



ECOLOGICAL DRIVERS OF COMMUNITY ASSEMBLY ACROSS TAXONOMIC GROUPS AND TROPHIC LEVELS

PhD thesis
Korhan Özkan

2013



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Author: Korhan Özkan
Institute: Aarhus University, Department of Bioscience,
Freshwater Ecology Group & Ecoinformatics and Biodiversity Group

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PhD supervisors: Professor Erik Jeppesen, Aarhus University, Department of Bioscience,
Freshwater Ecology Group
Professor Jens-Christian Svenning, Aarhus University, Department of Bioscience,
Ecoinformatics & Biodiversity Group

Assessment committee: Professor Tom Andersen, University of Oslo
Professor Luc De Meester, University of Ku Leuven
Assoc. Prof. Anders Barford, Aarhus University

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PREFACE

This thesis is the compilation of my PhD studies at Aarhus University between 2009 and 2013. We set out to understand how communities of organisms are assembled across space and time using two model systems: breeding birds in the Turkish Trace and plankton communities in Danish lakes. Community assembly is a very complex phenomenon and its conceptualization and analyses are manifold. We undertook five case studies from three main perspectives, community assembly across space (Papers 1 and 2), through time (Paper 3) and the role of biotic interactions (Papers 4 and 5). Overall, these studies showed that community assembly is regulated by the interplay of species sorting by local environment acting consistently across large spatial scales, metacommunity processes mediated by dispersal of organisms and ecological interactions across trophic levels, reflecting the complex nature of community assembly.

There are many people that I owe thanks. I feel very lucky to have great supervisors, Erik Jeppesen and Jens-Christian Svenning. Thank you very much Erik for your support for many years and also inviting me on-board for Greenland field trips, which were delightful. Thank you very much Jens-Christian for your constant encouragement and insightful discussions.

My PhD is mostly possible due to the availability of data from various sources, which were gathered through meticulous work of a large number of experts. I am grateful for all these dedicated anonymous work. I owe special thanks to Rikke Bjerring, Liselotte Sander Johansson and Lisbet Sortkjaer for their assistance with lake databases. I am also grateful to Anne Mette Poulsen for her great editorial aid in all manuscripts; Tinna Christensen and Juana Jacobsen for their beautiful touch on graphics including the layout of the present thesis; and Frankie Zea Henriksen as he was always welcomed me, whenever something in my computer refused to work. I am very grateful for the warm welcome from all shallow lakers, Erik Jeppesen, Torben Lauridsen, Martin Søndergaard, Frank Landkildehus, Rikke Bjerring, Liselotte Sander Johansson, Lissa Skov Hansen, Kirsten Landkildehus Thomsen, Dennis Trolle, Susanne Amsinck, Tommy Silberg and Lone Liboriussen. Tom Davidson and Frank Landkildehus, it was a real pleasure to share a single malt with you during long nights of Greenlandic fieldwork under the northern lights!

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There are always some that deserve some special thanks. Thank you very much Joachim Audet, Thomas Boll Christensen, Tom Davidson, Juliane Wischniewski, Shenglan Lu, Rosemberg Menezes, Daniel Graeber and Sandra Hille, your friendship is truly a pleasure.

The last and certainly not the least is my wife Keziban. She survived all the troubles of PhD life with me without even expecting to obtain a title for herself! Thank you darling, I love you.

Finally, thank you luck! I feel so grateful that I am blessed with an exceptionally supportive family and an amazing friend circle.

ENGLISH SUMMARY

This thesis aimed at elucidating ecological factors affecting community assembly in two study systems: (i) breeding bird communities sampled at 433 locations across north-west Turkey in 2009 and (ii) plankton communities sampled in c. 400 lakes across Denmark between 1989 and 2009. Community assembly is a complex phenomenon driven by the interplay between environmental species sorting, ecological interactions between species and dispersal, linking local communities with the surrounding communities as well as with their regional populations. Although the research on community assembly is century-old, current understanding is still poor, especially regarding the interaction of these factors across spatial and temporal scales. Five studies have been carried out in this thesis to obtain a better understanding of community assembly in space and time and the role of biotic interactions.

Local richness and community composition of forest birds in the Istranca forests were significantly related to forest structure, habitat diversity and altitude, while non-environmental spatial factors also had important, albeit weaker, effects, suggesting a secondary role of dispersal and/or biotic interactions. Local bird abundance was strongly linked with occupancy across the metacommunity (the bird communities in the Istranca landscape) as well as the species' regional population and range size across the western Palearctic. Null model analyses showed that bird occupancy was non-randomly related to species environmental niche. Furthermore, species abundance and occupancy across both the metacommunity and the whole western Palearctic were significantly related to an independent species specialization index calculated for the French birds. Together these results indicated that forest bird community assembly is primarily controlled by consistent environmental species sorting across spatial scales and geography locations.

Local environmental factors, mainly nutrients, were also the prime determinants of phytoplankton genera richness in 195 Danish lakes. The roles of lake water chemistry, lake morphology, land-use in lake catchments and climate in determining the richness of different phytoplankton groups reflect the differences in their ecology. There was spatial structure at local to landscape scale (< 30 km) in phytoplankton richness after accounting for the local environmental differences, probably reflecting catchment-scale connectivity among lakes mediating the dispersal of organisms as well as matter and energy. The analyses also showed that phytoplankton richness and productivity (nutrients) were unimodally related and that the role of nitrogen was stronger than that of phosphorus in driving phytoplankton diversity in Danish lakes.

Temporal analyses of 17 lakes, which were intensively monitored for 20 years, revealed synchronous changes in climate as well as in lake physico-chemical variables and plankton communities. Synchronous temporal changes in climate induced strong synchrony in lake physical variables (water temperature and stratification) that are those most affected by the atmospheric energy flux. The synchrony in lake chemistry and plankton communities were stronger for lakes in recovery from earlier eutrophication, characterised by a strong decrease in phytoplankton biomass accompanied by enhanced water transparency as well as a change in the phytoplankton community composition from *Chlorophyta* dominance towards more heterogeneous communities with an increase in the richness of both plankton groups. A common increasing trend in plankton richness was observed across all lakes, coinciding with positive trends in temperature and precipitation as well as negative trends in wind speed, total nitrogen, NO_3 and PO_4 concentrations.

Environmental control was not the only factor determining the plankton assembly in these 17 lakes. There was also significant congruence between phyto- and zooplankton genera richness and community composition, even to a greater extent than that driven by their environmental relationships. This indicated that trophic interactions may also influence the plankton community assembly and positively affect the diversity of both groups. The strength of congruence weakened in high-productivity lakes, suggesting that the trophic coupling between plankton groups weakens with eutrophication, most likely because of high predation pressure on zooplankton due to high abundance of planktivorous fish in eutrophic lakes.

Two events during the lake monitoring period enabled us to examine the cascading effects of the changes occurring at higher trophic levels on phyto- and zooplankton communities. Lake Engelholm was subject to biomanipulation in 1993; large amounts of planktivorous fish were removed from the lake to help recovery from eutrophication. An immediate effect was evident, the phytoplankton biomass decreased 10-fold indicating a released predation pressure on zooplankton. Trophic interactions between phyto- and zooplankton favoured the diversity of both groups. The second event was the invasion of zebra mussels (*Dreissena polymorpha*) to Lake Faarup in 1993. The invasion also resulted in a decrease in the phytoplankton biomass; however, the effect was more gradual and the shift occurred with a four-year delay, reflecting the time needed for zebra mussels to establish in the system. The invasion resulted in a decrease in the diversity of both phyto- and zooplankton, probably reflecting selective plankton feeding by the zebra mussels.

Overall, the analyses in this thesis reflect the complex nature of community assembly. The assembly of forest birds and plankton communities was strongly driven by environmental conditions. However, the local communities were also linked with the regional populations, with some evidence also for dispersal-related effects, and their assembly was strongly influenced by ecological interactions across trophic levels. Therefore, the interactions between environmental species sorting, dispersal and complex biotic interactions at different scales need to be further elucidated to obtain a better understanding of community assembly. Furthermore, studies aimed at understanding community response to global changes such as climate or habitat degradation should not only focus on the species direct response to the changing environment in isolation, but also include the potentially complex interactions among species as well as processes acting at regional scale.

DANSK RESUMÉ

Formålet med denne afhandling har været at frembringe ny viden om de miljømæssige faktorer, der styrer artssammensætningen i to undersøgte systemer, hhv. bestanden af ynglende fugle på 433 undersøgelseslokaliteter beliggende i det nordvestlige Tyrkiet i 2009 og planktonsamfundene i ca. 400 danske søer, monitoreret mellem 1989 og 2009. Artssammensætningen bestemmes af et komplekst sæt af faktorer, herunder samspillet mellem den miljømæssige artssortering, arternes interaktioner og arternes spredning, der knytter de lokale bestande til nabobestande såvel som til de regionale bestande. Selvom opbygningen af artssamfund har været genstand for forskning i mere end hundrede år, er nutidens viden om emnet stadig mangelfuld, især når det gælder de bestemmende faktorerers samspil på tværs af rumlige og tidslige skalaer. Denne afhandling inkluderer fem undersøgelser foretaget med henblik på at opnå større viden om artssamfunds opbygning i rum og tid, og den rolle biotiske interaktioner spiller herfor.

Den lokale artsrigdom og artssammensætning hos skovfugle i Istanca-skovene var signifikant relateret til skovstruktur, habitatdiversitet og terrænhøjde. Ikke-miljømæssige rumlige faktorer spillede ligeledes en betydelig, omend svagere, rolle, hvilket indikerer, at spredningsdynamik og/eller biotiske interaktioner spiller en sekundær rolle. Arternes lokale abundans var stærkt relateret til deres frekvens i meta-samfundet (fuglesamfundene i Istanca-landskabet) såvel som deres regionale udbredelse i det vestlige Palæarktis. Nulmodel-analyser viste, at arternes landskabsmæssige frekvens var relateret til deres miljømæssige niche på en ikke-tilfældig måde. Arternes lokale abundans og deres frekvens både i nærområdet og i hele det vestlige Palæarktis var signifikant relateret til et uafhængigt artsspecialiseringsindeks udviklet for franske fugle. Disse resultater indikerer til sammen, at opbygningen af skovfugle-samfund primært bestemmes via en konsistent miljømæssig artssortering på tværs af rumlige skalaer og geografisk lokalitet.

Lokale miljømæssige faktorer, først og fremmest næringsstoffer, var også afgørende for fytoplanktonets artsrigdom i 195 danske søer. Således spillede søvandets kemiske sammensætning, morfologi, arealanvendelsen i oplandet samt klimaet en rolle for udbredelsen af de forskellige fytoplanktongrupper og afspejlede dermed miljøforskellene søerne imellem. Efter at have taget højde for de lokale miljømæssige forskelle var der en signifikant effekt af den rumlige struktur fra lokal til landskabsskala (< 30km) i artsrigdommen. Disse afspejler formentlig, at søer ofte er forbundet i oplandet. Analyserne viste også, at fytoplanktonets rigdom og produktivitet (næringsstoffer) var unimodalt relateret, og at kvælstof tilsyneladende spillede en større rolle end fosfor for fytoplanktondiversiteten i danske søer.

Tidslige analyser af 17 søer, der har været genstand for intensiv overvågning i 20 år, viste synkrone ændringer i klimaet og søernes fysiske-kemiske parameter og planktonsamfund. Synkrone tidslige ændringer i klimaet førte til udpræget synkroni i søernes fysiske variable (vandtemperatur og stratifikation), som er de variable, der er mest påvirkede af den atmosfæriske energicirkulation. Synkronien mellem søkemi og planktonsamfund var stærkere for søer i bedring efter tidligere eutrofiering, karakteriseret ved et stort fald i fytoplanktonbiomassen efterfulgt af øget vandgennemslugtighed såvel som en ændring i fytoplanktonets sammensætning fra dominans af grønalger til mere heterogene samfund med stigende artsrigdom inden for begge planktongrupper. En general tendens til stigende artsrigdom blev observeret i alle søer, hvilket faldt sammen med tendenser til øget temperatur og nedbør såvel som tendenser til aftagende vindhastighed og mindre koncentrationer af totalkvælstof NO_3 og PO_4 .

Planktonsamfundene var ikke kun påvirket af omgivelsesvariable i de 17 søer. Der var et tydeligt sammenfald mellem artsrigdommen og artssammensætningen af fyto- og zooplankton, som ikke kunne tilskrives disse variable. Dette indiker, at også trofiske sammenhænge kan spille en betydelig rolle for artssammensætningen af planktonsamfundene og positivt påvirke artsdiversiteten i begge grupper. Sammenfaldet i artsrigdom aftog i højproduktive søer, sandsynligvis på grund af et højt prædationstryk på zooplanktonet fra planktivore fisk i eutrofierede søer.

To begivenheder i overvågningsperioden satte os i stand til at undersøge kaskadevirkningerne af ændringerne på de højere trofiske niveauer på fyto- og zooplanktonsamfundene. I 1993 blev Engelsholm Sø biomanipuleret, og et stort antal planktivore fisk blev opfisket for at fremskynde en forbedring af søens tilstand, efter at næringsstofflørslen var reduceret. En øjeblikkelig effekt blev observeret i form af et fald i fytoplanktonbiomassen til 10 % af udgangsniveauet, hvilket tilskrives et faldende prædationstryk på zooplanktonet. De ændrede trofiske samspil var gunstig for artsdiversiteten i både fyto og zooplanktonet. Den anden begivenhed var indvandring af zebamuslinger (*Dreissena polymorpha*) til Faarup Sø i 1993. Dette førte også til et fald i fytoplanktonbiomassen; ændringen skete dog mere gradvist, og et markant skift skete nogle år senere – den tid, det tog for vandremuslingen at etablere sig i systemet. Indvandringen af vandremusling førte til et fald i diversiteten af både fyto- og zooplankton, hvilket sandsynligvis skal tilskrives muslingens selektive planktonfødevalg.

Overordnet afspejler analyserne i denne afhandling den komplekse natur, som opbygningen af økologiske artssamfund har. Opbygningen af skovfugle- og planktonsamfund var stærkt drevet af miljøforhold. De lokale artssamfund var imidlertid også koblet til de regionale bestande, med visse indikationer på spredningsrelaterede effekter, og deres sammensætning var også markant påvirket af økologiske interaktioner mellem de trofiske niveauer. Følgelig er det nødvendigt bedre at belyse samspillet mellem miljømæssig artssortering, spredning og komplekse biotiske interaktioner på forskellige skalaer for at forbedre vores forståelse af artssamfunds opbygning. Desuden bør studier, der har til formål at klarlægge artssamfunds respons på *global change* såsom klimænderinger og habitatsforringelser ikke kun fokusere på arternes egne direkte respons på det foranderlige miljø, men også inddrage de potentielt komplekse interaktioner med andre arter såvel som processer, der forløber på regionalt niveau.

INTRODUCTION

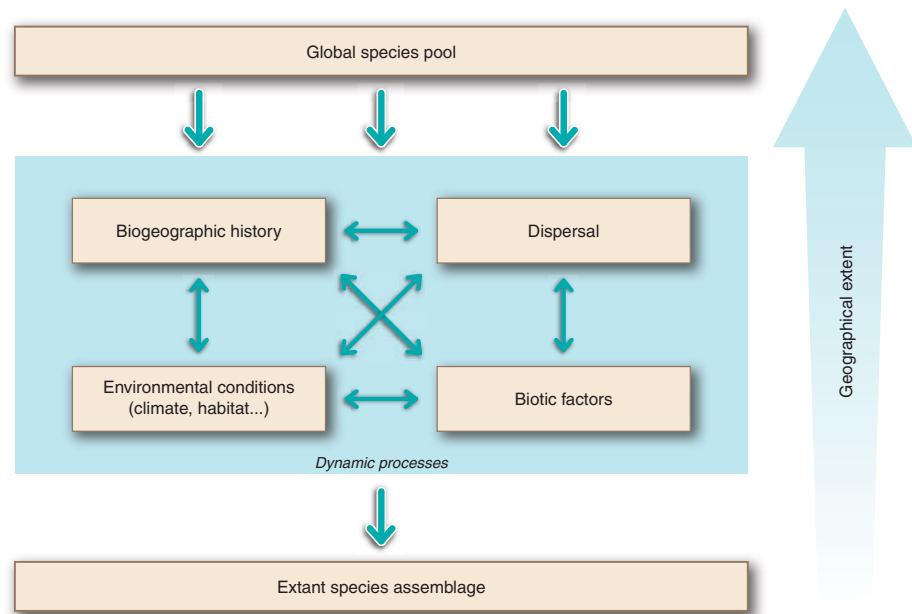
COMMUNITY ASSEMBLY

Ecologists have long been seeking to identify the patterns in species distribution, diversity and composition as well as the determinants behind. Understanding these patterns has always been an essential focus of ecology, but has now become crucial for management and conservation of biodiversity in the face of unprecedented change in climate and global landscape occurring in the last decades. Among the first patterns recognised by ecologists was the relationship between environmental conditions and species distributions. Merriam (1894) demonstrated that the distribution of terrestrial plants and animals is limited primarily by temperature, and Grinnell (1904, 1917) recognised the role of habitat selection in the distribution of birds in the beginning of the 20th century. Later, the importance of species interactions has been recognised mostly in the form of interspecific competition (MacArthur 1958, MacArthur and Levins 1967, Gause 2003). Definition of the niche concept, which is central to ecology, followed these paradigm shifts, evolved from the Grinnellian niche concept based on species habitat preferences to the Eltonian niche concept based on inter-specific interactions and later the more integrative form, the Hutchinsonian niche concept accounting for all ecological interactions of a species (Hutchinson 1957). Species interactions have also been used to conceptualise the community assembly across fragmented habitat patches (Diamond 1975). Diamond formulated that the composition of the bird community in a set of New Guinean islands was the outcome of non-random processes driven by interaction among species.

MacArthur and Wilson's (1967) seminal work on island biogeography was a cornerstone for the recognition of the effect of dispersal on community assembly. They conceptually modelled the species diversity of an island as a function of local extinction regulated by island size, balanced by immigration from the nearby mainland, which was regulated by the distance between island and the mainland. An important detail of island biogeography theory is that species ecological identities are not included in the conceptual model. Later, Hubbell (2001) controversially proposed that species occurring at the same trophic level are essentially equal in terms of their birth rates, death rates and ecological fitness and that their assembly is primarily driven by dispersal and demographic rates, lending support from tropical forest communities. Hubbell's proposal - he called his hypothesis a 'neutral theory of biodiversity' - emphasises dispersal as a key control of community structure, while species niche differences being absent or unimportant.

Recently, the roles of niche and dispersal processes in community assembly have been integrated into the metacommunity framework, in which local communities interconnected by dispersal are understood as being part of a wider metacommunity (Leibold *et al.* 2004). Within this perspective, the relative importance of niche and dispersal processes may vary from pure dominance of niche assembly (environmental species sorting) over intermediate situations (e.g., patch dynamics and mass effects) to complete dominance of dispersal assembly (neutral model). The metacommunity framework links local communities with regional populations through immigration and, consequently processes acting across large geographical regions, namely speciation, extinction and colonization (Ricklefs 1987, Zobel 1997). Ricklefs (2011) argues that a deeper understanding of local communities requires recognition of the fact that local communities are open assemblages consisting of overlapping regional populations of their constituent species. Supported

Figure 1. The main processes interacting across geographical scales in structuring local species assemblages. Taken from Wisz et al. (2013).



by the observation that the abundance and distribution of North American birds were mostly independent of the adaptations of individual species, he controversially speculated that co-evolution of species-specific parasite-host interactions was a potential mechanism in determining species distribution and abundance at the regional scale (Ricklefs 2011). Although local and regional processes have mostly been studied separately, the importance of both factors is recognised and the significance of regional processes has been supported by empirical evidence (Griffiths 1999, White and Hurlbert 2010). Impact of regional enrichment on local species diversity has been found for North American birds (White and Hurlbert 2010) and Fennoscandian freshwater phytoplankton communities (Ptacnik *et al.* 2010).

Overall, community assembly is a complex process shaped by the interplay of environmental species sorting, dispersal, biotic interactions as well as the biogeographical and evolutionary history of species (Figure 1). Although these mechanisms have been studied individually, current understanding is still poor and the relative roles of these processes and their interactions across scales require further investigation (Vellend 2010). Like many other aspects of ecology, all these processes are scale dependent (Levin 1992, Rahbek 2005). Local communities are assembled through the filtering of the local environment, while the dispersal of organisms links these local communities with neighbouring communities and with the regional populations. Biotic interactions have also been regarded as a local factor; however, effects of biotic interactions on species distribution may also be evident (Gotelli *et al.* 2010) and even more prominent (Kissling *et al.* 2010) at larger spatial scales (Wisz *et al.* 2013).

ANALYSING COMMUNITY ASSEMBLY

Understanding the changes in communities across space or through time is only possible if the communities are adequately monitored, i.e., presence or abundance of organisms is recorded within a standardised sampling unit representing a coherent community. To elucidate the extent that these changes are driven by changing environmental conditions, the environmental factors relevant to the ecology of these organisms should be sampled concurrently at each site. The

analyses of spatio-temporal data are particularly challenging because these data are not independent, as eloquently stated in Tobler's first law of geography: "Everything is related to everything else, but near things are more related than distant things" (Tobler 1970). This phenomenon, also called autocorrelation, violates the basic assumptions of many statistical approaches and renders the statistical significance and even estimates of conventional methods unreliable (Dormann *et al.* 2007). Distance-related biological processes such as dispersal, omission of an important spatially structured environmental determinant and the dependence of biological parameters like population size on the values prevailing at an earlier time-step are among the variety of factors responsible for spatio-temporal autocorrelation (Legendre and Fortin 1989, Legendre and Legendre 1998, Dormann *et al.* 2007). Therefore, spatially-explicit models or permutations and null model analyses that are robust to spatial dependence should be used with autocorrelated data (Dormann *et al.* 2007). However, autocorrelation should not be seen only as a statistical problem as the spatio-temporal structure often provides important ecological information, for instance on the effects of dispersal dynamics, density dependence and biotic interactions (Griffith and Peres-Neto 2006). Accordingly, statistical models allowing accounting for autocorrelation, such as simultaneous autoregressive models for spatial autocorrelation (Anselin 1988) and linear mixed effect models with autoregressive components for temporal autocorrelation (Pinheiro *et al.* 2012), have been commonly used (Legendre and Legendre 1998, Kissling and Carl 2008). Furthermore, autocorrelation structure in the data has been analysed to understand the underlying dynamics.

In this thesis, two community datasets have been used: (i) a dataset on breeding bird communities sampled at 433 locations within a forested landscape in north-west Turkey in 2009 (Figure 2) and (ii) a dataset on plankton communities sampled in c. 400 lakes across Denmark between 1981 and 2009 (Figure 3).

Breeding bird surveys are one of the most adequate method to study bird communities as breeding birds are strongly territorial and thus linked with the surrounding environment and communities (Bibby 2004). Breeding bird survey was conducted on the seaward side of the Istranca Mountains located at the Black Sea coast, north-west Turkey between 30 April and 6 July 2009 (Özkan 2009, Özkan 2011, Özkan *et al.* 2013c). The study site spanned c. 1200 km² and includes the highest point (1031 m) in the Turkish Thrace. The vegetation is primarily temperate deciduous forest, dominated by *Quercus* and *Fagus*, and is managed for timber production. Survey locations for bird counts were selected using a stratified random design with at least 200 m distance from the habitat edge. At each locality, sampling was conducted at three consecutive sites in a predetermined direction with an average inter-site distance of 308 m using circular plots modified after Bibby (2004). All birds identified by sight or sound during the 10 minute survey period were recorded in distance bands. Morning counts were conducted within the first 6 h after dawn, and sites were also visited after 10 p.m. for night counts of nocturnal bird species. Different habitat types were covered during the survey; however, only the forest sampling sites (c. 317) were included to ensure a coherent bird community.

Forest and undergrowth characteristics, like stand age and canopy closeness, which are relevant to forest bird ecology, were visually recorded at each site. Landscape characteristics, such as habitat richness, forest cover, average stand age and altitudinal range surrounding each sampling site, were also determined to elucidate their effect on forest bird communities. The National Forestry Registry Database (Ministry of Environment and Forest, unpubl. data) was used to analyse land use and forest characteristics, and a global digital elevation model (GDEM, ASTER GDEM is a product of METI and NASA) was used to analyse altitudinal range.

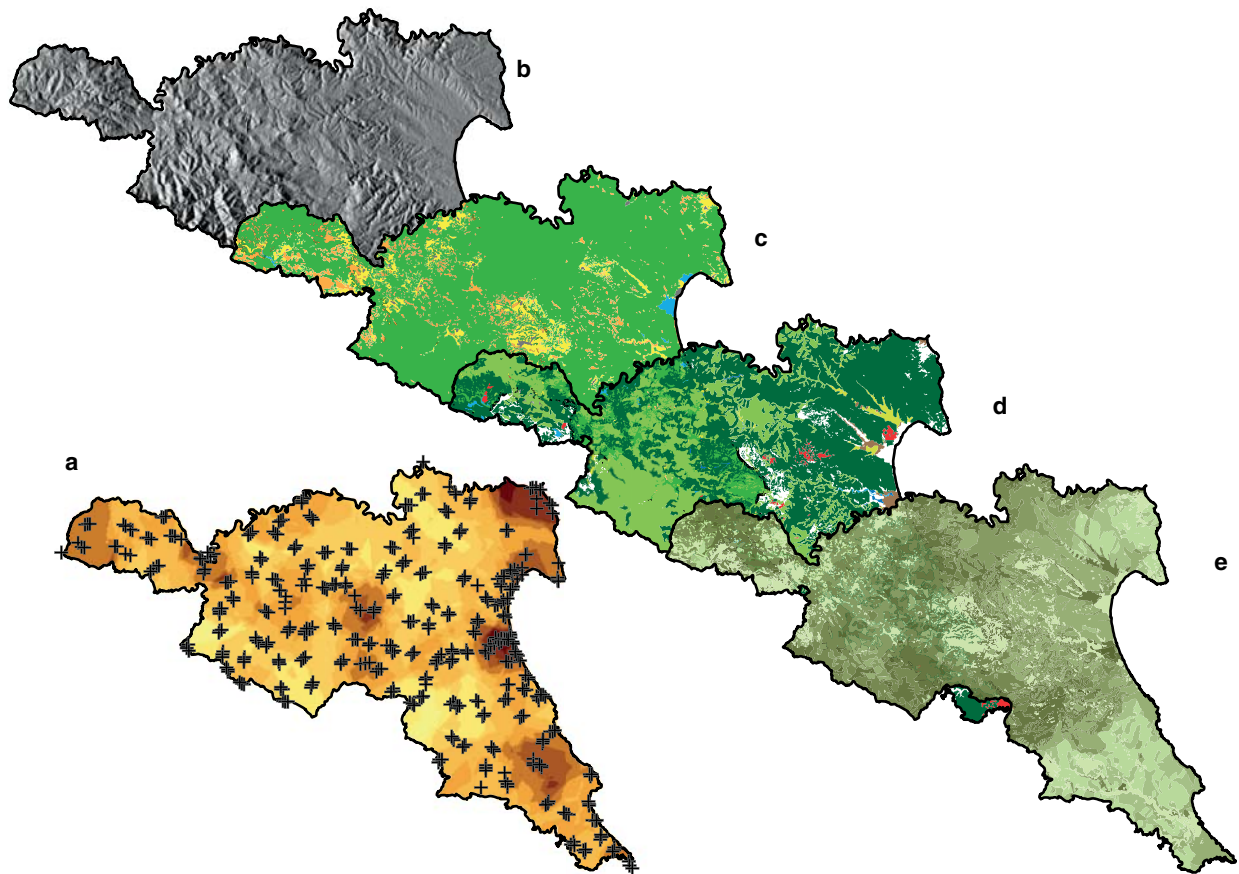


Figure 2. Overview of the dataset on breeding birds in the Istranca Mountains. Abundance of breeding birds was recorded at 433 circular plots using 10-minute standardised surveys (a). Important environmental factors on forest structure and landscape were gathered via on-site observations and analyses of landscape and remote sensing data. Variations in elevation (b), main habitat types (c), dominant tree genera (d) and stand age (e) are given as an overview.

Overall, the breeding bird dataset includes layers of data on local bird communities, local forest characteristics and environmental variation across the surrounding landscape (Figure 2). This permitted examination of the role of environmental factors in structuring local bird communities in the Istranca Forests in relation to other factors such as the role of spatial structure and the link between local and regional bird populations (Paper 1, Özkan *et al.* 2013c).

Lake plankton communities were sampled in c. 400 lakes between 1981 and 2009 as part of the Danish monitoring programme on the aquatic environment (Svendsen *et al.* 2004). Lake plankton constituted two trophic levels: phytoplankton as primary producers and zooplankton as primary grazers. Most of the lakes were sampled occasionally, however a smaller set of lakes (c. 20) were sampled intensively (c. 16 - 19 samples per year) during the monitoring period. The plankton samples were collected, identified, counted and their biomass was estimated using standardised methods (Ütermöhl 1958, Dumont *et al.* 1975, Bottrell *et al.* 1976, Edler 1979, Rott 1981, McCauley 1984, Svendsen *et al.* 2004).

Four main groups of environmental factors were identified as important determinants of plankton ecology in Danish lakes: lake water characteristics, lake morphology, land-use in lake catchments and climate. Lake water physico-chemical characteristics were sampled concurrently with plankton samplings. The water chemistry samples were analysed for different forms of key driving nutrients: phosphorus (P), nitrogen (N) and SiO_2 , as well as other relevant factors such as

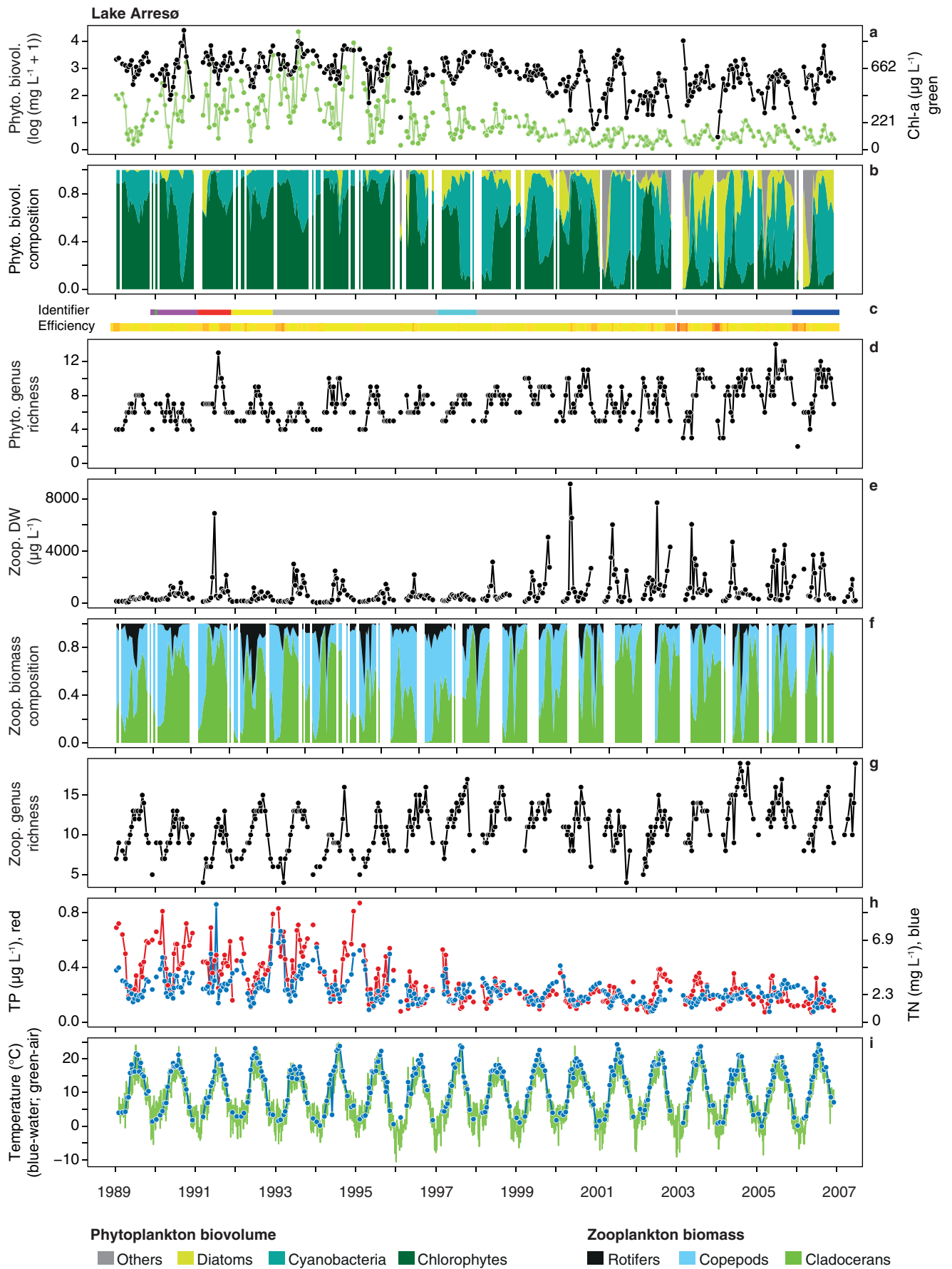


Figure 3. Changes in phyto- and zooplankton communities, water chemistry and climate in Danish Lake Arresø as an example of dataset on Danish lakes. More than 400 water bodies were sampled as part of the Danish monitoring programme on the aquatic environment. Most lakes were sampled extensively via “snap-shot” samples of plankton communities and environmental parameters. A smaller set of lakes (c. 20) were, however, monitored intensively to reveal long-term changes. a, phytoplankton biovolume and Chl-a; b, proportional biomass of main groups of phytoplankton; c, taxonomist performed phytoplankton identification and genus-level efficiency of identifications (the redder is the color, the lower is the efficiency); d, phytoplankton genus richness; e, zooplankton dry weight; f, proportional biomass of main groups of zooplankton; g, zooplankton genus richness; h, total phosphorus and total nitrogen concentrations; i, air and surface water temperatures.

chlorophyll-a (Chl-a) and pH (Svendsen *et al.* 2004, Lauridsen *et al.* 2007). Water transparency and the temperature profile across the water column were also measured at each sampling event. Information on other organisms such as submerged macrophytes and fish was also available, however occasionally. Data on lake morphology including lake area and maximum depth were also compiled. Land use in lake catchments was analysed for total cover of urban, agricultural, and forested land using a Danish land use map compiled during the late 1990s. Lastly, data on mean air temperature, wind speed, solar irradiation and precipitation (Danish Meteorological Institute) were compiled for each lake across the study period to represent climate.

Overall, the lake plankton dataset showed variation in both space and time thus allowing examination of how plankton communities assemble across space (Paper 2, Özkan *et al.* 2012) and time (Paper 3, Özkan *et al.* 2013a) in relation to environmental gradients (Figure 3). As the plankton data covered two trophic levels, primary producers and grazers, further analyses could be conducted on how trophic interactions affect community assembly, while accounting also for environmental factors (Paper 4, Özkan *et al.* 2013b). Furthermore, some of the lakes had undergone important interventions such as fish removal (biomanipulation) and species introduction (zebra mussel, *Dreissena polymorpha*) during the monitoring period, enabling examination of cascading effects in plankton communities triggered by changes at higher trophic levels (Paper 5, Jeppesen *et al.* 2012).

COMMUNITY ASSEMBLY IN SPACE

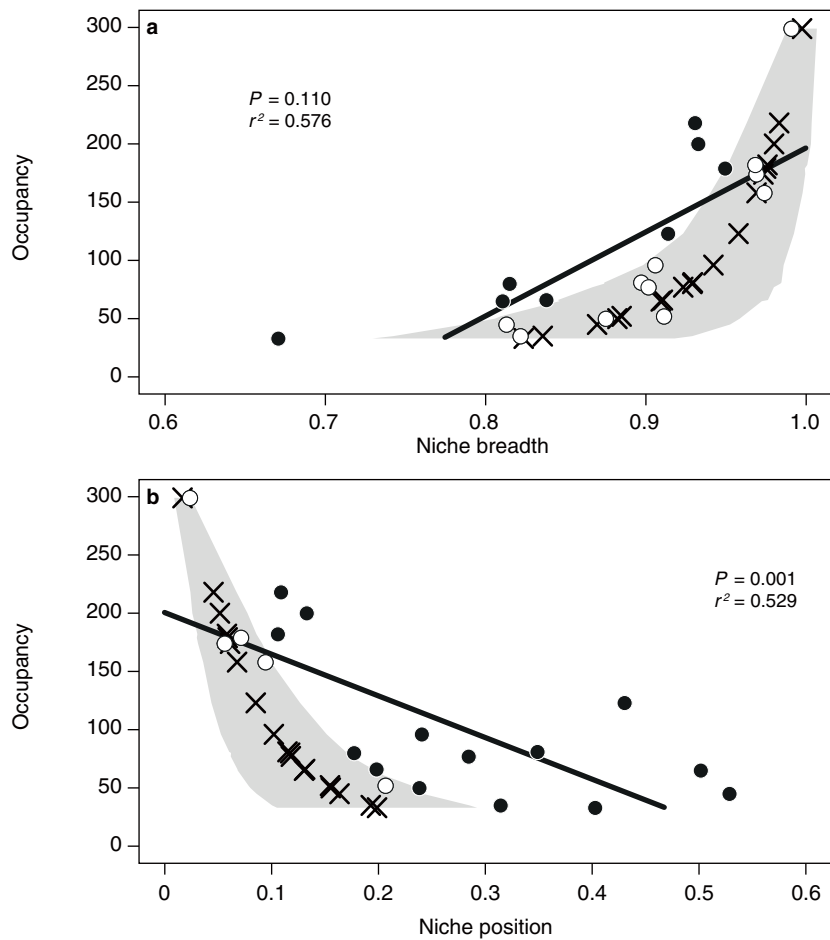
A meta-analysis of previous studies on spatial community assembly found that the majority of these revealed a dominant role of environmental species sorting (Cottenie 2005), but a potential role of dispersal has also been traced in terrestrial (Johnstone and Chapin 2003, Svenning and Skov 2004, Meynard *et al.* 2011, Svenning *et al.* 2011, Qian and Ricklefs 2012) and aquatic communities (Beisner *et al.* 2006, Capers *et al.* 2009, Palardy and Witman 2011). Previous studies have shown that the community structure and diversity of forest birds are closely influenced by vegetation structure and habitat heterogeneity (Thiollay 1992, Lampila *et al.* 2005, Meynard and Quinn 2008). A significant role of dispersal has also been recorded for bird communities assembled across isolated habitat patches and shallow environmental gradients (Driscoll and Lindenmayer 2009, Meynard *et al.* 2011). Furthermore, consistent abundance occupancy patterns in local and regional bird populations (Bock and Ricklefs 1983) and regional enrichment in local bird diversity (White and Hurlbert 2010) have also been observed. The importance of local environmental factors, notably inorganic nutrients, conductivity, pH and water column stratification, has long been recognised as drivers of the community assembly of lake phytoplankton (Reynolds 1984). Dispersal limitation has been

proposed to be weak or non-existent for microorganisms, including phytoplankton due to their high dispersal capacity (Finlay 2002). Previous studies have also established a dominant role of local species sorting in phytoplankton communities (Beisner *et al.* 2006, Vanormelingen *et al.* 2008, Astorga *et al.* 2012); however, experimental investigations have suggested a potential joint importance of dispersal (Drake 1991, Codeco and Grover 2001). The role of dispersal in the assembly of freshwater communities has been shown to be stronger for larger and less mobile freshwater organisms, including also zooplankton (Beisner *et al.* 2006, Astorga *et al.* 2012). However, dispersal-driven regional enrichment in local phytoplankton communities has also been proposed (Ptacnik *et al.* 2010). Overall, the interactions between environmental species sorting and dispersal in determining the local community assembly and the extent to which local and regional communities are linked are not yet fully understood for either bird or freshwater plankton communities and further research is thus required.

First, we set out to understand the role of environmental factors and dispersal in structuring local bird community assembly in the Istranca forests (Paper 1). This area located at the margin of wider western Palaearctic temperate-forest biome, which enabling a comparison between bird community structure at local and regional scales and testing whether consistency is found indicating the existence of mechanisms acting across scales. We used spatial regressions (Dormann *et al.* 2007) and Mantel tests (Legendre and Legendre 1998) to assess the relative importance of environmental and spatial factors as drivers of local species richness and composition within the metacommunity. Then, we analysed species abundance–occupancy relationship across the metacommunity to test if they are linked. Species occupancy (rarity) can be driven by their ecological niche, i.e., species with low tolerance or preference for marginal habitats would be rare and less abundant (Gaston *et al.* 2000). This relationship is, though, difficult to assess, as species niche characteristics are calculated using the environment, where the species occur, and the assessments are prone to sampling bias (Hartley 1998). That is, a rare species will show less tolerance and occupy a marginal niche position as its distribution samples the environment at limited number of locations, although species rarity might be regulated by other factors. To overcome this uncertainty we employed a null model approach in which the bird communities were randomised across the landscape (and thus across the environment) and niche breadth and position re-calculated in each randomization. Thereafter, the observed niche estimates were compared with randomised null expectations to test the significance of the observations. We further used generalised linear models to test for links between species' metacommunity-wide occupancy and their broader western Palaearctic regional populations and to assess whether these links are consistent with environmental species sorting.

Our results showed much stronger control by environmental than dispersal factors of local species richness and composition patterns within the metacommunity, similar to what has previously been documented for birds in Chile (Meynard and Quinn 2008) and many other organism groups (Cottenie 2005). However, dispersal has also been identified as an important control factor for birds, especially when they occupy isolated habitat fragments (Driscoll and Lindenmayer 2009) or when bird communities are analysed at large spatial scale with less steep environmental gradients (Meynard *et al.* 2011). Species occupancy in the Istranca forests correlated with their local abundance as well as with their range size and total abundance for the whole western Palaearctic. Positive abundance–occupancy relationships have been widely documented at metacommunity scale (Gaston *et al.* 2000) and a link between local and regional community has also been found for North American birds (Bock and Ricklefs 1983). This suggests that the same assembly mechanisms act consistently at local and regional scales (Ricklefs 1987,

Figure 4. Metacommunity-wide occupancy of bird species vs. niche breadth (a) and niche position marginality (b) in the Istranca Forests. Linear regression lines and associated r^2 s are shown. Niche breadth and position were only analysed for species with more than 10% occupancy and significance of the relationships was assessed by randomization. Crosses indicate mean niche breadth and position marginality in randomised communities, with the shaded area showing confidence intervals for estimated means. Black circles indicate significantly different niche breadth and position values ($P < 0.05$). Significance of the relationships in comparison with that of randomised communities is given.



Zobel 1997). Based on the finding that the abundance and distribution of North American bird populations did not appear to be shaped by competition or reflect the adaptations of individuals, Ricklefs (2011) proposed that species-specific parasite-host interactions could be a potential mechanism in determining species distribution and abundance at regional scale, implying weak local environmental sorting of the community assembly. However, species occupancy across the metacommunity of the Istranca forests was strongly determined by the marginality of species niche position (Figure 4). Furthermore, a species specialization index developed based on the habitat use of bird species across France, incorporating both niche position and breadth, showed a significant relationship with species occupancy and abundance at both metacommunity and western Palaearctic regional scale. Hence, the same niche-related assembly mechanisms appear to act consistently within the western Palaearctic region (Finlayson 2011).

To understand the factors determining lake phytoplankton diversity in the Danish landscape, we compiled a dataset consisting of plankton samples collected in 195 Danish lakes and ponds between 1994 and 2008 (Paper 2) as well as main environmental drivers. We modelled phytoplankton diversity using these environmental factors within a spatially-explicit statistical framework (Dormann *et al.* 2007). Phytoplankton is not a monophyletic classification, instead included organisms from bacteria to small plants with different ecological characteristics and analyses were thus run for all main groups of phytoplankton in order to examine potential differences in the role of the environment across groups.

The dataset posed some challenges for the analyses, one being that some lakes had been frequently sampled, while others had been sampled rarely or only once. To account for the uneven sampling of lakes in the monitoring data, we performed

two-layer permutations of the dataset and repeated all the analyses 1000 times. First, a random set of 131 lakes was assembled from 195 lakes to prevent dominance of the pattern by frequently sampled lakes. Second, a random sample was selected for each lake at each draw to be able to use all available information on each lake. An additional challenge was the fact that the samples were collected throughout the year, thus exhibiting seasonality, rendering comparison of phytoplankton from different seasons difficult. Therefore, only late summer samples (July 15 – September 30), providing a coherent ecological community under the influence of similar ecological factors, were used in the analyses. The third challenge was the taxonomic identifications to species level were not equally efficient across different samples. To minimise bias we used genus richness as our taxonomic unit and excluded samples with an identification efficiency (percentage of taxa identified to genus level) lower than 80%.

Lake water chemistry, mostly nutrients, was the prime determinant of phytoplankton genera richness in Danish lakes as forms of nitrogen (N) and phosphorus (P) were always selected in a model. Although total phosphorus (TP) has long been regarded as most important nutrient driver of lake ecosystems (Schindler 1977), we found that total nitrogen (TN) had a considerably stronger effect on phytoplankton richness in line with recent findings on joint importance of TN and TP in driving shallow lake ecosystems (Gonzalez Sagrario *et al.* 2005, James *et al.* 2005, Özkan *et al.* 2010). The spatial models indicated a weak, but significant spatial structure in phytoplankton richness within a 30 km neighbourhood, even after accounting for the effects of environmental factors, which corresponds well with the scale of the catchments. Therefore, connectivity among lakes might also influenced phytoplankton richness, not only through dispersal of organisms (Bergstrom and Jansson 2000, Lindstrom and Bergstrom 2004), but also through flows of energy and material (Leibold *et al.* 2004). Reflecting that phytoplankton is a polyphyletic conglomerate, separate analyses of the main groups of phytoplankton revealed contrasting patterns (Figure 5). For example, unlike other phytoplankton groups, the genus richness of *Bacillariophyceae* (diatoms) and *Dinophyceae* were proved to be strongly affected by lake morphology (depth and area), reflecting their ecology. Diatoms are heavy and susceptible to loss by sinking in deep-stratified lakes (Reynolds 1984). In contrast, *Dinophyceae* are motile, which allows them not only to stay in the surface waters (epilimnion) but also to migrate to the deeper waters (metalimnion/hypolimnion) during night to get access to nutrients and thereby gain a competitive advantage in deeper lakes (Reynolds 1984, James *et al.* 1992).

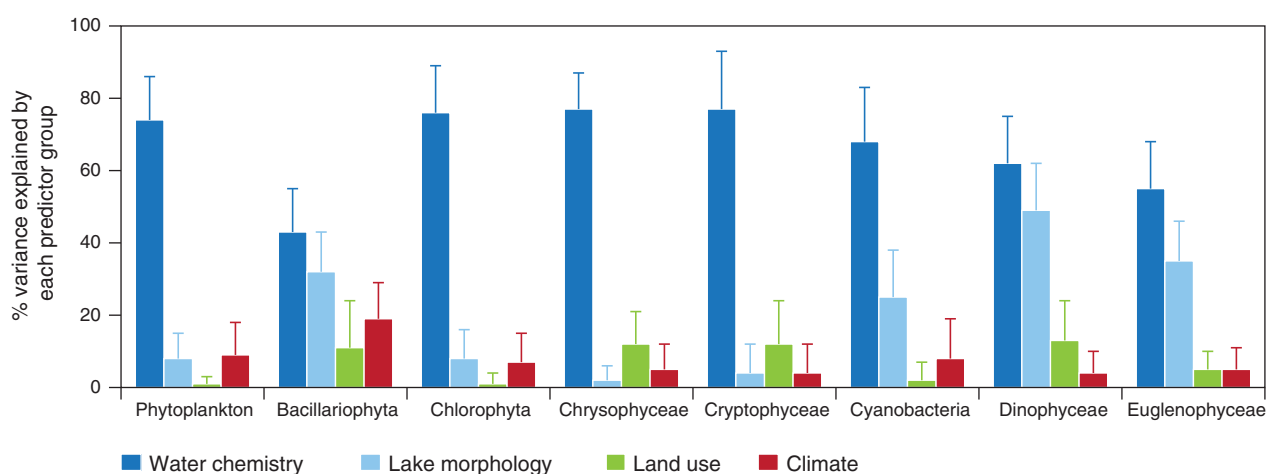


Figure 5. Variance explained by four main groups of environmental predictors relative to total variance explained by all selected predictors (means across 1,000 permutations with error bars for standard deviation) in models on genus richness of all main phytoplankton groups.

Overall, the analyses on both datasets traced a dominant role of environmental factors on the assembly of bird and phytoplankton communities in corresponding landscapes, in accordance with the high dispersal capacity of both groups (Finlay 2002, Meynard and Quinn 2008). However, significant but secondary role of spatial structure was also found in both analyses, indicating that dispersal also plays a role in the assembly of these communities (Driscoll and Lindenmayer 2009). Bird species occupancy in the Istranca forests was strongly consistent with bird population and range sizes in the wider western Palearctic biome, indicating a link to regional-scale processes (Ricklefs 1987, Griffiths 1999). Often such links would be equated with dispersal-driven effects (White and Hurlbert 2010). The observed patterns were, however, not compatible with regional dynamics independent of environmental control such as dispersal dynamics or host-parasite dynamics and their co-evolution (Ricklefs 2011), but rather suggested that the same niche assembly processes act consistently across spatial scales (Finlayson 2011). It was not possible to examine the role of regional processes in phytoplankton richness. Phytoplankton communities have long been regarded as being primarily driven by the local environment due to their small size, their cosmopolitan distribution and high reproductive capacity (Finlay 2002). However, a recent study on Fennoscandian lakes found that local phytoplankton diversity correlated only weakly with local productivity, with 70% of its variability being explained by regionally averaged productivity (nutrient level) on a spatial scale between 100 and 400 km. This suggests that high local phytoplankton diversity might have been sustained by dispersal from surrounding species-rich habitats (Ptacnik *et al.* 2010). Therefore, dispersal mediated regional processes might also be important for the phytoplankton community assembly.

COMMUNITY ASSEMBLY IN TIME

Temporal change in lake plankton has been intensively studied using paleolimnological analyses of sediment cores (Smol *et al.* 2005) and long-term lake monitoring studies (Jeppesen *et al.* 2000, Magnuson *et al.* 2005, Maberly and Elliott 2012) at different temporal scales. Striking changes in lake plankton communities have been observed due to climate change (George and Harris 1985, Smol *et al.* 2005), acidification (Fischer *et al.* 2001), anthropogenic eutrophication (Bergström and Jansson 2006) as well as re-oligotrophication (Anderson *et al.* 2005, Anneville *et al.* 2005, Pomati *et al.* 2011). However, the interactions of these long-term changes in driving lake ecosystems are still not fully understood (Arnott *et al.* 2003, Simpson and Anderson 2009, Vogt *et al.* 2011, Dong *et al.* 2012).

We analysed data on 17 Danish lakes monitored for two decades (1989-2008) to elucidate the temporal changes in climate, physical and chemical characteristics of lake water and plankton communities (Paper 3). We tested if the temporal change in each variable was synchronous (coherent) across the lakes and synchrony was calculated as mean pair-wise Pearson correlations across time-series of a variable in all lakes (Vogt *et al.* 2011). We also tested for long-term trends in any of these variables using Mann-Kendal trend test (Mann 1945). Climate has been regarded as a main factor inducing synchrony across lake ecosystems (Magnuson *et al.* 1990); however, the extent to which this synchrony cascades to biotic communities are not clear (Arnott *et al.* 2003, Vogt *et al.* 2011). In the last decades, intensive agriculture and untreated waste water have resulted in widespread eutrophication of aquatic ecosystems world-wide (Isermann 1990, Smith *et al.* 1999). Upon dramatic water quality crises, stringent measures have been applied to remedy this, and recovery has been observed (Cooke *et al.* 1993, Carpenter *et al.* 1999, Jeppesen *et al.* 2005). This extensive eutrophication/re-oligotrophication

might also induce synchronous temporal changes in lake ecosystems, especially in heavily urbanised and agricultural landscapes (Anneville *et al.* 2005, Pomati *et al.* 2011).

Strong synchrony (>0.7) was observed in climatic variables (Figure 6). Synchronies were also observed in the physico-chemical variables and were stronger for water temperature and stratification, which are most affected by atmospheric energy flux (0.3-0.5), and weaker for lake chemistry (0.1-0.3). Synchrony in plankton was also apparent to a similar degree as observed for lake chemistry. Notably, mean annual phyto- and zooplankton richness increased across most of the lakes, coincident with widespread increases in mean annual air temperature and precipitation, and decreases in wind speed, PO_4 , TN and NO_3 . As these temporal trends in climate and nutrient levels co-occurred and were mostly monotonic without shifts during the study period, it was not possible to perform a conclusive hypothesis test to identify the trend(s) responsible for the changes in plankton communities. Although speculative, a few explanations are plausible. The reduced nutrient loading might have led to a general increase in phytoplankton diversity via release of competitive pressure (Tilman and Pacala 1993), which likely cascaded to the level of zooplankton diversity, perhaps by allowing niche partitioning through trophic interactions (Tilman *et al.* 1982, Özkan *et al.* 2013b). Therefore, the common positive temporal trend observed in plankton richness might have been a response to the recent agricultural improvements across Danish landscape (Kronvang, Jeppesen, Conley *et al.*, 2005).

Six lakes exhibiting the strongest recovery from earlier eutrophication (S lakes, with significant and strong TP reduction) showed stronger synchrony (Figure 6) in both lake chemistry and plankton variables with consistent long-term trends compared to the remaining lakes. Phytoplankton biomass decreased and both phyto- and zooplankton richness increased in S lakes, while zooplankton biomass demonstrated no clear trend. The annual phytoplankton assemblages in S lakes exhibited a consistent temporal trend from *Chlorophyta* dominance towards more heterogeneous communities. The strong synchrony and consistent trends in the S lakes corroborated previous studies showing pronounced lake response to re-oligotrophication (Jeppesen *et al.*, 2005b; Köhler, Hilt, Adrian *et al.*, 2005; Anderson, Jeppesen and Søndergaard, 2005). Synchronies in lake chemistry and plankton were also observed in the remaining lakes (W and N lakes with fluctuating or unchanged TP concentrations, Figure 6), however weaker compared to S lakes, coinciding with decreases in PO_4 , TN and NO_3 .

Although no clear impact of climate on temporal trends and synchrony in lake chemistry and plankton variables was found in the present study, climate has pronounced influence on the richness and community structure of lake organisms acting through growing season, habitat availability (Douglas *et al.* 1994), thermal stratification (Sorvari *et al.* 2002), changes in the life history of organisms (Jeppesen *et al.* 2010) and nutrient dynamics (Jeppesen *et al.* 2009, Jeppesen *et al.* 2011) as well as plankton phenology (Thackeray *et al.* 2008, Meis *et al.* 2009). An analysis across European peri-alpine lakes found that significant changes in phytoplankton community composition occurred during the 1980s when a major shift in climatic conditions occurred during winter and early spring (Anneville *et al.* 2005). Furthermore, the increase in temperature was found to be one of the best predictors of the increase in phytoplankton richness in Lake Zurich, Switzerland, during 30 years (Pomati *et al.* 2011).

As the breeding bird survey was conducted only once, it was not possible to analyse temporal variation in bird communities. However, breeding bird surveys have been conducted for decades in many parts of the world and the role played by

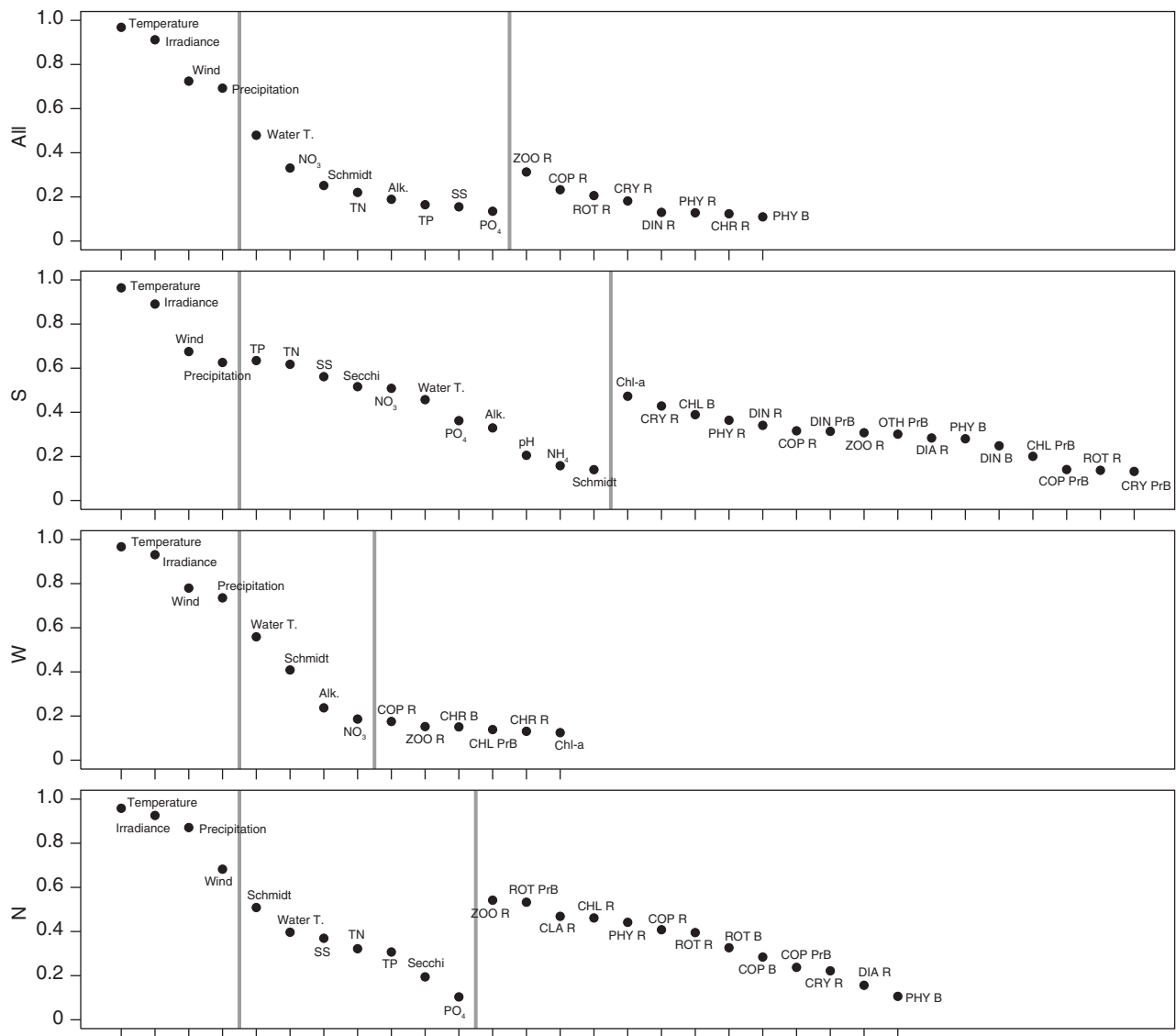


Figure 6. Summary of the synchrony in annual (excluding winter) time-series in climate, lake and plankton variables analysed for all lakes and lakes exhibiting strong recovery from earlier eutrophication (S) with nutrient fluctuations (W) and with no change in nutrients concentrations (N), separately. Only variables with $r > 0.1$ are shown, main variable groups (climate, lake physico-chemistry and plankton) are divided by vertical lines.

anthropogenic impacts and climate change in the temporal trends in birds is well documented (Wormworth and Sekercioglu 2011). Marked changes in phenology (Crick and Sparks 1999, Huin and Sparks 2000), range (Thomas and Lennon 1999) and population (Cunningham and Moors 1994, Barbraud and Weimerskirch 2001) of birds in response to climate change have been recorded. Furthermore, a significant population decline of most bird species occurred in Europe in the last quarter of 20th century (PECBMS 2007), the decline being most pronounced for farmland bird populations due to agricultural intensification (Donald *et al.* 2001).

