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Climate-induced ecological change in subarctic lakes

- Evidence from biological archives, long-term monitoring, and contemporary field studies

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Climate-induced ecological change in subarctic lakes

Abstract

Northern lakes have a unique flora and fauna and are sensitive ecosystems that experience rapid rates of change. Multiple environmental changes are currently affecting these lakes, including climate-induced oligotrophication, with nutrient levels decreasing in waters throughout the Scandinavian mountain range. In this thesis I have studied multiple lakes and used approaches that span over multiple time scales to study energy flows and community composition of primary producers and consumers in northern lake food webs, and how these respond to climate-induced environmental changes. Paleolimnological analyses showed that northern lake food webs have been closely connected to long-term climate fluctuations, and catchment vegetation during the last 5800 years. And further, that benthic primary production was the predominating carbon source to zooplankton biomass during warmer and drier conditions while pelagic primary production predominated during colder and wetter periods. Time-series analysis of contemporary data showed clear evidence of the current oligotrophication in all of the lakes with dramatic decreases in water concentrations of total phosphorus. Food web responses were, however, weaker than expected likely owing to a large dependence on benthic primary productivity in these systems. The results from a study on within- and among-lake variability in $\delta^{13}C$ and $\delta^{15}N$, for primary producers and consumers, showed that within- and among-lake stable isotope variability were similar. Which indicates that small-scale, within-lake processes, can be equally important as catchment-scale processes for stable isotope composition of primary producers and consumers.

Keywords: trophic interactions, subarctic, habitat, paleolimnology, spatial variability

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Klimatinducerade ekologiska förändringar i subarktiska sjöar

Abstract

Nordliga sjöar har en unik flora och fauna och är känsliga ekosystem som just nu genomgår snabba förändringar. Sjöarna påverkas av flera miljöförändringar, inklusive klimatinducerad oligotrofiering, med minskande näringshalter i sjöar längs hela den skandinaviska fjällkedjan. I arbetet med den här avhandlingen har jag studerat sjöar över flera tidsskalor för att undersöka hur energiflöden och organismsamhällen hos primärproducenter och konsumenter påverkas av klimatinducerade miljöförändringar. Paleolimnologiska analyser visade att de nordliga sjöarnas födovävar var nära kopplade till klimatets långsiktiga variationer och till vegetationen i avrinningsområdet. Analyserna visade också att bottenlevande alger var den dominerande kolkällan till djurplankton under varmare och torrare förhållanden, medan den pelagiska primärproduktionen dominerade under kallare och fuktigare perioder. Tidsserieanalys av miljöövervakningsdata visade tydliga bevis på den nuvarande oligotrofieringen i samtliga sjöar, med dramatiska minskningar av totalfosforkoncentrationen i vattnet. Påverkan på födovävarna var dock svagare än väntat vilket sannolikt berodde på att födovävarna i de här sjöarna är starkt beroende av bentisk primärproduktion. Resultatet från en studie om variation i $\delta^{13}C$ och $\delta^{15}N$ för primärproducenter och konsumenter visade att variationen i $\delta^{13}C$ och $\delta^{15}N$ var likartad inom och mellan sjöar. Det tyder på att småskaliga processer inom sjöar kan vara lika viktiga som processer i avrinningsområdet för sammansättningen av stabila isotoper hos primärproducenter och konsumenter.

Nyckelord: trofiska interaktioner, subarktisk, habitat, paleolimnologi, rumslig variabilitet

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Dedication

To my family and friends

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Belle, S., **Nilsson, J.L.**, Tõnno, I., Freiberg, R., Vrede, T., Goedkoop, W., (2019). Climate-induced changes in carbon flows across the plant-consumer interface in a small subarctic lake. Scientific Reports, 9 (17087)

II. **Nilsson, J.L.**, Belle, S., Goedkoop, W., Christoffersen, K. S., Freiberg, R., Kainz, M., Tõnno, I., Vrede, T. Variation in $\delta^{13}C$ and $\delta^{15}N$ for biofilm and invertebrate consumers among and within subarctic lakes - Implications for food web studies. (manuscript)

III. **Nilsson, J.L.***, Nilsson, K.A.*, Goedkoop, W., Belle, S., Byström, P., Holmgren, K., Olofsson, J., Power, M., Vrede, T. Long-term food-web responses to climate-induced oligotrophication in subarctic lakes. (manuscript) (*shared first author)

IV. **Nilsson, J.L.**, Belle, S., Goedkoop, W., Vrede, T. Trophic relationships of primary producers and invertebrate consumers across environmental gradients – A field study of high-elevation, subarctic lakes (manuscript)

Paper I is reproduced with the permission of the publishers.

The contribution of Jenny L. Nilsson to the papers included in this thesis was as follows:

I. JLN contributed to the planning of the study, was involved in the execution of field and laboratory work, and provided reviews of manuscript drafts.

II. JLN designed the study and planned the field work together with co-authors, was responsible for organizing and conducting field work and was involved in organizing and conducting laboratory work and sample preparation. She also performed data analysis, and was responsible for writing the manuscript with support from co-authors.

III. JLN was responsible for the collection of archived samples. JLN also planned and took active part in the field work and subsequent preparatory sample analysis. Together with KN, she was responsible for the writing of manuscript drafts for which they received inputs from the co-authors

IV. JLN planned the field work together with co-authors, was main responsible for execution of field work, sample preparation, data analysis, and the writing of manuscript drafts that were discussed with co-authors.

1. Introduction

Freshwater ecosystems are a prominent feature of the northern landscape (Vincent & Laybourn-Parry, 2008), with thousands of ponds, lakes, and rivers. These freshwater ecosystems are therefore closely connected with their catchments. They also constitute a refuge for cold-adapted animal and plant species, and provide food and drinking water for northerners (Lento et al. 2019).

Climate change and northern landscapes

While the Earth´s climate is changing rapidly, due to human activities, the air temperature, in subarctic and Arctic regions, is increasing twice as fast as anywhere else on Earth (AMAP, 2017; Masson-Delmote et al., 2021). In these high latitude regions the air temperature has increased 2°C since 1960, ice- and snow-cover duration has decreased, and extreme weather events are likely becoming more frequent (AMAP, 2017; Masson-Delmote et al., 2021). Temperature and precipitation are key drivers of many biotic and abiotic processes in the landscape. For example, changes in temperature and precipitation are affecting vegetation development (Berner et al., 2020; Elmendorf et al., 2012; Xu et al., 2013), ice- and snow-cover duration (AMAP, 2017; Lento et al., 2019), the depth and expansion of permafrost (Kokelj et al., 2015), and ultimately the biodiversity of lakes and rivers (Lento et al., 2019; Wrona et al., 2016). In large areas of the Arctic, permafrost thaw releases nutrients and carbon stored in the previously frozen soil which leads to increased turbidity of rivers (Kokelj et al., 2013; Levenstein et al., 2018) and increases nutrient loads to lakes (Ayala-Borda et al., 2021; Vonk et al., 2012). Permafrost thaw and nutrient loading then affect primary producers through changes in water quality and light penetration (Levenstein et al., 2018; Wauthy & Rautio, 2020) and may cause shifts in communities of primary producers and consumers (discussed in Vonk et al. 2015).

Permafrost is, however, sporadic in northern Scandinavia (AMAP) and ongoing climate change has, instead, resulted in large-scale oligotrophication (Huser et al. 2018; 2020). Greening of the tundra landscape (Elmendorf et al., 2012; Pouliot et al., 2009) and concurrent sequestration of nutrients in catchment vegetation and reductions in the nutrient run-off to lakes and rivers is believed to be the main driver of the ongoing oligotrophication. Marked declines in total phosphorus (TP) concentrations in many oligotrophic, high-elevation lakes in Scandinavia have thus resulted in ultra-oligotrophic conditions (Huser et al., 2020). Although these TP-declines have been going on for a few decades, the ecological effects of this oligotrophication on communities and trophic relationships have only been addressed in a few studies. (e.g., (Bergström et al., 2020; Isles et al., 2018) that primarily had a pelagic focus.

Energy flows through aquatic food webs are of fundamental importance for the functioning of lake ecosystems (Lindeman, 1942) and are a key to our understanding of their response to global changes. Carbon transfer through lake food webs is derived either from autochthonous primary production (i.e., phytoplankton and benthic algae, (Ask et al., 2009; Vadeboncoeur et al., 2003), from allochthonous detrital inputs and/or from remobilized dissolved organic carbon by bacteria (Jansson et al., 2007). As high-latitude lakes are typically nutrient-poor clear-water systems their food webs are mainly supported by benthic algae (Karlsson et al., 2009; Vadeboncoeur et al., 2003, 2006). While benthic algal productivity generally is unrelated to water nutrient concentrations, high phytoplankton biomass can shade and reduce the ambient light at higher nutrient concentrations (Björk-Ramberg, 1984; Vadeboncoeur et al., 2003). Obligate autotrophic phytoplankton, on the other hand, are likely to be further limited by the ongoing oligotrophication. Mixotrophic phytoplankton often predominate the phytoplankton community in oligotrophic lakes (Bergström et al., 2003; Waibel et al., 2019) as phagotrophy enables them to access nutrients and carbon from bacteria and other organic particles. Oligotrophication could therefore cause a lower relative importance of pelagic primary production and an increase in the predominance of mixotrophy in phytoplankton, while benthic pathways of nutrient and energy transfer would become even more important for higher trophic levels (i.e., invertebrate grazers and fish).

Food webs studies

Lakes consist of multiple, interconnected habitats that are important for their ecological functioning. For example, Arctic charr (*Salvelinus alpinus*) utilizes different habitats depending on the fish community composition. In lakes where charr is the only fish species the productive littoral zones are important feeding habitats, but in a warmer climate charr frequently coexists with trout which forces charr to feeds in the pelagic or profundal zone (Klemetsen et al. 2003 and references therein). Also, settling phytoplankton and zooplankton fecal pellets can constitute an important linkage between pelagic and benthic habitats (e.g., Goedkoop & Johnson 1996; Blumenshine et al. 1997; Hayden et al. 2019). However, plankton community composition is expected to change under future climate scenarios (AMAP, 2017; Schabhüttl et al., 2013) thus, affecting these linkages between habitats. Climate-induced changes will favor more warm adapted and disfavor more cold-adapted species which inevitably will lead to changes in lake communities and trophic interactions (Heino et al. 2020).

A common approach to study trophic relationships is the use of stable isotopes of key elements, especially 13-carbon ($\delta^{13}C$) and 15-nitrogen ($\delta^{15}N$) Stable isotopes are widely used as tracers of nutrient sources and habitat use (Cremona et al., 2014; Eloranta et al., 2015; Hayden et al., 2014; Vadeboncoeur et al., 2003), and for assessing the effects of climate-induced change (e.g., Hayden et al. 2019; Kivilä et al. 2019; Lau et al. 2014). The $\delta^{13}C$ of basal resources (e.g., benthic algae, plant detritus) shows limited fractionation per trophic transfer (≤1 ‰, Post 2002; Belle et al. 2020), and can therefore be used to quantify resource dependence of consumers. The $\delta^{15}N$ of basal resources, on the other hand, generally shows a more distinct fractionation per trophic transfer (Deniro & Epstein, 1981; Vander Zanden & Rasmussen, 2001), and can be used for assessing the trophic position of consumers.

Northern lakes have a unique flora and fauna, and are sensitive ecosystems that experience rapid rates of change (Lento et al., 2019). Although we have the tools to study these systems the logistic constraints caused by their remoteness contributes to them being poorly studied. In this thesis I have studied multiple lakes and used approaches that span over multiple time scales. The findings provided in this thesis will contribute to increasing the knowledge of the ecological effects of ongoing, climate-induced oligotrophication on the communities and food webs of subarctic lakes.

1.1 Objectives

The overall aim of this thesis was to investigate lake food web responses to past and ongoing climate change, with a specific focus on their ongoing oligotrophication. And to contribute to the knowledge of climate-induced ecological change in remote subarctic areas. This was done using three different approaches that cover different temporal and spatial scales and organism groups at different trophic levels. In **paper I** carbon flow to zooplankton under different climates and levels of landscape development was studied retrospectively, over millennial time scales. The idea was that paste change could contribute to predictions and understanding of ongoing and future change. Whereas the focus in **papers III** and **IV** was on the effects of the ongoing oligotrophication on communities and lake food. Since carbon and nitrogen stable isotope ratios constitute such a vital part of the conclusions drawn from these studies, and their within-lake spatial variability is not well studied (Syväranta et al., 2006; Thompson et al., 2018) we also studied within- and among-lake variability of stable isotopes of producers and consumers (**paper II)** to aid the interpretations of papers III and IV.

The specific objectives of this thesis work were to:

- Investigate relationships between climate change, landscape development, and carbon flows in lake food webs over long time scales (**paper I**)

- Aid to interpretations of food web responses to climate-induced environmental change by quantifying stable isotope within-lake variability (**paper II**)

- Investigate the effects of the ongoing, climate-induced oligotrophication on primary producers and consumers in lake food webs (**paper III, IV**)

2. Methodology

This thesis is based on field work, archived samples, and data extracted from long-term environmental monitoring from lakes located in the Swedish Arctic/alpine ecoregion (Nordic Council of Ministers, 1984), which is part of the global subarctic region. Lakes and sampling locations were chosen to be as similar as possible regarding lake and catchment characteristics (e.g., catchments with heath or mountain birch, wind exposed littoral habitats), while the lakes were selected along gradients in total phosphorus (TP) and total organic carbon (TOC) concentrations, using data from environmental monitoring and previous studies. In the following sections I provide a brief description of the study designs and an overview of the methods used. For more detailed descriptions see papers I-IV.

Study sites and study designs

The study lakes were all oligotrophic, clearwater lakes located at latitudes ranging from 62.23 to 68.47 °N, and with lake elevation ranging from 368 to 1066 m above sea level (Fig. 1, Tab1). The mean annual temperature is relatively low in the region (< 2 °C) and the growing season typically short (< 140 days) (Nordic Council of Ministers. 1984). Land cover in the catchments was generally predominated by heath, shrubs, mountain birch or were unvegetated (e.g., bare mountain) and the lakes were all unaffected by point source pollution, forestry, agriculture, and urban development which makes them ideal to study effects of climate-induced environmental changes.

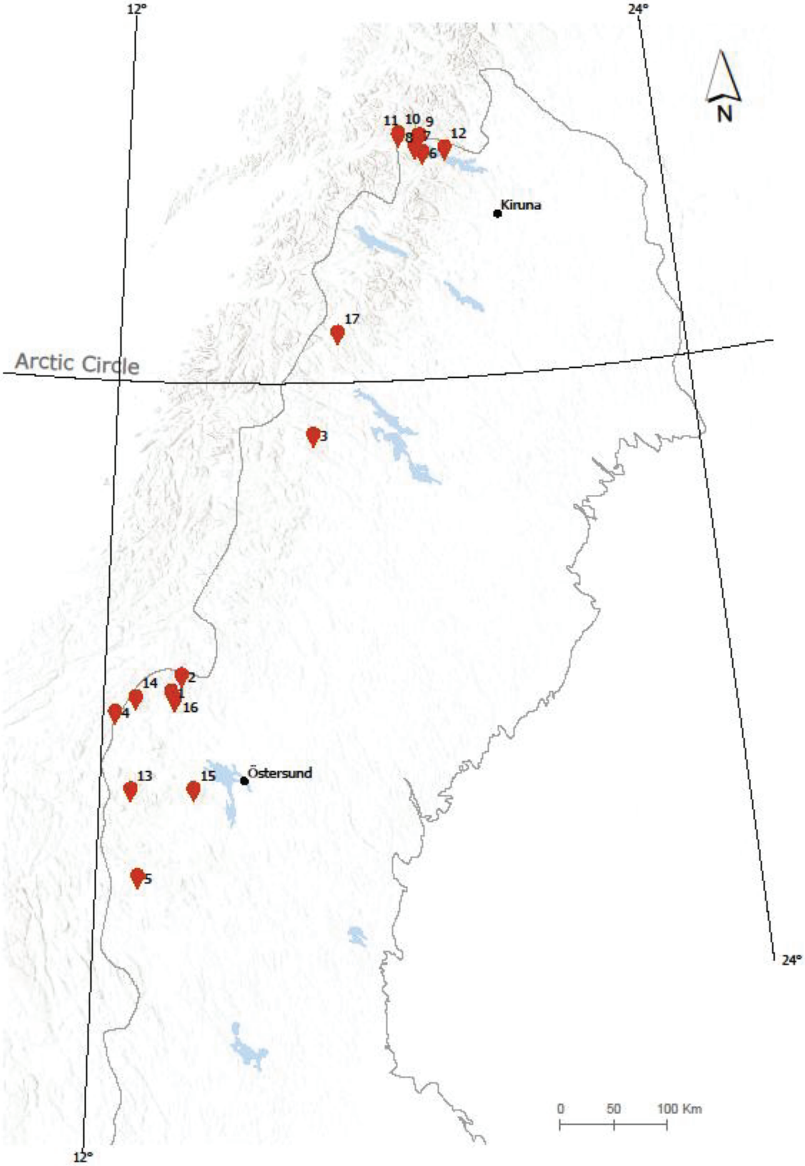

Figure 1. Map showing the geographical location of the study lakes in northern Sweden as well as the towns of Kiruna and Östersund, the 12° and 24° meridians, and the Arctic Circle. 1 = Västra Bijjie Bavlanjaevrieh, 2 = 1066, 3 = Stor-Tjulträsket, 4 = Stor-Björsjön, 5 = Övre Fjätsjön, 6 = Abiskojaure, 7 = Latnjajaure, 8 = Diktar Erik, 9 = BD12, 10 = BD01, 11 = BD02, 12 = BD15, 13 = Ljungsjöarna, 14 = Avundstjärnen, 15 = Gåtejaure, 16 = Bergsjön, and 17 = Njalakjaure.

Paper I describes a paleolimnological study using the sediment archive from Lake Diktar Erik (68.45 °N), and addresses the links between past climate, catchment vegetation, and carbon flows in the lake food web across large temporal scales (thousands of years). The study reports climate variability over 5800 years and how this affected algal communities and the lake food web (i.e., stable isotope composition of primary consumers). The sediment was characterized by organic matter content (OM) and carbon and nitrogen stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$). Photosynthetic pigment composition, in the sediment, was used as a proxy for algal community composition (Reuss et al. 2010), while the $\delta^{13}C$ of *Daphnia* (Cladocera, Crustacea) resting eggs provided information about the relative importance of allochthonous and autochthonous carbon sources in their diet.

