

Long-term resilience in microcrustacean communities despite environmental changes

M. EIE,¹ T. C. JENSEN,² B. WALSING,² A. J. EIE,³ AND D. O. HESSEN^{4,†}

¹County Governor of Agder, Box 788 Stoa, Arendal 4809 Norway

²Norwegian Institute for Nature Research, Sognsveien 68, Oslo 0855 Norway

³Seljeveien 8, Ski 1406 Norway

⁴Department of Biosciences and Centre for Biogeochemistry in the Anthropocene, University of Oslo, Box 1066, Blindern, Oslo 0316 Norway

Citation: Eie, M., T. C. Jensen, B. Walseng, A. J. Eie, and D. O. Hessen. 2021. Long-term resilience in microcrustacean communities despite environmental changes. *Ecosphere* 12(7):e03676. 10.1002/ecs2.3676

Abstract. The ability of communities to withstand stress or maintain their species composition over extended periods of environmental changes is a matter of major concern. Here, we utilize a dataset where microcrustacean communities were related to water chemistry in 82 boreal-alpine lakes over a 50-yr time span (1968 and 2016), with the same person involved in sampling both times ensuring identical sites and sampling protocols. Over this time span, there has been a 1–2°C increase in mean annual temperatures and prolonged growing seasons. Also, the strong changes in SO₄ deposition and acidification, peaking in the early 1980s, followed by a recovery period, have caused not only an increase in pH but also a pronounced browning (elevated DOC) and reduced levels of Ca over the past three decades. Despite this, the microcrustacean diversity and community composition have remained remarkably stable, and this holds both for the species-rich larger, boreal sites and the species-poor alpine sites. While likely there are interannual fluctuations in species abundance, and perhaps species may also be missing for certain periods, a combination of local refugia, resting egg seed-banks and a regional recolonization that may occur at the meta-population level, aims at maintaining a strong stability in community composition.

Key words: browning; calcium; lakes; resilience; richness; zooplankton.

Received 26 June 2020; revised 14 January 2021; accepted 1 February 2021. Corresponding Editor: Jonathan P. Doubek.

Copyright: © 2021 The Authors This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† **E-mail:** d.o.hessen@mn.uio.no

INTRODUCTION

Over the past 50 yr, lakes in many regions have experienced severe anthropogenic stressors besides temperature increase. For areas exposed to increased deposition of sulfuric or nitric acid, many of the lakes in poorly buffered catchments experienced acidification, often accompanied by loss of acid-sensitive species (Henriksen et al. 1989, Skjelkvåle and Henriksen 1995). Acidification peaked around the mid-1980s, and there has since been a pronounced recovery with

increasing pH as sulfur deposition has dropped (Garmo et al. 2014).

The current recovery from acidification of boreal regions on crystalline rocks has however revealed a somewhat paradoxical trajectory, characterized by striking declines of base cations, notably Ca (Likens et al. 1996, Jeziorski et al. 2008, Fölster et al. 2014). In parallel, there is a striking trend with increased levels of dissolved organic carbon (DOC), generally labeled “browning.” To a large extent, these changes are driven by the same mechanisms, such as

reductions in acid deposition (Monteith et al. 2007), climate change, and increased runoff (O'Reilly et al. 2015, de Wit et al. 2016), supported by increased terrestrial productivity (Finstad et al. 2011, Larsen et al. 2011, Kritzberg 2017). Increased forest biomass produces more organic matter from litterfall and root exudation, but also sequesters more of the available cations (Löfgren et al. 2017).

Over the past five decades, these stressors have likely affected the freshwater communities in many and partly contradictory ways. For microcrustaceans and zooplankton, it has been demonstrated that severe acidification has exerted a large toll on acid-sensitive species (Muniz 1990). Less is known about the microcrustacean community robustness over time toward multiple stressors such as acidification, post-acidification browning, and decreased levels of Ca. Browning implies in itself a suite of potential direct and indirect impacts on the biota (Creed et al. 2018) and not the least will the increased attenuation impact the entire food web via reduced primary production (Ask et al. 2009, Thrane et al. 2014). Decreased Ca in these soft-water systems will pose stress of Ca-demanding fauna, including certain microcrustacean species (Hessen et al. 2000, Jeziorski et al. 2008, Shapiera et al. 2011, Hessen et al. 2017). To fully reveal this issue, long-term microcrustacean data over several decades in a number of localities should be analyzed, together with relevant biotic and abiotic parameters. Such data are sparse for natural reasons, but comparison of relevant water chemistry and microcrustacean community data across a large time span could also offer insights at least in potential net changes in communities over time.

We have here utilized a dataset of 82 lakes, covering a wide gradient on lake size and altitude within a constrained area that was resampled with high precision with a nearly 50 yr' time span (1968–2016) and with the same person involved during both samplings to mimic the procedures. This period encompasses periods of acidification and recovery, as well as temperature increase, browning, and calcium decline. Both pelagic and littoral microcrustaceans were analyzed, and key parameters describing water color and organic C, calcium, and pH were included. The sites cover a wide span of area, ranging from ponds to lakes (0.1–20.0 ha), as well as an

altitudinal range from forested to alpine catchments (590–1225 m asl), yet all within a small region.