ROLE OF BIOTIC INTERACTIONS

Earlier studies in aquatic ecology have focused on bottom-up regulation of food webs, i.e., regulation of phytoplankton by nutrients and subsequent regulation of zooplankton by phytoplankton (Mcqueen *et al.* 1986) with few exceptions (Brooks and Dodson 1965). However, later studies have demonstrated that aquatic food webs may also be top-down controlled, i.e. zooplankton is regulated by fish and phytoplankton is regulated by zooplankton (Carpenter *et al.* 1985, Gulati *et al.* 1990, Carpenter and Kitchell 1993). This top-down control may also lead to a trophic cascade where the presence of a predator alters the behaviour and abundance of the lower trophic level and thereby release the next trophic level from predation/grazing pressure (Carpenter and Kitchell 1993). Although the strength of the trophic cascade on phytoplankton varies, widespread empirical support for its existence has emerged (Brett and Goldman 1996). Trophic interactions may also result in cross-taxon congruence between two trophic levels (Paine 1966, Tilman 1982, Kissling *et al.* 2007a). However, there is contradictory evidence for cross-taxon congruence in phyto- and zooplankton (Allen *et al.* 1999, Declerck *et al.* 2005a, Longmuir *et al.* 2007, Heino 2010, Padial *et al.* 2012).

We tested if phyto- and zooplankton communities are congruent in their diversity and compositional patterns over a 20-years monitoring period covering 17 lakes (Paper 4). Environmental control of biotic communities can promote cross-taxon congruence (Gaston and Williams 1996) as the diversity of different taxonomic groups will correlate across space and time if they respond to the same environmental gradients. Few studies have examined congruence in planktonic communities and contradictory evidence has emerged (Heino 2010). We examined the extent to which this congruence is driven by similar environmental needs of the two plankton groups and hypothesised that congruence beyond environmental control would indicate a role of trophic interactions. The genus richness and composition of phyto- and zooplankton were significantly congruent in 4958 samples from 17 lakes (Figure 7). Analyses with linear mixed effect models (Pinheiro and Bates 2000) showed that the predictive power of the models improved significantly if the richness of reciprocal plankton group was used as a predictor in models compared with the models including only environmental variables (R^2 from 6% to 12% for phytoplankton and 11% to 18% for zooplankton). Furthermore, partial Mantel tests (Legendre and Legendre 1998) showed that a large proportion of the congruence in genus composition was independent of environmental dissimilarity (Mantel r : 0.29 and partial Mantel r after accounting for environment: 0.17). These analyses suggested that the congruence in both genus richness and composition between two trophic levels of lake plankton was beyond the similar environmental requirements of the two groups.

Hutchinson (1961) was among the first to recognise that the established theories could not explain the diversity of planktonic life, where a homogeneous water column is to lead competitive exclusion of species, and termed it “the paradox of plankton”. Since the recognition of the problem, several mechanisms have been proposed to sustain planktonic biodiversity such as simultaneous limitation by multiple resources, heterogeneity in time or space, interspecific trade-offs in competition and colonization, non-equilibrium population dynamics and trophic interactions (Tilman and Pacala 1993), as well as chaos (Huisman and Weissing 1999). Facilitation between two trophic levels is especially relevant in the present case (Longmuir *et al.* 2007). Zooplankton can promote diversity in phytoplankton by grazing on dominant species, thereby preventing competitive exclusion (Paine 1966), especially through keystone species like *Daphnia* (McCauley and Briand 1979, Carter *et al.* 1995, Proulx *et al.* 1996, Sarnelle 2005). A microcosm experi-

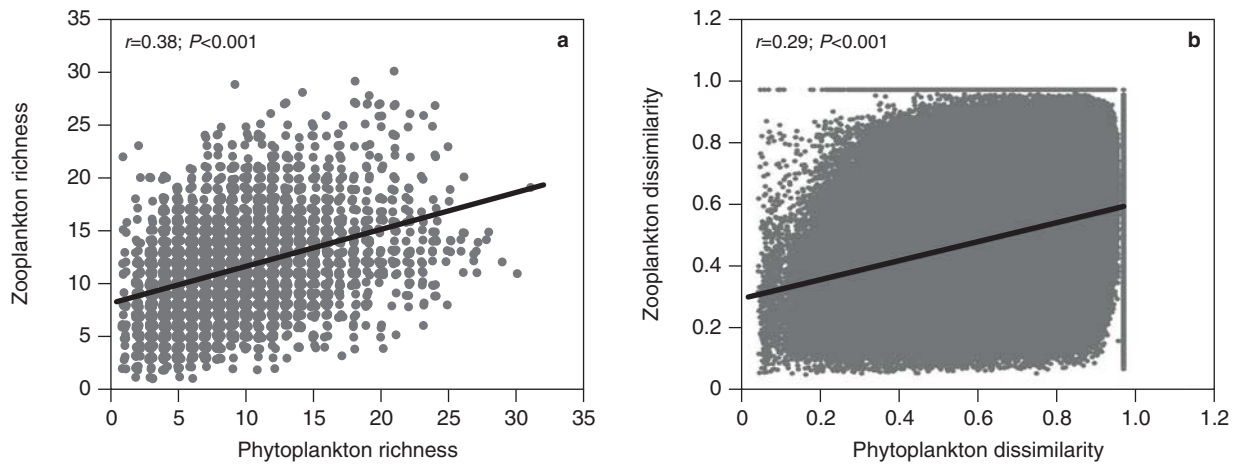


Figure 7. Correlation between phyto- and zooplankton genus richness (a) and pairwise phyto- and zooplankton compositional dissimilarities (b) in samples collected in 17 lakes during 20 years of monitoring.

ment conducted by Burgmer and Hillebrand (2011) also showed that zooplankton grazing delayed phytoplankton extinction and sustained higher phytoplankton richness. However, increasing grazer abundance might also lead to a decrease in primary producer diversity in nutrient-poor ecosystems where severe nutrient limitation reduces primary producer recruitment after grazing (Proulx and Mazumder 1998). Conversely, a diverse phytoplankton community may enable niche partitioning among zooplankton (Tilman *et al.* 1982). Analyses of zooplankton amino acid composition, identifying their food sources, have shown that the different zooplankton taxa have distinct food sources, and niche partitioning should therefore play an important role in their community assembly (Guisande *et al.* 2003). Furthermore, a recent microcosm experiment has shown that higher phytoplankton diversity promotes higher diversity in *Daphnia* cultures provided that phytoplankton biomass and elemental composition remain constant (Striebel *et al.* 2012). Modeling studies have also suggested that trophic interactions can promote diversity through assembly processes as colonization of a producer may facilitate colonization of a specialist consumer, which, in turn, may facilitate colonization of other competing producers (Grover 1994). The strength of the congruence varied among different groups of phyto- and zooplankton in a way consistent with ecological expectations, lending support to the hypothesis that trophic interactions play a role in sustaining the diversity of lake plankton. Rotifers, being the smallest zooplankton group, can only graze on a limited size spectrum of phytoplankton and showed the strongest congruence with phytoplankton, indicating its sensitivity to changes in phytoplankton composition (Padial *et al.* 2012). Notably, congruence was weakened with increasing productivity, suggesting that the strength of the trophic interactions between the two groups abates with eutrophication. This is likely due to changes in trophic dynamics (Jeppesen *et al.* 2000, Carpenter *et al.* 2001), where enhanced fish predation on large-bodied zooplankton with increasing nutrient levels results in reduced zooplankton grazing control of phytoplankton assemblages (Jeppesen *et al.* 2000, Jeppesen *et al.* 2003).

Two major biotic perturbations, namely the introduction of zebra mussels (*Dreissena polymorpha*) in Lake Faarup and mass removal of fish in Lake Engelsholm, occurred during the 20-year lake monitoring period enabling us to quantify the changes in plankton communities induced by these dramatic changes at the upper trophic levels (Paper 5, Chapter 6). Lake Engelsholm was monitored between 1989 and 2008 and mass removal of fish biomass (biomanipulation) was carried out in 1992-1993 to enhance recovery from eutrophication. Lake Faarup was

monitored between 1989 and 2004 and the first planktonic zebra mussel larvae were observed in 1993. First, we used change point analyses (Hinkley 1970) to test if any significant shift occurred in phytoplankton biomass in these two lakes and whether this change, if any, co-occurred with the perturbations. Upon verifying the shift, we compared the diversity and community structure of plankton communities before and after in order to elucidate the effects of these dramatic changes on phyto- and zooplankton assemblage.

The plankton community in Lake Engelsholm showed an immediate response to fish removal (Figure 8), thus providing a clear example of a trophic cascade, where the predator in a food chain suppress the abundance and change the community structure of their prey, thereby releasing the next lower trophic level from predation/grazing pressure (Hairston *et al.* 1960, Carpenter *et al.* 1985). A ten-fold decrease was observed in phytoplankton biomass with an increase in richness of both phyto- and zooplankton following biomanipulation. Significant shifts in the phytoplankton community occurred from dominance of *Cyanobacteria* before biomanipulation towards, first, stronger seasonal succession among *Chlorophyta*, *Cyanobacteria* and diatoms followed by larger total contribution of the other groups (Figure 9). Overall, the mass removal of fish probably reduced the predation pressure on zooplankton, in turn strengthening the trophic link between zooplankton and phytoplankton with positive effects on the diversity of both groups (Paper 4). The notable decrease in phytoplankton biomass and the positive effects on plankton diversity provide a clear example of the role of biotic interactions in the community assembly and evidence the utility of biomanipulation in aiding lake recovery from eutrophication (Paper 5).

Zebra mussel introduction also resulted in significant changes in plankton communities, however the response was more gradual and occurred with a delay (Paper 5). There was a significant shift in phytoplankton biomass in 1998, five years after the introduction, reflecting the time needed for invasive species to establish in the system. Zebra mussel introduction resulted in a 6-fold decrease in phytoplankton biomass, as expected (MacIsaac 1996, Caraco *et al.* 1997). A 50% decrease was also observed in zooplankton biomass, potentially indicating resource limitation due to decreasing phytoplankton biomass and zebra mussel grazing on small-sized zooplankton (MacIsaac 1996). The effect on the diversity of phyto- and zooplankton was negative. Although it is not possible to determine the exact cause of the negative effects of zebra mussel in the present case, a plausible explanation could be the differential grazing of zebra mussels on the different taxa and size classes of plankton (Winkel and Davids 1982, Caraco *et al.* 1997).

Overall, lake data analyses have demonstrated that trophic interactions are an important factor affecting how communities are assembled in freshwater systems. Aquatic ecology literature is especially rich for the effect of trophic interactions (Carpenter and Kitchell 1993), and many studies have shown that lake food webs may in part be top-down regulated (Carpenter *et al.* 1985, Jeppesen *et al.* 2000). However, biotic interactions are manifold and not limited to trophic interactions between producers and grazer. For example, fungal epidemics were found to be a major driver of diatom phenology and composition in Dutch Lake Maarsseveen (Ibelings *et al.* 2011). Furthermore, submerged macrophytes are central to the functioning of shallow lake ecosystems and mediate the diversity, abundance and interspecific interactions of all other groups of organisms (Carpenter and Lodge 1986, Moss 1990, Declerck *et al.* 2005b).

Even though the role of biotic interactions in structuring bird communities in the Istranca forests was not a subject of investigation in this thesis, bird communities can be strongly affected by biotic interactions (Kissling *et al.* 2007a, Gotelli *et al.* 2010),

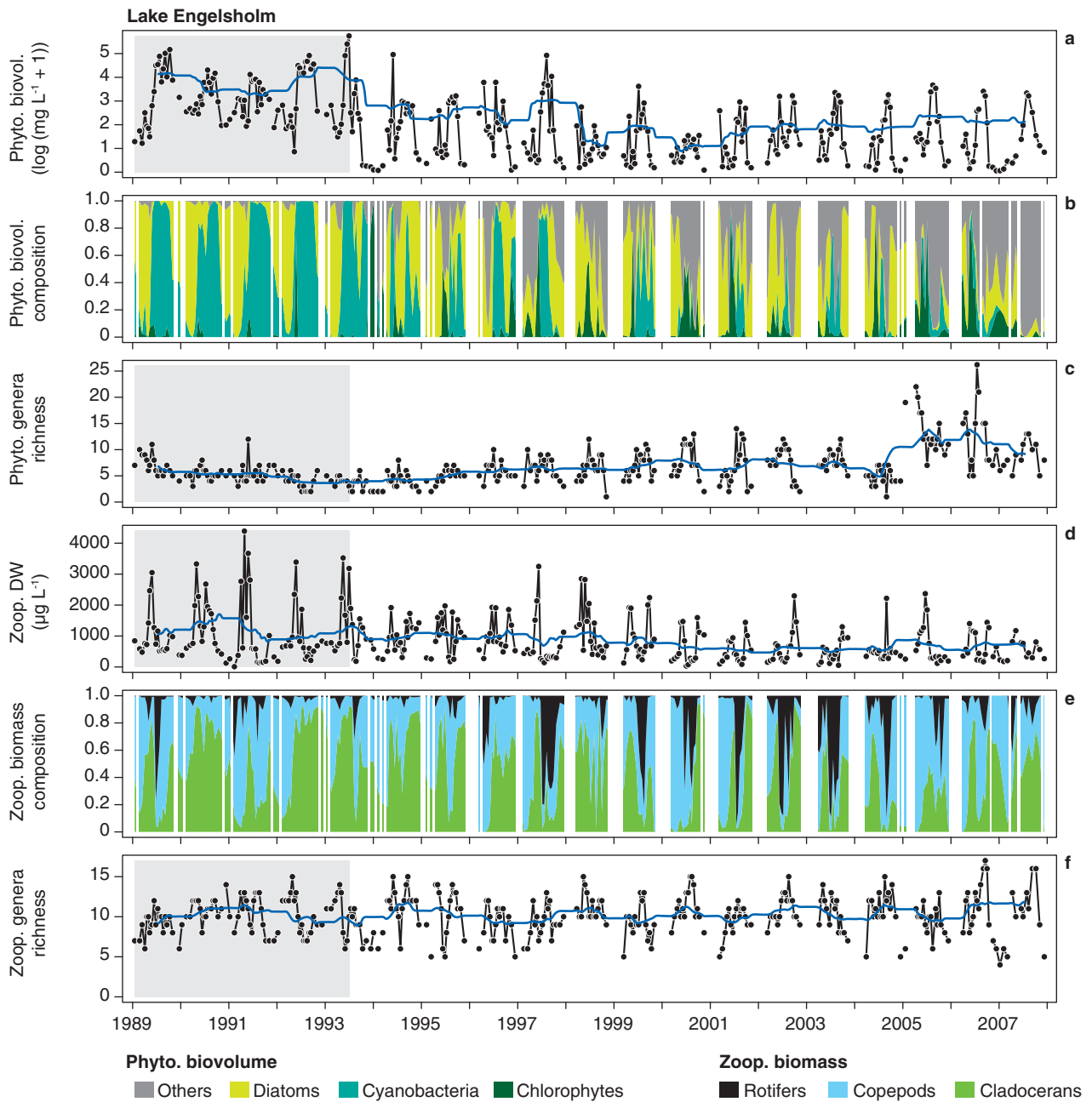


Figure 8. Time series for phytoplankton biomass (log-transformed, A), phytoplankton composition of four main groups (B), phytoplankton genera richness (C) and evenness (D), as well as zooplankton biomass (E), zooplankton biomass composition of three main groups (F), zooplankton genera richness (G) and evenness (H) in Lake Engelsholm, Denmark. Grey and white backgrounds indicate the shift in the phytoplankton biovolume trend identified by change point analysis, coinciding with the period of biomaniipulation. Blue lines in scatter plots denote yearly running means excluding winter samples. Lines in scatter plots and polygons in composition plots were left unconnected if two samples were more than 30 days apart.

and especially interspecific competition has long received considerable attention (MacArthur 1964, Diamond 1975). Although the role of biotic interactions has been widely regarded as a local factor (McGill 2010), accumulating evidence suggests that biotic interactions have large-scale influence on species' distributions (Wisz *et al.* 2013). A recent analysis of Danish avifauna revealed spatial segregation between ecologically similar species at spatial grains (up to 100 km²), which could not be ascribed to the patchy distribution of habitats or to gross differences in habitat utilization among ecologically similar species (Gotelli *et al.* 2010). Furthermore, analyses of New World passerine birds indicated that geographical range overlap between species was not driven solely by dispersal or environmental constraints;

rather rates of secondary sympatry was positively related to both the phylogenetic and the morphological distance between species, indicating that biotic factors may limit species distribution across large spatial and temporal scales (Pigot and Tobias 2013). Diversity of birds across large spatial scales might also be related to the diversity of other groups of organisms, such as food plants (Kissling *et al.* 2007b). Species richness of fruit-eating birds in sub-Saharan Africa was most strongly related to richness of *Ficus* trees, which are the main food resource for these birds (Kissling *et al.* 2007b). Similar relationship between vegetation and mammal diversity across large spatial scales has also been observed (Qian *et al.* 2009, Greve *et al.* 2012). Overall, these findings emphasise that complex ecological interactions between organisms should also be considered together with the environmental and dispersal control on community assembly in order to achieve a better understanding of species diversity and distribution dynamics (Meier *et al.* 2010, Kissling *et al.* 2011, Wisz *et al.* 2013).

CONCLUSIONS

The analyses of breeding birds in the Istranca forests and plankton in Danish lakes clearly showed that community assembly was driven by the complex interactions between environmental species sorting, trophic interactions and regional processes. Forest bird abundance, diversity and community structure were predominantly controlled by the environmental factors related to forest structure, habitat diversity and altitude. Local bird abundance was strongly linked with occupancy in the metacommunity as well as species regional population and range sizes in the western Palearctic, while bird abundance and occupancy at different spatial scales were non-randomly associated with species niche preferences. This indicated that similar niche sorting mechanisms function consistently across spatial scales in driving the forest bird assembly.

Plankton communities in Danish lakes also responded strongly to the environmental factors across space and time, mostly to anthropogenic eutrophication and re-oligotrophication. The analyses of environmental control of phytoplankton diversity in 195 lakes verified some common patterns such as a unimodal productivity-diversity relationship and a differential response of phytoplankton groups to different environmental factors. Notably, the diversity of phytoplankton in these lakes was more strongly affected by N than by P, the latter commonly accepted as the primary limiting nutrient in lake ecosystems. Furthermore, the spatial structure in the phytoplankton diversity suggested the potential joint role of catchment-scale connectivity in driving the phytoplankton community via exchange of organisms, mass and energy. Analyses of two-decade time series in 17 Lakes revealed that widespread nutrient reduction has led to synchronous temporal changes in biotic and abiotic characteristics of lakes and probably driven a widespread long-term increase in phyto- and zooplankton genera richness.

The plankton community assembly in Danish lakes was also controlled by trophic interactions, not only between two trophic levels of plankton but also by changes at higher trophic levels. Congruence in both genus richness and composition between phyto- and zooplankton was beyond the similar environmental relationships, indicating an independent role of trophic interactions in enhancing the diversity of both groups. The strength of the trophic interactions weakened with increasing TP concentrations, potentially reflecting a stronger predation on zooplankton by planktivorous fish in eutrophic lakes. We also found that mass removal of fish had an immediate positive impact on the diversity and seasonality of both phyto- and zooplankton. However, invasive zebra mussel had a more gradual and negative impact on phyto- and zooplankton diversity.

In-depth understanding of community assembly is essential in order to establish more reliable predictions concerning the consequences of global changes for the structure and functioning of ecosystems. However, this is only possible through a better understanding of how environmental control, ecological interactions and dispersal interact at different spatial scales. Therefore, studies aimed at understanding community responses to global changes should not solely rely on the response of species to a changing environment, but should also account for complex ecological interactions, dispersal and processes acting at regional scale.

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PAPER I

Environmental species sorting dominates forest-bird community assembly across scales

Korhan Özkan^{a,b}, Jens-Christian Svenning^b and Erik Jeppesen^{a,c,d}

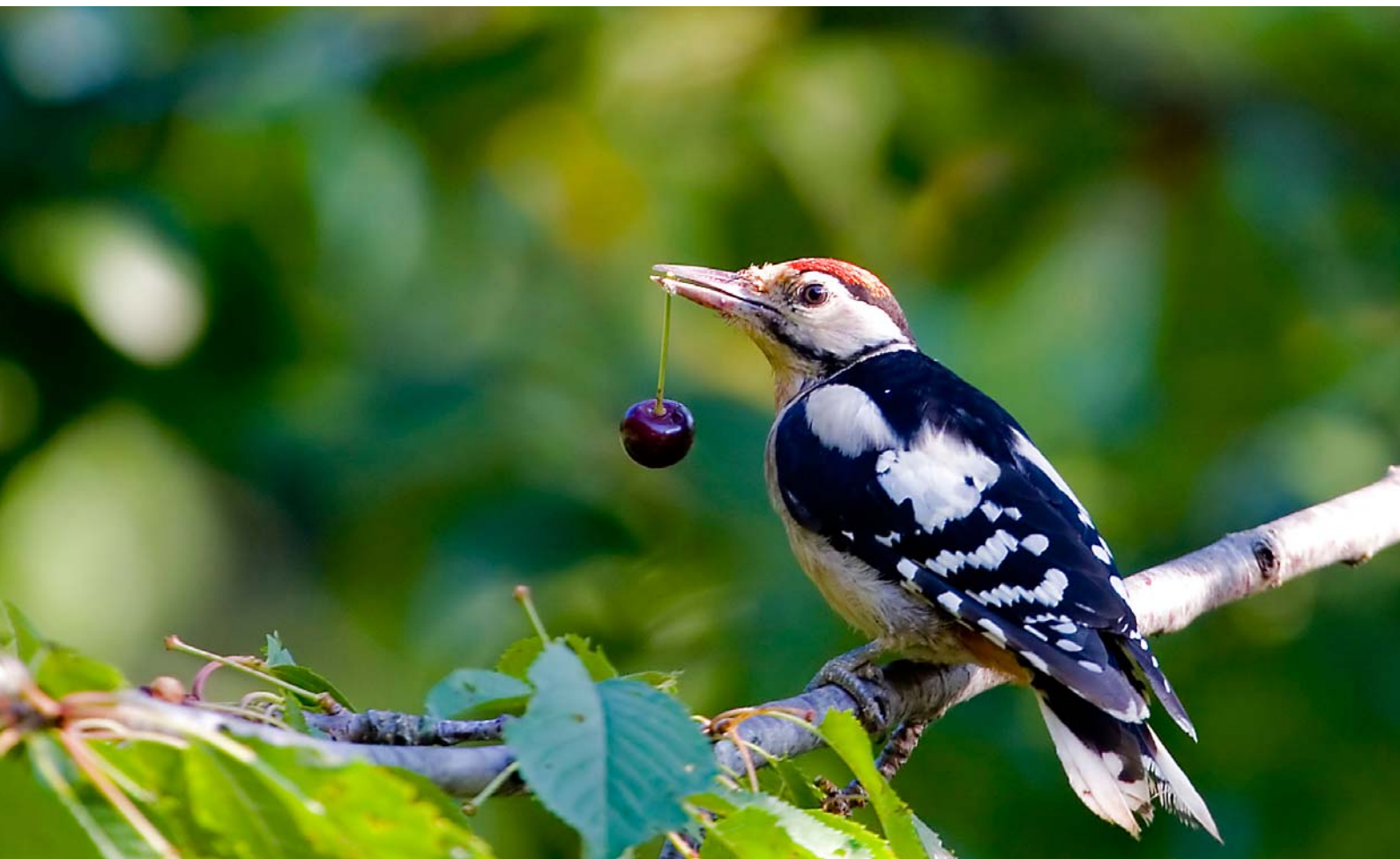
^a Freshwater Ecology Group, Department of Bioscience, Aarhus University, Silkeborg, Denmark

^b Ecoinformatics and Biodiversity Group, Department of Bioscience, Aarhus University, Aarhus, Denmark

^c Greenland Climate Research Centre, Greenland Institute of Natural Resources, Nuuk, Greenland

^d Sino-Danish Centre for Education and Research, Beijing, China

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Environmental species sorting dominates forest-bird community assembly across scales

Korhan Özkan^{1,2*}, Jens-Christian Svenning² and Erik Jeppesen^{1,3,4}

¹Freshwater Ecology Group, Department of Bioscience, Aarhus University, Silkeborg, Denmark; ²Ecoinformatics and Biodiversity Group, Department of Bioscience, Aarhus University, Aarhus, Denmark; ³Greenland Climate Research Centre, Greenland Institute of Natural Resources, Nuuk, Greenland; and ⁴Sino-Danish Centre for Education and Research, Beijing, China

Summary

1. Environmental species sorting and dispersal are seen as key factors in community assembly, but their relative importance and scale dependence remain uncertain, as the extent to which communities are consistently assembled throughout their biomes.

2. To address these issues, we analysed bird metacommunity structure in a 1200-km² forested landscape (Istranca Forests) in Turkish Thrace at the margin of the Western Palearctic (WP) temperate-forest biome. First, we used spatial regressions and Mantel tests to assess the relative importance of environmental and spatial factors as drivers of local species richness and composition within the metacommunity. Second, we analysed species' abundance–occupancy relationship across the metacommunity and used null models to assess whether occupancy is determined by species' environmental niches. Third, we used generalized linear models to test for links between species' metacommunity-wide occupancy and their broader WP regional populations and assessed whether these links are consistent with environmental species sorting.

3. There was strong environmental control on local species richness and composition patterns within the metacommunity, but non-environmental spatial factors had also an important joint role.

4. Null model analyses on randomized communities showed that species' occupancy across the metacommunity was strongly determined by species' environmental niches, with occupancy being related to niche position marginality.

5. Species' metacommunity-wide occupancy correlated with their local abundance as well as with their range size and total abundance for the whole WP, suggesting that the same assembly mechanisms act consistently across local to regional scales. A species specialization index that was estimated by bird species' habitat use across France, incorporating both niche position and breadth, was significantly related to species' occupancy and abundance at both metacommunity and WP regional scales. Hence, the same niche-related assembly mechanisms appear to act consistently across the WP region.

6. Overall, our results suggest that the structure of the Istranca Forest' bird metacommunity was predominantly controlled by environmental species sorting in a manner consistent with the broader WP region. However, variability in local community structure was also linked to purely spatial factors, albeit more weakly.

Key-words: bird metacommunity, niche breadth, niche position, occupancy, regional effects, temperate forest, Western Palearctic

Introduction

Niche processes have been recognized as important for community assembly for nearly a century, with emphasis on both abiotic and biotic factors (Grinnell 1917; Hutchinson 1959; Gotelli, Graves & Rahbek 2010).

However, the potential importance of dispersal has received increasing recognition since MacArthur & Wilson's (1967) seminal work on island biogeography. Controversially, Hubbell's (2001) neutral theory emphasizes dispersal as a key control of community structure, with species' niche differences being unimportant. Recently, niche and dispersal processes have been integrated in a framework, in which local communities are interconnected by dispersal as parts of a wider metacommunity (Leibold

*Correspondence author. E-mail: korhan_oz@yahoo.com

et al. 2004). Under this perspective, the relative importance of niche and dispersal processes may vary from pure dominance by niche assembly (environmental species sorting) over intermediate situations to complete dominance by dispersal assembly (neutral model). With greater importance of dispersal, speciation, extinction and colonization across large geographical regions may also influence metacommunity structure (Ricklefs 1987; Zobel 1997; Griffiths 1999; White & Hurlbert 2010). Ricklefs (2011) argued that local communities are open assemblages made up of the overlapping regional populations of their constituent species. Regional effects on communities may alternatively reflect niche-driven processes across large ecologically coherent regions under this perspective (also cf. Belmaker & Jetz 2012).

Considering bird community assembly, vegetation structure and habitat heterogeneity have emerged as important environmental factors (Thiollay 1992; Lampila, Mönkkönen & Desrochers 2005; Meynard & Quinn 2008). In contrast, less evidence exists for dispersal effects in bird community assembly (Meynard & Quinn 2008; Driscoll & Lindenmayer 2009). An analysis of bird communities across France provided support for the joint influence of environmental factors and dispersal (Meynard *et al.* 2011), while a study of bird communities across North America provided evidence for a moderate regional pool enrichment effect, consistent with the influence of dispersal, even though local environment was the dominant assembly factor (White & Hurlbert 2010). In addition, there is evidence that biotic interactions – even beyond those driven by environmental factors – may also affect bird community assembly, such as interactions within and among bird species (Gotelli, Graves & Rahbek 2010) as well as trophic and non-trophic interactions

with other organism groups, particularly plants (Kissling, Rahbek & Böhning-Gaese 2007; Qian & Kissling 2010). Presently, however, we still lack a comprehensive understanding of the factors driving bird community assembly and how these link from local assemblages to regional communities across whole biomes (Ricklefs 2011).

Here, we investigated forest-bird metacommunity structure and its underlying drivers across a 1200-km² forested landscape at the geographical margin of the Western Palearctic (WP, Europe, including Caucasus up to west Urals) temperate-forest biome. We assessed community structure and its potential drivers at three spatial scales, namely local communities, the metacommunity as a whole and the whole WP region. First, to understand drivers of local community assembly within the metacommunity, we analysed local species richness and composition. Second, to assess metacommunity scale assembly, we assessed whether species' occupancy across the metacommunity is linked to their niche breadth and/or niche position, as expected under niche assembly (Brown 1984; Hanski, Kouki & Halkka 1993). Third, we assessed whether occupancy across the metacommunity is related to species' range and abundance at the WP regional scale, which would indicate that consistent regional processes drive community assembly and tested whether these patterns are consistent with the environmental species sorting.

Materials and methods

STUDY AREA

The study was conducted in Istranca (Yıldız) Mountains (Fig. 1) and spanned *c.* 1200 km² including the highest point (1031 m) in Turkish Thrace. Average annual precipitation is 542 mm and

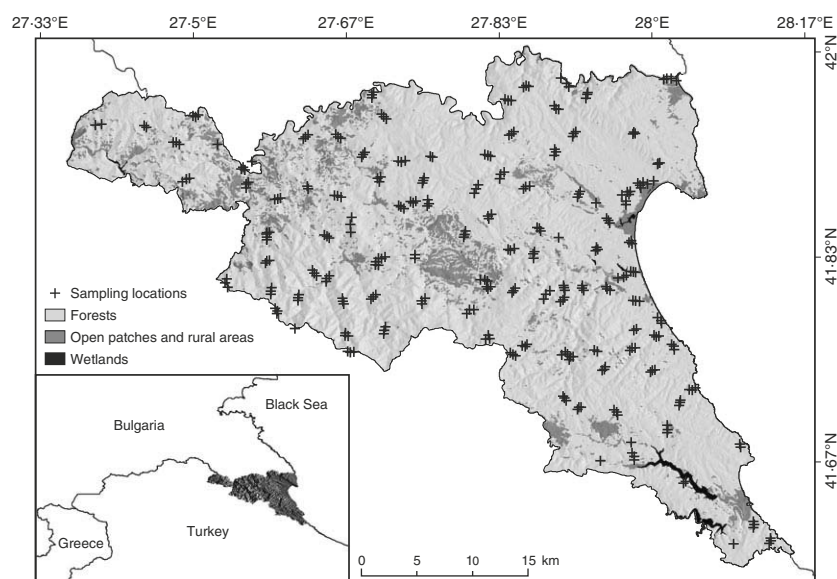


Fig. 1. Location of the survey area (Istranca Forests, Turkish Thrace) overlaid with the bird survey sites included in the present analysis. Hill-shade effect represents the terrain.

average annual temperature is 13 °C (Turkish State Meteorological Service, unpubl. data). The vegetation is primarily temperate deciduous forest, dominated by *Quercus* and *Fagus*. The forest is managed for timber production and most stands are homogeneous in age and species composition. Tree cover is fragmented by small openings, agricultural areas and villages.

BREEDING BIRD SURVEYS

Bird counts were conducted between 30 April and 6 July 2009 by four surveyors in two teams. Survey locations were selected using a stratified random design with at least 200-m distance from the habitat edge and with necessary logistic modifications. At each locality, sampling was conducted at three consecutive sites along a predetermined direction with an average inter-site distance of 308 m (Fig. 1), using circular plots modified after Bibby (2004). All birds identified by sight or sound during the 10-min survey period were recorded in four distance bands (≤ 10 , 11–20, 21–100, >100 m). Morning counts were conducted within the first 6 h after dawn, and sites were also visited after 10 p.m. for night counts of nocturnal bird species. Only sampling sites in forests (natural forests, including riparian forests, as well as plantations) were included in the present analyses (317 sites, 272 of which were also visited for night counts). Global average abundances for night birds were used for sites missing night counts. Birds flying above the canopy or exhibiting migratory behaviour, as well as *Cuculus canorus*, which migrate early in the breeding season, were excluded from the analysis.

ENVIRONMENTAL VARIABLES

Environmental variables were analysed as two groups: (i) on-site habitat characteristics and (ii) landscape characteristics surrounding each site. On-site habitat characteristics (dominant tree genera, tree genus richness, stand age, canopy cover and undergrowth characteristics) were recorded by visual observations at each site within c. 100 m. Three canopy cover (10–40–70–100% cover) and five stand age (0–8–16–25–50– >50 cm, diameter at breast height) classes were applied. If stands held mixed age groups of two consecutive age classes, a composite category was used (nine classes in total). Undergrowth was characterized by total cover and by the presence of logs-and-snags and *Rhododendron ponticum*. Pairwise correlations were small among on-site environmental variables ($r < 0.34$).

Landscape characteristics (altitudinal range, habitat richness, dominant tree genus richness, total forest cover, dominant stand age, average stand age and stand age class richness) were analysed in four different distance bands (250–500–1000–3000 m) around each site to avoid an arbitrary range selection. Landscape characteristics were computed in ArcMap (ESRI 2008) using National Forestry Registry Data base (Ministry of Environment and Forest, unpubl. data) and a global digital elevation model (GDEM, ASTER GDEM is a product of METI and NASA). Landscape characteristics were reduced to orthogonal factors by an exploratory factor analysis with varimax rotation (Legendre & Legendre 1998). Six factors were selected by a heuristic approach to achieve the best grouping of related environmental predictors. On-site altitude was correlated with altitudinal range and included in the Factor Analysis. Interpretation of the factor analysis is shown in Table 1, with further details in Appendix S2. The correlations among on-site and landscape characteristics were

Table 1. Interpretation of the factor analysis of the landscape characteristics around each sampling site in Istranca Forests, Turkey. For details see Appendix S2.

Factors	Interpretation
Stand age	Positively correlated with stand age
Habitat diversity	Positively correlated with habitat diversity and negatively correlated with forest cover
Tree genus richness	Positively correlated with dominant tree genus richness
Stand age diversity	Positively correlated with stand age class diversity
Altitude	Positively correlated with altitude and range of altitude
Canopy cover diversity	Positively correlated with canopy cover diversity

relatively low ($r = 0.59$ for on-site stand age and factor for stand age in the landscape; $r < 0.34$ for the remaining).

STATISTICAL ANALYSES

To assess community structure and its potential drivers at the three study scales, we first analysed local species richness and composition within the Istranca Forests' metacommunity by using spatial regressions with variance partitioning and Mantel tests, respectively. We then assessed species' occupancy–abundance relationships for the metacommunity as a whole using generalized linear models (GLM) and used null models to analyse whether occupancy is related to species' environmental niches. Niches were quantified as niche breadth (Brown 1984), representing environmental tolerance, and niche position marginality, representing how marginal a species' habitat use is in relation to habitat availability (Hanski, Kouki & Halkka 1993). Third, we analysed whether species' occupancy is related to their WP regional range and population size, which would indicate consistent regional processes driving community assembly. Finally, we used the species specialization index (SSI) from the French breeding bird survey (Julliard *et al.* 2006) to test whether this measure of species specialization from a distant part of the WP biome explains species occupancy across Istranca Forests as well as species' population and range sizes across the WP. A strong relationship would provide evidence for environmental species sorting acting consistently across the whole region.

VARIABILITY IN LOCAL COMMUNITY STRUCTURE

We analysed local species richness in relation to on-site and landscape characteristics using ordinary least squares (OLS) regression to assess how much variation is accounted for by the environmental factors. OLS models were simplified by stepwise Akaike Information Criterion (AIC) selection (Venables & Ripley 2002). Subsequently, we refit the final OLS models using a simultaneous autoregressive (SAR) model to assess the importance of spatial structure. SAR model accounts for spatial autocorrelation as an error term (Anselin 1988) and has performed well in simulation studies (Dormann *et al.* 2007; Kissling & Carl 2008). We also fit a null SAR model excluding all environmental variables to assess the effect of the spatial structure alone. We compared the OLS and SAR model fits to evaluate the relative roles of

environmental and spatial factors. Species richness was square-root transformed because of slight deviation from normality. All predictors were standardized to zero mean and unit standard deviation (Gelman 2008). Dominant tree genus as a categorical predictor was coded into binary variables with a contrast to *Quercus*, the dominant tree genus. Tree genus richness and canopy cover diversity in the landscape, as well as tree genus richness and all undergrowth-related variables on-site, were excluded by AIC selection. SAR model was constructed with a 3-km neighbourhood matrix and its fit was assessed with pseudo- R^2 (Nagelkerke 1991). All regressions were examined with diagnostics plots and standard methods; no violations of assumptions were found. Variance inflation factor was ≤ 1.41 , while the maximum difference in betas and fits were 0.76 and 1.1, respectively.

Local community species composition was analysed using partial Mantel tests (Legendre & Legendre 1998) to examine the relationship between community dissimilarity, geographical distance and environmental dissimilarity between each pair of sampling sites, where geographical distance represents dispersal or other non-environmental spatial processes (Svenning & Skov 2002). Species-site matrix, geographical coordinates and the environmental variables were converted into pairwise Bray–Curtis dissimilarity, log-transformed Euclidean distance and Gower dissimilarity matrices (Gower 1971), respectively, before Mantel tests. We used forward selection on environmental variables for Mantel Tests. Environmental variables were added into the pairwise-dissimilarity calculations until there was no increase in the correlation or the test became insignificant. Stand age diversity and tree genus richness in landscape as well as dominant tree and presence of *R. ponticum* and logs-and-snags on-site were excluded.

METACOMMUNITY-WIDE OCCUPANCY AND ABUNDANCE

We assessed whether species' occupancy across the Istranca Forests' metacommunity is related to local abundance. Local abundance was quantified as maximum and mean abundance in occupied plots. Species were classified into five habitat association groups (Appendix S1) following Cramp & Simmons (2006) in order to examine the relationships exclusively for forest-associated birds. We used negative binomial GLM with log-link to model occupancy because of overdispersion. Colonial breeders, post-breeding foraging groups and few species with high leverage (Cook's distance >1) were excluded from the analyses.

We assessed whether species' occupancy across metacommunity is determined by species' niche breadth and niche position marginality. Ten environmental variables selected in richness or community composition analyses were used as species' niche axes. Niche breadth of a species across an environmental variable was quantified as the range of an environmental variable at occupied sites divided by overall range of this environmental variable across all sites, so that each niche breadth was scaled between zero and one (i.e. niche breadth relative to available variation on each environmental axis). Mean range over ten environmental axes was calculated as niche breadth of each species. Niche position marginality was quantified as Euclidean distance in 10-dimensional environmental space between the means of environmental variables over all sites and over the occupied sites, that is, as niche position marginality relative to niche availability. Each niche position marginality axis was scaled by dividing with

the maximum possible distance for that environmental variable. Quantification of niche breadth and position on the categorical variable, dominant tree genus, was performed differently. Niche breadth was calculated as the number of different tree genera occupied by each bird species, and niche position was calculated as the Euclidean distance between tree genera composition over all sites and occupied sites for each species. As both niche breadth and position measures are mathematically constrained at lower sample sizes, only species with more than 10% occupancy (>32 sites) were included in the analysis. To assess the significance of the relationships between species niche traits and occupancy, we used null model analysis. We randomized the metacommunity over the landscape 1000 times with replacement, that is, species were randomly assigned to sampling sites, while their occupancy over the landscape was kept constant to test whether the observed relationship between species' niche traits and occupancy differs from random expectations. Niche breadth and positions were recalculated for each randomization.

LINK TO THE WESTERN PALEARCTIC REGION

We assessed whether species' occupancy across the Istranca Forests' metacommunity is related to their total breeding population (geometric means of minimum and maximum estimated population) and range sizes across WP (BirdLife International 2004), again using negative binomial GLM with log-link. Colonial breeders, post-breeding foraging groups and few species with high leverage (Cook's distance >1) were excluded from the analyses. We assessed whether any Istranca Forests-WP link reflects environmental species sorting by testing whether SSI explains species' occupancy across the Istranca Forests as well as their population and range sizes across the WP. SSI was calculated as variance of average densities among different habitat classes analysed across France (Julliard *et al.* 2006) and acts as a composite measure of niche breadth and position marginality. We used negative binomial GLM with log-link to model occupancy and gamma GLM with log-link to model WP population and range sizes. Species missing SSI data and *Dendrocopos medius* with high leverage (Cook's distance >1) were excluded from the analyses.

Data handling and statistical analysis were performed using R statistical software (R Development Core Team 2011) using vegan package (Oksanen *et al.* 2011) for Gower dissimilarity and Mantel test, MASS package (Venables & Ripley 2002) for GLM, spdep (Bivand 2011) and ncf packages (Bjornstad 2009) for SAR.

Results

A total of 67 breeding bird species were recorded in 317 sites across Istranca Forests (Appendix S1). Median species richness per sampling site was 8, ranging from 2 to 19.

VARIABILITY IN LOCAL COMMUNITY STRUCTURE

Environmental and non-environmental spatial factors explained 28% of the variation in species richness (Table 2). Variance partitioning revealed that 19% and 4% of the variation in local community richness were uniquely attributable to environmental variables and spatial factors, respectively, and 5% was shared. Environmental effects were mediated by both local habitat and

landscape characteristics, with dominant tree genus having the strongest role (Table 2).

Dissimilarity in local community species composition correlated with environmental dissimilarity ($r = 0.26$, $P < 0.001$) and less strongly with geographical distance ($r = 0.08$, $P < 0.001$) in partial Mantel tests, after the effect of one was corrected for the other.

METACOMMUNITY-WIDE OCCUPANCY AND ABUNDANCE

The studied metacommunity was dominated by a suite of widespread, locally abundant species. Species' occupancy was positively related to maximum abundance ($r_d^2 = 0.36$, $P < 0.001$; Fig. 2), but not significantly related to mean abundance ($P = 0.29$). When data were restricted to forest-associated birds, the relationship with maximum abundance became stronger ($r_d^2 = 0.39$, $P < 0.001$) and the relationship with mean abundance became significant and moderate ($r_d^2 = 0.19$, $P < 0.001$). Species' occupancy across the Istranca Forests' bird metacommunity increased with niche breadth ($r^2 = 0.58$) and decreased with niche position marginality ($r^2 = 0.53$; Fig. 3). However, only the relationship of niche position marginality differed significantly from randomized communities ($P < 0.001$), while of niche breadth did not ($P = 0.110$).

LINK TO THE WESTERN PALEARCTIC REGION

Occupancy across the Istranca Forests was related to total WP regional population size ($r_d^2 = 0.27$, $P < 0.001$, for all

species; $r_d^2 = 0.34$, $P < 0.001$, for forest species, Fig. 2) as well as more weakly related to WP regional breeding range size ($r_d^2 = 0.15$, $P < 0.001$ for all species; $r_d^2 = 0.17$, $P = 0.005$ for forest species). Species' occupancy ($r_d^2 = 0.15$, $P = 0.003$; Fig. 4) as well as species' WP population ($r_d^2 = 0.22$, $P < 0.001$) and range sizes ($r_d^2 = 0.15$, $P < 0.001$) decreased with SSI.

Discussion

While community assembly involves complex processes acting at different spatial scales, many ecological studies have focused on a single process or scale. In this study, we assess the roles of environmental species sorting and dispersal at different scales in forest-bird community assembly. Overall, bird assemblages in Istranca Forests constitute a metacommunity dominated by a suite of widespread, locally abundant species in a manner that is consistent with the patterns in the regional bird population across the broader WP region. Furthermore, we found strong environmental control on local community structure, metacommunity-wide occupancy and the link between the latter and the WP regional population and range sizes. Despite this, we also found evidence for a joint, albeit weaker role of non-environmental spatial factors for local community structure.

The Istranca Forests' metacommunity exhibited a strong positive abundance–occupancy relationship, as has been widely documented (Bock & Ricklefs 1983; Hanski, Kouki & Halkka 1993; Gaston *et al.* 2000). Furthermore, species' occupancy across the Istranca Forests' metacommunity significantly related to species' WP regional population and, to a lesser extent, range size, providing evidence for a close link between local and regional communities, as previously proposed for North American land birds (Bock 1987). This consistency in community structure across local to regional scales suggests that bird community patterns at smaller scales may be influenced by the factors acting on regional populations across large spatial scales (Ricklefs 2011).

Null model analyses showed that species' occupancy across Istranca Forests was clearly driven by species' environmental niche (Grinnell 1917). Although both niche breadth (Brown 1984) and niche position marginality (Hanski, Kouki & Halkka 1993) were correlated with occupancy, only the relationship of niche position marginality deviated from the relationship expected in randomized communities. In agreement with this finding, analyses on British breeding birds (Gregory & Gaston 2000) and invertebrate assemblages of tropical intertidal pools (Azeria & Kolasa 2008), as well as simulations with an individual-based model for the relationships between species ranges, speciation and extinction (Birand, Vose & Gavrillets 2012), have all found stronger support for niche availability than niche breadth hypothesis. Furthermore, SSI as a measure of species specialization from a distant part of the WP biome (France) correlated

Table 2. Final reduced spatial (SAR) and non-spatial (OLS) models for local bird species richness in Istranca Forests. Standardized regression coefficients, model R^2 and associated P values are given. R^2 for null model with no environmental variables were given in parenthesis for SAR model. Significant values are shown in bold ($P < 0.05$). F denotes factors from factor analysis (see Table 1) and DT denotes dominant tree genus in stands.

	Bird species richness			
	SAR		OLS	
R^2	0.278 (0.093)		0.240	
P	< 0.001		< 0.001	
	Coef.	P	Coef.	P
Intercept	0.023	0.606	0.019	0.614
F-stand age	−0.083	0.276	−0.106	0.110
F-habitat diversity	0.061	0.365	0.126	0.044
F-stand age diversity	0.146	0.012	0.145	0.007
F-altitude	−0.150	0.026	−0.154	0.007
Canopy cover on-site	−0.182	0.001	−0.209	< 0.001
Stand age on-site	0.209	0.004	0.204	0.004
DT - <i>Alnus</i>	0.047	0.703	0.062	0.610
DT - <i>Fraxinus</i>	0.491	0.002	0.495	0.003
DT - <i>Fagus</i>	−0.027	0.697	−0.027	0.693
DT - <i>Carpinus</i>	−0.137	0.318	−0.039	0.780
DT - <i>Pinus</i>	−0.521	0.001	−0.473	0.002
DT - <i>Populus</i>	−0.570	0.001	−0.534	0.002

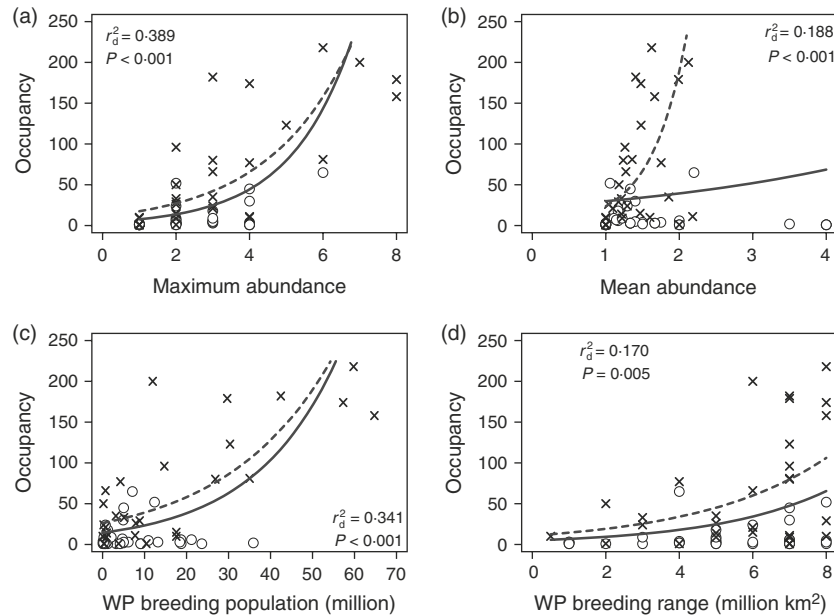


Fig. 2. Bird species occupancy vs. maximum (a) and mean abundance at occupied sites within the Istranca Forests' metacommunity (b), and their total population (c) and breeding range size (d) across the Western Palaearctic (WP) region. Forest-associated species (Appendix S1) are indicated by crosses and the remaining by circles. Generalized linear model regression lines are given for all species (solid) and separately for forest-associated species (dotted). Models fits as deviance ratio (r_d^2) are given for the latter.

with species' occupancy across Istranca Forests as well as species' population and range sizes across the whole WP, indicating consistent niche assembly mechanisms acting across the region. Therefore, our results suggest that the link between the local metacommunity and regional population reflects consistent environmental species sorting processes across a large ecologically coherent region. Species' abundance and occupancy may correlate across scales under a random assembly processes (Hartley 1998). However, both null model and SSI analyses clearly suggested non-random assembly linked to species' environmental niches. Such a broad-scale environmental structuring is consistent with the high dispersal capacity of birds but should not necessarily rule out supplementary dispersal-driven regional effects (Ricklefs 1987).

Overall, occupancy was not related to mean abundance, while it increased with maximum abundance. The relationship between mean abundance and occupancy became significant and moderately strong, when only forest-associated birds were analysed. One explanation for these patterns might be the mass effects (Leibold *et al.* 2004), which could weaken the relationship between occupancy and mean abundance for all birds by allowing non-forest species to sustain high mean abundance in forest stands through dispersal from neighbouring source habitats, like forest openings, agricultural fields or wetlands.

Our analysis indicated that the environmental species sorting and spatial factors jointly drive local community structure within the Istranca Forests' metacommunity,

but with the former having the dominant role. In line with our findings, a meta-analysis of metacommunity studies revealed that niche-based processes tend to dominate community assembly across a wide range of organism groups (Cottenie 2005). Environmental species sorting was also the dominant structuring force in Chilean temperate forest-bird assemblages up to 1000 km (Meynard & Quinn 2008). In contrast, an analysis of breeding bird communities across France (Meynard *et al.* 2011), where environmental gradients are less steep in comparison with the Chilean study, found a joint role of environmental and dispersal processes in community assembly. Moreover, Driscoll & Lindenmayer (2009) similarly found joint roles of environmental and neutral control across Australian bird assemblages in isolated woodland fragments. Istranca Forests span 1000 m in elevation and thus encompass strong environmental gradients within a relatively small geographic extent, probably enhancing the role of environmental control. Overall, dispersal-driven spatial processes might have a more pronounced role for bird communities, when the environmental gradients are not steep and isolation enhances dispersal limitation.

Spatial effects in ecological communities might reflect unmeasured spatially structured environmental variables. However, given our comprehensive environmental data, non-environmental spatial processes such as biotic interactions (Heikkinen *et al.* 2007; Kissling, Rahbek & Böhning-Gaese 2007; Gotelli, Graves & Rahbek 2010) and dispersal limitation (White & Hurlbert 2010) seem plausible underlying ecological mechanisms. Recent simulation studies have criticized the reliability of variation

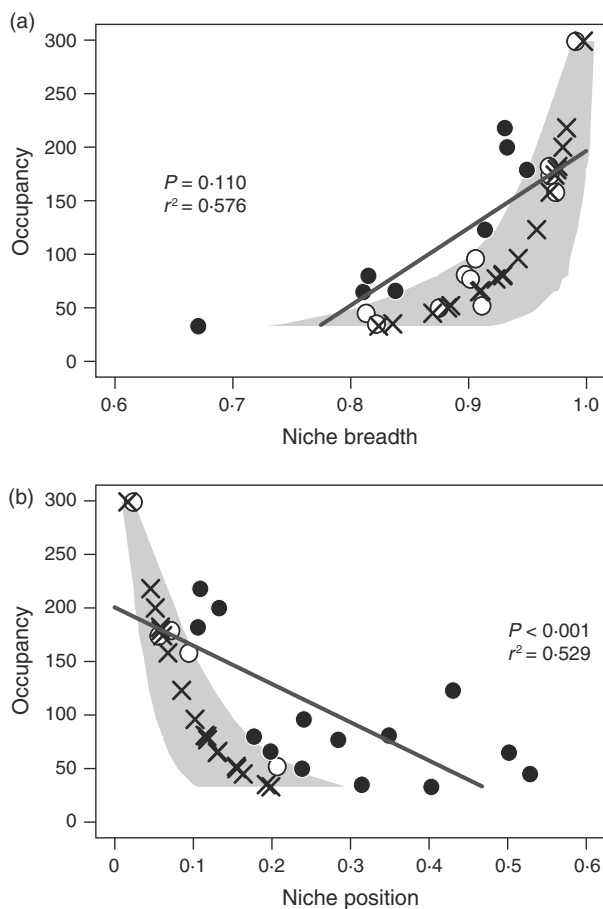


Fig. 3. Bird species' metacommunity-wide occupancy vs. niche breadth (a) and niche position marginality (b) in Istranca Forests. Linear regression lines and associated r^2 s are shown. Niche breadth and position were only analysed for species with more than 10% occupancy, and significance of the relationships was assessed by randomization. Crosses indicate mean niche breadth and position marginality in randomized communities, with the shaded area showing confidence intervals for estimated means. Black circles indicate significantly different niche breadth and position values ($P < 0.05$). Significance of the relationships in comparison with that of randomized communities is given.

partitioning in analysing different community assembly processes (Gilbert & Bennett 2010; Smith & Lundholm 2010). However, Tuomisto, Ruokolainen & Ruokolainen (2012) showed that these shortcomings are only important for data sets with large compositional gradients and high dissimilarity saturation (frequency of site pairs that do not share any species). Dissimilarity saturation in the present data set was only 2%.

In conclusion, we found that environmental species sorting played a dominant role in forest-bird community assembly across scales, while non-environmental spatial factors, perhaps dispersal or biotic interactions played a supplementary role in local community assembly. The Istranca Forests species' occupancy patterns were strongly consistent with population and range sizes across the WP region as well as linked to a measure of habitat specializa-

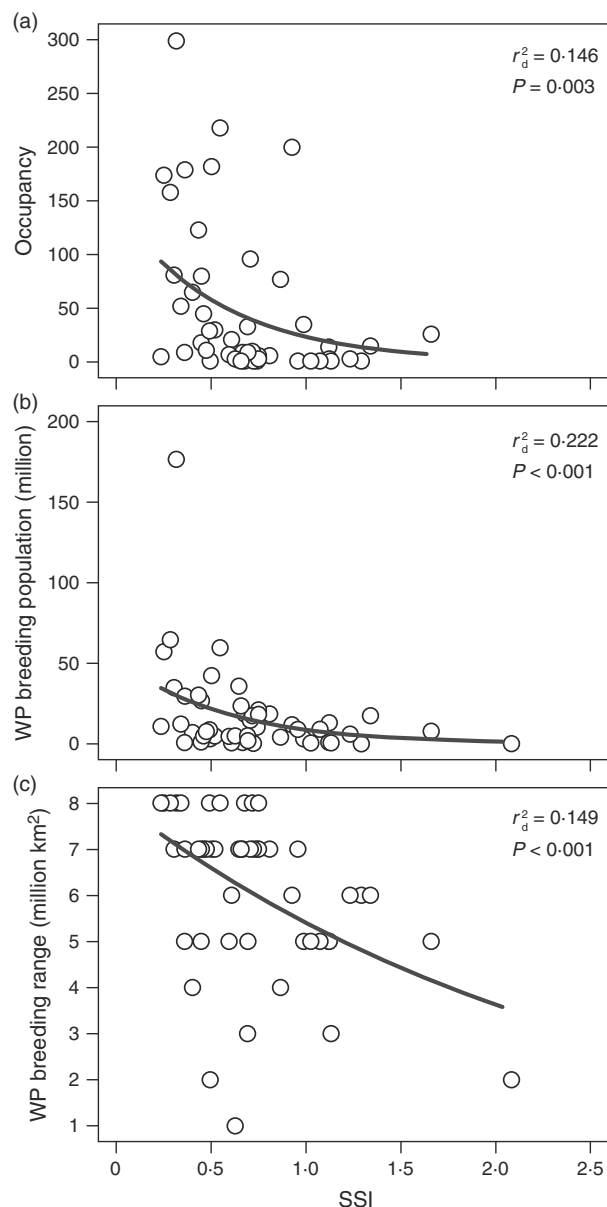


Fig. 4. Relationship between bird species specialization index (SSI) calculated as a composite measure for species' niche breadth and marginality in terms of habitat occupancy across France, and metacommunity-wide occupancy across Istranca Forests in Turkey (a) as well as total population (b) and breeding range size (c) for the Western Palaearctic (WP) region. Generalized linear model regression lines as well as statistical significances and models fits as deviance ratio (r^2_d) are given.

tion from a distant part of the biome, suggesting that community assembly is shaped by consistent environmental species sorting processes acting across the whole region as proposed by Finlayson (2011). Nonetheless, we also found a significant role of purely spatial factors in local community assembly. An important avenue for future research would thus be to further elucidate to what extent dispersal (White & Hurlbert 2010) and biotic interactions such as trophic interactions (Kissling, Rahbek &

Böhning-Gaese 2007), facilitation (Heikkinen *et al.* 2007) and conspecific attraction (Gotelli, Graves & Rahbek 2010) interact with environmentally driven niche processes to control bird community assembly across scales.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Species list.

Appendix S2. Factor Analysis.

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SUPPORTING INFORMATION

APPENDIX S1. SPECIES LIST

Bird species included in the present study with their occupancy and total abundance. The habitat association of each species was classified into five categories following Cramp and Simmons (2006): forest specialist (FS), forest preference (FP), inhabit forests (IF), inhabit forest edge (FE), indifferent to forest presence as long as other habitat requirements are met (I), avoid forests (A). FS and FP were grouped as forest-associated in the analyses.

