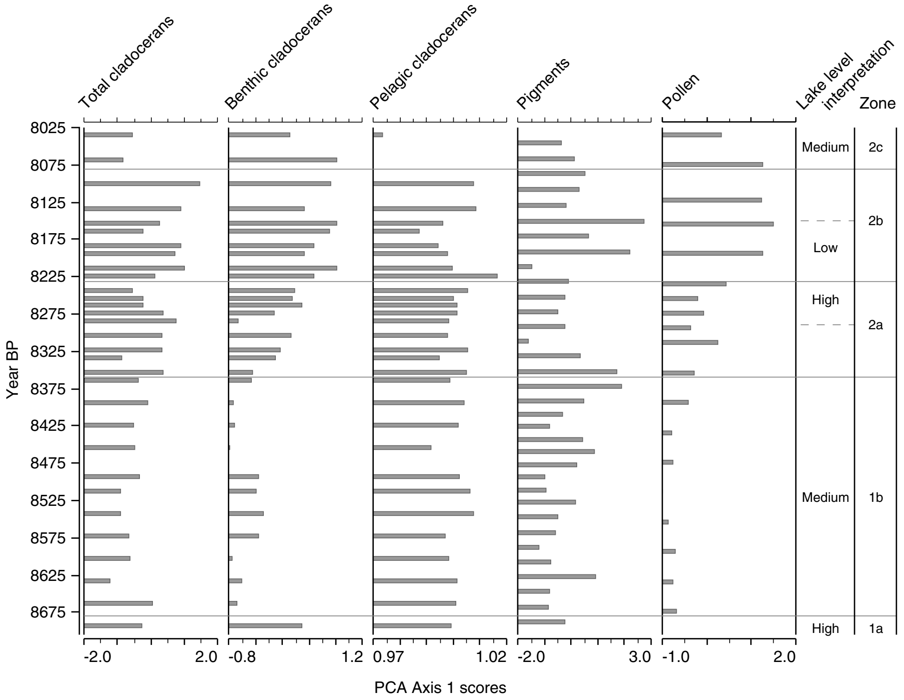

Fig. 8. Stratigraphical plot of rate of change of biological assemblages indicated by PCA axis 1 sample scores of total, benthic and pelagic cladoceran assemblage (arcsin transformed percentages), pigment accumulation assemblage (log_{10} transformed accumulation) and pollen assemblage (arcsin transformed percentages).

relating specific taxa or groups of taxa to $\delta^{13}C$. A significant shift in taxa composition and community assemblages occurred approx. 100 years before the extreme and synchronic changes in $\delta^{18}O$ and $\delta^{13}C$, identifiable as the 8.2 kyr. (Alley & Agustsdottir, 2005; Rohling & Palike, 2005). This suggests an earlier and longer climate deterioration than usually anticipated for the 8.2 kyr. event (Dansgaard et al., 1993; Thomas et al., 2007). The observed changes likely reflect a change in hydrology of the lake catchment rather than a lower temperature, as the amplitude of the isotopic changes (3-4 ‰) during the anormality was too high to represent temperature changes (1‰ change in $\delta^{18}O$ approximately corresponds to a change of 4°C (McDermott, Mattey & Hawkesworth, 2001; Hammarlund et al., 2002).

The timing and magnitude of the changes in $\delta^{18}O$ and $\delta^{13}C_{bulk}$ of Lake Sarup during the study period closely resembled those recorded by Hammarlund et al. (2003, 2005) in Lake Igelsjön, southern Sweden. Moreover, the direction of change at the two sites was identical for $\delta^{13}C_{bulk}$, whereas the opposite direction was observed for $\delta^{18}O$. The lakes have several similar characteristics as they both are without major inlets or outlets and mainly fed by groundwater (although the surface area of Lake Sarup is 14 times larger). Thus, we might at first glance expect Lake Sarup and Lake Igelsjön to show similar responses to the 8.2 kyr. event. However, the morphology of Lake Sarup and the topography of the surroundings complicate the interpretation of the observed stable isotopes as well as the comparison with results from Lake Igelsjön. The basin morphology of Lake Sarup resembles an inverted hat with a deep central part and a marginal shallow area (Fig. 1). This morphology was much more pronounced in the Early Holocene before deposition of the 15 m of sediment that now is found in the central part of the lake. The deep lake system was also indicated by the predominance of the pelagic species *Bosmina longirostris*, high abundance of planktivorous fish and low abundance of invertebrate predators, which may also explain the absence of changes in morphological *Bosmina* head types. (Kerfoot, 1981, 2006; Sanford, 1993). At low water levels Lake Sarup would occupy the central deep part with a resulting small surface:volume ratio. In contrast, at high water levels the lake likely included a large shallow marginal part and had a high surface:volume ratio. In the latter situation evaporation would be enhanced and this effect could possibly overrule any direct climatic influence on the moisture balance of the lake. Therefore, the special morphology of Lake Sarup may well explain the differences in isotope records between Lake Igelsjön and Lake Sarup.

Indications of water level increase prior to 8225 BP from isotopes, accumulation rates and biological proxies

Corresponding to the findings of Rohling & Pälike (2005) and Ally & Áugústdóttir (2005), the most likely scenario for Lake Sarup is an increase in precipitation prior to 8225 with high stable isotopic values. Firstly, the absolute maximum in SAR during the stable isotope maximum at 8225 BP coincided with a minimum of LOI. In addition, when LOI decreased, SAR and oSAR increased (Fig. 2), which indicates higher transport of allochthonous inorganic and organic matter from the lake catchment as expected when precipitation increases. During this period the sediment associated bottom-dwelling *Leydigia leydigii* (Flössner 2000) reappeared. Higher allochtonous input probably reduced water clarity, leading to observed abrupt decrease in anaerobic photosynthetic purple sulphur bacteria (okenone pigment concentration) that are known to thrive at or beneath the thermocline in deep lakes (Moss, 1998; Rodrigo; Vicente & Miracle, 2000). Changes in the preservation of okenone can be excluded as an explanatory factor for the decline in okenone as pigment preservation was relatively stable during the entire study period. The decreased accumulation of other algal pigments during *zone 2a* (Fig. 6) further suggests a decline in algal production, probably as a result of increased turbidity. Further indications come from the bryozoans, as the marked short-termed peak in the bryozoan *Plumatella fruticosa* (Fig. 4) appeared just prior to and during the indicated highest water level. This species occurs in highly coloured but non-eutrophic waters, growing on submerged branches of shoreline scrubs, wood substrate or floating-leaved macrophytes (Bushnell, 1974). Such habitats were probably increasing markedly during the water level increases in Lake Sarup (Fig. 1). In a subset of Norwegian lakes the distribution of *P. fruticosa* was mainly determined by poor aquatic vegetation abundance and summer temperatures higher than 11 ºC (Økland & Økland, 2002). Also, the increase in *Plumatella casmiana*, the most abundant bryozoan statoblast, supports the conclusion of higher turbidity since this species is known to survive well in turbid silty waters and grows on macrophytes, rock and sticks, and may form dense formations on wood substrates in shallow water *Typha* stands (Bushnell, 1974). Furthermore, the abundance of *Chaoborus* tended to be higher during the period with enriched stable isotopic values (Fig. 4). Increased abundance of this invertebrate was found to correlate with elevated levels of dissolved organic carbon in a study of 56 lakes (Wissel, Yan & Ramcharan, 2003), likely due to reduced fish predation when turbidity increased (Wissel, Boeing & Ramcharan, 2003; Wissel, Yan & Ramcharan, 2003). Also, the increase in Nymphaeaceae trichosclereids coincided with the increase in stable isotopes (approx. 8360 BP). Members of this family of floating-leaved plants would be expected to colonise the flooded areas with increasing water level (Dieffenbacher-Krall & Nurse, 2005). The increase in abundance of Nymphaeaceae is supported by an increase in bryozoans as well as cladocerans known to be related to floating-leaved macrophytes such as *Sida crystallina* (Flössner, 1972; Nilssen & Sandoy, 1990), *Ceriodaphnia* and *P. casmiana* (Massard, 1995). Finally, the sudden increase in the relative abundance of terrestrial *Tilia* and *Ulmus* pollen during (8350-8225 BP) further suggests a lake level increase. An expansion of these long-lived climax trees within a period of only 20-40 years is ecologically very unlikely and the increase in pollen frequency of these taxa most probably has a sedimentological cause. Both taxa thrive best on semi-humid deep mull soils that are likely to have occurred not far from the shore of the lake. The increase in *Ulmus* and *Tilia* pollen is probably the result of erosion of soils rich in these pollen types following an increase in water level.

Indication of a water level decrease following 8225 BP

The peak in *Salix* pollen and, especially, the pronounced peak in *Betula* pollen frequencies following 8225 BP (Fig. 5) indicate a decline in water level. Both are pioneer taxa that readily invade new suitable habitats. Due to the morphology of the basin, a lowering of the water level would have exposed a large, almost plain rim (border of the lake), open for invasion of plants and initial forest succession. The observed lag of about 60-80 years between the decrease in $\delta^{13}C$ and $\delta^{18}O$ values and the peak in *Betula* is consistent with the time elapsing for a succession from exposure of a lake floor to a shrub or forest of birch to become established. An alternative explanation for the expansion of *Betula* would be a temperature change affecting upland vegetation to change into a more boreal forest type. Such a change, however, would have required an excessive drop in temperature that would have affected a number of thermophilous plants as well. The continuous presence of fair amounts of *Tilia* pollen indicates that this was not the case.

A lower water level may lead to erosion of sediments in the littoral zone and a subsequent recycling of nutrients (Teeter et al., 2001). The increases in algal pigment accumulation and in LOI during or right after the abrupt change in isotopes may indicate an increase in lake productivity that may have been caused by a water level lowering. Supportingly, oSAR follows the trend of LOI during this period (*Zone 2b*) in contrast to the prior period (*Zone 2a*). The marked increase in Nymphaeaceae accumulation around 8225 is spurious, but may reflect washing in of remains from a drying-up shallow area. Combining the indications of all proxies, the majority of the responses support a lake-level decrease around 8225.

Lake changes 8150-8025 BP following the abrupt climate changes

Following the abrupt isotopic decrease, the system started to recover; the water level likely increased again (as indicated by the isotopes). Several factors, however, indicate that Lake Sarup did not recover but went through a regime shift towards a more productive system. Firstly, algal pigment accumulation seemingly was constantly higher than prior to the water level fluctuations, in particular for cyanobacteria-related pigments (Fig. 6), pointing to a more productive system after 8150 BP. This pattern cannot be explained by changes in sediment accumulation rates. Secondly, Nymphaeaceae values stayed remarkably after the fluctuations and may have benefited from a nutrient increase. Thirdly, the cladoceran community had a larger relative abundance of littoral-associated taxa, which can be attributed to early eutrophication (e.g. Johansson et al., 2005). Thus, the biological communities as well as water level (indicated from the isotopes) did not return to the state before the abrupt environmental changes (8350-8150 BP). This conclusion is supported by the results if the ordination analyses (cladocerans, pigments and pollen, the two latter ordination plots not shown).

In addition to the climate-related changes in the terrestrial environment, reflected by pollen assemblage change, vegetation changes seemed to have a separate, 40 years delayed (at the minimum), effect on the cladoceran assemblage. An overall change in the vegetation in close proximity to the lake during the period studied was the decline of *Corylus avellana* and an expansion of *Alnus glutinosa*. This development was accelerated around 8225 BP. *Alnus glutinosa* is known to effectively fix nitrogen through its symbiosis with the actimycete *Frankia alni* at a rate of about 50 kg N ha^{-1} (Dilly, 1999). The increased terrestrial productivity following an expansion of *Alnus* is likely to have had effects on the lake ecosystem as well, stronger and stronger the more mature and established the *Alnus* population would be. Such a slow terrestrial process may possibly explain the observed lagged response of cladoceran communities to vegetation changes. A similar process of lake eutrophication induced by an expansion of N-fixing *Alnus*-vegetation was observed in Alaska by Engstrom (2006), although in this case this was directly related to N-limited lakes.

Conclusion

Lake Sarup, underwent a climate-driven regime shift from a less productive state before the 8.2 kyr. event to a more productive state afterwards. The driving force likely was climate-induced changes in water level assisted by expansion of *Alnus*. The most pronounced responses were changes in sediment organic content, sediment accumulation rates of organic and inorganic material as well as accumulation rates and assemblage changes of the biological proxies (algal pigment concentration, cladocerans and pollen). These responses very likely indicated a humid period with pronounced climatic deterioration, beginning around 8375 as observed in several European studies (Rohling & Palike, 2005). This period was followed by a dry period as a consequence of the cool 8.2 kyr. event, leading to water level decrease in Lake Sarup. This supports Magny & Begeot (2004), but contradicts the interpretation of pollen and isotopic records from south central Swedish and Norwegian lakes (Seppa, Hammarlund & Antonsson, 2005; Nesje et al., 2006). However, the specific morphology of Lake Sarup complicates a comparison of isotopic signals from this lake with those from regular kettle-hole lakes. Moreover, the short 8.2 kyr. climatic event is sensitive to dating accuracy; thus, relatively small differences in dating could result in matches or mismatches between studies. The present study contains a very well dated chronology due to the presence of a floating series of varves anchored by wiggle-matched radiocarbon datings (Odgaard et al., in prep). The biological proxies responded to climatic-driven lake level changes, but never returned to the initial face of low-productive high water level during recovery within the time studied. These past hydrological changes may parallel future predictions of warmer but wetter winters in Denmark (Christensen & Christensen 2001), though effects of present-day intensive agriculture may hinder a reduction in production at higher precipitation and lake level increase.

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References

Alley, R.B. & Agustsdottir, A.M. (2005) The 8k event: cause and consequences of a major Holocene abrupt climate change. *Quaternary Science Reviews*, 24(10-11), 1123-49.

Alley, R.B., Mayewski, P.A., Sowers, T., Stuiver, M., Taylor, K.C. & Clark, P.U. (1997) Holocene climatic instability: A prominent, widespread event 8200 yr ago. *Geology*, 25(6), 483-86.

Anderson, N.J. (1995) Using the Past to Predict the Future - Lake-Sediments and the Modeling of Limnological Disturbance. *Ecological Modelling*, 78(1-2), 149-72.

Anderson, N.T. (2000) Diatoms, temperature and climatic change. *European Journal of Phycology*, 35(4), 307-14.

Battarbee, R.W. (1986). Diatom analysis. In *Handbook of Holocene Palaeoecology and Palaeohydrology* (ed B.E. Berglund), pp. 527-70. John Wiley & Sons Ltd.

Battarbee, R.W. (2000) Palaeolimnological approaches to climate change, with special regard to the biological record. *Quaternary Science Reviews*, 19(1-5), 107-24.

Bennett, K.D. (1996) Determination of the number of zones in a biostratigraphical sequence. *New Phytologist*, 132(1), 155-70.

Bennett, K.D. (2005). Documentation for psimpol 4.25 and pscomb 1.03. C programs for plotting pollen diagrams and analysing pollen data. . In, Uppsala University.

Björck, S.W., B. (2001). 14C chronostratigraphical techniques in palaeolimnology. In *Tracking Environmental Change Using lake sediments. Basin Analysis, Coring and Chronological Techniques.* (ed W.M.S. Last, J.P.), Vol. 1, pp. 205-45. Kluwer, Dordrecht, The Netherlands.

Buchaca, T.a.C., J. (2007) Factors influencing the variability of pigments in the surface sediments of mountain lakes. *Freshwater Biology*, 57(7), 1365-79.

Bushnell, J.H. (1974). Bryozoans (Ectoprocta). In *Pollution ecology of freshwater invertebrates* (ed C.W.a.F. Hart, S. L. H.), pp. 157-94. Academic Press, New York.

Carcaillet, C. & Richard, P.J.H. (2000) Holocene changes in seasonal precipitation highlighted by fire incidence in eastern Canada. *Climate Dynamics*, 16(7), 549-59.

Christensen, J.H.& Christensen, O. B. (2001). Regional Climate Scenarios – A study on Precipitation. In *Climate Change Research – Danish contributions* pp. 151-66. Gads Forlag, Copenhagen, Denmark.

Clarke, G.K.C., Leverington, D.W., Teller, J.T. & Dyke, A.S. (2004) Paleohydraulics of the last outburst flood from glacial Lake Agassiz and the 8200 BP cold event. *Quaternary Science Reviews*, 23(3-4), 389-407.

Dansgaard, W., Johnsen, S.J., Clausen, H.B., Dahl-jensen, D., Gundestrup, N.S., Hammer, C.U., Hvidberg, C.S., Steffensen, J.P., Sveinbjornsdottir, A.E., Jouzel, J. & Bond, G. (1993) Evidence for General Instability of Past Climate from a 250-Kyr Ice-Core Record. *Nature*, 364(6434), 218-20.

Dearing, J.A.F., I. D. L. (1986). Lake sediments and paleohydrological studies. In *Handbook of Holocene palaeoecology and palaeohydrology* (ed B.E. Berglund), pp. 67-90. John Wiley & sons, Chichester.

Dieffenbacher-Krall, A.C. & Nurse, A.M. (2005) Late-glacial and Holocene record of lake levels of Mathews Pond and Whitehead Lake, northern Maine, USA. *Journal of Paleolimnology*, 34(3), 283-310.

Dilly, O., Blume, HP, Kappen, L., Kutsch W.L., Middelhoff, U., Buscot, F., Dittert, K.,Bach, H.J., Mogge,m B., Pritsch, K. & Munch, J.C. (1999) Microbial processes and features of the microbiota in histosols from a black alder (*Alnus glutinosa* (L.) Gaertn.) forest. *Geomicrobiology Journal*, 16, 65-78.

Engstrom, D.R.F., S.C. (2006) Coupling between primary terrestrial succession and the trophic development of lakes at Glacier Bay, Alaska. *Journal of Paleolimnology*, 35(4), 873-80.

Fægri, K.a.I., J. (1989) *Textbook of Pollen Analysis*. John Wiley and Sons, New York.

Filby, S.K., Locke, S.M., Person, M.A., Winter, T.C., Rosenberry, D.O., Nieber, J.L., Gutowski, W.J. & Ito, E. (2002) Mid-Holocene hydrologic model of the Shingobee Watershed, Minnesota. *Quaternary Research*, 58(3), 246-54.

Flössner, D. (1972) *Kiemen - und Blattfüsser, Branchiopoda, Fischläuse, Branchiura* G. Fischer.

Flössner, D. (2000) *Die Haplopoda und Cladocera Mitteleuropas* Backhuys Publishers, Leiden, The Netherlands.