In **Paper II**, within-and among-lake variability in $\delta^{13}C$- and $\delta^{15}N$-values of zooplankton, littoral and sublittoral benthic macroinvertebrates, and biofilm was studied. Also, among- and within-lake variability in biofilm community composition was investigated through analysis of both photosynthetic pigment composition, using reversed-phase HPLC, and through microscopic analysis of benthic algae taxonomic composition. In addition, compound specific stable isotope analysis (CSIA) on algae specific fatty acids in biofilms was analysed.

In **paper III** time series analysis of several chemical and biological variables of five monitoring lake was conducted. This included surface water concentrations of TP and DIN, community composition and biovolume of phytoplankton, community composition of benthic macroinvertebrates, as well as $\delta^{13}C$ and $\delta^{15}N$ composition of Arctic charr (*Salvelinus alpinus*) and of key benthic macroinvertebrate taxa from archived samples. This was done to investigate the effects of climate induced oligotrophication on lakes' food webs.

Paper IV is a gradient study where we used a space-for-time approach to simulate the oligotrophication process to investigate its effects on lake food webs. 16 lakes were selected along gradients in TP and TOC, using data from environmental monitoring and previous studies. Analyses were conducted for water nutrient concentrations, plankton community composition, $\delta^{13}C$ and $\delta^{15}N$ composition of seston, zooplankton, biofilm, and littoral and sublittoral macroinvertebrates.

Table 1. Information on the study lakes arranged from north to south, including latitude (decimal degrees), longitude (decimal degrees), lake elevation (m.a.s.l.), lake surface area (km^2), dominating catchment types, and which paper(s) the lake is included in.

Lake	Latitude	Longitude	Elevation	Area	Catchment	Paper
BD01	68.473	18.125	458	0.04	Open land with vegetation, inland waters	IV
BD02	68.460	18.122	520	0.11	Open land with vegetation, inland waters	IV
Diktar Erik	68.445	18.606	368	0.09	Open land with vegetation, inland waters, mountain birch	I, II, IV
BD12	68.443	18.577	413	0.02	Open land with vegetation, open land without vegetation, inland waters	IV
Latnjajaure	68.351	18.488	976	0.72	Open land with vegetation, open land without vegetation, inland waters	III, IV
BD15	68.332	19.154	380	0.06	Open land with vegetation, inland waters, mountain birch	IV
Abiskojaure	68.306	18.652	486	2.7	Open land with vegetation, open land without vegetation	II, III, IV
Njalakjaure	66.8167	16.622	852	0.33	Bare rock, tundra vegetation	III

Stor-Tjulträsket	65.962	16.068	540	5.3	Open land with vegetation, open land without vegetation, mountain birch	II, III, IV
1066	63.936	13.473	1066	0.07	Open land with vegetation, open land without vegetation, inland waters	IV
Västra Bijjie Bavlanjaevrieh	63.797	13.281	696	0.23	Open land with vegetation, inland waters, wetland	IV
Avundstjärnen	63.739	12.612	553	0.26	Open land with vegetation. open land without vegetation, inland waters	IV
Bergsjön	63.732	13.354	408	3.3	Open land with vegetation, fir forest, wetland	IV
Stor-Björsjön	63.611	12.233	565	0.44	Open land with vegetation, wetland	II, III, IV
Gåtejaure	62.980	13.746	855	0.53	Open land with vegetation, open land without vegetation, inland waters	II, IV
Ljungsjöarna	62.963	12.580	935	0.88	Open land with vegetation, inland waters, wetland	IV
Övre Fjätsjön	62.235	12.765	746	2.3	Open land with vegetation, inland waters, fir forest	II, IV

2.1 Field work and sample collection

All field work was conducted during July-August 2017-2019, except for the collection of sublittoral samples in lakes Abiskojaure, Stor-Tjulträsket, Stor-Björsjön, and Övre Fjätsjön which was conducted at a separate sampling during autumn 2018. Table 1 provides information on which lakes were included in the different paper.

Pelagic samples

Water chemistry, seston, zooplankton and phytoplankton samples were collected in a mid-lake location in each lake (**papers II-IV**). Water was collected at every meter using a Limnoshämtare equipped with a thermometer. During stratified conditions, we conducted repeated sampling above the thermocline to acquire sufficient water for subsequent analyses. In unstratified lakes samples were collected every meter from the surface down to 8 m depth. Water samples from each meter were pooled in a larger container and thoroughly mixed for the collection of an integrated water sample. From this pooled sample, water for the seston samples was first pre-screened through a 40-µm mesh and then filtered on GF/F-filters. Quantitative phytoplankton and zooplankton subsamples, for analyses of community composition and biovolumes, were preserved using Lugols´solution. For the zooplankton samples a known volume of water was filtered through a 40-µm mesh and the concentrated water sample preserved. In mid-lake, qualitative zooplankton samples for stable isotope analysis were collected through repeated tows with a 120-µm plankton net over the same depths as applied for the water sample collection. Qualitative zooplankton samples were also collected along the shoreline, towing the net in the littoral zone either from a standing position at 1-m depth or from a rowboat.

Benthic samples

The sediment core, used for paleolimnological analysis, was collected from the deepest part of Lake Diktar Erik using a gravity corer (9 cm in diameter, UWITEC) (**paper I**). Benthic macroinvertebrates were collected from the littoral (0.5-1 m) and the sublittoral (4-6 m) zone (**papers II-IV**). In the littoral zone the samples were collected by kick-sampling and a hand-net (mesh size 0.5 mm) whereas an Ekman grab (surface area 250 cm^2) and a 0.5-mm sieve was used in the sublittoral zone. For all but four lakes the predominant taxa were sorted in the field. For lakes Abiskojaure, Stor-Tjulträsket, Stor-Björsjön, and Övre Fjätsjön, however, grabs were sieved and the invertebrates collected in bottles filled with lake water and shipped over night to our lab in Uppsala where the samples were sorted and then frozen (-20°C). Quantitative biofilm samples for taxonomic analysis were collected from five cobbles in the littoral zone (0.5-1 m depth) using a brush sampler (3.14 cm^2 in diameter, Peters *et al.*, 2005) and pooled to create a single sample. Qualitative samples were collected by scrubbing the cobbles thoroughly with a toothbrush and collecting the biofilm slurry (SS EN 13946:2014, **papers II-IV**).

Archived samples

In **paper III** we also analysed $\delta^{13}C$ and $\delta^{15}N$ composition on time series of freeze-preserved fish muscle samples (Arctic charr, *Salvelinus alpinus*) and ethanol preserved benthic macroinvertebrate samples from biobanks. The fish samples were collected from archives at the Swedish Museum of Natural History, Stockholm, and originated from their annual net fishing conducted for their monitoring program on contamination. Additional fishes, from Lake Abiskojaure for the years 2017-2019, were sampled from the annual, standardized fish sampling with multi-mesh gillnets (SS-EN 14757:2015, Appelgren 2000) performed by the Swedish University of Agricultural Sciences (SLU Aqua). A small sample of dorsal muscle was collected from besides the dorsal fin from approximately 10 fishes within a total length interval of 15-40 cm per lake and year. Ethanol-preserved samples of littoral and profundal *Gammarus lacustris* (Amphipoda), *Pisidium sp*. (Bivalvia), and predatory and deposit-feeding chironomids (Diptera) were collected from archived samples from the freshwater environmental monitoring program performed by the Swedish University of Agricultural Sciences (Depth. Aquatic Sciences and Assessment). Pooled samples composed of several individuals of benthic macroinvertebrates were used for stable isotope analysis.

2.2 Sample preparation and analysis

For the paleolimnological study (**paper I**) the core was first sliced vertically. The top 50 cm of one of the halves was then sliced horizontally in 1 cm slices, and the following analyses were then conducted on material from each slice.

Sediment organic matter (OM) content was determined through the loss-of-ignition method, and the results were expressed as percentage of dry weight. Sediment samples were also analyzed for carbon and nitrogen stable isotopes ($\delta^{13}C_{OM}$ and $\delta^{15}N_{OM}$), carbon and nitrogen concentrations (C_{org} and N_{tot}), and C/N weight ratios. Prior to analysis the samples were dried, ground, and transferred to tin capsules. The analysis was then conducted at SLU Stable Isotope Laboratory (Umeå, Sweden) and results were expressed using the delta (δ) notation using Vienna Pee Dee Belemnite and atmospheric nitrogen as standards: $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$; where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

Analysis of photosynthetic pigment composition, in the sediment, was conducted following Leavitt and Hodgson (2001) using reversed-phase HPLC on a Shimadzu Prominence® (Japan) series binary gradient system equipped with a photodiode-array (PDA) and fluorescence detector. Pigment peak identification and quantification were made using commercially available external standards from DHI Company (Denmark). Chlorophyll a (Chl *a*) and its derivative pheophytin a were selected to indicate the overall algal biomass (Leavitt & Hodgson, 2001; Waters et al., 2013). Taxon-specific indicator pigments were used to quantify the biomass of the following aquatic primary producer's classes: lutein for green algae (Chlorophyceae), fucoxanthin for diatoms (Bacillariophyceae), alloxanthin for cryptophytes (Cryptophyteae, (Waters et al., 2013) and canthaxanthin for cyanobacteria (Cyanophyceae, Deshpande et al., 2014). Pigment concentrations were expressed as nanomoles per gram of sediment organic matter (nmol g^{-1} OM).

For stable isotope analyses on *Daphnia spp*. resting eggs the sediment was deflocculated in NaOH (10%) solutions, pre-treated using washing with HCl (10%) solutions and sieved through a 100-μm mesh according to the standard protocol of Perga (2010). The resting eggs were sorted out using a dissection microscope until approximately 50 eggs or a mass of about 60 μg (minimal mass required for stable isotope analysis) was gained. If resting egg abundances in a single sediment layer were too low, then the next consecutive sediment layer was pooled to the sample. Carbon stable isotopic composition of *Daphnia* resting eggs ($\delta^{13}C_{Clado}$) was then analyzed and the results expressed according to the delta notation (see above).

In order to link sediment-depth to age, a relationship was established by analyzing radiocarbon dates in five bulk sediment samples using an accelerator mass spectrometer at the Poznan Radiocarbon Laboratory (Poland) and The Tandem Laboratory (Sweden). The age-depth model was then constructed using the results from the radiocarbon dating, the transition from proglacial to lacustrine sediment, that was clearly visible in the core, in combination with data on the deglaciation age (ca. 9500 ± 250 years BP) for the region (Barnekow et al., 1998; Bigler et al., 2003). The age-depth model was then constructed using linear interpolation.

The analyses of water chemistry, quantitative plankton samples, and stable isotope composition were the same for **papers II, III, and IV**. Analyses of water chemistry, phytoplankton and zooplankton community composition were conducted by our accredited laboratories at the Department of Aquatic Sciences and Assessment, SLU. Prior to analysis of $\delta^{13}C$ and $\delta^{15}N$, samples of zooplankton, seston, biofilm, littoral and sublittoral benthic macroinvertebrates, as well as that of Arctic charr muscle tissue were freeze-dried. All sample types except seston filters were ground before they were packed into tin capsules. The ethanol-preserved benthic macroinvertebrates (**paper III**) were instead dried before being ground and packed in tin capsules. Stable isotope results were expressed according to the delta (δ) notation (see above).

For **paper II** additional analyses of benthic algae community composition were performed through microscopic analysis of benthic algal taxonomic community composition and by analysis of photosynthetic pigment composition. For analysis of taxonomic community composition each sample was analyzed in four replicates using a 2.1-mL counting chamber and an inverted light microscope (Olympus®). Species with five or more individuals found in the chamber were included in the biovolume calculations. Results were presented as percentage of the total sample biovolume. Photosynthetic pigment composition was analyzed following slightly modified recommendations of Leavitt & Hodgson (2001) and Lie & Wong (2010). Just as for sedimentary pigments, commercially available external standards from DHI (Denmark) were used for peak identification and quantification. The following taxon-specific pigments were presented in the paper: lutein for Chlorophyta (Leavitt & Hodgson, 2001), fucoxanthin representing Bacillariophyta (Roy et al., 2011), alloxanthin representing Cryptophyta (Roy et al., 2011), and Canthaxanthin for Cyanophyta (Leavitt & Hodgson, 2001). The results were presented as percentage of total pigment concentrations.

Compound specific stable isotope analysis (CSIA) was conducted on algae specific fatty acids according to Kühmayer et al. (2020). The results were expressed according to the delta (δ) notation (see above). Due to low biomass of algae in some of the biofilm samples the concentrations of algae specific fatty acids were low, and we could only present the results for C20:5n-3, C22:6n-3 (ω3-FA) and C20:4n-6 (ω6-FA) in the paper.

The monitoring data used in **paper III** originate from the Swedish freshwater monitoring program which was initiated in the late 1980´s (see Fölster et al. 2014). Water chemistry and phytoplankton data constituted of epilimnetic water samples collected during Julian days 150-290 (ca. June-to mid-October). Benthic macroinvertebrate samples were collected in September-October each year using standardized methods.

Classification of phytoplankton into autotrophic and mixotrophic taxa in **paper IV** followed Rimet & Druart (2018), with the exception of Bacillariophyceae which we classified as autotrophic instead of mixotrophic. Hence, the mixotrophic taxa in **paper IV** were flagellates with the capacity for phagotrophy. In **paper III** the phytoplankton classes Cyanophyceae, Chlorophyceae, Bacillariophyceae and Synurophyceae were classified as autotrophic, whereas the classes Chrysophyceae, Dinophyceae, Cryptophyceae were classified as potentially mixotrophic.

2.3 Data handling and statistics

Among the statistical methods used in data analysis the following four constituted the most important, either because they reoccur in several papers or because they crucially contributed to the conclusions drawn in one of the papers.

Principal components analysis (PCA) was used to analyse gradients in sediment characteristics and pigment composition for **paper I**. While in **paper IV**, PCA was used to analyse gradients in water chemistry and to study correlations among $\delta^{13}C$ for seston, zooplankton, biofilm, littoral and sublittoral benthic macroinvertebrates.

Generalised additive models (GAM) were used for correlation analysis between $\delta^{13}C_{Clado}$ and the gradients in sediment characteristics and pigment composition (represented by the first PC-axis, $PCA1_{sed}$ and PCA_{pig}), respectively (**paper I**). In **paper III**, GAM was used for time series analysis. This approach was chosen since non-linear correlations between $\delta^{13}C_{Clado}$

and sediment and pigment composition, and for long-term changes in, for example, TP concentrations and phytoplankton biovolume were likely.

Analysis of Variance (ANOVA) was used in **paper II** to analyze among- and within-lake effects in stable isotope ratios of zooplankton, biofilm, littoral and sublittoral benthic macroinvertebrates. Among-lake variability was represented by the model whereas residual error was interpreted to represent within-lake variability.

Finally, linear correlation analysis, was used in **papers II, III and IV**. At the oligotrophic and ultra-oligotrophic conditions in our study lakes the relationship between water chemistry gradients and, for example, phytoplankton biovolume is expected to be linear. In more nutrient rich systems additional nutrients could potentially have adverse responses, whereas here any effects are likely linear.

All statistical analysis were conducted using the R programming language (R Core Team 2020).

3. Results and Discussion

In the following sections I will present and discuss the results from **papers I-IV**. The chapters are divided according to the research objectives to show the process of addressing each of them.

3.1 Relationships between climate change, landscape development, and carbon flows in lake food webs

One of many effects of the ongoing climate change is dramatically altered key characteristics of terrestrial-aquatic linkages such as run-off patterns and vegetation development (Masson-Delmote et al. 2021). The lack of long-term contemporary data means that it is still unclear how these landscape-level changes affect energy flows in lake food webs. Paleolimnological studies can provide us with information on how these lake ecosystems have responded to past climate changes, and the stable isotope composition of invertebrate remains in the sediment can be used to study climate-induced variations in the relative importance of different energy pathways under different climate scenarios.

Here I will present and discuss the results from **paper I** in which a paleolimnological study was conducted of sediment from Lake Diktar Erik, a small subarctic lake at 68.44°N in northern Sweden.

Description of temporal trends and climate

Previous studies in the region have shown that the landscape of northern Sweden developed relatively rapidly from a vegetation-free landscape into a forested landscape after the last deglaciation (ca 9500 ± 250 years BP). After this, the Holocene thermal maximum (ca 6500 to 3500 years BP) characterised a climatically stable period with a typically warmer and drier climate than the long-term mean, and pine forests spread in the catchment. From approximately 3500 years BP onwards the climate became gradually cooler and wetter and the pine forests were replaced by mountain birch forests similar to the ones present nowadays (Fig. 2D-H, Bigler et al. 2002; Barnekow 2000; Bigler et al. 2006).

Sediment analyses from Lake Diktar Erik showed that the sediment organic matter concentrations (OM), C_{org}, N_{tot}, and pigment concentrations peaked during the Holocene thermal maximum. The high concentrations of OM and C_{org}, and the high C/N ratio, in the sediment layers from this period, indicate a steady and high input of terrestrial organic matter to the lake, while the simultaneously high pigment concentrations showed that also autochthonous productivity was high during the Holocene thermal maximum. Analysis of *Daphnia* resting eggs ($\delta^{13}C_{Clado}$) showed values increasing from -30.6 at the beginning of the period (ca. 5800 years BP) to -28.8 ‰ around 3000 years BP (Fig. 2C). $\delta^{13}C_{OM}$ and $\delta^{15}N_{OM}$ ranged from -28.5 to -27.2 ‰ and 1.6 to 3.1 ‰, respectively, and gradually increased throughout the core, except for the top centimeters were again a decrease occurred. During the period from ca 3500 years BP to present day, the wetter and colder climate coincided with gradually lower concentrations of OM, C_{org}, N_{tot}, and pigment concentrations in the sediment. This shift towards a colder climate also coincided with markedly increasing $\delta^{13}C_{Clado}$ values, implying a shift in the *Daphnia* diet.