Family	Latin name	English name	Occup.	Abund.	Habitat association
Accipitridae	<i>Accipiter nisus</i>	Eurasian Sparrowhawk	1	1	FS
Accipitridae	<i>Buteo buteo</i>	Common Buzzard	9	11	FP
Aegithalidae	<i>Aegithalos caudatus</i>	Long-tailed Tit	11	24	FP
Alaudidae	<i>Lullula arborea</i>	Woodlark	9	11	I
Alcedinidae	<i>Alcedo atthis</i>	Common Kingfisher	4	7	I
Caprimulgidae	<i>Caprimulgus europaeus</i>	European Nightjar	24	31	I
Certhiidae	<i>Certhia brachydactyla</i>	Short-toed Treecreeper	33	40	FS
Certhiidae	<i>Certhia familiaris</i>	Eurasian Treecreeper	26	27	FP
Charadriidae	<i>Charadrius dubius</i>	Little Ringed Plover	2	7	A
Cinclidae	<i>Cinclus cinclus</i>	White-throated Dipper	2	2	I
Columbidae	<i>Columba oenas</i>	Stock Dove	1	1	FE
Columbidae	<i>Columba palumbus</i>	Common Wood Pigeon	52	55	FE
Columbidae	<i>Streptopelia turtur</i>	European Turtle Dove	45	60	I
Corvidae	<i>Corvus cornix</i>	Hooded Crow	5	7	A
Corvidae	<i>Garrulus glandarius</i>	Eurasian Jay	29	34	FS
Emberizidae	<i>Emberiza cirrus</i>	Cirl Bunting	1	4	FE
Emberizidae	<i>Emberiza citrinella</i>	Yellowhammer	1	2	FE
Emberizidae	<i>Emberiza hortulana</i>	Ortolan Bunting	1	1	IF
Emberizidae	<i>Miliaria calandra</i>	Corn Bunting	3	5	A
Falconidae	<i>Falco subbuteo</i>	Eurasian Hobby	1	2	FE
Fringillidae	<i>Carduelis carduelis</i>	Eurasian Goldfinch	6	12	I
Fringillidae	<i>Carduelis chloris</i>	Eurasian Greenfinch	6	7	FE
Fringillidae	<i>Coccothraustes coccothraustes</i>	Hawfinch	35	127	FS
Fringillidae	<i>Fringilla coelebs</i>	Common Chaffinch	299	979	FP
Hirundinidae	<i>Delichon urbicum</i>	Common House Martin	1	60	A
Laniidae	<i>Lanius collurio</i>	Red-backed Shrike	1	1	A
Meropidae	<i>Merops apiaster</i>	European Bee-eater	1	4	A
Motacillidae	<i>Motacilla alba</i>	White Wagtail	3	4	I

Motacillidae	<i>Motacilla cinerea</i>	Grey Wagtail	4	6	I
Muscicapidae	<i>Ficedula parva</i>	Red-breasted Flycatcher	1	1	FS
Muscicapidae	<i>Ficedula semitorquata</i>	Semi-collared Flycatcher	10	16	FS
Muscicapidae	<i>Muscicapa striata</i>	Spotted Flycatcher	10	10	FP
Oriolidae	<i>Oriolus oriolus</i>	Eurasian Golden Oriole	30	42	IF
Paridae	<i>Cyanistes caeruleus</i>	Blue Tit	179	356	FP
Paridae	<i>Parus major</i>	Great Tit	158	263	FP
Paridae	<i>Parus palustris</i>	Marsh Tit	77	135	FS
Passeridae	<i>Passer hispaniolensis</i>	Spanish Sparrow	1	4	I
Phalacrocoracidae	<i>Phalacrocorax carbo</i>	Great Cormorant	1	1	I
Picidae	<i>Dendrocopos leucotos</i>	White-backed Woodpecker	1	2	FS
Picidae	<i>Dendrocopos major</i>	Great Spotted Woodpecker	96	121	FS
Picidae	<i>Dendrocopos medius</i>	Middle Spotted Woodpecker	50	59	FS
Picidae	<i>Dendrocopos minor</i>	Lesser Spotted Woodpecker	21	23	FS
Picidae	<i>Dryocopus martius</i>	Black Woodpecker	14	17	FS
Picidae	<i>Picus canus</i>	Grey-headed Woodpecker	24	31	FS
Picidae	<i>Picus viridis</i>	European Green Woodpecker	9	9	FP
Rallidae	<i>Gallinula chloropus</i>	Common Moorhen	1	1	A
Scolopacidae	<i>Tringa ochropus</i>	Green Sandpiper	3	4	FE
Sittidae	<i>Sitta europaea</i>	Eurasian Nuthatch	200	425	FS
Strigidae	<i>Otus scops</i>	European Scops Owl	9	10	IF
Strigidae	<i>Strix aluco</i>	Tawny Owl	66	84	FP
Sturnidae	<i>Sturnus vulgaris</i>	Common Starling	2	2	FE
Sylviidae	<i>Hippolais icterina</i>	Icterine Warbler	2	3	FE
Sylviidae	<i>Hippolais pallida</i>	Eastern Olivaceous Warbler	3	4	I
Sylviidae	<i>Phylloscopus collybita</i>	Common Chiffchaff	182	256	FS
Sylviidae	<i>Phylloscopus sibilatrix</i>	Wood Warbler	15	22	FS
Sylviidae	<i>Sylvia atricapilla</i>	Blackcap	81	110	FS
Sylviidae	<i>Sylvia communis</i>	Common Whitethroat	1	1	I
Sylviidae	<i>Sylvia curruca</i>	Lesser Whitethroat	3	5	FE
Sylviidae	<i>Sylvia melanocephala</i>	Sardinian Warbler	3	3	IF
Troglodytidae	<i>Troglodytes troglodytes</i>	Winter Wren	123	182	FP
Turdidae	<i>Erithacus rubecula</i>	European Robin	218	354	FP
Turdidae	<i>Luscinia megarhynchos</i>	Common Nightingale	65	143	IF
Turdidae	<i>Phoenicurus phoenicurus</i>	Common Redstart	1	1	FP
Turdidae	<i>Turdus merula</i>	Common Blackbird	174	257	FP
Turdidae	<i>Turdus philomelos</i>	Song Thrush	80	99	FP
Turdidae	<i>Turdus viscivorus</i>	Mistle Thrush	7	8	FE
Upupidae	<i>Upupa epops</i>	Eurasian Hoopoe	18	21	I

APPENDIX S2. FACTOR ANALYSIS

Table 2. Loadings of landscape environmental variables in the Factor Analysis, with the factors named after the environmental characteristics that they most strongly describe*. Distance bands (m) are given in parentheses for each covariate. Correlation coefficients larger than 0.5 are shown in bold.

	Stand age	Habitat diversity	Tree genus richness	Stand age diversity	Altitude	Canopy cover diversity
Altitude on-site	0.33	-0.29	-0.01	0.09	0.67	0.18
Altitudinal range (250)	0.16	-0.13	-0.03	0.07	0.81	0.01
Altitudinal range (500)	0.17	-0.14	-0.02	0.07	0.90	0.00
Altitudinal range (1000)	0.20	-0.21	-0.04	0.05	0.93	0.01
Altitudinal range (3000)	0.31	-0.29	-0.07	0.03	0.75	0.05
Habitat richness (250)	-0.01	0.66	0.03	0.20	0.02	0.23
Habitat richness (500)	0.06	0.74	0.01	0.19	-0.10	0.27
Habitat richness (1000)	0.02	0.75	0.05	0.00	-0.14	0.08
Habitat richness (3000)	0.03	0.56	0.11	-0.07	-0.25	-0.02
Habitat evenness (500)	0.01	0.77	0.02	0.22	-0.09	0.17
Habitat evenness (1000)	0.04	0.97	0.00	0.11	-0.12	0.08
Habitat evenness (3000)	0.00	0.78	0.14	-0.01	-0.16	0.05
Dominant tree genus richness (250)	0.22	0.01	0.81	0.25	-0.03	0.07
Dominant tree genus richness (500)	0.34	0.08	0.73	0.25	-0.15	0.04
Dominant tree genus richness (1000)	0.39	0.07	0.56	0.19	-0.12	-0.07
Dominant tree genus richness (3000)	0.38	0.04	0.34	0.17	-0.24	-0.01
Dominant tree genus evenness (250)	0.17	-0.02	0.76	0.20	0.07	0.03
Dominant tree genus evenness (500)	0.26	0.05	0.90	0.19	0.01	0.02
Dominant tree genus evenness (1000)	0.40	0.15	0.83	0.10	-0.02	-0.05
Dominant tree genus evenness (3000)	0.52	0.12	0.62	0.05	0.01	0.04
Percent forest cover (1000)	-0.05	-0.95	0.03	-0.07	0.09	-0.02
Per cent forest cover (3000)	-0.03	-0.84	-0.01	0.05	0.13	-0.01
Dominant stand age (250)	0.87	0.08	0.19	0.07	0.07	0.09
Dominant stand age (500)	0.88	0.09	0.18	0.04	0.12	0.08
Dominant stand age (1000)	0.84	0.04	0.24	0.06	0.20	0.02
Dominant stand age (3000)	0.68	-0.10	0.18	-0.04	0.43	0.06
Average stand age (250)	0.94	0.06	0.20	0.12	0.12	0.07
Average stand age (500)	0.95	0.06	0.22	0.11	0.18	0.05
Average stand age (1000)	0.91	0.02	0.25	0.09	0.23	0.03
Average stand age (3000)	0.76	-0.11	0.26	0.06	0.33	-0.01

Stand age class richness (250)	0.01	0.14	0.13	0.82	0.05	0.15
Stand age class richness (500)	0.11	0.10	0.15	0.73	0.02	0.24
Stand age class richness (1000)	0.17	-0.10	0.21	0.43	0.07	0.25
Stand age class richness (3000)	0.10	-0.08	-0.14	0.01	0.00	0.19
Stand age class evenness (250)	0.04	0.15	0.12	0.80	0.07	0.07
Stand age class evenness (500)	0.07	0.17	0.15	0.93	-0.01	0.08
Stand age class evenness (1000)	0.10	0.15	0.22	0.75	0.05	0.15
Stand age class evenness (3000)	0.07	-0.16	0.23	0.33	0.10	0.17
Canopy cover class richness (250)	-0.03	0.32	0.05	0.15	0.07	0.73
Canopy cover class richness (500)	0.01	0.17	0.02	0.17	0.03	0.84
Canopy cover class richness (1000)	0.10	-0.09	0.05	0.19	0.16	0.66
Canopy cover class richness (3000)	0.22	-0.14	-0.11	0.13	0.20	0.36
Canopy cover class evenness (500)	0.00	0.42	0.04	0.20	-0.04	0.77
Canopy cover class evenness (1000)	0.03	0.56	0.03	0.16	-0.06	0.69
Canopy cover class evenness (3000)	-0.06	0.35	0.07	0.07	-0.11	0.60

*Sites along Turkey-Bulgaria border lacked landscape data in their Bulgarian part and the lacking data were replaced with the corresponding global average for the Turkish sites. Covariates with too skewed distributions in some distance bands were excluded from analysis (excluded distance bands: 250m for habitat evenness; 250 and 500 m for percent forest cover; 250 m for canopy cover class evenness). Canopy cover class dominance and average canopy cover were excluded from the analyses for all distance bands as they exhibited very limited variation.

PAPER II

Contrasting roles of water chemistry, lake morphology, land-use, climate and spatial processes in driving phytoplankton richness in the Danish landscape

Korhan Özkan ^{a,b}, Erik Jeppesen ^{a,c,d}, Martin Søndergaard ^a, Torben L. Lauridsen ^{a,d}, Lone Liboriussen ^a and Jens-Christian Svenning ^b

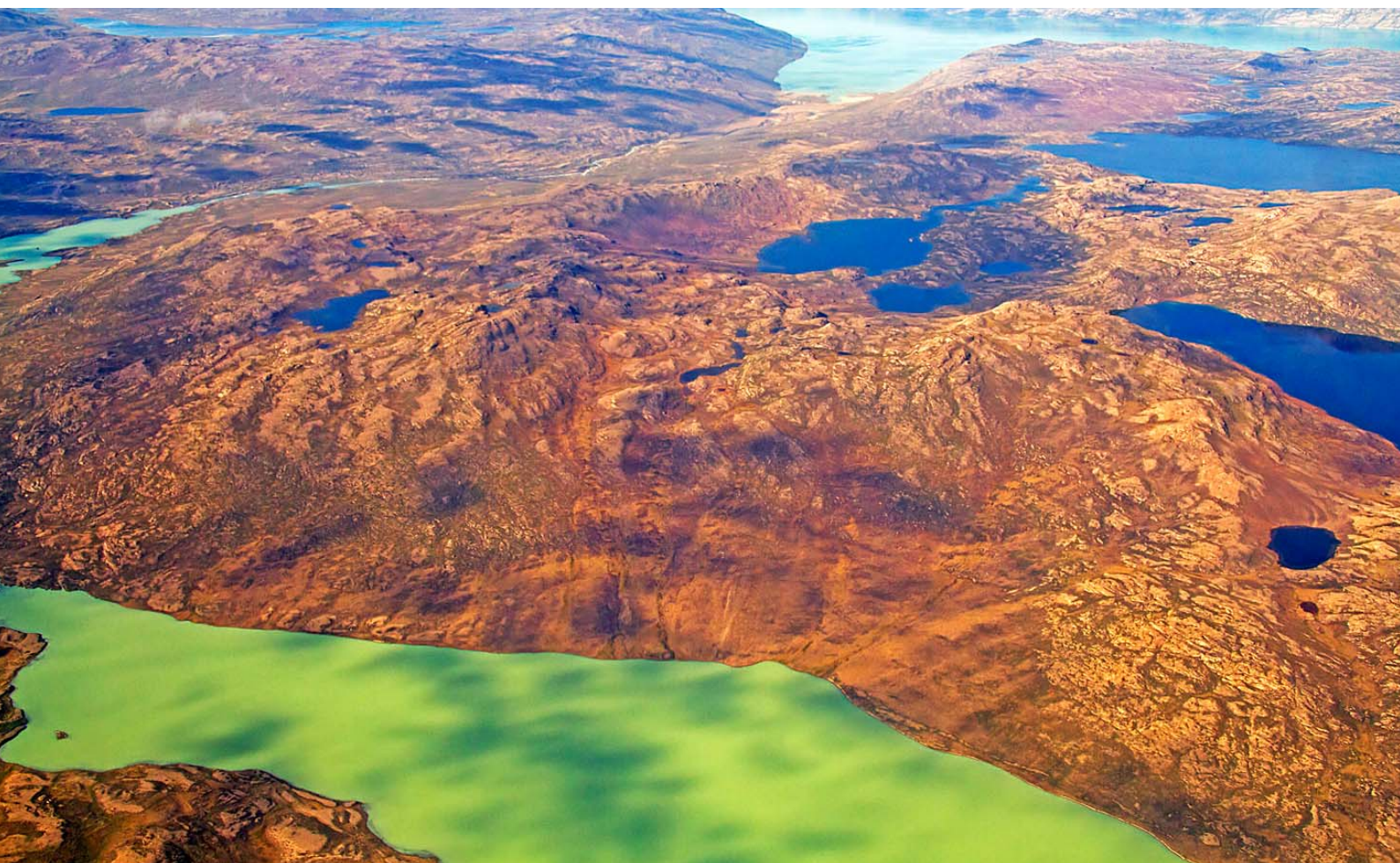
^a Freshwater Ecology Group, Department of Bioscience, Aarhus University, Silkeborg, Denmark

^b Ecoinformatics and Biodiversity Group, Department of Bioscience, Aarhus University, Aarhus, Denmark

^c Greenland Climate Research Centre, Greenland Institute of Natural Resources, Nuuk, Greenland

^d Sino-Danish Centre for Education and Research, Beijing, China

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Contrasting roles of water chemistry, lake morphology, land-use, climate and spatial processes in driving phytoplankton richness in the Danish landscape

Korhan Özkan · Erik Jeppesen ·
Martin Søndergaard · Torben L. Lauridsen ·
Lone Liboriussen · Jens-Christian Svenning

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Abstract Understanding of the forces driving the structure of biotic communities has long been an important focus for ecology, with implications for applied and conservation science. To elucidate the factors driving phytoplankton genus richness in the Danish landscape, we analyzed data derived from late-summer samplings in 195 Danish lakes and ponds in a spatially-explicit framework. To account for the

uneven sampling of lakes in the monitoring data, we performed 1,000 permutations. A random set of 131 lakes was assembled and a single sample was selected randomly for each lake at each draw and all the analyses were performed on permuted data 1,000 times. The local environment was described by lake water chemistry, lake morphology, land-use in lake catchments, and climate. Analysis of the effects of four groups of environmental factors on the richness of the main groups of phytoplankton revealed contrasting patterns. Lake water chemistry was the strongest predictor of phytoplankton richness for all groups, while lake morphology also had a strong influence on Bacillariophyceae, Cyanobacteria, Dinophyceae, and Euglenophyceae richness. Climate and land-use in catchments contributed only little to the explained variation in phytoplankton richness, although both factors had a significant effect on Bacillariophyceae richness. Notably, total nitrogen played a more important role for phytoplankton richness than total phosphorus. Overall, models accounted for ca. 30% of the variation in genus richness for all phytoplankton combined as well as the main groups separately. Local spatial structure (<30 km) in phytoplankton richness suggested that connectivity among lakes and catchment-scale processes might also influence phytoplankton richness in Danish lakes.

Keywords Freshwater algae · Metacommunity structure · Nitrogen concentration · Shallow lakes · Spatial structure

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K. Özkan (✉) · E. Jeppesen · M. Søndergaard ·
T. L. Lauridsen · L. Liboriussen
Freshwater Ecology Group, Department of Bioscience,
Aarhus University, Silkeborg, Denmark
e-mail: korhan_oz@yahoo.com

K. Özkan · J.-C. Svenning
Ecoinformatics and Biodiversity Group, Department of
Bioscience, Aarhus University, Aarhus, Denmark

E. Jeppesen
Greenland Climate Research Centre, Greenland Institute
of Natural Resources, Nuuk, Greenland

E. Jeppesen
Sino-Danish Centre for Education and Research (SDC),
Beijing, China

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Introduction

Understanding the factors driving phytoplankton community structure, assembly, and functioning in space and time is essential for the management of the world's water resources that are facing a wide range of anthropogenic stressors. The central role of lakes as a water resource and the increasing occurrence of water quality problems, including toxic algal blooms due to eutrophication (Huisman et al., 2005), have promoted research on phytoplankton–environment relationship, notably in terms of phytoplankton biomass and chlorophyll-*a* (chl-*a*) (McCauley & Murdoch, 1987). However, these measures do not comprehensively describe phytoplankton community function, including their toxicity (Scheffer et al., 1997) and quality as a food source (Sterner & Elser, 2002), as these strongly depend on phytoplankton taxa composition. On a general level, species diversity—both richness and composition—has been increasingly recognized to influence community dynamics and functioning, notably as regards to community stability and productivity (Tilman et al., 1996; McCann, 2000). Recent analyses indicate that resource use efficiency and diversity in phytoplankton communities are positively related (Ptacnik et al., 2008) in a similar way as in higher organisms (Hooper et al., 2005). Therefore, a more detailed understanding of the factors driving phytoplankton community richness and composition is needed.

Recent advances in metacommunity ecology have indicated that community assembly is a complex phenomenon driven by the interplay of local environmental sorting and dispersal processes (Leibold et al., 2004). Phytoplankton communities, like those of other micro-organisms, have long been regarded as being primarily driven by the local environment due to the high dispersal capacity and cosmopolitan distribution of some phytoplankton species (Finlay, 2002) assumed representative for the whole group. Inorganic nutrients like total phosphorus (TP) and total nitrogen (TN) and pH as well as physical habitat properties like lake morphology and water column stratification have been recognized to be among the primary local environmental predictors of phytoplankton richness (Reynolds, 1984). Furthermore, trophic relations, notably zooplankton grazing (Fott et al., 1980; Andersson & Cronberg, 1984; Jeppesen et al., 1998) and other biotic interactions, such as host-parasitism dynamics (Ibelings et al., 2011), also play an important role in

structuring phytoplankton communities. However, recent studies indicate that dispersal-driven regional processes may also be an important determinant of local phytoplankton richness through immigration from the regional species pool (Ptacnik et al., 2010).

We aimed at assessing the relative roles of four main groups of environmental factors, namely lake water chemistry (water chemistry hereafter), lake morphology, land-use in catchments (land-use hereafter) climate as drivers of phytoplankton genus richness across the Danish landscape. Furthermore, we used spatial multiple regressions to elucidate additional spatial effects on phytoplankton richness as a potential indicator of dispersal processes. We hypothesized that phytoplankton richness is primarily affected by water chemistry, while TP and TN have joint importance as primary nutrient sources. However, we also expected that lake morphology, land-use, and climate potentially drive phytoplankton richness and the relative importance of these potential driving factors would differ among the main phytoplankton groups given their different ecological requirements and life histories. All analyses were, therefore, also performed separately for each main phytoplankton group. However, we expected limited or no effect of large-scale dispersal in structuring phytoplankton richness, due to high dispersal capacity of phytoplankton and the limited spatial area covered by this study.

Methods

Phytoplankton database

The phytoplankton database contained information on 395 lakes sampled within the framework of the Danish Lake Monitoring Program since 1981. Phytoplankton samples were collected year-round from mid-lake surface water and counting was performed using a standardized method. Details of the monitoring program can be found elsewhere (Kronvang et al., 1993; Søndergaard et al., 2005). We re-organized the phytoplankton database to have a compatible dataset for the analysis. Only late-summer records (July 15–September 30) after 1994 were included in the analyses. We attempted to do similar analyses for other seasons; however, sufficient data was not available for the statistical randomization approach (explained below) for other seasons. To account for the possible

identification efficiency differences among the experts involved in the monitoring program ($n > 30$), we excluded samples identified by the experts who had consistently low phytoplankton genus richness counts. If more than 20% of the taxa in a sample were only identified to levels higher than genus, that particular sample was excluded. In addition, samples lacking late-summer TP measurements for the same year, lakes without depth or chl-*a* information, lakes on the island of Bornholm (due to its isolated location) were excluded. Last, acidified (median pH < 6) and brackish lakes (median salinity > 0.5 per mille or median conductivity > 1,600 mS m⁻¹ or maximum conductivity > 3,200 mS m⁻¹) were excluded. The remaining dataset contained 2,105 samples (collected between 1994 and 2008) from 195 lakes.

We chose genus richness as the main unit of phytoplankton diversity to reduce the possible influence of the identification skills of the taxonomists involved in this monitoring program. Phytoplankton species and genus richness were strongly correlated ($r = 0.98$) as in other studies (Ptacnik et al., 2010; Gallego et al., 2012). The occurrence of very rare species might be underestimated even in such long-term studies. However, sampling and identifications were performed using the same guideline, and thus such effect should be same for all lakes. Nonetheless, phytoplankton taxon richness used in the present analysis might reflect phytoplankton evenness to some extent, as the more abundant genera will be least affected by such under-sampling.

Environmental predictors

We compiled four groups of environmental predictors relevant for phytoplankton genus richness (phytoplankton richness hereafter): water chemistry; lake morphology, land-use, and climate. Water chemistry

including TP, TN, chl-*a*, and pH was sampled from mid-lake surface water at the same time as phytoplankton. Details of the chemistry analysis can be found elsewhere (Søndergaard et al., 2005). When a water chemistry variable was missing for a phytoplankton sample, it was replaced with the most relevant available data for the same lake: first, a sample from another station in the same sampling; second (if a substitute sample was not available at the first step) the mean value of the water chemistry variable for late summer in the same year; third, mean for late summer across all years (Table 1). Data on lake morphology including lake area and maximum depth were also compiled. If maximum depth was not available, depth in the middle of the lake was used (Table 1). A Danish land-use map compiled during late 1990s was used to characterize land-use in the corresponding catchments using ArcGIS (ESRI, 2008). Total cover of urban (all human-associated land, including settlements, industrial areas, and housings in the countryside), agricultural (excluding gardening and grazed areas), and forested land was calculated for each catchment. Last, data on mean air temperature, precipitation, wind speed, and solar radiation were compiled for 7 days before each sampling for all lakes (Danish Meteorological Institute, unpublished data). The summary of the environmental predictors included in the analysis is given in Table 2.

Permutations of the dataset

The lakes included in the Danish Lake Monitoring Program were sampled with varying intensity, which is common in long-term, broad-scale monitoring studies. Such differences in sampling effort make it difficult to utilize conventional statistical methods directly. Slightly more than half of the lakes included in the selected dataset were represented by a single

Table 1 Summary of data treatment for missing values in lake water chemistry and morphology

	TP	TN	Chl- <i>a</i>	pH	Max. depth
Specific sample	0.95	0.95	0.93	0.84	0.84
Lake average	0.02	0.02	0.02	0.02	–
Late summer average	0.03	0.03	0.04	0.04	–
Long term average	0.00	0.00	0.01	0.10	–

Frequencies of specific samples collected with phytoplankton samplings and replacements with different averages are given. The frequency of maximum depth measurements is given. When maximum depth measurement is missing, the depth in the middle of a lake was used

sample, while a smaller number of lakes had been sampled intensively throughout the years (minimum, median, and maximum 1, 1, and 75 samples per lake, respectively). To account for the uneven sampling effort among lakes, we used random permutations of the dataset. At each permutation, 131 lakes, which meet 10:1 subjects to predictors' ratio in multiple regressions (Harrell, 2001), were randomly drawn out of 195 lakes and one random sample for each lake was selected subsequently. All the statistical analyses (explained below) were performed for 1,000 permutations and the results were reported accordingly.

Relative roles of different environmental predictors in driving phytoplankton genus richness

Genus richness for all phytoplankton and each of the main groups (Bacillariophyta, Chlorophyta, Chrysophyceae, Cryptophyceae, Cyanobacteria, Dinophyceae, Euglenophyceae) was separately modeled with 13 environmental predictors related to water chemistry, lake morphology, land-use, and climate in ordinary least squares (OLS) multiple regressions. We also quantified the dominance of each main phytoplankton group in the total phytoplankton richness (% richness) as another set of response variables. Predictors were log-10 transformed when it was necessary to improve the distribution of the data (TN, TP, chlorophyll-*a*, lake area, lake depth, precipitation, and urban, agricultural and forested land cover in the catchments), and then standardized to zero mean and unit standard deviation. Response variables deviating too much from the normal distribution were also square-root transformed (genus richness for Chrysophyceae and Euglenophyceae; and percent genus richness for Chrysophyceae, Dinophyceae, and Euglenophyceae). Orthogonal second-order polynomials (Kennedy & Gentle, 1980) for TP, TN, and pH were included in multiple regressions to account for possible unimodal relationships. Models were simplified by backward stepwise selection based on Akaike Information Criterion (AIC) (Venables & Ripley, 2000). Model behavior was checked with diagnostic plots (for selected subsets of the data) and no strong deviations were observed. A few predictors were highly correlated ($r < 0.72$); however, there was no strong multicollinearity in the models (mean and max variance inflation factors (VIF) in the models across all permutations were 1.9 and 2.3).

To elucidate the relative roles of four groups of environmental factors in driving phytoplankton richness, we calculated the variance explained by each group relative to total variance explained by all predictors for all models across all permutations. After AIC selection for each model at each permutation, the selected predictors were divided into the four groups and the variance explained by each group was calculated relative to total explained variance by all selected predictors for that model as percentage. If none of the predictors belonging to a given group of environmental factors was selected in a model, the variance explained by that group was recorded as zero. Furthermore, selection frequencies of each main group of predictors (i.e., if any one of the predictors belonging to that group was selected) were also calculated for each model across all permutations.

To assess the significance of each environmental predictor, we reported their selection frequency and significance of their coefficients for all models across all permutations. We assessed if the coefficient of a predictor was significantly different from zero (i.e., confidence intervals do not include 0 with $\alpha = 0.05$) with three different calculations: (i) over just the final models that included the predictor; (ii) over all final models, after the predictor's coefficient was assigned to zero, when the predictor was excluded in AIC selection; and (iii) over all models without any selection.

Role of spatial structure in driving phytoplankton genus richness

We used simultaneous autoregressive regressions (SAR), which perform best in simulation studies (Kissling & Carl, 2008), to account for the spatial structure in multiple regressions. SAR models are built on OLS regressions by incorporating an additional term accounting for the spatial autocorrelation in the data (Anselin, 1988). Neighborhood in SAR models was calculated with the minimum distance to keep all lakes connected (minimum, mean, and maximum neighborhood distances of 26.6, 38.4, and 67.9 km across all permutations). As coefficient of determination is not supplied with SAR models, squared Pearson correlation coefficient was used in all models for comparison (Legendre & Legendre, 1998). To compare the variance explained by the spatial structure and environmental factors, we calculated the percentage increase in the total explained variance in SAR models

in comparison to the OLS models with all the selected environmental predictors. Furthermore, we used Moran's *I* autocorrelograms (with 30 km neighborhood) to visualize the spatial structure in the data. The spatial autocorrelation was reduced (Moran's $I < 0.05$) in the residuals of SAR models but not, however, completely removed.

Data handling and statistical analyses were performed with the R statistical software (R Development Core Team, 2008) using *spdep* (Bivand, 2009) and *ncf* packages (Bjornstad, 2009) for SAR.

Results

Lakes included in the analysis were mostly small and shallow (Table 2) with no altitudinal difference. A total of 276 phytoplankton genera were identified in the selected subset of the data. Chlorophyta was the richest group with 125 genera, followed by Bacillariophyceae (45) and Cyanobacteria (44), while the remaining groups were represented by 7–15 genera. Phytoplankton richness varied extensively with minimum, median, and maximum richness of 6, 35, and 82, respectively. The phytoplankton groups included in the analysis constituted on average 95% ($\pm 4\%$ SD) of total phytoplankton richness in the samples.

Relative roles of different environmental predictors in driving phytoplankton genus richness

Environmental predictors together explained almost one third of the variation in phytoplankton richness on average across 1,000 permutations (Table 3; Fig. 1). Water chemistry had the strongest impact on phytoplankton richness as at least one water chemistry

variable was selected in all permutations (Table 4), and selected water chemistry variables accounted for ca. 70% of the variation explained by all environmental variables (Fig. 2). Lake morphology and climate accounted for only ca. 10% of the total explained variation, but was selected in the majority of permutations. In contrast, a land-use variable was only selected in approximately half of the permutations and accounted for a negligible amount of total explained variation in phytoplankton richness.

Concerning the specific predictors, pH was selected in almost all permutations and significantly unimodally related to phytoplankton richness (Table 3). Similarly, chl-*a* was selected for the majority of models with a significant positive relationship. TN was selected in almost two-thirds of the permutations with a significant unimodal relationship. In comparison, TP was selected only in 379 permutations, with a negative relationship to phytoplankton richness. Both lake area and depth were selected in 789 and 583 permutations, with a positive and negative relationship, respectively. Total agricultural land area in catchments was the only land-use variable having significant coefficient estimates (negative); it was, however, selected only in 361 permutations. Considering climatic variables, both average air temperature and solar radiation were selected in 563 and 616 permutations, with a negative and positive relationship to phytoplankton richness, respectively.

Role of spatial structure in driving phytoplankton genus richness

Phytoplankton richness was spatially structured, with a positive spatial autocorrelation at small distances (ca. <30 km), but none or negative spatial

Table 2 Summary of water chemistry, morphology, and catchment area of the lakes, as well as average air temperature, precipitation, wind speed, and solar radiation measured for the week before each sampling

	TP (mg l ⁻¹)	TN (mg l ⁻¹)	Chl- <i>a</i> (mg l ⁻¹)	pH	Lake area (ha)	Max. depth (m)
Minimum	<0.001	0.210	0.001	4.90	0.1	0.3
Median	0.120	1.400	0.053	8.44	2.3	3.0
Maximum	4.100	10.300	1.100	10.25	399.7	33.0
	Catchment area (ha)	Air temperature (°C)	Precipitation (mm)	Wind speed (m s ⁻¹)	Solar radiation (W m ⁻²)	
Minimum	0.3	9.4	0	1.7	45.1	
Median	30.1	16.1	1.7	3.9	171.5	
Maximum	591.6	23.2	17.1	11.4	312.8	

Table 3 Summary of OLS regressions after selection across 1,000 permutations of the dataset

	Phytop.	Bacill.	Chloro.	Chryso.	Crypto.	Cyanob.	Dinoph.	Euglen.
<i>P</i>	*** (***/***)	*** (***/***)	*** (***/***)	*** (***/***)	** (***/*)	*** (***/***)	*** (***/***)	*** (***/***)
<i>R</i> ²	28 (18/39)	33 (21/44)	25 (15/35)	29 (19/39)	17 (8/27)	24 (14/34)	30 (19/41)	31 (20/41)
TP	379; −00	129; 000	303; 000	713; −0−	213; −00	426; 000	759; −0−	511; +0+
TP ^{^2}	379; 000	129; 000	303; 000	713; 000	213; 000	426; 000	759; 000	511; 000
TN	680; 000	697; −0−	596; 000	330; 000	742; 000	689; 00+	838; 000	208; −00
TN ^{^2}	680; −0−	697; 000	596; −0−	330; −00	742; −0−	689; 000	838; −0−	208; 000
Chl− <i>a</i>	986; +++	680; +0+	995; +++	284; −00	97; 000	835; +0+	353; +00	377; +00
pH	999; −−−	995; −−−	999; −−−	997; −−−	907; −−−	773; 000	307; 000	1,000; −−−
pH ^{^2}	999; −−−	995; −−−	999; 000	997; 000	907; −0−	773; −0−	307; 000	1,000; 000
Area	789; +0+	988; +++	465; +00	184; −00	310; +00	905; +++	252; 000	254; −00
Depth	583; −0−	749; −0−	692; −0−	596; −00	117; 000	126; 000	954; +++	990; −−−
Urban	102; 000	164; 000	85; 000	180; −00	600; −0−	110; 000	272; −00	80; 000
Agri.	361; −00	511; −00	164; −00	681; −0−	148; +00	163; −00	318; −00	693; −0−
Forest	103; 000	372; +00	167; 000	782; +0+	107; 000	140; 000	533; +00	122; 000
Wind	125; 000	150; 000	170; 000	127; 000	125; 000	118; 000	155; 000	455; −00
Temp.	563; −00	908; −−−	372; −00	288; 000	135; 000	425; −00	251; 000	265; 000
Preci.	155; 000	536; −00	170; 000	136; 000	105; 000	115; 000	137; 000	121; 000
Sol. R.	616; +00	549; +00	488; +00	380; +00	178; 000	405; +00	248; 000	283; 000
	% Bacill.	% Chloro.	% Chryso.	% Crypto.	% Cyanob.	% Dinoph.	% Euglen.	
<i>P</i>	*** (***/**)	** (***/*)	*** (***/***)	* (***/.)	*** (***/***)	*** (***/***)	*** (***/***)	*** (***/***)
<i>R</i> ²	28 (15/40)	17 (6/28)	30 (19/41)	12 (3/22)	30 (20/40)	29 (17/40)	31 (20/40)	
TP	235; +00	95; 000	628; −0−	166; 000	179; 000	660; −0−	679; +0+	
TP ^{^2}	235; 000	95; 000	628; 000	166; 000	179; 000	660; 000	679; 000	
TN	963; 00−	64; 000	174; 000	111; 000	799; +0+	671; −00	189; −00	
TN ^{^2}	963; +++	64; 000	174; 000	111; 000	799; 000	671; −0−	189; 000	
Chl− <i>a</i>	200; 000	729; +00	596; −00	893; −−−	224; 000	273; 000	167; 000	
pH	758; 000	836; −0−	963; −−−	301; +00	993; +++	327; 000	999; −−−	
pH ^{^2}	758; −0−	836; +0+	963; 000	301; 000	993; 000	327; 000	999; 000	
Area	782; +0+	267; −00	246; −00	126; 000	213; +00	232; −00	374; −00	
Depth	470; −00	722; −00	551; −00	349; +00	870; +0+	994; +++	988; −−−	
Urban	352; +00	95; 000	175; 000	244; −00	106; 000	353; −00	86; 000	
Agri.	137; 000	176; 000	499; −00	194; +00	67; 000	314; −00	590; −00	
Forest	272; +00	285; −00	698; +0+	99; 000	210; 000	499; +00	129; 000	
Wind	258; 000	143; 000	129; 000	85; 000	130; 000	117; 000	507; −00	
Temp.	780; −00	123; 000	196; 000	486; +00	139; 000	483; +00	339; +00	
Preci.	707; −00	123; 000	111; 000	125; 000	328; +00	164; 000	127; 000	
Sol. R.	225; 000	133; 000	272; 000	359; −00	162; 000	223; 000	229; 000	

Results are reported for all and the main groups of phytoplankton (upper panel) and also for the percentage richness constituted by each phytoplankton group in total phytoplankton richness (lower panel). Median model significance and R^2 are reported with 0.025 and 0.975 quartiles in parenthesis (., *, **, *** denote $P < 0.1$, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively). The selection frequencies in 1,000 permutations and significance of coefficients are reported for each predictor (3-digit codes after the semicolon). Plus and minus signs denote if the coefficient of a predictor is significantly positive or negative ($\alpha = 0.05$), while 0 denotes otherwise (i.e., 0 is included in the confidence interval), for three different confidence interval calculations in respective order: (i) only for the final models that included the predictor; (ii) for all final models, after a predictor's coefficient was assigned to zero, when the predictor was not selected; (iii) for all models without selection. ^{^2} denotes quadratic terms in orthogonal polynomials and Sol. R. denotes solar radiation

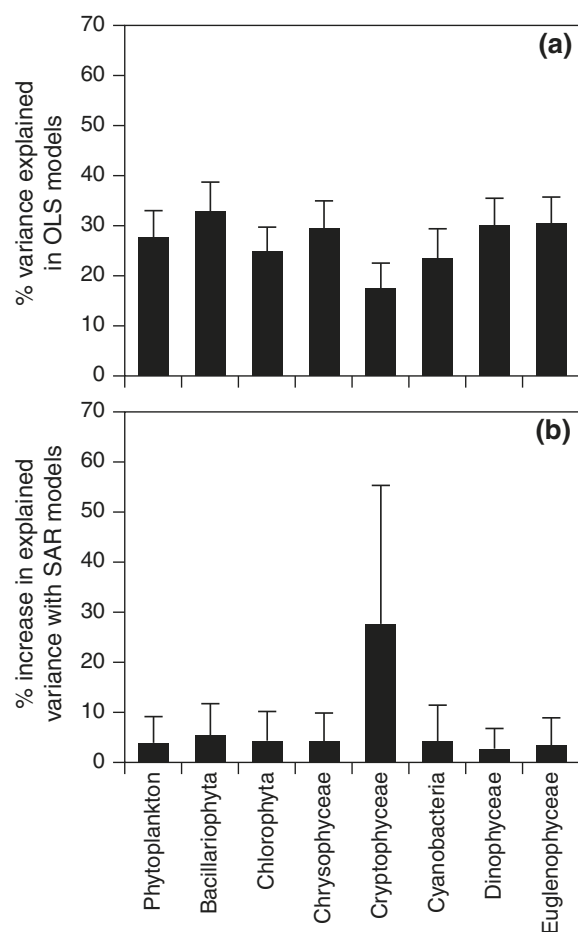


Fig. 1 Total variance explained in phytoplankton genus richness (means across 1,000 permutations with error bars for standard deviation) by OLS models (a) and percentage increase in total explained variance in SAR models relative to OLS models (b)

autocorrelation at greater distances (Fig. 3), i.e., reflecting small-scale clumping in phytoplankton richness. SAR models accounted for most of the spatial structure (average Moran's I in SAR residuals <0.05), but only contributed a ca. 5% increase in total explained variation over the OLS models (Fig. 1).

Variation in the response of different phytoplankton groups to environmental and spatial factors

Water chemistry was uniformly the strongest predictor for richness of all phytoplankton groups and accounted for ca. 70% of the total explained variation by all

environmental predictors (Fig. 2). The influence of water chemistry on Bacillariophyta, Cyanobacteria, Dinophyceae, and Euglenophyceae richness was relatively low, while lake morphology was a prominent richness predictor for these groups. In contrast, lake morphology had negligible influence on Cryptophyceae richness. Climatic variables were selected for Bacillariophyta richness in almost all permutations and accounted for almost 20% of the total variance explained. Climatic variables were also selected in 40–81% of the permutations for other phytoplankton groups; however, they had relatively small explanatory power. Similar to the models for overall phytoplankton richness, land-use had very limited influence on the richness of main phytoplankton groups. Nonetheless, land-use variables were selected in 61–86% of permutations and accounted for ca. 10% of the total explained variance for Bacillariophyta, Chrysophyceae, Cryptophyceae and Dinophyceae richness.

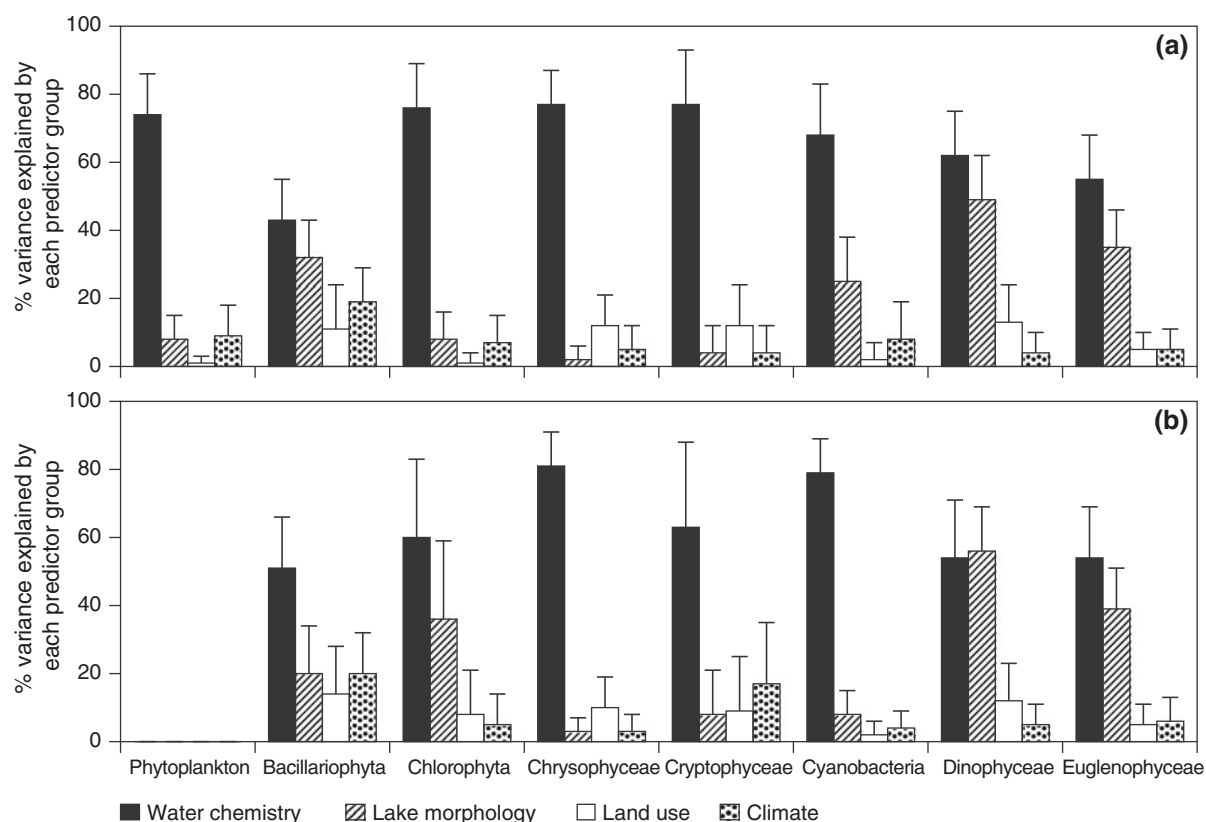
Concerning the specific drivers, pH was a strong and significant predictor of richness for all phytoplankton groups except Dinophyceae, and showed either a unimodal or negative relationship (Table 2). Chl-*a* was most strongly and positively related with Chlorophyta, but also positively related to the richness of other groups excluding Chrysophyceae and Cryptophyceae. Comparing TN and TP, TN had a stronger relationship with richness than TP for most of the phytoplankton groups. TN was selected in more than two-thirds of the permutations for Bacillariophyta, Chlorophyta, Cryptophyceae, Cyanobacteria, and Dinophyceae richness models with a unimodal or negative relationship, except for Cyanobacteria, which exhibited a positive relationship. On the other hand, TP had a stronger effect, compared to TN, on Chrysophyceae and Euglenophyceae richness with a negative and positive relationship, respectively. TP was also negatively related with Dinophyceae richness.

Lake area had a strong and positive relationship with the richness of main phytoplankton groups, excluding Chrysophyceae, Dinophyceae, and Euglenophyceae. Lake depth did not exhibit a strong relationship with Cryptophyceae and Cyanobacteria richness, while it had a strong positive influence on Dinophyceae richness and a relatively strong negative influence on the richness of the remaining groups.

Urban land cover in catchments was selected in a large number of permutations (600) only for

Table 4 Selection frequencies of four main environmental predictor groups, lake water chemistry (WC), lake morphology (LM), land-use in catchments (LU), and climate (CL) in OLS models for richness of all and the main phytoplankton groups

	Phytop.	Bacill.	Chloro.	Chryso.	Crypto.	Cyanob.	Dinoph.	Euglen.
WC	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00
LM	0.84	0.99	0.75	0.71	0.40	0.92	0.98	1.00
LU	0.49	0.61	0.38	0.86	0.68	0.35	0.72	0.74
CL	0.78	0.97	0.70	0.54	0.40	0.60	0.55	0.81
	% Bacill.	% Chloro.	% Chryso.	% Crypto.	% Cyanob.	% Dinoph.	% Euglen.	
WC	0.99	0.95	1.00	0.96	1.00	0.97		1.00
LM	0.84	0.86	0.71	0.40	0.93	1.00		1.00
LU	0.61	0.42	0.79	0.38	0.33	0.72		0.66
CL	0.95	0.40	0.45	0.61	0.54	0.64		0.86

**Fig. 2** Variance explained by four main groups of environmental predictors relative to total variance explained by all selected predictors (means across 1,000 permutations with error bars for standard deviation) in OLS models for genus richness of all and

the main phytoplankton groups (a), as well as the percentage richness constituted by each group in total phytoplankton genus richness (b)

Cryptophyceae richness, with a negative relationship. Agricultural land cover had a negative relationship with Bacillariophyta, Chrysophyceae, Dinophyceae,

and Euglenophyceae richness. In contrast, forest cover had a positive relationship with Chrysophyceae and Dinophyceae richness.

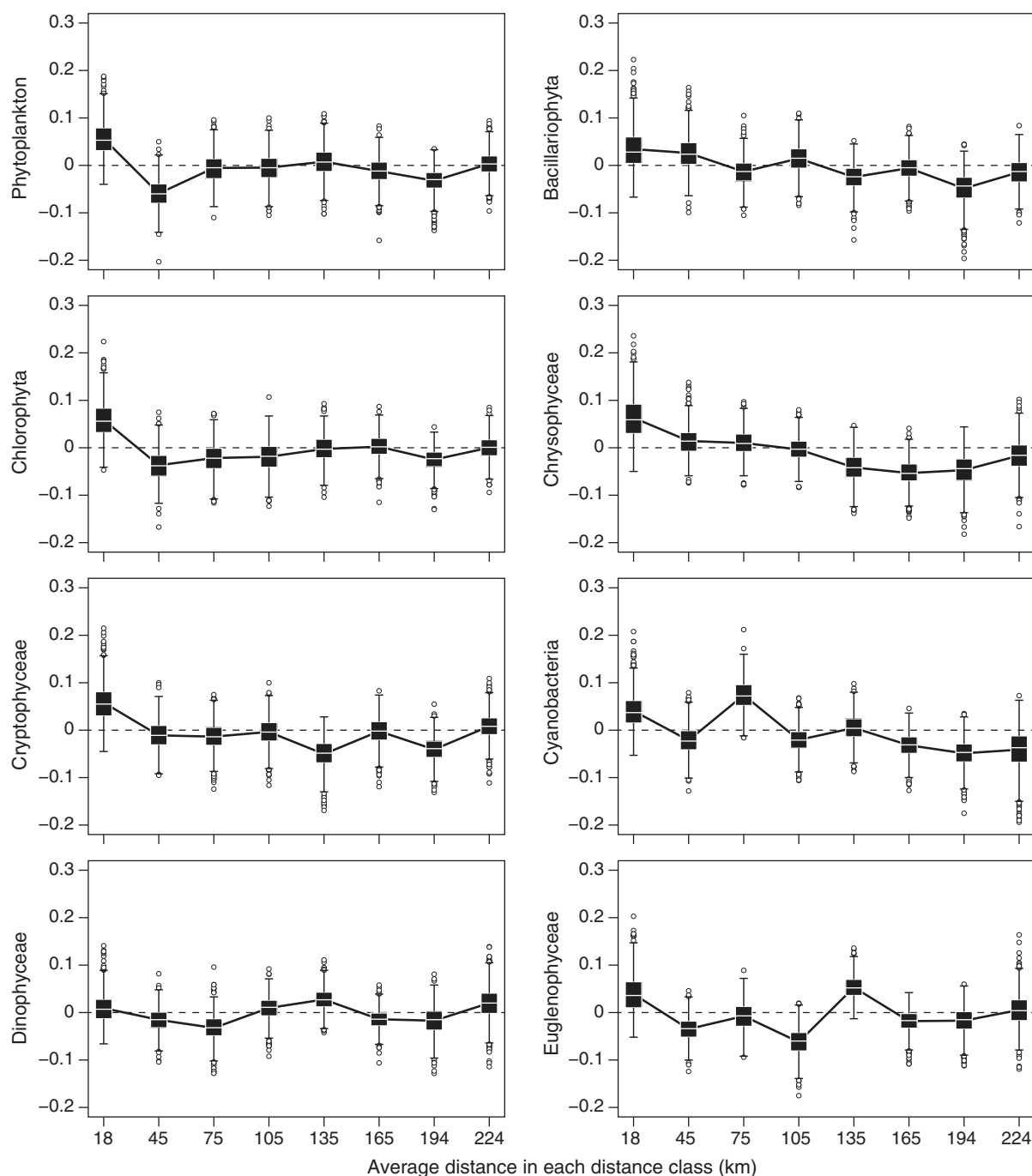


Fig. 3 Moran's I autocorrelograms for genus richness for all and the main groups of phytoplankton calculated across 1,000 permutations

Mean air temperature had a significant negative relationship with Bacillariophyta richness and a weaker negative relationship with Chlorophyta and

Cyanobacteria richness. Wind speed and precipitation had negative relationships with Bacillariophyta and Euglenophyceae richness, respectively. In contrast,

solar radiation had a positive relationship with Bacillariophyta, Chlorophyta, Chrysophyceae, and Cyanobacteria richness.

Considering the spatial structure, richness of all groups of phytoplankton other than Dinophyceae exhibited only small-scale positive autocorrelation (Fig. 3). Similar to overall phytoplankton richness, SAR models contributed only a small increase (ca. 5%) in total explained variance relative to the OLS models for all phytoplankton groups, except Cryptophyceae for which the SAR models contributed a ca. 30% increase in total explained variance (Fig. 1).

Discussion

Analysis of phytoplankton richness in Danish lakes revealed that water chemistry, lake morphology, land-use and climate have variable importance as drivers of genus richness of different phytoplankton groups. Water chemistry, mainly nutrients and pH, had pronounced effect on richness of all phytoplankton groups, while lake morphology also had a strong effect on Bacillariophyta, Cyanobacteria, Dinophyceae, and Euglenophyceae richness. Climate and land-use only weakly influenced phytoplankton richness, except for Bacillariophyta, for which their effect on richness was comparable to the effect of other predictor variables. Remarkably, TN had a stronger relationship with phytoplankton richness than with TP, which is often regarded as the main determinant of phytoplankton production, for most of the phytoplankton groups. Overall, one-third of the variation in richness of all and the main groups of phytoplankton was explained by OLS models, while SAR models exhibited only a small increase in total explained variation. Although weak, small-scale autocorrelation in phytoplankton richness suggested a possible role of connectivity among lakes and catchment-scale processes in driving the phytoplankton richness in the Danish landscape.

Phytoplankton richness in Danish lakes was primarily driven by water chemistry, which constitutes mostly productivity-related variables. Productivity (nutrients) has long been regarded as one of main determinants of diversity (Oksanen et al., 1981; Tilman, 1993). Although different response types and mechanistic explanations have been proposed to date (Mittelbach et al., 2001), a unimodal relationship has been regarded as the dominant response type.

However, the existence of the decreasing richness trends observed at the high end of the productivity scale is still subject to debate, as are the underlying mechanisms (Abrams, 1995). Decreasing heterogeneity of resources (Tilman, 1982) or increased competitive displacement (Huston, 1994) at high productivity have been proposed to be among the main drivers of this decrease. A recent experimental study on grassland plant communities indicated that host-specific soil microbes might also be an important determinant of diversity–productivity relationships (Schnitzer et al., 2011). TP has long been regarded as the primary limiting nutrient for lake ecosystems (Schindler, 1977; Elser et al., 2007), while a unimodal (Jeppesen et al., 2000; Ptacnik et al., 2010), linear or not significant (Declerck et al., 2007; Vanormelingen et al., 2008) relationship between TP and phytoplankton richness has been documented. Furthermore, submerged macrophytes, mostly abundant and diverse at low-to-intermediate nutrient concentrations (Rørslett, 1991), have been proposed to drive the unimodal relationship between productivity and phytoplankton diversity by keeping phytoplankton biomass low (Jeppesen et al., 1997) and thus mediating the negative effect of phytoplankton productivity on richness (Declerck et al., 2005, 2007). Accordingly, Kruk et al. (2009) found a positive relationship between phytoplankton richness and macrophyte abundance in subtropical Uruguayan lakes. However, high grazing pressure in lakes with high macrophyte coverage has also been proposed to limit phytoplankton richness (Muylaert et al., 2010) due to the direct effect of high grazing pressure (Pádisak, 1993), as well as low selectivity of herbivores in aquatic ecosystems (Hillebrand et al., 2001) and a shift in the phytoplankton community to very large organisms capable of avoiding grazing pressure (Muylaert et al., 2010). Muylaert et al. (2010) found a negative relationship between macrophyte coverage and phytoplankton genera richness across regional datasets from Denmark, Belgium/The Netherlands and, though more weakly, from southern Spain. The contrast between subtropical Uruguayan and temperate lakes is probably due to lower fish predation on the zooplankton, and thus higher grazing pressure on phytoplankton by zooplankton in northern European lakes (Meerhoff et al., 2007; Jeppesen et al., 2009; Muylaert et al., 2010).

We found a strong relationship between productivity and phytoplankton richness as either TN or TP

was selected in 93% of permutations. Notably, our results indicate that TN had a considerably stronger relationship to phytoplankton richness than TP, while TP was also of significant importance for Chrysophyceae, Euglenophyceae, and Dinophyceae richness. The prominent role of TN for phytoplankton richness accords with recent findings on joint importance of TN and TP in driving shallow lake ecosystems (Gonzalez Sagrario et al., 2005; James et al., 2005; Özkan et al., 2010; Jeppesen et al., 2011; Moss et al., 2012), considering that shallow lakes are the dominant lake type in our dataset. The relationship between TN and phytoplankton richness was mostly unimodal; however, a linear decrease or increase in richness of some groups with increasing TN or TP was also observed. An increase in Cyanobacteria richness and its dominance in total phytoplankton richness with increasing TN, as well as a positive relationship between TP and Euglenophyceae are to be expected (Reynolds, 1984; Søndergaard & Moss, 1998). pH was also a strong predictor of phytoplankton richness for most of the groups with an unimodal or negative relationship, while Cyanobacteria dominance in the phytoplankton richness pool was favored by high pH, which accords with previous findings (e.g., Shapiro, 1984; but see Jensen et al., 1994).

Concerning lake morphology, lake area generally favored phytoplankton richness, which accords with well documented diversity–area relationships (MacArthur & Wilson, 1967); however, it might also reflect better mixing of the water column in large lakes favoring the richness of some groups like Bacillariophyta (Reynolds, 1984). Phytoplankton richness for most groups decreased with lake depth, probably reflecting the high risk of loss by sinking in deep stratified lakes. Furthermore, deep lakes generally have a very small littoral zone, which might also lead to low phytoplankton richness due to less environmental heterogeneity and limited exchange with littoral communities. However, lake depth had a positive effect on Dinophyceae richness and promoted dominance of Cryptophyceae, Cyanobacteria, and Dinophyceae in total richness. Cryptophyceae and Dinophyceae are motile, allowing them not only to stay in epilimnion but also to migrate to the metalimnion/hypolimnion during night to get access to high nutrient concentrations, which provides competitive advantage to these groups in deeper lakes (Reynolds,

1984; James et al., 1992). The positive relationship between lake depth and Cyanobacteria probably reflects that stratification benefits Cyanobacteria, which are S-strategists and capable of developing buoyancy (Reynolds, 1984). In contrast, Bacillariophyceae richness was negatively affected by depth, which is to be expected as we focus on the late summer, when deep lakes are stratified, and Bacillariophyceae is prone to sedimentation into the hypolimnion under reduced turbulence (Reynolds, 1984).

Land-use had the strongest influence on Bacillariophyceae, Chrysophyceae and Dinophyceae richness, and the lowest significance for Chlorophyta and Cyanobacteria. This probably reflects that the former group has a competitive advantage at low to medium high nutrient concentrations (Reynolds et al., 2002; Pádisak et al., 2009) and thus they are more sensitive to the nutrient load from the catchment, which is dominated by non-point sources from agriculture in Denmark (Kronvang et al., 2005). Conversely, the positive effect of increasing forested land cover on richness of some phytoplankton groups most likely primarily reflects the decreased intensity of agriculture, when the size of forested land in lake catchments increases; however, increased DOC input might also have also added to the heterogeneity in resources. It must also be emphasized that lake water TN and TP in Danish lakes are strongly influenced by land-use, specifically agricultural land and forest cover (Jeppesen et al., 1999; Nielsen et al., 2012), which may explain the relatively weak effect of land-use, as it has probably been accounted for, in part, by including lake water TN and TP. However, these variables are further influenced by internal biological and chemical processes in lakes including internal loading, ultimately affecting phytoplankton richness.

There was a general tendency of a negative relationship between temperature and phytoplankton richness. Higher temperatures could result in stronger stratification, which provides fewer stable thermal habitats for phytoplankton (Longhi & Beisner, 2010) and thus might lead to competitive exclusion of species (Sommer et al., 1993; Muylaert et al., 2010). The negative effect of precipitation on Bacillariophyta richness, while promoting Cyanobacteria dominance, might potentially reflect increasing nutrient input from catchments due to surface run-off (Jeppesen et al., 2009, 2011). Wind speed related only negatively to richness of Euglenophyceae, which typically

dominates in relatively wind-protected small nutrient rich ponds and lakes. Last, the positive influence of solar radiation on the richness of a variety of phytoplankton groups might reflect its primary role as a limiting resource for plant life.

Most of the environmental variables had significant coefficient estimates in models for richness of different phytoplankton groups (especially if calculated for final models after selection or without selection), however, no environmental variables excluding chl-*a* and pH were selected in 95% of the permutations. Random permutations of lakes and samples might have resulted in shorter gradient lengths for an environmental predictor in some permutations and thus might have led to exclusion of the predictor from the model in those permutations. Furthermore, some variables with similar ecological effects on phytoplankton richness might have replaced each other in selections across permutations. For example, either TN or TP, as limiting nutrients, were selected in the model for phytoplankton richness in 93% of the permutations, although none of them had such selection frequency individually. Nonetheless, the ecology of phytoplankton is complex and different environmental factors have differential importance for the phytoplankton assembly at different temporal and spatial scales, a likely reason for contrasting results from different studies. Therefore, environmental predictors with significant coefficient estimates (in any of the three different calculations) probably indicate real ecological relationships, although only few environmental predictors were selected for a significant portion (0.95) of permutations.

Several studies have provided no support for the role of spatial structure in determining phytoplankton assemblages (Beisner et al., 2006; Soininen et al., 2007; Vanormelingen et al., 2008). However, an analysis of phytoplankton communities in Fennoscandia demonstrated a large-scale gradually-declining spatial structure in phytoplankton genus richness, which is explained by regional productivity, thus connecting local communities with regional processes (Ptacnik et al., 2010). In contrast to spatial patterns of phytoplankton richness in Fennoscandia, which span over three countries, the spatial structure in the phytoplankton richness in Danish lakes was local. This suggests that dispersal limitation in phytoplankton is of greater importance across larger spatial extents. Almost all phytoplankton groups had small-

scale positive autocorrelation in richness, with limited explanatory power of spatial structure in comparison to environmental factors, except for Cryptophyceae. Cryptophyceae has been shown to be strongly affected by the variation in abundance and community structure of fish in Danish lakes (Søndergaard et al., 2008), which might explain the weaker role of the selected environmental predictors and stronger role of spatial structure in explaining Cryptophyceae richness than for the other phytoplankton groups. A previous analysis of spatial autocorrelation in aquatic community richness in North America and Europe also found that there were no differences in spatial autocorrelation in richness of organisms ranging from diatoms to macro-invertebrates (Shurin et al., 2009). Nonetheless, local spatial structure in the present analysis (ca. <30 km) suggests a possible role of connectivity among lakes and catchments scale processes in driving phytoplankton richness. Especially, connected lakes in nearby catchments might transport materials and organisms (Loreau et al., 2003), thus promoting converging communities at local scale through priority or mass effects (Leibold et al., 2004). However, catchment characteristics that are not captured by land-use, like local geology and soil chemistry, might also contribute to the small scale autocorrelation.