Frey, D.G. (1959) The taxonomic and phylogenetic significance of the head pores of the Chydoridae (Cladocera). *Internationale Revue der gesamten Hydrobiologie*, 44, 27-50.

Fritz, S.C. (1996) Paleolimnological records of climatic change in North America. *Limnology and Oceanography*, 41(5), 882-89.

Fyns Amt (1995) *Sarup Sø 1983 -1993* Fyns Amt, Odense, Denmark.

Grootes, P.M., Stuiver, M., White, J.W.C., Johnsen, S. & Jouzel, J. (1993) Comparison of Oxygen-Isotope Records from the Gisp2 and Grip Greenland Ice Cores. *Nature*, 366(6455), 552-54.

Hammarlund, D., Barnekow, L., Birks, H.J.B., Buchardt, B. & Edwards, T.W.D. (2002) Holocene changes in atmospheric circulation recorded in the oxygen-isotope stratigraphy of lacustrine carbonates from northern Sweden. *Holocene*, 12(3), 339-51.

Hammarlund, D., Bjorck, S., Buchardt, B., Israelson, C. & Thomsen, C.T. (2003) Rapid hydrological changes during the Holocene revealed by stable isotope records of lacustrine carbonates from Lake Igelsjon, southern Sweden. *Quaternary Science Reviews*, 22(2-4), 353-70.

Hammarlund, D., Bjorn, S., Buchardt, B. & Thomsen, C.T. (2005) Limnic responses to increased effective humidity during the 8200 cal. Yr BP cooling event in southern Sweden. *Journal of Paleolimnology*, 34(4), 471-80.

Hammer, Ø., Harper, D. A. T., Ryan, P. D. (2006). PAST - PAlaeontological STatistics. In. Available at: http://folk.uio.no/ohammer/past.

Harrison, S.P., Prentice, I.C. & Guiot, J. (1993) Climatic Controls on Holocene Lake-Level Changes in Europe. *Climate Dynamics*, 8(4), 189-200.

IPCC. (2001) *Climate Change 2001: The Scientific Basis. Contribution of Working Group 1 to the Third Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, .

IPCC. (2007) *http://ipcc-wg1.ucar.edu/wg1/docs/WG1AR4_SPM_PlenaryApproved.pdf.* .

Jeppesen, E. (1998) *The Ecology of Shallow Lakes - Trophic Interactions in the Pelagial.* Doctor's dissertation (DSc). National Environmental Research Institute. NERI Technical Report 247

Jeppesen, E., Madsen, E.A., Jensen, J.P. & Anderson, N.J. (1996) Reconstructing the past density of planktivorous fish and trophic structure from sedimentary zooplankton fossils: A surface sediment calibration data set from shallow lakes. *Freshwater Biology*, 36(1), 115-27.

Johansson, L.S., Amsinck, S.L., Bjerring, R. & Jeppesen, E. (2005) Mid- to late-Holocene land-use change and lake development at Dallund So, Denmark: trophic structure inferred from cladoceran subfossils. *Holocene*, 15(8), 1143-51.

Kerfoot, W.C. (1981) Long-Term Replacement Cycles in Cladoceran Communities - a History of Predation. *Ecology*, 62(1), 216-33.

Kerfoot, W.C. (2006) Baltic Eubosmina morphological radiation: Sensitivity to invertebrate predators (induction) and observations on genetic differences. *Archiv für Hydrobiologie*, 167(1-4), 147-68.

Klitgaard-Kristensen, D., Sejrup, H.P., Haflidason, H., Johnsen, S. & Spurk, M. (1998) A regional 8200 cal. yr BP cooling event in northwest Europe, induced by final stages of the Laurentide ice-sheet deglaciation? *Journal of Quaternary Science*, 13(2), 165-69.

Korhola, A. & Rautio, M. (2001). Cladocera and other branchiopod crustaceans. In *Tracking Environmental Change Using Lake Sediments* (eds .P. Smol, H.J.B. Birks & W.M. Last), Vol. 4, pp. 1-37. Klüver Academic Publishers, Dordrecht, The Netherlands

Leavitt, P.R. & Findlay, D.L. (1994) Comparison of Fossil Pigments with 20 Years of Phytoplankton Data from Eutrophic Lake-227, Experimental Lakes Area, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, 51(10), 2286-99.

Legendre, P. & Legendre, L. (1998) *Developments in environmental modelling*, 2nd. edn. Elsevier, Amsterdam.

Magny, M. & Begeot, C. (2004) Hydrological changes in the European midlatitudes associated with freshwater outbursts from Lake Agassiz during the Younger Dryas event and the early Holocene. *Quaternary Research*, 61(2), 181-92.

Magny, M., Begeot, C., Guiot, J. & Peyron, O. (2003) Contrasting patterns of hydrological changes in Europe in response to Holocene climate cooling phases. *Quaternary Science Reviews*, 22(15-17), 1589-96.

Maher, L.J. (1981) Statistics for Microfossil Concentration Measurements Employing Samples Spiked with Marker Grains. *Review of Palaeobotany and Palynology*, 32(2-3), 153-91.

Massard, J.A.a.G., G. (1995) On the distribution of *Plumatella casmiana* in the European and Mediterranean parts of the Palaearctic region (Bryozoa, Phylactolaemata). *Bulletin de la Société des Naturalistes Luxembourgeois*, 96, 157-65.

McDermott, F., Mattey, D.P. & Hawkesworth, C. (2001) Centennial-scale holocene climate variability revealed by a high-resolution speleothem delta O-18 record from SW Ireland. *Science*, 294(5545), 1328-31.

Mingram, J., Negendank, J.F.W., Brauer, A., Berger, D., Hendrich, A., Kohler, M. & Usinger, H. (2007) Long cores from small lakes - recovering up to 100 m-long lake sediment sequences with a high-precision rod-operated piston corer (Usinger-corer). *Journal of Paleolimnology*, 37(4), 517-28.

Moss, B. (1998) *Ecology of Fresh Waters. Man and Medium, Past to Future*, Third edn. Blackwell Science Ltd., Oxford.

Muscheler, R., Beer, J. & Vonmoos, M. (2004) Causes and timing of the 8200 yr BP event inferred from the comparison of the GRIP Be-10 and the tree ring Delta C-14 record. *Quaternary Science Reviews*, 23(20-22), 2101-11.

Nesje, A., Bjune, A.E., Bakke, J., Dahl, S.O., Lie, O. & Birks, H.J.B. (2006) Holocene palaeoclimate reconstructions at Vanndalsvatnet, western Norway, with particular reference to the 8200 cal. yr BP event. *Holocene*, 16(5), 717-29.

Nesje, A. & Dahl, S.O. (2001) The Greenland 8200 cal. yr BP event detected in loss-on ignition profiles in Norwegian lacustrine sediment sequences. *Journal of Quaternary Science*, 16(2), 155-66.

Nilssen, J.P. & Sandoy, S. (1990) Recent Lake Acidification and Cladoceran Dynamics - Surface Sediment and Core Analyses from Lakes in Norway, Scotland and Sweden. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 327(1240), 299-309.

O'Sullivan, P.E. (1983) Anually-laminated lake sediments and the study of quaternary environmental changes - A review. *Quaternary Science Reviews*, 1, 245-313.

Quinlan, R., Douglas, M.S.V. & Smol, J.P. (2005) Food web changes in arctic ecosystems related to climate warming. *Global Change Biology*, 11(8), 1381-86.

Rasmussen, P., Bradshaw, E. & Odgaard, B.V. (2002) Fortidens miljø arkiveret år for år. Fund af varvige sedimenter i Sarup Sø på Fyn. *Naturens Verden*, 5, 34-40

Ricciardi, A. & Reiswig, H.M. (1994) Taxonomy, Distribution, and Ecology of the Fresh-Water Bryozoans (Ectoprocta) of Eastern Canada. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 72(2), 339-59.

Roberts, N. (1998) *The Holocene: An Environmental History.* Blackwell Publishing, Oxford.

Rodrigo, M.A., Vicente, E. & Miracle, M.R. (2000) The role of light and concentration gradients in the vertical stratification and seasonal development of phototrophic bacteria in a meromictic lake. *Archiv für Hydrobiologie*, 148(4), 533-48.

Rohling, E.J. & Palike, H. (2005) Centennial-scale climate cooling with a sudden cold event around 8,200 years ago. *Nature*, 434(7036), 975-79.

Røen, U.I. (1995) *Gællefødder og Karpelus.* Dansk Naturhistorisk Forening, Vinderup Bogtrykkeri A/S, Vinderup, Denmark.

Sanford, P.R. (1993) *Bosmina-Longirostris* Antennule Morphology as an Indicator of Intensity of Planktivory by Fishes. *Bulletin of Marine Science*, 53(1), 216-27.

Seppa, H., Hammarlund, D. & Antonsson, K. (2005) Low-frequency and high-frequency changes in temperature and effective humidity during the Holocene in south-central Sweden: implications for atmospheric and oceanic forcings of climate. *Climate Dynamics*, 25(2-3), 285-97.

Shuman, B. & Donnelly, J.P. (2006) The influence of seasonal precipitation and temperature regimes on lake levels in the northeastern United States during the Holocene. *Quaternary Research*, 65(1), 44-56.

Steenbergen, C.L.M., Korthals, H.J. & Dobrynin, E.G. (1994) Algal and Bacterial Pigments in Non-Laminated Lacustrine Sediment - Studies of Their Sedimentation, Degradation and Stratigraphy. *Fems Microbiology Ecology*, 13(4), 335-51.

Stockmarr, J. (1971) Tablets with spores used in absolute pollen analysis. *Pollen et Spores*, 13, 615-21.

Talbot, M.R. (1990) A Review of the Paleohydrological Interpretation of Carbon and Oxygen Isotopic-Ratios in Primary Lacustrine Carbonates. *Chemical Geology*, 80(4), 261-79.

Teeter, A.M., Johnson, B.H., Berger, C., Stelling, G., Scheffner, N.W., Garcia, M.H. & Parchure, T.M. (2001) Hydrodynamic and sediment transport modeling with emphasis on shallow-water, vegetated areas (lakes, reservoirs, estuaries and lagoons). *Hydrobiologia*, 444(1-3), 1-24.

ter Braak, C.J.F. & Šmilauer, P. (2002) *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination* version 4.5 edn. Microcomputer Power Ithaca New York, USA.

Thomas, E.R., Wolff, E.W., Mulvaney, R., Steffensen, J.P., Johnsen, S.J., Arrowsmith, C., White, J.W.C., Vaughn, B. & Popp, T. (2007) The 8.2 ka event from Greenland ice cores. *Quaternary Science Reviews*, 26(1-2), 70-81.

Vassiljev, J. (1998) The simulated response of lakes to changes in annual and seasonal precipitation: implication for Holocene lake level changes in northern Europe. *Climate Dynamics*, 14(11), 791-801.

Vassiljev, J., Harrison, S.P. & Guiot, J. (1998) Simulating the Holocene lake-level record of Lake Bysjon, southern Sweden. *Quaternary Research*, 49(1), 62-71.

Veski, S., Seppa, H. & Ojala, A.E.K. (2004) Cold event at 8200 yr BP recorded in annually laminated lake sediments in eastern Europe. *Geology*, 32(8), 681-84.

von Grafenstein, U., Erlenkeuser, H., Muller, J., Jouzel, J. & Johnsen, S. (1998) The cold event 8200 years ago documented in oxygen isotope records of precipitation in Europe and Greenland. *Climate Dynamics*, 14(2), 73-81.

Walker, I.R. (2001). Midges: Chironomidae and related Diptera. In *Tracking Environmental Change Using Lake Sediments. Zoological Indicators* (ed J.P. Smol, Birks, H. J. B., Last, W.M.), Vol. 4, pp. 43-66.

Wiersma, A.P. & Renssen, H. (2006) Model-data comparison for the 8.2 ka BP event: confirmation of a forcing mechanism by catastrophic drainage of Laurentide Lakes. *Quaternary Science Reviews*, 25(1-2), 63-88.

Wissel, B., Boeing, W.J. & Ramcharan, C.W. (2003) Effects of water color on predation regimes and zooplankton assemblages in freshwater lakes. *Limnology and Oceanography*, 48(5), 1965-76.

Wissel, B., Yan, N.D. & Ramcharan, C.W. (2003) Predation and refugia: implications for Chaoborus abundance and species composition. *Freshwater Biology*, 48(8), 1421-31.

Zillen, L., Snowball, I., Sandgren, P. & Stanton, T. (2003) Occurrence of varved lake sediment sequences in Varmland, west central Sweden: lake characteristics, varve chronology and AMS radiocarbon dating. *Boreas*, 32(4), 612-26.

Økland, K.A. & Økland, J. (2002) Freshwater bryozoans (Bryozoa) of Norway III: distribution and ecology of *Plumatella fruticosa*. *Hydrobiologia*, 479(1), 11-22.

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Using subfossils of cladocerans in surface sediments of 54 European shallow lowland lakes (latitude: 36-68 ºN) to assess the impact of climate on cladoceran community structure

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Short title: European climate gradient and zooplankton structure

Summary

1. This study describes the cladoceran community structure and environmental conditions of 54 shallow inland lakes along a European latitude gradient (36-68 ºN) with special focus on the impact of climate on cladoceran species composition and richness.

2. The cladoceran community structure was identified from subfossils enumerated from surface sediments. Multivariate methods, such as ordination and regression trees, were applied to explore the relationships between cladoceran species distribution and contemporary environmental variables.

3. A distinct difference was found in cladoceran community structure and body size structure along the latitude gradient, and the 54 lakes could thus be separated into three groups. The first group was composed of northern lakes (n=7) characterised by low summer temperature, conductivity and nutrient concentrations, and dominance by large-sized pelagic and occasionally acidic tolerant species. The second group mainly comprised southern eutrophic warm water lakes (n=5) with high conductivity and it was dominated by small-sized benthic-associated species. The third group mainly included lakes at intermediate latitudes and was characterised by cladoceran assemblages showing less overall species specific preferences towards habitat and environmental conditions, except for conductivity.

4. Taxa richness showed a unimodal relationship to latitude, being low in the northern-most lakes as well as in the southern-most and productive, macrophyte-rich lakes.

5. The proportion of cladoceran resting eggs relative to body shields was higher in the northern lakes, where the season is shorter, and was related to both climate variables and nutrient state.

6. In our study, latitude and, implicitly, temperature were strongly correlated to conductivity and nutrients, highlighting the difficulties of disentangling a direct climate signal from indirect effects of climate and human-related impacts when a latitude gradient is used as a climate proxy.

Introduction

In recent years, climate impact on ecosystems has received increasing attention due to the relatively rapid increase in global warming (IPCC, 2001, 2007). As many freshwater bodies are used as drinking water reservoirs and for agricultural irrigation and fishery, there is an acute need and demand for knowledge about the impact of global warming on these ecosystems. Overall, global warming is expected to alter the hydrology, chemistry and biology of lakes, rives and wetlands and their interactions. However, the interactions both within and between the systems are extremely complex, and the consequences of the changes are difficult to determine (Murdoch et al. 2000, Schindler 1997).

Lake sediments, containing a natural archive of subfossils of various lake organisms, offer an excellent potential for studying the impact of climate (Battarbee, 2000). In addition, this sedimentary archive provides an accurate and cost-effective tool for the assessment of parameters such as species richness and community structure, as spatial and seasonal species heterogeneity and year-to-year variations are integrated in the sediment records (Jeppesen et al., 2003; Brendonck & De Meester, 2003; Vanderkerkhove et al. 2004; 2005a,b). In contrast, conventional methods, being based on the sampling of active (living) communities, require costly repeated sampling multiple localities within the lake during an extended period of time to overcome the problems of species heterogeneity and between-year variations (Vanderkerkhove et al., 2005a).

In shallow lake ecosystems, cladocerans may play a key role by controlling phytoplankton and periphyton growth (Gliwicz, 2003) at low fish predation. Climate influences the cladoceran community directly through temperature-induced physiological changes (Moore et al., 1996; Goss & Bunting, 1983) and indirectly through changes in lake chemistry such as conductivity. Thus, most cladocerans are unable to survive at conductivities above 3000 µS cm^{-1} (Aladin, 1991; Frey, 1993; Sarma et al., 2006; Williams, 1981); yet, even below this threshold indirect responses through changes in fish predation may occur, for example at 2‰ salinity in northern temperate brackish lakes (Jeppesen et al., 1994, 2007).

Cladoceran subfossils have been applied to a wide variety of paleoecological studies assessing anthropogenic impact on lake ecosystems, climate-driven impacts provide no exception (Amsinck et al., 2007). Thus, cladoceran subfossils have proved to be useful as direct paleo-temperature indicators by the development of temperature transfer functions (Lotter et al., 1997; Korhola, 1999; Duigan & Birks, 2000). In addition, Jeppesen et al. (2003) have shown that the *Bosmina* ephippia to carapace ratio is a useful indicator of lake temperature. Climate change affecting salinity can be tracked directly by a zooplankton based salinity transfer function (Bos et al., 1999) or indirectly by tracking the cascading effects of changed salinity on the lake ecosystem via changes in cladoceran community structure (Amsinck et al., 2003). Increasing temperature will likely also impact the top-down control of fish (Jeppesen et al., 2005a,b) and the changes in fish predation pressure can be traced by cladoceran-based transfer functions of fish abundance (Jeppesen et al., 1996: Amsinck et al., 2005), the size (dorsal length) of *Daphnia* ephippia (Jeppesen et al., 2002) and the contribution of *Daphnia* to the total sum of *Daphnia* and *Bosmina* ephippia (Jeppesen et al., 2003).

In Europe, most cladoceran-based paleolimnological studies focussing on climate changes have been conducted on a restricted regional scale, such as the Alps (e.g. Lotter et al., 1997), European mountain lakes (Brancelj et al., 2007) or within single countries (e.g. Bennike, Sarmaja-Korjonen & Seppanen, 2004; Duigan & Birks, 2000; Sarmaja-Korjonen, 2003, 2004). In this study, cladoceran subfossils were recovered from the surfacial sediments of 54 shallow European lakes covering a wide latitude (36 - 68 ºN) and, implicitly, climate gradient (15 ºC difference in mean monthly temperature of the warmest month) as well as a wide nutrient gradient (TP: 6 to 470 $\mu g\ l^{-1}$). The overall aim was to explore cladoceran community composition, richness, ephippia production and body size structure and to identify key environmental factors structuring the cladoceran community composition along the north-south transect. Besides a direct effect of temperature and season length, we expect that the cladoceran community structure to be affected by increasing benthi-planktivorous fish predation with decreasing latitude (Dumont, 1994; Fernando, 1994; Gyllström et al., 2005) and by changes in conductivity, especially in the southern lakes (Beklioglu et al., 2007; Declerck et al., 2005, Vandekerkhove et al., 2005a.). We further expect the ephippia to body shield ratio to decline with decreasing latitude (Jeppesen et al., 2003).