Since we do not have data for intermediate years, we cannot really test species turnover, but rather whether potential end-point community changes could be related to size, altitude, or water properties, or to specific species. To ensure that water quality difference over this time span did not simply reflect arbitrary differences in weather conditions or seasonality, we used long-term monitoring data from lakes in the vicinity and the same climatic region to verify trends in browning and Ca decline (Finstad et al. 2016, Garmo and Skancke 2018). We hypothesized that over the actual time span, we would see declines in whole lake diversity related to the negative impacts of acidification (peaking between the two sampling periods) and a delayed recovery, browning (reducing primary production), and reduced Ca. The latter effect should primarily affect the most Ca-demanding species (e.g., *Daphnia* spp.). Elevated temperature and prolonged growth season were expected to manifest an altitudinal upward trend for at least certain species.

MATERIAL AND METHODS

In 1968, a regional study of the chemical and biological conditions in 100 lakes and ponds was initiated in Southern Norway (Eie 1974). The goal was primarily to assess the impact of humic content on aquatic ecosystems, using microcrustacean communities as indicators/biological response. The area was chosen as representative for the boreal part of Southern Norway in the *International Biological Program*. The 1968 survey also related microcrustacean community composition, species richness, and abundance data to water quality parameters such as pH, *hardness* (principally Ca and Mg contents), and color.

To make sure that all procedures and sites were mimicked, the person in charge of the sampling during the 1968 campaign did also accompany the sampling in 2016 when 96 of the localities were resampled with corresponding sampling equipment. A strength of this study is that both pelagic and littoral microcrustaceans are included, which is important since the latter group generally contributes the majority of

species (Walseng et al. 2006). To ensure comparability of the two datasets, some sites were excluded because either plankton or littoral sample were missing. In total, 82 sites entered the final comparative analysis. Data from a nearby climatological station (Nesbyen weather station) confirmed fairly similar summer conditions for the two years. Mean July temperature was 14.8° and 14.7°C for 1968 and 2016, respectively, while mean precipitation was 1.7 and 1.8 mm.

The study area is located in the central part of southern Norway (60°30' N 9°30' E). Covering an area of roughly 300 km², the study area can neatly be divided into a northern boreal cluster of lakes (37 sites, approximately 600–950 m asl) and a subalpine/alpine region (45 sites, 950–1250 m asl, and above the tree line). The bedrock geology in the study area is mainly composed of gneiss and granite. The vegetation in the forest catchments is dominated by spruce (*Picea abies*), pine (*Pinus silvestris*), and to a lesser extent birch (*Betula pubescens*). The alpine localities, being located above the tree line have a sparse vegetation, mainly consisting of lichens, mosses, heathers, and willows. The combination of crystalline rocks, a thin soil layer and sparse vegetation in the area, renders the localities in the study area low in ions and buffer capacity and with only sparse stands of littoral vegetation (*Carex rostrata*) in sheltered bays of some lakes.

Over the 50-yr time span, some of the larger lakes have been affected by multiple factors such as liming, restocking of fish populations, recreation (bathing, boat activities, fishing, etc.), and expansions of recreational cabins. However, judged from own observation and area maps, the impacts by recreational activities and physical alterations in the catchments have still been limited. With regard to liming, we have good records on the relevant events and sites (Appendix S1: Fig. S1).

Water samples for chemical analysis were taken at 1-m depth (over the deepest area) with a Ruttner water sampler. The samples were stored in dark and cold until analysis. pH, conductivity, and color were measured within 4–12 h after sampling, while total organic carbon (TOC), acid neutralizing capacity (ANC), total phosphorus (TP), and total nitrogen were analyzed at the University of Oslo or the Norwegian Institute for Water Research (NIVA), after the fieldwork was

finished. Samples for pH and conductivity were stored on airtight Winkler bottles, while the remaining chemical parameters were stored on 500- and 20-mL high-density polypropylene bottles.

Water color was in 1968 measured against a platinum standard by a comparator, and given as mg Pt/L, with a detection limit at 5 mg Pt/L. All analyses from 1968 that had the value of 0 mg Pt/L have been set at 1 mg Pt/L. The uncertainties of measurements seem to be the highest at very low (0–10 mg Pt/L) and very high (>120 mg Pt/L) values. For the 2016 survey, we both analyzed Pt color with the similar instrument used in 1968 (Hellige comparator), as well as analysis of total organic carbon (TOC). In these boreal lakes, >95% of TOC is typically in the dissolved form, as DOC. A methodological comparison and calibration for Pt vs. TOC was performed and gave a very good fit ($r^2 = 0.977$; Appendix S1: Table S2). For calcium concentrations, the 1968 data were obtained by EDTA titration for “hardness” (CaCO₃), while for 2016, standard ion chromatography was applied. For comparison of 1968 and 2016 samples, CaCO₃ was corrected for the contribution from CO₃. For 2016, TP was measured on an auto-analyzer as phosphate after wet oxidation with peroxodisulfate. TOC was measured by infrared CO₂ detection after catalytic high-temperature combustion (Shimadzu TOC-VWP analyzer). TN was measured by detecting nitrogen monoxide by chemiluminescence using a TNM-1 unit attached to the Shimadzu TOC-VWP analyzer. Chlorophyll *a* was measured spectrophotometrically after acetone extraction.