In conclusion, our analysis of phytoplankton genus richness in Danish lakes revealed that TN had a prominent role in driving phytoplankton richness in shallow lakes. The analysis revealed contrasting roles of water chemistry, lake morphology, land-use, and climate for different groups of phytoplankton. However, water chemistry and lake morphology were the most important drivers of phytoplankton richness. The functional role of phytoplankton communities in lake ecosystems closely depends on the structure of communities. Therefore, any management practice, like restoration of eutrophicated lakes, should preferentially consider the control of both TP and TN concentrations as well as climate and in-lake and catchment-scale processes including connectivity among lakes as potential drivers of phytoplankton communities.

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PAPER III

Long-term trends and synchrony in lake plankton and abiotic characteristics driven by re-oligotrophication and climate across 17 Danish Lakes

Korhan Özkan^{a,b}, Erik Jeppesen^{a,c,d}, Thomas A. Davidson^{a,b}, Rikke Bjerring^a, Liselotte S. Johansson^a, Martin Søndergaard^a, Torben L. Lauridsen^{a,e} and Jens-Christian Svenning^b

^aFreshwater Ecology Group, Department of Bioscience, Aarhus University, Silkeborg, Denmark

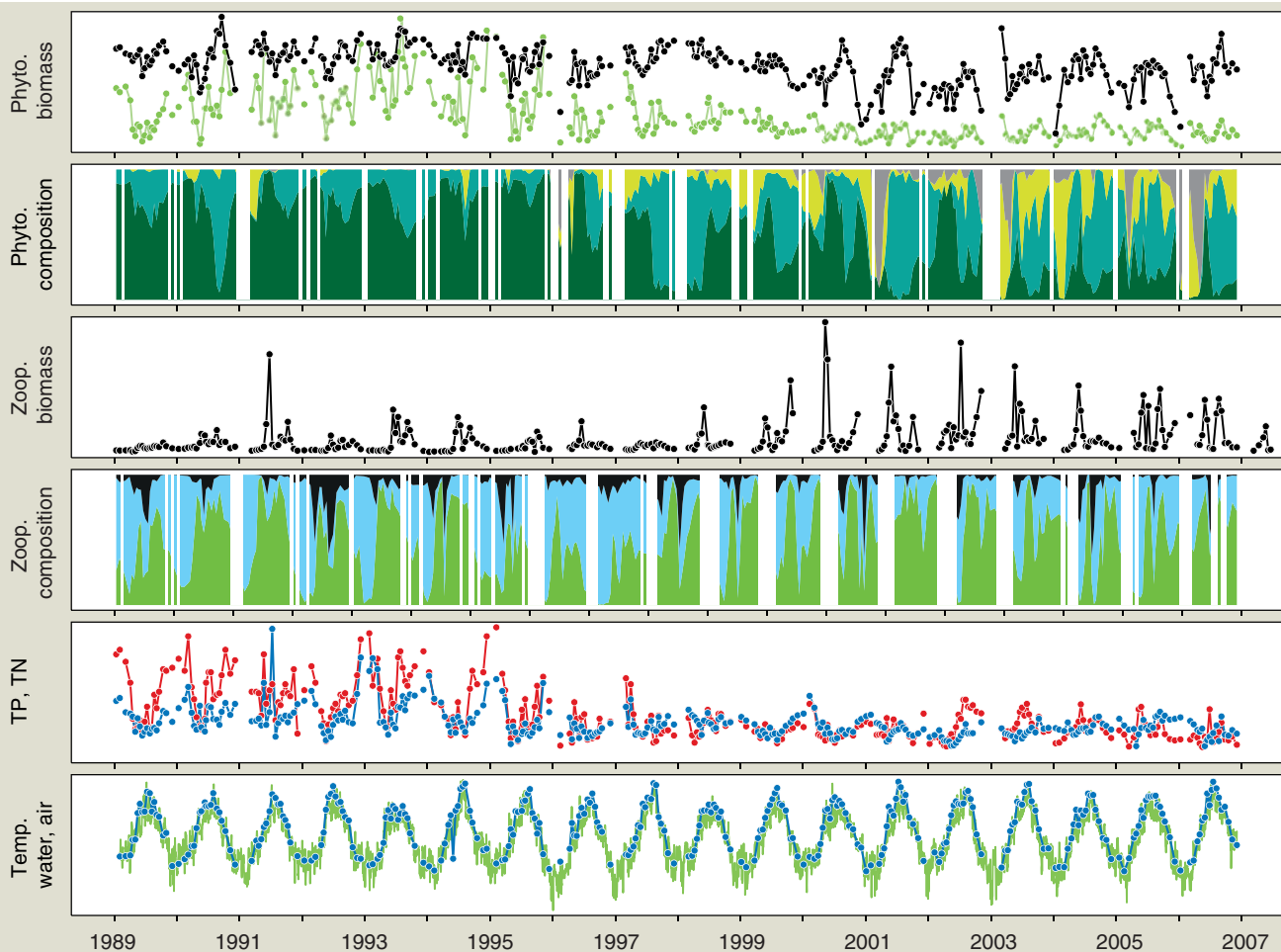
^bEcoinformatics and Biodiversity Group, Department of Bioscience, Aarhus University, Aarhus, Denmark

^cGreenland Climate Research Centre, Greenland Institute of Natural Resources, Nuuk, Greenland

^dSino-Danish Centre for Education and Research, Beijing, China

^eArctic Research Centre, Aarhus University, Aarhus, Denmark

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LONG-TERM TRENDS AND SYNCHRONY IN LAKE PLANKTON AND ABIOTIC CHARACTERISTICS DRIVEN BY RE-OLIGOTROPHICATION AND CLIMATE ACROSS 17 DANISH LAKES

Korhan Özkan^{a,b,*}, Erik Jeppesen^{a,c,d}, Thomas A. Davidson^{a,b}, Rikke Bjerring^a, Liselotte S. Johansson^a, Martin Søndergaard^a, Torben L. Lauridsen^{a,e}, Jens-Christian Svenning^b

^a Freshwater Ecology Group, Department of Bioscience, Aarhus University, Silkeborg, Denmark

^b Ecoinformatics and Biodiversity Group, Department of Bioscience, Aarhus University, Aarhus, Denmark

^c Greenland Climate Research Centre, Greenland Institute of Natural Resources, Nuuk, Greenland

^d Sino-Danish Centre for Education and Research, Beijing, China

^e Arctic Research Centre, Aarhus University, Aarhus, Denmark

* Corresponding author: korhan_oz@yahoo.com

SUMMARY

1. Two decade time-series in 17 Danish lakes on climate, water physico-chemistry and plankton (aggregated to genera) were analysed for synchronous temporal changes (coherence) across lakes and for significant temporal trends.
2. Synchrony was calculated as mean pair-wise Pearson correlations across time series and each time series was analysed using Mann-Kendal trend test. Annually-pooled plankton genera composition was also analysed using Non-metric Multidimensional Scaling and tested for directional temporal changes. Analyses were performed both on all lakes and then sub-sets of lakes grouped according to their trends in total phosphorus (TP) concentrations and each season was also analysed separately.
3. Strong synchrony (>0.7) was observed in climatic variables with significant increases in mean annual air temperature and precipitation, as well as a decrease in wind speed. Synchronies were also observed in the physico-chemical variables and were stronger for water temperature and stratification, which are most affected by atmospheric energy flux (0.3-0.5), and weaker for lake chemistry (0.1-0.3). Synchrony in the richness of plankton groups and phytoplankton biomass was apparent, to a similar degree as observed for lake chemistry.
4. Plankton richness increased across lakes, coincident with decreases in PO_4 and total nitrogen, as well as with the trends in climate. Although the widespread re-oligotrophication across lakes may be the driver of the increase in plankton richness, co-occurring trends precluded conclusive hypothesis testing.
5. Six lakes recovering from earlier eutrophication with significant and strong negative trend in their TP concentrations had considerably stronger synchrony in lake chemistry (<0.7) and plankton (<0.5). Phytoplankton biomass decreased and both phyto- and zooplankton richness increased in these lakes with a shift from *Chlorophyta* dominance towards more heterogeneous communities.
6. Long-term increase in air temperature and decrease in wind speed were consistent across seasons. However, the trend in air temperature resulted in a corresponding increase in surface water temperature only in summer with stronger synchrony both in surface water temperature and water column

stratification. Lake chemistry and plankton had stronger synchrony in spring with stronger negative trends in nutrients and phytoplankton biomass. The synchrony and trends in six lakes recovering from eutrophication had little variation across seasons. TP and PO₄ reduction in these six lakes were slightly weaker in summer indicating internal phosphorus loading, however, without affecting overall water clarity improvement.

7. Overall, coherent temporal changes and synchrony were observed during the two decades of monitoring largely driven by nutrient loading reduction across Danish landscape, which probably resulted in temporal increase in plankton richness. Surface lake water temperature and stratification had strong synchrony reflecting climatic control; however, with a significant trend only in the former in summer and without a clear signal in plankton.

Key-words: temporal coherence, zooplankton, phytoplankton, re-oligotrophication, lake recovery, plankton diversity

INTRODUCTION

Eutrophication and climate change are the two major global stressors of freshwater lakes in recent decades. Eutrophication is widely recognised as a serious problem (Isermann, 1990; Smith, Tilman and Nekola, 1999) due to notorious toxic algae blooms and low water clarity. Various restoration measures have been implemented in parts of the world (Cooke, Welch, Peterson *et al.*, 1993; Carpenter, Ludwig and Brock, 1999; Jeppesen, Søndergaard, Jensen *et al.*, 2005b). Climate change has also been widely recognised as an important factor affecting freshwater ecosystems (Kernan, Battarbee and Moss, 2010). Understanding the extent and coherence of long-term ecosystem response to the changes in these stressors is important for lake conservation, restorations and management (Schindler, 2001). Few studies have examined if temporal changes in lakes are coherent (Arnott, Keller, Dillon *et al.*, 2003; Magnuson, Benson and Kratz, 1990), i.e., synchronous (Vogt, Rusak, Patoine *et al.*, 2011; Baines, Webster, Kratz *et al.*, 2000) reflecting the scarcity of long-term multiple-lake datasets.

Climatic effects, especially in the form of energy flux (*i.e.*, temperature, irradiance) are uniform across large spatial scales and can induce coherent changes in physical characteristics of lake water, as well as, albeit weaker, changes in chemical and biological parameters (Vogt *et al.*, 2011; Arnott *et al.*, 2003; Rusak, Yan, Somers *et al.*, 1999). Factors affecting the exchange of matter, especially mediated through landscape have been recognised to weaken the synchrony in biotic and abiotic lake variables across large spatial scales due the catchment-specific variations (Magnuson *et al.*, 1990; Arnott *et al.*, 2003). However, changes in matter exchange, mostly with anthropogenic origin, like acidification (Fischer, Frost and Ives, 2001), eutrophication and re-oligotrophication (Pomati, Matthews, Jokela *et al.*, 2011; Anneville, Gammeter and Straile, 2005) can also induce synchrony in lakes, even at large scales.

We analysed long-term trends in phyto- and zooplankton as well as the lake water physico-chemistry and climate in 17 Danish lakes between 1989 and 2009. We first tested if the variables showed synchrony across the two decades using pair-wise Pearson correlations across lakes. Second, we assessed if variables had long-term trends across the study period using Kendall tests. In parallel, we also examined plankton community composition using Non-Metric Multidimensional Scaling (NMDS) and tested for directional temporal change in community space during the study period. Many of the study lakes are under recovery from previous

eutrophication (Jeppesen, Søndergaard, Kronvang *et al.*, 1999). We assumed that such a strong gradient in TP would be the strongest factor in temporal changes and probably mask the effects of other environmental variables and climate. Therefore, we grouped lakes into three subsets according to changes in their annual average TP concentrations: lakes with a dramatic decline (S), weak changes (W) and no change (N). The analyses were performed for all lakes together and each subset separately as well as whole year (excluding winter) and each season separately. We expected that the recovery from earlier eutrophication would induce strong long-term trends and synchrony in S lakes. We also expected consistent trends in all lakes or especially in N and W lakes would indicate other large-scale factors or climatic effect.

MATERIALS AND METHODS

Samplings and data treatment

seventeen lakes were monitored between 1989 and 2008 as part of the Danish monitoring programme of the aquatic environment (Svendsen, Bijl, Boutrup *et al.*, 2004). Winter samples (Dec. – Feb.) were excluded due to varying sampling intensity across years. Remaining samples had an average sampling interval of 14 days (Appendix). Phytoplankton was collected from mid-lake depth-integrated sample covering the photic zone. Depth-integrated zooplankton samples were collected at three stations placed randomly in areas representing 80% of maximum depth and subsequently pooled. Phyto- and zooplankton were counted and their biomass was estimated based on size and shape. The details of plankton sampling, identification and count can be found in Özkan, Jeppesen, Davidson *et al.* (2013). All taxa were aggregated to genus level due to varying intensity of species-level identification between different plankton groups and potential differences in identification skills of the taxonomists. We screened the plankton data for potential inconsistencies and made corrections with an inclusive approach (Özkan *et al.*, 2013).

Plankton biomass was calculated for whole sample as well as main groups (*Chlorophyta*-CHL, *Cyanobacteria*-CYA, *Bacillariophyceae*-DIA, *Cryptophyta*-CRY, *Chrysophyta*-CHR and *Euglenophyta*-EUG for phytoplankton; and *Cladocera*-CLA, *Copepoda*-COP and *Rotifera*-ROT for zooplankton). Additionally, proportional biomass for all groups was calculated to estimate their contribution (dominance). Proportional biomass excluding three most dominant groups (CHL, CYA and DIA) was also calculated to examine the changes in the rare groups together. Genera richness, rarefied richness (based on biovolume for phytoplankton and count for zooplankton) and Pielou's evenness (Pielou, 1975) were calculated as surrogates of plankton diversity. Richness of main groups of plankton was also calculated.

Lake water chemistry samples were collected simultaneously with the zooplankton samples and analysed for total phosphorus (TP), PO_4 , total nitrogen (TN), NO_3 , NH_4 , SiO_2 , chlorophyll-a (Chl-a), suspended solids (SS), pH and alkalinity and (Svendsen *et al.*, 2004; Lauridsen, Søndergaard, Jensen *et al.*, 2007). Temperature of the water column was recorded at one meter intervals at the deepest point of the lake. Water column stratification was calculated from temperature profile as Schmidt stability index (Idso, 1973). Matlab codes from Lake Analyser software (Read, Hamilton, Jones *et al.*, 2011) were adapted for the analysis. Calculations were made, only when temperature was recorded at least to the mid-depth of the lake and temperature profile was forced to be monotonic from bottom to top be-

fore calculations. When a lake variable was missing for a sample, it was replaced with the most relevant available data: first, the mean of observations in a seven-day temporal window; second (if a substitute sample was not available at the first step), the mean of observations in a 15-day window or, third, linear interpolation of neighbouring samples if they are less than 30 days apart (Appendix).

Mean air temperature, wind speed, solar irradiance (from daily averages interpolated to 20 km grids) and precipitation (from daily averages interpolated to 10 km grids and corrected for wind and altitude depending on the month of the year) were compiled from Danish Meteorological Institute. Main characteristics of the lakes are given in Table 1.

Table 1. Summary of lake morphology and nutrient concentrations in study lakes.

	Lake area (ha)	Average depth (m)	Max. depth (m)	TP (mg L ⁻¹)	TN (mg L ⁻¹)
Min.	12	0.8	1.8	<0.001	0.100
Median	37	2.7	6.0	0.075	1.770
Max.	3954	15.1	32.6	2.700	19.000

Statistical analyses

first, we assessed the synchrony in the long-term changes. Annual and seasonal means were calculated for each climatic, lake physico-chemical and plankton variable. Each time series was standardized to zero mean and unit standard deviation prior to the analyses. Synchrony of a variable was calculated as pair-wise Pearson correlation coefficient (r) across all pairs of time series across lakes (Vogt *et al.*, 2011). Synchrony was calculated for all lakes combined as well as separately for each subset of lakes.

Second we tested for significant monotonic trends in each variable over the two decades using Mann-Kendall rank correlation (Mann, 1945). We expected a monotonic fit would be adequate as most of the lakes experienced nutrient reduction (Jeppesen, Jensen and Søndergaard, 2002) and no shift of the trend in NAO index was observed (Figure 1) during the study period. Kendal correlation coefficient (τ) was taken as zero, when the test was insignificant. Many of the lakes were in recovery from earlier eutrophication. Therefore, we assumed that TP reduction would be the main driver of temporal changes and potentially preclude the detection of the effects of other factors, thus, lakes were classified into three subsets according to their trends in TP (Figure 2). Six lakes experienced significant and substantial decrease in TP with overall high concentrations ($\tau > 0.2$, annual mean TP 0.59-0.14 mgL⁻¹) and were grouped as S lakes. Six lakes had significant but weak changes with moderate-high concentrations ($\tau < \pm 0.2$, annual mean TP 0.40 – 0.05 mgL⁻¹) and they grouped as W lakes. Three lakes had no trend in TP with low concentrations (annual mean TP < 0.03 mgL⁻¹), and they are grouped as N lakes. The results are reported for each subset separately. Two lakes have also experienced significant decrease in TP with $\tau > 0.2$, however, the change was across a short TP gradient with overall low concentrations (annual mean TP 0.03-0.02 mgL⁻¹). Therefore, these 2 lakes were not included in any groups. However they are included, when the lakes analysed or reported together.

To elucidate if there is any directional changes in community composition of phyto- and zooplankton, NMDS ordinations (Minchin, 1987) were used. The ordinations were performed for combined annual and seasonal plankton communities separately. The number of plankton samples for whole year and each season var-

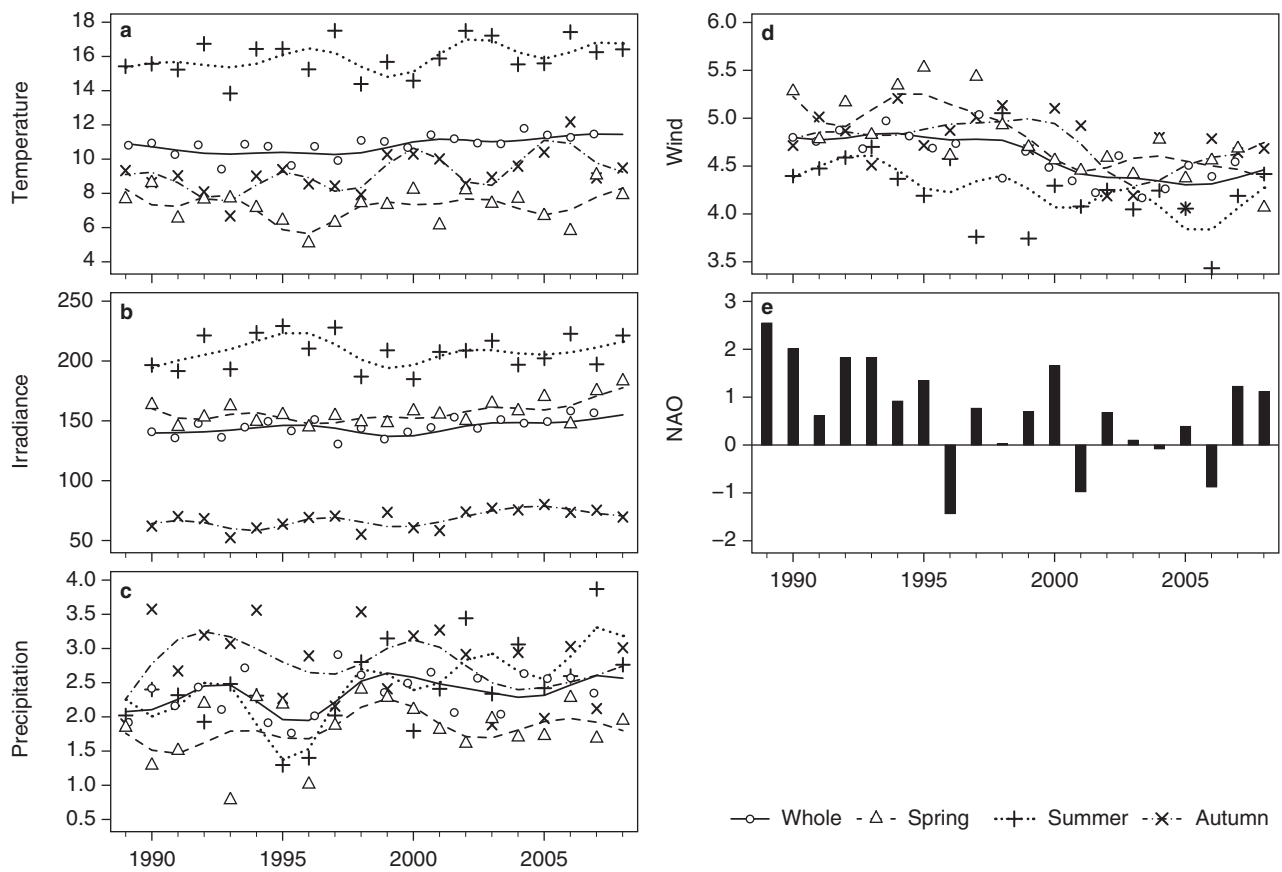
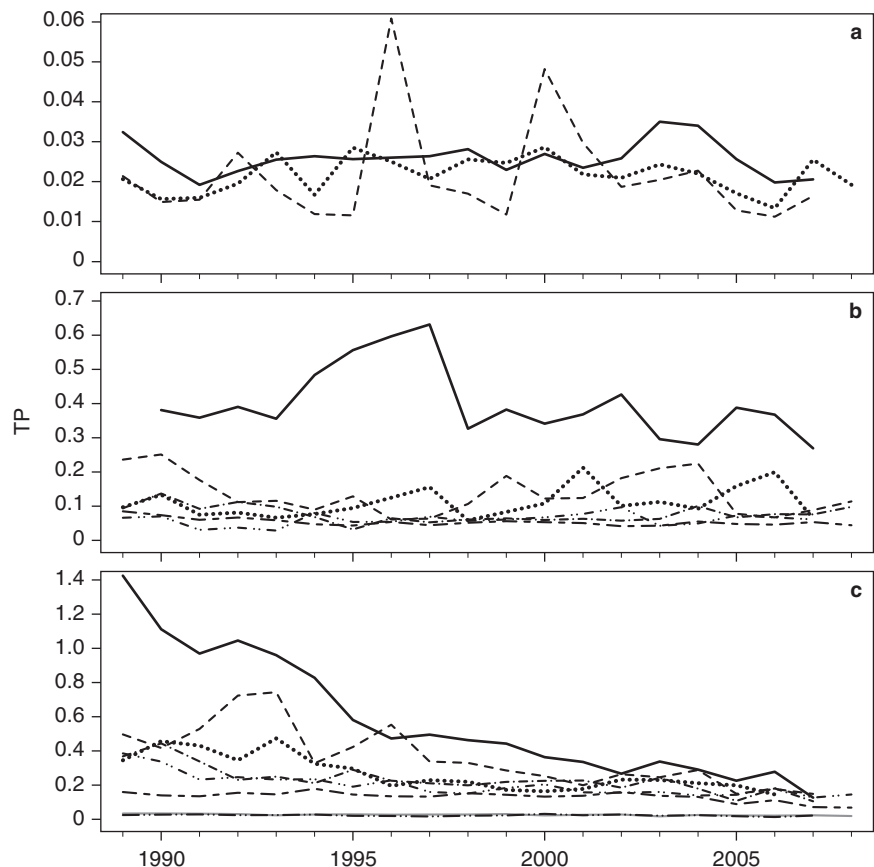


Figure 1. Two-decade trends in temperature (a), solar irradiation (b), precipitation (c) and wind speed (d) averaged over 17 lakes, as well as the North Atlantic Oscillation Index (e). Yearly means were calculated for the whole year excluding winters as well as separately for spring, summer and autumn. Generalized additive model annual smoothers (Wood, 2006) were overlaid to each dataset to summarize trends ($P < 0.05$ for all).

Figure 2. Annual mean total phosphorus (TP) change in lakes with no TP trend (N lakes, a), with weak TP trend (W lakes, b) and with strong TP decrease (S lakes, c); note that two lakes at the bottom of the plot was not included in S lakes, due to overall low TP concentrations).



ied across lakes. Therefore, 14, 3, 4 and 3 plankton samples were pooled (summed taxa biomass) as annual, spring, summer and autumn plankton communities for each lake and year. Lake-years without sufficient samples were discarded from the analyses and a random selection was performed in lake-years with excess number of samples. Ordination was performed for two axes based on Bray-Curtis distances between pair-wise plankton communities. The stress values were sub-optimal (0.27 and 0.24 for phytoplankton and zooplankton ordinations) as we tried to ordinate in two dimensions. Most of the models did not converge, and thus we used the best solution after 1000 trials. However, the conclusions were identical in different trials. Each lake was analysed if the annual plankton communities had significant directional change in the ordination space. The axes scores were modelled against years using multivariate linear model and significance was tested using the anova function. The direction of change was plotted with a vector for the lakes with significant change with end points corresponding to predicted axes scores by start and end years. An ellipse centred on mean axes scores of sites with radiuses corresponding to maximum distance of sites from the mean for each axes were plotted for lakes without a significant change.

All analyses were performed on time-series for the whole year (excluding winter) and each season (months 3-5, 6-8, 9-11 for spring, summer and autumn) separately. Statistical analyses were performed using R (R Development Core Team, 2011) with the vegan package (Oksanen, Kindt, Legendre *et al.*, 2011) for NMDS ordination, Kendall package (McLeod, 2011) for monotonic trend analyses in time series; mgcv package (Wood, 2006) for Generalized Additive Model smoothers.

RESULTS

Synchrony

climate variables exhibited strong synchrony ($r > 0.7$) both for all lakes and for the different subsets (Figure 3). The synchrony was less marked for wind and precipitation compared to air temperature and irradiance. Surface water temperature had consistent moderate synchrony (c. 0.5) across lakes. Synchrony in water column stability was lower than that of water temperature and varied across lake subsets, as a result of deep and shallow lakes being not evenly distributed across lake subsets. Lake chemistry and plankton had four-fold weaker synchrony than climate for all lakes and showed considerable variation across lake subsets. The strongest synchrony in lake chemistry was observed for S lakes (< 0.6), followed by N lakes (< 0.4), while only alkalinity and NO_3 showed weak synchrony in W lakes. Plankton variables had similar synchrony as lake chemistry in general, while stronger synchrony was observed for richness of main zooplankton groups, followed by phytoplankton groups across all lakes. A large number of plankton variables had moderate synchrony in S lakes (< 0.6), generally stronger for the phytoplankton variables. N lakes also had moderate synchrony for a large number of variables, however in contrast to S lakes it was stronger for variables related to zooplankton richness. A few plankton variables had weak synchrony in W lakes.

Synchrony in climatic variables was similar across seasons (Appendix). Synchrony in surface temperature and water column stratification was stronger in summer. Weaker synchrony was observed in lake chemistry and plankton variables in summer and autumn, compared to spring in all lakes, as well as W and N subsets. Synchrony in lake chemistry and plankton variables in S lakes were mostly consistent across seasons.

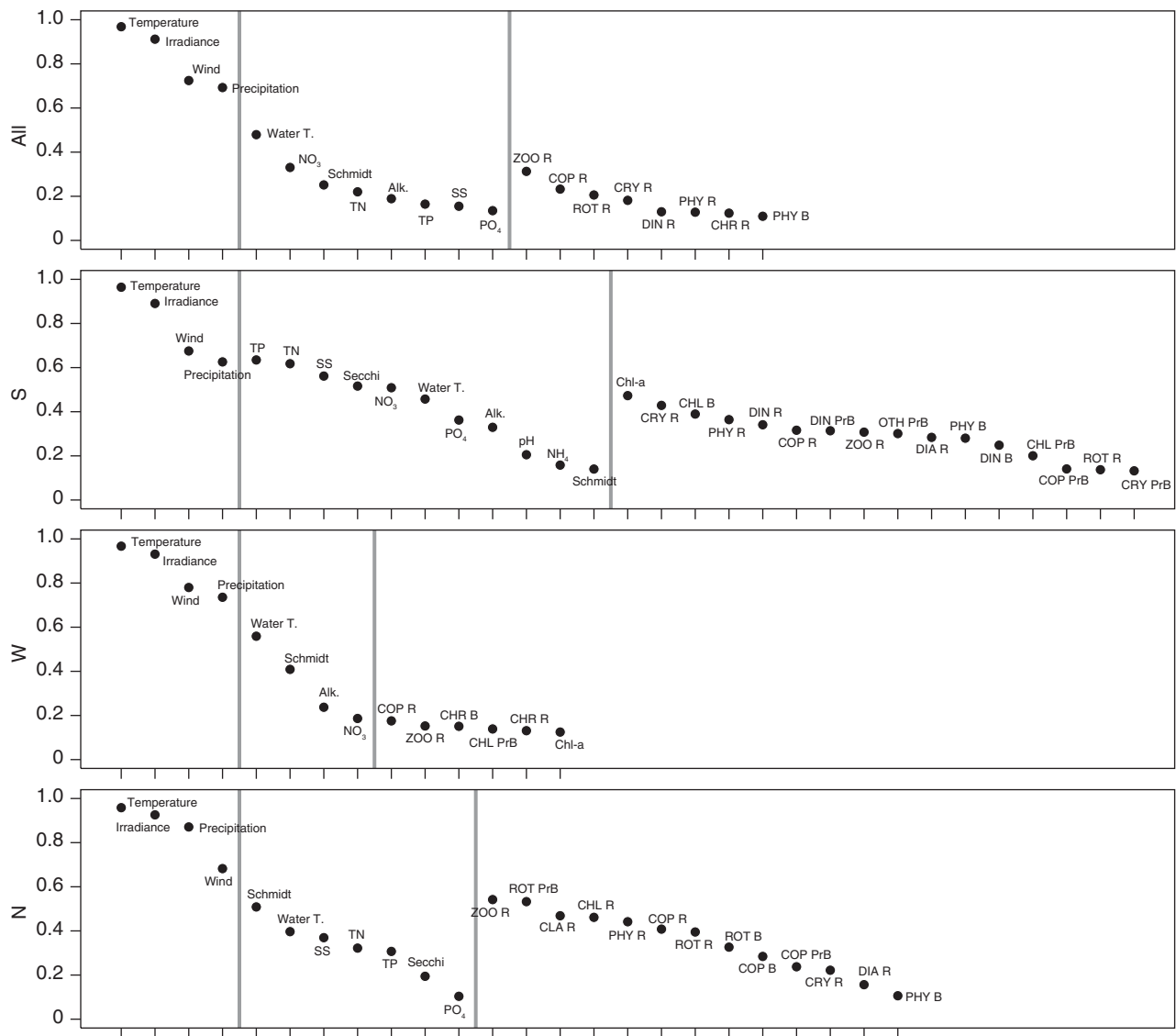


Figure 3. Summary of the synchrony in climate, lake physico-chemistry and plankton variables for the whole year excluding winters analysed for all lakes and S, W and N lakes separately. Only the variables with $r > 0.1$ are given and main variable groups were divided by vertical lines. Separate analyses for each season are given in appendix.

Monotonic trends

climatic parameters exhibited significant long-term trends over the two decades (Figure 4). Air temperature had a significant positive trend for the whole year (excluding winter) and each season separately ($r = 0.05 - 0.09$, $P < 0.01$), with the strongest trend in summer. Wind speed had a significant negative trend for the whole year and each season ($r = -0.05 - -0.10$, $P < 0.01$), with the strongest trend in spring. Precipitation had a significant positive trend for the whole year and in summer ($r = 0.02$ and 0.05 , $P < 0.05$). Light had significant positive trend only in spring ($r = 0.07$, $P < 0.001$). These trends coincided with a decrease in NAO index. Only the wind trend exhibited variation across lakes (Appendix).

Strong long-term trends were observed in annual time series of some of the lake physico-chemical variables, especially for S lakes (Figure 4). While water column stability and surface temperature had no significant trend, PO_4 had a decreasing trend in all lakes with the exception of three S and W lakes (Figure 4). TN had also a decreasing trend in all but two W lakes, with the strongest decrease in S lakes. NO_3 decreased in all W, two N and two S lakes. NH_4 , SiO_2 and pH showed

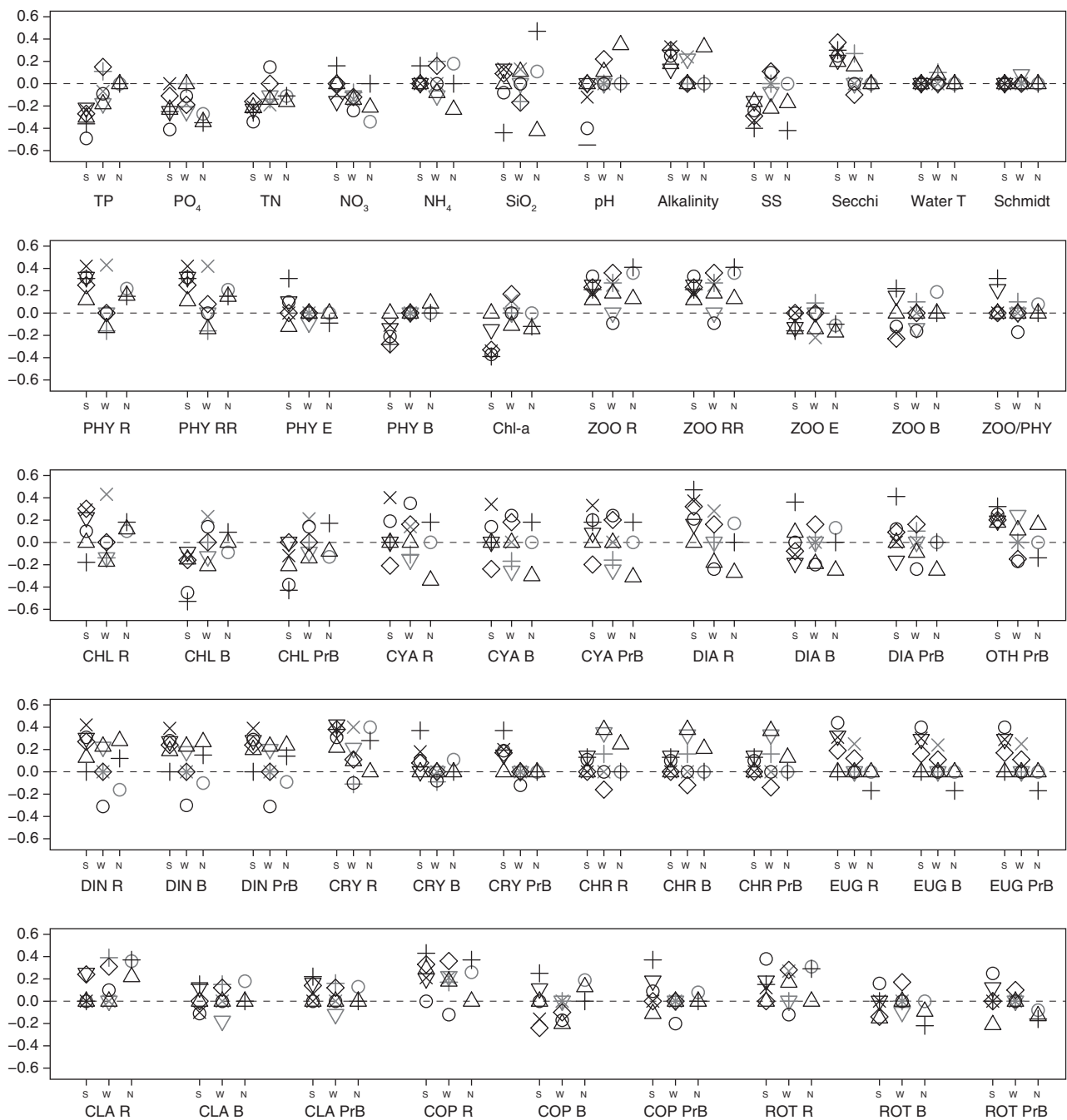


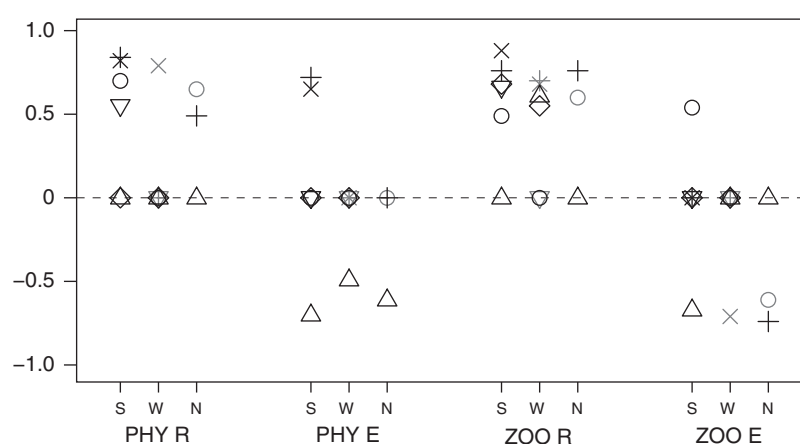
Figure 4. Summary of the monotonic long term trends in lake physico-chemistry and plankton variables. Trends were analysed using Kendall correlations and grouped for S, W and N lakes. Plotting symbols represents a unique lake for each lake group across different variables and grey colour represents deep lakes (mean depth < 3.5 m). R, richness; RR, rarefied richness; E, evenness; B, biomass; PrB, proportional biomass; CHL, *Chlorophyta*; CYA, *Cyanobacteria*; DIA, *Bacillariophyceae*; OTH, all phytoplankton groups excluding CHL, CYA, DIA; CRY, *Cryptophyta*; CHR, *Chrysophyta*; EUG, *Euglenophyta*; CLA, *Cladocera*; COP, *Copepoda*; ROT, *Rotifera*. Separate analyses for each season are given in appendix.

no clear trend between different lake groups; however, pH decreased for three S lakes. Alkalinity and Secchi depth increased and SS decreased for all S lakes. When analysed for seasonal time series (Appendix), surface temperature had a positive trend in 70% of lakes in summer. The main patterns were similar across seasons for the remaining lake chemistry variables. However, nutrient reduction was strongest in spring across all lakes. The negative trend in TP and PO_4 in S lakes was slightly weaker, while the increase in Secchi disk and decrease in SS were stronger in summer in S lakes.

The annual time series of plankton variables also exhibited strong long-term trends (Figure 4). Generally, trends in phytoplankton data were more pronounced in S lakes, while trends in the zooplankton were similar across all lakes. Total phytoplankton biomass and Chl-a strongly decreased in all S lakes except for one. Phytoplankton richness increased for all S and N lakes, but more strongly in the former. Notwithstanding increases in three S lake, no clear patterns were in phytoplankton evenness were evident. Zooplankton biomass did not show clear differences across lake subsets, whereas zooplankton richness increased in all but two W lakes, whilst evenness decreased in 65% of lakes. Richness of all the main groups of phytoplankton excluding CYA and CHR tended to increase in S lakes. CHL richness increased for all N lakes, while CRY richness increased for all but two W and one N lakes. Both CHL biomass and dominance decreased in S lakes, while the total contribution of all rare groups, as well as the biomass of DIN, CRY and EUG increased. CYA dominance increased in four S lakes. Richness of all the main groups of zooplankton increased in 90% of the lakes without a pattern in their biomass. However, CLA dominance increased in 6 lakes with decrease in only one lake, while ROT dominance decreased for all N lakes. When analysed for seasonal time series (Appendix), the negative trend in phytoplankton biomass was strongest in spring, while the positive trend in phytoplankton richness and the negative trend in Chl-a were strongest in summer in S lakes.

The general increasing trend in plankton richness was also observed in annual pooled plankton communities (Figure 5). Phyto- and zooplankton richness of pooled communities positively correlated with years in 40% and 75% of the lakes, respectively. When analysed with NMDS, phytoplankton communities differed strongly for S, W and N lakes (Figure 6) with a gradient from CHL and CYA dominance towards more heterogeneous communities. Most S lakes had significant temporal trend towards N lakes consistently across all seasons. Few W lakes had significant directional trend however their direction was not consistent. Zooplankton community composition also differed among S, W and N lake, the NMDS ordinations (Figure 7) revealed a gradient from ROT and COP dominance towards more heterogeneous communities with increasing CLA contribution. However, the temporal change in zooplankton composition was weak and inconsistent in direction, when compared with the phytoplankton communities.

Figure 5. Summary of temporal change (Pearson's correlation with years) in annual phyto- and zooplankton assemblage richness and evenness in S, W and N lakes. See Figure 4 for details.



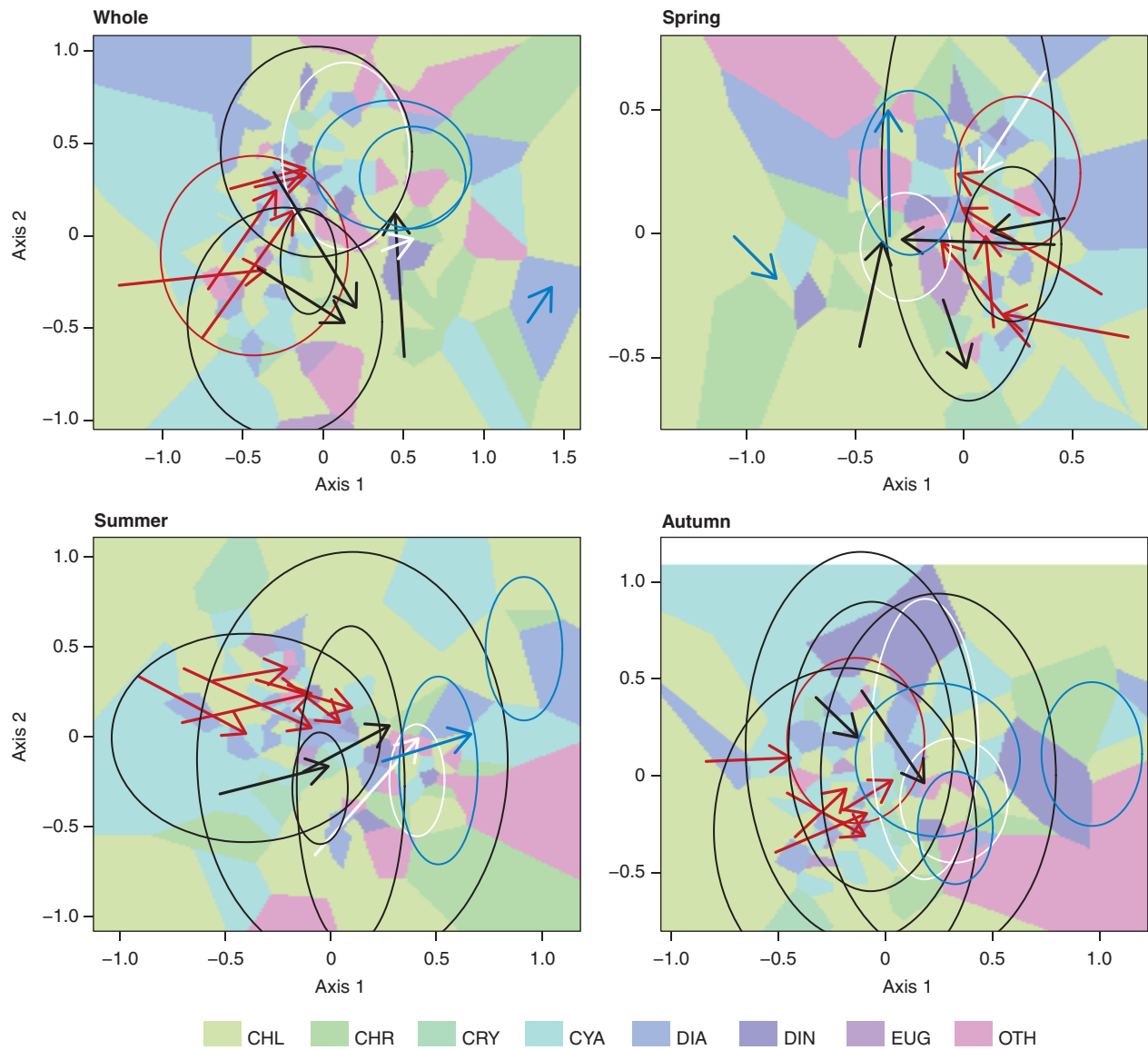


Figure 6. NMDS ordination of annual and seasonal phytoplankton assemblages. Temporal change in assemblage composition is given with an arrow for lakes with significant change across years. The compositional variation in lakes without a significant change was given with an ellipse. Taxa scores are aggregated for main groups and Voronoi polygons are plotted to represent the compositional surface. Blue, black and red are used for N, W and S lakes, respectively. Two lakes that were not included in any of the groups are given with white. For details see Figure 4.

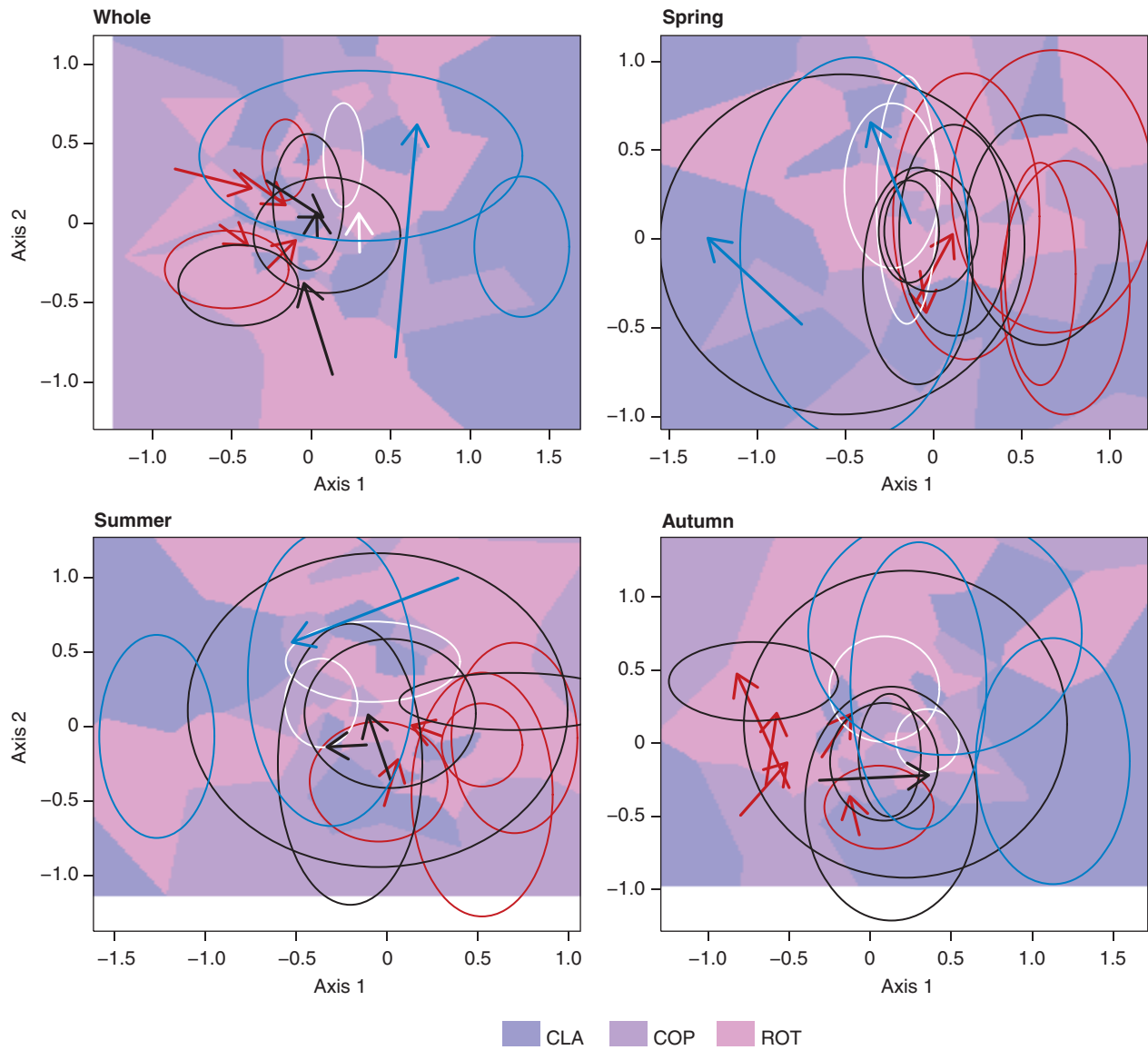


Figure 7. NMDS ordination of annual and seasonal zooplankton assemblages. See Figure 4 and 6 for details.

DISCUSSION

Analysing two-decade time series of 17 Danish lakes revealed widespread temporal synchrony in climate variables and moderate synchrony in lake physico-chemical and plankton variables. A widespread positive trend in phyto- and zooplankton richness was observed, which was coincided with a long term change in climate and widespread nutrient reduction. The substantial decrease in TP in S lakes, also accompanied by a decrease in TN, induced stronger synchrony in lake chemistry and plankton richness compared to other lake groups, which was accompanied by large decrease in phytoplankton biomass and increase in phyto- and zooplankton richness. The community change in S lakes was generally characterised by the decrease in biomass and dominance of CHL towards more heterogeneous communities.

The strong synchrony in climate variables across Danish lakes is to be expected considering the lack of altitudinal differences and small spatial scale (< 300 km).

Wind speed and precipitation had weaker synchrony (c. 0.7) and more variability across lakes compared to temperature and solar irradiance (c. 0.9), reflecting wind and precipitation are affected by local factors (Vogt *et al.*, 2011; Magnuson *et al.*, 1990). The synchrony in air temperature induced a corresponding synchrony in surface water temperature and water column stratification, which have a direct link to climate and have been shown to have stronger synchrony compared to lake chemistry (Vogt *et al.*, 2011). Interestingly, the positive trend in air temperature and negative trend in wind speed did not translate in a trend in surface water temperature and stratification in the complete time series and no clear effect of climate on lake chemistry was observed. Only in summer, when the increase in temperature was the strongest, a significant trend in surface water temperature was observed. This suggests that either the climatic changes were not strong enough to induce a trend in lake water, except for summer, or the effect of these variables were confounded by catchment-specific factors. However, analyses on 20 Danish lakes (some are also included in the present study) have found decrease in the depth of thermocline with a delay in autumn turnover (Jeppesen, Kronvang, Jørgensen *et al.*, 2013). Long-term warming trend has been documented in both north American (Arhonditsis, Brett, DeGasperis *et al.*, 2004; Coats, Perez-Losada, Schladow *et al.*, 2006) and European (Livingstone, 2003; Dokulil, Jägerskiöld, George *et al.*, 2006) deep lakes. Deep dimictic lakes with stable summer stratification are known to preserve long-term climatic trends, whereas frequently circulating polymictic lakes (like most in the present study) are least influenced (Gerten and Adrian, 2001).

The annual means of water chemistry variables were also synchronous across lakes, there was, however, four-fold decrease in comparison to climate variables. Weaker synchrony in lake chemistry than climate reflects confounding influence of catchment scale local factors or internal dynamics (Magnuson *et al.*, 1990; Vogt *et al.*, 2011). Moderate synchrony in NO₃, TN, TP and PO₄ accompanied by an inter-annual decrease in PO₄, TN and NO₃ across most of the lakes (80, 90 and 75 %, respectively) reflected a widespread nutrient reduction in Danish lakes (Jeppesen *et al.*, 2005b). There was weak synchrony in SS, with negative trends in 70% of the lakes probably associated with water quality improvements. However, no consistent trends in Secchi depth, Chl-a and phytoplankton biomass have been observed across all lakes. The widespread decrease in nutrients is consistent with the fact that the Danish landscape is largely used by agricultural activities (c. 60%, Nielsen, Trolle, Sondergaard *et al.*, 2012) and recent reduction in fertilizer use and changes in agricultural activities has resulted in a decrease in nutrient load from the catchments (Kronvang, Andersen, Borgesen *et al.*, 2008; Jeppesen, Kronvang, Olesen *et al.*, 2011). Annual mean alkalinity was also synchronous with a positive long-term trend in 70% of the lakes suggesting the role of re-oligotrophication and consequent long-term decrease in ecosystem autotrophy and potentially a shift to heterotrophy, as previously shown in four Danish lakes (Trolle, Staehr, Davidson *et al.*, 2012).

Previous studies suggested lake biotic variables are less synchronous than abiotic variables (Arnott *et al.*, 2003; Vogt *et al.*, 2011; Magnuson *et al.*, 1990; Bloch and Weyhenmeyer, 2012; George, Talling and Rigg, 2000). Here, plankton displayed a similar level of annual synchrony as lake chemistry. Notably, richness of phyto- and zooplankton, as well as many of their main groups exhibited synchrony and positive long-term trends across all lakes. Furthermore, richness of annually-pooled phyto- and zooplankton communities increased across years. Change in identification efficiency of taxonomists across time may potentially drive the observed trends in plankton richness (Straile, Jochimsen and Kümmerlin, 2013; Nöges, Mischke, Laugaste *et al.*, 2010). However, the increasing trend was consistent in most plankton groups including CLA, which has no special difficulties in genus-level identification. This widespread increase in plankton richness coincided with the

positive trends in temperature and precipitation and negative trend in wind speed, as well as negative trends in PO_4 , TN and NO_3 that are common to most of the lakes. As the trends in climate and nutrients have co-occurred and they were mostly monotonic without a shift during the study period, it was not possible to perform a conclusive hypothesis test to elucidate which of these trends was responsible for the widespread increase in plankton. Although speculative, few explanations are plausible. Widespread nutrient reduction across Danish landscape (Kronvang, Jeppesen, Conley *et al.*, 2005) in most of the lakes could potentially affect plankton richness positively. N and P have been recognised as co-limiting factors in shallow lake ecosystems (Özkan, Jeppesen, Johansson *et al.*, 2010; Gonzalez Sagrario, Jeppesen, Gomá *et al.*, 2005; James, Fisher, Russell *et al.*, 2005) and significantly related to the diversity of phytoplankton (Özkan, Jeppesen, Søndergaard *et al.*, 2012) and submerged macrophytes (James *et al.*, 2005). Therefore, the widespread nutrient reduction in the study lakes might have increased phytoplankton richness by releasing competitive pressure (Tilman and Pacala, 1993) and this in turn might enhance the zooplankton richness by allowing niche partitioning (Tilman, Kilham and Kilham, 1982; Özkan *et al.*, 2013; Guisande, Bartumeus, Ventura *et al.*, 2003; Striebel, Singer, Stibor *et al.*, 2012). Analyses of 30 years data in Lake Zurich also demonstrated a strong association of phytoplankton and zooplankton richness indicating the role of food-web interactions in sustaining diversity across trophic levels (Pomati *et al.*, 2011). Improved water quality might also increase the planktonic diversity mediated by the return of submerged macrophytes (Declerck, Vandekerckhove, Johansson *et al.*, 2005; Declerck, Vanderstukken, Pals *et al.*, 2007; Kruk, Rodriguez-Gallego, Meerhoff *et al.*, 2009; Muylaert, Perez-Martinez, Sanchez-Castillo *et al.*, 2010). However, no strong indication was found for widespread water quality improvements in Chl-a or Secchi depth across all lakes except for a widespread decrease in SS. Climatic trends, especially the increase in temperature and wind might also affect plankton richness (Pomati *et al.*, 2011). However, the direct effect of air temperature on surface water temperature was limited and only significant in summer and no trend in stratification was observed. Previous studies have also shown that nutrient changes may have stronger influence on lake plankton than long-term climatic trends (Dong, Bennion, Maberly *et al.*, 2012; Salmaso, 2010). However, joint effect of nutrient reduction and climate in driving an increase in both taxonomic and functional richness of phytoplankton has also been documented (Pomati *et al.*, 2011). Although no clear effect of climatic trends on plankton has been found in the present analyses, climatic control of phytoplankton phenology (Thackeray, Jones and Maberly, 2008; Meis, Thackeray and Jones, 2009; Blenckner, Adrian, Livingstone *et al.*, 2007) and community composition (Anneville *et al.*, 2005; Winder, Reuter and Schladow, 2009), as well as the effect of extreme climate events on plankton ecology (Pomati *et al.*, 2011) have previously been shown. Overall, although the conclusive test was not possible, widespread nutrient reduction across Danish landscape might have been responsible for the temporal increase in plankton richness.

Recovery from earlier eutrophication in S lakes resulted in high levels of synchrony in lake water chemistry and plankton, as well as strong and consistent long-term trends of water quality improvements with decrease in phytoplankton biomass and a shift from CHL dominance towards more heterogeneous and richer phytoplankton communities. Analyses of the earlier phase of the monitoring program found that 10 lakes, which experienced nutrient reduction prior to 1999 had an immediate response with decreases in Chl-a (Jeppesen *et al.*, 2002). Decrease in phytoplankton biomass and a shift towards phytoplankton flora more associated with oligotrophic conditions in response to nutrient reduction have been widely documented (Jeppesen *et al.*, 2005b; Köhler, Hilt, Adrian *et al.*, 2005; Anderson, Jeppesen and Søndergaard, 2005). However, weak or inconsistent response in phytoplankton biomass and community composition to nutrient reduction has also

been observed (Anneville *et al.*, 2005; Moss, Barker, Stephen *et al.*, 2005; Nöges *et al.*, 2010). Although a dramatic decline in TP and phytoplankton biomass is expected to lead a corresponding decline in zooplankton biomass (Köhler *et al.*, 2005; Phillips, Kelly, Pitt *et al.*, 2005), no clear response was observed in zooplankton biomass in S lakes corroborating some of previous observations (Straile and Geller, 1998). Earlier analyses on Danish lakes in recovery from eutrophication have also found weak or insignificant response of zooplankton biomass to re-oligotrophication, suggesting confounding bottom-up and top-down affects (Jeppesen *et al.*, 2002; Jeppesen, Jensen, Søndergaard *et al.*, 2005a).

W and N lakes reflected the patterns observed across all lakes in general. Although N lakes had no change in their TP levels, they experienced a decrease in PO_4 , TN and NO_3 , which caused a moderate synchrony in water chemistry and substantial synchrony in plankton with an increase in the richness of both plankton groups. Almost no synchrony in lake water chemistry was observed in W lakes, reflecting inconsistent trends in their TP and TN concentrations. However, the weak synchrony in NO_3 and, decreasing trends in NO_3 and PO_4 might have resulted in a weak synchrony in few plankton variables and increase in zooplankton richness in 4 W lakes. The weaker synchrony and less consistent long-term trends in W lakes indicated that confounding catchment-scale processes weakens the synchrony in temporal changes in lake chemistry and plankton as previously shown (Magnuson *et al.*, 1990; Arnott *et al.*, 2003; Vogt *et al.*, 2011).

The pattern in synchrony and long-term trend varied across seasons. Synchrony in water chemistry, as well as nutrient reduction, Secchi depth improvement and phytoplankton biomass decrease were strongest in spring, however, increase in plankton richness was strongest in summer across all lakes. Synchrony in S lakes was consistent across seasons across seasons, reflecting the overriding effect of re-oligotrophication. The negative trend in TP and PO_4 in S lakes was slightly weaker in summer. This has also been observed in previous analyses of Danish lakes (Søndergaard, Jensen and Jeppesen, 2005), British and German lakes (Köhler *et al.*, 2005; Phillips *et al.*, 2005) and attributed to internal TP loading from the sediment in summer (Søndergaard *et al.*, 2005; Søndergaard, Bjerring and Jeppesen, 2012). However, the increase in Secchi depth and the decrease in SS and Chl-a were strongest in summer in S lakes, indicating water clarity improvement in summer, although the strongest decrease in phytoplankton biomass occurred in spring. Eight Danish lakes (five of them included in the present study) had no significant decline in their phytoplankton biomass in summer and autumn between 1989 and 2001, probably reflecting that the internal loading was stronger in the earlier period of nutrient reduction (Jeppesen *et al.*, 2005a).