The GAM conducted to correlate $\delta^{13}C_{Clado}$ with gradients in sediment characteristics and photosynthetic pigment composition ($PCA1_{sed}$ and PCA_{pig}, respectively, Fig. 2A-B) showed a monotonically, positive relationship between $\delta^{13}C_{Clado}$ and $PCA1_{sed}$. The most negative $\delta^{13}C_{Clado}$ values coincided with the most organic-rich sediment that also had the lowest values of $\delta^{13}C_{OM}$ and $\delta^{15}N_{OM}$. The correlation between $\delta^{13}C_{Clado}$ and $PCA1_{pig}$, on the other hand, was unimodal. As described above, pigment concentrations decreased throughout the core while $\delta^{13}C_{Clado}$ values increased during the Holocene thermal maximum and then decreased during the late Holocene (from ca. 3000 years BP onwards). During the Holocene thermal maximum $\delta^{13}C_{Clado}$ correlated negatively with the decreasing pigment concentrations (i.e., increasing $\delta^{13}C_{Clado}$ with decreasing pigment concentrations). During the more recent colder and wetter climate, i.e., during the late Holocene, however, the correlation instead turned positive with simultaneous decreases in $\delta^{13}C_{Clado}$ and pigment concentrations. (Fig. 2)

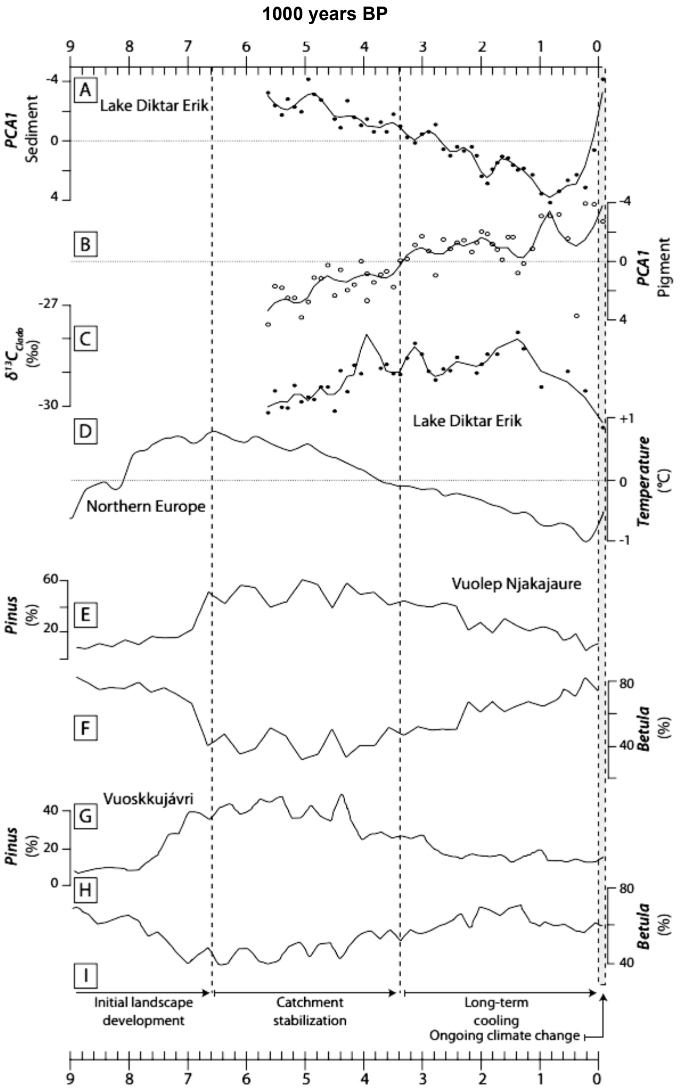

Figure 2. (**A**) Sediment PCA1 scores, (**B**) pigment PCA1 scores, (**C**) $\delta^{13}C_{Clado}$ composition from Lake Diktar Erik, in ‰, (**D**) pollen-based temperature variability for Northern Europe from (Seppa et al., 2009), percentage of pollen from (**E**) *Pinus sylvestris* and (**F**) *Betula pubescens* in a sediment core from Lake Vuolep Njakajaure (Bigler et al., 2002), percentage of pollen from (**G**) *Pinus sylvestris* and (**H**) *Betula pubescens* in a sediment core from Lake Vuoskkujávri (Barnekow, 2000), and dashed lines dividing the stratigraphy into four phases: initial landscape development (ca. 9500-6600 years BP), catchment stabilization (ca. 6600-3400 years BP), long-term cooling (ca 3400-0 years BP) and ongoing climate change (adapted from Meyer-Jacob et al. (2017)). Figure adapted from Belle et al. (2019).

Connecting Daphnia $\delta^{13}C$ to variability in climate and catchment vegetation

Firstly, the finding that δ^{13}C of *Daphnia* resting eggs (range -30.6 to -27.2 ‰) were more negative than that of sedimentary organic matter (range -28.5 to -27.0 ‰) implies that *Daphnia* assimilated a carbon source with a more negative δ^{13}C than bulk sedimentary organic matter. $\delta^{13}C_{OM}$ has commonly been used as a proxy for quantifying the relative share of organic matter from aquatic and terrestrial origins (Meyers & Ishiwatari, 1993). The results from **paper I** then mean that *Daphnia* in Lake Diktar Erik selectively utilized carbon associated with aquatic primary producers, suggesting that cladoceran zooplankton consumers in subarctic lakes, also in the long-term perspective, have been mainly fueled by autochthonous primary production (Cazzanelli et al., 2012; Mariash et al., 2014; Rautio & F. Vincent, 2007; Wenzel et al., 2012).

Secondly, the observed shift in the relationship between pigment composition and $\delta^{13}C_{Clado}$ as the climate changes from warmer and drier to colder and wetter indicates a close connection between aquatic primary production and *Daphnia* feeding habits. During the Holocene thermal maximum $\delta^{13}C_{Clado}$ increased while the photosynthetic pigment concentrations decreased. Then after ca 3500 years BP pigment concentrations continued to decrease and $\delta^{13}C_{Clado}$ started to decrease again. Combining these results with those of previous studies from the region (Bigler et al. 2002; Barnekow 2000; Bigler et al. 2006; Rosén 2005; Rantala et al. 2015) suggests that this dual relationship can be explained by climate effects on the catchment and the terrestrial-aquatic linkages. During the Holocene thermal maximum (ca. 5800-3500 years BP) $\delta^{13}C_{Clado}$ correlated positively and strongly with decreasing pigment concentrations. This suggests a decline in autochthonous primary production during a warm period when catchment vegetation had developed markedly. This also implies an increase in the relative incorporation of terrestrial organic matter to *Daphnia* biomass. After the onset of a cooler and wetter climate during the late Holocene (from ca 3500 years BP), however, $\delta^{13}C_{Clado}$ started to decrease again while pigment concentrations continued to decrease (Fig. 2B-C). The colder and wetter climate induced a change in catchment vegetation, from pine to mountain birch (Fig. 2E-H), and generated a higher transport of DOC to the lakes which. The increased DOC concentrations reduced the photic zone and favored pelagic primary productivity over benthic. Thus, the changed climate during the late Holocene induced a shift from benthic- to pelagic-dominated algal assemblages (Rantala et al., 2015; Rosén, 2005). As phytoplankton usually is more negative in $\delta^{13}C$ than both benthic algae (France, 1995; Hecky & Hesslein, 1995) and terrestrial organic matter (Meyers & Ishiwatari, 1993), this indicates that the observed decrease in $\delta^{13}C_{Clado}$ during the late Holocene means a higher contribution of planktonic algae to zooplankton biomass.

Conclusions

The results of this study provide evidence of a close and complex relationship between climate, vegetation development, and subarctic lake food webs. Our finding that benthic primary production predominates the carbon contribution to zooplankton biomass during periods with warmer and drier conditions, whereas the importance of pelagic primary productivity increases during colder and wetter conditions, suggests that the current climate warming, and the ongoing oligotrophication, would induce a shift back towards a higher incorporation of benthic derived carbon. Browning has currently not reached these oligotrophic high-elevation lakes (**paper III**) which implies that the initial effect of increased temperature would be an increased primary production mainly in the benthic since water nutrient concentrations are decreasing substantially (Huser et al., 2018, 2020). However, when landscape greening, and the increasing precipitation, finally raise the input of terrestrial DOC to the lake the incorporation of pelagic primary production to zooplankton biomass can be expected to increase again.

3.2 Within- and among-lake variability in $\delta^{13}C$ and $\delta^{15}N$ of primary producers and consumers

Although paleolimnological studies can provide important information on linkages between climate change/fluctuations and lake food webs over long timespans, the low sedimentation rate in these oligotrophic lakes prevents a more fine-scale resolution of the processes that govern different energy pathways. Contemporary studies are therefore necessary to understand in more detail how these aquatic ecosystems function. We therefore performed time series analysis on data from selected monitoring-lakes and conducted a field study across gradients in water chemistry including 16 subarctic lakes. However, while paleo-data originate from a single integrated lake sample (i.e., core collected at the deepest part of the lake) and integrate variability over large spatial scales, contemporary studies are more vulnerable to the effects of spatial variability within the lake. Such variability in $\delta^{13}C$ and $\delta^{15}N$ composition play a crucial role in the interpretations of lake food web interactions but is frequently disregarded. So far only a few studies (as far as we know) have addressed within-lake variability in $\delta^{13}C$ and $\delta^{15}N$ (Mbabazi et al., 2010; Syväranta et al., 2006; Thompson et al., 2018) which motivated a study on stable isotope spatial variability.

In the following sections I present and discuss the results from **paper II**. In addition, I will then discuss their implications for **papers III** and **IV**, where we prioritized to increase the number of studied lakes over more intensive sampling in a fewer number of lakes.

Variability in $\delta^{13}C$ and $\delta^{15}N$ in primary producers and consumers

Within-lake variability ($\delta^{13}C$: 0.1-7.8 ‰, $\delta^{15}N$: 0.2-4.8 ‰) was as large as among-lake variability ($\delta^{13}C$: 0.7-9.8 ‰, $\delta^{15}N$: 0.5-2.5 ‰), a finding that has implications for interpretations of results from food web studies (Tab. 2). For example, although the inclusion of baseline stable isotope variability greatly improves the accuracy of mixing model outputs (Ward et al., 2010), variability of almost 10 ‰ can be expected to reduce the predictive strength of a mixing model if this variability severally reduces the separation between baselines (Vander Zanden & Rasmussen, 2001). In addition, the impact of variable baseline stable isotope values should increase the higher number of food sources (i.e., baselines) are included in the mixing model as this would increase the risk of stable isotopic overlap among baselines.

The results of **paper II** also showed that among- and within-lake δ^{13}C-variability covered larger ranges than those for δ^{15}N. While δ^{15}N-variability often was < 1 ‰ both among and within lakes, δ^{13}C-variability could be as large as 9.8 ‰ in the most extreme cases. The difference in variability between δ^{15}N and δ^{13}C was attributed to the naturally larger variability among different carbon sources than among nitrogen sources. For example, Vander Zanden & Rasmussen (1999) reported an average δ^{15}N difference of 3.6‰ between littoral and profundal primary consumers while the average difference in primary consumer δ^{13}C was 6.7‰ between the habitats. Observed δ^{15}N-variability of benthic macroinvertebrates exceeding 1 ‰, in **paper II**, could often be attributed to the diverse taxonomic groups used (e.g., Trichoptera, Chironomidae). While the higher-level taxonomic resolution for many of the benthic macroinvertebrates was a necessary consequence of the low overlap in taxa found among- and within-lakes, it likely affected the results strongly owing to the impact of different feeding strategies on consumer isotopic signals (e.g., Vander Zanden & Rasmussen (2001).

Table 2 Among- and within-lake stable isotope variability for zooplankton, biofilm, and benthic macroinvertebrates. Among-lake variability is the maximum difference between lake mean isotope ratios, within-lake variability the difference between the highest and lowest isotope ratio within a lake. If the taxon was collected in more than one lake, the min and max within-lake variability is given. N equals the number of lakes from which we have collected the taxa in at least two locations in a habitat type. n.d = not determined.

	N	**Habitat**	**δ^{13}C Among**	**δ^{13}C Within min/max**	**δ^{15}N Among**	**δ^{15}N Within min/max**
Bosmina sp.	4	Planktonic	3.1	0.1 / 1.4	1.7	0.3 / 1.8
Daphnia	2		1.1	0.4 / 1.9	0.5	0.2 / 0.3
Diaptomids	1		n.d.	0.9	n.d.	0.8
Heterocope sp.	1		n.d.	1.2	n.d.	0.8
Holopedium sp.	1		n.d.	1.4	n.d.	0.6
Biofilm bulk	6	Littoral	3.2	1.1 / 4.0	1.2	0.2 / 0.8
Biofilm ω3-FA	6		6.1	1.2 / 5.9	n.d.	n.d.
Biofilm ω6-FA	6		9.8	0.3 / 5.9	n.d.	n.d.
Chironomidae	5		6.8	0.5 / 4.4	1.0	0.3 / 1.9

Coleoptera	1		n.d.	1.8	n.d.	0.2
Diptera	1		n.d.	1.1	n.d.	0.0
Ephemeroptera	3		1.8	2.9 / 6.1	1.1	0.2 / 2.3
Gammarus lacustris	2		0.7	2.6 / 3.0	1.0	0.5 / 1.0
Oligochaeta	3		1.4	1.3 / 3.4	0.8	0.1 / 1.0
Plecoptera	1		n.d.	1.4	n.d.	0.7
Tipulidae	1		n.d.	2.5	n.d.	0.5
Trichoptera	5		4.9	0.1 / 5.4	2.5	0.0 / 4.8
Chironomidae	4	Sublittoral	7.9	1.7 / 7.8	2.2	0.2 / 1.9
Gammarus lacustris	1		n.d.	4.2	n.d.	0.3
Hirudinida	1		n.d.	0.5	n.d.	0.6
Sialis	1		n.d.	6.1	n.d.	0.4

Beside differences in invertebrate-δ^{13}C caused by a lack of taxonomic resolution also δ^{13}C for bulk biofilm, biofilm ω3-FA (C20:5n-3, C22:6n-3) and ω6-FA (C20:4n-6) was strikingly variable (Fig. 3). For example, bulk biofilm δ^{13}C within-lake variability was 1.1 ‰ in Lake Stor-Björsjön but 4.0 ‰ in Lake Abiskojaure, whereas among-lake variability for ω3-FA was 6.1 ‰ (Tab. 2). If the complex matrix of biofilms (Battin et al., 2016; Quesada et al., 2008) was the only reason for the observed variability in bulk biofilm stable isotope signals, the ω3-FA δ^{13}C (within-lake variability 1.2 – 5.9 ‰) and ω6-FA δ^{13}C (within-lake variability 0.3 – 5.9 ‰) would be less variable than bulk biofilm δ^{13}C (within-lake variability 1.1 – 4.9 ‰) as these FA mainly originated from algae (Hixson et al., 2015; Twining et al., 2016). This was, however, not the case, as δ^{13}C in the algae-specific FA was even more variable than that of the bulk biofilm samples. Some of this variability likely originates from smaller sample sizes and the low fatty acid concentrations in some of the samples, which inherently leads to lower analytical precision. But again, this is likely not the sole explanation.

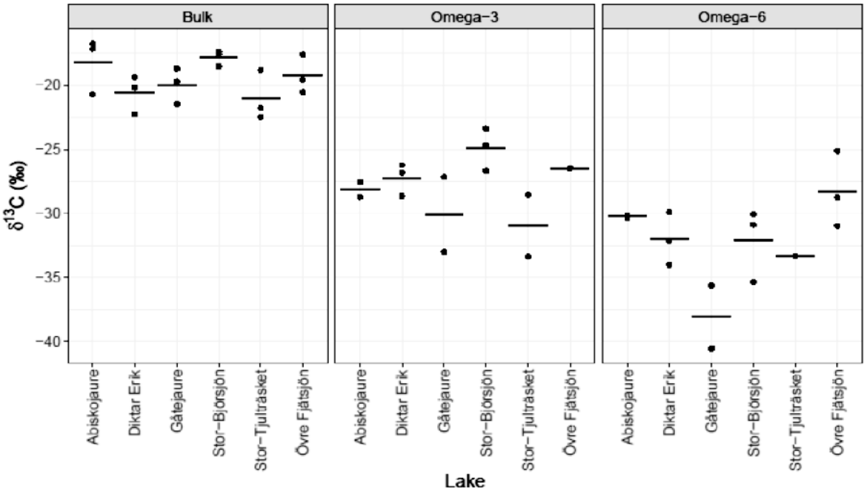

Figure 3. δ^{13}C -values of bulk epilithic biofilm samples (left panel), epilithic algae long-chain ω3-fatty acids (middle panel) and long-chain ω6 fatty acids (right panel) by lake. Dots represent values for different sampling locations within each lake while the line gives the lake mean

Besides the sources of variability addressed in our study, also shading can potentially contribute to biofilm stable isotope variability (Hill et al., 2008; MacLeod & Barton, 1998). However, as the catchments for the study lakes in **paper II** were generally dominated by forest-free vegetation types (Tab. 1) it is unlikely that varying degrees of shading would be a driving factor for the observed $\delta^{13}C$ within-lake variability.

The taxonomic composition of primary producers in biofilms might also be a significant source of variation in isotopic composition among and within lakes. Different algae taxa contain differing amounts of the algae specific fatty acids (Ahlgren et al., 1990; Galloway & Winder, 2015) and our results in **paper II** show that ω6-FA $\delta^{13}C$ was significantly more negative than ω3-FA $\delta^{13}C$. This suggests that variability in algal community composition also contributed to the observed variability in biofilm $\delta^{13}C$ (bulk and specific fatty acids) in our study. However, the results for both photosynthetic pigment composition and benthic algal taxonomical composition of biofilm samples showed that neither of these variables correlated strongly to bulk biofilm $\delta^{13}C$, ω3-FA $\delta^{13}C$ or ω6-FA $\delta^{13}C$. The only significant correlation was between pigment composition and ω6-FA $\delta^{13}C$ ($p = 0.01$) whereas no other correlations were found between biofilm $\delta^{13}C$ pigment composition or taxonomical community composition ($p > 0.05$). Probably the difference in $\delta^{13}C$ among fatty acids was not large enough to elicit detectable differences in biofilm $\delta^{13}C$ from variable algal communities, especially so when the differences in algal community composition were relatively small within lakes (Fig. 4). Instead, I suggest that small-scale variability in physical and/or chemical variables among sampling locations could be attributed to the observed variability in biofilm $\delta^{13}C$.