Materials and methods

Study sites

The study was based on a subset (44 European lakes) of the ECOFRAME data set, six south Spanish lakes from the BIOMAN data set and four Greek lakes from the EUROLIMPACS data set. In these former studies, lake surface sediment samples were taken and environmental variables measured in 2000 (except for one Finnish sediment surface sample taken in winter 2003) (ECOFRAME), 2000 or 2001 (BIOMAN) and 2005 (EUROLIMPACS). The study lakes were located in nine European countries and eleven different regions (Fig. 1): Sweden (northern SN, southern SS), Finland (FIN), Estonia (EST), Poland (PL), Denmark (DK), United Kingdom (UK), Germany (D), Greece (G) and Spain (northern EN, southern ES). In each region four to six lakes were sampled.

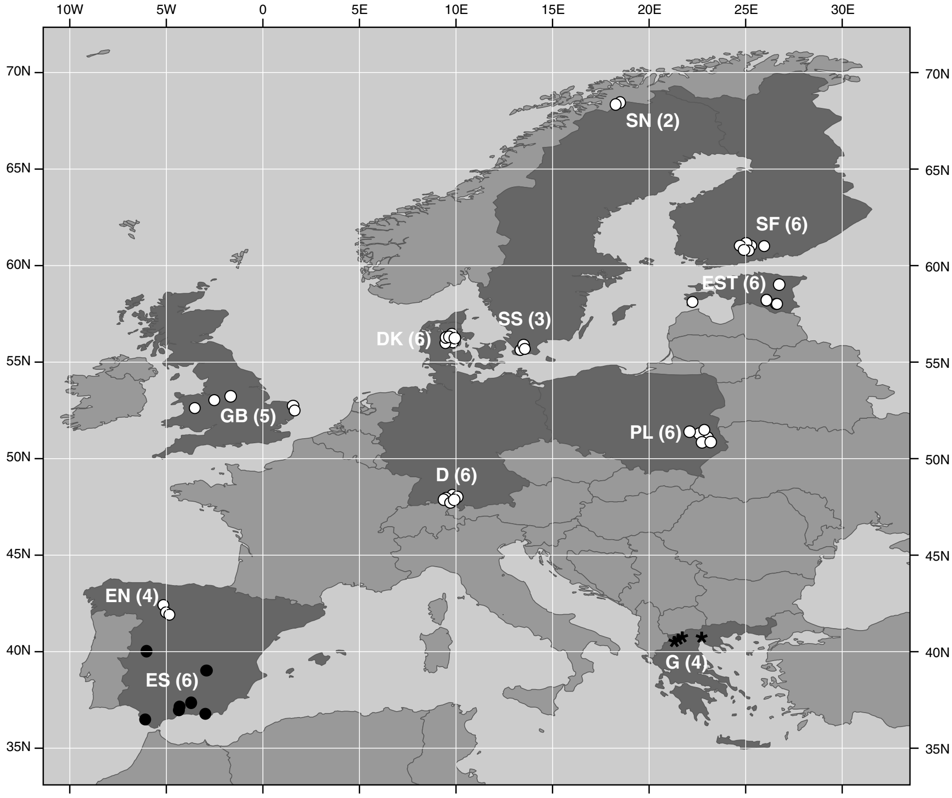


Figure 1.Geographical location of the 54 European study lakes. Capital letters denote country, subscript S= southern, N= northern. Numbers of study lakes are given in brackets. ○ ECOFRAME data set, ● BIOMAN data set, * EUROLIMPACS data set.

Table 1. Summary statistics of environmental variables from the 54 European study lakes.

Parameter	Mean	Median	25 % percentile	75 % percentile	Min	Max	N	Transformation
Latitude (ºN)	51	53	42	58	36	68	54	Log$_{10}$ x
Longitude	13	12	4	23	-6	27	54	Log$_{10}$ (x+10)
Area (ha)	782	24	9	60	1	27000	54	Log$_{10}$ x
Mean depth (m)	1.92	1.60	1.20	2.50	0.47	6.00	54	Log$_{10}$ x
Total phosphorous (µg L^{-1})	107	71	32	141	6	470	54	Log$_{10}$ x
Total nitrogen (µg L^{-1})	1936	1365	992	2690	239	7710	54	Log$_{10}$ x
Chl *a* (µg L^{-1})	47	24	8	58	1	331	54	Log$_{10}$ x
Secchi depth (m)	1.5	1.1	0.6	2.2	0.2	5.6	54	Log$_{10}$ x
Secchi/mean depth	0.9	0.6	0.4	1.1	0.1	4.6	54	Log$_{10}$ x
Conductivity (µS cm^{-1})	775	313	141	585	9	7229	54	Log$_{10}$ x
pH	8.0	8.1	7.7	8.4	5.1	9.5	54	-
PVI submerged plants (%)	15	5	1	14	0	87	44	Log$_{10}$ (x+1)
Piscivorous fish biomass (kg net^{-1} night^{-1})	0.902	0.259	0.023	1.054	0	4.479	35	$x^{0.5}$
Planktivorous fish biomass (kg net^{-1} night^{-1})	2.282	0.908	0.102	3.922	0	11.141	35	$x^{0.5}$
Mean air temperature of the warmest month of the year (ºC)	18.7852	17	16.5	21	12	26.4	54	$x^{0.5}$
Mean annual temperature (1961-90) (ºC)	8	8	6	10	-3	16	54	$(x+10)^{0.5}$

Sampling and laboratory procedure

For each of the 54 lakes surface sediment samples from the top 0-1 cm to 0-3 cm were taken using a Kajak surface corer in the deepest part of the lake. Approximately 5 g (wet weight) of homogenised surface lake sediment was boiled in 50 ml of 10 % KOH for 20 minutes to remove the organic content after which the samples were kept cold (4 ºC) for maximum two weeks until counting was performed. Cladoceran fragments >80 µm were identified according to Frey (1959), Røen (1995), Flössner (2000) and Alonso (1996) using a binocular microscope (100x, Leica MZ12) and an inverted light microscope (320x, Leitz Labovert FS). Remains withdrawn on a 140 µm mesh sieve were quantified for the entire sub-sample, whereas the remaining fragments withdrawn on an 80 µm mesh sieve were sub-sampled and, depending on the density of the remains, 2.5 to 40 % counted. A total of 74,634 remains were identified from the 54 surface samples, the sample median of remains counted being 1367 (min: 269; max: 2547). Counting of remains was adjusted to represent individuals (e.g. number of carapace halves/2, number of headshields/1), and only the most abundant and most representative fragment of a species or taxa was used for data analysis.

The sampling of environmental variables (three physical and five chemical variables plus macrophyte abundance) followed a standardised protocol described in detail by Moss et al. (2003) (ECOFRAME and EUROLIMPACS lakes) and Declerck et al. (2005) (BIOMAN lakes). A further description of chlorophyll *a* and nutrient (total phosphorous (TP) and total nitrogen (TN)) analyses can be found in Nõges et al. (2003). Water samples for chemical analyses were sampled twice from the centre of the lake during summer 2000 with a depth-integrating tube sampler. Water temperature and Secchi depth (20 cm disc) were measured from the boat and pH and conductivity were measured in unfiltered water using electronic pH and conductivity meters. Plant volume inhabited (Canfield et al., 1984) of submerged macrophytes (PVIsub) was measured once (late summer) by estimating plant coverage and height using water glass along transects from the lake shore to the centre of the lake. Where visibility was low, random samples were taken with a rake at each transect point. Ten percent of the lake area was scanned. Data on annual mean air temperature were obtained from meteorological records (1961-1990) (New et al., 2000), while mean air temperature of the warmest month of the year (air temperature) was calculated in accordance to Moss et al. (2003) and obtained from the websites; http://www.inm.es and http://www.hnms.gr.

Statistical analyses

Prior to the statistical analyses environmental data were transformed (Table 1) to obtain the best approximation to normal distribution. Chemistry variables were an average of the two measurements in 2000 for the ECOFRAME data set. A combined variable, SecDep, was created by dividing Secchi depth with mean depth as a surrogate for the light exposure to the sediment. Accordingly, mean depth and Secchi depth were excluded as environmental variables. Concentrations of remains (no. per g dw sediment) were converted into relative percentage abundance since accumulation rates to adjust for site specific sediment accumulation were not available. In multivariate analyses relative abundances were arcsin transformed to stabilise variance (Sokal & Rohlf, 1997).

Taxa richness (total number of taxa) and the taxa diversity estimate Hill's N2 (Hill 1973) were calculated for each lake and related to climate (T_{summer} and latitude).

The proportion of gametogenetic reproduction versus parthenogenetical reproduction was estimated for *Bosmina* and Chydoridae as the percentage constituted by ephippia abundance of the sum of parthenogenetic carapaces and ephippia according to Jeppesen et al. (2003). As male carapaces cannot be distinguished from female carapaces, these were included in the parthenogenetical carapaces. The ephippia ratios were $\log_{10}$ +1 transformed, and linear and multiple linear regressions were performed including contemporary environmental variables.

Ordinations

Redundancy (colinearity) among the environmental variables was explored by principal component analysis (PCA) on environmental variables exclusively and by variance inflation factors (VIF) estimated using canonical correspondence analysis (CCA), including both environmental and species data. To determine whether linear or unimodal ordinations would be most appropriate to conduct, detrended canonical analysis (DCA, detrending by segments) as well as detrended canonical correspondence analysis (DCCA) were applied. Correspondence analysis (CA) was used to determine the main directions of variance in the species data among the lakes and to estimate the full variance in species composition across the data sets. The unconstrained (DCA, CA) and the constrained ordinations (CCA, DCCA) were performed on the full species data set (DAT1: 59 taxa, 54 lakes) and for a reduced data set comprising species occurring in minimum five lakes (DAT2: 38 species, 54 lakes), as rare species may have an unduly large influence in ordinations (ter Braak & Smilauer, 2002). In addition, ordinations (DCA, CA, CCA, DCCAs) were performed on a subset of lakes (n=44) with data on plant filled volume (PVIsub (%) available. Furthermore, DCCA and redundancy analyses (RDA) on the group of lakes remaining after excluding the most distinct groups of lakes as revealed by the multivariate regression trees (MRT) analysis (see below) were conducted. Monte Carlo permutation significance test (significance level: 5%) was performed with 999 permutations All ordinations were performed in CANOCO version 4.5 (ter Braak & Smilauer, 2002).

Multivariate regression trees

Multivariate regression trees (Deáth, 2002) using the same combinations of data sets as for the ordinations, except for the data set including PVIsub, were applied to determine the thresholds of the most important environmental variables structuring the taxa community of the 54 lakes into clusters. In contrast to the ordination analyses, MRT can be used to analyse complex ecological data with linear as well as non-linear relationships between environmental variables and high-order interactions (Deáth, 2002). MRT forms clusters of species and sites modelled from species and environmental relationships by repeated splitting of the data. Each split minimises the dissimilarity (sum of squared Euclidian distances, SSD) of the species and sites within clusters (Deáth and Fabricus, 2000). The overall fit of a tree is given by the relative error (RE: SSD in clusters divided by SSD in unsplit data), whereas the predictive accuracy is specified as cross validated relative error (CVRE) (Breiman et al., 1984; Deáth, 2002). The model with the minimum cross validated error was selected as the final tree (Deáth and Fabricus 2000), 1000 cross validations were applied. To further establish the significance of the selected model, a non-parametric analysis of similarity of differences between and within groups (ANOSIM) was carried out with 1000 permutations. The ANOSIM R-statistics ranges from 0, representing a random distribution of objects between groups, whereas 1 indicates complete dissimilarity between groups. Species characteristics for a given cluster defined by the MRT analysis were identified by using an indicator species index (INDVAL) calculated by the product of relative abundance and the relative frequency of occurrence within the cluster (Dufrene & Legendre, 1997). An INDVAL value of 1 indicates that the species is only abundant in one particular cluster, whereas a value of zero indicates a wide distribution among clusters. Significance of taxa association to the cluster was tested by permutation with 500 random iterations. Taxa with an indicator value larger than 0.25 and with $p<0.01$ were considered indicator species according to Dufrene & Legendre (1997). MRT was carried out in R (The R Foundation for Statistical Computing Version 2.2.0) using the mvpart package (Multivariate partitioning), ANOSIM by using the vegan library and INDVAL analyses were performed applying the labdsv package (Dynamic Synthetic Vegephenomenology).

Comparisons between MRT clusters
Significant differences in medians between groups of lakes based on separation by MRT analysis with respect to influential environmental variables for the cladoceran community assemblage were tested by ANOVA (on transformed variables, Table 1) (significance at the 5% level, with Tukey's test of multiple comparisons to separate groups). Prominent variables for the cladoceran species distribution were those identified both by MRT analysis and by the ordination analyses. In addition, ephippia abundance (log-transformed), species richness and diversity (square-root transformed) were analysed for between-MRT-group differences by ANOVA. Additionally, cladocerans were divided into three habitat groups (pelagic, macrophyte/sediment-associated and sediment-associated taxa) as well as into three size classes: large (taxa $\geq$ 1 mm), medium (taxa between 0.5-1 mm) and small (taxa <0.5 mm) in accordance to Alonso (1996), Flössner (2000), and Røen (1995). The relative distribution of these between MRT-groups was tested statistically by ANOVA on arcsin-transformed percentage data for pelagic taxa, small and large-sized taxa. Generally, where variance-heterogeneity appeared in analyses using Bartlett's test of equal variance, Welsch's ANOVA was applied.

Results

Environmental characteristics of study lakes
The study lakes included 54 inland lakes distributed along a broad north-south transect across Europe, ranging from latitude 36°N to 68 °N (Fig. 1). Mean annual temperature ranged from -3 to 16 °C (Table 1). The sampled lakes were mainly shallow (0.5-6m), covering a wide range of surface areas, nutrient concentrations, conductivity and submerged macrophyte abundances (Table 1).

The PCA based on ten environmental variables exclusively showed that all environmental variables were highly correlated with the first axis, indicating pronounced redundancy (colinearity) among the variables, excepting Secdep, which correlated with the second axis. The PCA axis 1 explained 89% of the variation in the lakes, while the PCA axis 2 accounted for only 7% of the variation. PCA on the environmental subdata set including PVIsub (n=44 lakes) ($\lambda_1=0.870$, $\lambda_2=0.076$) revealed similar patterns. In this ordination, PVIsub, as did SecDep, correlated closely with PCA axis 2.

Taxa richness and diversity
In total, remains of 59 cladoceran taxa were recorded in the surface sediment from 54 lakes. The most common taxa were *Chydorus* spp. and *Ceriodaphnia* spp. occurring in all 54 lakes and in 53 lakes, respectively (Fig. 2). In contrast, *Bosmina longirostris* showed by far the highest abundance (relative as well as absolute) summed over all 54 lakes, *Chydorus* spp. being the second most abundant. Twenty one taxa were found in less than five lakes (Fig. 2). Median taxa richness was 21, the maximum of 33 taxa being found in a Polish lake (PL_5) and the minimum of four taxa in a southern Spanish lake (ES_11). Lakes with low numbers of taxa additionally had a low Hill's N2 diversity, as Hill's N2 correlated positively with number of taxa (Pearson r=0.58, p>0.0001). Although approximately the same amount of sediment was analysed in the samples, evenness correlated negatively with taxa number (Pearson r=-0.41, p=0.0020), and we cannot exclude that increased sample sizes may change the relation between diversity and taxa number.

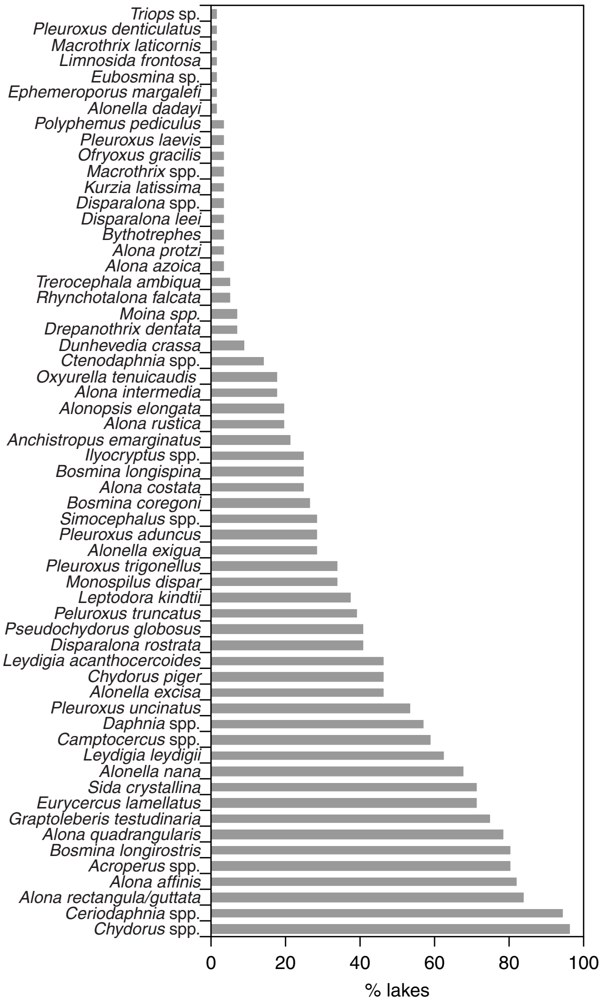


Figure 2. Frequencies of taxa observations in the 54 European study lakes.

Square root transformed taxa richness as well as Hill's diversity showed a unimodal tendency when related to latitude (Fig. 3). In correspondence, when dividing the data into two subsets with break point 50 ºN, taxa richness of lakes with latitude below 50 ºN correlated significantly positively with latitude (Pearson r=0.81, p<0.0001, n=20), whereas lakes of higher latitude (>50 ºN) correlated significantly but negatively with latitude (Pearson r=-0.37, p=0.0381, n=34). Similar tendencies were present when relating taxa richness to T_{summer} (southern: Pearson r=-0.78, p<0.0001, n=20; northern: Pearson r=0.62, p<0.0001, n=34). The unimodal tendency of Hill's diversity was, however, not significant for either latitude or T_{summer}.