Overall, this widespread nutrient reduction across Danish landscape led to moderate synchrony in lake chemistry and plankton, and probably resulted in a widespread increase in plankton richness in 17 lakes. The synchrony was strongest and trends were most consistent for the lakes recovering from eutrophication, demonstrating the prominent role of re-oligotrophication, increasing plankton diversity and altering plankton composition. The potential effect of climate on lake chemistry and plankton, even in summer, when it is strongest, was probably confounded by widespread nutrient reduction. The present study indicates that anthropogenic changes across agricultural landscapes might act like a large scale forcing and induce synchrony in ecosystems.

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APPENDIX

Table 1. Summary of data treatment for missing values in environmental variables. Frequencies of samples collected with plankton samplings and replacements with different averages are given.

	Original	One week	Two weeks	One month	Missing
TP	0.97	0.015	0.005	0.005	0.005
PO ₄	0.968	0.015	0.005	0.005	0.006
TN	0.969	0.015	0.005	0.006	0.006
NO ₃	0.966	0.015	0.005	0.004	0.01
NH ₄	0.968	0.015	0.005	0.006	0.006
pH	0.97	0.015	0.004	0.005	0.005
Alkalinity	0.956	0.015	0.005	0.007	0.018
Chl-a	0.964	0.015	0.005	0.007	0.009
SS	0.949	0.013	0.005	0.007	0.026
Secchi	0.963	0.006	0.004	0.005	0.021
SiO ₂	0.967	0.015	0.005	0.007	0.007
Water temperature	0.956	0.006	0.005	0.009	0.024
Schmidt	0.884	0.006	0.004	0.01	0.096

Table 2. Monotonic long-term trends using Kendall correlations in climate averaged for all lakes.

	Air Temperature	Irradiance	Precipitation	Wind Speed
whole	0.05 ***	0.01 ns	0.02 *	-0.07 ***
spring	0.05 ***	0.07 ***	-0.01 ns	-0.10 ***
summer	0.09 ***	-0.00 ns	0.05 ***	-0.08 ***
autumn	0.04 **	0.01 ns	0.00 ns	-0.05 **

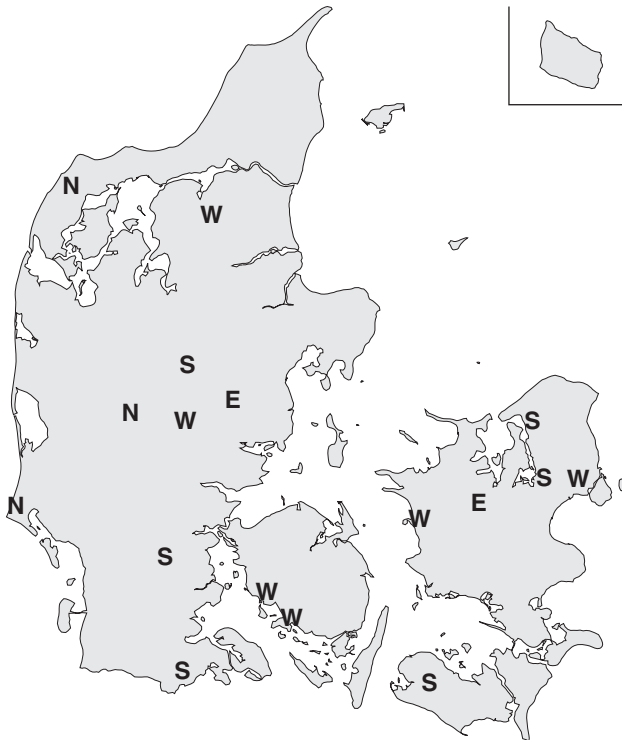


Figure 1. Location of the study lakes. S, W, N denotes for lake types and E denotes for lakes not included in any group.

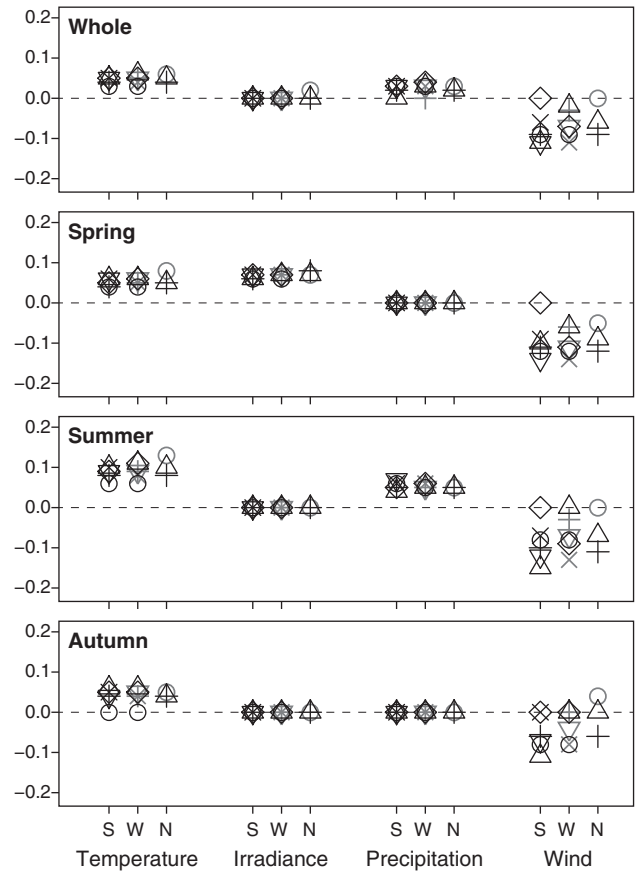


Fig 2. Summary of the monotonic long term trends in climatic parameters in each lake analysed for whole year and separate seasons. For details see Figure 2 and 4.

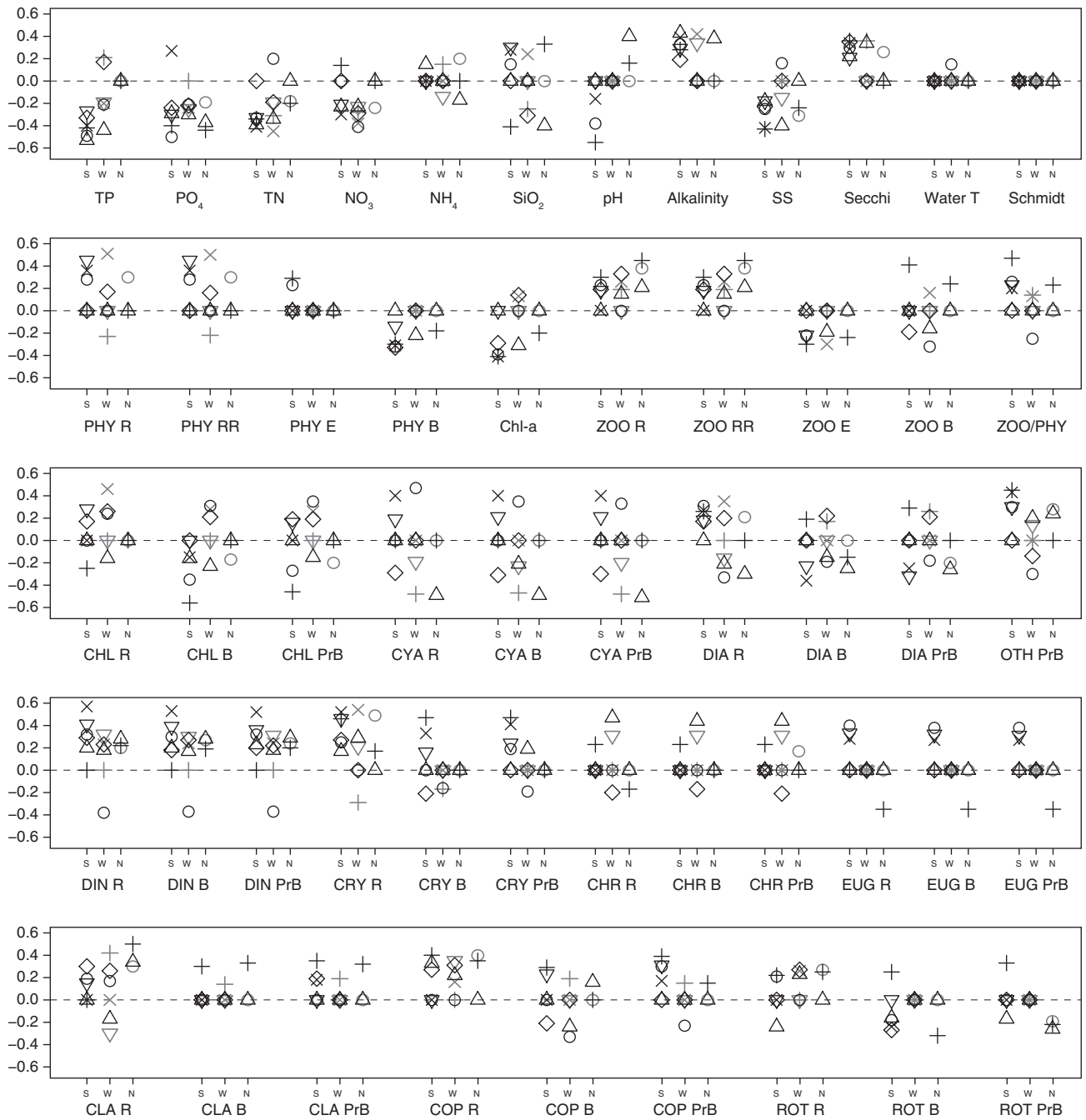


Figure 3. Summary of the monotonic long term trends in lake and plankton variables analysed for spring. For details see Figure 4.

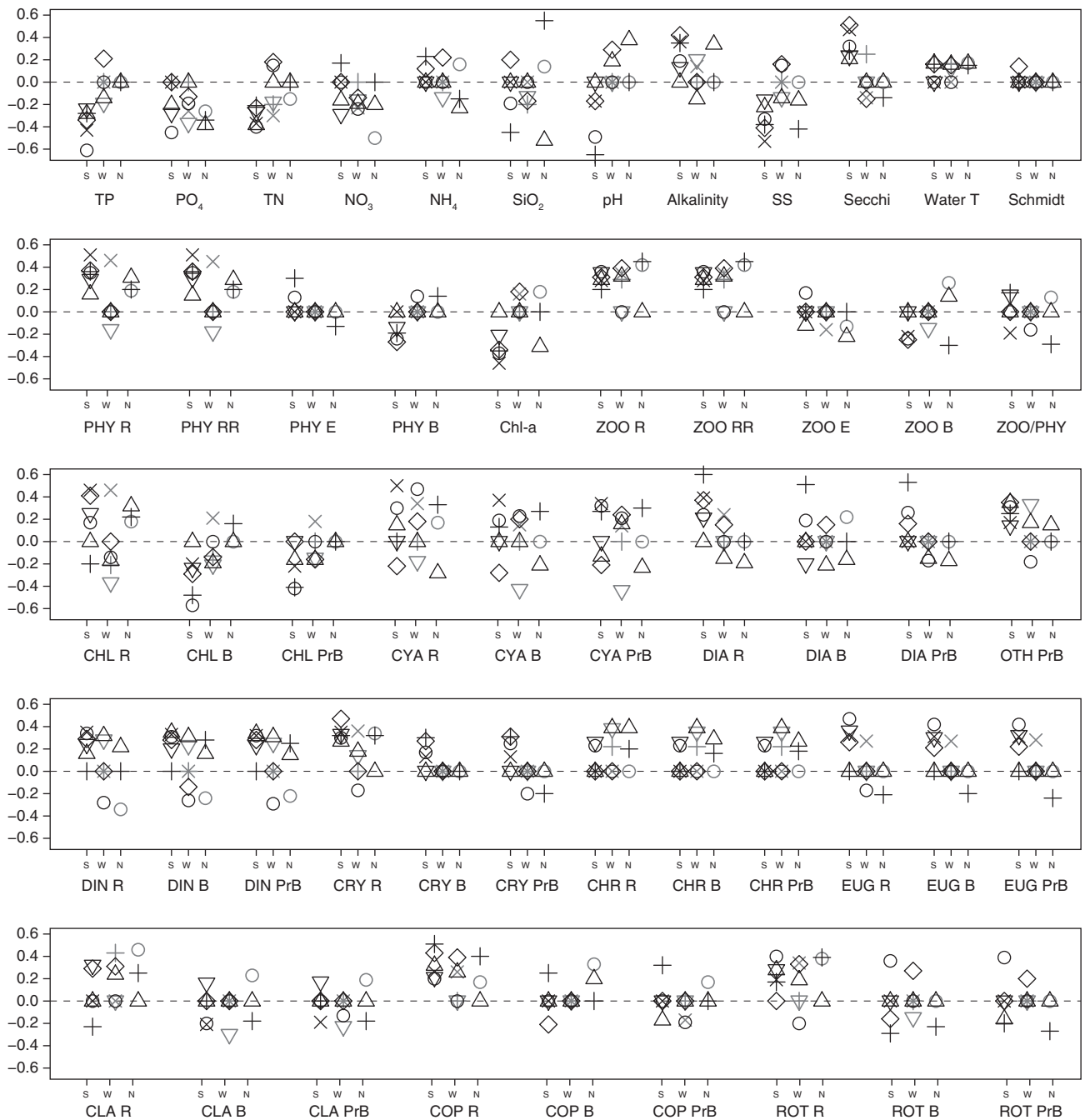


Fig 4. Summary of the monotonic long term trends in lake and plankton variables analysed for summer. For details see Figure 4.

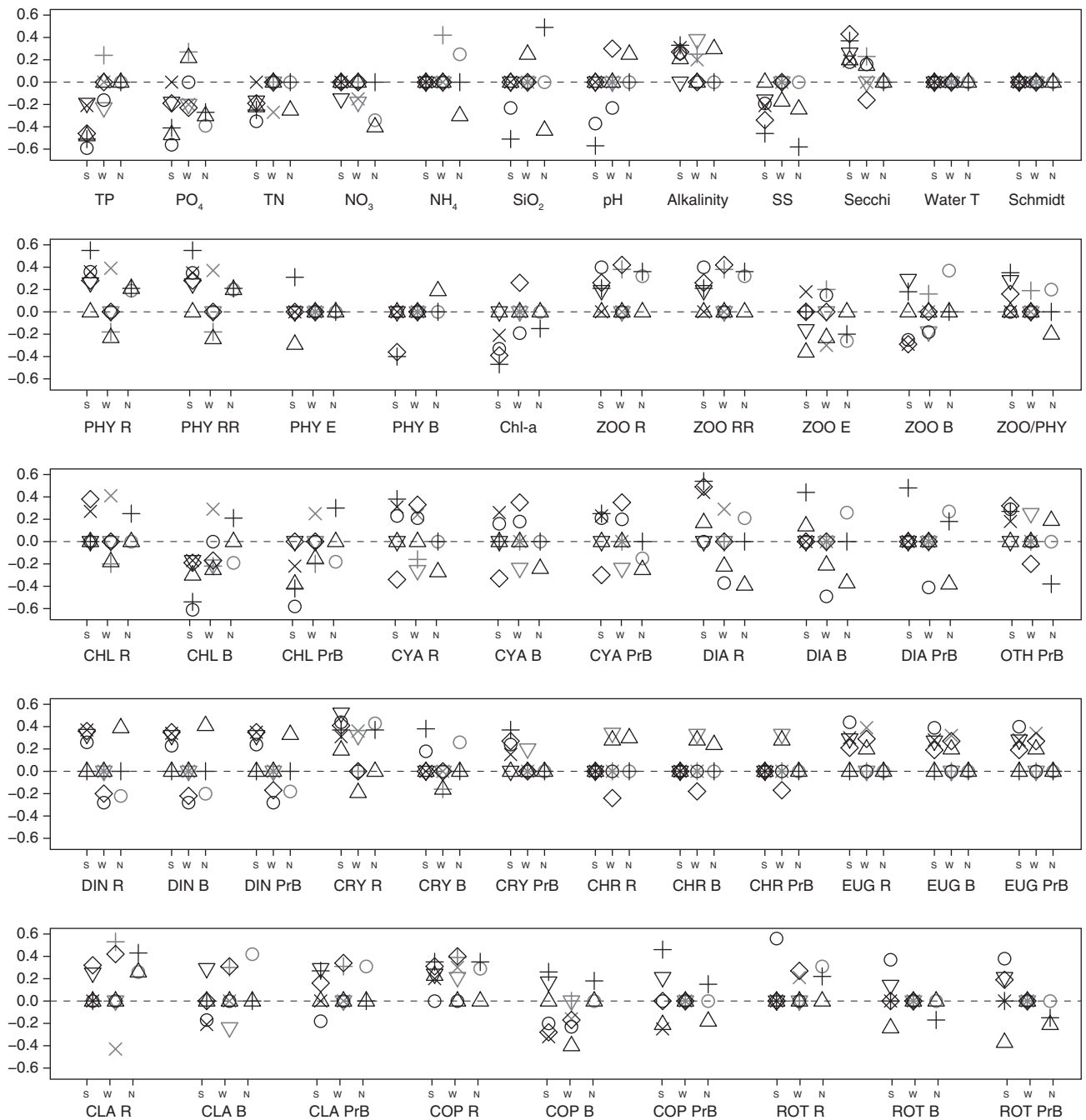


Fig 5. Summary of the monotonic long term trends in lake and plankton variables analysed for autumn. For details see Figure 4.

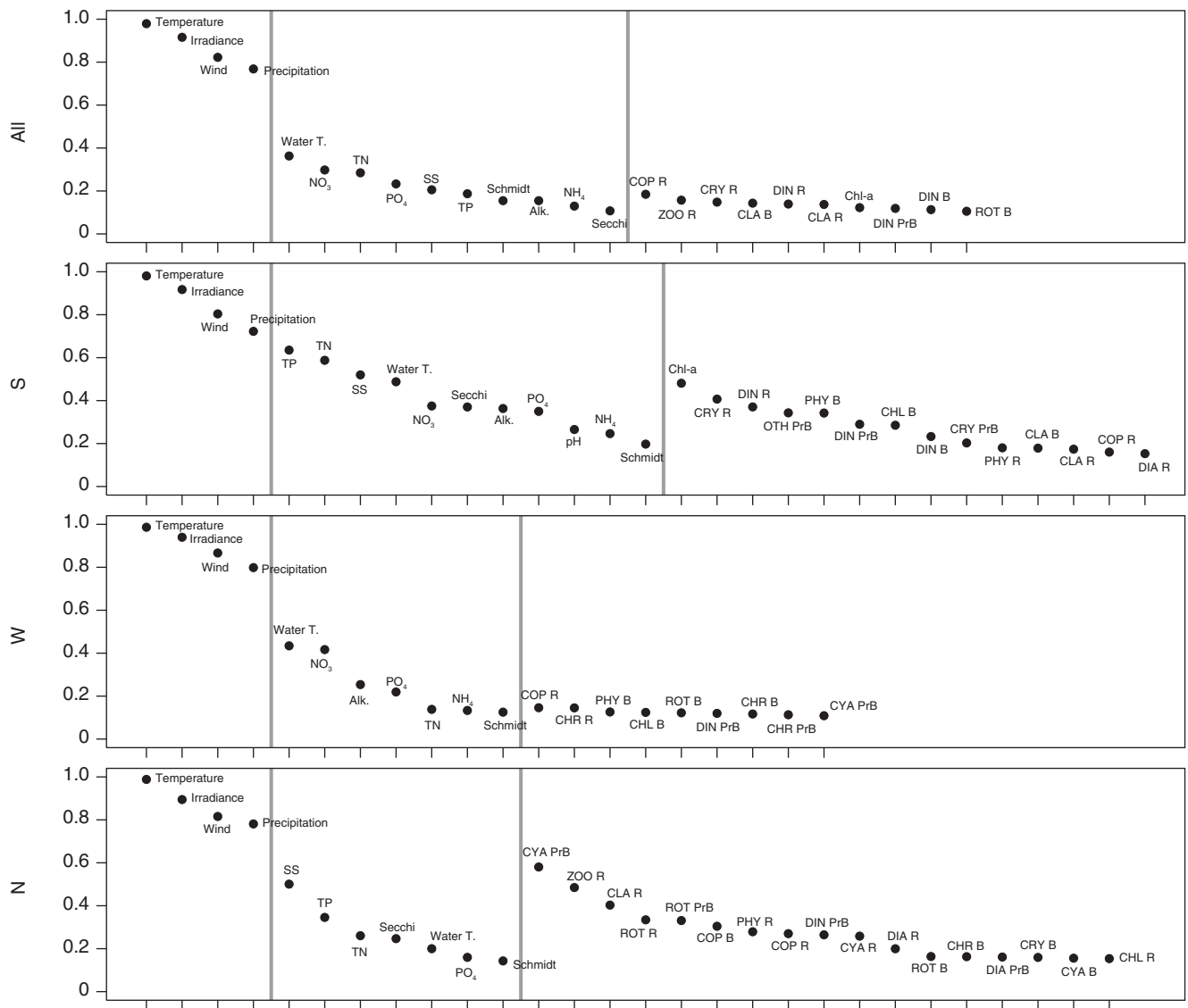


Fig 6. Summary of the synchrony in climate, lake and plankton variables in spring analysed for all lakes and S, W and N lakes separately. For details see Figure 3.

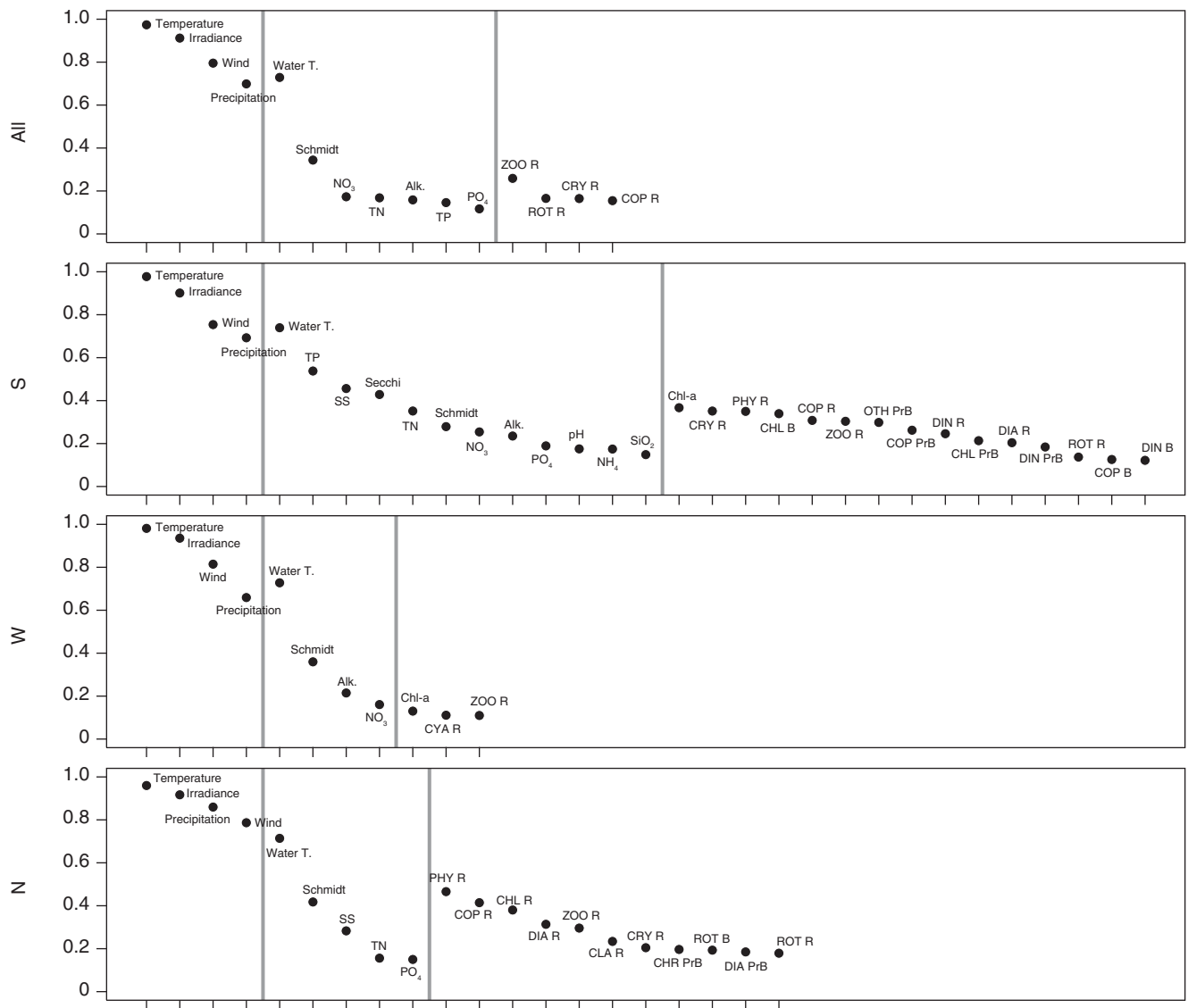


Fig 7. Summary of the synchrony in climate, lake and plankton variables in summer analysed for all lakes and S, W and N lakes separately. For details see Figure 3.

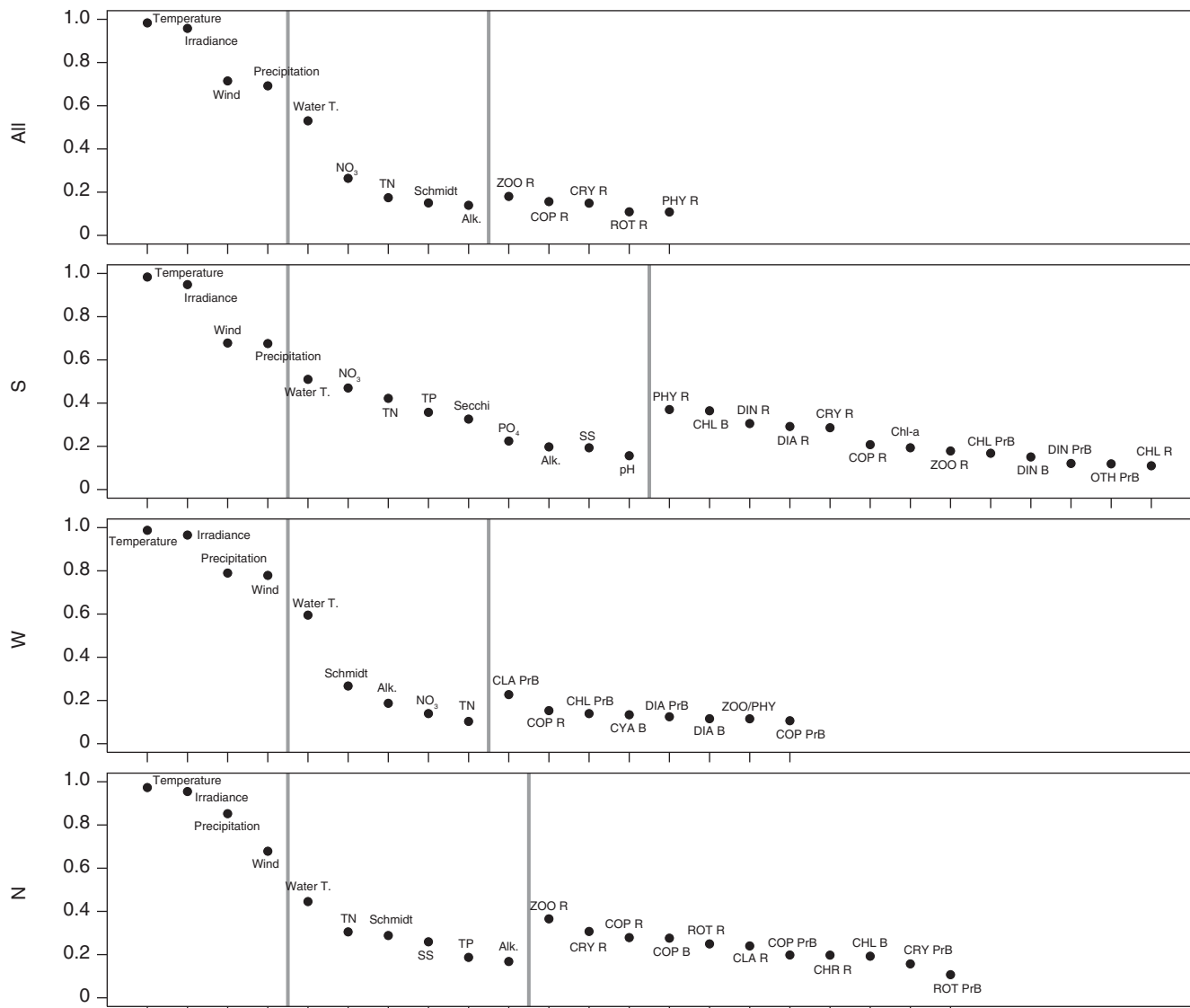


Fig 8. Summary of the synchrony in climate, lake and plankton variables in autumn analysed for all lakes and S, W and N lakes separately. For details see Figure 3.

PAPER IV

Cross-taxon congruence in lake plankton largely independent of environmental gradients

Korhan Özkan ^{a,b}, Erik Jeppesen ^{a,c,d}, Thomas A. Davidson ^{a,b}, Martin Søndergaard ^a, Torben L. Lauridsen ^{a,e}, Rikke Bjerring ^a, Liselotte S. Johansson ^a and Jens-Christian Svenning ^b

^a Freshwater Ecology Group, Department of Bioscience, Aarhus University, Silkeborg, Denmark

^b Ecoinformatics & Biodiversity Group, Department of Bioscience, Aarhus University, Aarhus, Denmark

^c Greenland Climate Research Centre, Greenland Institute of Natural Resources, Nuuk, Greenland

^d Sino-Danish Centre for Education and Research, Beijing, China

^e Arctic Research Centre, Aarhus University, Aarhus, Denmark

To be re-submitted to Ecology.

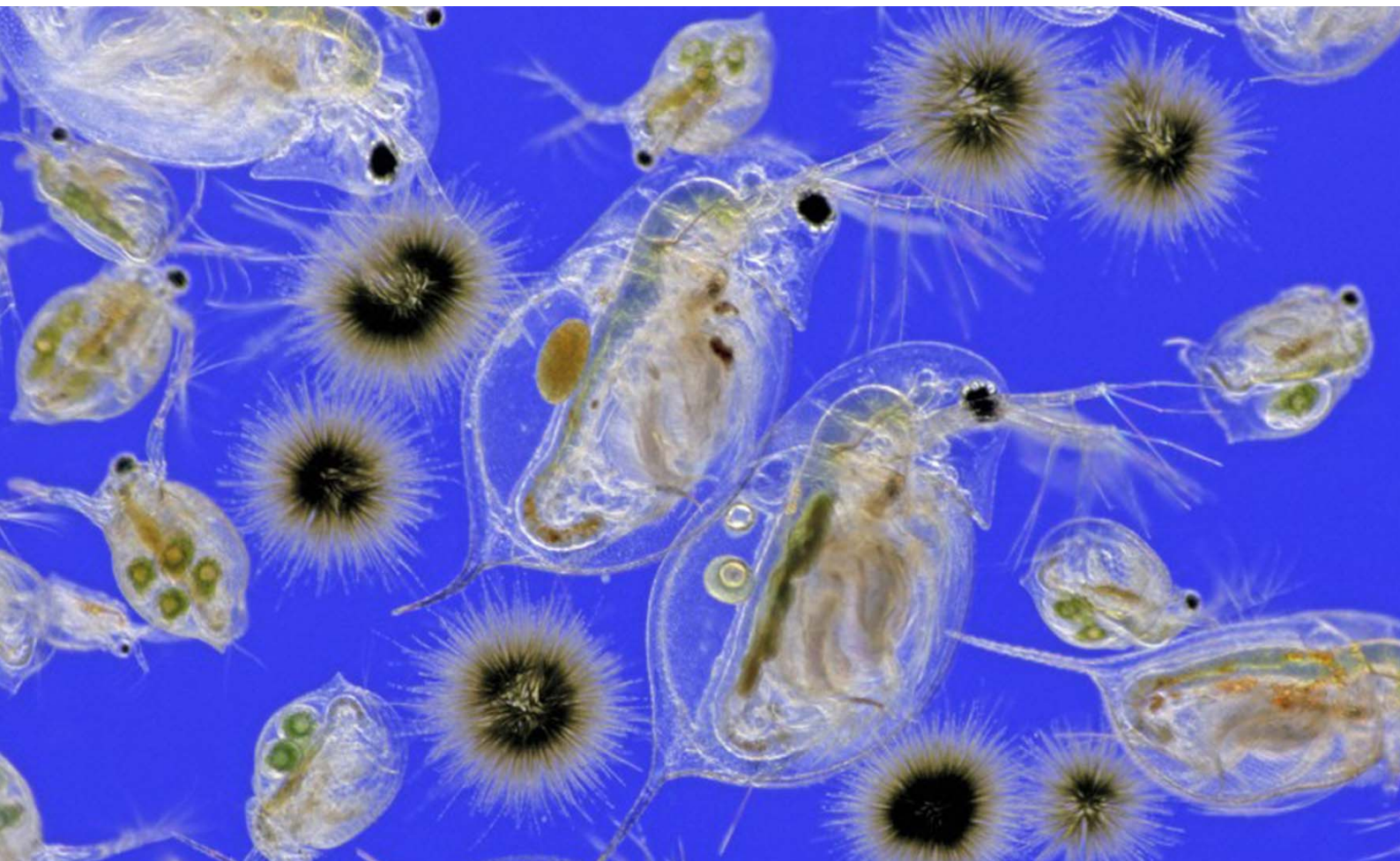


Photo: Spike Walker, Wellcome Images.

CROSS-TAXON CONGRUENCE IN LAKE PLANKTON LARGELY INDEPENDENT OF ENVIRONMENTAL GRADIENTS

Korhan Özkan^{a,b*}, Erik Jeppesen^{a,c,d}, Thomas A. Davidson^{a,b}, Martin Søndergaard^a, Torben L. Lauridsen^{a,e}, Rikke Bjerring^a, Liselotte S. Johansson^a and Jens-Christian Svenning^b

^a Freshwater Ecology Group, Department of Bioscience, Aarhus University, Silkeborg, Denmark

^b Ecoinformatics & Biodiversity Group, Department of Bioscience, Aarhus University, Aarhus, Denmark

^c Greenland Climate Research Centre, Greenland Institute of Natural Resources, Nuuk, Greenland

^d Sino-Danish Centre for Education and Research, Beijing, China

^e Arctic Research Centre, Aarhus University, Aarhus, Denmark

* Corresponding author: korhan_oz@yahoo.com

ABSTRACT

Groups of organisms often have congruent patterns of diversity or community structure due to similar environmental requirements. However, ecological interactions across trophic levels may also promote congruence independent of environmental drivers through selective predation, niche partitioning or facilitation. We examined congruence between phytoplankton and zooplankton communities using 20 years of monitoring data from 17 Danish lakes, most of which were in recovery after a period of eutrophication due to reduction of external nutrient loading. Linear mixed effect models and partial Mantel tests were used to elucidate the extent to which congruence in genus richness and composition was driven by environmental factors. Congruence not explained by environmental controls might indicate ecological interactions across trophic levels of lake plankton. Genus richness and composition of phyto- and zooplankton were significantly congruent. Environmental factors had limited power to explain the genus richness of phyto- and zooplankton (R^2 : 6 % and 12 %, respectively). Including richness of the reciprocal group among the predictors in both cases markedly improved the explanatory power (R^2 : 11 % and 18 %, respectively). Similarly, a large proportion of the congruence in genus composition was independent of environmental dissimilarity (Mantel r : 0.29 and partial Mantel r after accounted for environment: 0.17). The strength of the congruence varied among different groups of zooplankton and phytoplankton as expected from trophic interactions. Remarkably, congruence was strongest for the smallest group of zooplankton, the rotifers, which graze on a limited size spectrum of phytoplankton. Moreover, congruence was stronger in low-nutrient lakes, suggesting that the strength of the trophic interactions between the two groups weakens with eutrophication. This is likely due to changes in trophic dynamics, where enhanced fish predation on large-bodied zooplankton with increasing nutrient level results in reduced zooplankton grazing control of phytoplankton assemblages.

Keywords: phytoplankton, zooplankton, diversity, community composition, community concordance, coherence, biotic interactions, ecological interactions, trophic interactions.

INTRODUCTION

Understanding the processes shaping the spatiotemporal patterns of biodiversity is a fundamental aim of ecological research and is, furthermore, essential for management and conservation purposes. Studies at different scales have shown that spatial patterns of biodiversity across different groups of organisms are often non-randomly associated, a phenomenon termed cross-taxon congruence (Gaston 2000). Congruence in diversity across different groups has important implications for defining conservation areas, ecological impact assessments and environmental monitoring (Heino 2010).

Environmental control of biotic communities can promote cross-taxon congruence (Gaston and Williams 1996) as the diversity of different taxonomic groups will correlate across space and time if they respond to the same environmental gradients. The diversity of different taxonomic groups may also be correlated even if they respond to different environmental factors, provided that these are inter-correlated. However, spatial sampling bias, where a number of sites are sampled more intensively for multiple organism groups, may create positive correlations across the diversity of different taxonomic groups (Gaston 2000).

Several studies examined lake time-series to quantify if the abiotic and biotic variables changed coherently through time in a set of lakes, which might be attributable to large scale factors like climate change, acidification or eutrophication/re-oligotrophication (Magnuson et al. 1990, Fischer et al. 2001, Anneville et al. 2005). Coherent temporal changes in abiotic variables due to climatic changes have been documented (Magnuson et al. 1990, Arnott et al. 2003, Preston and Rusak 2010). Coherent temporal changes in productivity or plankton biomass (Magnuson et al. 1990, Arnott et al. 2003, Preston and Rusak 2010, Vogt et al. 2011) as well as plankton richness or composition (Arnott et al. 2003, Anneville et al. 2005, Bloch and Weyhenmeyer 2012) have also been observed, albeit typically weaker. A weaker temporal coherency in biotic variables has been mostly attributed to the fact that the biota responds more strongly to system specific local factors (Magnuson et al. 1990, Kratz et al. 1995).

Cross-taxon congruence may also be promoted through trophic interactions. Consumers can promote diversity at lower trophic levels by asymmetrically affecting dominant species and thereby preventing competitive exclusion (Paine 1966, Greve et al. 2012). Furthermore, a diverse producer community can promote diversity at the higher trophic levels by enabling niche partitioning (Tilman et al. 1982) or simply via productivity effects (cf. Currie 1991). Biotic interactions such as mutualism and parasitism may similarly promote congruent diversity patterns with diversity in one group promoting diversity in interacting groups (Kissling et al. 2007). Moreover, trophic interactions may promote diversity through assembly processes as colonization by one group may facilitate the colonization of another (Grover 1994). If such interactions across trophic levels play an important role in shaping diversity, cross-taxon congruence across trophic levels (Longmuir et al. 2007) will exceed that attributable to environmental control (Kissling et al. 2007, Greve et al. 2012).

Few studies have revealed significant cross-taxon congruence between lake phyto- and zooplankton (Allen et al. 1999b, Bowman et al. 2008, Padial et al. 2012) and the observed associations were complex and not consistent between the different groups and studies. However, a number of studies have found diversity and composition of zooplankton and phytoplankton to be independent of each other (Allen et al. 1999a, Declerck et al. 2005, Tolonen et al. 2005, Longmuir et al.

2007). Overall, there is therefore contradictory evidence of whether lake plankton diversity patterns are congruent across trophic levels (Heino 2010), and the extent to which the observed congruence reflects ecological interactions versus environmental forcing is not well understood.

We analyzed a unique, comprehensive data set of phyto- and zooplankton counts from 17 lakes monitored for 20 years during which many of the lakes were in recovery from past eutrophication. We tested for congruence in genus richness and genus composition between planktonic primary producers (phytoplankton) and grazers (zooplankton). Upon verifying significant congruence, we examined the extent to which this was driven by environmental factors. We hypothesized that interactions across trophic levels would cause strong congruence beyond that is driven by environmental factors. We also hypothesized that the strength of the congruence would differ between different subgroups of phyto- and zooplankton, reflecting their ecological characteristics, most importantly zooplankton size, as smaller zooplankton can handle a narrower spectrum of particles and thus potentially are more responsive to the changes in phytoplankton. We further expected that the trophic link between zooplankton and phytoplankton would weaken along an eutrophication gradient as fish control of large-bodied zooplankton increases with eutrophication.

METHODS

Samplings and data treatment

From 1989 to 2008 phyto- and zooplankton samples were collected year-round as part of the Danish monitoring programme of the aquatic environment (Svendsen et al. 2004). Seventeen lakes without major internal disturbances (biomanipulation, etc.) were included (Figure 1). Winter samples (December, January, February) were excluded due to varying sampling intensity across years. The remaining 4958 samples had an average sampling interval of 14 days (Appendix). The phytoplankton was collected from mid-lake depth-integrated sample covering the photic zone. Depth-integrated zooplankton samples were taken with a heart-valve sampler at three stations placed randomly in areas representing 80% of maximum depth and subsequently pooled. Depending on the TP level, 0.9 or 1.8 L water was taken for counting nauplii and rotifers and 4.5 or 9 L water was filtered through a 90 µm net for larger zooplankton. Phyto- and zooplankton were fixed in Lugol's iodine.

At least 100 and 75 individuals of each of the dominant species were counted for phyto- and zooplankton, respectively. Phytoplankton biovolume was calculated by fitting each taxon to simple geometric forms (Ütermöhl 1958, Edler 1979, Rott 1981). If possible, up to 25 individuals of each zooplankton species were measured and biomass was calculated using length-weight relationships (Dumont et al. 1975, Bottrell et al. 1976, McCauley 1984). All taxa were aggregated to genus level due to varying intensity of species-level identification between different plankton groups and potential differences in identification skills of the taxonomists. We screened the plankton data for potential inconsistencies and employed an inclusive approach, i.e., if a taxon was present in the data set with partially missing information (e.g., biomass being registered without count), the missing information was recalculated using original information or a representative average for the taxon in question.

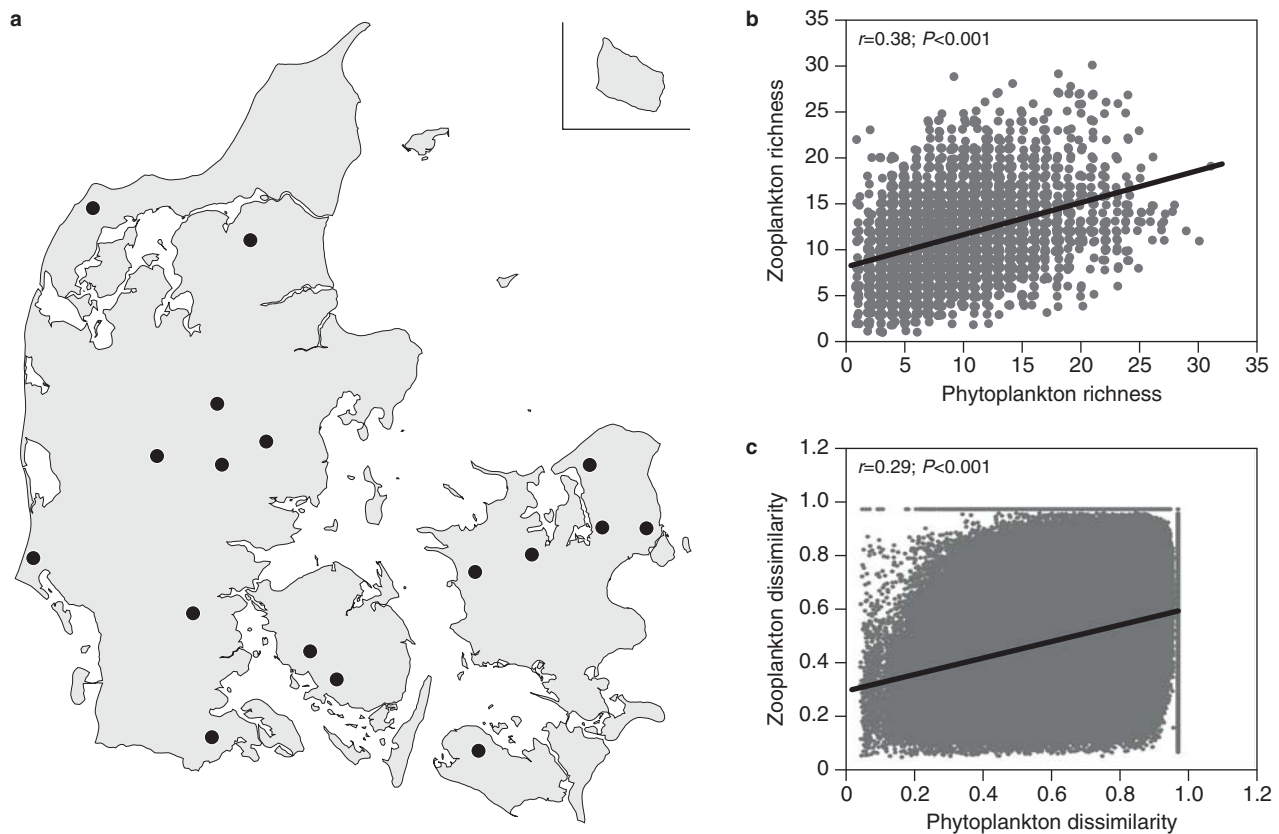


Figure 1. Location of the lakes across Denmark (a), correlation between phyto- and zooplankton genus richness (b) and pairwise phyto- and zooplankton compositional dissimilarities across samples (c). Mantel correlations is reported for compositional dissimilarities and linear regression lines are plotted.

We compiled a data set of lake water chemistry and climatic parameters as potential environmental predictors of plankton communities. Lake water chemistry samples were collected simultaneously with the zooplankton samples. The water chemistry samples were analyzed for total phosphorus (TP), soluble reactive phosphorus (SRP), total nitrogen (TN), NO_{3-2} , NH_4 , SiO_2 , chlorophyll-a (Chl-a) and pH (Svendsen et al. 2004, Lauridsen et al. 2007). TP and SRP as well as TN and NO_{3-2} were strongly correlated ($r > 0.84$) and thus only TP and TN were used in the analyses. When a water chemistry variable was missing for a sample, it was replaced with the most relevant available data: first, the mean of observations in a seven-day temporal window; second (if a substitute sample was not available at the first step), the mean of observations in a 15-day window or, third, linear interpolation of neighboring samples if they are less than 30 days apart (Appendix). Mean air temperature, wind speed, solar radiation (from daily averages interpolated to 20 km grids) and precipitation (from daily averages interpolated to 10 km grids and corrected for wind and altitude depending on the month of the year, Danish Meteorological Institute) were calculated for 15 days before each sample. From 1993 and onwards, submerged macrophyte abundance was estimated once a year in August at maximum biomass. It was calculated as percentage of plant volume infested (PVI, Canfield et al. 1984) using plant coverage and height. During 1993-2003, lakes were divided into sub-areas and plant coverage and height were measured at minimum 10 sites in depth intervals of 0.25 or 0.5 m. From 2004 onwards, observation points were arranged on transects evenly placed perpendicular to the longitudinal axis of the lake. Depth intervals were equally represented in each transect. Observations were performed by using a water glass in shallow areas and a plant rake or diver in deeper areas. Characteristics of the lakes are given in Table 1. All predictors were scaled to zero mean and unit standard deviation prior to analyses.

Table 1. Summary of morphology and water chemistry of 17 Danish lakes monitored from 1989 to 2009. The winter samples were excluded due to varying sampling intensity across years.

	Lake area (ha)	Average depth (m)	Max. depth (m)	TP (mg/l)	TN (mg/l)	pH	Chl-a (mg/l)
Min.	12	0.8	1.8	< 0.001	0.100	3.930	< 0.001
Med.	37	2.7	6.0	0.075	1.720	8.300	0.027
Max.	3954	15.1	32.6	2.700	12.549	10.60	1.000

Statistical analyses

Congruence in richness

We tested the correlation between richness of phytoplankton and zooplankton across all samples. Next, we used Linear Mixed Model (LMM, Pinheiro and Bates 2000) to elucidate whether phytoplankton and zooplankton richness responds to the same environmental gradients by applying the modeling framework suggested in Zuur (2009). Lake identity was modeled as random factor, while temporal dependence was modeled as correlation using the *corArma* function (Pinheiro et al. 2012) nested within each lake. The performance of random factor and correlation structure was assessed via model comparisons based on the likelihood ratio (LR). We did not perform model selection as there is no consensus on a robust methodology for LMM (Zuur 2009) and our sample size is large. To account for potential nonlinear relationships, we included the quadratic terms for all environmental predictors one-by-one and retained the quadratic terms if they significantly improved the models.

To elucidate the role of zooplankton and phytoplankton in predicting each other's richness in the presence of environmental predictors, we included first the biomass and then the richness of a group in reciprocal models and examined the improvement in model fits. Model comparisons were based on LR and model fits were estimated as pseudo- R^2 calculated using *ImmR2* function (Maj 2011).

Autoregressive models have previously been used to quantify temporal coherence between species groups, while accounting for main environmental gradients (Ives 1995, Fischer et al. 2001). We repeated the analyses with autoregressive models fitted for each lake and the results are reported in the appendix. The findings were largely similar and we prefer to present LMM results as they enable us to analyze all lakes together and draw general conclusions.

Congruence in genus composition

We used Mantel tests to determine congruence in phyto- and zooplankton genus composition (Legendre and Legendre 1998) followed by partial Mantel tests to elucidate the extent to which any such congruence was driven by environmental differences. The species-site and environmental-site data matrices were converted into matrices of pair wise Bray-Curtis dissimilarity and Euclidean distance, respectively, for the Mantel tests. Genera observed in <1 % of the samples were excluded and plankton biomass was log transformed to reduce the influence of very rare and dominant species. A forward selection procedure was used to select the environmental variables used in the Mantel tests: environmental variables were added into the pairwise-dissimilarity matrix until there was no increase in their correlation with the species dissimilarity matrix or the test became insignificant.

The richness and community congruence analyses were repeated for each lake, year, and month to examine the robustness of correlations. We also repeated the

analyses for the main taxonomic groups of zooplankton and phytoplankton separately. Moreover, we analyzed the correlations across different lake productivity levels (0-0.05; 0.05-0.10 and >0.10 mg l⁻¹ TP) to elucidate the effect of eutrophication on lake plankton richness congruence as well as between shallow and deep lakes (> 3.6 m). Furthermore, we analyzed the correlations across three levels (0-5, 5-15, >15 %) of PVI. Lastly, we analyzed the correlations for a gradient in proportional abundance (0-10, 10-50, >50% of total phytoplankton biovolume) of potentially inedible phytoplankton (>35 µm, Cyr and Curtis 1999) to examine if phyto- and zooplankton congruence increases with phytoplankton edibility. Proportional abundances were calculated only if at least 75% of the total phytoplankton biovolume contained size information (82 % of the samplings).

All statistical analyses were performed using *r* (R Development Core Team 2011) with the *vegan* package (Oksanen et al. 2011) for Mantel test, the *nlme* package (Pinheiro et al. 2012) for LLM and the *lmmfit* package (Maj 2011) for pseudo-*R*² calculations.

RESULTS

Median phyto- and zooplankton genus richness per sample were 8 and 11, respectively, during 20 years sampling of 17 study lakes (Table 2). Chlorophytes and rotifers were the most diverse groups of phyto- and zooplankton, respectively (Table 2).

Table 2. Minimum, median and maximum genus richness of the main groups of phyto- and zooplankton during 20 years of sampling of 17 study lakes.

Phytoplankton	Min.	Med.	Max.	Zooplankton	Min.	Med.	Max.
All	1	8	31	All	1	11	30
Chlorophyta	0	3	16	Rotifers	0	5	17
Cyanobacteria	0	1	10	Cladocerans	0	3	12
Diatoms	0	1	8	Copepods	0	2	7

Congruence in richness

Phyto- and zooplankton genera richness were significantly correlated in the 17 lakes studied during the 20-year study period ($r = 0.38$, $P < 0.001$, Figure 1). This correlation also consistently appeared in separate analyses for each month (mean $r = 0.33$, $P < 0.001$ for all), year (mean $r = 0.35$, $P < 0.01$ for all) and lake (mean $r = 0.32$, $P < 0.05$ for 16 of the 17 lakes). When analyzed across years, richness congruence first increased and then decreased from 2000 onwards (Figure 2). When the correlations were analyzed for different zooplankton groups, the strongest correlations with phytoplankton richness were observed for rotifers, followed by cladocerans and then copepods (Table 3). When the correlations were analyzed for different phytoplankton groups, the strongest positive correlations with zooplankton richness were observed for cyanobacteria, followed by diatoms and then chlorophytes (Table 3). The correlation between zooplankton and phytoplankton richness was stronger in deep ($r = 0.51$, $P < 0.001$) compared to shallow lakes ($r = 0.34$, $P < 0.001$). The correlation was also stronger in low-TP lakes ($r = 0.46, 0.37, 0.33$, for low, medium and high TP; respectively; $P < 0.001$ for all, Figure 3). The correlation was strongest in lakes with intermediate macrophyte abundance ($r = 0.29, 0.65, 0.05$, for low, medium and

Figure 2. Temporal changes in plankton congruence in genus composition (a) and richness (b) as well as changes in total phosphorus concentrations (c) across years. Only the years, where all lakes were sampled, were analyzed (1991-2006, appendix).

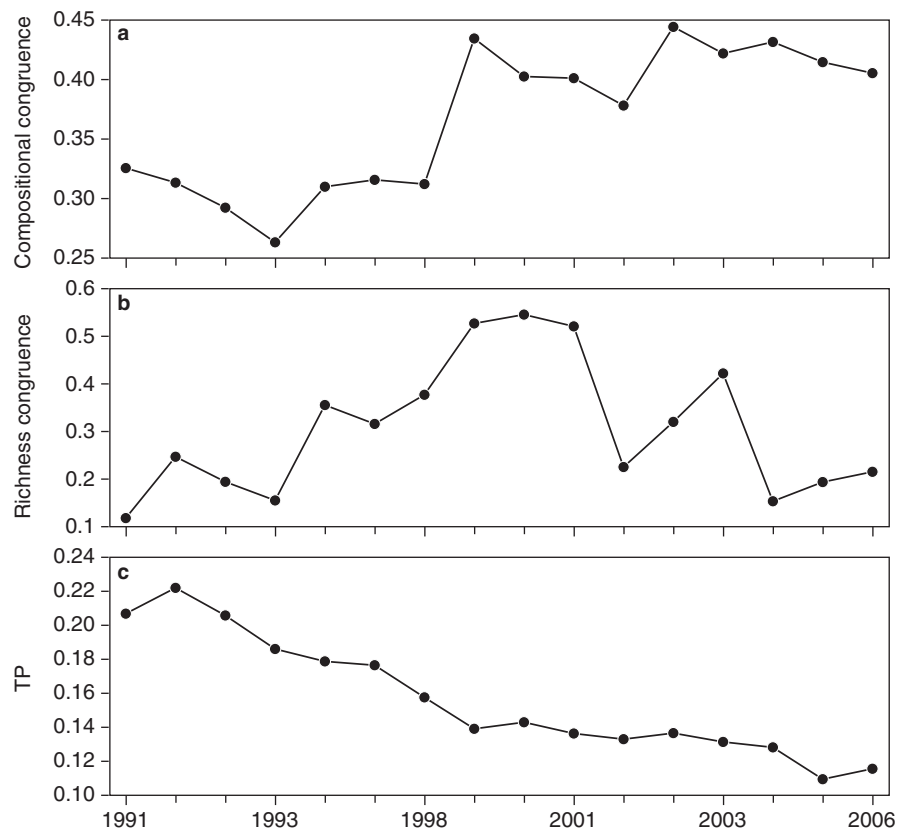


Table 3. Congruence in richness (Pearson r , upper) and community composition (Mantel r , lower) across the main groups of phytoplankton and zooplankton genera. P: <0.05, *; <0.01, **; <0.001, ***; ns, not significant.

	Pearson r	Phytoplankton	Cyanobacteria	Diatoms	Chlorophyta
Zooplankton		0.38 ***	0.30 ***	0.25 ***	0.11 ***
Rotifers		0.38 ***	0.24 ***	0.31 ***	0.09 ***
Cladocerans		0.23 ***	0.26 ***	0.02 ns	0.14 ***
Copepods		0.18 ***	0.16 ***	0.16 ***	-0.00 ns
	Mantel r	Phytoplankton	Cyanobacteria	Diatoms	Chlorophyta
Zooplankton		0.29 **	0.17 **	0.13 **	0.21 **
Rotifers		0.27 **	0.10 **	0.09 **	0.15 **
Cladocerans		0.17 **	0.09 **	0.06 **	0.16 **
Copepods		0.13 **	0.12 **	0.09 **	0.08 **

high PVI; respectively; $P < 0.001$ for all). The correlations tended to be stronger at higher proportional abundance of potentially inedible algae ($r = 0.24, 0.24, 0.43$, for low, medium and high abundance; respectively; $P < 0.001$ for all). We repeated the analyses for PVI and potentially inedible algae across different groups of phyto- and zooplankton and the patterns were largely similar.

LMM models for both phytoplankton and zooplankton richness yielded largely similar results. pH was unimodally related to both groups (Table 4). The nutrients were non-linearly or negatively related to the richness of both groups, while mean air temperature, solar radiation and precipitation were positively related. However, the explanatory power of the environmental models was low ($R^2 = 0.06$ for phytoplankton richness and $R^2 = 0.12$ for zooplankton richness).

Table 4. Summary of Linear Mixed models of phytoplankton and zooplankton genus richness with environmental factors as predictors. R^2 values are reported for models with environmental variables (E) only, for models with environmental variables and biomass of the other trophic level (E+B) and for models with environmental variables and biomass and richness of the other trophic level (E+B+R). Quadratic terms are denoted by superscript and significant variables are given in bold.

	Phytoplankton model		Zooplankton model	
R^2_E	0.06		0.09	
R^2_{E+B}	0.06		0.10	
R^2_{E+B+R}	0.11		0.17	
	Coefficient	P value	Coefficient	P value
Recip. richness	0.479	<0.001	0.540	<0.001
Recip. Biomass (log)	-0.384	<0.001	0.058	0.371
TP	-0.075	0.587	-0.347	0.004
TP ²	<0.001	0.992	0.020	0.279
TN	-0.521	<0.001	-0.362	<0.001
NH ₄	-0.273	<0.001	0.029	0.506
NH ₄ ²	0.014	0.008	-	-
SiO ₂	-0.030	0.769	-	-
SiO ₂ ²	-0.002	0.959	-	-
pH	0.215	0.050	0.137	0.206
pH ²	-0.069	0.222	-0.069	0.215
Temperature	0.449	<0.001	0.721	<0.001
Solar radiation	0.199	0.015	0.112	0.102
Precipitation	0.094	0.014	0.124	0.001
Wind speed	0.032	0.531	-0.059	0.229

Both plankton models improved when reciprocal plankton biomass was included in the predictor set but no change was observed in explanatory power ($\delta AIC = 37$, $P < 0.001$ and $R^2 = 0.07$ for phytoplankton model; $\delta AIC = 4$, $P = 0.015$ and $R^2 = 0.12$ for zooplankton model). However, including reciprocal plankton richness improved the models and increased the explanatory power of both plankton richness models ($\delta AIC = 52$, $P < 0.001$ and $R^2 = 0.11$ for phytoplankton model; $\delta AIC = 52$, $P < 0.001$ and $R^2 = 0.18$ for zooplankton model).

Congruence in composition

Phytoplankton and zooplankton communities were significantly correlated in the Mantel test ($r = 0.29$, $P < 0.001$; Figure 1). The correlations appeared consistently in separate analyses for each month (mean $r = 0.33$, $P < 0.001$ for all), year (mean $r = 0.36$, $P < 0.001$ for all) and lake (mean $r = 0.27$, $P < 0.001$ for all). When analyzed across years, the Mantel correlations tended to increase following the decrease in average TP across lakes (Figure 2). Mantel correlations with phytoplankton composition were strongest for rotifers, followed by cladocerans and copepods (Table 3), whereas Mantel correlations with zooplankton composition were strongest for chlorophytes followed by cyanobacteria and diatoms. The Mantel correlations were stronger in shallow ($r = 0.35$ and 0.25 for shallow and deep lakes, respectively, $P < 0.001$ for all) and low-TP lakes ($r = 0.37$, 0.23 , 0.27 for low, medium and high TP, respectively, $P < 0.001$ for all). The correlation was strongest for intermediate macrophyte abundance ($r = 0.26$, 0.39 , 0.16 , for low, medium and high PVI, respectively, $P < 0.001$ for all). The correlations tended to be stronger at higher proportional abundance of potentially inedible algae ($r = 0.21$, 0.30 , 0.39 , for low, medium and

high abundance, respectively, $P < 0.001$ for all). We repeated the analyses for PVI and inedible algae across different groups of phyto- and zooplankton and the patterns were widely similar.