Microcrustacean sampling

Vertical net hauls (90- μ m mesh size) were used to sample pelagic crustacean microcrustaceans. The net was hauled from the approximately greatest pond/lake depth 2–3 times. In ponds with maximum depth <1 m, horizontal net hauls from the open water (4–5 m) were used as a substitution. Littoral samples (4–7 m length) for microcrustaceans were taken as 2–3 horizontal net hauls from near-shore areas (<1.0 m depth).

When sparse populations of *Carex* occurred, littoral samples were also taken inside these stands. Sampling was performed during daytime, and the same protocols were followed in

1968 and 2016. Since there was a major overlap in species between pelagic and littoral net hauls, and the smaller localities in fact could be classified as entirely “littoral,” pelagic and littoral net hauls were merged for statistical analysis (see below for further explanation). Previous tests on separate and merged pelagic and littoral samples do in fact provide evidence for more robust statistical predictions from pooled samples (Walseng 2002).

To reduce the possibility of species contamination between different water bodies, the sampling net was carefully cleaned between each sampling. Samples were fixed on formaldehyde (1968) or Lugol (2016); however, this should not impact the species identification. All cladocerans and copepods were identified to species, with the exception of copepod nauplii, copepodite stages I-III. Cladocerans and copepods were identified according to Sars (1903, 1918), Smirnov (1971), Herbst (1976), Flössner (2000), Einsle (1993) and Bledzki and Rybak (2016). In general, entire samples were counted. However, in high-density samples, successive subsamples were examined until at least 200 organisms were identified and counted. The rest of the sample was scanned for the presence of rare species. Most species are rare, and there is a very skewed distribution of species abundances both for pelagic and for littoral samples (cf. Walseng et al. 2006). Hence, relative abundance for each species was assigned to four categories: absent, <1%, 1–10%, and >10% of total number. The “absent” category may of course still be present in low numbers in the lake, although not represented in the samples. For any given species, the highest recorded relative abundance in the two samples from each locality (pelagic and littoral) was used to represent each species.

Statistical analysis

A paired *t*-test was applied to test whether water chemical parameters and species richness differed between the two datasets. In cases with non-normal distribution, a non-parametric Wilcoxon signed-rank test was applied. The relationships between sampling year and other environmental variables and microcrustacean species composition were analyzed using unconstrained and constrained ordination techniques. Initially, we explored the impact of sampling

year on microcrustacean species composition by nonmetric multidimensional scaling. We examined whether communities had changed from 1968 to 2016 by testing significant differences of Bray-Curtis similarity indices between the two years by one-way analysis of similarities (ANOSIM). To test for a potential bias by zero values, we also examined community differences between years testing significant differences of Jaccard similarity indices (which ignores double zeros) between the two years by one-way analysis of similarities. This test confirmed predictions from Bray-Curtis NMDS. To further explore how sampling year and other environmental variables impacted species composition, constrained ordination was applied. Detrended correspondence analysis (Hill and Gauch 1980) showed that the first axis spanned gradient lengths of 2.4 SD units, justifying the use of redundancy analysis (RDA) for these data (Økland 1990).

The relationship between species and environmental variables was judged by the significance of the canonical axes together with a Monte Carlo permutation test. A minimal adequate model was developed by forward selection of environmental variables with a Monte Carlo test (499 permutations). Only variables that made significant independent contributions to species abundance ($\alpha = 0.05$ level) were included in the model. Multicollinearity of the environmental variables was assessed by checking their variance inflation factors (VIFs) using a limit of VIF >10 to exclude variables (Borcard et al. 2011). All environmental variables had VIFs below this limit and were thus included in the analysis.

Frequencies of microcrustacean taxa (calculated from abundance) were categorized into four dominance classes reflecting the dominance of species/taxa based on their relative frequencies (Walseng et al. 2006). The highest dominance score (pelagic/littoral) of the different taxa was used as input data for the ordination analyses. Two separate RDAs were conducted. Both included altitude, depth, area, pH, Ca concentration, and color as environmental variables. As a separate case, species richness for the 1968 and 2016 datasets was also analyzed with RDA using species richness as the only response variable (ter Braak and Šmilauer 2012). In 2016, some additional variables were measured. Hence, to gain further insight into the environmental control of

the microcrustacean community, we also conducted an RDA of the 2016 samples separately, including these additional variables. This RDA included depth, area, water temperature, fish presence, Chaoborus presence, pH, Ca concentration, color, total nitrogen, total phosphorus, and chlorophyll *a*.