Ordinations - all 54 lakes

CA and CCA were applied as gradient lengths of DCAs as well as those of DCCAs were ≥ 3.0 standard deviation (S.D.) units in DAT1 and DAT2, implying that most taxa are assumed to show a unimodal response to the underlying ecological gradients (ter Braak, 1995). The eleven environmental variables captured 41% of the total variation in the taxa assemblage (DAT 1), the eigenvalues of the CCA being $\lambda1=0.415$ and $\lambda2=0.266$ and thus close to those of the CA ($\lambda1=0.548$, $\lambda2=0.369$). However, VIF showed that latitude was highly correlated with T_{summer} (VIF= 36 and 20, respectively, the remaining variables ranged from 2-9) and latitude was therefore excluded from further analyses.

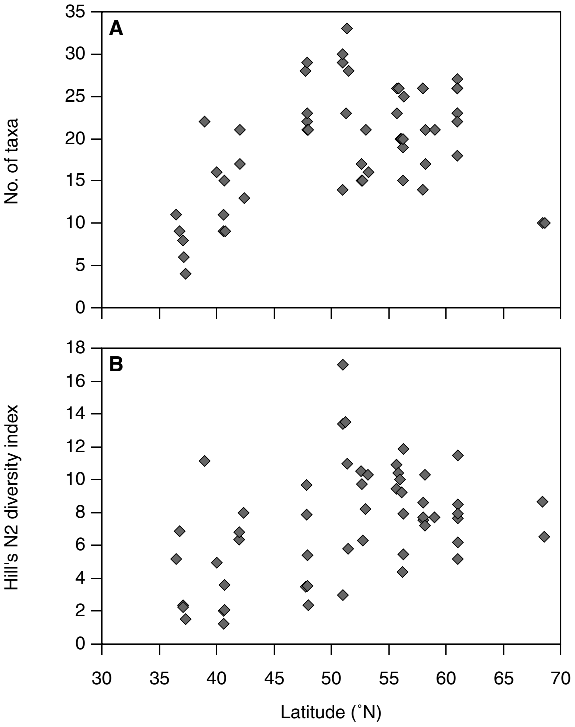
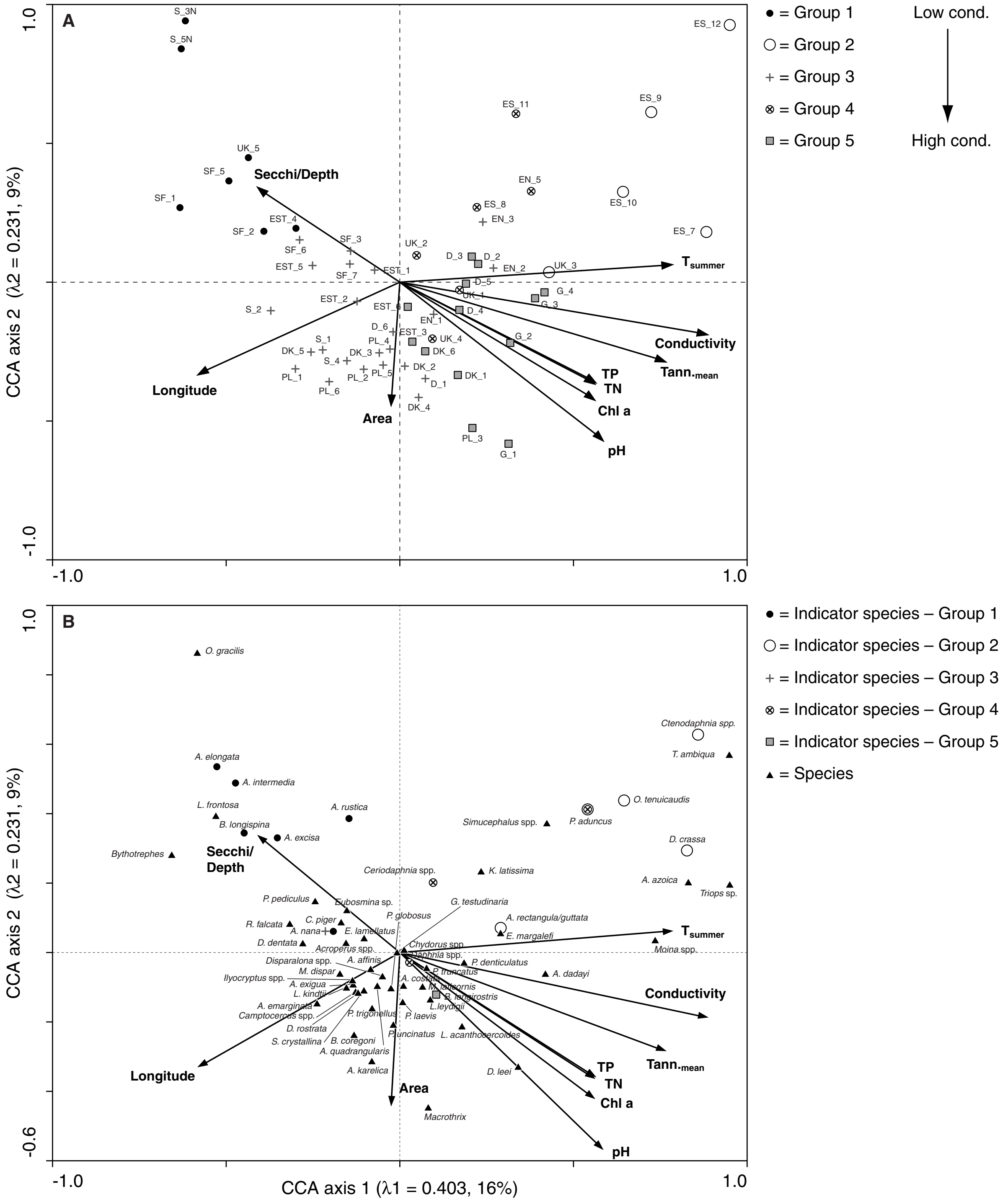


Figure 3. Taxa richness (observed taxa per lake) and Hill's N2 diversity index in relation to latitude.

The resultant CCA (n=10 environmental variables) explained in total 39% of the taxa variation (sum of all ´s=1.014, total inertia=2.600), most of the variance being explained by CCA axis 1 (16%, 1=0.403 and 9%, 2=0.231 for axis 2). This axis closely correlated positively with conductivity, T_{summer}, $T_{ann.mean}$ and negatively with longitude (Fig. 4), the four variables contributing significantly to the taxa variance after Bonferroni correction and explaining 13%, 10%, 11% and 8%, respectively, of the variation. For



CCA ordination plot of the 54 European lakes, including 10 environmental variables. Sites (A) and 59 cladoceran taxa (B). Site symbols and species symbols refer to the MRT-division in groups and identified indicator-species (Fig. 5). Taxa and country abbreviations identical with figure 1 and 2, respectively.

the CCA of the DAT2 data set, 42% of the total variation in the taxa assemblage ($\lambda1=0.370$ and $\lambda2=0.215$) was explained by the ten environmental variables. Bonferroni-adjusted forward selection in CCA showed conductivity, pH and longitude to be significant for the taxa assemblage, explaining respectively, 15%, 10% and 9% of the variation uniquely. T_{summer} was only marginally significant after Bonferroni correction, explaining 11% of the variation uniquely.

CCA ($\lambda1=0.305$, $\lambda2=0.094$) conducted on the data set with macrophyte cover data available (n=44 lakes) showed PVIsub to contribute significantly to the variation in the cladoceran assemblages, explaining 12% as sole explanatory variable. Also conductivity, T_{summer} and longitude contributed significantly, explaining 14%, 10% and 15%, respectively, of the assemble variation as sole variables. Again latitude was excluded due to high VIF (17, range 2-8). PVIsub correlated closely and positively with T_{summer} and negatively with longitude in the ordination plot (not shown). All three variables correlated to CCA axis 2.

MRT analyses - all 54 lakes

MRT analyses including the ten environmental variables produced a three-leaved tree (Fig. 5A1) (DAT1: CVRE=0.914, DAT2: CVRE=0.195) explaining 66.6% (DAT1) and 66.3% (DAT2) of the taxa variation. As for ordination the splits were defined by conductivity, the first split reducing the deviance by the largest amount, separating seven lakes (SN3, SN5, FIN1, FIN2, FIN3, EST4, UK5) with conductivity < 46 (μS cm^{-1}) (Fig. 5A2). Close surrogate variables were pH (threshold: < 6.9, $r^2=0.981$), TP (threshold: < 10 µg L^{-1}, $r^2=0.926$) and T_{summer} (threshold: < 15.7ºC, $r^2=0.926$), and several taxa associated with oligotrophic and/or acidic water (e.g. *Bosmina longispina, Alona intermedia, Alonella excisa, Alona rustica*) were among the indicator taxa for these lakes.

As in the first split, the second split was defined by conductivity, separating five mainly warm water lakes with conductivity above 2210 µS cm^{-1} (ES7, ES9, ES10, ES12, UK3) (Fig. 5A) with the surrogate split variables $T_{ann.mean}$ (threshold: >= 23.6ºC, $r^2=0.936$) and Chl *a* (threshold: < 137 µg l^{-1}, $r^2=0.936$). Macrophyte associated taxa dominated within this group of lakes, whereas taxa indicators for the remaining 42 lakes were *Bosmina longirostris* and two sediment associated species (Fig. 5A). The ANOSIM R statistics of 0.75 (P< 0.001) showed significant difference between MRT designated groups of DAT1 and DAT2.

Ordination and MRT – high and low conductivity lakes excluded

An additional ordination was conducted in order to investigate whether grouping occurred among the remaining 42 lakes with intermediate conductivity (REST, Fig. 5B2). RDA was performed (latitude and $T_{ann.mean}$ being excluded due to high VIFs), as the largest gradient of the DCCA was 1.7 S.D. units. The nine environmental variables explained in total 49% of the taxa assemblage variation, SecDep being the single significant variable (Bonferroni corrected), explaining 13% of the variation, whereas T_{summer} was found to be marginally significant. RDA with exclusion of taxa occurring in less than three lakes revealed similar results.

The best predictive mode of MRT on cladoceran data from the 42 lakes did not reveal a split (Fig. 5B1). In accordance to Breiman et al. (1984), the rule of selecting the most complex tree within 1 standard error of the best predictive tree was applied with the constraint that the smallest resulting group contained more than three lakes. The resulting three-leaved MRT (CVRE=1.04) (Fig. 5B2) explained 69.4% of the community variance including the ten environmental variables. The first split divided the 42 lakes across ecoregions with reference to conductivity < 344 µS cm^{-1}, in correspondence with the results from the RDA analysis. Surrogate splits were T_{summer} (thres-hold: < 22.0ºC, $r^2=0.714$), TN (threshold: 1167 µg l^{-1}, $r^2=0.690$), TP (threshold: < 84.5 µg l^{-1}, $r^2=0.667$), Chl *a* (threshold: < 34 µg l^{-1}, $r^2=0.667$) and SecDep (threshold: >= 0.25, $r^2=0.643$).

Alonella nana was significantly associated with the 23 low-conductivity lakes (Fig. 5B2). The second split was attributed to longitude and separated six east-European lakes with lower trophic level, pH and lake size than the remaining lakes, indicated by surrogate splits (Chl *a*: threshold: < 12 µg l^{-1}, $r^2=0.947$; pH: threshold: < 8.0, $r^2=0.895$; SecDep: threshold: > 0.72, $r^2=0.895$ and lake area: threshold: < 3.2 ha, $r^2=0.789$) (Fig. 5B2). Larger pelagic cladoceran taxa dominated the indicator taxa of these lakes, whereas the smaller pelagic species *Bosmina longirostris* was significantly associated with group 5 (Fig. 5B2). The ANOSIM analysis confirmed a significant difference between groups 3-5 (R=0.40, P< 0.001). Performing MRT and ANOSIM on the 42 lakes excluding taxa occurring in less than three lakes revealed similar results.

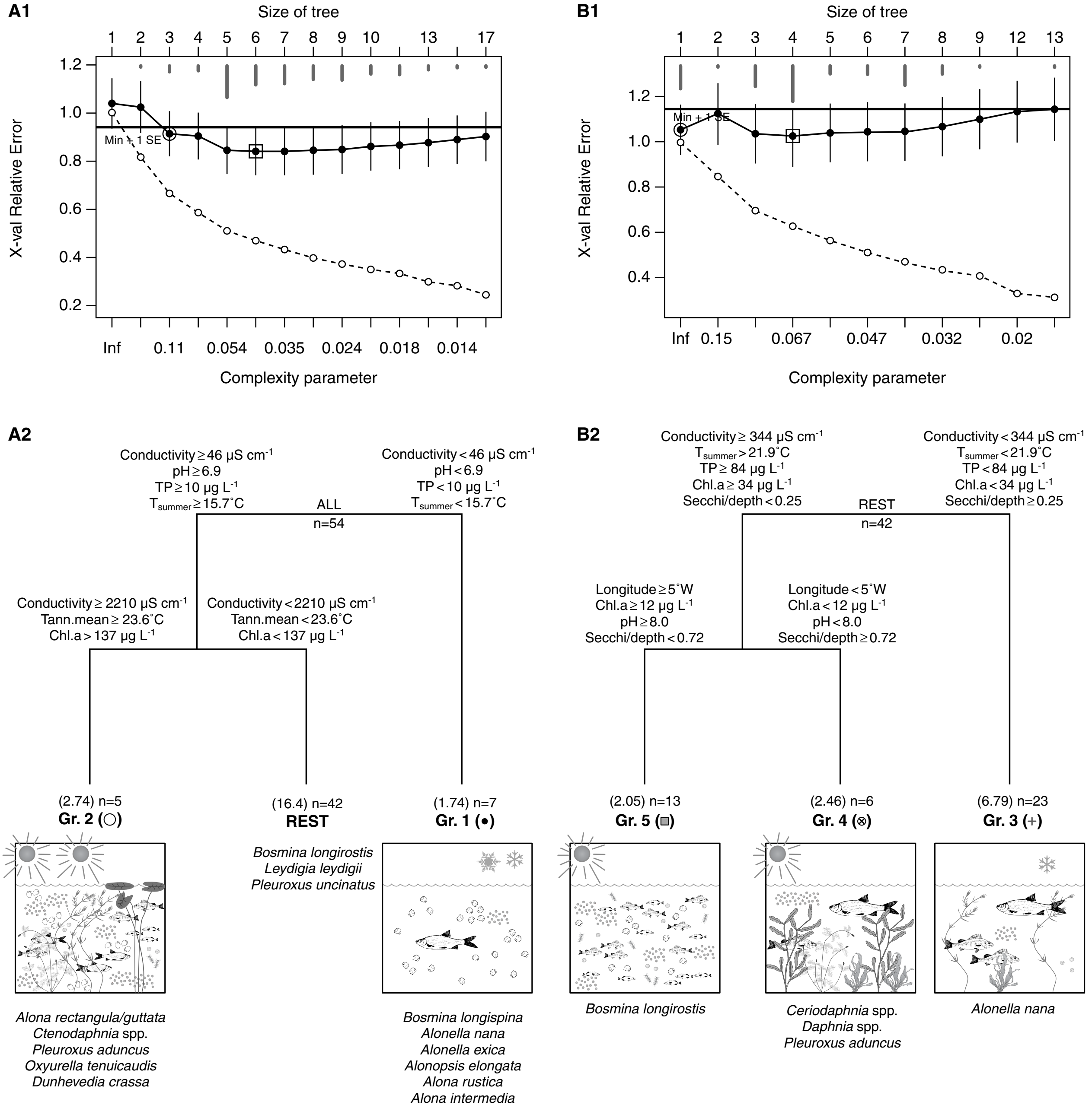


Figure 5. Cross-validation of a multivariate regression tree based on cladoceran remains from A1: all 54 European lakes and B1: with the exclusion of low- and high-conductive lakes (groups 1 and 2). The lower line shows the explanatory power, the upper line the predictive power and the solid horizontal line the one standard distance error from the best model. The circle shows the model with greatest cross-validated accuracy, the square shows the most complex tree within 1 standard error of the best mode. The selected multivariate regression trees was A2: all 54 European lakes, with greatest cross-validated accuracy, B2: with the exclusion of low- and high-conductive lakes, the three-leaved tree within 1 standard error. Number of lakes per group (n) and indicator taxa are given for each group, deviance (SSD) given in brackets.

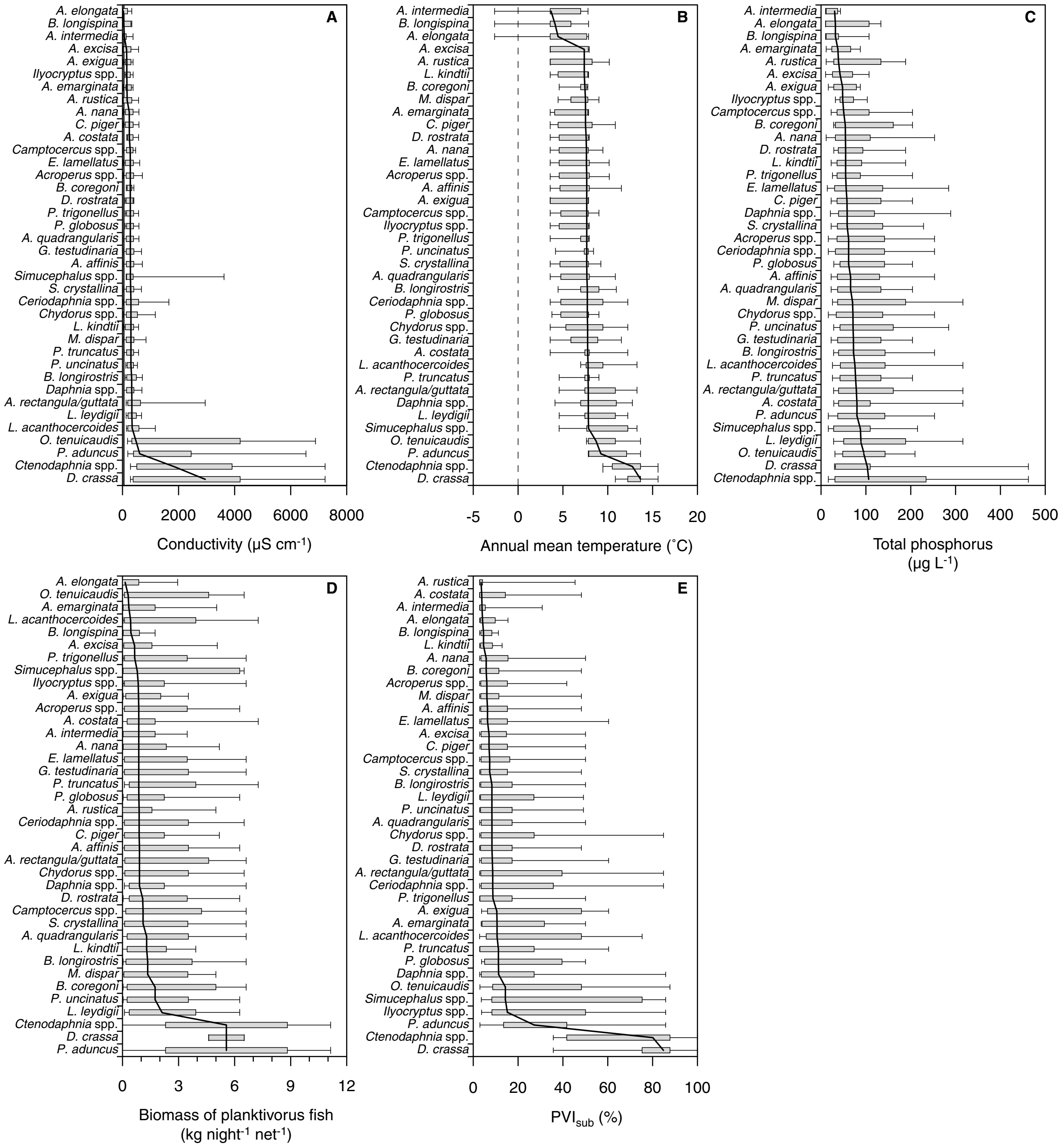


Figure 6. Distribution of taxa (present in ≥ 3 lakes) with respect to A) conductivity (µS cm^{-1}), B) annual mean temperature (1961-1990) (ºC), C) total phosphorous (µg L^{-1}), D) biomass of planktivorous fish (kg net^{-1} night^{-1}) and E) submerged macrophyte filled volume (%). The taxa (see Fig. 2) are sorted by increasing median value (solid vertical line), the boxes represent 25 and 75 percentiles, and whiskers show 10 and 90 percentiles.