TP and pH were the only environmental variables selected after the forward selection in Mantel tests for both phyto- and zooplankton genus composition. Mantel correlations between phyto- and zooplankton were largely independent of dissimilarities in TP and pH (partial Mantel test $r = 0.17$, $P < 0.001$).

DISCUSSION

We found clear congruence between phyto- and zooplankton communities in the 17 Danish lakes monitored over 20 years. Notwithstanding that phyto- and zooplankton richness and composition responded similarly to the environmental gradients, the degree of congruence exceeded that explained by the environment. An outcome to be expected if the plankton community assembly is influenced by cross-trophic interactions. Congruence with phytoplankton was strongest for smaller zooplankton (rotifers) likely due to the fact that they graze on a narrow size spectrum and are thus potentially more strongly coupled with phytoplankton in contrast to the larger zooplankton with a wider size selection of prey, confirming ecological expectations (Longmuir et al. 2007). Moreover, several of the copepod genera are predators leading to reduced coupling with phytoplankton. Congruence tended to be weaker at higher productivity levels, suggesting that eutrophication might weaken the trophic interactions between phyto- and zooplankton.

Previous studies analyzing congruence in lake plankton have reported inconsistent findings (Heino 2010). A recent study of floodplain lakes in Brazil found significant cross-taxon congruence between phyto- and zooplankton, but it was weak and inconsistent across groups (Padial et al. 2012). Bowman et al. (2008) found strong association between phyto- and zooplankton communities in eight Canadian lakes; the pattern was not, however, robust across years and lakes. Allen et al. (1999b) found that the composition of zooplankton and sedimentary diatom communities were significantly associated in 186 northeastern U.S. lakes, but evinced no association in richness patterns (Allen et al. 1999a). Furthermore, the cyanobacterial composition in Lake Blaarmeersen, Belgium, was significantly related to zooplankton composition (Gremberghe et al. 2008). Lastly, a recent microcosm experiment showed that higher phytoplankton diversity promoted higher diversity in *Daphnia* cultures, where phytoplankton biomass and elemental composition were kept constant (Striebel et al. 2012). By contrast, phyto- and zooplankton richness and composition were independent from each other and responded to different environmental gradients in 31 Canadian lakes (Longmuir et al. 2007). Similarly, a study across 27 sites in a Finnish lake system revealed no significant association between phyto- and zooplankton diversity measures (Tolonen et al. 2005). Another study from western Europe showed no richness associations between lake plankton groups (Declerck et al. 2005). Overall, there is no consistent support for the occurrence of congruence in lake plankton diversity patterns across trophic levels.

Our results corroborate those previous studies documenting congruence across lake phyto- and zooplankton communities, but provides stronger support than in these studies, as congruence appeared to be consistent for both richness and composition and in separate analyses across months, years and lakes. Lack of congruence between aquatic primary producers and herbivores has been proposed to reflect the fact that aquatic zooplankton are generalist grazers (Irigoiien et al.

2004). However, the lack of congruence in some of the previous studies might also simply reflect the limited spatial and temporal data availability. Plankton communities are extremely dynamic and snap-shot or even regular but low resolution sampling thus includes a large amount of noise, which may easily mask ecological patterns. Furthermore, common inaccuracies in sampling and counting procedures may introduce extra noise. Therefore, data covering a limited spatial and temporal scale might prevent the detection of congruence. The present data set is of exceptional quality, consisting of biweekly monitoring data on 17 lakes covering 20 years, enhancing the probability of detecting ecological patterns against the high stochasticity in plankton communities, as was indeed found.

Environmental gradients were partially responsible for congruence in lake plankton diversity patterns across trophic levels as the effects of environmental variables were similar for the richness and composition of both groups. Coherent temporal changes in climate (Magnuson et al. 1990), productivity (Anneville et al. 2005) and acidity (Fischer et al. 2001) have previously been showed to result in coherent changes in lakes, especially for parameters that have a direct link with these factors, like abiotic variables or productivity (Vogt et al. 2011). Coherent temporal changes in plankton richness and community composition have also been shown, however often weaker (Arnott et al. 2003, Anneville et al. 2005, Vogt et al. 2011). Productivity has long been regarded as a main determinant of diversity, often showing unimodal relationships (Jeppesen et al. 2000, Mittelbach et al. 2001, Özkan et al. 2012), as was the case in our study where nutrient concentrations reflecting productivity were unimodally related to plankton richness (except for TN). pH was one of the main environmental factors affecting both plankton communities (Tilman et al. 1982); however, pH is strongly influence by photosynthesis and likely represents seasonal change in productivity in the present analyses. Climatic parameters also probably reflected seasonal changes. However, the fit of the models to predict zooplankton and phytoplankton richness solely from environmental variables was poor. The models were significantly improved by including richness of the reciprocal trophic level. Similarly, most of the congruence between phyto- and zooplankton communities remained after accounting for the environmental drivers using the partial Mantel tests. Therefore, the plankton congruence across trophic levels strongly suggests linked dynamics beyond those forced by similar responses to environmental gradients.

Hutchinson (1961) was among the first to recognize that the established theories could not explain the diversity of planktonic life with the environmental characteristics of the water column and termed it “the paradox of plankton”. The pelagial water column is regarded as homogeneous and competitive exclusion is, thus, expected to lead to dominance of few organisms, whereas pelagial waters support a surprising diversity of plankton. Since the recognition of the problem, several mechanisms have been proposed to sustain planktonic biodiversity, such as simultaneous limitation with multiple resources, heterogeneity in time or space, interspecific trade-offs in competition and colonization, non-equilibrium population dynamics and trophic interactions (Tilman and Pacala 1993), as well as chaos (Huisman and Weissing 1999). Trophic interactions are especially relevant in the present case as congruence across trophic levels is an indication of potential facilitation between trophic levels (Longmuir et al. 2007). Zooplankton can promote diversity in phytoplankton by grazing on dominant species, thereby preventing competitive exclusion (Paine 1966). Several studies have documented a positive effect of zooplankton grazing, especially by key-stone species like *Daphnia*, on phytoplankton diversity (McCauley and Briand 1979, Carter et al. 1995, Proulx et al. 1996, Sarnelle 2005). Furthermore, in a microcosm experiment Burgmer and Hillebrand (2011) showed that zooplankton grazing delayed phytoplankton extinction and sustained higher phytoplankton richness. However, increasing grazer

abundance might also lead to a decrease in primary producer diversity in nutrient-poor ecosystems, where severe nutrient limitation reduces primary producer recruitment after grazing (Proulx and Mazumder 1998). Conversely, a diverse phytoplankton community may enable niche partitioning among zooplankton (Tilman et al. 1982). Analyses of zooplankton amino acid composition, identifying their food sources, have shown that the different zooplankton taxa have distinct food sources, and niche partitioning should therefore play an important role in their community assembly (Guisande et al. 2003). Furthermore, modeling studies have suggested that trophic interactions can promote diversity through assembly processes as colonization of a producer may facilitate colonization of a specialist consumer, which, in turn, may facilitate colonization of other competing producers, especially due to keystone predation (Grover 1994).

Analyses of congruence across different groups of zooplankton and phytoplankton showed differences consistent with ecological expectations. Rotifers, being the smallest group of zooplankton, exhibited the strongest correlations with phytoplankton for both richness and community composition. Rotifers, in contrast to larger microcrustaceans, only graze on a narrow size window of phytoplankton and are thus more sensitive to changes in the phytoplankton community (Padial et al. 2012). The lowest level of congruence was generally found for copepods which is to be expected as several of the cyclopoid species present in Danish lakes are predators or facultative predators in their advanced stages (Hansen and Jeppesen 1992). Overall, differences in congruence across different groups of lake plankton, especially across different groups of zooplankton with different grazing size spectrum, indicate that the observed congruence reflects trophic interactions.

Congruence across trophic levels of lake plankton, both richness and community composition, weakened with increasing productivity. Furthermore, the strength of both community and richness congruence tended to increase until c. 2000, during when strongest decrease in TP was observed (Figure 2) However, after 2000, the increase in community congruence stopped and a decrease in richness congruence was observed. This is probably because strong TP-reduction drove the change in the strength of congruence in the early period of recovery, while other lake-specific factors expectedly became more important for congruence following the slowing down in TP-reduction after 2000. The weakening of congruence with increasing TP (productivity) might be mediated through two factors: (i) eutrophic lakes are often characterized by high abundance of plankti-benthivorous fish (Jeppesen et al. 2000, Carpenter et al. 2001), while trophic control of planktivorous fish by piscivores is higher at intermediate and low TP (Persson et al. 1992, Carpenter et al. 1997). High planktivorous fish abundance at higher lake productivity may, in turn, yield a strong grazing pressure on large-bodied zooplankton (Jeppesen et al. 1997, Jeppesen et al. 2003). Therefore, the weakening of congruence with increasing TP may indicate uncoupling of trophic interactions between zooplankton and phytoplankton generated by a strong top-down predation pressure on zooplankton by fish in eutrophic lakes. Lack of fish abundance data for most of the years precluded a thorough testing of this assertion but studies of Danish lakes have shown increasing predation control by fish and reduced grazing pressure on phytoplankton with increasing nutrient level (Jeppesen et al. 2000, Jeppesen et al. 2003). (ii) Eutrophication may also result in dominance of a few strongly competitive and often toxic algae (mostly cyanobacteria), which are less palatable for most zooplankton. Therefore, the weakening of congruence with increasing TP levels may also be indicative of uncoupling of trophic interactions between zooplankton and phytoplankton due to a compositional change in the phytoplankton community towards inedible forms. However, neither richness nor community congruence of plankton showed a pattern supporting this suggestion across a gradient in the proportional abundance of potentially inedible algae.

Consistent bias in the survey effort may also create a congruent pattern, especially regarding diversity measures (Gaston 2000). We expected no such effect in the present survey as the monitoring program employed the same sampling procedure in all lakes and as several taxonomists counted the individual lake samples during the 20-year monitoring period. It may be suggested that the congruence beyond the environmental control arises due to omitted environmental factors. However, we compiled a comprehensive environmental data set including productivity, climate and water chemistry, which are the main generally recognized drivers of phytoplankton community assembly (Tilman et al. 1982). It is, therefore, expected that omitted environmental gradients would increase the explanatory power of the environmental models rather than reduce the variance explained by congruence between zooplankton and phytoplankton. Stratification is an important driver of lake plankton that we omitted in the present analyses as the data were incomplete for some shallow lakes. However, we repeated the analyses for stratification and the conclusions were largely similar (Appendix). Furthermore, the abundance of submerged macrophytes may also affect lake plankton congruence (Declerck et al. 2005) as they mediate multiple interactions across organisms. The congruence tended to be highest at intermediate macrophyte PVI which is to be expected as both planktonic and macrophyte-associated species and communities contribute to plankton assemblages. However, PVI was not used in the LMM models as it was sampled for a limited set of lakes during only part of the study period (since 1993) and only once a year.

Strong cross-taxon congruence may enable researchers and managers to use one group as a surrogate for another. This is especially useful in biodiversity surveys as well as for conservation and management purposes where resources are limited. Although we found significant congruence in lake plankton, the correlations were too weak to have reliable predictive power (Heino 2010). Therefore, our results, as well as those of others (Longmuir et al. 2007, Heino 2010, Padial et al. 2012), indicate that it is necessary to perform extensive monitoring of lake plankton at different trophic levels to obtain reliable predictions for management and conservation, especially when dealing with ecosystem change (Jeppesen et al. 2011).

In conclusion, we found significant congruence among lake plankton groups across trophic levels for both richness and community composition patterns. A similar response of zooplankton and phytoplankton communities to environmental gradients only partially explained the observed congruence. A large proportion of the congruence was independent of environmental control and consistent with ecological interactions across trophic levels acting as driving mechanisms, for instance selective predation, niche partitioning and facilitation. Hereby, the present study adds to the emerging evidence in literature of the role of cross-trophic interactions as an important driver of cross-taxon congruence in diversity patterns (Kissling et al. 2007, Greve et al. 2012). The general role played by trophic interactions in shaping diversity patterns across organism groups, ecosystems and spatiotemporal scales remains poorly elucidated, though, and is therefore an important focus point for future research.

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APPENDIX

Figure 1. Temporal distribution of samples for each lake.

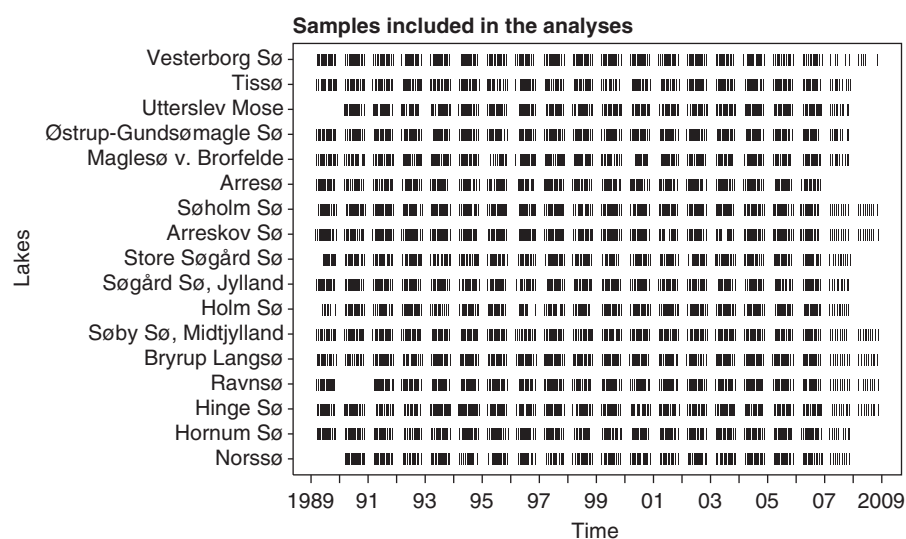


Table 1. Summary of data treatment of missing values in environmental variables. Frequency of samples collected with plankton samplings and replacement with different averages are given. Plant volume inhabited (PVI) frequency was measured as lake-years.

	Original sample	7 days	15 days	31 days	NA
TP	0.970	0.015	0.004	0.005	0.006
TN	0.970	0.015	0.004	0.005	0.006
NH ₄	0.967	0.014	0.004	0.005	0.009
Chl a	0.963	0.015	0.004	0.007	0.011
pH	0.968	0.015	0.004	0.005	0.008
SiO ₂	0.967	0.015	0.004	0.006	0.008
Stratification (Schmidt)	0.874	0.007	0.004	0.01	0.105
PVI	0.508	–	–	–	–

Table 2. Summary of linear mixed models of phytoplankton and zooplankton genus richness with water column stratification (Schmidt stability index) as a predictor. For Mantel tests, stratification was excluded from the environmental variables set by the forward selection (see Methods). See Table4 in the manuscript for table details.

	Phytoplankton model		Zooplankton model	
R^2_E	0.06		0.13	
R^2_{E+B}	0.06		0.12	
R^2_{E+B+R}	0.11		0.19	
	Coefficient	P value	Coefficient	P value
Zoop. richness	0.467	<0.001	–	–
Phyto. richness	–	–	0.535	<0.001
Zoop. biomass (log)	–0.387	<0.001	–	–
Phyto. biomass (log)	–	–	0.063	0.347
TP	–0.046	0.748	–0.353	0.005
TP ²	–0.005	0.828	0.021	0.280
TN	–0.491	<0.001	–0.347	<0.001
NH ₄	–0.295	<0.001	0.079	0.072
NH ₄ ²	0.019	0.006	–	–
SiO ₂	–0.016	0.876	–	–
SiO ₂ ²	0.001	0.983	–	–
pH	0.159	0.125	0.187	0.067
pH ²	–0.056	0.279	–0.107	0.035
Temperature	0.464	<0.001	0.710	<0.001
Solar radiation	0.220	0.010	0.107	0.133
Precipitation	0.097	0.015	0.123	0.001
Wind speed	0.023	0.652	–0.053	0.29
Stratification	–0.148	0.113	0.061	0.427

Table 3. Summary of autoregressive models (Ives 1995, Fischer et al. 2001) between phyto- and zooplankton genus richness for each lake. Richness of a plankton group was modeled using linear model with autoregressive component (response with one-time-step lag, AC1), richness of reciprocal group and environmental variables in the predictor set. Only the environmental variables included in LMM models for both phyto- and zooplankton were used (see Table 4 for details). Winter period was excluded in the study and the models were constructed accordingly. That is the first sample of the spring was not predicted from the last sample of the previous fall. P: <0.10, .: < 0.05, *: < 0.01, **: < 0.001, ***. The results of autoregressive models are in accord with the LMM models. Reciprocal richness of phyto- and zooplankton was significant for at least one comparison in 11 lakes out of 17, while the relationship was positive in 29 of 34 models. Reciprocal richness of the groups in general had more significant and stronger coefficients than the environmental predictors.

Lake	Response	AC1	Recip. rich.	TP	TN	NH ₄	pH	T°C	Light	Precip.	Wind
1009	Phy. rich.	0.68 ***	0.44 *	2.37	2.99	-1.21	0.20	0.24	0.69 *	0.24	0.21
1009	Zoo. rich.	0.61 ***	0.50 *	4.05	0.62	0.57	-1.93 *	0.63 .	0.84 **	0.39 .	0.22
13002	Phy. rich.	0.75 ***	0.44 *	-0.00	-2.16 **	0.17	-0.23	-0.19	0.16	0.05	0.05
13002	Zoo. rich.	0.76 ***	0.27 .	-0.46	-0.01	0.64 .	0.34	-0.55 **	0.44 **	0.27 .	-0.44 *
21278	Phy. rich.	0.79 ***	0.46	-1.80 .	-0.21	-0.30	0.39	0.09	1.20 ***	0.02	0.70 .
21278	Zoo. rich.	0.43 ***	0.60 ***	0.31	-0.32	-0.29	0.58	0.75 **	-0.41	0.23	-0.76 **
21321	Phy. rich.	0.65 ***	0.82 **	-0.11	-1.11 *	-1.89 *	-1.40 *	-0.60 *	0.95 **	0.41 *	0.01
21321	Zoo. rich.	0.36 ***	0.74 ***	-3.84	0.64 .	-0.04	1.16 *	0.73 **	-0.06	0.23	-0.34
21324	Phy. rich.	0.70 ***	0.97 ***	-1.51	-1.15 **	-0.98 .	-0.51	-0.75 .	1.13 **	0.14	0.29
21324	Zoo. rich.	0.48 ***	1.12 ***	0.06	0.74 *	-0.58	-0.18	0.47	-0.17	0.14	-0.18
25044	Phy. rich.	0.70 ***	0.46 **	8.36 .	0.87	-1.91	-0.71	0.05	0.46 .	0.10	0.08
25044	Zoo. rich.	0.68 ***	0.57 *	5.33	0.53	-0.09	0.24	0.07	0.31	0.21	-0.11
30005	Phy. rich.	0.52 ***	0.29 *	-0.39	-1.08	0.05	-0.06	0.24	-0.02	0.07	0.08
30005	Zoo. rich.	0.39 ***	0.87 *	0.55	2.95 .	-0.45 *	-0.08	0.20	0.01	0.08	0.49 *
36005	Phy. rich.	0.87 ***	0.10	-0.70 *	-0.38 *	0.09	0.79 *	-0.31	0.45 .	0.30 .	0.08
36005	Zoo. rich.	0.37 ***	0.13	-0.29	-0.05	-0.18	-0.36	0.76 **	0.43 *	0.14	0.06
42003	Phy. rich.	0.62 ***	0.30	-0.16	-0.33	-0.53 .	-1.30 *	0.18	0.76 *	0.01	0.88 **
42003	Zoo. rich.	0.30 ***	0.42 **	-0.09	-0.04	-0.04	0.54	0.13	0.22	0.15	-0.12
45003	Phy. rich.	0.82 ***	0.26	-0.27	0.18	-0.19 .	0.38	-0.27	0.11	0.23	-0.33
45003	Zoo. rich.	0.50 ***	0.29 .	-1.41 **	-0.50	0.04	1.62 ***	0.32	0.07	0.13	0.02
46007	Phy. rich.	0.68 ***	-0.03	-1.60	-0.56	-0.58 *	-0.81	-0.24	0.51 *	-0.07	0.24
46007	Zoo. rich.	0.41 ***	-0.01	-3.89 **	0.35	0.33	0.47	0.00	-0.40 .	-0.18	-0.04
49001	Phy. rich.	0.49 ***	0.53 **	-0.62 **	0.45 *	-0.09	-0.31 .	0.18	-0.02	-0.04	-0.17
49001	Zoo. rich.	0.55 ***	1.16 ***	-0.05	-0.19	-0.01	0.38 .	0.26	0.25	0.37 **	-0.05
51004	Phy. rich.	0.54 ***	-0.25	1.25	0.49	-1.37 *	-0.85	0.63 **	0.34	0.16	0.48 *
51004	Zoo. rich.	0.37 ***	-0.15	-4.48	-0.25	-0.11	-0.31	0.16	0.29	0.33 *	0.16
52005	Phy. rich.	0.88 ***	-0.01	-0.03	-0.32	0.20	-0.58	-0.29	0.47 .	-0.06	0.04
52005	Zoo. rich.	0.50 ***	0.20 .	-0.16 .	-0.06	-0.24	-1.40 ***	0.26	0.17	0.15	0.29
53017	Phy. rich.	0.71 ***	0.35	0.00	0.33	-0.19 .	-0.27	0.19	0.09	0.15	-0.01
53017	Zoo. rich.	0.32 ***	0.41 *	-0.21	-0.68 *	0.27 **	-0.22	0.08	0.52 **	-0.06	0.16
55002	Phy. rich.	0.55 ***	0.30	0.05	0.29	-0.73 *	0.24	0.24	-0.07	-0.09	-0.06
55002	Zoo. rich.	0.55 ***	0.62 **	0.21	-0.38 .	0.24	2.13 ***	-0.23	0.39 .	0.04	-0.18
62003	Phy. rich.	0.72 ***	0.11	-0.33	0.09	-0.38 *	-0.95	0.42	0.82 ***	0.17	0.00
62003	Zoo. rich.	0.51 ***	0.14	-0.40	0.19	-0.25	-0.17	0.76 **	0.31 .	0.17	-0.06

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PAPER V

Biomanipulation as a Restoration Tool to Combat Eutrophication: Recent Advances and Future Challenges

Erik Jeppesen^{*†‡1}, Martin Søndergaard^{*}, Torben L. Lauridsen^{*‡}, Thomas A. Davidson^{*}, Zhengwen Liu^{§¶}, Nestor Mazzeo^{||,¶¶}, Carolina Trochine[#], Korhan Özkan^{*}, Henning S. Jensen^{**‡}, Dennis Trolle^{*‡}, Fernando Starling^{††}, Xavier Lazzaro^{##55}, Liselotte S. Johansson^{*}, Rikke Bjerring^{*}, Lone Liboriussen^{*}, Søren E. Larsen^{*}, Frank Landkildehus^{*}, Sara Egemose^{**}, Mariana Meerhoff^{*||,¶¶}

^{*}Department of Bioscience, Aarhus University, Vejlsøvej, Silkeborg, Denmark

[†]Greenland Climate Research Centre (GCRC), Greenland Institute of Natural Resources, Kivioq, P.O. Box 570, 3900 Nuuk, Greenland

[‡]Sino-Danish Centre for Education and Research (SDC), Beijing, China

[§]Chinese Academy of Sciences, Nanjing Institute of Geography & Limnology, Nanjing, China

[¶]Department of Ecology and Hydrobiology, Jinan University, Guangzhou, Guangdong, China

^{||}Department of Ecología & Evolución, Centro Universitario de la Región Este-Facultad de Ciencias, Universidad de la República Burnett s/n, Maldonado, Uruguay

[#]Laboratorio de Limnología, Instituto de Investigaciones en Biodiversidad y Medioambiente-Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional del Comahue, Bariloche, Argentina

^{**}Institute of Biology, University of Southern Denmark, Campusvej, Odense M, Denmark

^{††}Companhia de Saneamento Ambiental do Distrito Federal-CAESB, Superintendência de Monitoramento e Controle de Recursos Hídricos, PHI, SAIN, Área Especial, R1, CAESB, Brasília-DF, Brazil

^{##}UMR7208 Borea, CNRS, IRD, UPMC, MNHN, CP 53, Paris cedex 5, France

⁵⁵ULRA/UMSS, Cochabamba, Bolivia

^{¶¶}South American Institute for Resilience and Sustainability Studies (SARAS), Maldonado, Uruguay

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Mariana Meerhoff^{*,||,¶,¶}

^{*}Department of Bioscience, Aarhus University, Vejlsøvej, Silkeborg, Denmark

[†]Greenland Climate Research Centre (GCRC), Greenland Institute of Natural Resources, Kivioq, P.O. Box 570 3900, Nuuk, Greenland

[‡]Sino-Danish Centre for Education and Research (SDC), Beijing, China

[§]Chinese Academy of Sciences, Nanjing Institute of Geography & Limnology, Nanjing, China

[¶]Department of Ecology and Hydrobiology, Jinan University, Guangzhou, Guangdong, China

^{||}Department of Ecología & Evolución, Centro Universitario de la Región Este-Facultad de Ciencias, Universidad de la República Burnett s/n, Maldonado, Uruguay

[#]Laboratorio de Limnología, Instituto de Investigaciones en Biodiversidad y Medioambiente-Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional del Comahue, Bariloche, Argentina

^{**}Institute of Biology, University of Southern Denmark, Campusvej, Odense M, Denmark

^{††}Companhia de Saneamento Ambiental do Distrito Federal—CAESB, Superintendência de Monitoramento e Controle de Recursos Hídricos, PHI, SAIN, Área Especial, R1, CAESB, Brasília-DF, Brazil

^{‡‡}UMR7208 Borea, CNRS, IRD, UPMC, MNHN, CP 53, Paris cedex 5, France

^{§§}ULRA/UMSS, Cochabamba, Bolivia

^{¶¶}South American Institute for Resilience and Sustainability Studies (SARAS), Maldonado, Uruguay

¹Corresponding author: e-mail address: ej@dmu.dk

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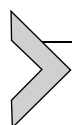
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Abstract

Eutrophication resulting from high nutrient loading has been the paramount environmental problem for lakes world-wide for the past four decades. Efforts are being made in many parts of the world to reduce external nutrient loading via improved wastewater treatment or diversion of nutrient-rich inflows. However, even after a reduction of the external phosphorus loading, the effects obtained may be unsatisfactory. This may reflect an insufficient reduction in the external nutrient loading to effectively limit phytoplankton growth. However, the lack of success may also be due to chemical or biological within-lake inertia preventing or delaying improvements. To overcome the resilience and thereby reinforce recovery, a number of physico-chemical and biological restoration methods have been developed.

In this chapter, we describe recent developments of biological restoration methods related to eutrophication, their short-term and long-term effects, and discuss the possibility of using combined physico-chemical and biological methods to improve the long-term stability of restoration and to reduce restoration costs. As comprehensive reviews of the effect of fish manipulation in cold temperate lakes are numerous, for these waterbodies, we highlight recent results, including effects on biodiversity and metabolism, and present new approaches of biomanipulation. Our particular focus is, however, directed at biomanipulation in warm lakes and on combined treatments which are far less well described in the literature.



1. INTRODUCTION

As the demand for food and other ecosystem goods and services increases in line with the growing global human population and improved welfare, the pressure on all natural marine, terrestrial and freshwater alike,

has intensified dramatically and is expected to reach unprecedented levels by the end of this century ([Millennium Ecosystem Assessment, 2005](#)), with consequences over both ecological and evolutionary timescales ([Hagen et al., 2012](#); [Moya-Larano et al., 2012](#)). Freshwaters, in particular, face a multitude of anthropogenic stressors (e.g. [Hladysz et al., 2011](#); [Layer et al., 2010, 2011](#)), which have led to the development of numerous bio-assessment, biomonitoring and restoration schemes to mitigate or, even reverse, these human impacts, that have had varying degrees of success ([Feld et al., 2011](#); [Friberg et al., 2011](#)). Although the effects of climate change have recently become an area of increasing research activity in aquatic ecology ([Ledger et al., 2012](#); [Meerhoff et al., 2012](#); [Mintenback et al., 2012](#); [Mollmann and Dieckmann, 2012](#); [O’Gorman et al., 2012](#); [Peck et al., 2012](#)), eutrophication as a result of high nutrient loading has been the paramount environmental problem for lakes world-wide for the past four decades, and it will continue to be a major issue for the foreseeable future ([Carpenter et al., 1999](#)). This has led to turbid water, often toxic cyanobacteria blooms, biodiversity loss, and changes in biological community structure and ecosystem functioning ([Jeppesen et al., 2000](#)). During the past decades, measures have been taken in many parts of the world to reduce external nutrient loading via improved wastewater treatment or diversion of nutrient-rich inflows. Nutrient loading is, however, still increasing, particularly in developing countries and, thus, the most serious problems may not yet have been manifested on a global scale ([Le et al., 2010](#)).

Reducing the external nutrient loading is the key measure to restore eutrophied lakes and should have the highest priority ([Cooke et al., 2005](#)). A multi-faceted approach is often needed to attain sufficiently low external nutrient loading to improve the ecological status of lakes, particularly in more densely populated areas. Such approaches include phosphorus (P) stripping and occasionally nitrogen (N) removal at sewage works, sewage diversion, greater use of phosphate-free detergents, higher demands for animal fertiliser storage capacity and strict fertilisation plans and green cover of agricultural fields in winter. In addition, nutrient retention and N loss in lake catchments can be enhanced by re-establishing wetlands, stabilising river banks to reduce erosion, re-establishing a natural riparian zone and by allowing flooding of riverine areas ([Jeppesen et al., 2011](#)).

Notwithstanding significant reductions in nutrient loading, chemical or biological within-lake resilience can delay the establishment of clear water even when the external nutrient loading is sufficiently low to limit

phytoplankton growth. Chemical resilience is largely due to P release from the sediment pool accumulated during high loading (Søndergaard et al., 2003). Depending on the loading history and release mechanisms, this internal P loading typically persists for 10–15 years after the loading reduction until new equilibrium conditions are established (Jeppesen et al., 2005). Biological resilience can emerge through (i) resilience in the fish community and (ii) delayed return of submerged macrophytes. Zooplanktivorous and bottom-feeding fish species often dominate in nutrient-rich turbid lakes and may prevent a shift to a clear water state after nutrient loading reduction as they disturb the sediment when foraging and by preying on the zooplankton. Submerged macrophytes are important for creating and maintaining stable clear water conditions, particularly in temperate and cold areas (Jeppesen et al., 1998; Moss, 1990; Scheffer et al., 1993; Vanderstukken et al., 2011), but they typically disappear or decline significantly in abundance when nutrient loading is high. Their recovery following nutrient loading reduction may be delayed (Lauridsen et al., 2003) due to high turbidity (caused by fish and resuspension of loose sediment), insufficient seeds or turion banks in the sediment and grazing by waterfowl (Barko and Smart, 1986; Søndergaard et al., 1998).

Several physico-chemical and biological restoration methods related to eutrophication have been developed to overcome this ecological inertia and thereby reinforce recovery (for an extensive overview, see Cooke et al., 2005; Moss et al., 1996). In this chapter, we discuss the recent development of biological restoration methods (Table 1, Diagram 1), their effects and the prospects for using combined physico-chemical and biological methods to improve the long-term stability of restorations with a reduction in cost. As the effect of fish manipulation in cold temperate lakes has been comprehensively reviewed (Benndorf, 1995; Cooke et al., 2005; Hansson et al., 1998; Jeppesen and Sammalkorpi, 2002; Lathrop, 2007; Mehner et al., 2004; Meijer et al., 1999; Moss et al., 1996; Perrow et al., 1997; Sierp et al., 2009; Søndergaard et al., 2008), we focus on more recent results, including the effects on biodiversity and lake metabolism, and discuss new approaches to biomanipulation for cold temperate lakes. The main emphasis is given to biomanipulation in warm and warming lakes and new methods, including combined chemical and biological restoration. We also include a number of case studies, mostly from temperate Danish lakes, as a set of specific examples of more general phenomena. A brief description of these lakes and restoration methods used is given in Table 2 ordered as they appear in the text.

Table 1 Overview of key biological restoration methods and main targets

Method	Processes involved	References
Removal of zooplankton-eating fish to control phytoplankton	Zooplankton- and/or benthic invertebrate-eating fish are removed to increase the number of large zooplankton and their grazing on phytoplankton	McQueen (1998); Søndergaard <i>et al.</i> (2008)
Removal of benthic omnivorous fish to control phytoplankton	Benthic filter-feeding omnivorous fish (typically warm water herbivores-detritivores, for instance, common carp, tilapia, <i>Prochilodus</i> sp.), which naturally reach high carrying capacity, are removed to decrease sediment bioturbation and internal nutrient recycling	Starling <i>et al.</i> (2002); Lazzaro <i>et al.</i> (2003); Lazzaro and Starling (2005)
Stocking of predatory fish to control phytoplankton	Fish-eating fish are added to decrease the number of zooplankton-eating fish and improve zooplankton numbers and phytoplankton grazing	Benndorf (1995); Skov and Nilsson (2007)
Stocking of pelagic herbivorous fish to control phytoplankton	Pelagic filter-feeding herbivorous fish (e.g. bighead carp, silver carp) are stocked to remove phytoplankton, notably cyanobacteria	Arcifa <i>et al.</i> (1986); Starling (1993); Starling <i>et al.</i> (1998)
Macrophyte transplantation and protection	Submerged macrophytes are established and protected from plant-eating birds or fish to maintain high macrophyte coverage	Lauridsen <i>et al.</i> (2003); Qiu <i>et al.</i> (2001)
Stocking with herbivorous fish to control macrophytes	Plant-eating fish (grass carps) are added to reduce excessive growth of submerged macrophytes	Shireman and Maccina (1981); Hanlon <i>et al.</i> (2000)
Introduction of mussels	Mussels are introduced to increase filtration of the water and create clearer water	Roy <i>et al.</i> (2010); Gulati <i>et al.</i> (2008)

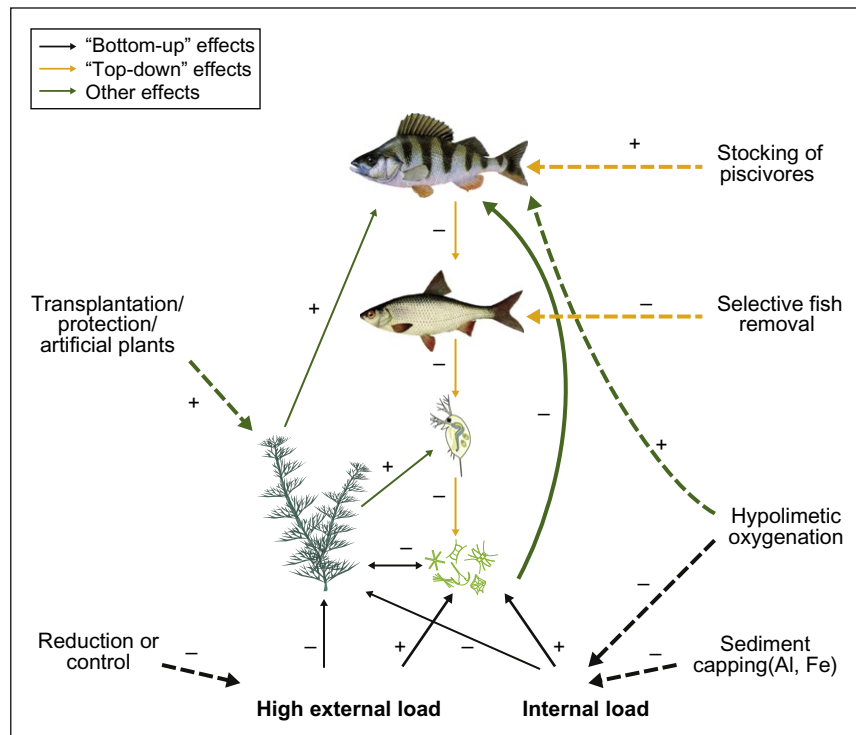


Diagram 1 Conceptual model on the interactions between nutrients and the main components of a lake trophic web as affected by classic biomanipulation to enhance trophic cascade effects and other restoration measures. The nature of the interactions, whether positive or negative, bottom-up (via nutrients), top-down (consumption) or others (e.g. mechanical, chemical), is highlighted. The simultaneous application of both biomanipulation and a treatment to reduce internal nutrient loading may generate synergistic effects.

2. FISH MANIPULATION

In this chapter, we give an overview of fish manipulation methods and their effects on the short- and long-term ecology of temperate lakes where they have been used extensively. We then discuss their applicability to warm lakes and illustrate this with two case studies.

2.1. Fish manipulation in temperate lakes

This section focuses on fish removal, piscivorous fish stocking and effects of fish manipulations and discusses the use of repeated fish manipulation. We also discuss how the sediment record may help elucidating the effects of fish manipulation.

Table 2 Overview of the case studies presented

Lake, country	Position	Area (km ²)	Mean depth (m)	Type of manipulation	Analysed
Lake Christina, USA	46°05'N, 95°44'W	16	4.3	Repeated fish removal	Sediment subfossils
Lake Vesijärvi, Finland	61°0'N, 25°35'E	26	6.8	Fish removal	Sediment subfossils
Lake Væng, Denmark	56°02'N, 9°39'E	0.16	1.2	Repeated fish removal	Water chemistry and biota Metabolism
Huizhou West Lake, China	23°06'N, 114°23'E	1.6	1.0	Fish removal, macrophyte planting	Water chemistry and biota
Lake Paranoá, Brazil	15°47'S, 47°48'W	38	14	Repeated fish management, algecides and others	Water chemistry and biota
Lake Kollelev, Denmark	55°47'N, 12°27'E	0.05	1.8	Fish removal with and without sediment capping	Water chemistry
Lake Fure, Denmark	55°48'N, 12°24'E	9.32	13.5	Hypolimnion oxygenation and fish removal	Water chemistry and biota
Lake Engelsholm, Denmark	55°43'N, 9°18'E	0.44	2.4	Fish removal	Water chemistry and biota Biodiversity Metabolism
Lake Faarup, Denmark	55°43'N, 9°24'E	0.99	5.6	Colonisation by zebra mussels	Water chemistry and biota Biodiversity Metabolism

2.1.1 Removing zooplanktivorous and benthivorous fish

The most frequently used biomanipulation method (Table 1) is removal of plankti-benthivorous fish. This method has been extensively used over the past 20 years in northern temperate lakes in Europe (Søndergaard et al., 2007). It has been most successful in small shallow lakes but has had variable long-term effectiveness. Removal of a high proportion of the planktivorous

and benthivorous fish stock during a 1- to 2-year period has been recommended to avoid re-growth of the original stock and to stimulate the growth of young specimens of fishes that potentially become piscivores when they reach a sufficient size (Hansson et al., 1998; Jeppesen and Sammalkorpi, 2002). A simple, feasible strategy of fish removal is to catch non-moving fish with active gear and active moving fish with passive gear using information on the seasonal behaviour of fish, such as spawning or foraging migration and shoaling of the target species. In addition, ice fishing during winter, when fish may aggregate near the bottom (Ventelä et al., 2007), and removal of fish seeking winter refuge in adjacent streams (Brönmark et al., 2010) have also been used (Annadotter et al., 1999). A drastic method is to apply a piscicide (typically rotenone) treatment to part or the entire lake and its tributary streams (Eilers et al., 2011; Hanson and Butler, 1994). Rotenone affects all fish species, as well as invertebrates, and thus requires ethical considerations, and legal permissions may be difficult to obtain. Nonetheless, rotenone treatment has been used to regulate fish communities for sport fishing and to eliminate invasive species (McClay, 2000) as well as native but aggressive species that are assumed to pose a danger to fishermen (e.g. piranha, *Serrasalmus* spp., see Lazzaro et al., 2003).

An efficient reduction of the zooplanktivorous fish biomass generally achieves dramatic, short-term cascading effects in eutrophic lakes. This takes the form of a shift to dominance by large zooplankton, reduced phytoplankton biomass (and much less noxious cyanobacteria), and improved transparency (Hansson et al., 1998; Meijer et al., 1999), an increase in benthic feeding and herbivorous waterfowl (Allen et al., 2007) and a higher proportion of piscivorous fish (e.g. perch, *Perca fluviatilis* L., and pike, *Esox Lucius* L.), in part promoted by the development of submerged macrophytes. Strong cascading effects of fish removal have also been found in ponds, also leading to a decrease in cyanobacteria (Peretyatko et al., 2012).

In some temperate lakes, however, a reduction in cyanobacterial biomass and improved water clarity have been achieved after biomanipulation without a trophic cascade, which has been attributed to reduced P release from the sediment, not least due to less fish foraging at the bottom following the biomanipulation (Horppila et al., 1998). Biomanipulation only achieves the desired results when fish removal is sufficiently extensive (Fig. 1, Meijer et al., 1994; Jeppesen and Sammalkorpi, 2002; Søndergaard et al., 2008), and Jeppesen and Sammalkorpi (2002) estimated the annual amount of fish removal required to significantly improve water quality in shallow temperate lakes based on biomanipulation experiments as: $\text{Catch-required} = 6.9 \text{ TP}^{0.52}$ with catch in kg ha^{-1} and TP in $\mu\text{g L}^{-1}$.

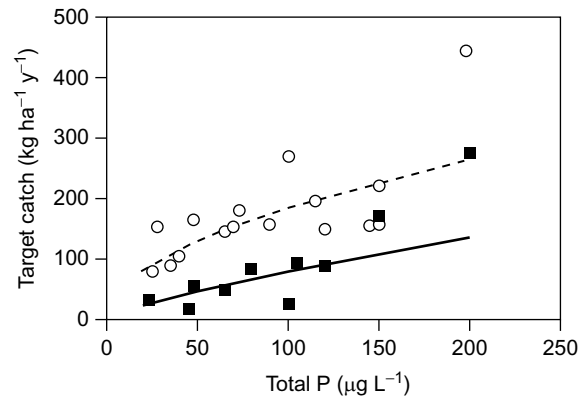


Figure 1 The catch-need of fish removal versus total phosphorus in eutrophic European lakes dominated by planktivorous and benthivorous fish. White circles denote the annual catch in cases with effective fish removal and an improvement of water quality (increased transparency, decline in cyanobacteria) at least in the short term or an increase in the numbers of piscivorous perch. Black squares denote cases in which the fish removal was too inefficient to elicit an effect on water quality or fish density. The curves are regressions lines (log-transformed data) through the two sets of points (broken line, successful cases; full line unsuccessful cases) (from Jeppesen and Sammalkorpi, 2002).

This target catch per year is higher than the estimates of fish biomass based on TP concentrations for deep stratified lakes (Hanson and Leggett, 1982), indicating that lower catches are likely needed to create a significant increase in water clarity in such lake types (Jeppesen and Sammalkorpi, 2002).

2.1.2 Stocking of piscivorous fish

An alternative or supplementary method to fish removal is stocking of potential piscivores (Benndorf, 1995; Berg et al., 1997; Drenner and Hambright, 1999; Ha et al., 2012), which may enhance water clarity through a trophic cascade (Carpenter and Kitchell, 1993) and/or a behavioural cascade (Romare and Hansson, 2003). By preying on zooplanktivorous and benthivorous fish, piscivores diminish prey fish abundance, resulting in lower fish-induced resuspension, lower predation on large zooplankton and lower translocation of nutrients from sediments to water via feeding and excretion. However, stocking of pelagic foraging piscivores, such as zander (*Sander lucioperca* L.), may also affect water clarity more indirectly through a behavioural cascade. Prey fish may be forced to take refuge in the littoral zone, which would not only release predation on pelagic zooplankton (Braband and Faafeng, 1994; Romare and Hansson, 2003)

but also decrease nutrient translocation from the littoral zone to open water (Braband et al., 1990; Kairesalo et al., 1999).

The basic tools are stocking with nursery or pond-raised fingerlings, often accompanied by catch and mesh size limits for fishing (Jeppesen and Sammalkorpi, 2002; Mehner et al., 2004). The most often used piscivores in northern temperate lakes are pike, zander, walleye (*Stizostedion vitreum* (Mitchill)), various trout species and largemouth bass (*Micropterus salmoides* (Lacepede)) (Drenner and Hambright, 1999).

The effects of introducing piscivorous fish have varied considerably but have often been poor compared with the effects of fish removal (Drenner and Hambright, 1999). Pike has been used in numerous biomanipulation experiments, often with high stocking densities (1000–4000 fish ha⁻¹) of fry in spring, aiming at almost instantaneous removal of 0⁺ planktivores (Berg et al., 1997; Prejs et al., 1994). The seasonal timing of the 0⁺ pike stocking might influence the efficiency of pike controlling the 0⁺ cyprinids (Skov, 2002), and the strongest effect is expected if the stocking coincides with the appearance of the newly hatched larvae of the dominant prey species (Prejs et al., 1994; Skov, 2002). While strong cascading effects of stocking large numbers of pike have been observed in Polish Lake Wirbel (Prejs et al., 1994) and Danish Lake Lyng (Berg et al., 1997; Søndergaard et al., 1997), the effects have been weak in many other lakes (Skov, 2002). Pike stocking may be most successful in systems where they have gone extinct and other strong pelagic predator species, such as perch or pikeperch, are present, as was the case in Lake Lyng (Berg et al., 1997), thereby creating a high predation risk on prey fish in both the pelagic and the littoral zone. Pike stocking has also been used to control exotic bullfrogs (*Lithobates catesbeianus* (Shaw)) in Australian ponds, which also host mostly small and planktivorous fish (e.g. pumpkinseed, *gibbosus* L., and topmouth gudgeon, *Pseudorasbora parva* Temminck and Schlegel) (Louette, 2012).

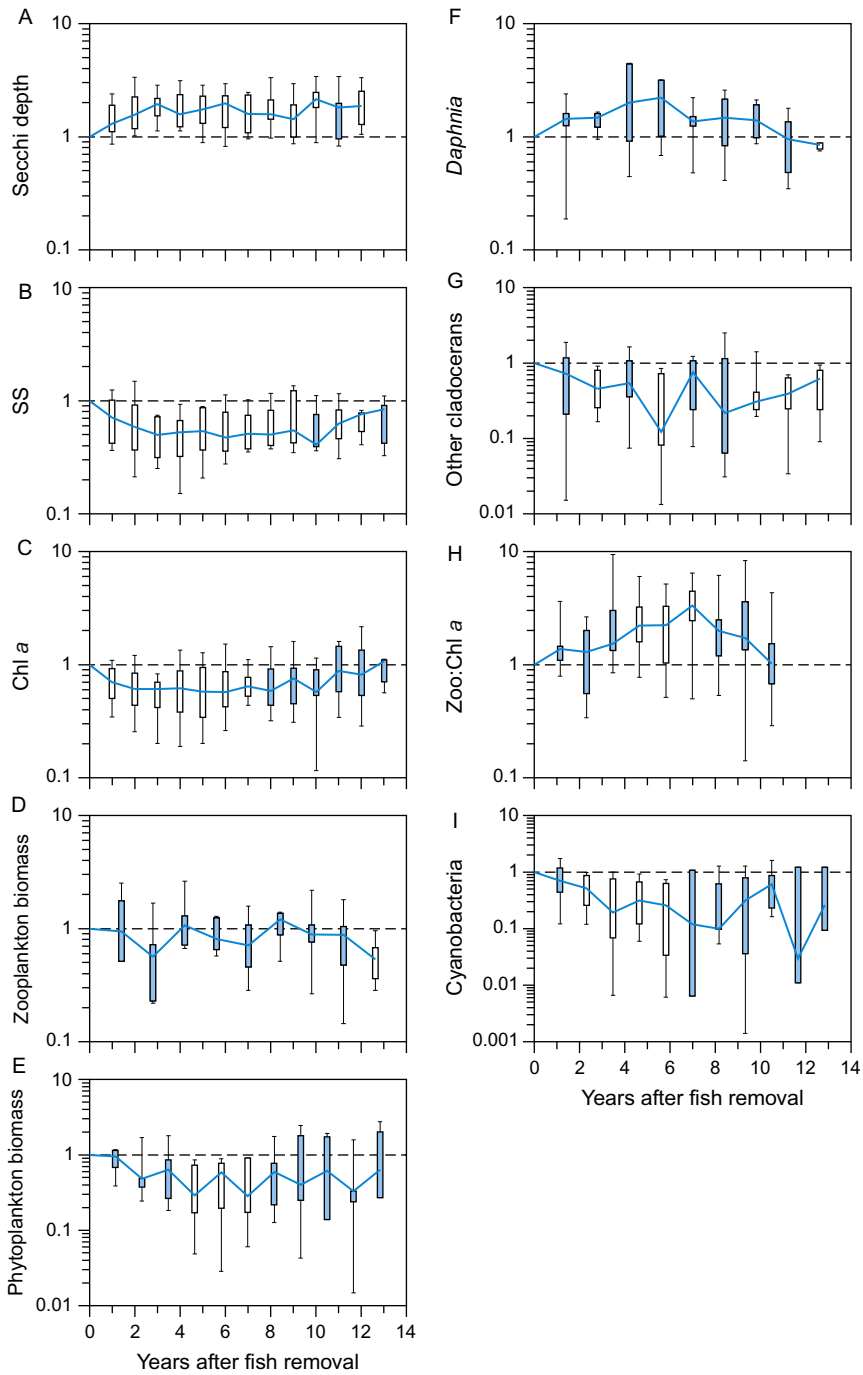
Zander in Europe and walleye in the USA have also been popular species to stock for the control of planktivores (Benndorf, 1995; Lathrop et al., 2002). As an example, Potthoff et al. (2008) stocked walleye in a series of shallow lakes in Minnesota (USA) to control fathead minnows (*Pimephales promelas* Rafinesque) and shift the lakes to a clear water state. Six lakes were stocked with walleye fry (12,000 individuals or 0.05 kg ha⁻¹), six with walleye age-1 and older (21–130 individuals and 6–8 kg ha⁻¹), while six lakes served as controls. Stocking of fry decreased fathead minnow and increased cladoceran abundance. Phytoplankton chlorophyll *a* (Chl *a*) also decreased at the fry-treated sites. By contrast, stocking of age-1 and older walleye induced few changes at the stocking densities tested. The success of

walleye fry appeared to be partly attributable to their rapid diet shift to piscivory and their ability to consume and suppress all life stages of planktivores (fry, juveniles and adults). For zander, stocking with fingerlings (up to 200 fish ha⁻¹) in summer has been recommended. However, later age-0 juveniles are a better alternative (up to 20 fish ha⁻¹), but since they are stocked in late autumn, a minimum fish length of 15 cm is recommended to prevent high overwinter mortality (Mehner et al., 2002). Most responses to walleye and zander fry additions have been short-lived, however, which may be in part attributed to gape limitation of these two species, leaving bigger planktivores or benthivores (e.g. bream, *Abramis brama* (L.), common carp, *Cyprinus carpio* L.) with size refuges (Lammens, 1999). Repeated stocking has therefore been used and recommended (Benndorf, 1995). Moreover, repeated stocking of piscivores in combination with size and bag limits for recreational fisheries had a strong positive effect on zooplankton body size and water clarity in a number of reservoirs in Germany (Scharf, 2007). Other piscivores used with at least short-term effects are brown trout (*Salmo trutta* L.) (e.g. Scharf, 2007) and rainbow trout (*Oncorhynchus mykiss* Walbaum) (Ha et al., 2012); however, these species may be non-native to the location they are introduced to, entailing undesirable conservation and biodiversity consequences.

An alternative or a supplement to stocking is to adjust the size structure of the population of piscivores. For example, Sharma and Borgström (2008) removed large pike in Lake Årungen, Norway, aiming to achieve a higher contribution of small pike and large perch in the fish community. There was a consequent decline in the abundance of small perch and roach (*Rutilus rutilus* L.), as well as a habitat shift of small specimens towards the littoral, releasing the predation pressure on the pelagic large-bodied zooplankton. However, long-term consequences remain unclear.

2.1.3 Longevity of the fish manipulation effects

There are only a few long-term studies (>10 years) of the effects of fish manipulation and the results are ambiguous. However, in most studies where strong short-term effects were seen, a gradual return to the turbid state and higher abundance of zooplanktivorous fish have occurred after 5–10 years. In particular, the trophic cascade effects in the pelagic seem to weaken in the long term due to return of zooplanktivorous fish; in contrast, the impact on benthic fish apparently lasts longer and may even be permanent. The most comprehensive comparative study of long-term responses so far is from 27 Danish Lakes (Fig. 2, Søndergaard et al., 2008). In



this study, abundance of benthivores, such as bream, was demonstrated to remain lower than before the restoration, creating significantly reduced levels of suspended matter and improved water clarity in the long term. However, the grazing effect, as indicated by the zooplankton:phytoplankton biomass ratio, returned to pre-manipulation levels and cyanobacteria returned and were dominant, indicating almost complete nullification of the strong trophic cascade in the pelagic observed 10–15 years after the fish manipulation, likely reflecting a return of planktivorous roach. A similar response with long-term effects on bream and mainly short-term effects on roach has been observed in bio-manipulated Cockshoot Broad, UK (Hoare et al., 2008).

Nonetheless, some examples exist of lakes with persistent effects, especially on water quality (see, for instance, examples below). A necessary prerequisite for obtaining a clear water state in the long term is an adequate external nutrient loading reduction, which corresponds to annual mean TP concentrations $<0.05 \text{ mg P l}^{-1}$ for shallow temperate lakes (Jeppesen et al., 2000) and $<0.02 \text{ mg P l}^{-1}$ for deep temperate lakes (Sas, 1989). However, internal loading may remain high for decades after loading reduction (Jeppesen et al., 1991; Søndergaard et al., 2003). A drawback of the fish manipulation is that lakes retain more P in the clear water state after manipulation, leaving more sediment P available for internal loading if the system returns to the turbid state than if fish manipulation had not been conducted (Søndergaard et al., 2007).

2.1.4 Repeated measures—fish manipulation in Lake Væng, Denmark

Repeated fish removal may be a way to maintain the clear-water state if lakes are deemed likely to shift back to the turbid state. We anticipate that the repeated manipulation effort does not have to be as comprehensive as the first for several reasons: (a) as shown for temperate Danish lakes, some of the large benthivorous fish show slow recovery (Søndergaard et al., 2008), probably due to food limitation and in some lakes also extensive growth of macrophytes; (b) small potential piscivores, such as perch, are

Figure 2 The effects of removal of more than $200 \text{ kg fish ha}^{-1}$ relative to the levels less than 3 years before intervention on Secchi depth (A), suspended solids (B), chlorophyll *a* (C) in 27 Danish shallow lakes (summer means). Also shown are biomass of total zooplankton (D), *Daphnia* (F) and other cladocerans (G) the zooplankton:chlorophyll *a* biomass ratio (H), phytoplankton biomass (E) and the relative biomass share of cyanobacteria (I) (number of lakes = 10). Light boxes show significant differences from the pre-removal situation. The boxes show 10% and 90% fractiles (outer lines) and 25% and 75% fractiles (boxes) (modified from Søndergaard et al., 2008).

typically more abundant after the first biomanipulation and tend to shift to piscivory more quickly; (c) presence of submerged macrophytes, seeds or turions may facilitate faster development of plants compared to the first manipulation and (d) the mobile pool of P in the sediment has likely declined (buried deeper or released via the outlet).

The shallow Lake Væng in Denmark (Table 2) is an example of repeated biomanipulation. The lake catchment area is 9 km² and consists of agricultural and forested land and a gravel pit. The water residence time is 15–25 days. Following sewage diversion in 1981 the lake remained in a turbid state (Søndergaard et al., 1990). In an attempt to restore the lake, approximately four tonnes of benthivorous and zooplanktivorous fish (mainly bream and roach) were removed during the years 1986–1988 by use of gill, fyke and pound nets as well as electrofishing.

Following fish removal, phytoplankton Chl *a* and water turbidity decreased substantially (Fig. 3). Two years after the biomanipulation Secchi depth improved, submerged macrophytes recolonised, first *Potamogeton crispus* L. and then *Elodea canadensis* Rich., with the latter completely covering the lake within 1–2 years (Lauridsen et al., 1994). However, from 1998 macrophytes disappeared or occurred only in low densities, coinciding with an increase in the abundance of roach (Fig. 4), and small perch became dominant. Immediately after the fish removal, seasonal TP in the lake changed considerably (Fig. 3). When submerged macrophytes were abundant, Chl *a* and TP were also low. Later, when macrophytes disappeared and the water turned turbid, TP reached high summer concentrations again.

In an attempt to shift the lake back to the clear state, 2.7 tonnes (68% of the first biomanipulation effort) of fish consisting of bream and roach were removed during 2007–2009. Catch per unit effort in gillnets (CPUE) decreased from ca. 5–7 kg net⁻¹ before the fish removal to 2–3 kg net⁻¹ afterwards (Fig. 4). Following the second biomanipulation, mean summer Chl *a* was reduced, Secchi depth increased, TP and TN both declined markedly and coverage of submerged macrophytes increased substantially (Fig. 3). Macrophyte recovery thus followed the pattern observed after the first fish removal in the 1980s, with fast re-growth after improved water clarity. The macrophyte community became completely dominated by *E. canadensis* a few years after the first manipulation. Biomasses of roach and bream have been low since 2009 compared to the years before fish manipulation (Fig. 4).

Although the longevity of the new restoration is obviously unknown, the effort needed to bring the lake back to the clear state was clearly much

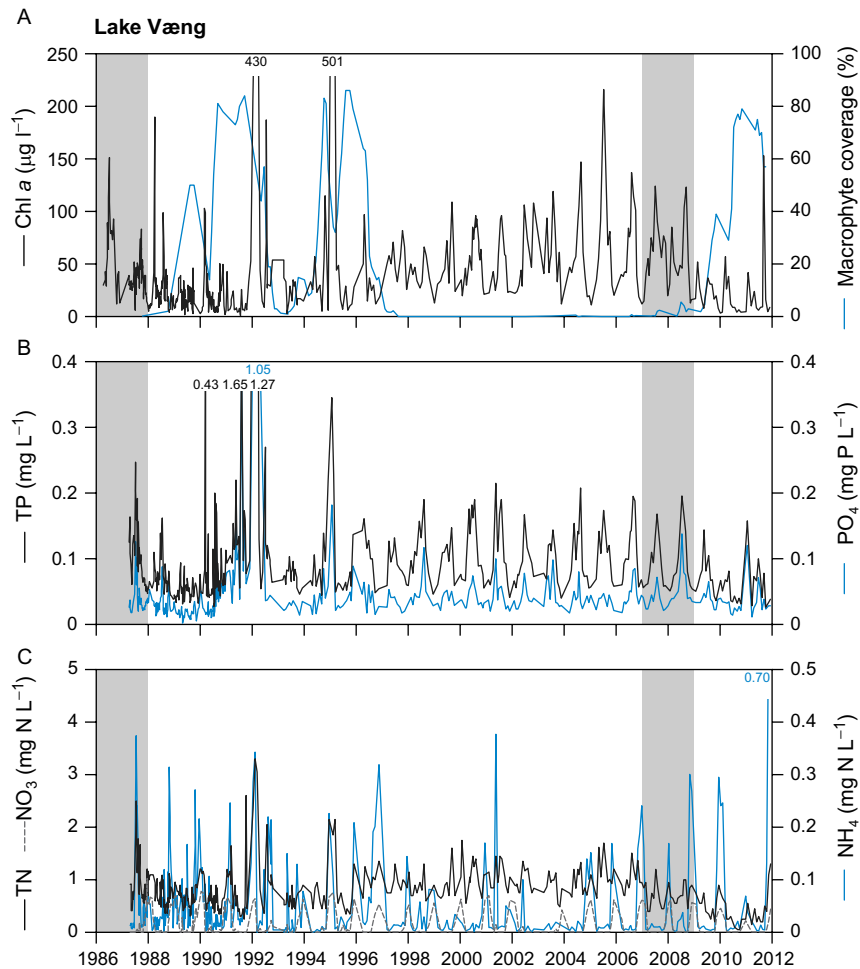


Figure 3 Changes in chlorophyll *a* and macrophyte coverage (A), various phosphorus (B) and nitrogen (C) specimens in Lake Væng, Denmark. Fish removal was undertaken during 1986–1988 and 2007–2009, respectively.

less extensive than that for the first biomanipulation, which is in line with our expectations.