To address the unique effects of altitude and shared effects of lake/pond size (depth and area) and water chemical variables (pH, Ca concentration, and color) on species richness, a two-variance partitioning analysis (VPA) using partial redundancy analysis and partial correspondence analysis was conducted (Borcard et al. 1992). This may be used to separate variation in ecological datasets between two or three environmental variables (or groups of environmental variables, e.g., Liu 1997). In this type of analysis, the total variation and the unique contribution of the variables and their joint effects are obtained in several steps. For further description of this method, see for example Liu (1997). As a separate case, species richness for the 1968 and 2016 datasets was also analyzed with RDA using species richness as the only response variable (ter Braak and Šmilauer 2012).

Statistical analyses were conducted in SPSS Statistics 24 (IBM 2017), PAST 3.1.8 (Hammer et al. 2001), and CANOCO 5.0 (ter Braak and Šmilauer 2012).

RESULTS

Environmental changes

The localities span a wide range in area and depth (Table 1). However, the majority are small, <4 ha in area and <4 m deep. The presence of fish in the lakes is to a large extent determined by depth, with shallow sites being devoid of fish. In the 2016 dataset, color was strongly correlated with total organic carbon (TOC; Appendix S1: Table S2), allowing for a direct comparison of color between the two years. Altitude was strongly inversely correlated with TOC (Fig. 1; Appendix S1: Table S2). At lower altitudes, the variation in TOC was pronounced with the highest values in lakes situated around 800 m above sea. Above the tree line, TOC decreased strongly. The Ca concentration showed a corresponding decline with altitude, being substantially lowest in the alpine

Table 1. Altitude, depth, area, and water chemistry in 82 lakes/ponds in the Vassfaret area in South-Central Norway.

Variable	1968			2016		
	Mean	Min.	Max.	Mean	Min.	Max.
Altitude (m)	1006	590	1225	–	–	–
Depth (m)	4.2	0.3	27.5	–	–	–
Area (ha)	2.8	0.1	20.0	–	–	–
pH	4.9	4.4	6.0	5.2	4.2	6.8
Ca (mg/L)	0.54	0.16	1.48	0.63	0.08	2.69
Color (mg Pt/L)	22	1	137	35	5	150
TN (µg/L)	–	–	–	143	4	338
TP (µg/L)	–	–	–	5.9	1.0	28.0
Chl. <i>a</i> (µg/L)	–	–	–	1.8	0.3	5.9

lakes, except for the limed localities. pH increased with altitude (Fig. 1; Appendix S1: Table S2). These general patterns were similar for 1968 and 2016, yet with a general upward trend for TOC and pH and a downward trend for Ca. The 2016 dataset with additional water chemical measurements and temperature measurements also revealed an altitudinal gradient with decreasing pelagic productivity and water temperature at higher altitude (Appendix S1: Table S2).

The water chemistry of the study sites changed considerably between the two surveys. The average pH 4.9 in 1968 had increased significantly to 5.2 in 2016 (Table 2). Likewise, the color also increased significantly over these five decades from a mean of 22 mg Pt/L in 1968 to 35 mg Pt/L in 2016. The change in average Ca concentration was not significant (0.54 mg/L in 1968 and 0.63 mg/L in 2016). However, excluding limed localities (30), the Ca concentration dropped significantly, from 0.57 mg/L in 1968 and to 0.37 mg/L in 2016. The trends for pH and color remained similar for limed and un-limed lakes.

Microcrustacean communities

A total of 44 species were recorded in the total dataset (Appendix S1: Table S3). Of these, 42 were recorded in 1968 and 40 in 2016. Three species found in 1968 were not recorded in 2016, while there were two “newcomers” in 2016. These are rare species, and absence or presence might simply reflect stochastic sampling impacts. Hence, the vast majority, and all the common species, were recorded in both years and basically in the same