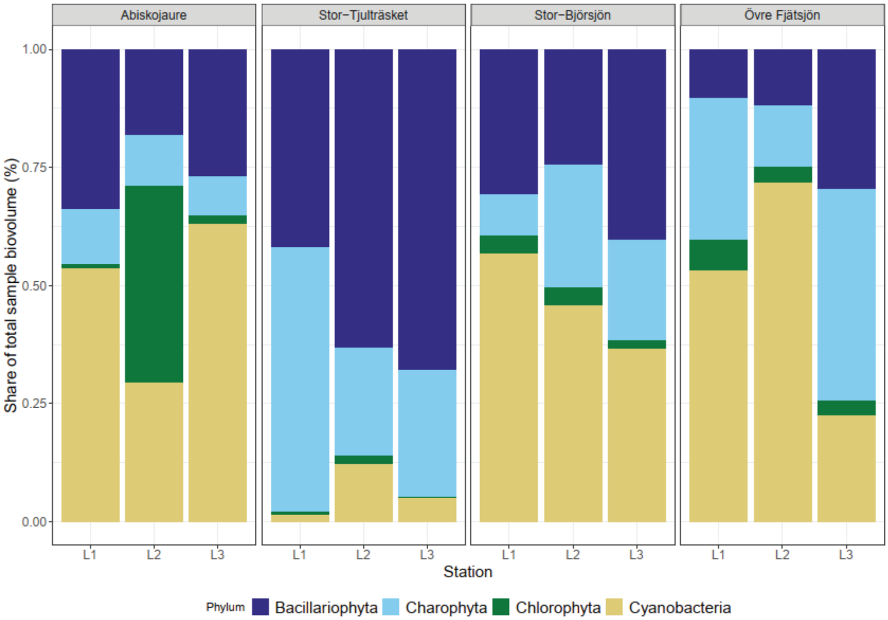

Figure 4. Taxonomic analysis (relative proportions by phyla) of quantitative epilithic biofilm samples for each of three locations in four lakes.

Littoral benthic macroinvertebrates δ^{13}C-variability was also unaffected by lake- and taxon-specific effects ($p > 0.05$), just as the different components of the biofilm ($p > 0.05$). As biofilm constitutes an important food source for benthic macroinvertebrates (e.g., Lau et al. 2014) it is likely that biofilm δ^{13}C-variability would influence that of littoral benthic macroinvertebrates (e.g., see Vesterinen et al. 2022). Therefore, in order to test if biofilm δ^{13}C-variability correlated with that of littoral benthic macroinvertebrates correlation analysis between residual errors ($\delta^{13}C_{\text{lake mean}}$ - $\delta^{13}C_{\text{location}}$) was conducted. By correlating the residual errors instead of the stable isotope values directly we removed the variability caused by lake- and taxon-differences. This correlation analysis showed a weak positive correlation between littoral benthic macroinvertebrate δ^{13}C and bulk biofilm δ^{13}C (p = 0.002, Adj. R^2 = 0.145), a weak negative correlation with ω6-FA δ^{13}C (p = 0.04, Adj. R^2 = -0.0790), whereas no correlation was found between littoral benthic macroinvertebrate δ^{13}C and ω3-FA δ^{13}C (p = 0.660, Adj. R^2 = -0.0205).

We identified several factors that could have contributed to this lack of consistent correlations between benthic macroinvertebrates and biofilm δ^{13}C.

Firstly, some biofilm samples contained relatively high amounts of inorganic matter (21.4-99.3 % of dry weight) which resulted in relatively low concentrations of the different algae-specific ω3-FA and ω6-FA. This complicated the analyses since concentrations sometimes were close to, or below, the detection limit. Secondly, different algae contain varying amounts of algae specific fatty acids (Ahlgren et al., 1990; Galloway & Winder, 2015), and the same for different food sources (Goedkoop et al., 2000), which could have contributed to a mismatch between consumer and producer $\delta^{13}C$. A third possible explanation could be the difference in $\delta^{13}C$ fractionation for foods of different quality (e.g., Vander Zanden & Rasmussen 2001; Belle et al. 2020). If various food sources within the biofilms were fractionated differently, this could lead to an altered total stable isotope composition of the consumer compared to if all food sources were fractionated equally, which in turn could explain the poor correlation with biofilm stable isotope composition.

Conclusions and implications

Our study shows that $\delta^{13}C$ values of primary producers and consumers can vary substantially among similar habitats within a lake, and especially so for biofilm and littoral benthic macroinvertebrates. Although our results emphasize that this variability exists, it was not possible to unravel its causes, as the variability was neither explained by lake- or taxon-specific effects, nor by algal community composition. Further, these findings stress that this variability should be taken into account in studies of trophic dependence and food web structure in order to avoid misinterpretations (Ward et al., 2010), for example, of predator-prey interactions or ontogenetically segregated habitat use. $\delta^{15}N$-variability, on the other hand, was often considerably lower, and was often explained by differences among lakes or taxa.

In addition, the result that among- and within-lake variability were similar suggest that local-scale processes, within lakes, can be equally important as catchment-scale processes for stable isotope values of producers and consumers. The results further highlight the fact that there are still unknown factors influencing $\delta^{13}C$ and $\delta^{15}N$ values which significantly can affect the outcome of food web studies if not properly addressed.

However, even though $\delta^{13}C$-values were quite variable within lakes there was no overlap in $\delta^{13}C$ between primary producers (i.e., seston, biofilm), and also $\delta^{13}C$ of consumer organism groups (e.g., zooplankton, littoral benthic macroinvertebrates) were well separated. This suggests that $\delta^{13}C$ within-lake-variability should not interfere with interpretations of food web interactions in lakes where isotopic baselines are distinctly separated, for example, where there is a distinct difference in $\delta^{13}C$ between seston and biofilm. From this I conclude that as long as **papers III** and **IV** show clear patterns over time, or over the studied water chemistry gradients, the patterns likely represent true trends.

3.3 Effects of climate-induced oligotrophication on subarctic lake food webs and community composition

In **papers III** and **IV**, we studied how the food webs and communities of subarctic lake ecosystems are affected by climate-change induced oligotrophication. Below I present and discuss the results and findings from **papers III** and **IV**.

Water chemistry trends

Long-term data, especially in these high-latitude regions, are uncommon (Lento et al., 2019). The Swedish environmental monitoring data are therefore a valuable source of information to analyse trends that date back to the 1980´s. Using this data, one of the clearest results from **paper III** was the dramatic decrease in water concentrations of TP and DIN in all five of the study lakes (Fig. 5), clearly illustrating the ongoing oligotrophication in the region. Since the late 1980´s TP concentrations have decreased by 45-80 % and those of DIN by 30-65 %. These results add to similar reports from Finland (Arvola et al., 2011) and Canada (Yan et al., 2008) showing how greening in northern regions can affect lake water chemistry (see Huser et al. 2020). Although browning is a common process in boreal regions (Monteith et al., 2007), no temporal increases in TOC were detected in the time series for these subarctic lakes in **paper III**. This lack of trend in TOC is likely a consequence of the sparse vegetation coverage in the catchments of these lakes (Tab. 1) resulting in a low inflow of terrestrial organic matter.

For our space-for-time study in **paper IV**, a PCA of water chemistry from all 16 study lakes was conducted. This analysis showed two separated water chemistry gradients among the studied lakes, a PCA1 that correlated with base ions (i.e., water hardness) and a PCA2 that was interpreted as a gradient in nutrient and TOC concentrations. The gradient in water hardness was primarily due to the inclusion of a few lakes in the county of Jämtland with high proportions of limestone in their catchments. As the focus of this thesis is on oligotrophication, I will primarily address correlations with the second PC-axis. The nutrient concentrations along PCA2 (e.g., TP 1.2-9.9 µg TP L^{-1}) were similar to those reported in the time series in **paper III**.

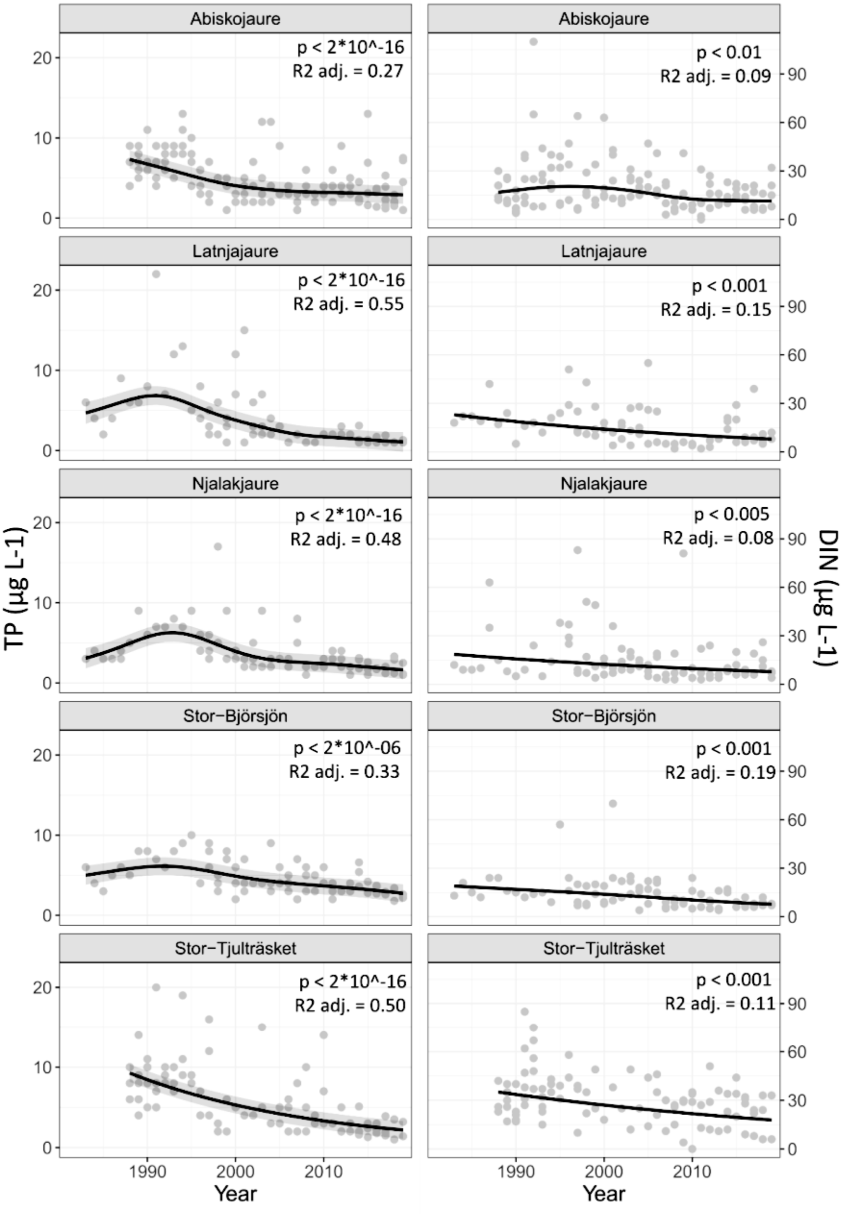

Figure 5. Long term trends (late 1980´s – 2017) in total phosphorus (TP, left panels) and dissolved inorganic nitrogen (DIN, right panels) concentrations from the monitoring data in paper III. Plot symbols show individual measurements, while the line is the fit of the GAM-model, and the shadowed area is standard error of the fit. P-values represent the significance of splines. The fit was calculated on $\log_{10}$-transformed data but is here back-transformed to match the original data scale.

Quantitative trends for primary producers and consumers

The space-for-time approach in **paper IV** showed that total phytoplankton biovolume decreased substantially with decreasing nutrient and TOC concentrations (i.e., $8.8* 10^9$ times across the nutrient gradient). However, despite the observed dramatic declines in TP-concentrations the time series analysis (**paper III**) did not detect any temporal trends in total phytoplankton biovolume for any of the lakes, except for a small increase in Lake Stor-Tjulträsket. Surprisingly, correlations between the time series for total phytoplankton biovolume and those for TP and DIN, respectively, showed no correlations, except for a negative correlation between phytoplankton biovolume and DIN in Lake Stor-Tjulträsket (i.e., higher biovolume at lower DIN, **paper III**).

Mixotrophic taxa constituted a large part (27.5 – 99-0 %, **paper IV**) of the phytoplankton community in all of the study lakes, but the proportion of mixotrophs correlated neither with TP nor DIN concentrations (**papers III, IV**). Mixotrophs often constitute a large part of the phytoplankton community in nutrient-poor lakes (Bergström et al., 2003) likely owing to their competitive advantage over obligate autotrophs under these conditions (Anneville et al., 2005; Waibel et al., 2019). Our interpretation of this lack of correlations was therefore that the nutrient range among our study lakes was too narrow to affect the phytoplankton community composition. However, the long-term monitoring data provided an interesting observation, namely that a single genus (i.e., the ochrophyte *Pseudopedinella spp.*) increased considerably in all five study lakes (Fig. 6) and was the main driver that caused the observed increases in mixotrophic biovolume (**paper III**). *Pseudopedinella* are considered highly effective bacterivores and are only found in oligotrophic environments (Gerea et al., 2016).

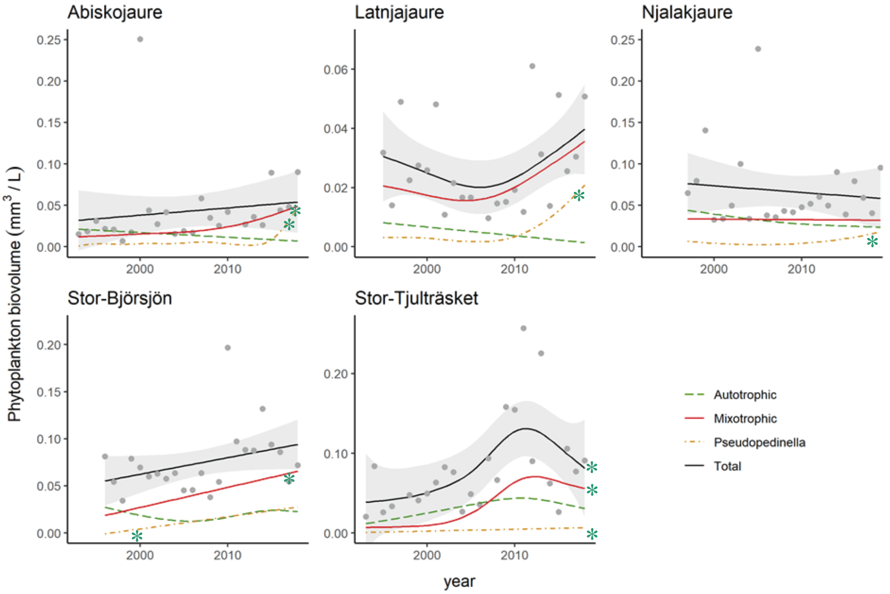

Figure 6. Time series of phytoplankton annual mean biovolume (mm^3 L^{-1}) with total biovolume, autotrophic groups, mixotrophic groups including the genus *Pseudopedinella spp.* and *Pseudopedinella spp.* separately. Fitted GAMs are shown with significant splines indicated with *. Note the different scales of the y-axes.

Zooplankton biovolume ranged between 0.025 mm^{3}L^{-1} and 10.3 mm^{3}L^{-1} among the lakes in our space-for-time study (**paper IV**) but correlated neither with nutrients and TOC concentrations (i.e., PCA2) nor with phytoplankton biovolume or the proportion of mixotrophs (**paper IV**).

Total benthic macroinvertebrate biomass declined in the two northernmost lakes in **paper III**, lakes Abiskojaure and Latnjajaure. This decline was found for both the sublittoral (sampling depth 4.6 ± 1.0 m, mean + sd) and the profundal (13.5 ± 2.1 m) in Lake Abiskojaure, and for the profundal (12.8 ± 3.4 m) in Lake Latnjajaure (sublittoral sampling not included in monitoring). In Lake Abiskojaure the trend was driven by decreases in Bivalvia and Oligochaeta, while Diptera displayed more of a hump-shaped pattern. In Lake Latnjajaure there was a decrease in all groups, while Diptera predominated the benthic macroinvertebrate community. No general trends in benthic macroinvertebrate biomass were found for the other three lakes, but individual groups (e.g., oligochaetes) increased in two of the lakes.

Fish biomass data was also available for lakes Abiskojaure, Stor-Björsjön, and Njalakjaure (**paper III**). Arctic charr is the only fish species residing in lakes Abiskojaure and Njalakjaure, while Arctic charr and brown trout (*Salmo trutta*) co-exist in Lake Stor-Björsjön. This community difference was visible also in the biomass time series, where Arctic charr in lakes Abiskojaure and Njalakjaure predominantly were caught in benthic nets while the charr in Lake Stor-Björsjön mainly was caught in pelagic nets (Fig. 7). This is a common pattern for Arctic charr, which often prefers the littoral habitat in allopatric lakes, but moves into the pelagic or deeper benthic habitats in sympatric lakes (Klemetsen et al. 2003 and references therein). The benthic biomass of Arctic charr increased in both lakes Abiskojaure and Njalakjaure, but the time series displayed large among year variability in both lakes. Pelagic biomass decreased over time in lakes Njalakjaure and Stor-Björsjön, whereas the decrease in Lake Stor-Björsjön was largely due to a large catch in the first year. Brown trout displayed no temporal trends in Lake Stor-Björsjön. No fish monitoring is conducted in Lake Stor-Tjulträsket. However, the fish population consists of a range of species, including Arctic charr, several species which have been introduced or spread there in the last decades (Filipsson, 1994).