Taxa distribution along environmental gradients

Ranking the cladoceran taxa abundance medians along the enviromental gradients measured revealed a close relationship between cladoceran taxa distribution and conductivity and climate ($T_{ann.mean}$) (Fig. 6A, B). Species occurring at low temperature and conductivity regimes were *Alonopsis elongata* (n=11 lakes), *Alona intermedia* (n=10 lakes) and *Bosmina longispina* (n=14 lakes), whereas *Oxyrella tenuicaudis* (n=10 lakes) and *Pleuroxus aduncus* (n=16 lakes) primarily occurred at both high conductivity and in productive lakes (high Chl *a* concentration) (Fig. 6A, C). Taxa primarily found in warm water lakes were *Dunhevedia crassa*, *Ctenodaphnia*, *Pleu-*

roxus aduncus, Simocephalus spp. and *Oxyrella tenuicaudis* (Fig. 6B). These taxa were additionally mainly found in lakes with high planktivorous biomass and PVIsub (Fig. 6D, E). Additionally, eight of the 21 taxa occurring in less than five lakes were found solely in the southern lakes (EN, ES, G), and at least three of these are known to be related to macrophytes (Flössner 2000, Alonso 1996). Three of the four species found only in North-Swedish or Finnish lakes were pelagic.

Ephippia to carapace ratio

The most abundant ephippia were those of *Bosmina*, appearing in 46 of the 49 lakes inhabited by this taxa. The *Bosmina* ephippia to carapace ratio ranged from 0-33%. Chydoridae ephippia were present in 50 lakes and the chydorid ephippia to carapace ratio ranged from 0-15%. The proportion of resting eggs compared to body shields was highest in the two northernmost lakes for both *B. longirostris* (33 and 40%) and Chydoridae (10 and 15%) and was generally lowest in the most southern lakes (EN, ES, G). Thus, among the most northern lakes (SN, SF) more than half of the lakes had a *Bosmina* ephippia ratio larger than 6%, and ¾ of the lakes had a chydorid ephippia ratio larger than 1.3%. Correspondingly, 66% and 70% of the EN, ES and G lakes had an ephippia ratio<0.5% for *Bosmina* and chydorids, respectively. Both ephippia ratios were closely linearly negatively related to climate variables: T_{summer} (F=15.14, P=0.0003; F=24.13, P<0.0001), $T_{ann.mean}$ (F=20.82, P<0.0001; F=32.51, P<0.0001) and Chl *a* (F=22.67, P<0.0001; F=11.59, P=0.0013). When excluding the two northernmost lakes with maximum ephippia (S_N), the linear relations were still significant except for the chydorid ephippia to carapace ratio and Chl *a*. Fish biomass data were available for 35 lakes. Multivariate linear regression including some key factors controlling ephippia production: Chl *a* (feeding), $T_{ann.mean}$, T_{summer}, latitude (climate) and planktivorous and piscivorous fish biomass (predation) identified $T_{ann.mean}$ as a significant variable for both the *Bosmina* and the chydorid ephippia to carapace ratio (t value=-3.88, p=0.0006, t value=-5.59, p<0.0001, respectively) and Chl *a* as being marginally significant for the *Bosmina* ephippia to carapace ratio (t value=-2.17, p=0.0393) (T_{summer} was excluded due to high VIF).

Characteristics of the different MRT groups of lakes

The MRT-identified groups of lakes (DAT 1, DAT 2) differed with respect to several of the investigated variables (Fig. 7). All groups were significantly different with respect to conductivity. The low-conductive lakes were additionally characterised as cold with low nutrient conditions as well as low Chl *a* and submerged macrophyte abundance. Fish biomass was low and piscivorous species prevailed and, correspondingly, the cladoceran community was dominated by large-sized pelagic taxa. Moreover, ephippial production was high (Fig. 7K, L). In contrast, the high-conductive lakes were warm-water lakes with high abundance of primary producers and low Secchi depth and a tendency to high planktivorous fish biomass and with a submerged macrophyte coverage ranging from 34-100% (mean 72%). Unfortunately, PVIsub was only measured for one of these lakes (6%), making tests including PVIsub on this subdata set inappropriate. The cladoceran community in this group was dominated by small and medium-sized macrophyte associated and macrophyte-sediment associated taxa (Fig. 7N-R). The three remaining groups of lakes (REST) differed significantly in conductivity (Fig. 7A), but not in temperature ($T_{ann.mean}$) and TP (Fig. 7B, D). However, group 5 tended to have higher Chl *a* and lower Secchi depth as well as lower PVIsub (Fig. 7E-G). This group of lakes clearly deviated from group 3 and 4 by major dominance of pelagic cladoceran taxa as well as low species diversity. Also *Bosmina* ephippial production was generally low (Fig. 7K). The cladoceran community of group 3 and 4 resembled each other with respect to habitat group. Indeed, the only significant variable separating these groups was conductivity, although tendencies to a lower Chl *a* and a higher SecDep and PVIsub in group 4 were observed (Fig. 7E-G).

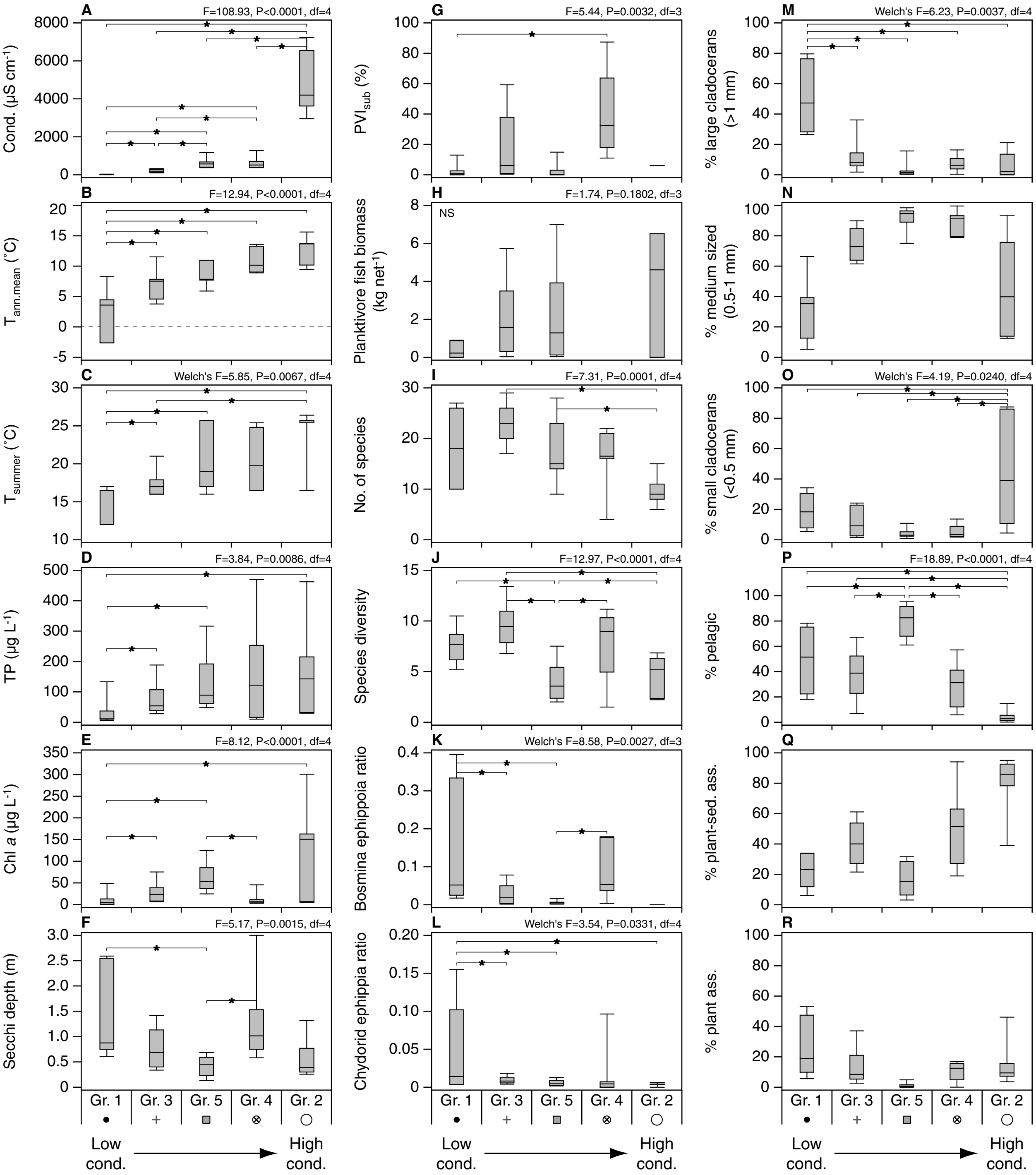


Figure 7. The distribution (median, 25 and 75 percentiles (boxes), 10 and 90 percentiles (whiskers)) of selected variables divided into lake groups defined by MRT, group numbers and symbols refer to those in Fig. 5. A) Conductivity (µS cm^{-1}), B) annual mean temperature (1961-1990) (ºC), C) mean monthly air temperature of the warmest month (ºC), D) total phosphorus (µg L^{-1}), E) chlorophyll *a* (µg L^{-1}), F) Secchi depth (m), G) volume of submerged macrophytes (PVI) (%), H) biomass of planktivorous fish (kg net^{-1} night^{-1}), I) taxa richness (no.), J) Hill's N2 species diversity, K) the ratio of *Bosmina longirostris* ephippia to *Bosmina longirostris* ephippia + body shields, L) the ratio of chydorid ephippia to chydorid ephippia + body shields, M) The relative distribution of large-sized cladocerans (> 1 cm) (%), N) medium-sized cladocerans (0.5-1 cm) (%) and O) small cladocerans (< 0.5 cm) (%), P) the relative distribution of pelagic cladocerans (%), Q) plant-and sediment associated cladocerans (%), and R) plant-associated cladocerans (%). F denotes ANOVA test, where variance heterogeneity occurred, Welch's F-test was applied. * denotes significant difference (α=0.05) between groups (Tukey's multiple comparisons). NS= no significant differences between groups. Arcsin-transformation was applied to percentage data before statistical tests.

Discussion

The present study demonstrated clear differences in the cladoceran community structure, taxa richness and ephippia to body shield ratio along the European latitude gradient. However, close correlation between latitude, implicitly temperature, was found to conductivity and nutrients, precluding a clear differentiation of a direct climate signal from the indirect effects of climate and human-related impact. This was demonstrated by both the multivariate ordination analyses, showing temperature and conductivity to explain almost equally significant amount of variation in the entire cladoceran species data as well as the MTR analysis, indicating temperature and nutrients and pH to be close surrogate variables for conductivity.

Distinct differences in cladoceran community structure were identified by the MRT analysis, dividing the 54 study lakes into three groups. The first group consists of seven low-conductivity lakes (pH 5-7) and was characterized by species typical for acidic lakes (Røen, 1995; Flössner, 2000). Likewise, de Eyto et al. (2003) found pH and latitude, to be the most important variables for the contemporary littoral chydorid assemblage in 59 European lakes of which 44 lakes are included in the present study. Moreover, they found a significantly negative correlation between pH and the abundance of five species, three of which (*Alonopsis elongata, Alonella excisa* and *Alona rustica*) were indicator species of the acidic, low conductive lakes in our study. The low-conductivity lakes were characterised by low TP and Chl *a* concentrations, high light penetration, low PVI of submerged macrophytes and relatively low fish abundance. High transparency likely results in high benthic production of algae and mosses (Liboriussen & Jeppesen, 2003; Vadeboncoeur et al., 2003), which explains the relatively large abundance of macrophyte and macrophyte/sediment-associated cladocerans despite low PVI in these lakes.

The second group consisted of five high-conductivity lakes located in the southernmost Spain (except for UK-3) and was characterised, in particular, by the total absence of *Bosmina* and the presence of small eutrophic and macrophyte-sediment associated taxa, including *Dunhevedia crassa*, *Oxyrella tenuicaudis* and *Pleuroxus aduncus* (Fig. 4 & 6). High conductivity is indeed an important structuring variable for inland Mediterranean lakes and has been proposed to act as one of the WFD lake classification variables by Boix et al. (2005). Their threshold of 5000 µS cm^{-1} was exceeded in two of the five lakes in the high conductivity group. However, adverse effects on hatching of zooplankton (Brock, Nielsen & Crossle, 2005) and on the abundance and reproduction of both pelagic and benthic cladocerans (Sarma et al., 2006) are found below this threshold. The high-conductivity lakes were meso-hyper-trophic and, unlike the northern temperate shallow lakes of similar trophic states, they were characterised by high macrophyte cover (34-100%, although only 6% in UK-3). Dominance of small species even in the macrophyte rich lakes is in accordance with previous findings that aquatic macrophytes do usually not provide adequate refuge to zooplankton in Mediterranean (Castro, Marques & Goncalves, 2007) and in subtropic shallow lakes (Meerhoff, 2007) because of high fish density even within macrophyte beds (Castro, Marques & Goncalves, 2007); Ortega-Mayagoitia et al., 2000; Blanco et al., 2003; Romo et al., 2004). By contrast, two of the high conductivity ES lakes were fishless and had the highest observed relative abundance of large-sized Ctenodaphnia (2 and 10%). Species belonging to the Ctenodaphnia group (*D. magna*, *D. mediterranea*) are recognised as salt- and nutrient tolerant (Boronat, Miracle & Armengol, 2001; Goncalves et al., 2007), which fits well with the lake characteristics of the high-conductivity lakes.

Even when shortening the conductivity gradient by excluding the low and high conductivity lakes (MRT group 1 and 2), conductivity still appeared as a prominent factor structuring the zooplankton community, it being, however, closely correlated to T_{summer}, TP Chl *a* and SecDep in the MRT analysis. The indicator species of the group of relatively low conductivity, TP and temperature (Group 3, Fig. 5B2) was the small sized *Alonella nana*. This species is associated with medium TP levels (25-40 µg l^{-1}) and often with macrophyte habitats (Flössner, 2000; Brodersen et al., 1998). The remaining 19 warmer and more productive lakes were separated with respect to Chl *a* and turbidity. Thus, the low Chl *a,* warmer lakes (group 4, median Chl *a*=7 µg l^{-1}) were characterised by planktonic as well as plant associated taxa and tended to have a larger percentage of large taxa than group 5. The warmer, low Chl *a* lakes consisted of ES, EN and UK lakes, whereas the lakes with higher Chl *a* (group 5, median Chl *a*=53 µg l^{-1}) were characterised by total dominance of the small pelagic *B. longirostris* (Fig. 5B2), which is known to be abundant in nutrient rich temperate lakes with high planktivorous fish predation pressure (Dahl-Hansen, 1995; Jeppesen et al., 1996). In accordance with this, the relatively high TP levels (median 88 μg l^{-1}) of these lakes indicate sub-optimal growth conditions for submerged macrophytes and therefore less benthic habitat diversity (Scheffer et al., 1993); Søndergaard et al., 2005). The latter group (group 5) included lakes from DK, EST, PL, four D lakes and all G lakes. The high-productive high-conductive lakes (group 4) seemed to have higher TP but lower Chl *a*, higher Secchi depth, higher macrophyte cover, less pelagic but more macrophyte and sediment associated cladocerans than the low-productive, low-conductivity lakes (group 3).

The PVI of submerged macrophytes in our study lakes correlated positively with T_{summer} and $T_{ann.mean}$, thus potentially providing increased habitat availability for plant-associated taxa in warmer lakes. This pattern was also seen in the constrained ordination based on the subset of 44 of the study lakes. Climate variables have been found to explain a larger fraction of the variance in depth of maximum macrophyte biomass than water transparency along a latitudinal gradient (mean at 42°, 164 lakes) including 45 low to mesotrophic lakes (Secchi depth median around 3-4 m) (Durate & Kalff, 1987). Additionally, Rooney & Kalff (2000) found a positive relationship between temperature and macrophyte biomass in five relatively deep (3-10 m), low productive lakes (3-26 μg hl *a* l^{-1}) (45°N'18) due to an earlier onset of the growing season. Accordingly, cladoceran communities in the warmer lakes may potentially show higher taxa richness as an indirect climate response through increased macrophyte cover. However, taxa richness tended to be unimodally related to latitude, with low richness in the most southern high-conductivity lakes than in all other MRT-groups, except for the most northern lakes. Lakes with less than 10 taxa in our study were all G or ES lakes (n=6 lakes), and the measured macrophyte cover ranged from 34-100% (no data for G-lakes). The unimodal response we observed corresponds well with the findings of (de Eyto et al., 2003) in their study of contemporary chydorid distribution in 56 European lakes. Moreover, a study investigating the biodiversity of several organisms at different levels in the food chain in 30 Danish, 30 Dutch and 30 Spanish lakes revealed that the associations between submerged macrophyte cover and taxa richness varied among geographical regions – being positively related to macrophyte cover in Danish and Dutch lakes, but not in southern Spanish lakes (Declerck et al., 2005). Overall, strong evidence of a latitudinal gradient exists, showing increasing species richness in freshwater systems towards the equator (Mittelbach et al., 2007). This was also the general finding when applying a meta-analysis of species richness and latitudinal gradient including almost 600 studies, although the gradients of freshwater studies were weaker than for marine and terrestrial studies (Hillebrand, 2004). Our data show that the Mediterranean study lakes overall have low taxa richness, likely due to high conductivity and fish predation, indicating that taxa richness in European lowland lakes peaks at intermediate latitudes.