2.1.5 Effects of fish manipulation judged from paleoecological studies

The limited number of suitable case studies of biomanipulation makes it difficult to judge the long-term effects. However, considerable information is stored in the sediment, and this ecological archive could be extremely

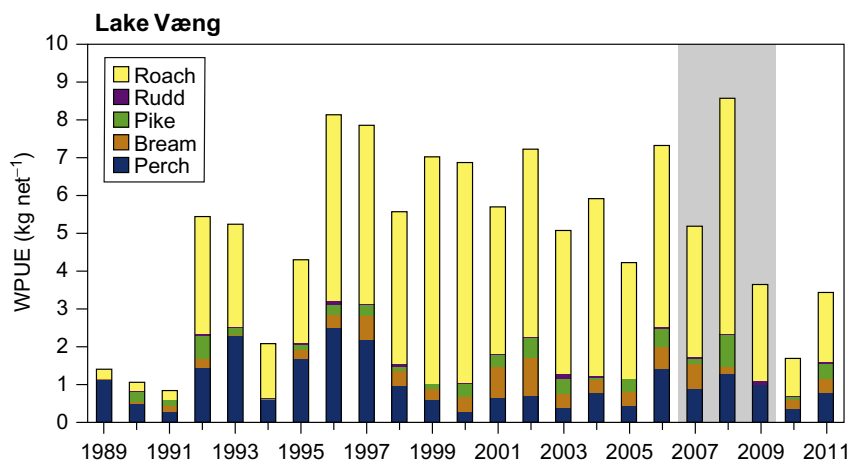


Figure 4 Fish weight per unit effort (WPUE) in Lake Væng, Denmark. Biomanipulation was undertaken during 1986–1988 and 2007–2009, respectively (highlighted).

valuable as most biomanipulation experiments were conducted in the 1980s and 1990s, that is, a sufficiently long time ago to enable detection of changes in the sediment provided that mixing is not too severe.

Among the few existing studies, the shallow Lake Christina in the USA (Table 2) (Hanson and Butler, 1994) has been biomanipulated three times since 1987, each time with only short-term (5–10 years), but dramatic effects (shifts to clear water with abundant macrophytes). The long-term changes in the lake were evident from changes in diatoms and cladocerans in the sediment record (Hobbs et al., 2012). However, the periodic shifts between clear and turbid states generated by biomanipulation are not as clearly reflected by the sediments as by the contemporary data, which may reflect sediment disturbance due to the large size and shallowness of the lake.

One of the best proxy indicators of changes in fish abundance in the sediment record is the abundance and size of *Daphnia* taxa (Jeppesen et al., 2001, 2002), in particular, relative to the abundance of smaller pelagic taxa such as *Bosmina* (Davidson et al., 2010), due to size selective predation by fish (Brooks and Dodson, 1965). An example of biomanipulation and its effects accurately recorded in lake sediment comes from Nykänen et al. (2010) who studied changes in subfossil cladocerans in annually layered sediment in the Enonselkä basin of Lake Vesijärvi, Finland (Table 2). Measures to restore the Enonselkä basin included reduction of the external nutrient loading and mass removal of planktivorous and benthivorous fish during 1993–1996 (Kairesalo et al., 1999). Water clarity increased and the lake changed from

a eutrophic to a mesotrophic state, but some signs of increased turbidity were observed after 5–10 years of recovery. After the removal of fish, the mean size of *Daphnia* ephippia and *Eubosmina crassicornis* ephippia and carapaces, and the ephippia ratio of *Daphnia*/(*Daphnia* + *E. crassicornis*), increased significantly, all indicating reduced fish predation (Nykänen et al., 2010) (Fig. 5). Expanding littoral vegetation along with improved water clarity owing to

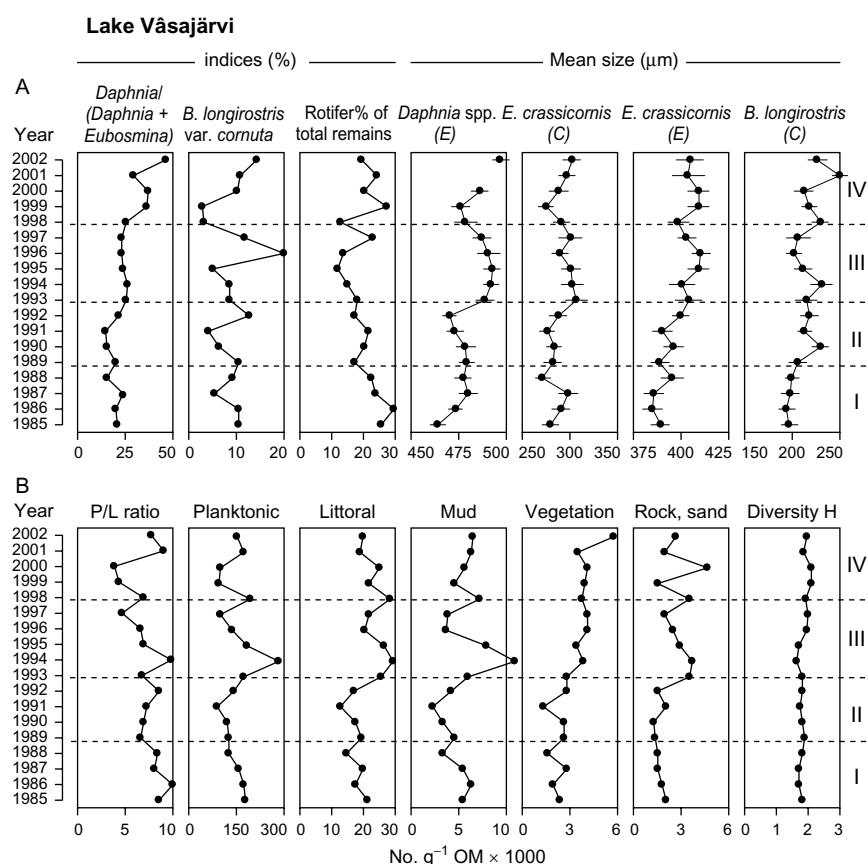


Figure 5 Sediment record of subfossil cladocerans in Lake Väsajärvi, Finland, before and after fish removal conducted to restore the lake (modified from Nykänen, 2010). (A, left) The ratio *Daphnia*/(*Daphnia* + *E. crassicornis*) (based on ephippia), the proportion of the cornuta form to the straight antennule form of *Bosmina longirostris*, and the proportion of rotifer resting eggs to cladoceran remains as percentages, all proxies for fish predation. (A, right) Mean size of *Daphnia* ephippia, *E. crassicornis* ephippia and carapaces and *B. longirostris* carapaces (ephippia E, carapaces C). *Daphnia* ephippia in varve 2001 were damaged and therefore not measured. (B) Planktonic/littoral (P/L) ratio, total concentration of planktonic and littoral remains per unit organic matter (OM), concentrations of mud-, plant- and rock-/sand-associated littoral species, as well as Shannon's diversity index (H) for the entire subfossil community.

higher contribution of littoral species was clearly detected. Moreover, the species diversity index for the entire subfossil community increased.

Effects of fish stocking and removal have also been demonstrated in several studies by analysing remains of cladocerans, chironomids and/or pigments (Buchaca et al., 2011; Leavitt et al., 1989; Sayer et al., 2012; Verschuren and Marnell, 1997), in several cases showing strong effects of the trophic cascade and the resulting change in the pelagic zooplankton community and increased or decreased algal crop. The accuracy with which the sediment can record these events is dependent on a number of factors, including lake size, depth and fetch, which will dictate the degree of temporal smoothing of the sediment record, the size of the disturbance and perhaps the nutrient status of the sites. However, a clear-cut response to a change in the fish predation pressure in terms of abundance and size of *Daphnia ephippia*, not to mention the rapid evolutionary response, was recorded from the sediment record of a small lake, Oud Heverlee Pond, Belgium (Cousyn et al., 2001). Furthermore, a number of more naturally occurring fish kill events and their ecological consequences have been tracked via the sediment record (Amsinck et al., 2005; Carl Sayer et al., unpublished data). Carl Sayer et al. (unpublished data) found a sharp shift in the sediment record reflecting a step change in ecology. Thus, the sediment record holds great potential to assess the longevity of success of biomanipulation and whether the shift back to more turbid conditions is rapid or gradual, and it may perhaps be used to detect early warning signals as well. In agreement with contemporary observations (e.g. Søndergaard et al., 2007), the limited number of studies using the lake sediment record points to short-term, decadal scales effects of extensive changes in fish biomass on lake ecology.

Some further perspectives on the ecological condition of biomanipulated systems without contemporary data may be provided by the sediment record. Plant macrofossils can record the shifts in the dominant components of the macrophyte flora and provide some indication of the abundance of certain taxa, such as charophytes (Davidson et al., 2005). Other indicators may reveal trophic shifts through change in the relative abundance of benthic and pelagic taxa (Davidson et al., 2010; Vadeboncoeur et al., 2003).

2.2. Fish manipulation in warm lakes

In warm locations (Mediterranean, subtropical and tropical lakes), where omnivory among fishes is common (González-Bergonzoni et al., 2012), stocking of pelagic filter-feeding fish has, at times, been used as a

biomanipulation tool. Typically, various carp and tilapia species are introduced (Arcifa et al., 1986; Jones and Poplawski, 1998; Starling, 1993) to reduce noxious filamentous cyanobacteria, rather than total algae biomass, through direct feeding. This practice seems inefficient when fish reach a high carrying capacity. In such circumstances, nutrient recycling by fish may outperform their filtration of phytoplankton, resulting in phytoplankton enhancement with a frequent shift from edible to inedible forms (Attayde and Hansson, 2001; Figueredo and Giani, 2005). Examples are limited, but several characteristics suggest that biomanipulation is a more complex task in warm lakes than in cold and temperate areas.

In this section, we briefly describe how fish assemblages in warm lakes differ from those in northern temperate lakes and discuss the challenge that this presents to biomanipulation, followed by two case studies from warm lakes in China and Brazil.

2.2.1 Fish assemblages in warm lakes

It is debatable whether the fish manipulation approach used in northern temperate lakes can be used with success in warm lakes (Jeppesen et al., 2005; Lazzaro, 1997) due to several key differences in fish community structure between warm (i.e. tropical, subtropical and Mediterranean) and cold or temperate fish assemblages, as partly highlighted in recent reviews (i.e. Jeppesen et al., 2010; Meerhoff et al., 2012). The occurrence of similar patterns in the structure of fish assemblages in different geographic locations suggests that they are generally more related to climate than to biogeographic history (Meerhoff et al., 2012).

Firstly, fish species richness is often higher in warm lakes, with the exception of lakes with high hydrological stress such as some tropical closed-basin African lakes (Lévêque, 1997). A global meta-analysis has shown increasing fish species richness, in particular, of omnivores, in shallow lakes towards lower latitudes (González-Bergonzoni et al., 2012). A consistent pattern emerges at latitudinal continental scales in shallow and deep lakes in Europe (Brucet et al., submitted for publication) and North America (Griffiths, 1997; Mandrak, 1995) as well as in cross-comparison studies (Meerhoff et al., 2007a; Teixeira-de Mello et al., 2009). The same pattern of higher species richness in warmer locations was found in altitudinal studies worldwide (Amarasinghe and Welcomme, 2002). Recent investigations suggest that littoral and benthic production promote fish diversity in lakes (Vander Zanden et al., 2011), which might contribute to explain the higher diversity in the typically plant-associated

fish assemblages in warm lakes (Conrow et al., 1990; Delariva et al., 1994; Meerhoff et al., 2003, 2007a; Teixeira-de Mello et al., 2009).

Secondly, the fish assemblages in many warm lakes, independent of trophic state, are often more functionally diverse (Moss, 2010) and dominated by omnivorous species (Lazzaro, 1997; Teixeira-de Mello et al., 2009). In addition, top-down control by piscivorous fish is apparently weaker in warm lakes than in temperate lakes, as suggested by a study on a series of subtropical lakes (Gelós et al., 2010). However, in shallow lakes of the Pantanal floodplains, it seems that drastic changes in the relative abundance within fish species assemblage (subtropical area) may promote turbid or clear water states (Mormul et al., 2012).

Thirdly, biomass and density of fish assemblages seem to increase with increasing ambient temperature, as found in a series of shallow lakes of varying nutrient concentrations along a climate gradient in Europe (Gyllström et al., 2005), and supported by cross-comparison studies conducted in both freshwater (Meerhoff et al., 2007a,b; Teixeira-de Mello et al., 2009) and brackish shallow lakes (Brucet et al., 2010).

Fourthly, in many warm lakes, fish reproduction occurs throughout the year (Fernando, 1994; Paugy and Lévêque, 1999), and an increase in the number of reproductive events with decreasing latitude (i.e. increasing temperature) has been reported for some species (Goyenola et al., 2011). In temperate systems, in contrast, most freshwater fish species have seasonal reproduction, with peaks in spring or summer (Wootton, 1984). Small, short-lived species with generally opportunistic strategies are more abundant at low latitudes, whereas large, longer-lived species predominate at high latitudes, as found in a very exhaustive research on North American fishes (Mims et al., 2010).

Finally, fish assemblages in warm lakes are typically characterised by smaller individuals than in temperate and cold lakes (reviewed in Meerhoff et al., 2012), as detected in several studies in freshwaters, both along latitudinal gradients (North America, Griffiths, 1997; South America, Lacerot, 2010; Europe, Brucet et al., submitted) and in cross-comparison studies (Brucet et al., 2010; Teixeira-de Mello et al., 2009).

As small fishes are more zooplanktivorous and have a much higher energy demand per unit of biomass than large fish (Kalf, 2002), the dominance of small fishes in such high abundances leads to a higher predation pressure on large zooplankton than in similar colder lakes, where the effect of juvenile fish is typically strongest in mid-late summer (Jeppesen et al., 1998). Moreover, the diurnal refuge potential of aquatic plants for large zooplankton against visual-feeder fishes, which is often seen in temperate shallow lakes (Burks et al., 2002; Timms and Moss, 1984), is almost absent in warm lakes (Iglesias et al., 2007; Meerhoff et al., 2006, 2007b; Tavsanoğlu et al., 2012).

Supporting the hypothesis of higher fish predation as a key factor in shaping the zooplankton size structure in warm lakes (Fernando, 2002; Jeppesen et al., 2007; Meerhoff et al., 2007a,b), occurrence of large *Daphnia* spp. in subtropical lakes, has been associated with the absence of fish (Iglesias et al., 2011). The control of phytoplankton by large zooplankton, typically observed in temperate lakes, is rarely found in warm lakes, as indicated by the low zooplankton:phytoplankton biomass ratio found in several latitudinal gradient studies (reviewed in Meerhoff et al., 2012), and as explained by theoretical models analysing fish reproduction in warm systems (Van Leeuwen et al., 2007). This predation pressure on zooplankton may represent a further limitation of the usefulness of the classic biomanipulation in tropical and subtropical lakes.

2.2.2 Fish manipulation examples in warm lakes: Huizhou Westlake, China

Huizhou West Lake is a shallow lake in tropical China (Table 2, Fig. 6). The lake has a total surface area of about 1.6 km². The lake is divided into several basins connected through waterways. Water temperature in the lake ranges from 12 to 35 °C over the season and the hydraulic retention time is ca.

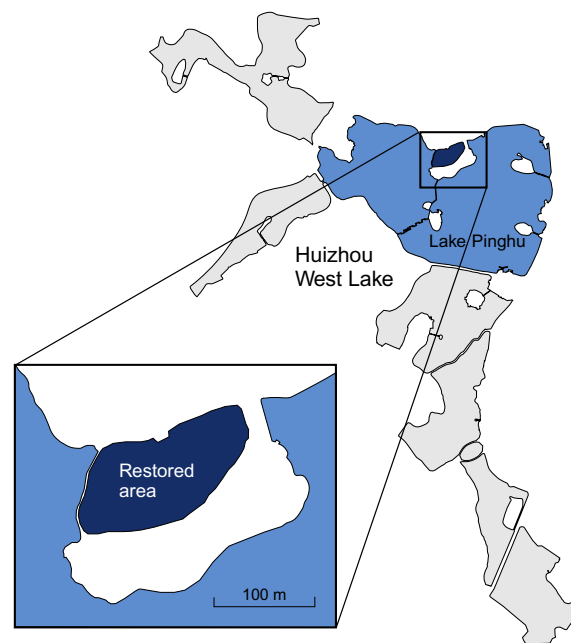


Figure 6 Drawing of Huizhou West Lake China showing the restored area.

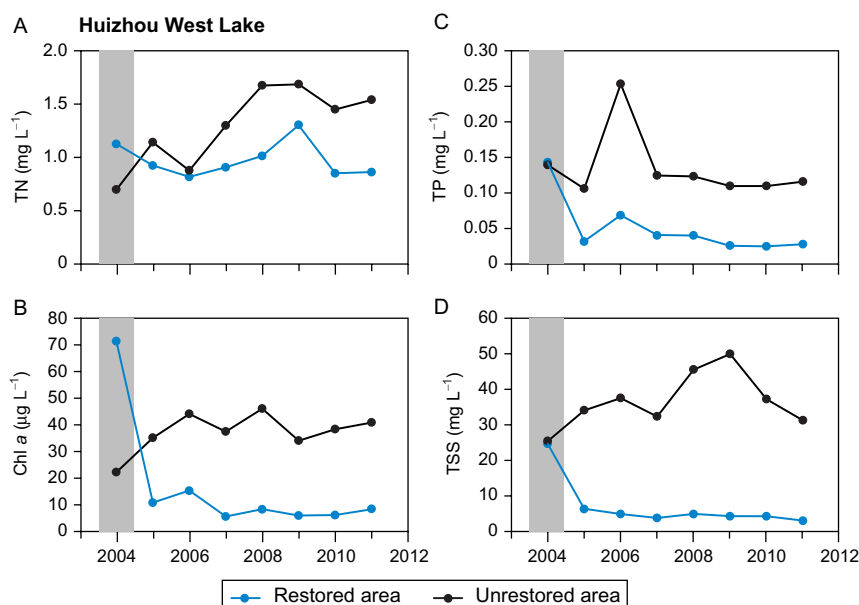


Figure 7 Comparison of total nitrogen (A), chlorophyll *a* (B), total phosphorus (C) and total suspended solids (D) in the restored and unrestored area in Huizhou West Lake, China. The highlighted period in grey shows pre-restoration levels.

3 months. The lake was dominated by submerged macrophytes before the 1960s. Following the initiation of fish aquaculture in the lake during the 1970s and increased waste water input, the lake became eutrophic and submerged macrophytes have been absent since the 1980s (Li, 2009).

In spite of restoration efforts, including effluent diversion and sediment removal (Li, 2009), the lake has remained eutrophic and turbid (Fig. 7). Furthermore, fish stockings have occasionally been conducted, the fish community being dominated by omnivorous and benthivorous species including Nile tilapia (*Oreochromis niloticus* L.), common carp and Crucian carp (*Carassius auratus* L.).

In order to improve water quality, a large-scale biomanipulation experiment was conducted in a 1-ha basin (restoration area, Fig. 6) of the lake at the end of 2004. The biomanipulation included fish removal, followed by submerged macrophyte transplantation and stocking of piscivorous fish. After isolating the area from the rest of the lake, the water level was lowered to ca. 60 cm and ca. 200 kg ha⁻¹ of fish, including tilapia, common carp, Crucian carp, mud carp (*Cirrhina molitorella* Cuvier et Valenciennes), silver

carp (*Hypophthalmichthys molitrix* (Valenciennes)) and bighead carp (*Aristichthys nobilis* (Richardson)) were removed over a 2-month period. Submerged macrophytes, including *Hydrilla verticillata* Royle, *Vallisneria natans* L. and *Myriophyllum spicatum* L., were planted and coverage reached 60% in May–June, 2005, and > 80% in summer 2006. Some piscivorous fish were stocked after macrophyte transplantation, including snakehead (*Channa argus* Cantor) and mandarin fish (*Siniperca chuatsi* Basilewsky).

Annual mean TN was reduced to less than 1.0 mg L^{-1} and TP to less than 0.050 mg L^{-1} . Fish removal likely reduced sediment resuspension, while submerged macrophytes protected the sediment from resuspension, and macrophytes and benthic algae may have reduced the nutrient release from the sediments (Zhang et al., 2012).

Annual mean concentration of Chl *a* also dropped to less than $10 \text{ } \mu\text{g L}^{-1}$ in the restored area, while total suspended solids were $\ll 5 \text{ mg L}^{-1}$, respectively, reflecting a decrease in both inorganic suspended solids and phytoplankton in the restored part. Densities of planktonic crustaceans, both cladocerans and copepods, were extremely low in both the restored and unrestored areas, suggesting that zooplankton grazing played a minor role in controlling phytoplankton in the restored area. So, the biomanipulation in this tropical shallow lake did not increase the number of zooplankton grazers as seen in north temperate lakes (Søndergaard et al., 2008), likely reflecting high predation by the abundant small fish including fish fry. Many fishes can spawn several times a year in this climate region, as is the case for species such as tilapia and Crucian carp which are abundant in the lake (Z. Liu, unpublished data).

The restoration study of Huizhou West Lake demonstrated that fish removal and transplantation of submerged macrophytes can restore the clear water state in a tropical eutrophic lake via enhanced bottom-up control and reduced sediment resuspension, even without increasing zooplankton grazing on phytoplankton. The experimental design does not allow us to disentangle the relative strength of these two mechanisms nor can we evaluate the long-term impacts of the restoration methods yet.

2.2.3 Fish manipulation examples in warm lakes: Lake Paranoá, Brazil

Lake Paranoá is a tropical urban reservoir in Brasilia (Table 2, Fig. 8). During 1970–1990, the release of partially treated domestic wastewater led to deterioration of the lake water quality, particularly in two basins (Banal and Riacho Fundo basins, Secchi depth $< 0.5 \text{ m}$, and Chl *a* $> 40 \text{ } \mu\text{g L}^{-1}$, Fig. 9).

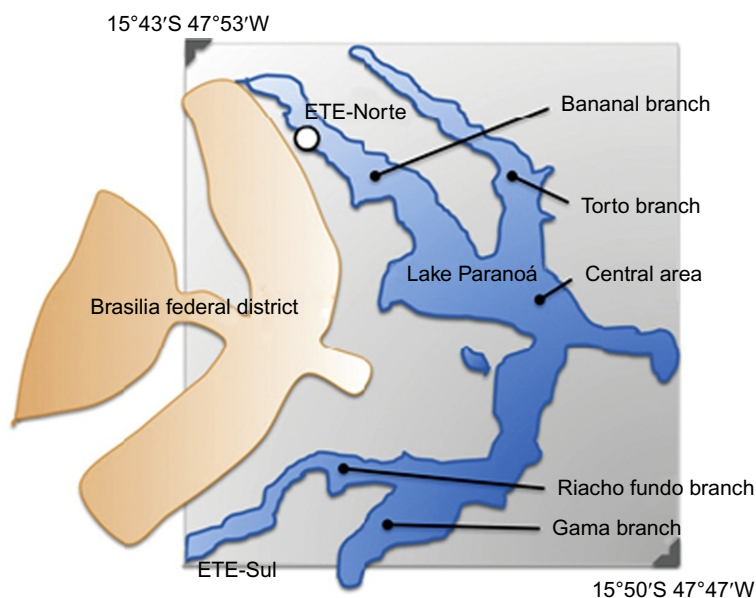


Figure 8 Drawing of Lake Paranoá, Brazil—eutrophic Bananal and Riacho Fundo branches and water treatment plants (ETE-Norte and ETE-Sul).

Eutrophication intensified until early 1990, with recurrent cyanobacteria blooms of the colonial *Microcystis aeruginosa*, and a permanent bloom of the filamentous cyanobacteria *Cylindrospermopsis raciborskii*, associated with high TP ($0.03\text{--}0.08\text{ mg L}^{-1}$) and TN ($1.8\text{--}2.5\text{ mg L}^{-1}$) concentrations (Branco and Senna, 1996; Mattos et al., 1992). The Brasília Water and Sewage Corporation (CAESB) treated the surface blooms with periodical applications of copper sulphate.

Omnivorous Nile tilapia, Congo tilapia (*Tilapia rendalli* (Boulenger)) and common carp dominated the fish assemblage (average $>500\text{--}790\text{ kg ha}^{-1}$ in the eutrophic areas) during the 1970–1990s (Starling, 1998). The highest fish biomasses occurred in both basins (Lazzaro et al., 1998; Lebourges-Dhaussy et al., 1999a,b) despite a 300-tonnes fish removal following two major episodes of fish kill in 1997. These cyanobacteria bloom-induced fish kills were regarded as an “accidental biomanipulation”, since water quality during the period following the massive removal of dead fish improved noticeably (Starling et al., 2002).

A 10-year research programme was initiated in 1987 including fish stock assessments and laboratory and *in situ* mesocosm experiments to quantify the role of different key species of filter-feeding omnivorous fish (Nile tilapia,

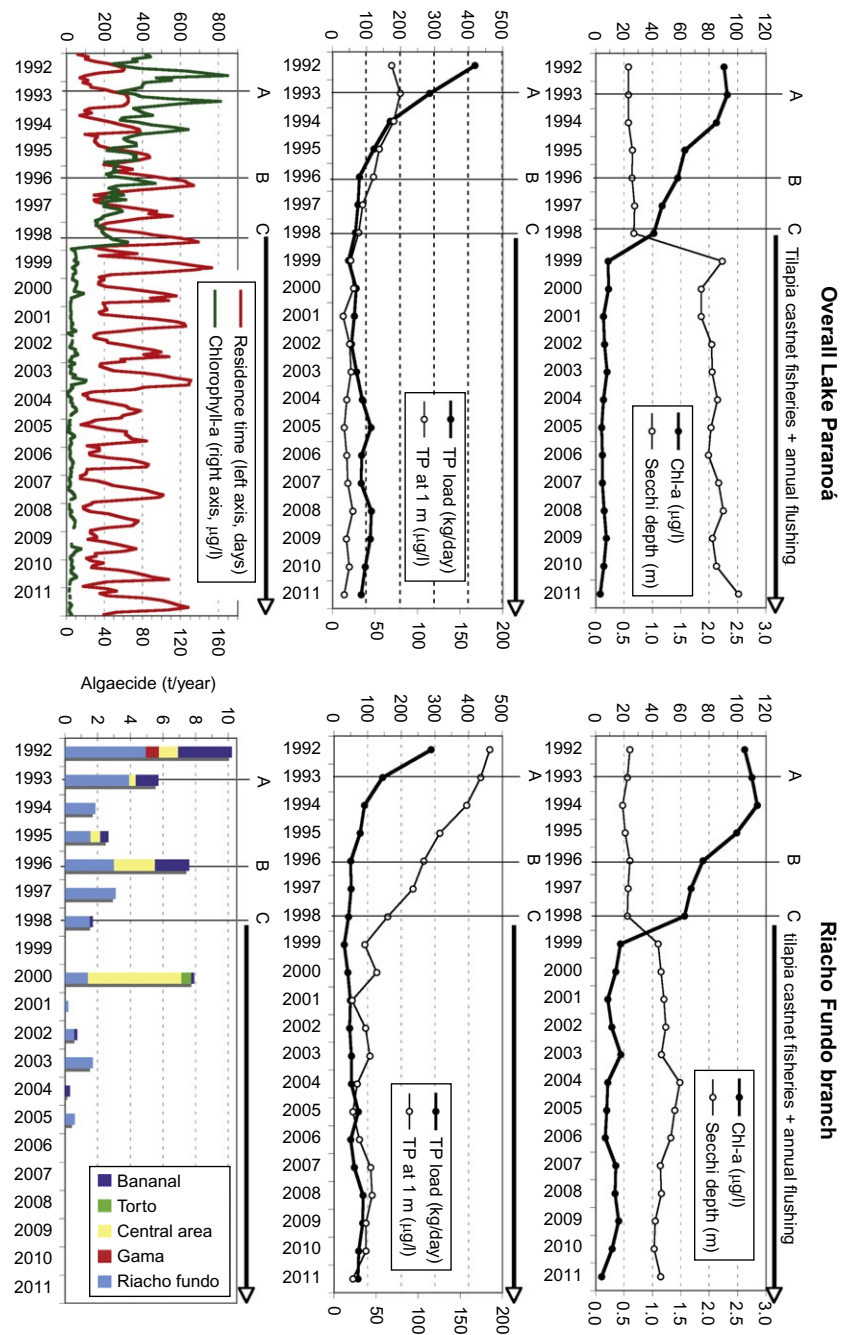
common carp and silver carp) with the aim to develop effective measures to restore the lake. The focus was on fish–cyanobacteria interactions and whether a large-scale fish manipulation could contribute to significantly improve the water quality (Lazzaro and Starling, 2005). A major result was that omnivorous Nile tilapia, owing to its benthivorous habits, was a major P recycler contributing to N limitation, which is favourable to cyanobacteria growth. In contrast, omnivorous silver carp, as an obligate filter feeder, effectively suppressed cyanobacterial biomass without detrimental reduction of the zooplankton grazing pressure because of the typically small size of the zooplankton herbivores. For more details, see Starling and Lazzaro (1997) and Lazzaro and Starling (2005).

The restoration of Lake Paranoá involved several steps. Starting in 1993, the external nutrient loading was drastically reduced (90% of N and P removal from raw sewage, resulting in a 70% reduction in P external loading) but did not lead to a drastic improvement in water quality. Thus, at the end of the 1998 dry season, the reservoir was flushed by opening the dam gates. As a result, the Chl *a* concentration dropped from 90 $\mu\text{g L}^{-1}$ in 1992 to 9 $\mu\text{g L}^{-1}$ in 1999, Secchi depth transparency reached 2.5 m and green algal species progressively replaced *C. raciborskii* (Burnett et al., 2001) (Fig. 9). From 1999 and onwards, less dramatic yearly flushing has been performed.

Since 1998, the biomanipulation has involved removal of omnivorous tilapias and common carp from the Bananal and Riacho Fundo branches by professional cast-net fishery. Yet, the average yield (8 tonnes per month in 1999–2001) has been declining since then. The contribution to lake internal P by the removed fish can be estimated in terms of P release rates to reach the same magnitude as the external P load to this area.

Analysing temporal series, Angelini et al. (2008) assessed that the activation of the water treatment plants (1993) had no effect on the Chl *a* and TP concentrations, whereas both significantly declined after proper operation of these plants (1996), and water transparency increased notably after the 1998 flushing event and following biomanipulation.

Currently, the lake shows oligo-mesotrophic characteristics (Batista, 2011), with higher phytoplankton richness, lower phytoplankton primary productivity, reduced fisheries yield and increased fish biodiversity in the captures, including a strong reduction of tilapia and re-occurrence of the native cichlid *Geophagus* sp. The best outcome of this restoration programme is that Lake Paranoá is currently being certified for its appropriate water quality to become a major drinking water supply source for the Brasilia Federal District.



The restoration has thus been successful, although it remains difficult to disentangle the effect of the different measures (flushing and biomanipulation).



3. MACROPHYTE ESTABLISHMENT AND PROTECTION

Submerged macrophytes play a fundamental structuring role in shallow lakes (Jeppesen et al., 1998; Moss et al., 1996), and their re-establishment and protection are thus of key importance for long-term recovery. Therefore, exclosures to protect macrophytes against herbivory have been used as an alternative or supplementary restoration tool to fish manipulation (Table 3). Such exclosures allow the macrophytes to grow in a grazer-free environment from where they may expand colonisation across the entire lake.

There are many examples where submerged macrophytes do not readily return to former levels (with respect to both abundance and species composition) despite reduced external nutrient loading and favourable light conditions (Bakker et al., 2012; Hilt et al., 2006; Sand-Jensen et al., 2008). This may be due to lack of sufficient propagules and low spreading potential or limited connection with other aquatic systems acting as source (Strand and Weisner, 2001). However, herbivory by fish (Prejs, 1984; Körner and Dugdale, 2003; Hutorowicz and Dziedzic, 2008), crayfish (Rodriguez et al., 2004) and waterfowl (Lauridsen et al., 1993; Marklund et al., 2002; Søndergaard et al., 1996) may also hamper their recovery (Lodge et al., 1998), although it may also promote higher macrophyte diversity under certain conditions (Hidding et al., 2010; Sandsten and Klaassen, 2008).

Here, we first describe macrophyte manipulation in temperate lakes where many experiments have been performed; then, we highlight the few available case studies from warm lakes, followed by a brief discussion about the potential and need for measures in warm lakes. Finally, we discuss the future perspectives of macrophyte manipulation.

Figure 9 Changes in yearly means of key environmental variables as an average for the lake (left panel) and the most eutrophic Riacho Fundo Branch (right panel) from 1992 to 2011. The three key events that may be responsible for the oligotrophication process of Lake Paranoá are shown: (A) activation of two water-treatment plants (Jan 1993), (B) proper operation of these plants (Jan 1996) and (C) major flushing event (sudden release of water in Nov. 1998). To reduce cyanobacterial blooms, the algacide copper sulphate was applied in various amounts until 2006 (lower right panel).

Table 3 Overview of natural and man-made macrophyte re-establishment following restoration attempts in shallow lakes

Natural or man-made plant establishment	Methods and effects	Temperate/ Lake name warm, T/W and country	References
Natural	- Fish manipulation <i>E. canadensis</i>	T L. Zwemlust, The Netherlands	van Donk and Otte (1996)
	- Fish manipulation <i>E. canadensis</i>	T L. Væng, Denmark	Lauridsen <i>et al.</i> (1993)
	- Fish kill Establishment of a diverse submersed flora	T L. Arreskov, Denmark	Sandby and Hansen (2007)
	- Repeated, low-percentage fish removal. Establishment of a more diverse submersed flora	L. Røgbølle, Denmark	Fugl and Myssen (2007)
	- Fish manipulation, establishment of submersed macrophytes - Protection of submersed macrophytes	T L. Ringsjön, Sweden	Strand (1999)
	No significant effect due to abundant natural vegetation	T L. Björkesåkrasjön, Sweden	Marklund <i>et al.</i> (2002)

Table 3 Overview of natural and man-made macrophyte re-establishment following restoration attempts in shallow lakes—cont'd

Natural or man-made plant establishment	Methods and effects	Temperate/ Lake name warm, T/W and country	References
Man-made	- Protection of submerged macrophytes (birds or fish)	T	Hanlon et al. (2000) ; Lauridsen et al. (1996) ; Shireman and Maceina (1981)
	- Transplantation and protection of submerged macrophytes from bird and fish grazing	T L. Engelsholm, Denmark	Lauridsen et al. (2003)
	- Introduction of shoots, seeds and seed pods of <i>Vallisneria americana</i> and protection against grazing	T Freshwater tidal area, USA	Moore et al. (2010)
	- Removal of herbivores, transplantation and protection of plants (large scale)	W Wuli Bay, China	Chen et al. (2009) , Ye et al. (2011)
	- Removal of herbivorous fish and transplantation of macrophytes (large scale)	W L. Qinhu, China	Z. Liu (unpublished data)
	- Transplantation in combination with stocking of piscivorous fish (large scale)	W L. Huizhou, West Lake, China	Z. Liu (unpublished data)
	- Establishment of artificial plant beds	T Lake Stigsholm, Lake Væng	Schou et al. (2009) and Boll et al. (2011)

The table includes only studies where full-scale manipulations or natural fish community changes have occurred, plus small/larger scale experiments in which the aim was to establish submerged macrophytes by protecting areas from bird and/or fish grazing.

3.1. Macrophyte manipulation in north temperate lakes

Studies in UK (Chaichana et al., 2011; Irfanullah and Moss, 2004), Denmark (Lauridsen et al., 2003; Søndergaard et al., 1996) and Germany (Hilt et al., 2006) have shown higher survival and number of plants and longer total shoot length in enclosures that prevent bird access. A large-scale experiment conducted in Danish Lake Engelsholm, where three submerged macrophyte species were planted in three 250 m² exclosures (protected from bird grazing, Lauridsen et al., 2003), demonstrated an up to fourfold increase in plant coverage in two growth seasons. Furthermore, the experiment revealed that submerged macrophytes were capable of colonising non-transplanted but protected areas. No macrophytes were observed outside the protected areas, illustrating the high grazing pressure, despite relatively few herbivorous birds in the lake. The importance of macrophyte establishment outside the maximum diving depth of herbivorous birds was a key factor for the macrophyte re-establishment in the lake.

Transplantation and protection of submerged macrophytes are a time- and resource demanding restoration process, particularly in large systems. Many eutrophic submerged macrophyte species have a very large growth potential and are able to completely overgrow shallow lakes within a few seasons (e.g. Lauridsen et al., 1993; Rørslett et al., 1986). *Elodea* species can develop from absence to complete cover within 1–2 years (Meijer et al., 1994; Søndergaard et al., 1998). It therefore remains an open question under which circumstances it would actually be necessary to transplant and protect macrophytes from grazing. In a recent review, Bakker et al. (2012) concluded that after abiotic conditions for plant macrophyte growth are reached it is imperative to study the role of propagule availability and herbivory more closely in order to define what exactly can be restored and what cannot. There may be unfavourable conditions that can explain why submerged vegetation does not return naturally if clear water conditions have been established, as suggested by Marklund et al. (2002) and Lauridsen et al. (2003). Once these uncertainties have been clarified, transplantation would mainly be relevant where propagule availability and spreading/colonisation potential are both low. However, protection from herbivory would be expected to be most important in shallow lakes which often need to recover from very low (or zero) macrophyte abundance. Waterfowls are also important, and in a recent meta-analysis on their impacts, Wood et al. (2012) found a positive linear relationship between waterfowl abundance and the reduction in plant standing crop when the waterfowl density was expressed as kg ha⁻¹, but not when expressed as individuals per hectare, emphasising the importance of species body mass.

If the restoration goal includes the re-introduction of species typically dominating in nutrient poor lakes, or species which may have become regionally extinct or have allelopathic effects to enhance clear water conditions, species-specific macrophyte transplantations may be relevant. The need to reintroduce species to areas where they have become extinct has been a subject of recent discussion, and a whole new research field on this topic is emerging (Seddon et al., 2007). In Danish Lake Fure, for instance, the number of macrophyte species almost returned to pre-enrichment levels after an external loading reduction, but short angiosperms, mosses and characeans disappeared along with all other vegetation in deeper waters (5–8 m) and have only partly recovered recently (Sand-Jensen et al., 2008). Instead, tall angiosperms became dominant and only 4 out of 10 characean species originally present reappeared. The review by Bakker et al. (2012) showed that the macrophyte community in none of six European lakes studied had recovered completely, but all lakes showed a different community composition with fewer species compared with the situation 20–100 years ago. It is important to emphasise that a lack of recovery of plants may also reflect excessive N loading after P loading reduction, as several studies indicate that plant recovery and species richness are sensitive to high N (Barker et al., 2008; James et al., 2005; Jeppesen et al., 2005; Moss et al., 2012).

An alternative way forward to restore the macrophytes is to spread seeds or whole shoots. The restoration success of *V. americana* was studied in different types of transplantations in Virginia, USA, by Moore et al. (2010), who found that whole shoot transplants resulted in the most rapid cover of the bottom, whereas direct dispersal of individual seeds or intact seed pods was effective, but slower. They also concluded that protection of the plants from herbivory was critical to restoration success, and for large-scale restorations, they suggested that large founder colonies may be needed to withstand grazing pressures for expansion outside herbivore exclosures. The success of transplantation also depends on suitable substrate conditions, with sufficient organic content and limited toxic substances (Bornette and Puijalon, 2011; Smolders and Roelofs, 1996; Van der Welle et al., 2007).

If plants do not respond immediately either naturally or to the above transplantations methods, then artificial plants may initially help to create and stabilise a clear water state by serving as a daytime refuge for zooplankton against predation by fish. Schou et al. (2009) found that a ca. 3–5% lake area covered by artificial plants doubled zooplankton densities in shallow Lake

Stigsholm, Denmark. Moreover, these plants could serve as a substrate for plant-associated macroinvertebrates. [Boll et al. \(2012\)](#) found plant-associated macroinvertebrate densities of 342,000 and 133,000 ind. m⁻² of lake bottom at high- and low artificial plant density (80% and 40% PVI, plant volume inhabited), respectively, which was substantially higher than the densities of benthic macroinvertebrates (3500 ind. m⁻²) in sediment without plants. As submerged vegetation may serve as profitable feeding grounds for small perch, it is likely that artificial plant beds can enhance recruitment to the piscivorous stage, as when submerged vegetation is expanding ([Hargeby et al., 2005](#)). The optimal plant density for efficient foraging by small, non-piscivorous perch has yet to be determined.

3.2. Macrophyte manipulation in warm lakes

While in temperate systems the introduction and development of aquatic plants are considered key steps in restoration ([Moss et al., 1996](#)), in the tropics and subtropics, many aquatic plants, particularly exotics, are often considered a nuisance and subject to eradication measures. Plant beds in nutrient-enriched lakes may become so abundant that they impede transportation, hydroelectricity generation and fisheries and reduce the recreational value for anglers ([Bini et al., 1999](#); [Cilliers et al., 1996](#); [Moreira et al., 1999](#)). Moreover, excessive growth of several species, like *M. spicatum*, or the free-floating *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart) Solms (the latter two native to South America) in many lakes and reservoirs in the USA, South America, Asia and Africa, or the North American *E. canadensis* in Europe, may alter lake ecosystems and constitute a serious threat to the native flora and fauna. Biomanipulation by stocking of herbivorous grass carp (*Ctenopharyngon idella* Valenciennes) or the introduction of specialist phytophagous insects, such as weevils (*Euhrychiopsis lecontei*) and water veneer moth (*Acentria ephemerella*), have been used to control some of these plants ([Cooke et al., 2005](#)).

Manipulation of dense mats of free-floating plants has been conducted in an attempt to remove suspended solids and nutrients from artificial systems (e.g. from sewage or industrial runoff, [Dellarossa et al., 2001](#)), as well as to complement other restoration measures (e.g. Lake Rodó, Uruguay, [Rodríguez-Gallego et al., 2004](#)). The need for rapid transport of the typically huge volume of removed biomass (to avoid nutrients from returning to the system) might impose logistic problems for this method.

In contrast to temperate lakes, few comprehensive full-scale studies on the use of macrophytes for restoration are available for subtropical lakes.

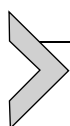
The experimental results demonstrate the usefulness of plant refuges as a restoration tool in small shallow lakes (Chen et al., 2009; Ye et al., 2011). In the studies by Ye et al. (2011) and Chen et al. (2009), herbivorous fish were excluded from a 5-ha shallow area followed by transplantation of submerged macrophytes into the same area. In one area, there was an increase in submerged macrophyte coverage, whereas in another area, a decrease was observed due to overgrowth by floating-leaved macrophytes (Ye et al., 2011), with transplanted submerged plants having limited success (Chen et al., 2009). Consequently, it was concluded that transplantation of submerged macrophytes may not necessarily be successful in itself in this particular case, but in combination with emergent and floating-leaved macrophytes, they had a positive impact on water quality. Also in Lake Qinhu, China, a relatively large-scale successful transplantation of macrophytes following removal of herbivorous fish has been performed (Z. Liu, unpublished data). In Lake Qinhu, macrophytes have expanded and have a positive effect on the system, contributing to maintaining a newly established clear water state.

For regulated lakes, alteration in water level may help promote the submerged macrophytes (Cooke et al., 2005), as it may improve the light environment for macrophyte growth (Blindow, 1992; Nöges and Nöges, 1999; Coops et al., 2003; Beklioğlu et al., 2006) depending on lake morphology (Beklioğlu et al., 2006). Accordingly, mesocosm studies in a Turkish shallow lake showed that macrophytes could resist higher nutrient loading due to a high, evaporation-triggered, water level reduction during summer, overriding the deleterious effect of periphyton- and phytoplankton-induced turbidity (Bucak et al., 2012; Özkan et al., 2010).

3.3. Macrophyte manipulation: Future perspectives

The use of macrophyte establishment and protection as a lake restoration method will remain an important measure in the future, but there are many unsolved issues: under which circumstances are these measures needed? can the methods be optimised? and how can long term effects be assured? A possible conflict between recreational users and nature conservation remains as promotion of indigenous submerged macrophytes is an important measure to restore shallow lakes but may, if plants become very abundant, cause nuisance to boating and swimming and obstruct the water flow (van Nes et al., 2002). In many densely populated and agricultural countries, nutrient levels in lakes will remain at such high levels that both situations may occur. The predicted increase in eutrophication in the future can boost both

turbidity-tolerant submerged and free-floating plants, since they are not affected by the underwater light regime. The choice of the best plant species to promote will thus be of key importance.



4. ENHANCEMENT OF ALTERNATIVE HERBIVORES: STOCKING MUSSELS

Stocking of aquatic mussels may potentially help induce clear water in lakes after nutrient loading reduction (Gulati et al., 2008), although full-scale stocking experiments have not, to our knowledge, been conducted yet. Here, we overview the potential role that naturally occurring and introduced mussels may play in lake ecosystems and discuss how they might restore eutrophied lakes.

Mussels can have an important impact on shallow lake ecosystems via several mechanisms, including suspension feeding (e.g. Strayer et al., 1999), deposit feeding (Vaughn et al., 2004), grazing (Hill et al., 1992; Karatayev et al., 2009a,b), predation (e.g. Howells et al., 2006), biodeposition (Stewart et al., 1998), bioturbation (e.g. Karatayev et al., 2007) and shell production (e.g. Gutierrez et al., 2003).

Large unionid mussels, such as *Anodonta*, *Unio* and *Hyridella* spp., are sometimes abundant in well-mixed macrophyte-dominated systems and can filter the entire water volume in a few days (Ogilvie and Mitchell, 1995; Strayer et al., 1994; Welker and Walz, 1998). Ogilvie and Mitchell (1995) observed that the *Hyridella menziesii* population of Lake Tuakitoto (New Zealand) filtered a volume of water equal to that of the lake (826,000 m³) once every 32 hours. Unionid mussels are also capable of filtering and ingesting cyanobacteria (Bontes et al., 2007; Dionisio Pires et al., 2007). However, these mussels often disappear from turbid lakes, probably due to predation of their larvae by fish (Gulati et al., 2008). The re-introduction of these species may therefore potentially be a useful tool for the restoration of the clear water state, but so far, this has received little attention. If a lake is in a turbid state and fish are abundant, however, re-introduction of mussels without removing fish is, in fact, unlikely to have any effect, unless the introduction involves large quantities of adult mussels that can escape fish predation.

The zebra mussel (*Dreissena polymorpha*), which is native to the lakes of southeast Russia, could be another candidate. It has become an invasive species in many countries (e.g. it was discovered in North America around 1986, Herbert et al., 1989) and affects sediment chemistry, grain size and

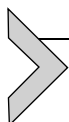
organic matter content via bioturbation. It also increases light penetration into the water column due to filter feeding, induces changes in near bed flows and shear stress due to the presence of shells and provides colonisable substrate and refuges by their shells.

Zebra mussels may, through filter feeding, markedly reduce phytoplankton while increasing water clarity (Caraco et al., 1997; Karatayev et al., 1997; Reeders et al., 1993) and thus often trigger a shift in primary production from the phytoplankton-dominated towards a macrophyte-dominated community (MacIsaac, 1996; Phelps, 1994). Ricciardi et al. (1998), however, demonstrated competition between zebra mussels and North American endemic bivalve species (Unionoida), increasing the risk of extinction of the latter, and the former are a nuisance for recreational boating and commercial shipping as well as for raw water using industries, potable water treatment plants and electric power stations (see Ludyanskiy et al., 1993; Roberts, 1990; Sousa et al., 2009).

Changes in the composition of phytoplankton and zooplankton communities following zebra mussel establishment have been observed in many systems (Bastviken et al., 1998; Ten Winkel and Davids, 1982). For zooplankton, Pace et al. (1998) showed that zebra mussels caused a strong size-dependent decline in microzooplankton in the Hudson River. How zebra mussels affect the potential toxic cyanobacteria is debatable. Invasion of North American lakes by zebra mussels has been argued to cause the widely observed increase, in particular, of species of toxigenic cyanobacteria, *M. aeruginosa* (Knoll et al., 2008; Raikow et al., 2004; Sarnelle et al., 2010; Vanderploeg et al., 2001). In contrast, a decline of *Microcystis* has been observed in other studies (Baker et al., 1998; Dionisio Pires et al., 2004, 2005; Smith et al., 1998). Dionisio Pires et al. (2005) suggested that the mussels may be used as a biofilter for the removal of harmful cyanobacterial blooms in shallow (Dutch) lakes where the mussels are already present and not a nuisance.

Cascading effects on water clarity, macrophyte growth and the nutrient levels have been observed in many lakes. For example, studies in Oneida Lake in North America showed a clear improvement of light climate and macrophyte vegetation extending to deeper areas in the presence of zebra mussels (Idrisi et al., 2001; Mayer et al., 2002) and macrophyte diversity increased (Zhu et al., 2006). There is therefore no doubt that stocking of zebra mussels has promoted a clear water state in several eutrophic lakes. However, it is an invasive species to most countries, and serious effects on other components of the local food webs have been described. It is therefore important

to test native species for their potential in lake restoration and the considerable diversity of bivalves in warm regions represents an interesting opportunity.



5. COMBINED TREATMENTS

The high probability of a return to the turbid state some years after biomanipulation calls for supplementary methods. One such possibility is to combine biomanipulation with chemical treatment of the water to precipitate phosphorus and to reduce internal phosphorus loading and thus phytoplankton growth. The resulting higher water clarity may potentially reinforce recovery by altering the top-down control of zooplankton by fish through a trophic and/or a behavioural cascade. An alternative is to combine biomanipulation with oxygenation of the hypolimnion, which may not only result in reduced internal loading but also affect the fish community and eventually the entire pelagic ecosystem. If oxygen appears in the hypolimnion, the conditions for macroinvertebrates improve. In addition, piscivorous fish species may be favoured. Perch, in particular, may obtain better foraging possibilities in the benthos, thereby allowing them to pass from consuming zooplankton through a macroinvertebrate eating stage before becoming piscivores (pass the “macroinvertebrate bottleneck”, [Persson et al., 1991](#)), facilitating a higher control of planktivorous fish. A behavioural cascade is also expected if water clarity increases in the epilimnion, as it enhances grazing and thus reduces sedimentation and thereby the need for oxygenation in the hypolimnion. The hypothesis is therefore that a combined treatment may be more effective than individual application of methods, due to synergistic effects ([Diagram 1](#)), and may reduce the costs of restoration and perhaps the need for subsequent intervention.

Here, we describe dual techniques, including biomanipulation combined with chemical treatment and with hypolimnion oxygenation, respectively, and give two case studies from Danish lakes. We further highlight the need for controlled experiments within this field.

5.1. Biomanipulation combined with chemical treatment

Chemical restoration aims at supplying new sorption sites for phosphate onto the surface sediment (sediment capping) (e.g. [Cooke et al., 2005](#); [Koschel et al., 2006](#)). Phosphate adsorbs readily to calcite (CaCO_3), hydroxides of oxidised iron (Fe^{3+}) and aluminium (Al^{3+}). Aluminium addition has been used for restoration in some 150 lakes in the USA and Europe,

and the reported longevity of positive effects varies from a few to 10–15 years, seemingly depending on dosage and on how well external P loading is controlled. Lanthanum (La) and the La-modified bentonite can also be used (Meis *et al.*, 2011). The commercial La product PhoslockTM was introduced in the late 1990s and treatment has been reported for ~10 lakes or basins, but a long-term positive effect has yet to be documented.

The precipitation of P and a reduction in internal loading followed by chemical restoration result in higher water clarity, which, in turn, may reinforce recovery by altering the top-down control of zooplankton by fish through a trophic and behavioural cascade. Following treatment with aluminium in deep Danish Lake Nordborg, a change in the fish community structure, abundance and habitat selection occurred within a year or less (Lund *et al.*, 2010). The proportion of perch increased in overnight gill net catches, at the expense of roach. Similarly, growth and average size of all key species increased. The habitat distribution of perch and roach changed from a high proportion in the upper pelagic zone towards increasing proportions in the deeper pelagic and profundal zones, even though the abundance of large-bodied zooplankton increased and the abundance of benthic macroinvertebrates decreased (E. Jeppesen *et al.*, unpublished results). These results indicate that enhanced risk of predation from piscivorous perch was more important for the habitat distribution change of the fish than food availability, resulting in less predation on zooplankton, higher grazing on phytoplankton and thus higher water clarity than expected from the aluminium treatment. They also demonstrated that chemical restoration may rapidly and strongly affect the trophic structure and dynamics of the system.

It is therefore likely that a combination of aluminium treatment or other chemical restoration methods with biomanipulation may have stronger and perhaps also more long-lasting effects than when the various methods are applied individually and that synergistic effects may even reduce the cost of restoration as a less extensive chemical and less fish manipulation effort will be needed. However, evidence of this is so far still limited.

5.1.1 Dual treatment: chemical treatment and fish manipulation in Lake Kollelev, Denmark

Shallow Lake Kollelev (Table 2) is an example of combined chemical and biological treatment. The lake is divided into three basins connected by channels. Until 1998, the lake received waste water or storm water with overflow, but after waste water diversion, the lake remained hypertrophic. Different in-lake measures were applied in the period 1999–2005 to

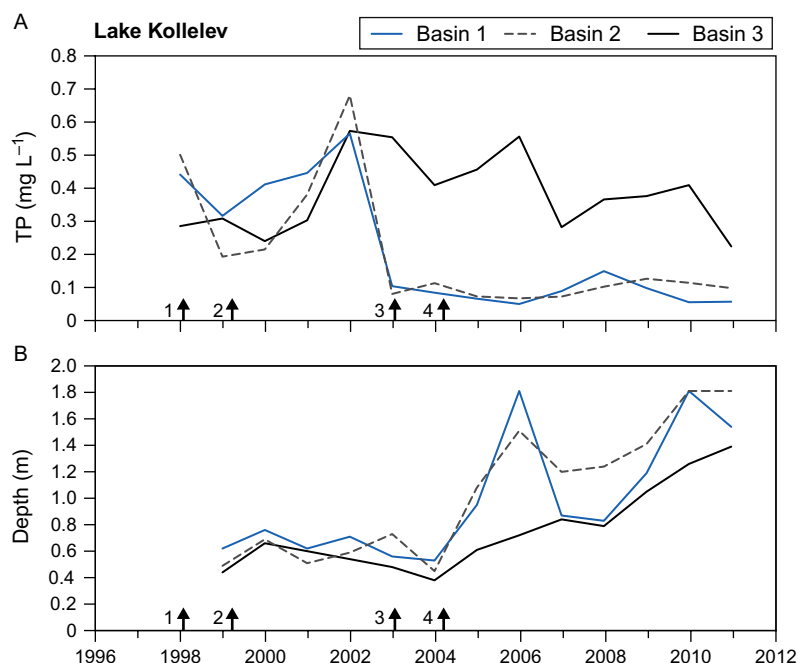


Figure 10 Total phosphorus (summer average for May 1–October 1) (A) and summer average Secchi depth (B) in the three basins of Lake Kollerlev, Denmark. Maximum water depth is 180 cm. Arrow 1: Iron addition to bind P in the sediment in basin 2 and 3. Arrow 2: First biomanipulation in all basins. Arrow 3: Aluminium treatment in basin 1 and 2. Arrow 4: Second biomanipulation in all three basins.

improve water clarity (Fig. 10, Appendix). Iron addition to two of the basins in 1998 had little effect. Biomanipulation including cyprinid removal and perch stocking in all basins in 1999 was also ineffective, while aluminium treatment of basins 1 and 2 in 2003 immediately resulted in lowered lake water TP. Meanwhile, no improvements in Secchi depth were observed in any of the basins. A new biomanipulation in all three basins resulted in an immediate and strong improvement in water clarity in the aluminium-treated basins, but a much less pronounced and only gradual improvement of the Secchi depth in the untreated basin, coinciding with a gradual decrease in TP.

This case study indicates that only the combined treatment with aluminium (bottom-up control) and biomanipulation (top-down control) ensured a rapid improvement in water clarity. However, as this experiment is not replicated, we cannot exclude that other factors may be involved, and

controlled follow-up experiments are needed before any firm conclusions can be drawn.

5.2. Biomanipulation combined with hypolimnion oxidation

Another possibility restricted to (summer-) stratified lakes is to combine biomanipulation and addition of oxidisers to an otherwise anoxic hypolimnion. Oxygenation (addition of pure oxygen) has been used worldwide (for recent reviews, see [Cooke et al., 2005](#); [Liboriussen et al., 2009](#)) in summer-stratified lakes. It improves the redox sensitive sorption of phosphate to iron in the sediment and thereby reduces the internal P loading. Alternatively, electron acceptors such as nitrate may be used as alternative oxidisers. When using nitrate, a liquid solution of nitrate is added by stirring it into the upper sediment layer or by injecting it into the water just above the sediment ([Ripl, 1976](#); [Søndergaard et al., 2000](#)). The advantage of using nitrate is that it will penetrate the sediment to a greater depth than oxygen, although the oxygen concentration in the hypolimnion will be less affected.

5.2.1 Dual treatment: oxygenation and fish removal in Lake Fure, Denmark

Lake Fure is a deep lake ([Table 2](#)) with a hydraulic retention time of 10.6 years. The annual external P loading to the lake peaked in 1969 (ca. 37 tonnes in 1969) followed by a major decline due to improved sewage treatment and sewage diversion. Since an intensive monitoring programme was established in 1989, both the total annual P and N loading as well as annual/summer mean TP, TN and Chl *a* have decreased in the lake ([Appendix, Fig. 11](#)). To reinforce recovery, a combined biomanipulation–oxygenation restoration initiative was initiated in 2003. Biomanipulation was conducted from March 2003 to June 2006. In total, 213 tonnes of coarse fish (226 kg ha^{-1}) were removed from the lake and adjacent waters using different types of pound nets and dragnets. Of the total catch, 173 tonnes were target species, particularly roach and small bream. There was a clear change in the composition of the fish community towards a much higher percentage in both abundance and biomass of piscivorous fish, primarily perch ([Frederiksborg County, 2006, Table 4](#)). Oxygenation of the hypolimnion (method described in [Appendix](#)) has been conducted during stratification from June to November since 2003 ([Rambøll, 2012](#)).