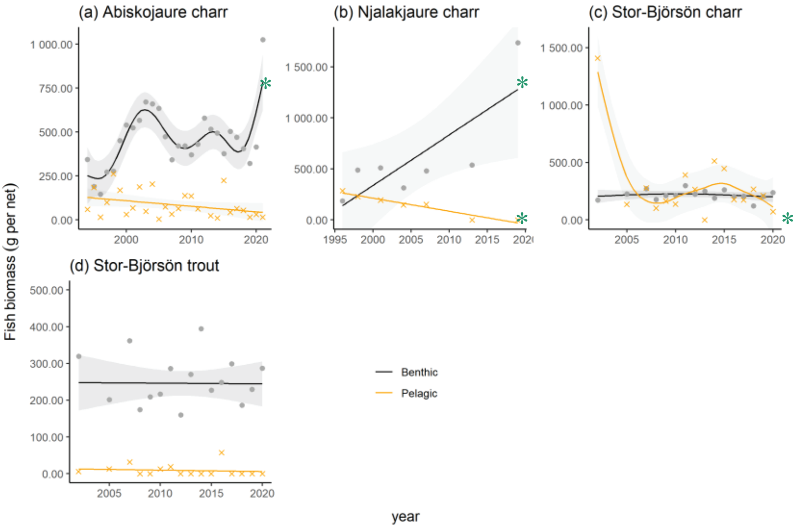

Figure 7. Time series of pelagic and benthic fish biomass for a) Arctic charr in Lake Abiskojaure, b) Arctic charr in in Lake Njalakjaure, c) Arctic charr in Lake Stor-Björsjön, and d) for brown trout in Lake Stor-Björsjön. Significant fits are indicated with *.

Trends in $\delta^{13}C$ and $\delta^{15}N$ for primary producers and consumers

PCA of δ^{13}C of seston, zooplankton, bulk biofilm, littoral and sublittoral benthic macroinvertebrates identified two distinct energy pathways and a third, intermediate pathway (Fig. 8, **paper IV**). Seston and zooplankton were closely associated, representing “the pelagic pathway”, as were bulk biofilm and littoral benthic macroinvertebrates that represented “the littoral pathway”. Sublittoral benthic macroinvertebrates were placed intermediate to the first two pathways and represented something that could be referred to as “the sublittoral pathway”.

The pelagic pathway primary producer (i.e., seston) δ^{13}C and δ^{15}N ranged 3.3 and 8.6 ‰, respectively, among lakes but did not correlate with the nutrients and TOC gradient (i.e., PCA2, **paper IV**). This means that the inorganic nutrient sources utilized by seston originate from similar sources along the nutrient and TOC gradient. The observation that zooplankton δ^{13}C did not correlate with seston δ^{13}C, indicates that bulk seston is not a good proxy of zooplankton food sources in these lakes (**paper IV**).

The littoral primary producer (i.e., bulk biofilm) stable isotopic composition also did not correlate with changes in nutrient and TOC concentrations, while biofilm δ^{13}C correlated positively with water hardness (i.e., PCA1, **paper IV**). In other words, the isotopic signal of this basal resource seems to be affected more by inorganic carbon than by organic carbon (TOC). In contrast to the poor correlations between the residual errors of littoral benthic macroinvertebrate δ^{13}C and those of bulk biofilm δ^{13}C, biofilm ω6-FA δ^{13}C, and biofilm ω3-FA δ^{13}C in **paper II**, littoral benthic macroinvertebrate δ^{13}C did correlated with biofilm δ^{13}C in the space-for-time study **(paper IV**) showing more positive δ^{13}C values at higher water hardness (i.e., PCA1). The time series of δ^{13}C or δ^{15}N for littoral benthic macroinvertebrates generally did not show any trends (**paper III**), showing that the littoral primary consumer stable isotope composition was stable also over time, in addition to the lack of correlation with the nutrient and TOC gradient in **paper IV**.

As the sublittoral pathway (i.e., sublittoral benthic macroinvertebrates) were placed intermediate of the pelagic and the littoral pathways correlation analyses were run with the primary producers from both habitats to determine the influencing sources to sublittoral stable isotope composition. Surprisingly, sublittoral benthic macroinvertebrate stable isotope composition was not correlated with δ^{13}C or δ^{15}N for either of the primary producers (i.e., seston or bulk biofilm, **paper IV**). There was, however, a near significant negative correlation between sublittoral benthic macroinvertebrate $\delta^{13}C$ and the nutrient and TOC gradient ($p = 0.056$, R = -0.542) in **paper IV**. Unfortunately, we lack the data to determine if the pattern is due to changes in baseline $\delta^{13}C$, or changes in relative contribution to consumer biomass. It is, however, likely that higher TOC concentrations would cause shading for benthic primary production and thus increase the relative role of heterotrophic processes, resulting in a more negative $\delta^{13}C$.

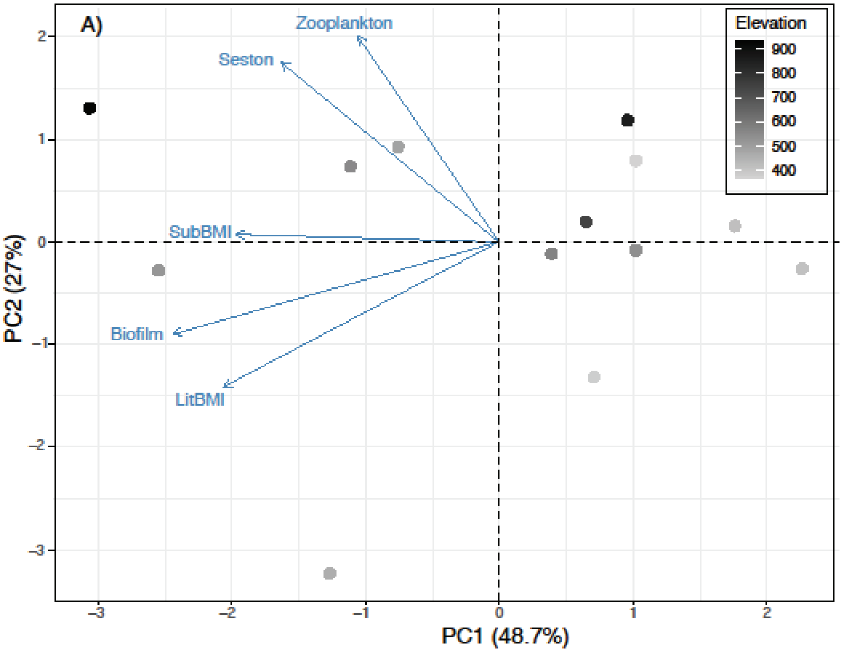

Figure 8. PCA of $\delta^{13}C$ for zooplankton, seston, bulk biofilm, littoral and sublittoral benthic macroinvertebrates from **paper IV**. Points represent individual lakes, and the color gradient shows lake elevation (as m.a.s.l.).

Temporal trends for Arctic charr $\delta^{13}C$ and $\delta^{15}N$ were studied using archived fish samples for lakes Abiskojaure, Stor-Tjulträsket, and Stor-Björsjön (Fig. 9, **paper III**). These data showed that $\delta^{13}C$ decreased markedly over time in lakes Stor-Tjulträsket and Stor-Björsjön (both sympatric lakes). As benthic macroinvertebrate $\delta^{13}C$ generally were stable over time, this was attributed to interactions among different fish species where the introduction of a fish species or increasing abundance of a competing fish species would lead the

Arctic charr to relocate (Klemetsen et al., 2003). Charr $\delta^{15}N$, on the other hand, significantly increased in lakes Abiskojaure and Stor-Tjulträsket whereas trends were not significant for the shorter time series of charr $\delta^{15}N$ in Lake Stor-Björsjön. These increasing trends in charr $\delta^{15}N$ are likely caused by different processes in the two lakes. In Lake Stor-Tjulträsket, we speculate that the increase in $\delta^{15}N$ likely was the result of interactions with other fish species. The large pelagic predatory fish Lake trout (*Salvelinus namaycush*) was introduced to a nearby lake and spread to Lake Stor-Tjulträsket around the year 1980 (Filipsson, 1994). Additionally, Eurasian minnow (*Phoxinus phoxinus*) spread to Lake Stor-Tjulträsket in the 1980's and has increased in abundance in later years. It is possible that high numbers of Eurasian minnow in the shallow habitats, in combination with an introduction of grayling (*Thymallus thymallus*), may have forced Arctic charr to gradually again shift habitat to the pelagic and/or profundal zone. This habitat shift would result in the charr feeding to a larger degree on predatory zooplankton and sublittoral/profundal benthic macroinvertebrates, who had higher $\delta^{15}N$ than littoral benthic macroinvertebrates in these lakes (see Figs. 5 and 7 in **paper III**), thus supporting this conjecture. In Lake Abiskojaure charr is the only fish species, but the observed increase in $\delta^{15}N$ could still be caused by a proportional increase of zooplankton in their diet. Sublittoral and profundal benthic macroinvertebrate biomass have decreased markedly in the lake (see above), while stomach content data have shown increasing abundance of smaller prey and zooplankton in charr stomachs from 1985 to 2020 (Skarp, 2020). This indicates that the charr has transitioned to a more zooplankton rich diet when the benthic macroinvertebrate biomass decreased.

A simple two-source mixing model, with seston and bulk biofilm $\delta^{13}C$ as endmembers, showed that Arctic charr pelagic resource use was variable over time in Lake Abiskojaure. Charr pelagic resource use was lowest during the 1980´s at approximately 40 %, this was immediately followed by the period with the highest pelagic resource use (ca 62 %) during the early to mid-1990´s, while after this the charr in Lake Abiskojaure stabilized at intermediate pelagic resource use of around 58 %. Arctic charr pelagic resource use correlated neither with TP nor DIN concentrations in Lake Abiskojaure, even though the periods with the highest TP concentrations coincided with those with highest pelagic resource use. In Lake Stor-Tjulträsket, pelagic resource use of charr increased from 69 % at the start of the time-series, via 54 % (at peak littoral resource use around the year 1990) to 82 % at the end of the time series. In Lake Stor-Tjulträsket pelagic resource use correlated positively with TP ($p < 0.001$, r = 0.63), whereas no temporal trends were found for pelagic resource use in Lake Stor-Björsjön (70-86 %). These results are in line with the conjectures above with species interactions influencing the habitat use of Arctic charr in Lake Stor-Tjulträsket, whereas for charr in Lake Abiskojaure the diet instead appear to be more influenced by prey abundances. Also, neither of the lakes had general temporal trends for primary consumer $\delta^{13}C$ or $\delta^{15}N$ (**paper III**), or for stable isotope composition for the benthic macroinvertebrates over the nutrient and TOC gradient (i.e., PCA2, **paper IV**) strengthening the argument of a changed feeding behaviour for the charr as the stable isotope composition of their prey did not change in lakes Abiskojaure, Stor-Tjulträsket, or Stor-Björsjön respectively.

Conclusions

The dramatically declining temporal trends in TP clearly show that oligotrophication is strongly affecting the nutrient concentrations in these subarctic lakes (**paper III**, Fig. 5). Still, however, the food web responses are less clear and hard to link to declining TP-concentrations (**paper III, IV**).

The finding that phytoplankton biovolume increased with increasing nutrient and TOC concentrations (i.e., PCA2) in our space-for-time study (**paper IV**) corroborates the findings of both gradient (e.g., Vadeboncoeur et al. 2003) and fertilization studies (Holmgren, 1984; Rydin et al., 2008), whereas the time series analysis show no effect of nutrient depletion on phytoplankton biovolume (**paper III**, Fig. 6). We attributed this lack of effect, at least partially, to the high proportion of mixotrophic taxa in the phytoplankton community. However, since our space-for-time study showed that the biovolume of mixotrophs increased when the total phytoplankton biovolume increases in response to higher nutrient and TOC concentrations (**paper IV**) this cannot be the sole explanation. In other words, the high proportion of mixotrophic taxa (i.e., increased phagotrophy) in the phytoplankton community might mitigate some of the negative effects of decreasing nutrient concentrations, on phytoplankton biovolume. However, this is likely is not the full explanation to the lack of temporal response in phytoplankton biovolume (**paper III**) since the biovolume of mixotrophs increased together with the total biovolume of phytoplankton with increasing nutrient and TOC concentrations in our space-for-time study (**paper IV**).

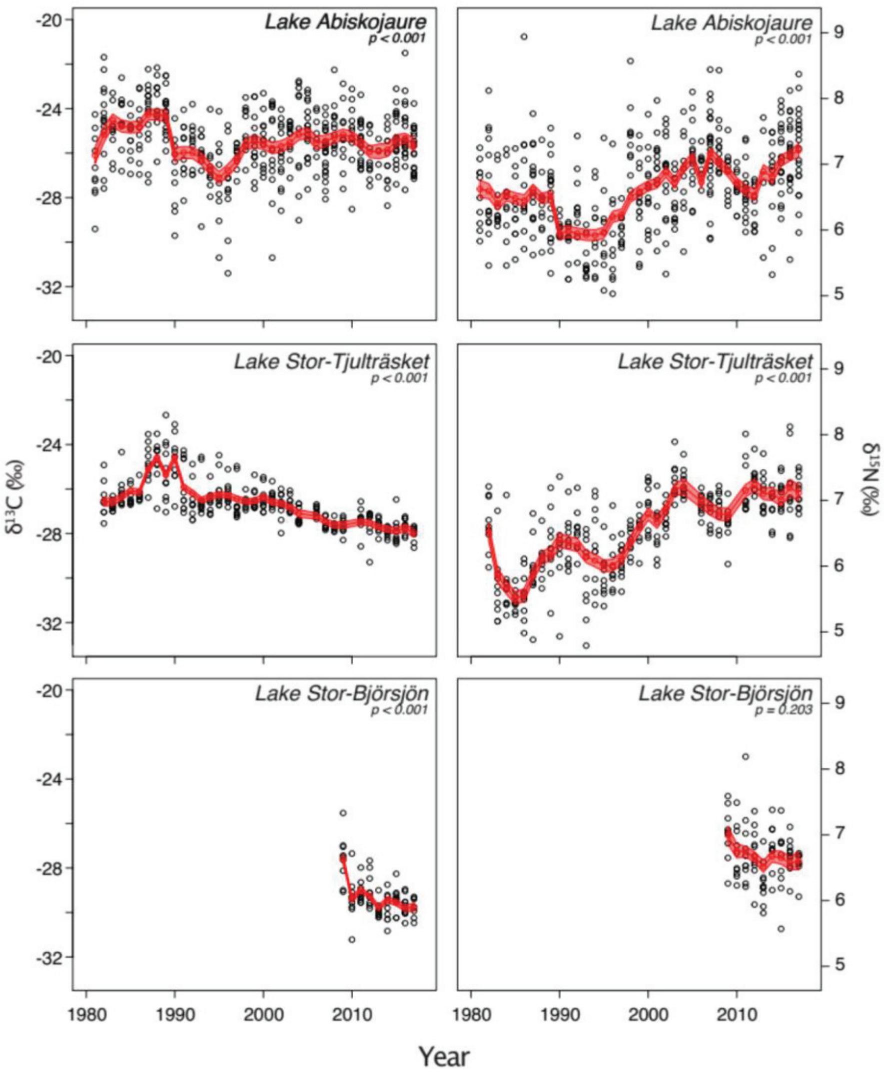

Figure 9. Time series $\delta^{13}C$ (left) and $\delta^{15}N$ (right) in Arctic charr from Lakes Abiskojaure (top panels), Stor-Tjulträsket (middle), and Stor-Björsjön (bottom). Dots represent individual fish, while the red line shows the fit of the GAM-model, and the red shade the standard error of the fit.

Also, phytoplankton production may be stimulated by increased nutrient concentrations (e.g., Rydin et al. 2008; Vadeboncoeur et al. 2003), while this not necessarily implies an increased biovolume. As an example, Rydin et al. (2008), found the strongest effects of whole lake fertilization on fish biomass, which shows that the effects on intermediate trophic levels might have been obscured by increased fish predation.

The finding that zooplankton biovolume did not respond to changes in phytoplankton biovolume or community composition (**paper IV**) in combination with the observed lack of correlation between zooplankton $\delta^{13}C$ and that of seston (**paper IV**), implies that seston is a poor predictor of zooplankton diet in these lakes. A seston samples is a bulk sample that contain particles of different size and food quality from which different zooplankters ingest different fractions (Berggren et al., 2015; Sommer & Sommer, 2006). In addition, previous studies have reported that benthic primary producers constitute an alternative basal resource for zooplankton (Karlsson & Säwström, 2009; Vadeboncoeur et al., 2002, 2005). These factors could thus explain the lack of zooplankton response to relatively strong changes in for example phytoplankton biovolume (400 times difference, **paper IV**).

Arctic charr pelagic and benthic biomass showed no strong temporal trends in lakes Abiskojaure, Njalakjaure, and Stor-Björsjön, as the observed patterns often depended on one or a few diverging years (**paper III**, Fig. 7). Arctic charr stable isotope composition did, however, change over time in all three lakes (**paper III**, Fig. 8). Charr $\delta^{13}C$ significantly decreased in lakes Stor-Tjulträsket and Stor-Björsjön, while $\delta^{15}N$ increased for charr in lakes Abiskojaure and Stor-Tjulträsket. Since the time series of benthic macroinvertebrates stable isotope composition did not show any general trends, this likely means that the charr partly altered its feeding habits/habitat (**paper III**). This is further supported by time series analysis of Arctic charr stomach contents, from Lake Abiskojaure, that showed a relative increase in the share of zooplankton over time (Skarp, 2020). This is interesting since neither charr biomass data nor pelagic resource use for the charr showed any temporal trends in Lake Abiskojaure (**paper III**). The Arctic charr population of Lake Stor-Tjulträsket were the charr population that appeared to respond most strongly to decreased nutrient concentrations, with pelagic resource use positively correlated with TP concentrations (**paper IV**). Likely, however, this correlation is merely spurious as the lake has had several introductions of fish species (e.g., Lake trout (*Salvelinus namaycush*), Eurasian minnow (*Phoxinus phoxinus*)) which has forced the charr to switch habitat.

The findings from **papers III** and **IV** together suggest that the food webs of oligotrophic subarctic lakes are relatively robust to decreasing water nutrient concentrations. This should not be interpreted as if no changes occurred, however. Benthic macroinvertebrate biomass decreased over time in the two northern most lakes (**paper III**), one with and one without fish excluding the possibility that this decrease is caused by increased fish predation. It is, however, not straight forward to explain the connection between water nutrient concentrations and benthic macroinvertebrate biomass in these lakes as the phytoplankton community to a large extent consists of phytoplankton species that are generally not considered to settle (i.e., Cryptophytes and Chrysophytes, Goedkoop and Johnson 1992). However, Rydin et al. (2008) reported increased sedimentation in the alpine Lake Mjölkvattnet during fertilization, while the phytoplankton community was dominated by Chrysophyceae (Persson et al., 2008). They reported increased biomass of both phytoplankton and zooplankton after fertilization, and it is therefore likely that the increased sedimentation of organic matter, in their study, was a combination of settling phytoplankton and of zooplankton excrements. Our two lakes where time series analysis showed declining benthic macroinvertebrate biomass are situated relatively close to each other, but with very different altitudes and catchments characteristics (Tab. 1). Neither of the lakes were extreme in terms of nutrient concentrations, but they did show a stronger decline in nutrient concentrations than lakes Njalakjaure and Stor-Björsjön which were sampled at comparable depths and had higher phytoplankton biovolumes (**paper III**), implying some sort of connection (direct or indirect) between benthic macroinvertebrate biomass and water nutrient concentrations.