The proportion of *Bosmina* resting eggs compared to body shields in the two northernmost lakes (0.33 and 0.4) was similar to the mean ratio (0.34) of arctic and sub-arctic lakes from Greenland (Jeppesen et al., 2003). Likewise, the most southern lakes generally showed a low ratio, in particular for *Bosmina*. Multivariate regressions revealed that T_{summer} was the most important variable determining variations in the egg:carapace ratio. However, for *Bosmina*, Chl *a* also seemed important. Thus, the most northern lakes (S_N, SF, EST) generally also had the lowest Chl *a* and the lowest mean T_{summer} and $T_{ann.mean}$. Accordingly, both climate (length of growing season) and low food availability could be responsible factors for the high proportion of resting eggs.

In summary, the species composition of cladoceran subfossils in the surface sediments of 54 shallow lakes showed significant changes along the European latitude, ranging from northern Sweden to southern Spain. In addition, a clear relationship between taxa richness to latitude was identified, being low in the northern-most lakes as well as in the southern-most productive and vegetation-rich lakes. Moreover, the ephippia production was found to be higher in northern lakes, where the season is shorter, and was related to both climate variables and nutrient state. Yet, the correlative nature of the data highlighted the difficulties of disentangling a strict climate signal from indirect effects of climate and human-related impact when the European latitude gradient is used as a climate proxy.

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References

Aladin, N. V. 1991, Salinity tolerance and morphology of osmoregulation organs in Cladocera with special reference to Cladocera from the Aral Sea. Hydrobiologia 225: 291-299.

Alonso, M. 1996, Fauna Iberica, Crustacea, Branchiopoda, vol. 7. Museo National de Ciencias Naturales Consejo Superior de Investigaciones Científicas, Madrid

Amsinck, S.L., Jeppesen, E., Verschuren, D., 2007. Cladoceran resting eggs and anthropogenic changes. In Diapause in aquatic invertebrates: role for ecology, physiology and human uses. Eds. Alekseev, V., De Stasio, B., - Cluwer Publisher. 257p.

Amsinck, S.L., Jeppesen, E., Landkildehus, F., 2005. Relationships between environmental variables and zooplankton subfossils in the surface sediments of 36 shallow coastal brackish lakes with special emphasis on the role of fish. J. Paleolimnol. 33: 39-51.

Amsinck, S.L., Jeppesen, E., Landkildehus, F. , 2003. Cladoceran stratigraphy in two shallow brackish lakes with special reference to changes in salinity, macrophyte abundance and fish predation. Journal of Paleolimnology 29: 495-507.

Battarbee, R. W. 2000, Paleolimnological approaches to climate change, with special regard to the biological record. Quarternary Science Reviews 19: 107-124

Beklioglu, M., Romo, S., Kagalou, I., Quintana, X., Becares, E. 2007, State of the art in the functioning of shallow Mediterranean lakes: workshop conclusions. Hydrobiologia 584: 317-326

Bennike, O., Sarmaja-Korjonen, K., Seppänen, A., 2004. Reinvestigation of the classic late-glacial Bølling Sø sequence, Denmark: chronology, macrofossils, Cladocera and chydorid ephippia. Journal of Quaternary Science 19(5): 465-478.

Blanco, S., Romo, S., Villena, M. & Martínez, S. 2003, Fish communities and food web interactions in some Mediterranean lakes. Hydrobiologia 506-509: 473-480

Boix, D., S. Gascon, et al. 2005, A new index of water quality assessment in Mediterranean wetlands based on crustacean and insect assemblages: the case of Catalunya (NE Iberian peninsula). Aquatic Conservation Marine and Freshwater Ecosystems 15(6): 635-651.

Boronat, L., M. R. Miracle, et al. 2001, Cladoceran assemblages in a mineralization gradient. Hydrobiologia 442(1-3): 75-88

Bos, D. G., Cumming, B. F. & Smol, J. P. 1999, Cladoceran and Anostraca from the Interior Plateau of British Columbia, Canada, as paleolimnological indicators of salinity and lake level. Hydrobiologia 392: 129-141

Brancelj, A., Kernan, M., Jeppesen, E., Manca, M., Rautio, M., Stuchlik, E. 2007, Pan-European Cladocera remains from remote mountain lakes Archiv für Hydrobiologie Supplementum

Breiman, L., Friedman, J. H., Olshen, R. A. & Stone, C. G. 1984, Classification and regression trees. Wadsworth International Group, Belmont, California, USA

Brendonck, L. & De Meester, L., 2003, Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. Hydrobiologia 491: 65-84

Brock, M.A., Nielsen, D.L., Crossle, K. 2005, Changes in biotic communities developing from freshwater wetland sediments under experimental salinity and water regimes. Freshwater Biology 50: 1376-90

Brodersen, K. P., Whiteside, M. C., Lindegaard, C. 1998, Reconstruction of trophic state in Danish lakes using subfossil chydorid (Cladocera) assemblages. Canadian Journal of Fishery and Aquatic Science 55: 1093-1103

Canfield, D. E., Shireman, J. V., Colle, D. E., Haller, W. T., Watkins, C. E., Maceina, M.J. 1984, Prediction of chlorophyll a concentrations in Florida Lakes - Importance of aquatic macrophytes. Canadian Journal of Fisheries and Aquatic Sciences 41: 497-501.

Castro, B. B., S. M. Marques, et al. 2007, Habitat selection and diel distribution of the crustacean zooplankton from a shallow Mediterranean lake during the turbid and clear water phases. Freshwater Biology 52(3): 421-433.

Dahl-Hansen, G. A. P. 1995, Long-term changes in crustacean zooplankton – effects of a mass removal of Arctic charr, *Solvalinus alpinus* (L.), from an oligotrophic lake. Journal of Plankton Research 17: 1819-1933

de Eyto, E., Irvine, K., Garcia-Criado, F., Gyllstrom, M., Jeppesen, E., Kornijow, R., Miracle, M.R., Nykanen, M., Bareiss, C., Cerbin, S., Salujoe, J., Franken, R., Stephens, D., Moss, B. 2003, The distribution of chydorids (Branchiopoda, Anomopoda) in European shallow lakes and its application to ecological quality monitoring. Archiv für Hydrobiologie 156: 181-202

Deáth, G. 2002, Multivariate regression trees: A new technique for modeling species-environment relationships. Ecology 83 (4): 1105-1117

Deáth, G. & Fabricius, K. E. 2000, Classification and Regression Trees: A Powerful Yet Simple Technique for Ecological Data Analysis. Ecology 81 (11): 3178-3192

Declerck, S., Vandekerkhove, J., Johansson, L., Muylaert, K., Conde-Porcuna, J.M., Van der Gucht, K., Perez-Martinez, C., Lauridsen, T., Schwenk, K., Zwart, G., Rommens, W., Lopez-Ramos, J., Jeppesen, E., Vyverman, W., Brendonck, L. & De Meester, L. 2005, Multi-group biodiversity in shallow lakes along gradients of phosphorus and water plant cover. Ecology 86(7), 1905-15

Dufrene, M. & Legendre, P. 1997, Species Assemblages and Indicator Species: The Need for a Flexible Asymmetrical Approach. Ecological Monographs 67 (3): 345-366

Duigan, C. A. & Birks, H. H. 2000, The late-glacial and early-Holocene palaeoecology of cladoceran microfossil assemblage at Kråkenes, western Norway, with a quantitative reconstruction of temperature changes. Journal of Paleolimnology 23: 67-76

Dumont, H. J. 1994, On the diversity of the Cladocera in the Tropics. Hydrobiologia 272: 27-38

Durate, C. M. & Kalff, J. 1987, Latitudinal influences on depths of maximum colonization and maximum biomass of submerged angiosperms in lakes. Canadian Journal of Fisheries and Aquatic Science 44 (10): 1759-1764

Fernando, C. H. 1994, Zooplankton, fish and fisheries in tropical freshwaters. Hydrobiologia 272: 105-123

Flösner, D. 2000, Die Haplopoda und Cladocera Mitteleuropas. Backhuys Publishers, Leiden, The Netherlands

Frey, D. G. 1993, The penetration of cladocerans into saline waters. Hydrobiologia 267: 233-248

Frey, D. G. 1959, The taxonomic and phylogenetic significance of headpores of the Chydoridae Cladocera. Internationale Revue der Gesamten Hydrobiologie 44: 27-50

Gliwicz, Z.M. 2003, Between Hazards of Starvation and Risks of Predation: The Ecology of Offshore Animals. Excellence in Ecology, Vol. 12. International Ecology Institute, Oldendorf/Luhe. 379 pp

Goncalves, A. M. M., B. B. Castro, et al. 2007, Salinity effects on survival and life history of two freshwater cladocerans (*Daphnia magna* and *Daphnia longispina*). Annales De Limnologie-International Journal of Limnology 43(1): 13-20.

Goss, B. L. & Bunting, D. L. 1983, *Daphnia* development and reproduction: Responses to temperature. Journal of Thermal Biology 8: 375-380.

Gyllström, M., Hansson, L. A., Jeppesen, E., Garcia-Criado, F., Gross, E., Irvine, K., Kairesalo, T., Kornijow, R., Miracle, M.R., Nykänen, M., Noges, T., Romo, S., Stephen, D., Van Donk, E., Moss, B. 2005, The role of climate in shaping zooplankton communities of shallow lakes. Limnology and Oceanography 50(6): 2008-21

Hill, M. O. 1973, Diversity and evenness: a unifying notion and its consequences. Ecology, 54: 427-432.

Hillebrand, H. 2004, On the generality of the latitudinal diversity gradient. American Naturalist 163(2): 192-211.

IPCC. 2001, *Climate Change 2001: The Scientific Basis. Contribution of Working Group 1 to the Third Assessment Report of the Intergovernmental Panel on Climate Change* Cambrigde University Press, Cambridge, United Kingdom and New York, NY, USA

IPCC. 2007, http://ipccwg1.ucar.edu/wg1/docs/WG1AR4_SPM_PlenaryApproved.pdf. .

Jeppesen, E., Søndergaard, M., Pedersen, A. R., Jurgens, K., Strzelczak, A., Lauridsen, T. L., Johansson, L. S. 2007, Salinity induced regime shift in shallow brackish lagoons. Ecosystems 10(1): 47-57

Jeppesen, E., Søndergaard, M., Mazzeo, N., Meerhoff, M., Branco, C., Huszar, V., Scasso, F., 2005a, Lake restoration and biomanipulation in temperate lakes: relevance for subtropical and tropical lakes. Chapter 11 in: (Ed. M.V. Reddy), Tropical eutrophic lakes: their restoration and management: 331-359

Jeppesen, E., Meerhoff, M., Jakobsen B. A, Hansen, R. S., Søndergaard, M., Jensen, J. P., Lauridsen, T. L., Mazzeo, N., Branco, C., 2005b, Restoration of shallow lakes by nutrient control and biomanipulation – the successful strategy depends on lake size and climate. Hydrobiologia. In press

Jeppesen, E., Jensen, J. P., Lauridsen, T., Amsinck, S. L., Christoffersen, K., Søndergaard, M., Mitchell, S. F. 2003, Sub-fossils of the cladocerans in the surface sediment of 135 lakes as proxies for community structure of zooplankton, fish abundance and lake temperature. Hydrobiologia 491: 321-330

Jeppesen, E., Jensen, J. P., Amsinck, S. L., Landkildehus, F., Lauridsen, T., Mitchell, S. F. 2002, Reconstructing the historical changes in *Daphnia* mean size and planktivorous fish abundance in lakes from the size of *Daphnia* ephippia in the sediment. Journal of Paleolimnology 27: 133.143.

Jeppesen, E., Madsen, E. A. & Jensen, J. P. 1996, Reconstructing past density of planktivorous fish and trophic structure from sedimentary zooplankton fossils: a surface sediment calibration data set from shallow lakes. Freshwater Biology 36: 115-127

Jeppesen, E., Søndergaard, M., Kanstrup, E., Petersen, B., Eriksen, R. B., Hammershøj, M., Mortensen, E., Jensen, J. P., Have, A. 1994, Does the Impact of Nutrients on the Biological Structure and Function of Brackish and Fresh-Water Lakes Differ. Hydrobiologia 276: 15-30

Liboriussen, L. & Jeppesen, E. 2003, Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and a turbid shallow lake. Freshwater Biology 48(3): 418-431

Lotter, A.F., Birks, H.J.B., Hofmann, W., Marchetto, A., 1997, Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. 1. Climate. Journal of Paleolimnology 18: 395-420

Korhola, A. 1999, Distribution patterns of Cladocera in subarctic Fennoscandian lakes and their potential in environmental reconstruction. Ecography 22: 357-373

Meerhoff, M., Iglesias, C., Teixeira De Mello, F., Clemente, J.M., Jensen, E., Lauridsen, T.L. & Jeppesen, E. 2007, Effects of habitat complexity on community structure and predator avoidance behaviour of littoral zooplankton in temperate versus subtropical shallow lakes. Freshwater Biology 52: 1009-1021.

Mittelbach, G. G., Schemske, D. W., Cornell, H. V., Allen, A. P., Brown, J. M., Bush, M. B., Harrison, S. P., Hurlbert, A. H., Knowlton, N., Lessios, H. A., McCain, C. M., McCune, A. R., McDade, L. A., McPeek, M. A., Near, T. J., Price, T. D., Ricklefs, R. E., Roy, K., Sax, D. F., Schluter, D., Sobel, J. M. & Turelli, M. 2007, Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecology Letters 10(4): 315-331

Moore M. V., Folt, C. F., Stemberger, R. S. 1996, Consequences of elevated temperatures for zooplankton assemblages in temperate lakes. Archiv für Hydrobiologie 135: 289-319

Moss, B., Stephen, D., Alvarez, C., Becares, E., Van de Bund, W., Collings. S. E., Van Donk, E., De Eyto, E., Feldmann, T., Fernandez-Alaez, C., Fernandez-Alaez, M., Franken, R. J. M, Garcia-Criado, F., Gross, E. M., Gyllstrom, M., Hansson, L. A., Irvine, K., Jarvalt, A., Jensen, J. P., Jeppesen, E., Kairesalo, T., Kornijow, R., Krause, T., Kunnap, H., Laas, A., Lille, E., Lorens, B., Luup, H., Miracle, M. R., Noges, P., Noges, T., Nykanen, M., Ott, I., Peczula, W., Peeters, E. T. H. M., Phillips, G., Romo, S., Russell, V., Salujoe, J., Scheffer, M., Siewertsen, K., Smal, H., Tesch, C., Timm, H., Tuvikene, L., Tonno, I., Virro, T., Vicente, E. & Wilson, D. 2003, The determination of ecological status in shallow lakes - a tested system (ECOFRAME) for implementation of the European Water Framework Directive. Aquatic Conservation: Marine and Freshwater Ecosystems 13 (6): 507-549

Murdoch, P.S., Baron, J.S., Miller, T.L. 2000, Potential effects of climate change on surface-water quality in North America. Journal of the American Water Resources Association 36:347-366

New, M., Humble, M., Jones P. D. 2000, Global 30-year mean monthly climatology, 1961-1990 (Internet). Oak Ridge, Tennessee: Oak Ridge National Laboratory Distributed Archive Center. Data set available from http://www.daac.ornl.gov. Accessed May 2007

Noges, P., Noges, T., Tuvikene, L., Smal, H., Ligeza, S., Kornijow, R., Peczula, W., Becares, E., Garcia-Criado, F., Alvarez-Carrera, C., Fernandez-Alaez, C., Ferriol, C., Miracle, R. M., Vicente, E., Romo, S., Van Donk, E., van de Bund, W., Jensen, J. P., Gross, E. M., Hansson, L. A., Gyllstrom, M., Nykanen, M., de Eyto, E., Irvine, K., Stephen, D., Collins, S.& Moss B. 2003, Factors controlling hydrochemical and trophic state variables in 86 shallow lakes in Europe. Hydrobiologia 506 (1-3): 51-58

Ortega-Mayagoitia, E., Armengol, X., Rojo, C. 2000, Structure and dynamics of zooplankton in a semi-arid wetland, the national park Las Tablas De Daimiel (Spain). Wetlands 20: 629-638

Romo, S., Miracle, M. R., Vellena, M., Rueda, J., Ferriol, C., Vicente, E. 2004, Mesocosm experiments on nutrient and fish effects on shallow lake food webs in a Mediterranean climate. Freshwater Biology 49: 1593-1607

Rooney, N. & Kalff, J. 2000, Inter-annual variation in submerged macrophyte community biomass and distribution: the influence of temperature and lake morphometry. Aquatic Botany 68: 321-335

Røen, U. I. 1995, Gællefødder og karpelus. Danmarks Fauna 85. Dansk Naturhistorisk Forening, Vinderup Bogtrykkeri A7S, Vinderup, Denmark

Sarma, S. S. S., Nandini, S., Morales-Ventura, J., Delgado-Martinez, I., Gonzalez-Valverde, L. 2006, Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans). Aquatic Ecology 40(3): 349-360

Sarmaja-Korjonen, K. 2003, Chydorid ephippia as indicators of environmental change - biostratigraphical evidence from two lakes in southern Finland. Holocene 13(5): 691-700

Sarmaja-Korjonen, K. 2004, Chydorid ephippia as indicators of past environmental changes - a new method. Hydrobiologia 526: 129-136

Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B. & Jeppesen, E. 1993, Alternative Equilibria in Shallow Lakes. Trends in Ecology & Evolution 8(8): 275-279

Schindler, D. W., 1997, Widespread effects of climatic warming on freshwater ecosystems in North America. Hydrological Processess 11: 1043-1067

Sokal, R.R & Rohlf, F.F. 1999, Biometry. The principles and practice of statistics in biological research. 3rd edition. W.H. Freeman and company, New York. 887 pp

Søndergaard, M., Jeppesen, E., Jensen, J.P. & Amsinck, S.L. (2005) Water framework directive: Ecological classification of danish lakes. *Journal of Applied Ecology*, **42**(4), 616-29

ter Braak, C. J. F & Smilauer, P. 2002, CANOCO Reference manual and CanoDraw for Windows. User's guide. Software for Canonical Community Ordination (version 4.5). Microcomputer Power (Ithaca, New York, USA), 500 pp

ter Braak, C. J. F. 1995, Ordination. In Data analysis in community and landscape ecology. Edited by R. H. G. Jongman, C. J. F. ter Braak and O. F. R. van Tongeren. Cambridge University Press, Cambridge, England, pp. 91-173

Vadeboncoeur, Y., Jeppesen, E., Vander Zanden, M. J., Schierup, H. H., Christoffersen, K., Lodge, D. M. 2003, From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. Limnology and Oceanography 48(4): 1408-1418

Vandekerkhove, J., Declerck, S., Jeppesen, E., Conde-Porcuna, J.M., Brendonck, L., De Meester, L., 2005a, Dormant progagule banks integrate spatio-temporal heterogeneity in cladoceran communities. Oecologia 142: 109-116.