The combined restoration led to major changes in key physico-chemical variables. The hypolimnion became oxic during the entire stratification period, and concentrations of ammonia, *ortho*-phosphate and, with those,

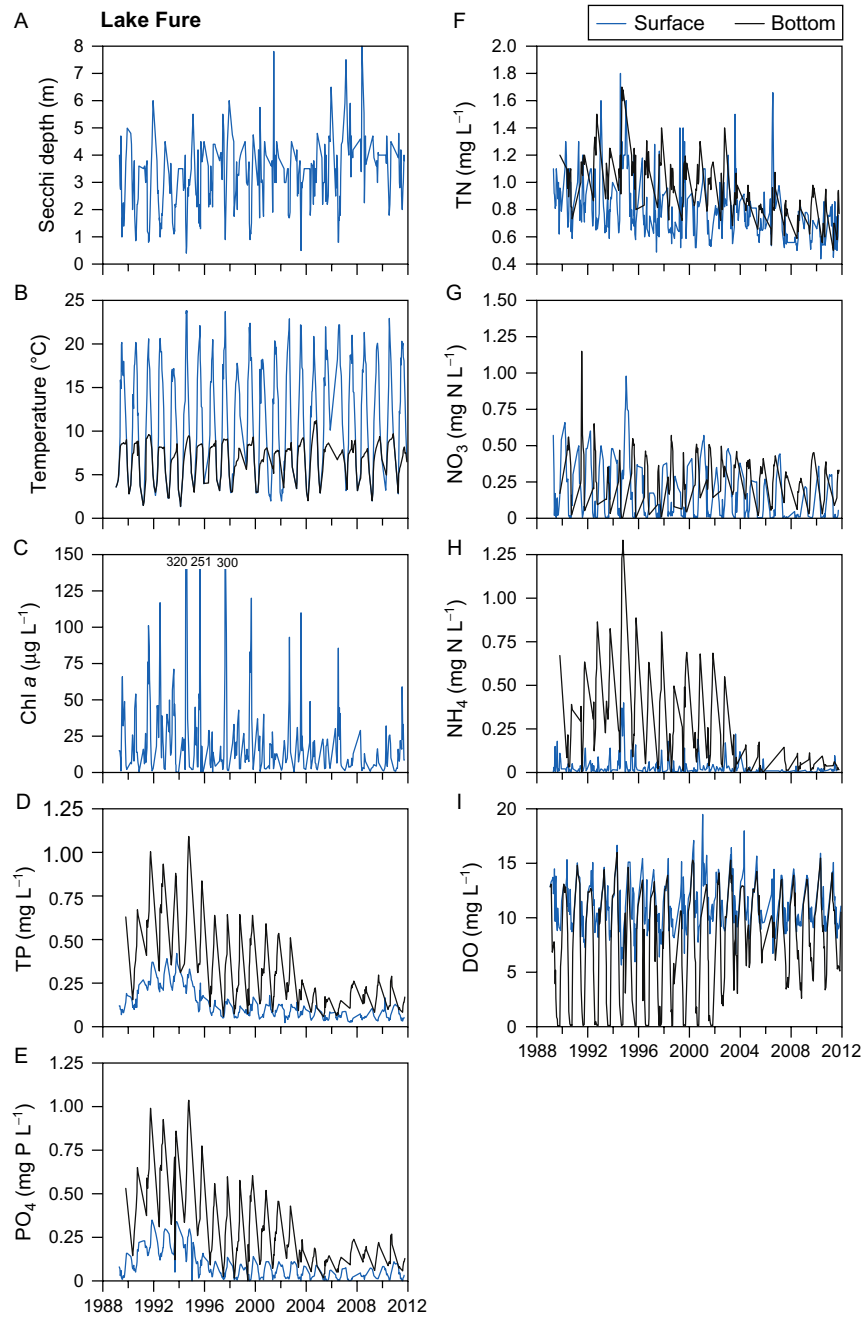


Figure 11 Time series of Secchi depth (A), chlorophyll *a* (C), total phosphorus (D), *ortho*-phosphate (E), total nitrogen (F), nitrate (G) and ammonia (H) in the surface water and hypolimnion (data include the stratification period only) in Lake Fure from 1989 to 2011. Also shown are (I) and temperature in the surface water (B) and near the sediment at a mid-lake deep station. Oxygenation started in 1993 and fish removal occurred between 2003 and 2006.

Table 4 Change in fish abundance in Lake Fure before, during and after dual restoration

	1991	1996	1999	2002	2003	2006
CPUE (no.)	155 (15%)	62 (39%)	87 (38%)	77 (51%)	96 (44%)	86 (38%)
CPUE (g)	7086 (10%)	3669 (35%)	5031 (30%)	4293 (49%)	5296 (62%)	7372 (26%)
Mean body mass (g)	45.7	59.1	57.9	55.7	54.1	85.7

Shown are total Catch Per Unit Effort of fish in gillnets in terms of numbers (no) and weight (g WW), percentage contribution of perch (in parenthesis) and average body mass (g WW) of fish in surveys in late summer in the main basin of the lake during 1991–2006.

also TN and TP decreased markedly (Fig. 11; Liboriussen et al., 2009) and immediately, while Chl *a* decreased, though with periodic peaks in concentrations after 2006, and vice versa for Secchi depth.

Unfortunately, only few fish surveys are available in the years following the fish manipulation, making it difficult to judge the effect of this measure. No clear effect on CPUE was found, but apparently the individual biomass of fish increased, being much higher in 2006 than in any of the surveys prior to 2003 (Table 4), but the total CPUE in terms of weight was also high. A fish survey in 2006 revealed several species, including large piscivores in the hypolimnion below 15 m, after oxidation.

Zooplankton individual biomass and the proportion of *Daphnia* among cladocerans are valuable indicators of fish predation pressure in lakes as fish select for large species (Brooks and Dodson, 1965; Jeppesen et al., 2011). Summer means of these ratios followed largely the changes in fish CPUE, being low in the early 1990s when the smelt population and total CPUE of fish by weight were high, peaked in 2003–2004 when the lake was bio-manipulated, but decreased again in the following years. The changes in zooplankton were overall mirrored in the phytoplankton Chl *a*, which was lower in years where large-bodied zooplankton were dominant (Fig. 12), emphasising the role of top-down control in the system. The immediate response to oxygenation of nutrient concentrations and the more slow response of zooplankton size and Chl *a* following three years of fish manipulation suggest a combined effect of the two restoration measures as hypothesised. However, like for Lake Kollelev, the study does not allow firm disentangling of the effects of the treatments or evaluation of potential synergistic effects.

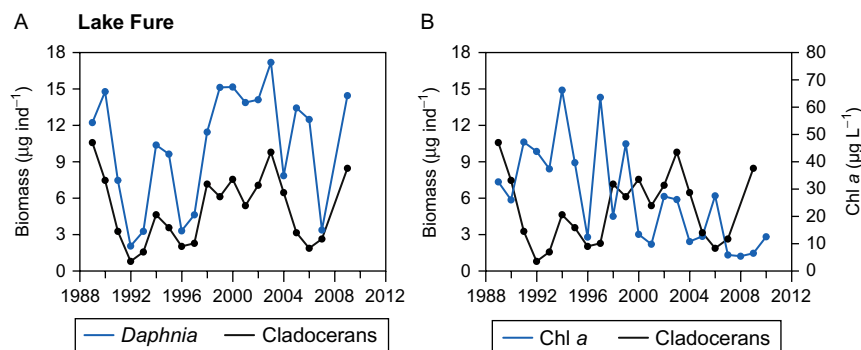


Figure 12 Changes in summer mean (May 1–Oct 1) individual biomass of cladocerans and of *Daphnia* (A) and summer mean chlorophyll *a* and individual biomass of cladocerans (B) in Lake Fure from 1989 to 2011.

6. EFFECT OF BIOMANIPULATION ON BIODIVERSITY

Shifts in trophic structure may trigger complex changes in biodiversity. In theory, a more balanced fish community after fish manipulation and less top-down control by planktivorous fish should cascade to higher diversity at the lower trophic level (zooplankton and phytoplankton) unless key-stone species (such as large *Daphnia*) completely dominate the zooplankton and benthic invertebrates. Moreover, provided that submerged macrophytes re-colonise and become more abundant after biomanipulation, habitat complexity in the lake littoral will increase, which would lead to more diverse microhabitats and an increase in biodiversity. A comprehensive study of 96 European lakes covering organisms from fish to bacterioplankton showed that taxon richness was best associated with abundance of submerged macrophytes (Declerck et al., 2005).

Here, we present analyses of changes in richness of phytoplankton and zooplankton due to biomanipulation by analysing data from before and after fish manipulation in Lake Engelsholm and zebra mussel introduction in Lake Faarup (Denmark). Main results are given below, while a more thorough analysis including statistics can be found in the [Appendix](#). We expected that fish removal would lead to enhanced biodiversity of the plankton following a shift to a more diverse fish assemblage and reduction in nutrients and that mass development of zebra mussels would, in contrast, lead to a reduction due to selective removal of some taxa of both zooplankton and phytoplankton despite an expected reduction in the nutrient level.

6.1. Effects of fish manipulation on biodiversity: Lake Engelsholm, Denmark

Shallow Lake Engelsholm (Table 2) was biomanipulated in 1992–1993 to reinforce its recovery after nutrient loading reduction (Søndergaard et al., 2007). Nineteen tonnes of cyprinids were removed and the estimated biomass of cyprinids subsequently decreased from 675 to 150–300 kg ha⁻¹ (Møller, 1998). Biomanipulation led to a substantial reduction of Chl *a*, TP and TN as well as an increase in Secchi depth (Fig. 13). Change point analysis (Hinkley, 1970) identified a shift ($p < 0.01$) in phytoplankton biomass trends in July 1993, immediately after the fish biomanipulation (Fig. 14). There was a 10-fold decrease in both median and minimum phytoplankton biomass (10th percentile) following the shift (Table 5). Time series analysis identified no trend in phytoplankton biomass before biomanipulation and a negative trend afterwards (Fig. 14). Mean within-year variation in phytoplankton biomass almost doubled after the shift. Both median phytoplankton richness and evenness increased after the shift (Table 5), whereas within-year variation increased for richness, but decreased for evenness, indicating a year-round higher evenness of phytoplankton. Following biomanipulation, phytoplankton composition shifted from year-round dominance of cyanobacteria to, first, stronger seasonal succession among chlorophytes, cyanobacteria and diatoms, followed by higher dominance of the remaining groups (Fig. 14).

There were also marked changes in zooplankton in the lake. Median zooplankton biomass as well as its mean within-year variation decreased after biomanipulation (Table 5). There was no trend in the zooplankton biomass before and a negative trend after biomanipulation. Zooplankton richness and its within-year variation increased slightly with a positive trend after the shift. The fraction of rotifers in zooplankton biomass increased, while cladocerans decreased following biomanipulation (Fig. 14).

6.2. Effects of mussel invasion on biodiversity: Lake Faarup, Denmark

Lake Faarup is a stratified lake (Table 2) with a water residence time around 0.5–0.7 year. The majority of the nutrient loading of both N and P is diffuse (large part via groundwater input) from the cultivated catchment. Zebra mussels were observed for the first time in 1993 and veliger larvae in the plankton from 1998 and onwards (Andersen, 2009). The density in 2000 was recorded 1300 m⁻². Since 1995, a major decrease has occurred

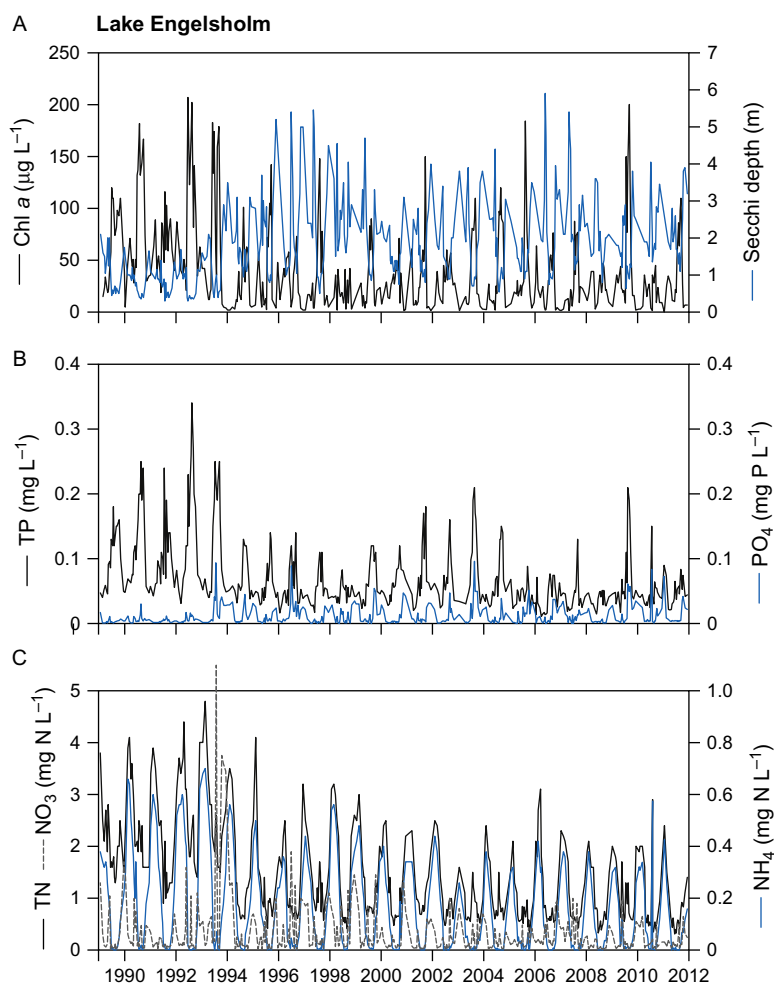
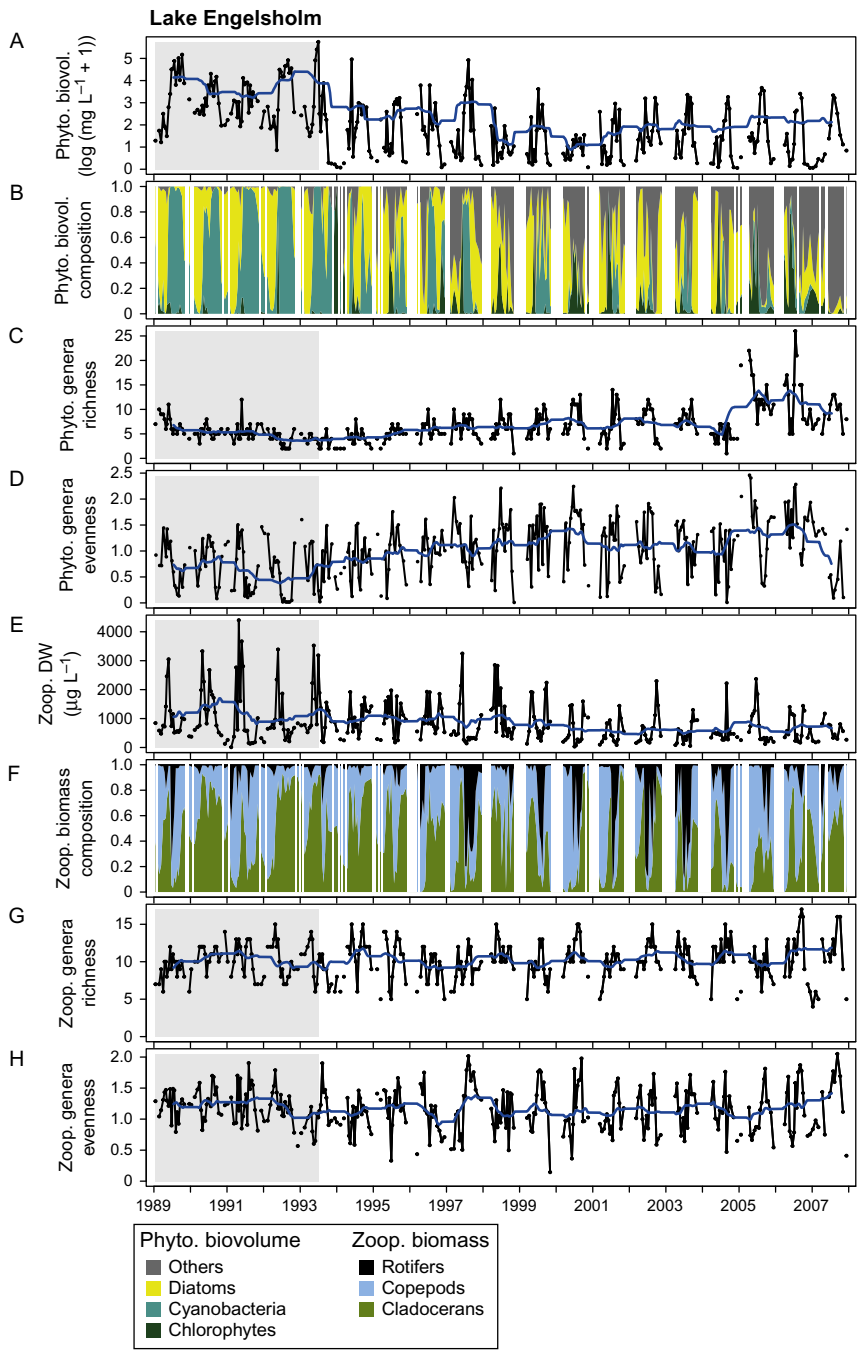


Figure 13 Time series of chlorophyll *a* and Secchi depth (A), total phosphorus and *ortho*-phosphate (B), total nitrogen, nitrate and ammonia (C) in Lake Engelsholm, Denmark, from 1989 to 2011. Fish removal was conducted in 1992–1993.

in summer mean Chl *a*, TP and TN. Accordingly, Secchi depth has increased (Fig. 15). As the external loading of TN and TP has not changed during the study period, the drastic changes can most likely be attributed to the colonisation and a gradual increase in zebra mussel densities.

Compared to fish-manipulated Lake Engelsholm, the changes in the Lake Faarup planktonic communities were more gradual (Fig. 16). Although zebra mussel was first observed in 1993, the change point analysis identified a shift



($p < 0.01$) in phytoplankton biomass trends in August 1997 (Fig. 15), coinciding with the observation of high densities of zebra mussel larvae in plankton. Median phytoplankton biomass decreased sixfold, and both phytoplankton genera richness and evenness decreased as well by 40% (Table 5). Both phytoplankton biomass and diversity measures showed a decreasing trend after the shift. Also mean within-year variation in phytoplankton biomass and diversity measures increased considerably after the shift. The change in phytoplankton community composition between these periods was mostly reflected by a decrease in cyanobacteria dominance and a gradual increase in heterogeneity accompanied by an increase in abundance of diatoms and the remaining groups, with little change in chlorophytes in the latter period.

Median zooplankton biomass in Lake Faarup decreased by 50% after 1997, while its mean within-year variation increased (Fig. 16). Both zooplankton genera richness and evenness were higher before 1997 and within-year variation in both zooplankton genera richness and evenness increased after 1997. Considering community composition, cladoceran dominance gradually decreased, whereas the fraction of rotifers of the zooplankton biomass rose after the shift (Fig. 16).

Judged from the results obtained from Lake Engelsholm and Lake Faarup, changes in top-down control via fish removal enhanced the diversity of phytoplankton and zooplankton (but also led to a reduction in cladoceran biomass, contrary to expectations), while mass development of zebra mussel reduced the diversity of both planktonic communities as predicted and enhanced within year variation. The latter is supported by other studies (Caraco et al., 1997; Pace et al., 1998; Ten Winkel and Davids, 1982), showing a differential strong impact of zebra mussels on specific phytoplankton and zooplankton taxa and size classes. However, more studies are clearly needed to draw any firm conclusions about the generality of our findings concerning the effect of biomanipulation on biodiversity.

Figure 14 Time series for phytoplankton biomass (log-transformed, A), phytoplankton biomass composition across four main groups (chlorophytes, cyanobacteria, diatoms and others, B), phytoplankton genera richness (C) and evenness (D), as well as zooplankton biomass (E), zooplankton biomass composition across three main groups (cladocerans, copepods and rotifers, F) zooplankton genera richness (G) and evenness (H) in Lake Engelsholm, Denmark. Grey and white backgrounds indicate the shift in the phytoplankton biovolume trend identified by change point analysis. Blue lines in scatter plots denote yearly running means excluding winter samples. Lines in scatter plots and polygons in composition plots were left unconnected if two samples were more than 30 days apart.

Table 5 Effects on biodiversity in Lake Engelsholm and Lake Faarup, Denmark

	Phytoplankton before	Phytoplankton after	Zooplankton before	Zooplankton after	Lake
Biomass	6.27–31.89– 116.15	0.65–3.40– 21.45	393.99–942.08– 2590.40	237.27–624.93– 1481.48	E
CV	0.65	1.08	0.7	0.62	E
Richness	3.31–5.02– 7.30	3.58–6.57– 11.68	7.38–10.29– 12.58	7.79–10.36– 12.89	E
CV	0.25	0.3	0.16	0.18	E
Evenness	0.12–0.61– 1.17	0.46–1.10– 1.67	0.97–1.22– 1.53	0.79–1.15– 1.62	E
CV	0.67	0.38	0.17	0.25	E
Biomass	1.99–7.83– 22.82	0.21–1.36– 4.90	227.19–510.42– 1111.35	64.90–231.19– 834.09	F
CV	0.79	1.02	0.69	0.9	F
Richness	5.11–7.31– 10.00	3.00–4.86– 8.52	7.29–10.58– 12.85	5.00–7.63– 10.36	F
CV	0.19	0.33	0.19	0.26	F
Evenness	0.73–1.30– 1.58	0.53–0.98– 1.42	0.89–1.23– 1.71	0.75–1.08– 1.60	F
CV	0.25	0.31	0.25	0.3	F

Phytoplankton and zooplankton biomass, richness and evenness before and after the shift date in phytoplankton biovolume trends identified by change point analysis in Lake Engelsholm (E) and Lake Faarup (F). 10, 50 and 90 percentiles in both periods were reported for each parameter. CV is mean yearly coefficient of variation within a period.



7. EFFECT OF BIOMANIPULATION ON LAKE METABOLISM

The effect of biomanipulation on ecosystem metabolism is debatable and not well studied. It is expected that phytoplankton production is reduced due to the enhanced grazer control of phytoplankton. However, this may be compensated by an increase in production by benthic algae, submerged macrophytes and epiphyton due to an increase in water clarity. Studies of lakes with contrasting nutrient levels have shown only slightly higher system production in nutrient-rich phytoplankton-dominated systems than in oligo-mesotrophic lakes dominated by benthic production

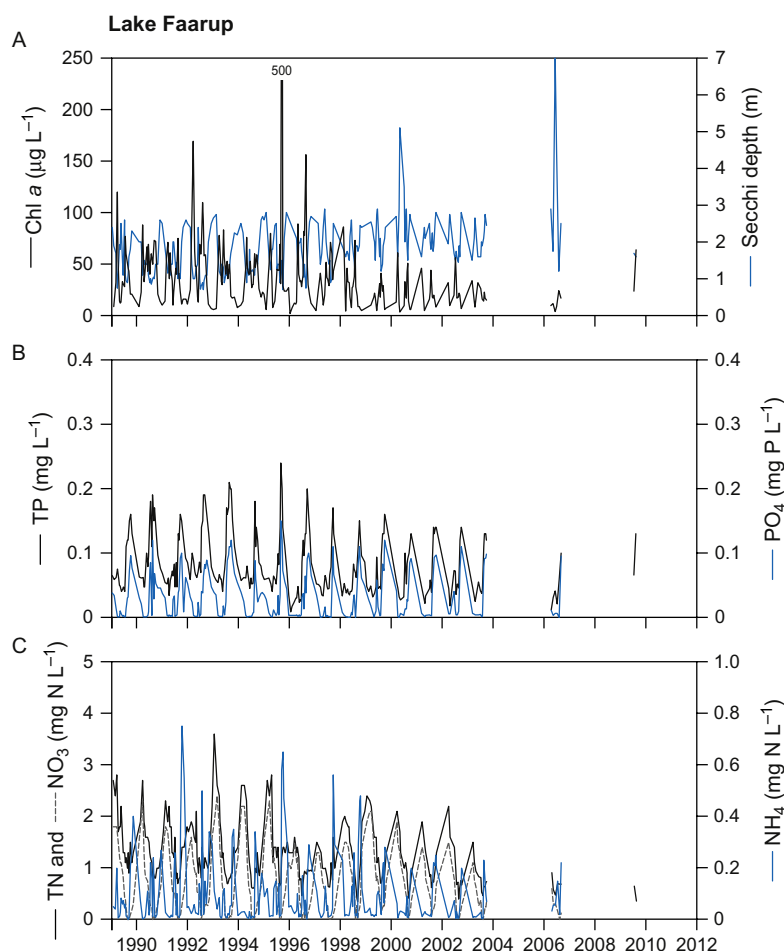
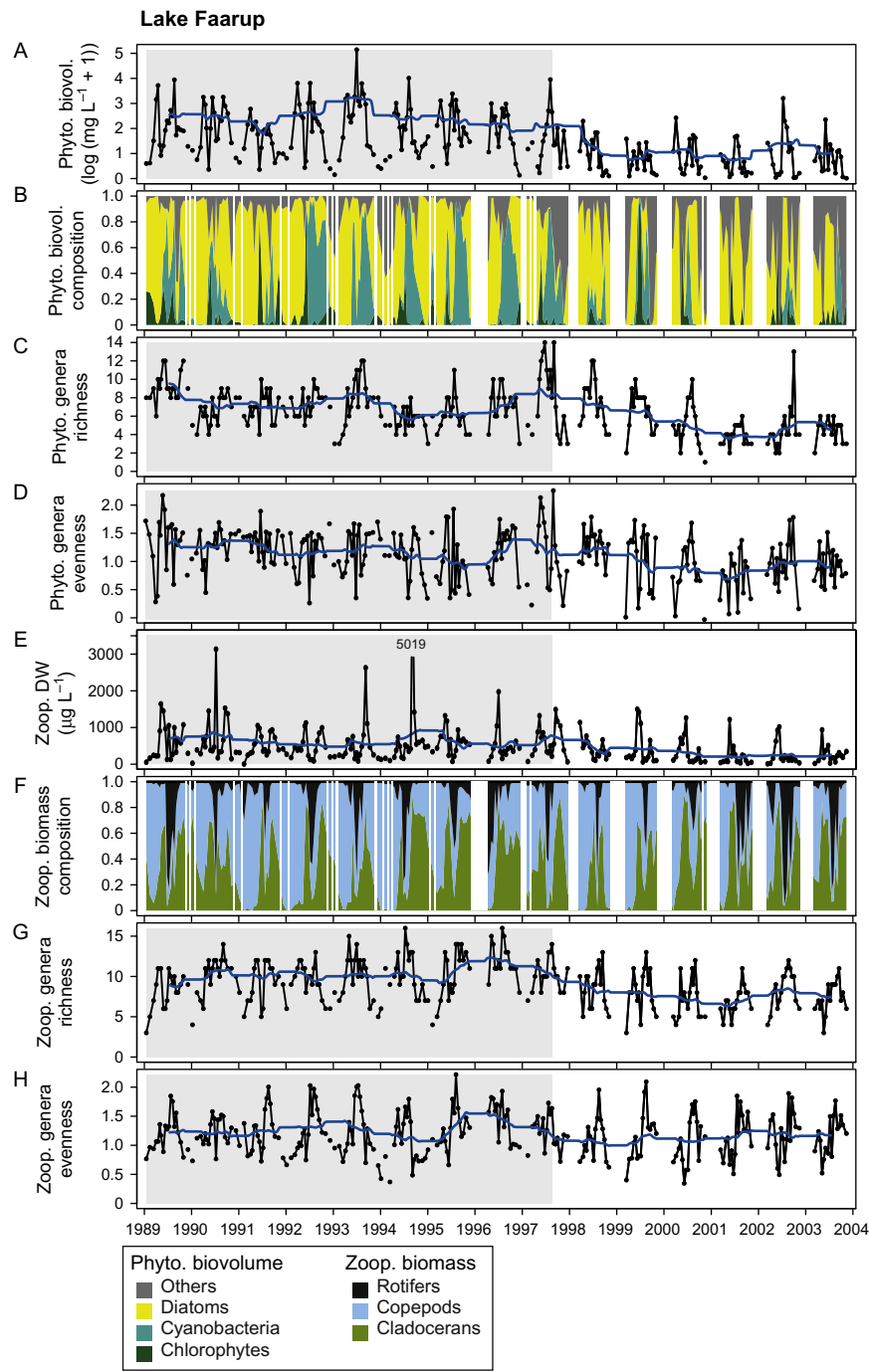


Figure 15 Time series of chlorophyll *a* and Secchi depth (A), total phosphorus and ortho-phosphate (B), total nitrogen, nitrate and ammonia (C) in Lake Faarup from 1989 to 2010. Zebra mussels were first observed in 1993, reaching high densities in the early 2000s.

(Vadeboncoeur et al., 2003; Liboriussen and Jeppesen, 2003) or macrophytes and associated epiphyton (Vadeboncoeur *et al.*, 2003). A modelling study by Genkai-Kato et al. (2012) indicates that the phosphorus loading threshold for a shift in dominance from benthic to pelagic production decreases with increasing mean depth, and also that the system production decreases with increasing mean depth (mean depth range 1.6–3.6 m), particularly at high nutrient loading where phytoplankton dominates. So, the effect of fish manipulation on ecosystem metabolisms and thus oxygen



state of the lake will depend on the degree of compensatory benthic production following a decline in Chl *a*, being highest in shallow lakes. In shallow lakes with high biomass of benthic fish, fish manipulation may further enhance production as light limitation, triggered by fish-induced high concentration of suspended matter, is diminished.

Here, we provide three examples of the effects of biomanipulation on the metabolism in lakes, from fish-manipulated Lake Væng and Lake Engelsholm, and from Lake Faarup, subjected to invasion by zebra mussels. The oxygen metabolism in Lake Væng has been followed 4 years during and after the second biomanipulation event (see [Section 2.1.4](#)) using high-frequency (every 15 min) sampling of oxygen, temperature and Chl *a* (methods in [Appendix](#)). As predicted, the major decline in Chl *a* had comparatively minor effects on gross production and respiration ([Fig. 17A](#)). Net production (March 1–Nov 15) ranged between 0.49 and 0.52 mg O₂ L⁻¹ d⁻¹ in 2007 and 2008 became negative in 2009 (−0.65 mg O₂ L⁻¹ d⁻¹) and increased to 0.23 mg O₂ L⁻¹ d⁻¹ in 2010, coinciding with extensive growth of *Elodea* ([Fig. 17 B](#)). A major decline in net production was found in autumn 2010 following a drastic decline in the macrophytes, so the amplitude in net production tended to vary more over the seasons after restoration ([Figs. 3 and 17B](#)).

As in Lake Væng, drastic reductions in Chl *a* in Lake Engelsholm and Lake Faarup have not resulted in changes (regressed against year, $p > 0.5$, [Fig. 18](#)) in oxygen saturation (averages for Mar 1–Dec 1), and the saturation ranged between 115% and 128% in Lake Engelsholm and between 112% and 161% in Lake Faarup after the respective shifts in trophic structure. Given the strong reduction in planktonic biomass, the high saturation values suggest a shift from pelagic to benthic production, as also evidenced by modelling of primary production in Lake Engelsholm ([Genkai-Kato et al., 2012](#)). Potentially, the oxygen supersaturation may also reflect production of submerged macrophytes together with their attached periphyton. Plant colonisation of Lake Engelsholm gradually occurred, but plant coverage remained low (maximum in 2010: 12.5% coverage, but only 2.5% of the lake volume inhabited by plants (PVI)). Likewise, plant coverage in Lake Faarup was below 7.4% and PVI < 0.5%. Macrophytes can therefore only marginally have contributed to

Figure 16 Time series for phytoplankton biomass (log-transformed, A), phytoplankton biomass composition across four main groups (chlorophytes, cyanobacteria, diatoms and others, B), phytoplankton genera richness (C) and evenness (D), as well as zooplankton biomass (E), zooplankton biomass composition across three main groups (cladocerans, copepods and rotifers, F), zooplankton genera richness (G) and evenness (H) in Lake Faarup, Denmark (see legend of [Fig. 14](#) for further details).

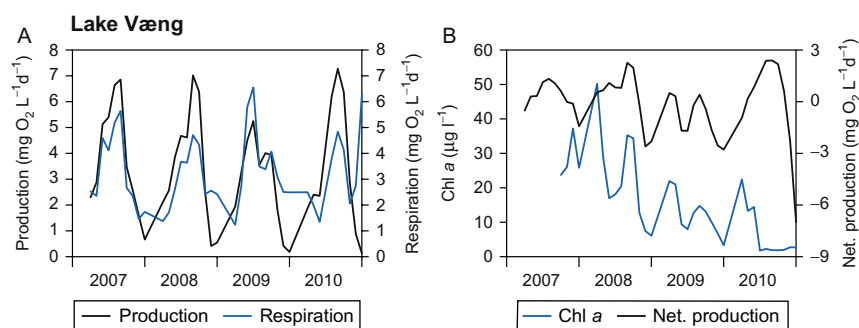


Figure 17 Seasonal variation in (A) system gross production (GPP) and respiration (R) and (B) net production and chlorophyll *a* in Lake Væng, Denmark, during March 1–November 15 before (2007–2008) and after (2009–2010) the second biomanipulation event. Based on high-frequency measurements of oxygen and temperature (four times per hour, monthly means shown).

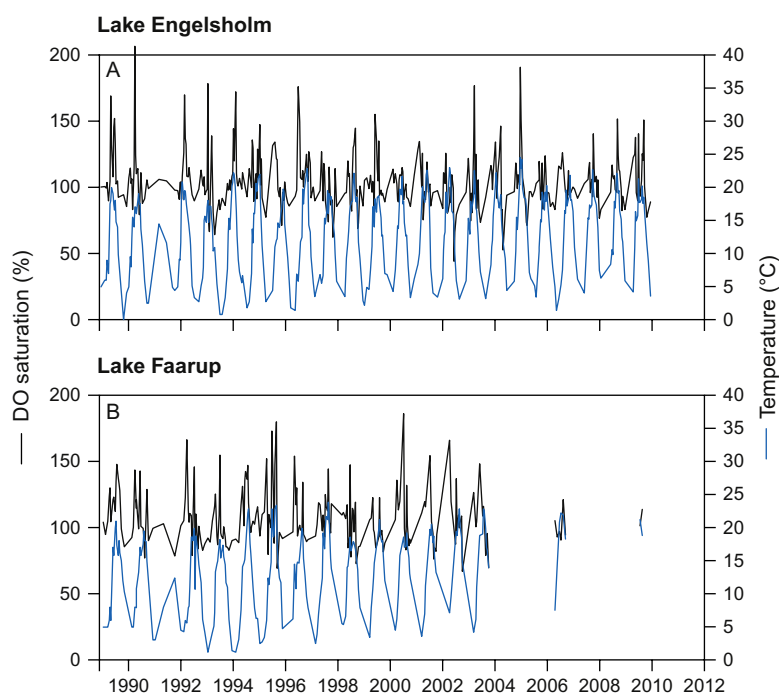


Figure 18 Time series for oxygen and temperature in biomanipulated Lake Engelsholm and Lake Faarup, colonised by zebra mussels.

the lake metabolism in these two lakes, and the high summer production may therefore be attributed to an increase in production by benthic algae.

Judged from these three examples, all from relatively shallow lakes, oxygen metabolism is overall marginally or only shortly affected by biomanipulation of fish or mass development of zebra mussels, though seasonality might change (higher net production in summer and lower in winter) if macrophytes become abundant. A different scenario is likely to prevail in deep lakes where loss of pelagic production may not be fully compensated by an increase in benthic/macrophyte production leading to lower net production, at least in the short term, where respiration of previously settled organic matter (from the time when production was high) may be important.



8. FUTURE CHALLENGES FOR BIOLOGICAL LAKE RESTORATION

We are currently facing an increasing pressure on aquatic systems, which is expected to worsen considerably in the future. One of the challenges is the global development resulting in a substantially higher human population. This will entail a much higher agricultural production, a stronger need for fertilisation and, consequently, potentially higher eutrophication of waterbodies (Howden et al., 2007). In addition, climate warming will overall lead to higher eutrophication or worsening of eutrophication symptoms of lakes (Jeppesen et al., 2009, 2010; Moss et al., 2011, but see Nöges and Nöges, 1999) due to higher external and/or internal loading of nutrients and a shift in trophic structure and dynamics in lakes (Jeppesen et al., 2009, 2010, 2012; Meerhoff et al., 2007a,b, 2012).

It is also to be expected that warming and enhanced precipitation in Central and Northern Europe, as well as in other parts of the globe, induced by climate change, will enhance the allochthonous carbon inflow to lakes and reduce carbon burial, leading to higher in-lake dissolved organic concentrations and often a higher humic content (Sobek et al., 2007; Tranvik et al., 2009), which may affect the trophic dynamics in lakes (Karlsson et al., 2009). Moreover, higher winter survival of young fish due to reduced ice cover (Jackson et al., 2007) may lead to enhanced fish predation pressure on the zooplankton (Balayla et al., 2010; Ruuhijärvi et al., 2010) and, consequently, to lower grazing on phytoplankton. Accordingly, it will be more difficult to maintain or obtain a high ecological quality of lakes to fulfil the criteria of minimum good ecological status stipulated by, for example, the European Water Framework Directive, without substantial control of the nutrient input to the lakes. This enhances the demand for additional within-lake restoration measures to improve the ecological state of lakes.

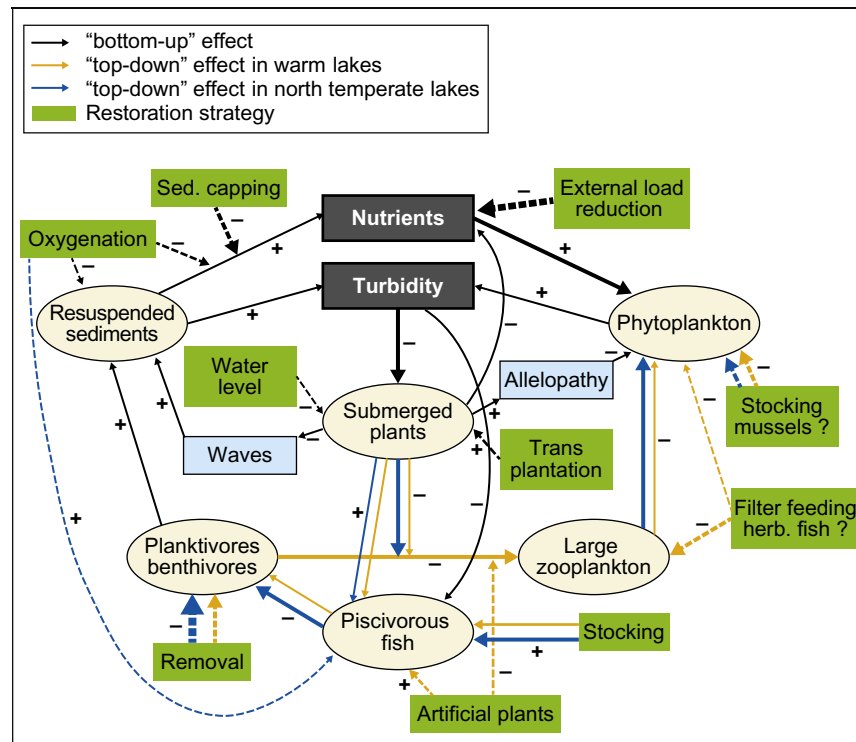


Diagram 2 Main feedback loops responsible for the existence of different states in lakes (turbid or clear water) and the potential effects of different restoration measures under different climates. The relative strength of the connections (including the expected success of restoration measures) is indicated by the width of the arrows. Different responses, evidenced by empirical investigations, due to climatic differences are highlighted by arrows with colour codes for warm (red or light grey) and cold (blue or dark grey) lakes. The potential climate effects on other in-lake processes remain a topic for further research. The qualitative effect of each route in the diagram can be determined by multiplying the signs along the connections. Based on the schemes for alternative states of shallow lakes proposed by [Scheffer et al. \(1993\)](#) and modified by [Meerhoff and Jeppesen \(2009\)](#).

9. DISCUSSION OF METHODS AND RECOMMENDATIONS

Here, we discuss further the biological and dual restoration for use in temperate and warm lakes and provide recommendations. A conceptual scheme on the main in-lake processes and potential success of the different restoration methods for lakes is given in [Diagram 2](#) and will form the basis for the discussion.

9.1. Temperate lakes

While there are examples of positive effects of removing a large proportion of fish, the lakes often return partly or fully to the state before manipulation after 10–15 years. This lack of long-term success may well have contributed to the reduction in the number of biomanipulation projects initiated during the recent decade. In Denmark, for example, biomanipulation activities peaked in 1995–1996 (Søndergaard et al., 2007). With the future challenges of climate effects exacerbating eutrophication, time has therefore come to re-think the theory and methodology. Repeated measures combined with detection of early warning on relapse offer one way forward. The earlier the adjustments are made, the less effort is required. Such measures may be combined with protection of crucial size classes of piscivores from angling or commercial fishing, and stocking of native piscivores.

A promising alternative or supplement is the dual treatment combining biomanipulation with sediment capping or oxygenation of the bottom water (the latter in stratified lakes only). While oxygenation is costly and needs to be continued for a very long time period (Liboriussen et al., 2009), sediment capping is comparatively cheap in the long term. However, also here adjustment may be needed as relapses have been observed after 5–10 years in most of the restoration case studies. We argue that combined and repeated (based on early warning signals) sediment capping and biomanipulation treatment likely are a way forward. With dual treatment, it is, however, impossible to elucidate the specific role of the different treatments or determine whether synergistic effects occur. Controlled experiments are therefore needed, where single and dual treatments are run at the same time, and with untreated controls. Such experiments are easily done for sediment capping and biomanipulation, while it is more difficult, though not impossible at large scales, to undertake combined hypolimnion oxygenation–biomanipulation experiments. Experiments of this kind cannot stand alone, however, as scale also matters (Schindler, 1998). Therefore, a greater range of full-scale dual treatment experiments are required before any firm conclusions can be drawn, and the optimal sets of methods adapted to lake type and nutrient level can be assessed. Ideally, such experiments should be based on combined single and dual treatment in the same lake.

There are still many unresolved issues regarding the use of macrophyte establishment and protection as a lake restoration method, and research gaps to address. However, based on previous experiences, it is shown that macrophytes may re-establish if a macrophyte source is available (via seed banks

or connectivity to other waterbodies), and the plants are tolerant to eutrophic conditions, since such plants are typically fast growing and able to overgrow a potential grazing pressure from herbivores. If a potential macrophyte source is unavailable, however, natural re-establishment may be prevented. In such cases, introduction of macrophytes by simple transplantations may be a solution, although large-scale or full-scale experiments are still lacking. In most cases, where transplantations have been successful, macrophytes have been protected from herbivores. Artificial plants or other structured items are an alternative to be considered if plant establishment is delayed, at least in small lakes, in order to maintain a clear water state after, for instance, fish removal until the plants appear. However, more research is needed within this field to find the optimal cost effective solution.

9.2. Warm lakes

So far, few studies have investigated the applicability of biomaniipulation theory, based on the trophic cascade (i.e. with the ultimate aim to enhance zooplankton grazing on phytoplankton) to tropical and subtropical freshwater lakes (e.g. [Scasso et al., 2001](#)). Due to the characteristics of the typical fish assemblage in warm lakes described above, the “classical biomaniipulation” model (i.e. introduction of piscivores and/or removal of planktivores) has serious limitations. It is therefore likely that a removal-induced reduction of the biomass of omnivorous or planktivorous fishes will be quickly compensated by the adjustment of the remaining population, and the impact of fish manipulation will therefore be of only very brief duration ([Jeppesen et al., 2005, 2009](#)), as found in subtropical and tropical lakes after massive fish kills ([Iglesias et al., 2011](#); [Nagdali and Gupta, 2002](#)). However, removal of fish can also promote a reduction in nutrient loading in warm lakes. In subtropical Lake Apopka (Florida, USA), it was estimated that, in the short term, most of the P demand of phytoplankton is met through recycling of P, which greatly exceeds external P loading. Depending on population biomass, phosphorus excretion by a resident fish population was similar in magnitude to the P release by diffusive flux from the sediments ([Schaus et al., 2010](#)), an indication that a reduction in the fish stock might enhance nutrient control of phytoplankton.

Widespread omnivory potentially allows the fish stock in warm locations to attain a higher carrying capacity than obligate zooplanktivores or strictly pelagic fishes, which augments the potential control of the large zooplankton. Many of the fish species show partial niche overlap, which expectedly

increases predator control of prey items (Aguilaro and Caramaschi, 1998; Lazzaro, 1997). Other factors could also affect the success of biomanipulation in warm lakes but are poorly elucidated. There is scarce knowledge of the trophic role of many fish species, given the much larger number of species typically present in warm areas (González-Bergonzoni et al., 2012). This implies a potentially large number of fish species to be controlled, but also a window of opportunity to test several species or alternative trophic groups in a biomanipulation programme. For instance, periphyton-feeding fishes could potentially facilitate the establishment of submerged macrophytes via removal of their epiphyton competitors; however, we have found no experimental test of this.

Most of the existing experimental studies in warm areas have examined food web interactions in mesocosms in eutrophic lakes and reservoirs, with the aim to control cyanobacterial blooms via enhanced grazing by omnivorous filter-feeding fish (Arcifa et al., 1986; Jones and Poplawski, 1998; Northcote et al., 1990; Starling, 1993). Caution must be taken in the choice of the manipulative species, however, due to the side effects associated with the release of competition within the phytoplankton community (for the preferential consumption of certain phytoplankton taxa) and the increased rate of nutrient recirculation, such as that associated with the use of tilapia (Menezes et al., 2010). For instance, as a result of the consumption of both phytoplankton and zooplankton, silver carp (ideally non reproductive) could be used for biomanipulation only when the primary aim is to reduce nuisance blooms of cyanobacteria that cannot be effectively controlled by large herbivorous zooplankton, such as in tropical nutrient-enriched lakes (Radke and Kahl, 2002).

For most fish species, management practices are still under discussion in the scientific literature. Incorrect timing for the harvest of silver carp from the system could result in the release of grazing on phytoplankton and the boosting of blooms (Xiao et al., 2010). Similarly, overstocking of silver and bighead carps could enhance the predation pressure on zooplankton rather than on phytoplankton (Ke et al., 2008). Besides, the few existing whole-lake biomanipulation examples in the subtropics (e.g. Scasso et al., 2001) have often removed fish biomass at levels lower than those typically successful in temperate lakes, thus limiting the extent of their conclusions regarding the effort needed and long-term effects.

The absence of a native piscivorous fish culture in many tropical countries precludes the application of biomanipulation. Generally, aquaculture has so far focused on a few species (i.e. tilapia, common carp and grass carp),

mostly for protein production that typically has negative effects on water quality and biodiversity. It is remarkable that in many countries, state institutions are responsible for the introduction and dispersal of fish species with—in many cases—known undesirable effects on water quality and biodiversity (Agostinho et al., 2004). A change in the management paradigm seems crucial for successful restoration and biomanipulation of warm lakes.

Other biomanipulation alternatives, such as those described here, have to our knowledge not yet been seriously applied in warm lakes, perhaps in part due to the high cost (e.g. of some of the chemical restoration methods). The typical scarcity of large zooplankton as phytoplankton grazers highlights the relevance of focusing on other grazers. As in temperate zones, in warmer areas, there are several native and exotic filter-feeding bivalves that could control phytoplankton biomass. There are several case studies on the effects of the zebra mussel in the temperate zone, while in South America, studies on the effects of the food web of the invasive *Corbicula fluminea* and *Limnoperna fortunei*, and a few on native species, such as *Diplodon* spp, are still scarce.

The management of aquatic vegetation represents another potential alternative to restoration in warm areas, given the large diversity of species and of functional groups and their potential effects on water clarity. Despite the fact that submerged plants do not seem to offer similar refuge for large zooplankton in warm lakes as in temperate lakes due to high fish aggregation among the plants (Iglesias et al., 2007; Meerhoff et al., 2003, 2006, 2007b), submerged plants may improve water clarity by competing for nutrients with phytoplankton (Kosten et al., 2009) and by releasing allelopathic substances (Vanderstukken et al., 2011). We thus urge for more research on these promising topics of high relevance under the scenario of increasing eutrophication, particularly in developing countries with warm climates.

The few study cases available raise some interesting research topics and questions for the future, that is, (a) shifting a warm eutrophic lake towards an alternative oligotrophic state seems possible if the manipulation pressure is strong and encompasses both external nutrient loading and internal recycling; but (b) the recuperation process of warm lakes may be faster than in the temperate zones due to lower accumulation of organic matter and nutrients (higher metabolism); (c) the resilience associated with the internal nutrient load might therefore be lower in warm systems, whereas the resilience associated with fish might be higher than in the temperate zone and (d) the success of flushing indicates that repeated measures might be needed in order to maintain the effectiveness of the biomanipulation (as also highlighted for temperate lakes in the previous sections). The effort required,

the frequency of application, the success of dual treatments, and the duration of effects of any methods remain open topics of high interest for research.

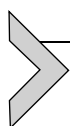


10. CONCLUSIONS

While short-term effects of biomanipulation have often been successful, provided that they have been sufficiently strong, the long-term perspectives have for various reasons been less positive. Repeated measures may maintain the initial positive effect of biomanipulation, but more research is needed to optimise the frequency and extent of such events. A promising alternative is combining biological and physico-chemical techniques to make the restoration more robust and perhaps less expensive due to synergistic effects of the treatments. We encourage controlled experiments and large-scale field studies and modelling to elucidate the effects of such dual methods, as the scientific evidence is so far poor. More research is particularly needed to determine the potential of biomanipulation or dual methods in warm lakes. It is important to emphasise, though, that the key measure to restore eutrophied lakes is the reduction in external nutrient loading. In-lake restoration only serves the purpose of reinforcing recovery, treating symptoms or improving/maintaining a high environmental quality temporarily until the external loading can be significantly reduced.

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APPENDIX. OVERVIEW OF DATA, METHODS AND SPECIFIC ANALYSIS FOR HITHERTO UNPUBLISHED STUDIES

Section 2.1.4: Biweekly to monthly samples during all seasons using standard methods

Sections 4, 5 and 6.1/6.2: Data are derived from the National Monitoring Programme on Lakes in Denmark with typically sampling 19 times per year,

most intensively in summer (Jeppesen et al., 2000; Kronvang et al., 1993; Søndergaard et al., 2003).

Section 5.1.1: Iron and aluminium salts form hydroxides through acid producing hydrolysis steps when dissolved in water. It is the neutral hydroxides that precipitate phosphorus. Most often the salts are spread uniformly in the surface water from a boat. The product is normally concentrated to ~25% in hydrochloric or sulfuric acid.

The shallow Lake Køllev (Table 2) is divided into three basins connected by channels. Basin 2 is upstream and basin 3 is downstream the main basin 1. Until 1942, the lake received untreated or mechanically treated wastewater and has until 1998 received storm water with overflow of waste water. After 1998, the annual average inlet P concentration was $<0.1 \text{ mg P L}^{-1}$, but the lake remained hypertrophic. Therefore, different in-lake measures were applied in the period 1999–2005 to improve water clarity (Fig. 10): (1) In 1998, 24 g m^{-2} iron was added to basins 2 and 3 in order to bind P in the sediment. Although lake water P dropped slightly in basin 2, iron rapidly became reduced in the sediment and high P levels returned. (2) In 1999, cyprinid fish (2500 kg in total) were removed in all three basins and perch (300 kg) were added, but with no improvements in Secchi depth or lower TP (Fig. 10). (3) In spring 2003, basins 1 and 2 were treated with aluminium (54 and 32 g Al m^{-2}) which immediately resulted in lowered lake water TP, an effect that has lasted in the following years. A similar drop in lake water TP was not observed in basin 3, although gradual improvement has been observed until 2011. This is likely a result of the downstream position and a water retention time of ~4 months in basin 3 (compared to retention times of ~2 years in basins 1 and 2). Meanwhile, no improvements in Secchi depth were observed in any of the basins in 2003 or 2004. (4) In 2004–2005, a new biomanipulation (removal of 2200 kg cyprinid fish) was conducted in all three basins. In basins 2 and 3, there was an immediate and strong improvement in water clarity lasting until 2011. In basin 3, a much less pronounced and gradual improvement of the Secchi depth has occurred coinciding with a decrease in TP.

Section 5.2.1: Lake Fure: Oxygen is supplied from an oxygen tank at the shore through distribution pipes to 100-m long perforated diffusers placed in star formation (each consisting of between 10 and 21 diffusers) at the three deepest places in the lake. The oxygen added amounted to 50 and 500 tonnes per year. Linear regression showed that both the total annual P and N loading have decreased significantly with time ($r^2=0.70$, $F_{1,19}=45.72$, $p<0.001$ and $r^2=0.54$, $F_{1,19}=22.10$, $p<0.002$,

log-transformed data) and so have annual mean concentrations of TP ($r^2=0.67$, $F_{1,20}=40.54$, $p<0.001$), TN ($r^2=0.67$, $F_{1,20}=40.58$, $p<0.001$) and Chl *a* ($r^2=0.49$, $F_{1,20}=19.58$, $p<0.001$) as well as summer concentrations ($r^2=0.66$, $F_{1,20}=39.48$, $p<0.001$; $r^2=0.37$, $F_{1,20}=11.52$, $p<0.003$; $r^2=0.50$, $F_{1,20}=20.81$, $p<0.001$, respectively, for TP, TN and Chl *a*).

Section 6: Data are derived from the National Monitoring Programme on Lakes in Denmark (Jeppesen et al., 2000; Kronvang et al., 1993; Søndergaard et al., 2003). Data were linearly interpolated for Julian days, while winter samples (Nov 15–March 15) were excluded from the analyses due to uneven winter sampling intensity across years, while the figures include all data. Taxonomic identifications were aggregated to genus level, whenever possible. We performed time series analysis for monotonic trends based on Kendall rank correlation (Mann, 1945) before and after the shift. Significant Kendall trends were denoted with a star sign if robust for serial autocorrelation after a block bootstrap test. Within-year variations in plankton biomass and diversity measures were calculated as coefficients of variation per year averaged across years within a period. All the analyses were performed by R (R Development Core Team, 2011) using the Kendall package (McLeod, 2011) for monotonic trend analyses in time series; the changepoint package (Killick and Eckley, 2012) for changepoint analyses and the vegan package (Oksanen et al., 2012) for Shannon evenness calculation.

Section 6.1: There was a 10-fold decrease in both median and minimum phytoplankton biomass (10th percentile) following the shift (Table 5). Time series analysis identified no trend ($p=0.411$) in phytoplankton biomass before biomanipulation and a negative trend ($\tau=-0.10$, $p=0.018$; afterwards Fig. 14). Mean within-year variation in phytoplankton biomass almost doubled after the shift. Both median phytoplankton richness and evenness (Shannon diversity, Magurran, 1988) increased after the shift (Table 5), whereas within-year variation increased for richness, but decreased for evenness, indicating a year-round higher evenness of phytoplankton. Both richness and evenness exhibited a negative trend before biomanipulation ($\tau^*=-0.49$, $p<0.001$ for richness and $\tau=-0.22$, $p=0.006$ for evenness), which was replaced by a positive trend ($\tau^*=0.39$, $p<0.001$ for richness and $\tau=0.12$, $p=0.006$ for evenness). Following biomanipulation, phytoplankton composition shifted from year-round dominance of cyanobacteria to, first, stronger seasonal succession among chlorophytes, cyanobacteria and diatoms, followed by higher dominance of the remaining groups (Fig. 14).

There were also marked changes in zooplankton in the lake. Median zooplankton biomass as well as its mean within-year variation decreased after

biomanipulation (Table 5). There was no trend in the zooplankton biomass before ($p=0.563$) and a negative trend after biomanipulation ($\tau^*=-0.17$, $p<0.001$). Zooplankton richness and its within-year variation increased slightly with no trend ($p=0.499$) before and a positive trend ($\tau=0.10$, $p=0.031$) after the shift. Zooplankton evenness decreased slightly, but its within-year variation increased before the shift, while the negative trend ($\tau=-0.20$, $p=0.012$) was replaced by no trend ($p=0.27$) following the shift. The fraction of rotifers in zooplankton biomass increased, while cladocerans decreased following biomanipulation (Fig. 14).

Section 6.2: The density of zebra mussels was recorded in 2000 to 1300 m⁻². Since 1995 a major decrease has occurred in log-transformed values of summer mean Chl *a* ($r^2=0.52$, $F_{1,11}=11.95$, $p<0.005$), mean TP ($r^2=0.32$, $F_{1,11}=5.29$, $p<0.04$), mean TN ($r^2=0.56$, $F_{1,11}=13.75$, $p<0.004$) and annual mean Chl *a* ($r^2=0.77$, $F_{1,9}=31.37$, $p<0.001$), but not in annual mean TP and TN ($p>0.05$). Accordingly, Secchi depth has increased (Fig. 15). As the external loading of TN and TP has not changed during the study period ($p>0.21$ for both TN and TP), the drastic changes can most likely be attributed to the colonisation and a gradual increase in zebra mussel densities

Median phytoplankton biomass decreased 6-fold, and both phytoplankton genera richness and evenness decreased as well by 40% following the shift (Table 5). Before the shift, there were no significant trends in phytoplankton biomass and evenness ($\tau=-11$, $p=0.067$ and $\tau=-10$, $p=0.074$, respectively), although ($\tau=-12$, $p=0.035$) phytoplankton richness declined. However, both phytoplankton biomass and diversity measures showed a decreasing trend after the shift ($\tau=-0.17$, $p=0.011$; $\tau^*=-0.27$, $p<0.001$ and $\tau=-0.16$, $p=0.018$, respectively).

Mean within-year variation in phytoplankton biomass and diversity measures increased considerably after the shift. The change in phytoplankton community composition between these periods was mostly reflected by a decrease in cyanobacteria dominance and a gradual increase in heterogeneity accompanied by an increase in abundance of diatoms and the remaining groups, with little change in chlorophytes in the latter period. Median zooplankton biomass in Lake Faarup decreased by 50% after 1997, with no trend ($p=0.104$) before and a negative trend ($\tau^*=-0.28$, $p<0.001$) after the shift, while its mean within-year variation increased (Fig. 16). Both zooplankton genera richness and evenness were higher before 1997 with a positive trend ($\tau^*=0.20$, $p<0.001$ for richness; $\tau=0.12$, $p<0.045$ for evenness). However, zooplankton genera richness exhibited no trend ($p=0.223$) and

evenness had an increasing trend ($\tau=0.14$, $p=0.038$) after 1997 (Fig. 16). Within-year variation in both zooplankton genera richness and evenness increased after 1997.

Section 7: Gross primary production (GPP) and respiration (R) were estimated from the formula given by Erlandsen and Thyssen (1983) and Kelly et al. (1983):

$$\begin{aligned} \left[\frac{dDO}{dt} \right] &= [K_2 1.0241^{(T-20)} (DO_{\text{sat}} - DO_t)] - [\rho_{20} 1.07^{(T-20)}] \\ &\quad + \left[\pi_{20} 1.035^{(T-20)} \frac{I_t}{\eta + I_t} \right] \\ [\text{Oxygen change}] &= [\text{Air water exchange}] \\ &\quad - [\text{Temperature-dependent respiration}] \\ &\quad + [\text{light and temperature-dependent production}] \end{aligned} \quad [1]$$

where

T : water temperature °C

DO_{sat} : dissolved oxygen saturation concentration mg l^{-1}

DO : dissolved oxygen concentration mg l^{-1}

I_t : light intensity $\text{Einst m}^{-2} \text{ 30 min}^{-1}$

K_2 : is the air–water transport coefficient (cm h^{-1}) at 20 °C, depending on wind speed. Several empirical relations between the transport coefficient and wind speed have been established:

a	$k = 2.07 + 0.215 U^{1.7}$	Cole and Caraco (1998)
b	$k = 0.72 U$	Crusius and Wanninkhof (2003)
c	$k = 0.168 + 0.228 U^{2.2}$	Crusius and Wanninkhof (2003)

where U is the average wind speed (m s^{-1}) recorded at a standard height of 10 m. We used an average value for the transport coefficient derived from the three empirical relations.

ρ_{20} , π_{20} and η are parameters estimated (using the secant non-linear method, PROC NLIN, in the statistical software SAS 9.3). We used this method instead of the simple Odum method commonly used in recent lake metabolism research, as Eq. (1) takes into account that respiration is temperature dependent and that temperature can vary substantially on a diel basis, not least in the shallow lakes encompassed by this study. Moreover, simultaneous calibration of the parameters ρ_{20} , π_{20} and η (respiration and photosynthesis parameters) has been shown to be

a robust method by stream researchers (Mahlon et al., 1983). Net ecosystem production was derived as the difference between GPP and R as a daily accumulated value ($\text{mg O}_2 \text{ day}^{-1}$) and DO was given as the dissolved oxygen percentage (DO%) relative to saturation levels, calculated as a function of T (Lin, 2001):

$$\text{DO}_{\text{sat}} = 14.652 - 0.41022T + 0.0079910T^2 - 0.000077774T^3 \quad [2]$$

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