Overall, however, oligotrophic clear-water lakes have a strong dependence on benthic primary and secondary productivity (Karlsson & Byström, 2005). This, in combination with the high degree of mixotrophy in the primary producers, are likely contributing factors to the relatively robust food web responses to decreasing nutrient concentrations (**paper III**, **IV**).

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4. Conclusions and Outlook

Major conclusions of this thesis are:

- Subarctic lake food webs are closely connected to climate and catchment vegetation. During periods with warmer and drier conditions benthic primary production predominates the carbon contribution to zooplankton biomass, whereas the importance of pelagic primary productivity increases during colder and wetter conditions (**paper I**).
- Although the allochthonous contribution to *Daphnia* biomass increased during periods with lower aquatic primary production, autochthonous primary production has constituted the most important food source to Daphnia biomass over the last 5800 years in Lake Diktar Erik (**paper I**).
- Within-lake $\delta^{13}C$-variability can be substantial, especially for biofilm and littoral benthic macroinvertebrates. Although our study emphasizes the existence of this variability, it was not possible to unravel its causes as it was neither explained by lake- or taxon-specific-effects, nor by algal community composition (**paper II**).
- Within-lake variability can be as large as among-lake variability for $\delta^{13}C$ and $\delta^{15}N$ in primary producers and consumers in subarctic lake food webs. This implies that local, within-lake, processes can be equally important as catchment-scale processes for stable isotope values of producers and consumers. This also highlights that there are still unknown factors that influence $\delta^{13}C$ and $\delta^{15}N$ values that significantly can affect the outcome of food web studies (**paper II**).

- The findings that zooplankton biovolumes correlated neither to phytoplankton biovolumes nor to community composition, while zooplankton $\delta^{13}C$ values were uncorrelated to those of seston, indicate that bulk seston samples are a poor proxy for zooplankton diet in these lakes (**paper IV**).
- While the dramatically declining temporal trends in TP clearly show that oligotrophication is strongly affecting the nutrient concentrations in these subarctic lakes (**paper III**) the food web responses are less clear and hard to link to oligotrophication (**paper III, IV**). A strong dependence on benthic primary and secondary production, in combination with a high degree of mixotrophy in primary producers likely lessens the impacts of decreasing nutrient concentration on the lake food web.
- The observed temporal changes in Arctic charr $\delta^{13}C$ in lakes Stor-Tjulträsket and Stor-Björsjön, and those of $\delta^{15}N$ values in lakes Abiskojaure and Stor-Tjulträsket likely originate from declines in benthic macroinvertebrate biomass (Lake Abiskojaure) and species interactions that force charr to switch to pelagic/profundal habitats (lakes Stor-Tjulträsket and Stor-Björsjön) (**paper III**).

Figure 10 shows the relationship between the lakes' TOC and TP and illustrates the trajectory of future change under continued warming, where ongoing oligotrophication drives them toward ultraoligotrophic conditions (see also Huser et al. 2018, **paper III**). This process is primarily driven by the ongoing greening of catchments (Elmendorf et al., 2012; Jenkins et al., 2020), i.e., increased growth of shrubs and mountain birch forests, and increased nutrient sequestration in terrestrial vegetation and subsequent declines in run-off. Indeed, correlations between catchment NDVI and water concentrations of TP for a set of lakes in this study are highly significant (Goedkoop et al., unpublished). This means that these mountain lakes will experience more oligotrophic conditions until forest growth and soil development will induce increased run-off of terrestrial TOC and associated nutrients and ultimately counteract the trend.

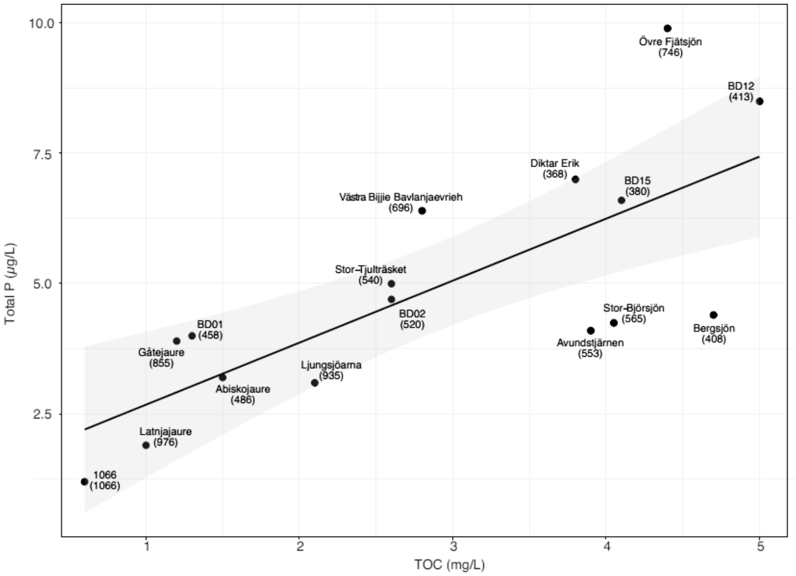

Figure 10. Relationship between total organic carbon (TOC) and total phosphorus (TP) water concentrations for the field work lakes in this thesis project (R = 0.75, $p < 0.001$). The shaded area represents the 95%-confidence limits. Numbers in parentheses is lake elevation.

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Popular science summary

The current climate change is rapidly altering temperature and precipitation regimes, resulting in more extreme weather events and higher mean temperatures. Temperature and the hydrological cycle (for example, how often it rains, if precipitation comes as rain or snow) are controlling factors for all chemical, physical, and biological processes in freshwater ecosystems. This means that climate change in a fundamental way changes the way our ecosystems function. Subarctic and Arctic regions also harbor unique, and sensitive, cold-adapted species that have evolved to live in these low-temperature environments. Subarctic and Arctic regions are, however, also the places on Earth where climate change is progressing fastest. This results in rapid environmental change and changing living environments for animals and plants.

Another consequence of climate change is increased productivity. Increasing temperatures makes plants grow faster, while at the same time more moisture and nutrients are becoming available to the plants when the previously frozen soil thaws. The landscape in subarctic and Arctic regions often have naturally low productivity, due to low temperatures and a short season for terrestrial vegetation growth. The current increasing temperatures and altered hydrological regimes now are increasing productivity (a.k.a. “greening”). When more of the of the soil nutrients are being incorporated into plant biomass this leads to less nutrients following rain- and meltwater into rivers and lakes which, in turn, leads to decreasing nutrient concentrations in the lakes (a.k.a. “oligotrophication”).

The aim of this thesis was to study how subarctic lake food webs respond to climate change, with a special focus on the effects of oligotrophication. We used long-term environmental monitoring data sets and stable isotope composition of food items and consumers to quantify climate-induced change on food webs. Analysis of carbon and nitrogen stable isotopes are commonly used in food web studies, to analyze interactions among different organisms. The carbon stable isotope ratio usually differs between different sources of carbon, for example between benthic algae and phytoplankton. The nitrogen stable isotope ratio instead changes when nitrogen from food is incorporated into consumer biomass.

In the first study we analyzed sediment composition, the photosynthetic pigment composition, and the stable carbon isotope composition for remains of the water flee *Daphnia sp*. over geological time spans. This gave us information on changes in the phytoplankton community composition, and the relative proportions of terrestrial and aquatic carbon in the diet of *Daphnia* over the last 5800 years. We then combined our data with that from other studies that have reconstructed temperature fluctuations and vegetation over the same time period. By doing so we could show that during warmer periods the daphniids mostly fed on bottom living algae whereas they predominantly consumed planktonic algae from the open water during colder and wetter periods. Regardless of the climate, algae always constituted a larger part of the *Daphnia* diet than terrestrial organic matter did.

In the second study we collected samples of algae and benthic macroinvertebrates from three different locations in each of five lakes and quantified within- and among-lake variability in carbon and nitrogen stable isotope ratios. We found that within-lake was as large as among-lake variability for both carbon and nitrogen stable isotopes. This indicates that small-scale, within-lake, processes are as important as catchment-scale processes for the carbon and nitrogen stable isotope composition of algae and benthic macroinvertebrates. We also found that variability in nitrogen stable isotope ratios often could be explained by differences between lakes or different organism groups, whereas carbon stable isotope variability often was larger and could not be attributed to differences between lakes, organisms, or even species.

In the last two studies we focused specifically on the effects of climate-induced oligotrophication on subarctic lake food webs. In one of the studies, we analyzed time series of environmental monitoring data and archived biological samples to determine temporal trends in the size and composition of algae and consumer communities. In the second study we instead sampled water nutrient concentrations, benthic algae, phytoplankton and benthic macroinvertebrates from 16 lakes along gradients in water nutrient concentrations to simulate the oligotrophication process. Time series analysis of water chemistry data clearly showed strong effects of oligotrophication on water nutrient concentrations. The concentrations of total phosphorus were approximately half in 2017 compared to those at the end of the 1980´s. However, neither time series analysis nor the gradient study revealed any strong, coherent effects on the lake food webs. We found decreasing biomass of benthic macroinvertebrates in the deep sediment of two lakes. We also found indications of altered feeding habits of Arctic charr in a few of the lakes. However, since we did not find any clear, similar trends occurring in a majority of the lake food webs we suspect that lake characteristics and species interactions influenced food web interactions and therefore the response to oligotrophication. Typically, the food web in nutrient-poor, clear-water lakes are very dependent on the benthic communities for energy and nutrients. This could also explain the relatively weak responses to the dramatic decreases in water nutrient concentrations, as the benthic communities usually are less dependent on retrieving nutrients from the water.

In this thesis I have studied multiple lakes and used approaches that span over multiple time scales. The findings provided in this thesis will contribute to increasing the knowledge of the ecological effects of ongoing, climate-induced oligotrophication on the communities and food webs of subarctic lakes.

Populärvetenskaplig sammanfattning

De pågående klimatförändringarna orsakar snabba förändringar i temperatur och nederbörd, vilket i sin tur resulterar i högre medeltemperaturer och mer högfrekvent extremväder. Temperaturen och den hydrologiska cykeln (till exempel, hur ofta det regnar, om nederbörden kommer som regn eller snö) kontrollerar alla kemiska, fysiska och biologiska processer i sötvattensekosystem. Det innebär att klimatförändringarna på ett grundläggande sätt förändrar hur våra ekosystem fungerar. I de subarktiska och arktiska regionerna finns många unika och känsliga växter och djur som är anpassade för att leva i låga temperaturer. Men de subarktiska och arktiska regioner är också de platser på jorden där klimatförändringarna går som snabbast, vilket leder till snabba förändringar av livsmiljön för växter och djur.

Ytterligare en konsekvens av klimatförändringarna är ökad produktivitet. Ökande temperaturer gör att växter växer snabbare, samtidigt som mer fukt och näring blir tillgängligt för växterna när mark som tidigare varit frusen tinar. Landskapet i subarktiska och arktiska regioner har ofta en naturligt låg produktivitet på grund av de låga temperaturerna och en kort sommarsäsong. Men på grund av de nuvarande temperaturökningarna och den förändrade hydrologiska cykeln ökar nu produktiviteten i området (så kallad "greening"). När mer av markens näringsämnen tas upp i växtbiomassa leder det till att mindre näringsämnen följer med regn- och smältvatten ner i älvar och sjöar, vilket i sin tur leder till minskande näringskoncentrationer i sjövattnet (så kallad oligotrofiering).

Syftet med den här avhandlingen var att studera hur födoväven i subarktiska sjöar reagerar på klimatförändringar, och särskilt på effekterna av oligotrofiering. Vi analyserade långa tidsserier av miljöövervakningsdata och den stabila isotopsammansättningen av föda och konsumenter för att kvantifiera hur det förändrade klimatet påverkar sjöarnas födovävar. Analys av stabila isotoper av kol och kväve används ofta i studier av födovävar för att analysera hur olika organismer interagerar med varandra. Ration mellan olika kolisotoper skiljer sig vanligtvis åt mellan olika kolkällor, till exempel mellan bottenlevande alger och växtplankton medan kvoten av olika kväveisotoperna istället ändras när kväve från födan inkorporeras i konsumenternas biomassa.

I den första studien analyserade vi sammansättningen av sediment och fotosyntetiska pigment, och den stabila kolisotopsammansättningen för viloägg av vattenloppan *Daphnia sp*. över geologiska tidsperioder. Det gav oss information om hur växtplanktonsamhällets sammansättning och de relativa proportionerna av kol från land och alger varierat i *Daphnias* kost under de senaste 5800 åren. Sedan kombinerade vi resultatet från vår studie med data från andra studier där forskare rekonstruerat hur temperaturen och vegetation varierat i området under samma tidsperiod. På så vis kunde vi visa att *Daphnia* under varmare perioder mest livnärde sig på bottenlevande alger, medan växtplankton från den öppna vattenmassan var en viktigare födokälla under kallare och blötare tidsperioder. Oavsett klimat utgjorde dock alltid alger en större del av *Daphnias* diet än organiskt material från land.

I den andra studien samlade vi in prover av alger och ryggradslösa bottenlevande djur från tre olika platser i var och en av fem sjöar och kvantifierade hur den stabila isotopsammansättningen av kol och kväve varierade inom och mellan sjöar. Vi fann att variationen inom sjöarna var lika stor som variationen mellan sjöarna för både kol- och kväveisotoperna. Det indikerar att småskaliga processer inom sjön är lika viktiga som processer i avrinningsområdet för den stabila isotopsammansättningen av kol- och kväve hos alger och bottenlevande ryggradslösa djur. Vi fann också att variationen i sammansättningen av kväveisotoperna ofta kunde förklaras av skillnader mellan sjöar eller olika organismgrupper, medan variationerna i kolisotopsammansättningen ofta var större och inte kunde förklaras av skillnader mellan sjöar, organismer eller ens arter.

I de två sista forskningsstudierna fokuserade vi specifikt på effekterna av klimatinducerad oligotrofiering på födovävarna i subarktiska sjöar. I den ena studien analyserade vi långa tidsserier av miljöövervakningsdata och arkiverade biologiska prover för att studera trender över tid i storleken och artsammansättningen av alg och konsumentsamhällen. I den andra studien tog vi istället prov på näringskoncentrationerna i vattnet, och på bottenlevande alger, växtplankton och bottenlevande ryggradslösa djur från 16 sjöar längs gradienter i vattennäringskoncentrationer för att på så vis simulera oligotrofieringsprocessen. Tidsserieanalys av vattenkemidata visade tydligt starka effekter av oligotrofiering på näringsnivåerna i sjövattnet. Halterna av totalfosfor var ungefär hälften år 2017 i jämförelse med nivåerna i slutet av 1980-talet. Men varken tidsserieanalysen eller gradientstudien visade några starka, gemensamma effekter på sjöarnas födovävar. Våra resultat visade minskande biomassa av ryggradslösa djur på de djupa bottnarna i två sjöar. Vi hittade också indikationer på förändrade födoval hos röding i några av sjöarna. Men eftersom vi inte hittade några tydliga trender som var gemensamma för en majoritet av sjöarna, misstänker vi att sjöarnas individuella sammansättning arter, och sjöarna fysiska egenskaper (t.ex. storlek och djup) påverkade födovävarna och på så vis också födovävarnas respons på oligotrofiering. Dessutom är födovävarna i näringsfattiga klarvattensjöar mycket beroende av energi och näring från bottenlevande alger och ryggradslösa djur vilket också skulle kunna förklara den relativt svaga responsen på den dramatiska minskningen av näringskoncentrationerna i sjöarnas vatten. Bottenlevande växter och djur är nämligen mindre beroende av näringsämnen från vattnet.

I den här avhandlingen har jag studerat ett flertal sjöar och använt mig av metoder som spänner över flera tidsskalor. Resultaten i den här avhandlingen kommer att bidra till att öka kunskapen om hur den pågående, klimatinducerade oligotrofieringen påverkar födoväven i subarktiska sjöar.

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Climate-induced changes in carbon flows across the plant-consumer interface in a small subarctic lake

Simon Belle[1*], Jenny L. Nilsson[1], Ilmar Tõnno[2], Rene Freiberg[2], Tobias Vrede[1] & Willem Goedkoop[1]

Reconstructions of past food web dynamics are necessary for better understanding long-term impacts of climate change on subarctic lakes. We studied elemental and stable isotopic composition of sedimentary organic matter, photosynthetic pigments and carbon stable isotopic composition of *Daphnia* (Cladocera; Crustacea) resting eggs ($\delta^{13}C_{Clado}$) in a sediment record from a small subarctic lake. We examined how regional climate and landscape changes over the last 5800 years affected the relative importance of allochthonous and autochthonous carbon transfer to zooplankton. Overall, $\delta^{13}C_{Clado}$ values were well in line with the range of theoretical values of aquatic primary producers, confirming that zooplankton consumers in subarctic lakes, even in the long-term perspective, are mainly fuelled by autochthonous primary production. Results also revealed greater incorporations of benthic algae into zooplankton biomass in periods that had a warmer and drier climate and clearer water, whereas a colder and wetter climate and lower water transparency induced higher contributions of planktonic algae to *Daphnia* biomass. This study thus emphasizes long-term influence of terrestrial-aquatic linkages and in-lake processes on the functioning of subarctic lake food webs.