Vandekerkhove, J., Declerck, S., Brendonck, L., Conde-Porcuna, J. M., Jeppesen, E., Sander Johansson, L., De Meester, L., 2005b, Uncovering hidden species: hatching diapausing eggs for the analysis of cladoceran species richness, Limnology and Oceanography: Methods 3: 399-407

Vandekerkhove, J., Declerck, S., Vanhove, M., Brendonck, L., Jeppesen, E., Conde-Porcuna, J.M., De Meester, L., 2004, Use of ephippial morphology to assess richness of anomopods: potentials and pitfalls. Journal of Limnology 63: 75-84

Williams W. D. 1981, The limnology of saline waters in western Victoria. A review of some recent studies. Hydrobiologia 82: 223-259

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Description of the subfossil head shield of *Alona protzi* Hartwig 1900 (Anomopoda, Chydoridae) and the environmental characteristics of its finding sites

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Abstract

This paper gives a description of the head shield of *Alona protzi*, a rare species of Cladocera (water fleas) whose separated head shield has not yet been described in detail. Subfossil head shields of *A. protzi* were found in sediment cores taken from lakes in Denmark, Sweden, Finland, Estonia and Poland. Despite the rarity of the species this suggests a wide distribution of *A. protzi* in northern Europe. The ecology of *A. protzi* is poorly known. The environmental spectrum of the finding sites was wide and ranged from relatively nutrient poor clear water lakes to eutrophic turbid water lakes, indicating that *A. protzi* is not narrowly restricted. Most of the lakes were, however, meso-eutrophic with neutral to high pH, and with a relatively low abundance of submerged macrophytes. However, we cannot exclude the possibility that *A. protzi* mainly lives in groundwater and is only occasionally transported into lakes.

Introduction

Chydoridae, a diverse family of Cladocera (water fleas), appear commonly in freshwater habitats. Most of the European chydorid fauna was already described in the early 20th century. In identification literature, the intact animals are depicted from the side and the shape of the head shield is thus not clearly shown. The head shield and carapace of living animals are seamlessly attached, implying that the shape of the posterior margin of the head shield is invisible. When the animal dies or molts, the head shield is detached from the carapace by a special ecdysial suture (molting seam).

The chitinous remains of chydorids (e.g. head shields, carapaces and postabdomens) are usually well-preserved in lake sediments and can be used to reconstruct past limnological conditions (Frey 1986, Korhola, Rautio 2001). This particular field of paleolimnology developed in the latter half of the 20th century when David Frey (1958, 1959) described "flat", detached head shields. Their characteristic pore configurations and shapes of the posterior margin enabled their identification in lake sediment studies. Separate description of subfossil remains is necessary, because some of the characteristics of living animals, for instance the outer membranes forming part of the surface sculpturing, are not always preserved.

Since Frey's pioneer work (1958, 1959), the subfossil remains of most European chydorids have been described. However, some of the rarest spe-

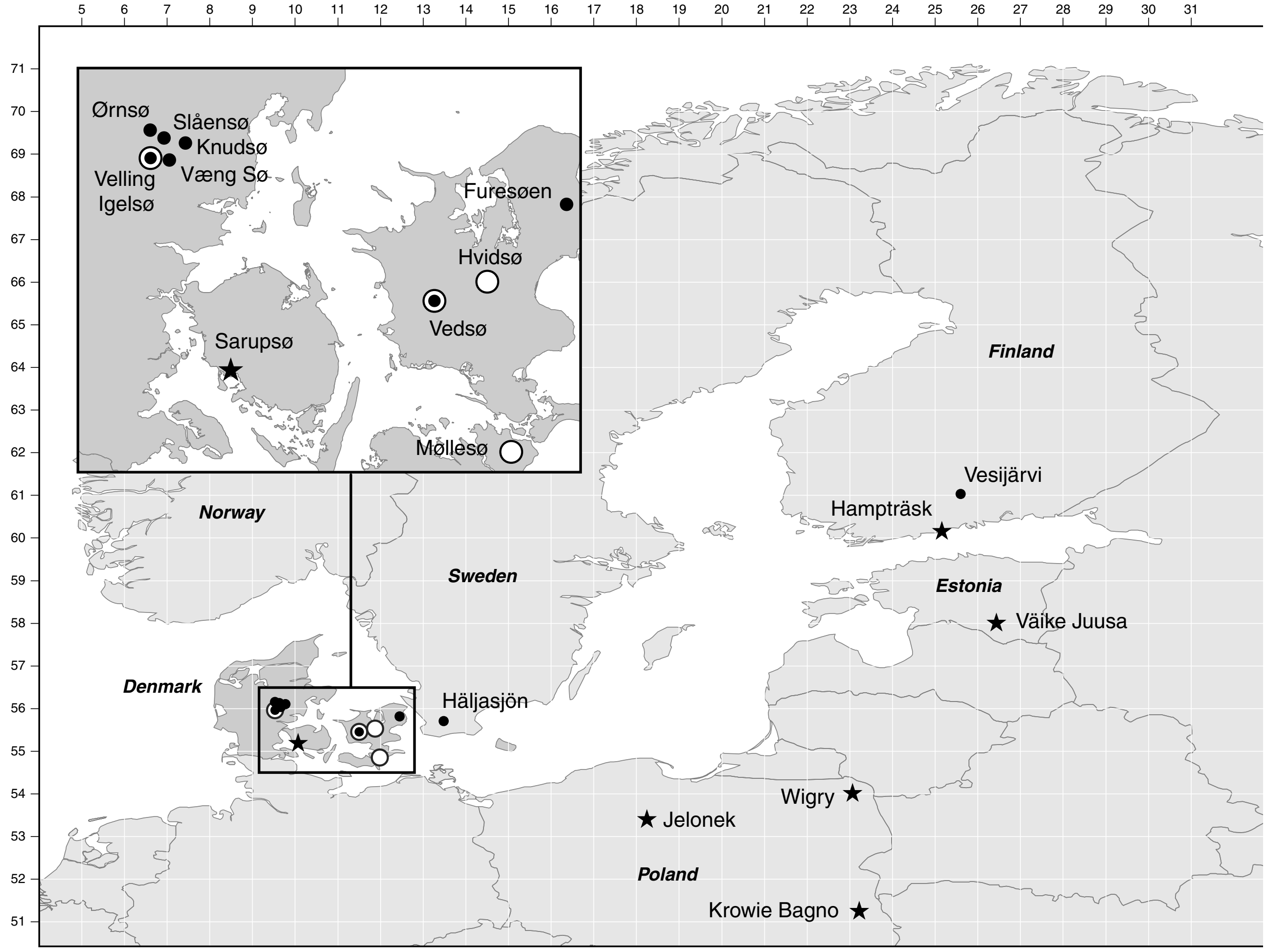

Fig. 1. The 17 finding sites of *A. protzi* subfossil head shields in Northern Europe. ● Findings in recent sediment (1986-2002 BC), ○ findings in sediment dated AD 1850-1950, * findings in old sediments (6600 BC – AD 1300).

cies, including *Alona karelica* Stenroos 1897 and *Alona protzi* Hartwig 1900, still puzzle palaeolimnologists. The carapace of *A. protzi* can be identified from its characteristic denticles on the posterior-ventral corner of the shell (e.g. Smirnov 1974, Dumont 1983, Røen 1995, Flössner 2000), but the shape of its head shield has not yet been described in detail. Furthermore, the ecological demands of this rare species are poorly known.

In recent years, the present authors found unknown chydorid head shields in lake sediments from Denmark, Sweden, Finland, Estonia and Poland. Not until specimens with head shield and carapace still attached were found, the previously undetermined head shields could be identified as belonging to *A. protzi*. Flössner (2000) presented a somewhat sketchy drawing of the head shield of *A. protzi*, lacking several features characteristic to the subfossil specimens. In the present paper, we give a detailed description of the subfossil head shield and an overview of the environmental characteristics of the lakes in which they were found. We aimed to examine whether *A. protzi* has specific environmental demands that may have indicator value in paleolimnological research, assuming that no evolutionary adaptation of demands have occurred.

Sites and laboratory methods

Subfossil head shields of *A. protzi* were discovered in sediments from 17 lakes located in Denmark, Finland, Sweden, Estonia and Poland (Fig. 1). The findings were divided into three groups according to sediment type: surface sediment (AD 1986-2002) with contemporary water chemistry data, sediment accumulated in recent time (AD 1850-1950) and older sediments (6600 BC – AD 1300).

All samples were heated in 10% KOH and washed on a sieve (Korhola, Rautio 2001). Two different methods were applied. In the first method, 42-50-µm mesh size was used and the samples were counted on slides under light microscope (samples from Finland, Estonia and Poland) (Korhola, Rautio 2001). In the other method, fragments > 80 µm were counted in water under magnifying glass and inverted light microscope (samples from Denmark and Sweden). The number of cladoceran remains counted varied between samples and analysts: 700-2800 (Danish lakes), 200-250 (Lake Väike Juusa, Estonia), 450 (Hampträsk, Finland), and 300-1000 (Polish lakes). One head shield was found in Krowie Bagno (Poland) during a screening of more than 20 slides containing hundreds of cladoceran remains. In Lake Vesijärvi (Finland) minimum 400 individuals (converted from remains) were counted per sample.

Results and discussion

Subfossil remains of A. protzi

Findings of subfossil remains

We found 84 head shields distributed in 53 sediment samples from 17 lakes (the first finding was made in October 2002) (Table 1). All head shields had a peculiar shape with a notched posterior margin and a short, broadly rounded rostrum (Fig. 2).

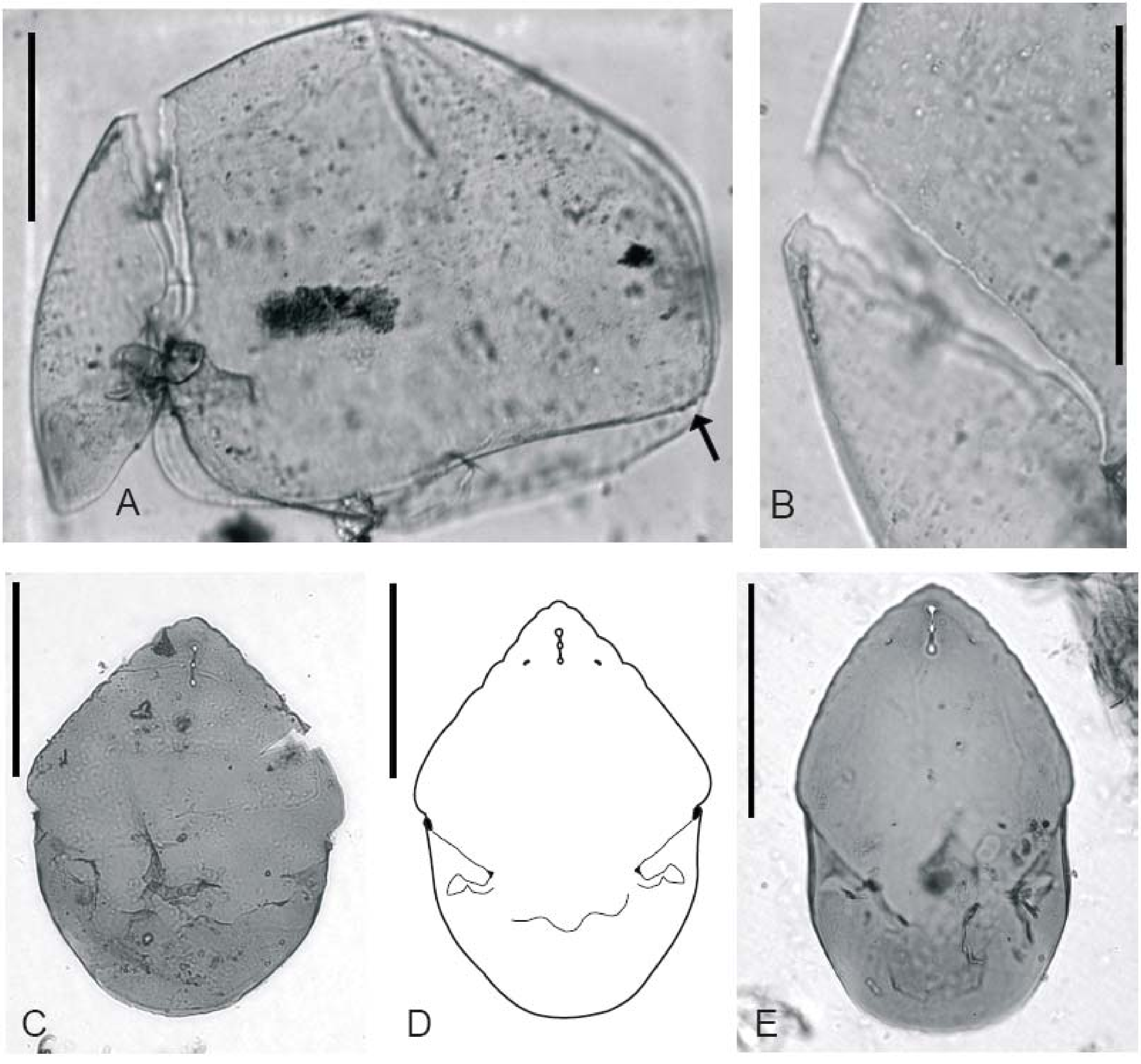

Fig. 2. The subfossil head shield and carapace of *Alona protzi* from Lake Sarup, Denmark. An arrow indicates the denticles on the posterior-ventral corner of the carapace. B) A detail of the opened molting seam between the head shield and carapace of *A. protzi*, showing the head pores and the notched posterior margin of the head shield and the corresponding notched margin of the carapace. C) *A. protzi* head shield, Lake Jelonek, Poland. D) Drawing of *A. protzi* head shield, original from Lake Väike Juusa, Estonia. E) *A. protzi* head shield, Lake Krowie Bagno, Poland, the curvature of the head shield makes it look exceptionally narrow. Scale bar = 100 µm.

Table 1 Characteristics of the finding sites and the abundance data on *A. protzi*. For Lake Vesijärvi contemporary data were available for each of the 7 samples. The mean value was used in order not to skew the results (ranges shown in brackets). For the remaining lakes, contemporary data was available only for one sample (surface sediment). The percentage of *A. protzi* head shields from all chydorid head shields (abundance %), and the number *A. protzi* head shields per sample encountered during counting, are given as a mean value per lake (with ranges in brackets, if found in more than three samples). DK=Denmark, EST=Estonia, FIN=Finland, PL=Poland, SE=Sweden; S = surface sediment (AD 1986-2002), R= recent sediment (AD 1850-1950), O= old sediment (6600 BC – AD 1300), W=water sample; H=head shield, C=carapace, I=intact animal.

Lake	Country	Sediment age	Fragment found	Area ha	Max depth m	Mean depth m	Secchi depth m	Total N µg L^{-1}	Total P µg L^{-1}	Chl *a* µg L^{-1}	Alkalinity mmol L^{-1}	Conductivity µS cm^{-1}	pH	PVI %	Abundance %	Number of head shields per sample	Number of samples
Velling Igelsø	DK	S, R	H	8.8	14		2.5	605	15	9.7	0.22		7.5		1.6	2.3	3
Knudsø	DK	S	H	1.4	29	17	3.7	2246	27	11.8	1.94		8.5	2.4	0.1	1.0	1
Ørnsø	DK	S	H	0.4	10	4	1.2	1344	101	62.8	0.81		7.9	0	1.2	1.0	1
Ved Sø	DK	S, R	H	5	3.5	2.8	0.8		125						0.4	1.5	2
Vængsø	DK	S	H	16		1.2	0.4	1300	161	80.5	1.29	281	8.1	0	0.4	1.0	1
Furesøen	DK	S	H	7.3	37.7	16.5	2.5	1028	245	66.2	2.1		8.7	1.9	0.1	1.0	1
Slåensø	DK	S	H	0.2	11.5	7.3	3.8							4.3	0.9	1.0	1
Häljasjön	SE	S	H	19.6		5.6	2.5	1346	39	23.7	2.18	300	7.8	0	0.6	2.0	1
Vesijärvi, the Enonselkä basin	FIN	S	H, C	2600	33	6.8	2.1 (1.5-2.4)	548 (505-703)	31 (25-50)	11.9 (7.5-23.2)	0.55 (0.52-0.57)	123 (120-130)	7.8 (7.7-7.9)		0.7 (0.4-1.1)	1.1 (1-2)	7
Hvidsø	DK	R	H												0.7	2.0	1
Mølle Sø	DK	R	H												0.2	1.0	2
Sarup Sø	DK	O	H, C												1.8 (0.7-4.2)	1.8 (1-4)	21
Hampträsk	FIN	O	H													1.0	1
Väike Juusa	EST	O	H												1.6 0.9-2.6	1.4 1-2	5
Krowie Bagno	PL	O	H												# 0.2	5	3
Jelonek	PL	O	H												# 0.1	1	1
Wigry	PL	O	H												# 0.1	3	1
R. Valkjärvi *	FIN	S	C	8	9	4.5	2.3	340	15	5.8	0.05	25	6.2				
Valvatus *	FIN	S	C	303	7.5		1.1	830	42	31	0.66	150	7.4				
Lovonjärvi *	FIN	S, W	C, I	5	17.5	7.7	1	872	49	28	0.51	129	7.2				
Sylvöjärvi *	FIN	W	I	235	5.5	1.9	1.1	700	38	7	0.45	93	7				
Mean				**247**	**16.2**	**6.8**	**1.9**	**1014**	**74**	**30.8**	**0.98**	**157**	**7.6**	**1.4**	**0.7**	**1.3**	
Median				**8**	**11.5**	**5.6**	**2.1**	**872**	**40.5**	**23.7**	**0.66**	**129**	**7.8**	**0.95**	**0.6**	**1**	
Min				**0.2**	**3.5**	**1.2**	**0.4**	**340**	**15**	**5.8**	**0.05**	**25**	**6.2**	**0**	**0.1**	**1**	
Max				**2600**	**37.7**	**17**	**3.8**	**2246**	**245**	**80.5**	**2.18**	**300**	**8.7**	**4.3**	**1.6**	**2.3**	

* Nykänen & Sarmaja-Korjonen 2007

The percentage of head shields of all counted chydorids remains in the sample (not included in mean and median abundance)

The shape resembled that of *A. phreatica* in Alonso (1996), a closely related and rare species with a relatively narrow distribution within Europe (Dumont 1987, 1995, Alonso 1996, Dumont, Negrea 1996). However, when compared to the drawing of *A. phreatica* in Alonso (1996), the notched structure of the head shield appeared more pronounced and symmetric. Intact *A. phreatica* was first described by Dumont (1983) and Sabater (1987) (male) and was reported to be similar to *A. protzi,* but lacking the denticles on the posterior-ventral corner of the carapace. *A. phreatica* is entirely limited to a groundwater mode of life (stygobitic) (Dumont 1983, 1987, 1995, Dumont, Negrea 1996).