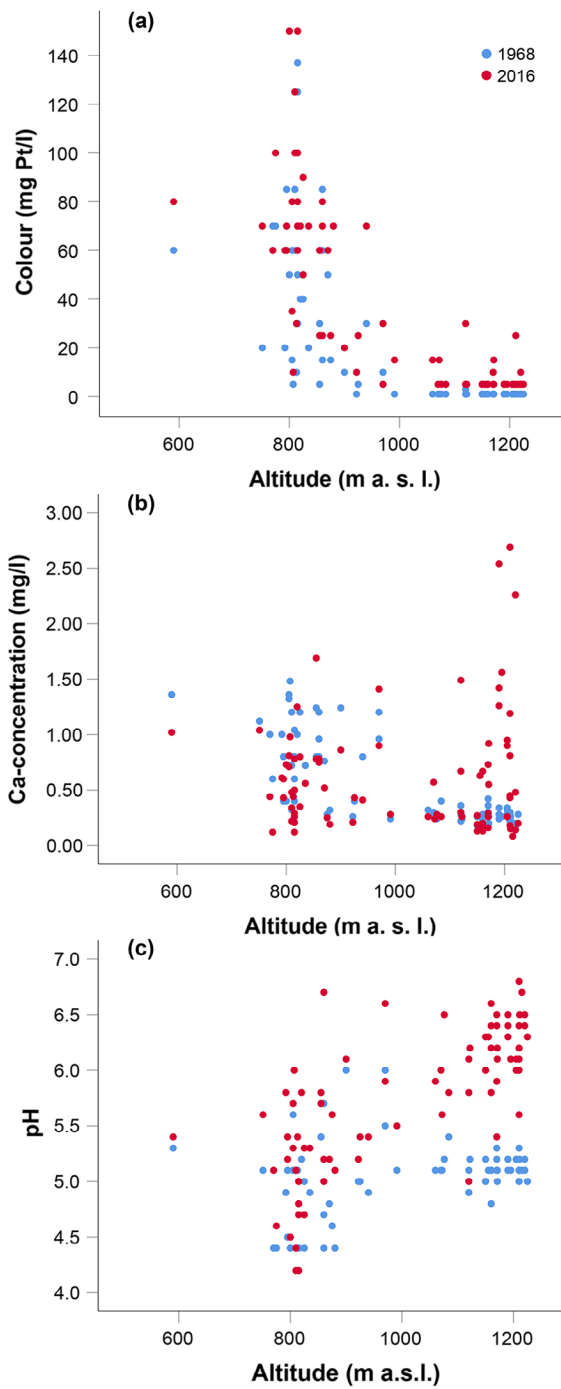


Fig. 1. Scatterplot of altitude vs color, Ca concentration, and pH.

localities. The average species richness of microcrustacean species did not change significantly from 1968 to 2016 (paired *t*-test, $t = -1.933$, $P = 0.057$; Fig. 2). Species richness was 9.9 ± 0.49

Table 2. Comparison of water chemical from 1968 to 2016 in 82 lakes/ponds in the Vassfaret area in the central part of Southern Norway.

Dataset	Variable	Test	<i>t</i> / <i>Z</i>	<i>P</i>
Full dataset	pH	Paired <i>t</i> -test	-13.383	<0.0001
	Color	Wilcoxon signed-rank test	-6.377	<0.0001
	Ca	Wilcoxon signed-rank test	-0.599	0.549
Excluding limed sites	pH	Paired <i>t</i> -test	-9.192	<0.0001
	Color	Wilcoxon signed-rank test	-4.464	<0.0001
	Ca	Wilcoxon signed-rank test	-5.537	<0.0001

Note: Comparison of water chemical changes between years was done with paired *t*-test (pH) and Wilcoxon signed-rank test (color and Ca concentration).

(mean \pm SE) and 10.7 ± 0.48 in 1968 and 2016, respectively. In the analysis of the 1968 dataset, the “minimal adequate model” resulting from the forward selection in the RDA of species richness included the explanatory variable Ca concentration (pseudo- $F = 88.1$, $P = 0.002$). In the analysis of the 2016 dataset, the “minimal adequate model” included the explanatory variables altitude (pseudo- $F = 59.6$, $P = 0.002$) and pH (pseudo- $F = 5.2$, $P = 0.014$). A full species list and an account on species-specific responses to ambient variables in the RDA are provided as supplementary information (Appendix S1: Tables S3, S4 and Fig. S2).

Species richness decreased with increasing altitude and increased with increasing Ca concentration, except in the limed localities (Appendix S1: Fig. S1). The VPA of the 1968 dataset including altitude, size, depth, area, and water chemistry (Ca concentration, color, and pH) demonstrated that the combination of altitude and water chemistry explained most of the variation in microcrustacean species richness followed by the pure effect of water chemistry. In the 2016 data, altitude per se explained the largest fraction of the variation, yet with significant contributions of the combination altitude and water chemistry (Table 3). There was a strong overlap in the microcrustacean species composition between 1968 and 2016 (Fig. 3; Table 4), and the community composition was not significantly different (one-way ANOSIM, $R = -0.001697$, $P = 0.5422$).