Arctic/subarctic ecosystems are exposed to rapid and extensive changes driven by accelerated warming[1], including changes in vegetation cover, biomass and productivity (i.e. the greening of the Arctic[2];), and permafrost thaw[3]. These climate-driven landscape transformations dramatically affect run-off patterns, biogeochemical cycles and organic matter dynamics, potentially triggering cascading effects acting at regional and global scales[4,5]. Arctic/subarctic landscapes are also characterized by a high density of lakes and water courses, exceeding 4% of the total land area[6]. Direct effects of warming on lakes affect both their physical (e.g. timing and duration of ice cover[7]), chemical (e.g. changes in organic matter inputs and nutrients run-off[8,9]) and biological (e.g. community composition[10]) properties. In northern landscapes, surface waters also play a key role in the transport of detrital materials, nutrients and organic matter[11,12], thus indirectly determining the relative role of allochthonous (terrestrial) and autochthonous food resources available for aquatic consumers.

Energy flows through aquatic food webs are of fundamental importance for the function of lake ecosystems[13], and are a key to our understanding of their response to global change. Biological communities in arctic/subarctic lakes are often species-poor and have simpler food webs than those of temperate lakes[14]. Carbon transfer through food webs is derived either from autochthonous primary production (i.e., phytoplankton and benthic algae[15,16],), from allochthonous detrital inputs and/or from remobilized dissolved organic carbon by bacteria[17]. However, due to the lack of long-term contemporary data of subarctic lakes, it is still unclear to what extent climate change affects the relative importance of these trophic processes. Hence, past dynamics of aquatic ecosystems from paleo-data provide key information that can contribute to our understanding of lake responses to changes[18].

Recent paleolimnological studies conducted on subarctic lakes have shown complex relationships between changes in terrestrial vegetation in the watershed, inputs of allochthonous organic matter, changes in in-lake light regime and taxonomic shifts in the assemblages of aquatic primary producers[19–21]. However, identification of past energy pathways within subarctic lake food webs has been less extensively studied[21]. Innovative approaches using stable isotope composition (mainly carbon, nitrogen and hydrogen) of invertebrate remains archived in lake sediment records have recently been developed to reconstruct past energy flows to aquatic primary consumers[22–24]. Specifically, Cladocera (Crustacea) form an important trophic link between basal resources (i.e.,

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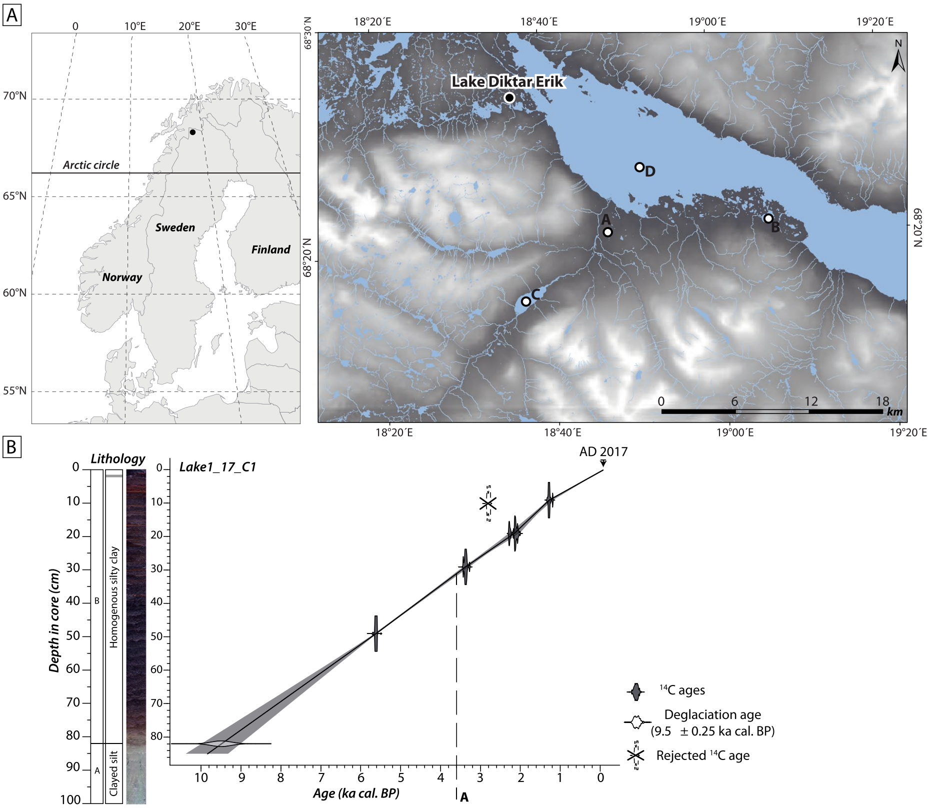

Figure 1. (**A**; left) Location of the study region showing the sampling site (black circle; Lake Diktar Erik). (right) Elevation map over the study region showing the major lakes, streams and rivers. Black circle marks the sampling site (Lake Diktar Erik), whereas open ones indicate locations of selected paleolimnological studies from the area (**A**: Vuolep Njakajaure, **B**: Vuoskkujávri, **C**: Abiskojaure and **D**: Torneträsk). (**B**) Picture, lithological description and age–depth model (linear interpolation) of the sediment core retrieved from Lake Diktar Erik. Letter "A" refers to major changes reported in sediment, pigment and cladoceran data (see Fig. 5).

primary producers, bacteria) and fish, and stable isotopic composition of their sclerotized remains (e.g. resting eggs or ephippium) is thus a good indicator of long-term changes in aquatic food webs[25]. These paleolimnological approaches help to provide insight in future trajectories of lake food webs under ongoing climate and land-cover change in Arctic and subarctic regions.

The main objective of this study was to investigate the past relationships between climate change, landscape development and carbon flows to zooplankton in a subarctic lake. We analysed sediment composition and photosynthetic pigments, as well as carbon stable isotope composition of *Daphnia* (Cladocera; Crustacea) resting eggs to reconstruct past dynamics of sedimentary organic matter, autochthonous primary production and carbon resources available to Cladocera under different climatic conditions. Results were then compared with those of previous paleolimnological studies that focused on climate and landscape changes to estimate the relative importance of in-lake impacts *vs.* catchment-mediated processes on energy flows through subarctic food webs.

Methods

Study site. Lake Diktar Erik (68°26′43″N, 18°36′50″E) is a small lake (0.1 km^2) located in northern Sweden (Fig. 1A). The bedrock geology in the region is predominated by granite and its metamorphic products, while the prevailing catchment vegetation consists of mountain birch forest (*Betula pubescens* ssp. *tortuosa*). The lake is located at 375 m a.s.l. and has a maximum water depth of 16 m (Secchi depth of 6 m). The lake is oligotrophic and slightly humic, with a pH of 6.3, conductivity of 14.7 µS.cm^{-1}, and concentrations of total organic carbon of 3.6 mg.L^{-1}, total phosphorus (TP) of 5 µg P L^{-1} and total nitrogen (TN) of 206 µg N L^{-1} recorded in 1997[26]. Similar

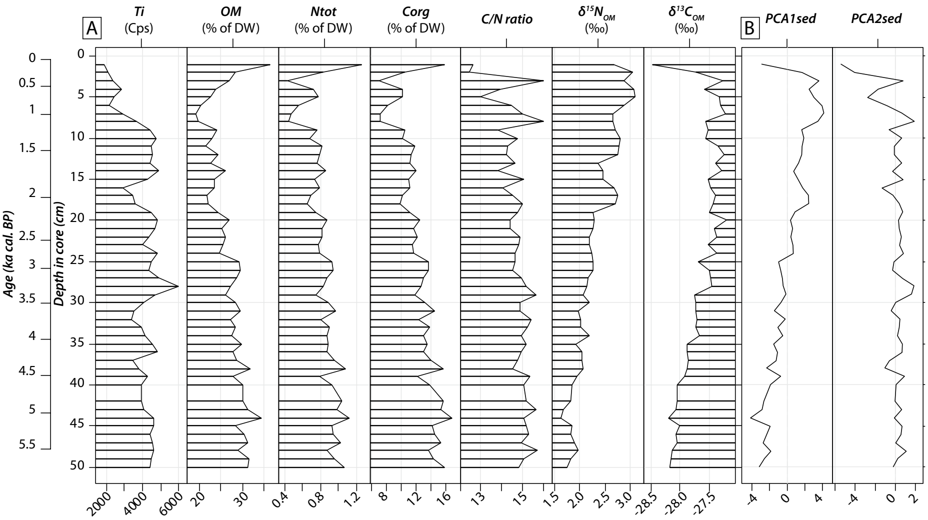

Figure 2. (**A**) Trends in titanium intensities in lake sediments (Ti; cps), organic matter concentration (OM; % of dry weight), total nitrogen (N_{tot}; % of dry weight), total organic carbon (C_{org}; % of dry weight), atomic ratio of organic carbon to total nitrogen (C/N), stable nitrogen isotopic composition of sedimentary organic matter ($\delta^{15}N_{OM}$; ‰), and stable carbon isotopic composition of sedimentary organic matter ($\delta^{13}C_{OM}$; ‰). (**B**) Temporal trends in scores of first and second principal component analysis axes ($PCA1_{sed}$ and $PCA2_{sed}$) performed on sediment data. Age and depth scales are both given on y-axes.

concentrations of DOC: 4.2 mg L^{-1}, total phosphorus: 4 µg TP L^{-1} and total nitrogen: 270 µg TN L^{-1} for this lake were also reported by Karlsson *et al.*[27].

Deglaciation of the surroundings of Lake Diktar Erik was reported at approximately 9.5 ka cal. BP[28–30]. Then, favourable climatic conditions allowed rapid transition from a vegetation-free landscape to forest vegetation during the early Holocene (*ca.* 9.5–6.5 ka cal. BP)[29–31]. This initial phase of landscape development was followed by a climatically stable and warm period during the Holocene Thermal Maximum (*ca.* 6.5–3.5 ka cal. BP), marked by widespread developments of pine forests. The late Holocene period (*ca.* 3.5–0.05 ka cal. BP) was instead characterised by a long-term marked cooling and wetter conditions inducing a gradual replacement of pine forest by a mountain birch forest similar to that present nowadays[30–32]. Previous paleoecological studies from the area have not identified distinguishable environmental impacts in response to early human activities[33]. During the 20th century, rail tracks and road constructions have occurred along the southern, downstream shore of the lake. Thus, Holocene development of northern Sweden provides an exceptional natural experiment to study how climate and landscape changes affected terrestrial-aquatic linkages and carbon flows across the plant-consumer interface in subarctic lakes.

Sediment sampling. In August 2017, a 100-cm sediment core was retrieved from the largest depth of the lake using a gravity corer (9 cm of diameter; UWITEC). As terrestrial macrofossils were absent in the collected core, radiocarbon dates were determined on five bulk sediment samples measured using an accelerator mass spectrometer at the Poznan Radiocarbon Laboratory (Poland) and The Tandem Laboratory (Sweden; Table 1). In addition, we constrained the marked transition between proglacial and lacustrine sediments (Fig. 1B) using the deglaciation age (*ca.* 9.5 ± 0.25 ka cal. BP) reported by previous paleolimnological studies conducted in the region[34,35]. Age-depth modelling, combining calibrated radiocarbon dates and deglaciation age, was performed using simple linear interpolation (Clam package for R[36];).

Sedimentological and sedimentary pigment analysis. The sediment core was vertically split in two halves, and one split core surface was covered with Ultralene® foil to avoid desiccation and contamination, and scanned every 3 mm using an ITRAX XRF Core Scanner at the University of Stockholm (Cox Analytical Systems). XRF measurements were carried out using a Mo tube, set at 30 kV and 30 mA, for 60 s to detect relative concentrations of selected major elements (Ti, Fe, Mn, Si, Al). Elemental intensities were expressed as counts per unit time per unit area (cps).

The other split core was continuously and horizontally sliced down to 50 cm depth into 1 cm thick sub-samples that were subjected to further analyses. Organic matter concentration (OM) was analysed using the loss-on-ignition method, and results were expressed as percentage of dry weight (hereafter; % of dry weight). In

Depth (cm)	Lab code	Material	yr BP	Model
9	Ua-62416	Bulk	1356 ± 31	I
10	Poz-99924	Bulk	2685 ± 30	R
19	Ua-62417	Bulk	2147 ± 31	I
29	Poz-99925	Bulk	3145 ± 30	I
49	Poz-99926	Bulk	4885 ± 35	I

Table 1. Radiocarbon dates (±1 standard deviation) of the sediment sequence (Lake1_17_C1) from Lake Diktar Erik. In column Model, "R" refers to rejected date, and "I" to included dates.

addition, sediment samples were analysed for carbon and nitrogen stable isotopes (depicted as $\delta^{13}C_{OM}$ and $\delta^{15}N_{OM}$, respectively), carbon and nitrogen concentrations (C_{org} and N_{tot}), and C/N weight ratios. Prior to analysis, sediment samples were dried (at 60 °C for 72 h), ground, and 3 mg of dried sediments were transferred to tin capsules. $\delta^{13}C_{OM}$ and $\delta^{15}N_{OM}$ were analysed using an Isotope Ratio Mass Spectrometer interfaced with an Elemental Analyser (EA-IRMS) at our Stable Isotope Laboratory (Umeå, Sweden). Results were expressed as the delta notation with Vienna Pee Dee Belemnite and atmospheric nitrogen as standards: $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$; where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Sample measurement replications from internal standards (wheat and maize flour) produced analytical errors (1σ) of ±0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$ values ($n = 24$).

Photosynthetic sedimentary pigments were analysed as a paleo-proxy of aquatic primary production[20], following the method by[37]. Briefly, sediment samples were freeze-dried, and pigments were extracted at −20 °C in the dark during 24 h using a solution of acetone and methanol (80:20 V:V). Extracts were then clarified by filtration through a 0.45 µm Millex-LCR hydrophilic PTFE membrane filter before chromatographic analysis. Reversed-phase high–performance liquid chromatography (RP-HPLC) was applied to separate pigments. A Shimadzu Prominence (Japan) series binary gradient system with a photodiode array (PDA) and fluorescence detectors was used (see[38] for details). Peak identification and quantification were made by commercially available external standards from DHI Company (Denmark). Chlorophyll *a* (Chl *a*) and its derivative pheophytin *a* (Phe *a*) were selected to indicate the overall algal biomass[37,39]. The Chl *a*/ Phe *a* ratio was used to assess pigment preservation in lake sediments, and ratios are expected to remain relatively stable over time if stabile preservation conditions occur[40]. Taxon-specific pigments were used to indicate the biomass of aquatic primary producer's classes: lutein (Lut) for green algae (Chlorophyceae), fucoxanthin (Fuco) for diatoms (Bacillariophyceae), alloxanthin (Allo) for cryptophytes (Cryptophyteae[39],) and canthaxanthin (Cantha) for cyanobacteria (Cyanophyceae[41],). Pigment concentrations are expressed as nanomoles per gram of sediment organic matter (nmol g^{-1} OM).

Carbon stable isotope analysis of cladoceran remains.

Stable isotope analyses were performed on resting eggs of *Daphnia* ssp. morphotype retained from lake sediment layers and identified using the photograph book of Szeroczyńska and Sarmaja-Korjonen[42]. Sediment samples were deflocculated in NaOH (10%) solutions, pre-treated using washing with HCl (10%) solutions and sieved through a 100-µm mesh according to standard protocol of Perga[22]. Resting eggs were sorted out under a dissection microscope until approximately 50 eggs or a mass of about 60 µg (minimal mass required for stable isotope analysis) was gained. If resting egg abundances in a single sediment layer were too low, then the next consecutive sediment layer was pooled to the sample. Carbon stable isotopic composition of cladoceran remains ($\delta^{13}C_{Clado}$) was then analysed using an EA-IRMS at INRA Nancy (Champenoux) expressed according to the delta notation (see above). Replication of sample measurements from internal laboratory standards produced analytical errors (1σ) of ±0.2‰ ($n = 15$).

Data analysis.

Two separate principal component analyses (PCA) were performed on sediment and pigment data, respectively. PCA axis significance was checked using the broken-stick model[43]. Pigment concentrations and sediment composition were expected to have significant, and potentially non-linear, influences on food resources for zooplankton and, therefore, $\delta^{13}C_{Clado}$ values. Statistical relationships between $\delta^{13}C_{Clado}$ values and PCA1 scores performed on sedimentological and pigment data ($PCA1_{sed}$ and $PCA1_{pig}$, respectively) were examined using a generalized additive model (GAM; fitted using the *mgcv* package for R[44];) approach, with a continuous-time, first-order autoregressive process to account for temporal autocorrelation[45]. Significance of fitted trends was checked using standard statistical inferences for GAM. All statistical analyses and plots were performed using the R 3.5.2 software[46].

Results

Past changes in sediment composition.

The transition from proglacial to lacustrine sedimentation could be well observed in our sediment core as a colour-change from dark grey at the bottom to dark brown towards the surface (Fig. 1B). All calibrated radiocarbon ages consistently increase with depth in core, except at 10 cm (Table 1), and this radiocarbon age was therefore not included in the final model as it would lead to age-reversal and/or abrupt change in sedimentation rates not supported by sedimentological observations (Fig. 1B). This radiocarbon age also conflicted with another date at 9 cm depth (Table 1) which fits well the age model. Hence, 81 cm of sediments from Lake Diktar Erik covered the last *ca*. 9500 years, corresponding to an average sedimentation rate of about 0.08 mm.yr^{-1}. Titanium intensities in lake sediments were relatively stable at approximately 4500 cps from 50 to 10 cm sediment depth, and then gradually decreased to 2000 cps at the sediment surface (Fig. 2A). Organic matter concentration (OM) ranged 19.1–36.5%, while C_{org} and N_{tot} concentrations in sediments ranged 6.9–16.9% and 0.5–1.2%, respectively (Fig. 2A). Overall, OM, C_{org} and N_{tot} concentrations

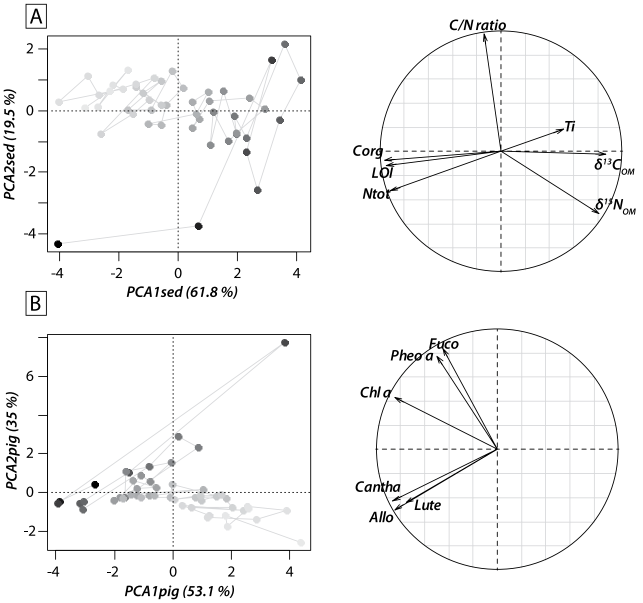

Figure 3. Factorial map of principal component analyses (PCA1 *vs*. PCA2) performed on (**A**) sedimentological data and (**B**) individual sedimentary pigment data. A grey-scale was used to identify the sample age: light-grey colours correspond to the oldest samples, whereas black symbols represent the youngest samples. Correlation circles representing variable contributions to the first two axes of the PCA.

were higher in the oldest part of the record (*ca*. 5.8–3.5 ka cal. BP), and followed a gradual decrease over time (from *ca*. 3.5 to 0.05 ka cal. BP). Interestingly, however, these declining trends reversed and showed conspicuous peaks in the most recent sediment layers. The sediment weight C/N ratio ranged 12.5–16.0 with the highest values observed from *ca*. 5.8 to 3.5 ka cal. BP (Fig. 2A). $\delta^{15}N_{OM}$ values ranged 1.6–3.1‰, and those of $\delta^{13}C_{OM}$ ranged from −28.5 to −27.2‰. $\delta^{13}C_{OM}$ and $\delta^{15}N_{OM}$ temporal trends were similar, with gradual increases over time, except for the uppermost samples where instead decreases were noted (Fig. 2A).