Identification of the head shield remained uncertain until the finding of five specimens with head shield and carapace still attached (Fig. 2A,B). Two specimens clearly exhibited a carapace with three characteristic denticles in the posterior-ventral corner (Smirnov 1974, Røen 1995, Flössner 2000) and a surface sculpture of horizontal lines typical to *A. protzi* (Kay van Damme, pers. communication). The carapace closely resembled the picture and description of the subfossil *A. protzi* carapace in Nykänen, Sarmaja-Korjonen (2007). Two other specimens exhibited at least one and two denticles, respectively, but no visible horizontal lines. The exact number of denticles was impossible to determine because of debris covering them on the permanent (mounted in glycerol gelatine) slide. The fifth specimen had neither lines nor denticles, but the shape of the carapace closely resembled those in Nykänen, Sarmaja-Korjonen (2007). According to Flössner (2000), denticles may be missing on rare occasions.

Description of A. protzi head shield

The head shield of *A. protzi* (Fig. 2B-E) is small, only ca. 200 µm long (the measured head shields ranged from 194 to 230 µm; n=15). Its width is difficult to estimate due to the frequently occurring curvature of the head shield on sample slides, which creates a false impression of it being narrower than in reality (Fig. 2E). Three specimens appeared entirely "flat" (Fig. 2C-D), two of which were 167 µm and one 170 µm wide.

The posterior margin is notched and more tapered than for other small European *Alona* species. The notches begin slightly anterior to the first median pores and the lateral pores. The depth of the notches varies between specimens. Three median pores are narrowly connected and situated close to the posterior margin. The postpore distance (the distance between the posterior pore and the posterior margin) is smaller than the interpore distance (the distance between the anterior and posterior pores). Two minor pores are situated laterally at approximately the level of the anterior pore. In subfossil head shields the minor pores appear as narrow oblong depressions at the same angle as the posterior margin. The head shield is widest just behind the fornices. The rostrum is short and very broadly rounded, sometimes almost flat. Chitin appears thickened in the anterior region and in many specimens the posterior edge of the thickening is undulating.

Abundance of A. protzi head shields in sediments

Generally, *A. protzi* is referred to as a rare species (Dumont 1983, Røen 1995, Flössner 2000). Most zooplankton investigations and monitoring programs focus on pelagic samples and do not encompass the littoral zone, which may partly explain the rarity of the species in contemporary samples. However, in paleolimnological studies, as well as in investigations where living individuals have been sampled directly in the littoral zone, *A. protzi* has also been rare, even in studies including numerous lakes (Smyly 1958, Whiteside 1970, Jones 1989, Cotten 1985, Eyto *et al.* 2003, Bjerring *et al.* unpublished, Nykänen *et al.* unpublished). Admittedly, in our samples the abundance of subfossil *A. protzi* head shields was low, constituting a median of only 1% and 0.6% of the total subfossil Chydoridae head shields per sample (n=47 samples) and per lake (n=13 lakes, Table 1), respectively. Generally, the percentage was lower than 0.5 % of all counted cladoceran remains in the samples (n=45). To our knowledge, with one exception (Nykänen, Sarmaja-Korjonen 2007), comparable abundance data have not been reported in the literature. The low abundance has prevented the inclusion of this species in studies of the relationship between cladocerans and their environment, even in multi-lake studies (>70 lakes) (e.g. Whiteside 1970, Jones 1989).

Environmental characteristics of the lakes

Characteristics of the sites with contemporary findings

Contemporary (1986-2002) morphological and limnological data were available for 6-13 lakes depending on the variable in question (Table 1). Additionally, we had contemporary data for 4 lakes in which *A. protzi* has previously been found in the form of subfossil carapaces in the sediment or as intact animals in the littoral zone (Nykänen, Sarmaja-Korjonen 2007). The lakes varied widely in area and depth, exhibiting no clear pattern. This is in contrast to Røen (1995) who claimed that *A. protzi* prefers small clear water lakes. Most of the discovery sites were meso- to eutrophic (Table 1), although two findings were made in lakes (Lake Velling Igelsø and Lake Riikoisten Valkjärvi) with relatively low phosphorus (15 µg total P L^{-1}) and low chlorophyll *a* concentrations (≤10 µg chl *a* L^{-1}). These two lakes also had low alkalinity (≤0.2 mmol L^{-1}), while alkalinity was moderate (median: 0.7 mmol L^{-1}) and pH values predominantly neutral to high (6.2-8.7; median 7.8) in the other lakes. Thus, for most contemporary variables one or two measurements were in the low or high end of the spectrum (Table 1), indicating that *A. protzi* may be rather widely distributed seen from an ecological perspective.

Due to the use of different sampling protocols there were no consistent and comparative data on macrophytes between sites. However, six lakes investigated for submerged macrophytes all showed very low or no plant-filled volume of coverage. However, area-based coverage may be larger in some lakes owing to small macrophyte inhabitants, such as isoetids.

Characteristics of the sites with findings in older sediments

In 4 Danish lakes *A. protzi* head shields were found in 6 sediment samples (1850-1950 AD). Recently, i.e. in year 2000, these lakes differed as to nutrient state, alkalinity and land cover of catchments. The diatom-inferred epilimnetic total phosphorous (DI-TP) level in concurrent, old samples varied widely, from 14 to 164 µg TP L^{-1} (Bradshaw *et al.* 2006, Amsinck *et al.* 2003). In two lakes, the dominance of *Chydorus sphaericus*, and in one lake *Alona quadrangularis*, indicated relatively high trophic conditions. One lake (DI-TP 14-18 µg L^{-1}) was dominated by *Alonella excisa* and *Acroperus* spp. In this lake, as well as in one *Chydorus sphaericus* dominated lake, *A. protzi* head shields occurred also in the surface sediment. These two lakes differed greatly in DI-TP values (18 and 152 µg L^{-1}, respectively), but shared the feature of a relatively constant DI-TP through 1850-2000 AD (Amsinck *et al.* 2003).

In five lakes, *A. protzi* remains were found in sediments older than 1300 AD. One head shield was found in Lake Hampträsk, Finland (Fig. 1, Table 1) (Nevalainen, unpublished), where the depth of the sample (44 cm) corresponded to the 14th century. The concurrent cladoceran assemblage suggested relatively low trophy. However, the dominance of *C. sphaericus* and the presence of *Disparalona rostrata* suggested that Lake Hampträsk was probably mesotrophic, the latter species being untypical for Finnish oligotrophic lakes (TP < 10 µg L^{-1}). Seven head shields were found in Lake Väike Juusa, Estonia (Fig. 1, Table 1) (Koff *et al.* 2005), with an approximate time range from 2000 BC to AD 1000. The cladoceran assemblage (e.g. *Alona rectangula*, *Leydigia* spp. and *Pleuroxus* spp.) indicated eutrophy. The disappearance of the species was likely connected to the transformation of the lake shore into a mire.

Nine head shields were found in Poland (Fig. 1, Table 1). Five of them occurred in Krowie Bagno Basin (ca. 7000-6300 BC) before it turned into a mire, and the concurrent faunal assemblages suggested eutrophic conditions (Szeroczyńska 2003). Three head shields were found in Lake Wigry (ca. 6300 BC) in a sample indicating mesotrophic conditions (Zawisza, Szeroczyńska 2007). The head shield from Lake Jelonek corresponded to ca. AD 1000, and the cladoceran assemblage indicated meso/eutrophic conditions (Zawisza, unpublished).

Ecology of A. protzi

Our results showed that *A. protzi* occurs under various environmental conditions and has no clear preference to, for instance, lake area or depth. The species appeared at a wide range of nutrient levels, but was not found in lakes with TP < 14 µg L^{-1} or pH < 6. This suggests that the species prefers meso-eutrophic lakes with neutral or high pH.

Generally, *A. protzi* is described as a pelophilic and phytophilic species living in silt, on algae-covered stones, or among macrophytes (Røen 1995, Dumont, Negrea 1996, Flössner 2000). In correspondence with this, two intact individuals of the species were found on a sampling site with rocky bottom and only sparse vegetation in Lake Sylvöjärvi, Finland (Nykänen, Sarmaja-Korjonen 2007). In Lake Lovonjärvi, Finland, *A. protzi* inhabited artificial substratum placed among submerged littoral macrophytes (Uimonen 1985). However, the 6 lakes investigated for submerged macrophytes in this study, all showed very low or no plant-filled volume of coverage (Table 1). At our finding sites, the overall submerged plant-filled volume seemed insignificant for *A. protzi*, although submerged plants generally are an important habitat for a number of chydorid species (Whiteside & Harmsworth 1967, Whiteside 1970). Furthermore, *A. protzi* abundance correlated significantly ($p<0.05$, n=21 samples) with the abundance of the sediment-associated species *Leydigia leydigi* and *Pleuroxus uncinatus,* as well as with the sum of all sediment-associated Cladocera species found in the old sediment of Lake Sarup (Denmark) (Bjerring *et al.*, unpublished).

The obvious rarity of *A. protzi* and the relatively wide environmental spectrum of finding sites (Table

1) may have two explanations: (i) unknown species specific requirements or (ii) the proposed connection of *A. protzi* to groundwater, which implies that *A. protzi* only occasionally appears in open fresh water or streams (Dumont 1983; 1987; 1995; Dumont, Negrea 1996). Six of the 10 Danish finding sites and at least 2 of the Finnish sites containing *A. protzi* head shields or carapaces are to some extent groundwater fed (Bradshaw *et al.* 2006, Nykänen, Sarmaja-Korjonen, Bjerring, unpublished data). Therefore, we cannot exclude the possibility that the species mainly lives in groundwater and is only occasionally transported into lakes.

Conclusions

In this study, we described the subfossil head shield of *Alona protzi*, which can be distinguished by its characteristic shape with a short rounded rostrum and a tapering, notched posterior margin. The head shield of *A. protzi* closely resembles that of *Alona phreatica* in Alonso (1996), although the notches of *A. protzi* seem more pronounced and symmetric.

We found *A. protzi* head shields and carapaces in lake sediments from Denmark, Sweden, Finland, Estonia and Poland, and *A. protzi* is thus relatively widely distributed in the northern part of Europe. Despite its wide distribution, the numbers were low. The environmental spectrum of the finding sites was wide, ranging from relatively nutrient poor clear water lakes to highly eutrophic, turbid lakes. Most lakes, however, were meso-eutrophic with neutral to high pH and relatively low abundance of submerged macrophytes. Therefore, provided that the occurrence of *A. protzi* in lakes is not merely occasional due to a groundwater mode of life (further studies are needed), its remains in lake sediments could tentatively be used as indicators of higher trophy and pH.

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References

Amsinck S.L., Johansson L.S., Bjerring R., Jeppesen E., Søndergaard M., Jensen J.P., Jensen K., Bradshaw E., Anderson N.J., Nielsen A.B., Rasmussen P., Ryves D., Stavngaard B., Brodersen K., McGowan S., Odgaard B.V., Wolin J. 2003. The Waterframework Directive and Danish lakes. Part 2: Paleolimnological studies (original: Vandrammedirektivet og danske søer. Del 2: Palæoøkologiske undersøgelser). Danmarks Miljøundersøgelser. 120 s. Faglig rapport fra DMU. nr. 476 (in Danish).

Alonso M. 1996. Fauna Iberica, Crustacea, Branchiopoda, vol. 7. Museo National de Ciencias Naturales Consejo Superior de Investigaciones Científicas, Madrid. 486 pp. (in Spanish)

Bradshaw E.G., Nielsen A.B., Anderson N.J. 2006. Using diatoms to assess the impacts of prehistoric, pre-industrial and modern land-use on Danish lakes. Regional Environmental Change 6, 17-24.

Cotten C.A. 1985. Cladoceran assemblages related to lake conditions in eastern Finland. PhD thesis. Department of Biology. Indiana University. 70 pp.

De Eyto E., Irvine K., Garcia-Criado F., Gyllström M., Jeppesen E., Kornijow R., Miracle M.R, Nykänen M., Bareiss C., Cerbin S., Salujõe J., Franken R., Stephens D., Moss B. 2003. The distribution of chydorids (Branchiopoda, Anomopoda) in European shallow lakes and its application to ecological quality monitoring. Archiv für Hydrobiologie 156, 181-202.

Dumont H.J. 1983. Discovery of groundwater-inhabiting Chydoridae (Crustacea: Cladocera), with the description of two new species. Hydrobiologia 106, 97-106.

Dumont H.J. 1987. Groundwater Cladocera: A synopsis. Hydrobiologia 145, 169-173.

Dumont H.J. 1995. The evolution of groundwater Cladocera. Hydrobiologia 307, 69-74.

Dumont H.J., Negrea S. 1996. A conspectus of the Cladocera of the subterranean waters of the world. Hydrobiologia 325, 1-30.

Flössner D. 2000. Haplopoda and Cladocera (without Bosminidae) in Central Europe (original: Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas). Backhuys Publishers, Leiden, The Netherlands (in German).

Frey D.G. 1958. The late-glacial cladoceran fauna of a small lake. Archiv für Hydrobiologie 54, 209-275.

Frey D.G. 1959. The taxonomic and phylogenetic significance of the head pores of the Chydoridae (Cladocera). Internationale Revue der gesamten Hydrobiologie 44, 27-50.

Frey D.G. 1986. Cladocera analysis. In: Berglund, B.E. (ed.), Handbook of palaeoecology and palaeohydrology, 667-692. John Wilwey & Sons Ltd. Chichester.

Jones D.H. 1989. The ecology of some microcrustacea from standing waters in Tayside, Scotland. Journal of Natural History 23, 375-406.

Koff T., Punning J.-M., Sarmaja-Korjonen K., Martma T. 2005. Ecosystem response to early and late Holocene lake-level changes in Lake Juusa, southern Estonia. Polish Journal of Ecology 53, 553-570.

Korhola A., Rautio M. 2001. Cladocera and other branchiopod crustaceans. In: Smol J.P, Birks H.J.B, Last W.M. (eds.), Tracking environmental change using lake sediments. Volume 4. Zoological indicators, 5-41. Kluwer Academis Press, Dordrecht.

Nykänen M. & Sarmaja-Korjonen K. 2007. Findings of *Alona protzi* Hartwig 1900 (Branchiopoda: Anomopoda, Chydoridae) in Finland. Studia Quaternaria 24: 73-77.

Røen U.I. 1995. The Fauna of Denmark, Crustaceans V (Original: Danmarks Fauna, Krebsdyr V). Danmarks Fauna 85. Dansk Naturhistorisk Forening, Copenhagen, 358 pp. (in Danish).

Sabater F. 1987. On the interstitial Cladocera of the River Ter (Catalonia, NE Spain), with a description of the male of *Alona phreatica*. Hydrobiologia 144: 51-62.

Smirnov N.N. 1974. Fauna of the U.S.S.R. Crustacea. Volume 1, No. 2. Chydoridae. Israel Program for Scientific Translations. Jerusalem. (Translated from Russian). 1-644 pp.

Smyly W.J. 1958. The Cladocera and Copepoda (Crustacea) of the tarns of the English Lake District. The Journal of Animal Ecology 27, 87-103.

Szeroczyńska K. 2003. Cladoceran succession in lakes and peat bogs of Leczna-Wlodawa District. Limnological Review 3, 235-242.

Uimonen P. 1985. Cladoceran remains in the varves of 1959-1981 in Lake Lovojärvi sediment (Original: Kalvoäyriäisten (Cladocera) jäänteet Lammin Lovojärven sedimentissä vuosien 1959-1981 lustoissa). MSc thesis. Department of Zoology, University of Helsinki, 55 pp. (in Finnish).

Whiteside M.C., Harmsworth R.V. 1967. Species Diversity in Chydorid (Cladocera) Communities. Ecology 48, 664-667.

Whiteside M.C. 1970. Danish Chydorid Cladocera: Modern ecology and core studies. Ecological Monographs 40, 79-118.

Zawisza E., Szeroczyńska K. 2007. The development history of Wigry
Lake as shown by subfossil Cladocera. Geochronometria vol. 27 (in press)

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Lake response to global change:
nutrient and climate effects using cladoceran (*Crustacea*) subfossils as proxies

Lake development is explored on a decadal to millennial scale on different lakes based on Cladocera subfossils analyses in lake sediment cores. Eutrophication was found to have occurred during centuries – or even millennia - in many Danish lakes. The effect of climate on lake ecosystems was investigated using a European latitudinal gradient as a climate proxy, showing a complex pattern of larger and occasionally acid tolerant species in northern cold, low nutrient and low conductivity lakes, whereas dominance of small and benthic-associated species prevailed in southern warm, nutrient rich and high conductivity lakes. Taxa richness was found to be highest at intermediate latitudes. Additionally, climate response was explored through changes in pollen and Cladocera subfossils during a cold event period 8200 years before present in a core from Lake Sarup, which indicated lake level to play a key role.

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