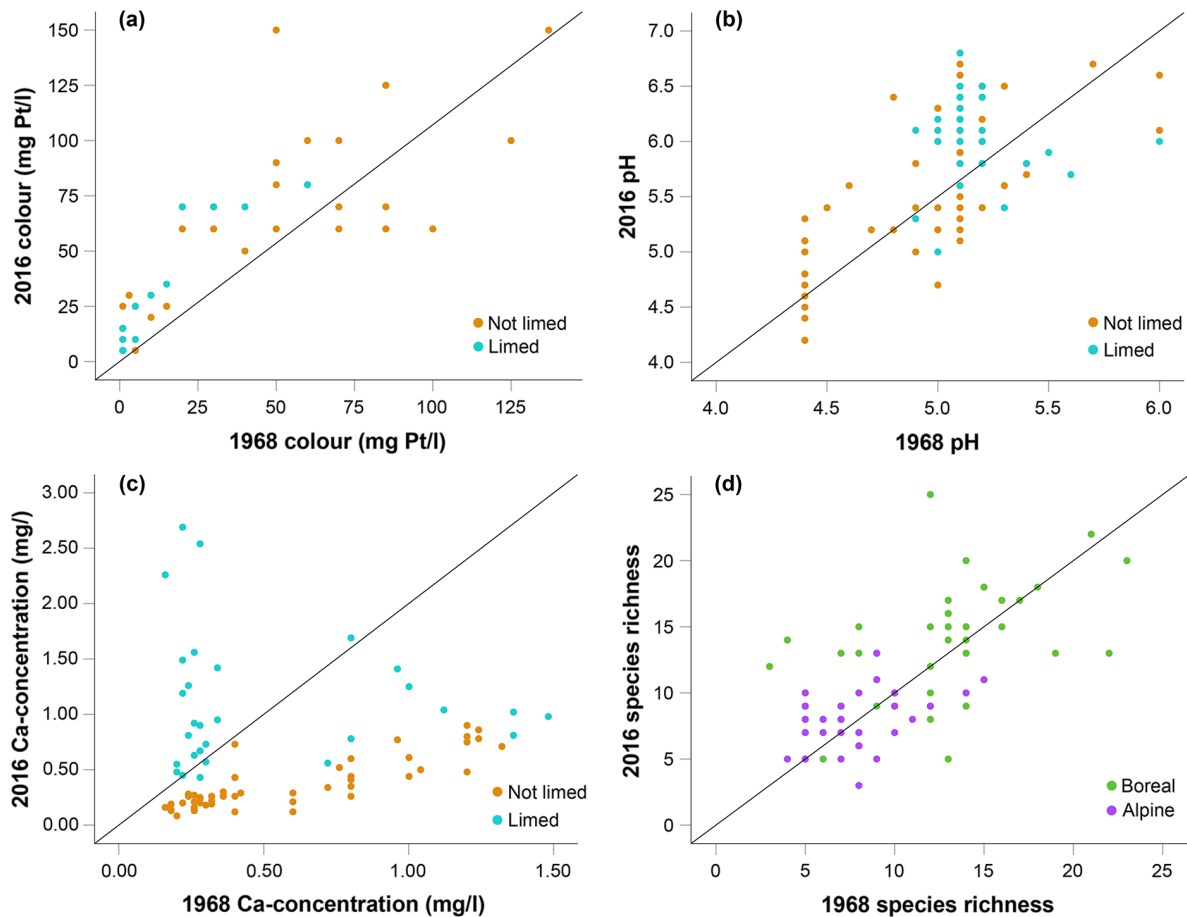


Fig. 2. Scatterplot of 1968 values vs. 2016 values of (a) color, (b) Ca concentration, (c) pH, and (d) species richness. The 1:1 lines are indicated in the scatterplots. In panels a, b, and c, it is indicated whether lakes were limed between 1968 and 2016. In panel d, altitudinal zone (boreal or alpine) is indicated. The scatterplots in panel (a) and (d) appear to have fewer data points, because some points overlap.

Overall, the RDA of the microcrustacean community showed that the most important drivers of microcrustacean species composition were altitude, Ca concentration, depth, pH, and color (Appendix S1: Table S4; Fig. 4). The two first RDA axes in the ordination in the 1968 dataset had eigenvalues of 0.1619 and 0.0850, respectively, and explained 24.69% of the variation in the species data and 83.61% of the variation in the species–environment relationship (Appendix S1: Table S4). There was a significant relationship between the set of environmental variables (altitude, depth, area, pH, Ca, and color) and species composition (i.e., all canonical axes, pseudo- $F = 5.4$, $P = 0.002$). The “minimal adequate model” resulting from the forward selection

included the explanatory variable altitude (pseudo- $F = 12.0$, $P = 0.002$), depth (pseudo- $F = 8.9$, $P = 0.002$), color (pseudo- $F = 3.7$, $P = 0.002$), and Ca concentration (pseudo- $F = 3.3$, $P = 0.002$).

In 1968, the microcrustacean communities were distributed mainly along a gradient of altitude and Ca along RDA axis 1 (Appendix S1: Table S4; Fig. 4). Lakes at high altitude and low Ca had low axis 1 scores and sites of lower altitude with higher color had high axis 1 scores. RDA axis 2 was mainly correlated with depth and color (Appendix S1: Table S4, Fig. 4). Lakes of low depth and high color had low axis 2 scores, while sites with high depth and low color had high axis 2 scores.

Table 3. Variance partitioning analysis (altitude, size [depth/area], and water chemistry [pH, Ca concentration, and color]) of species richness in 1968 and 2016, respectively.