The first two PCA axes explained 61.8% and 19.5%, respectively, of the total variance of sediment data. $PCA1_{sed}$ axis was strongly correlated with OM, N_{tot}, C_{org}, $\delta^{15}N_{OM}$ and $\delta^{13}C_{OM}$ (Fig. 3A). Negative values on $PCA1_{sed}$ axis represented organic-rich sediment layers with low $\delta^{15}N_{OM}$ and $\delta^{13}C_{OM}$ values. $PCA2_{sed}$ axis predominantly explained C/N ratios (Fig. 3A), with negative values representing samples with low C/N ratios. $PCA1_{sed}$ scores followed a gradual increase over time, switching from negative to positive values at *ca*. 3.4 ka cal. BP (Fig. 2B), whereas $PCA2_{sed}$ scores showed no specific temporal trend, except a conspicuous decrease observed from 8 cm and upward (Fig. 2B).

Trends of sedimentary pigment concentrations. Chl *a*/Phe *a* ratios showed high values in deep sediment layers, but gradually declined from 50 to 13 cm depth in the core (Fig. 4A). The highest photosynthetic pigment concentrations, 132.0 nmol g^{-1} OM and 43.1 nmol g^{-1} OM, respectively, were found for Phe *a* and Chl *a* (Fig. 3A). Allo and Lute, indicating cryptophytes and green algae, respectively, were the most concentrated taxon-specific pigments (ranging 2–9 nmol g^{-1} OM, Fig. 4A). Temporal trends of pigments were almost consistently similar, higher during the oldest part of the record (*ca*. 5.8–3.5 ka cal. BP), and then decreasing gradually to present day. However, Fuco instead showed slightly higher values from *ca*. 3 ka cal. BP to present-day, indicating an increase in diatoms. The first two axes of the PCA applied to sedimentary pigment data accounted for 53.1% and 35.0% of the total variance, respectively. $PCA1_{pig}$ axis explained Chl *a*, Allo, Cantha and Lute (Fig. 3B). Negative values on the $PCA1_{pig}$ axis represent pigment-rich sediment layers. $PCA2_{pig}$ axis predominantly explained Pheo *a* and Fuco (Fig. 3B), with positive values representing sediment samples with high Fuco and Pheo *a* concentrations. $PCA1_{pig}$ scores followed a gradual decrease over time, switching from positive to negative values around 3.5 ka cal. BP (Fig. 4B) suggesting a decline in concentrations of Chl *a*, Allo, Cantha and Lute, whereas $PCA2_{pig}$ scores followed a small increase over time (Fig. 4B).

Stable C isotope in cladoceran resting eggs. $\delta^{13}C$ values of *Daphnia* resting eggs ranged from −30.6 to −27‰ (Fig. 5C), and the lowest $\delta^{13}C_{Clado}$ value was found for the uppermost sediment layer (−30.6‰; Fig. 5C). Based on the temporal trends, two distinct patterns were identified in the uppermost 50 cm of the core. The

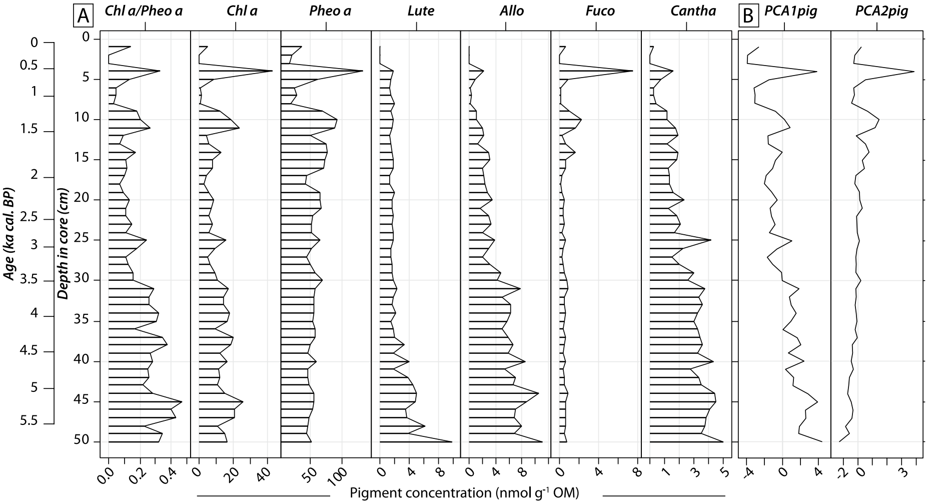

Figure 4. (**A**) The stratigraphic diagram shows temporal trends of investigated sedimentary pigments. Individual pigments are expressed in terms of nanomoles per gram of organic matter (nmol g^{-1} OM). Pigment names are abbreviated as follows (from left to right): chlorophyll *a*: Chl *a*, pheophytin *a*: Pheo *a*; lutein: Lut, alloxanthin: Allo, fucoxanthin: Fuco, canthaxanthin: Cantha. (**B**) Temporal trends in scores of first and second principal component analysis axes ($PCA1_{pig}$ and $PCA2_{pig}$) performed on sedimentary pigment data. Age and depth scales are both given on y-axes.

$\delta^{13}C_{Clado}$ values first increased from −30.6 to −28.8‰ between 5.8 and *ca.* 3 ka cal. BP, and then decreased to values around −30.2‰ after *ca.* 3 ka cal. BP. GAM showed that $PCA1_{sed}$ and $PCA1_{pig}$ covariates explained 56.9% of the overall variability of $\delta^{13}C_{Clado}$ values (*p*-value < 0.001). Relationships between $\delta^{13}C_{Clado}$ values and covariates were non-linear (Fig. 6). $PCA1_{sed}$ showed a monotonic and positive relationship with $\delta^{13}C_{Clado}$ values (F = 7.2; *edf* = 1.39; Fig. 6A), whereas $PCA1_{pig}$ scores were unimodally related to them (F = 7.9; *edf* = 1.89; Fig. 6B). The $PCA1_{pig}$ fitted function showed a positive relationship with $\delta^{13}C_{Clado}$ values for negative $PCA1_{pig}$ scores, and a negative relationship with $\delta^{13}C_{Clado}$ values for positive $PCA1_{pig}$ scores. Therefore, major shift in pigment response curve occurred for $PCA1_{pig}$ scores observed at *ca.* 3.5 ka cal. BP (Figs. 5 and 6).

Discussion

We reconstructed the long-term development of Lake Diktar Erik over the last 5800 years based on sedimentary organic matter and photosynthetic pigment analyses. Our results showed that during the Holocene Thermal Maximum (5.8–3.5 ka cal. BP), steady organic matter inputs from the surrounding watershed were revealed by high OM and C_{org} concentrations and high C/N ratios in Lake Diktar Erik sediment core (Fig. 2). Moreover, the highest pigment concentrations in the Lake Diktar Erik record were also observed during this period (Fig. 4), a period that otherwise showed a general decline in all pigment concentrations (Fig. 4). This decline in autochthonous primary production was likely driven by the onset of the cooling phase around 5 ka cal. BP in Fennoscandia (Fig. 5D[45]; but see also[21,28,29]). Then, the climate during the late Holocene period (*ca.* 3.5–0.05 ka cal. BP) was characterised by long-term cooling and wetter conditions (Fig. 5D)[47], inducing a gradual replacement of pine forest by a mountain birch forest similar to that present nowadays (Fig. 5E–H)[30–32]. These results were well in line with successional developments of similar lakes in northern Sweden (see also[28,30,32]), and showed three distinct development phases. Our results also showed a strong decline in OM and pigment concentrations (Figs. 2 and 4), suggesting marked decreases in both terrestrial and aquatic productivity, as previously reported from pigment and diatom dynamics[21,29] and sedimentological records from other subarctic lakes[30,31]. Moreover, the ongoing trend towards more oligotrophic conditions revealed from long-term monitoring of Swedish lakes[11] corroborate this observation. Whereas the overall trend in $PCA1_{sed}$ scores followed a gradual increase over time, switching from negative to positive values, the uppermost sediment sample instead showed the most negative $PCA1_{sed}$ value of the time series (Fig. 5A), likely due to the effects of diagenetic alteration of both elemental and isotopic compositions of sedimentary organic matter (see also[48,49]).

The $\delta^{13}C$ values of *Daphnia* resting eggs ($\delta^{13}C_{Clado}$) ranged from −30.6 to −27‰, and were lower than those of organic matter (Fig. 5C), implying that zooplankton utilised an isotopically lighter food source than the overall sedimentary organic matter. $\delta^{13}C_{OM}$ values have been widely used as a reliable proxy of sediment composition, reflecting the relative contribution of organic matter from aquatic and terrestrial origins, as autochthonous primary producers usually exhibit lower $\delta^{13}C$ values than those of terrestrial organic matter[50]. Therefore, *Daphnia* of

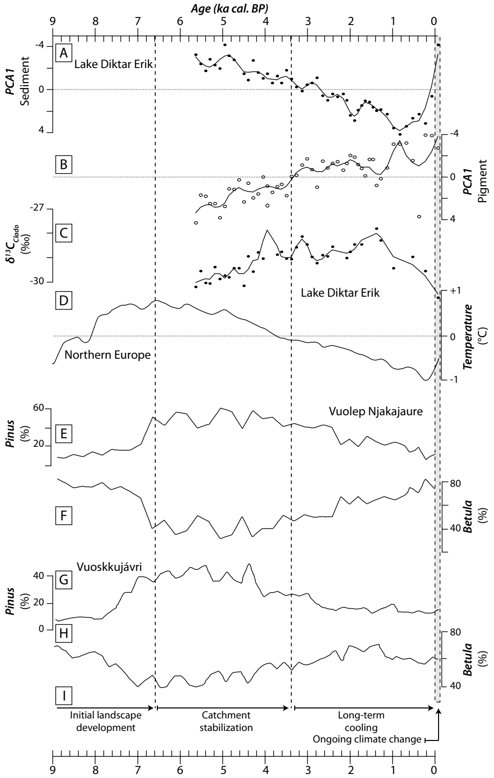

Figure 5. (**A**) Sediment PCA1 scores, (**B**) pigment PCA1 scores, (**C**) carbon stable isotopic composition of cladoceran remains in sediment core Lake1_17_C1 from Lake Diktar Erik ($\delta^{13}C_{Clado}$; ‰), (**D**) pollen-based temperature variability for Northern Europe[47], percentage of pollen from (**E**) *Pinus sylvestris* and (**F**) *Betula pubescens* in sediment core of Vuolep Njakajaure (letter A in Fig. 1[30]), percentage of pollen from (**G**) *Pinus sylvestris* and (**H**) *Betula pubescens* in sediment core of Vuoskkujávri (letter B in Fig. 1[31]), and (**I**) vertical dashed lines dividing the stratigraphy into 4 phases: initial landscape development (*ca*. 9.5–6.6 ka cal. BP), catchment stabilization (*ca*. 6.6–3.4 ka cal. BP), long-term cooling (*ca*. 3.4–0 ka cal. BP) and ongoing climate change (adapted from[28]).

Lake Diktar Erik likely has preferentially assimilated ^{13}C-depleted aquatic primary producers, and our study thus confirms that zooplankton consumers in subarctic lakes, even in the long-term perspective, have been mainly fuelled by autochthonous primary production (see also[51–54]). The $\delta^{13}C_{Clado}$ values were also closely correlated to changes in sediment composition ($PCA1_{sed}$ scores) and in-lake primary production ($PCA1_{pig}$ scores), but we found a major change in their relationships with in-lake primary production occurring at *ca*. 3.5 ka cal. BP (Figs. 5B and 6B). This pattern suggest a strong influence of autochthonous primary production on *Daphnia* feeding habits, and these results could strengthen previous findings that zooplankton diet and algal dynamics are closely linked in subarctic food webs.

Based on long-term trends in Lake Diktar Erik and previous paleolimnological investigations of other lakes from the area, we identified the potential mechanisms of these observed patterns. Between 5.8 and *ca*. 3.5 ka cal. BP, $\delta^{13}C_{Clado}$ values increased from −30.6 to −28.8‰, and were positively correlated to a decrease in autochthonous primary productivity (Fig. 6A). This increase in $\delta^{13}C_{Clado}$ values could thus reflect a slight increase in the relative contribution of ^{13}C-enriched terrestrial organic matter to *Daphnia* biomass. Our study thereby strengthens previous findings that the relative contribution of allochthonous organic matter to consumer biomass largely increased in unproductive lake food webs (Fig. 6[55,56]). After *ca*. 3.5 ka cal. BP, $\delta^{13}C_{Clado}$ values decreased and correlated negatively with pigment data (Fig. 6B). Several studies of similar subarctic lakes during this period have demonstrated a taxonomic shift in algal assemblage composition (from benthic- *vs*. pelagic-dominated algal assemblages) induced by increased inputs of terrestrial DOC and a decline in the phototrophic zone of the lake[19,21]. Specifically, there is evidence that cooler and wetter climate conditions reported in Fennoscandia

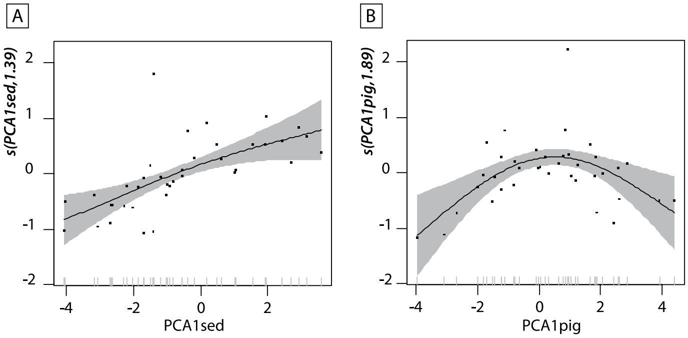

Figure 6. Fitted smooth function between explanatory variables (**A**: PCA1$_{sed}$ and **B**: PCA1$_{pig}$) and $\delta^{13}C_{Clado}$ values from a generalized additive model (GAM), with a continuous-time first-order autoregressive process to account for temporal autocorrelation. Grey surface marks the 95% uncertainty interval of the fitted function. On the x-axis, black ticks show the distribution of observed values for variables. Numbers in brackets on the y-axis are the effective degrees of freedom (*edf*) of the smooth function.

during the late Holocene induced a change in catchment vegetation, increased transport of DOC to lakes and a subsequent functional predominance of benthic to pelagic algae[19,21]. Our observed trends in sediment composition and pigment concentrations (Figs. 2 and 4) are typical for this type of lakes[19,21], and we therefore conjecture that a similar shift from a benthic- to a pelagic-dominated algal assemblages occurred in Lake Diktar Erik during this period. As phytoplankton usually is more ^{13}C-depleted than benthic algae[57,58] and terrestrial organic matter[50], an observed decrease in $\delta^{13}C_{Clado}$ values would therefore indicate a higher contribution of planktonic algae to zooplankton biomass. Results suggested that the diet of *Daphnia* in subarctic lakes, even in the long-term perspective, depends on both availability (i.e. standing stock) and quality of food resources (i.e. allochthonous *vs*. autochthonous; benthic *vs*. pelagic), as previously demonstrated at seasonal scale (see also[59]). Our study thus revealed the long-term influence of terrestrial-aquatic linkages and in-lake processes on the functioning of subarctic food webs.

In this study, we examined how regional climate and landscape changes over the last 5800 years affected the relative importance of allochthonous and autochthonous carbon transfer to zooplankton in a subarctic lake. Our study revealed complex interplays between climate-induced change in in-lake (through algal productivity and assemblage composition) and catchment-mediated (through changes in allochthonous DOC and vegetation composition) processes in the functioning of planktonic food webs of a small subarctic lake. The results showed greater incorporations of benthic primary production into zooplankton biomass in periods that had a warmer and drier climate and clearer water, whereas colder and wetter climates and lower water transparency led to higher relative scontributions of planktonic algae into zooplankton biomass. Hence, our results demonstrate that paleolimnological studies can be a powerful approach for further exploring impacts of climate change on biogeochemical cycles and terrestrial-aquatic linkages in subarctic environments.

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Author contributions

S.B., T.V., J.N. and W.G. designed the study. S.B. analysed all samples with the exception of pigment analysis performed by I.T. and R.F. S.B. wrote the paper with substantial contribution from all co-authors.

Competing interests

The authors declare no competing interests.

Additional information

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Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2022:47

Northern lakes are currently experiencing rapid rates of change. This thesis explored the relationship between climate and trophic interactions in lake food webs over several temporal and spatial scales. The results of this study showed a close and complex relationship between lake food webs and climate fluctuations. While climate-induced oligotrophication dramatically decreased total phosphorus concentrations lake food web responses were weak and none uniform among lakes. The results further showed that stable isotope variability was similar within and among lakes.

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