Variable	1968		2016	
	Percentage of explained	Percentage of all	Percentage of explained	Percentage of all
Altitude	-0.1	-0.1	49.8	21.7
Depth/area	-0.3	-0.1	-2.1	-0.9
Water chemistry	34.4	14.1	6.7	2.9
Altitude and depth/area	-1	-0.4	5.1	2.2
Depth/area and water chemistry	-3.7	-1.5	-0.3	-0.1
Altitude and water chemistry	69.3	28.4	43.6	19
Altitude, depth/area and water chemistry	1.3	0.6	-2.8	-1.2
Total explained	100	40.9	100	43.7
All variation		100	-	100

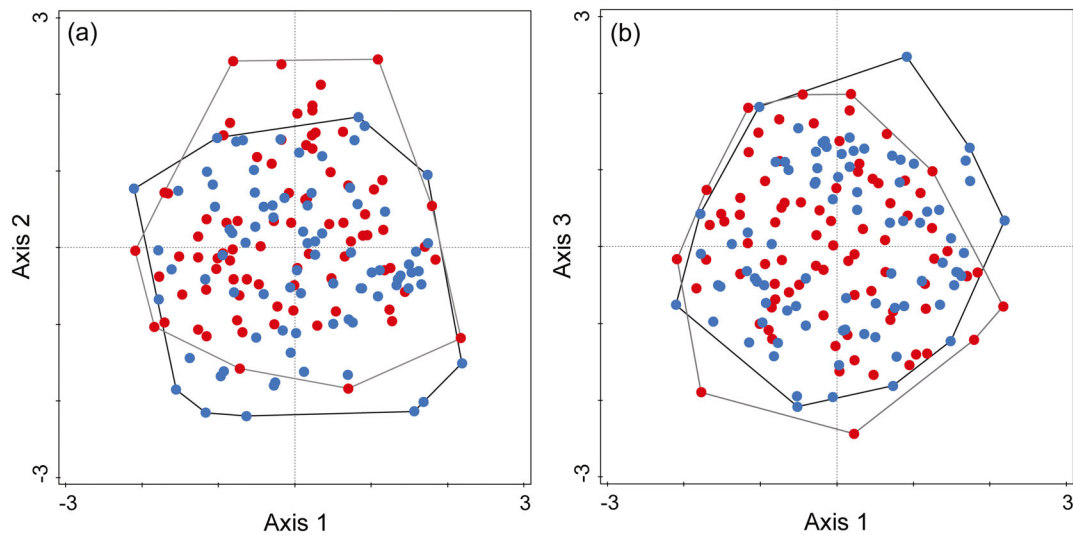


Fig. 3. Sample scores from nonmetric multidimensional scaling (three-dimensional solution) using Bray-Curtis dissimilarities between microcrustaceans communities. (a) Axes 1 and 2; (b) axes 1 and 3. Sites sampled in 1968 indicated as blue circles enveloped by solid black line; lakes and ponds sampled in 2016 indicated by red circles enveloped by solid gray line.

Table 4. Nonmetric multidimensional scaling (three-dimensional solution) using Bray-Curtis dissimilarities between microcrustacean communities from lakes and ponds sampled in 1968 and 2016.

Statistical parameters	Axis 1	Axis 2	Axis 3
Eigenvalues	0.427	0.297	0.276
Explained variation (cumulative)	42.7	72.4	100

In the RDA of the 2016 dataset, the two first RDA axes had eigenvalues of 0.1576 and 0.0623, respectively, explaining 21.98% of the variation in the species data and 80.14% of the variation in the species–environment relationship (Appendix S1: Table S4). Overall, the RDA showed that the most important drivers of microcrustacean species composition were altitude, color, pH, and depth (Fig. 4;

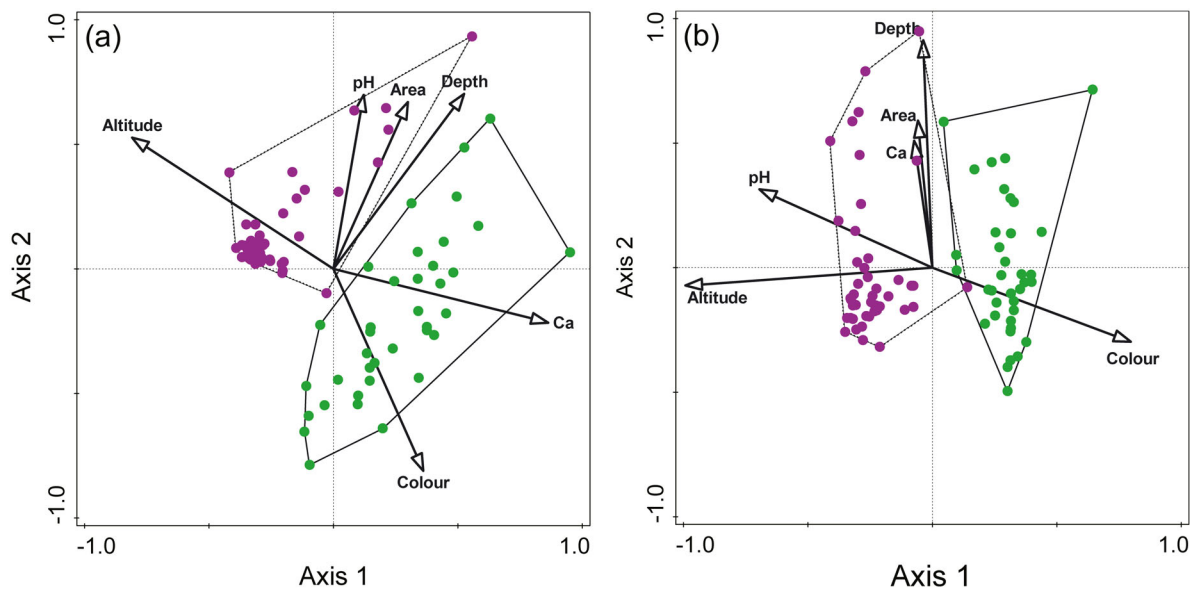


Fig. 4. Redundancy analysis (RDA) of microcrustacean communities in the 82 lakes and ponds (a) RDA ordination sample plot of the 82 study lakes sampled in 1968. Environmental variables included in both plots are both significant (altitude, depth, color, and Ca) and non-significant variables (pH and area). (b) RDA ordination sample plot of the 82 study lakes sampled in 2016. Environmental variables included in both plots are both significant (altitude, depth, and pH) and non-significant variables (area, Ca, and color). In both sample plots, lakes in the boreal zone are indicated as green circles enveloped by solid black line; lakes and ponds in the alpine zone are indicated by purple circles enveloped by dotted black line.

Appendix S1: Table S4). There was a significant relationship between the set of environmental variables (altitude, depth, area, pH, Ca, and color) and species composition (i.e., all canonical axes, pseudo- $F = 4.7$, $P = 0.002$). The “minimal adequate model” resulting from the forward selection included the explanatory variable altitude (pseudo- $F = 14.7$, $P = 0.002$), depth (pseudo- $F = 5.6$, $P = 0.002$), and pH (pseudo- $F = 2.4$, $P = 0.004$).

In 2016, the microcrustacean communities were distributed mainly along a gradient of altitude and color/pH along RDA axis 1 (Fig. 4; Appendix S1: Table S4). Lakes at high altitude and high pH/low color had low axis 1 scores and sites of lower altitude with higher color/pH had high axis 1 scores. RDA axis 2 was mainly correlated with depth (Fig. 4; Appendix S1: Table S4). Lakes of low depth had low axis 2 scores, while sites with high depth had high axis 2 scores.

In both 1968 and 2016, the combination of altitude and water chemistry was the main contributor to the variation in species composition

(Table 5). Altitude did however explain a larger fraction of the variation in 2016 than in 1968. The fractions explained by the pure effect of depth, the pure effect of water chemistry, and the combined effect of depth and water chemistry had decreased somewhat from 1968 to 2016 (Table 5). Besides the fact that alpine lakes had fewer species, there were some distinctive altitudinal patterns at the species level. The calanoid copepod *Mixodiaptomus laciniatus* and the cladoceran *Chydorus sphaericus* were strictly associated with low axis 1 scores, that is, alpine lakes above the tree line with low Ca. On contrary, the cladocerans *Ophryoxus gracilis* and *Holopedium gibberum*, *Diaphanosoma brachyurum* and the copepods *Cyclops scutifer* and *Macrocyclus albidus* were mainly found in boreal lakes below the tree line with higher Ca. While there was no overlap in species with a dominant alpine vs boreal preference between years, there were some changes notably in species with high boreal scores between the two years of sampling, reflecting that there were differences in within-lake

Table 5. Results from the variance partitioning analysis (altitude, size [depth/area], and water chemistry [pH, Ca concentration, and color]) of species composition in 1968 and 2016, respectively.

Variable	1968		2016	
	Percentage of explained	Percentage of all	Percentage of explained	Percentage of all
Altitude	5	1.2	27.8	6
Depth/area	21	5.1	15	3.2
Water chemistry	18.2	4.4	10.5	2.3
Altitude and depth/area	1.8	0.4	3.4	0.7
Depth/area and water chemistry	11.5	2.8	7.4	1.6
Altitude and water chemistry	42.2	10.3	39.1	8.4
Altitude, depth/area and water chemistry	0.2	<0.1	-3.2	-0.7
Total explained	100	24.4	100	21.6
All variation		100	-	100

communities, despite a high regional stability (see Appendix S1 for more information on the species level).

The RDA of the 2016 dataset including the additional environmental variables gave further insight into the environmental controls of the community. Hence, the altitudinal gradient covaried with pelagic productivity (higher total N, total P, and chlorophyll *a*, respectively) and temperature (see Appendix S1: Table S5 and Fig. S3 for further detail).

DISCUSSION

This comparative analysis of water quality and microcrustacean communities in boreal and alpine lakes revealed major changes in water quality in terms of “browning,” increased pH, and decreased levels of Ca over a nearly 50-yr time span. Despite this, the microcrustacean communities remained largely unchanged. The altitudinal patterns remained similar with a steep decrease in TOC (color) and a major increase in pH with altitude, and a more scattered relation between Ca and altitude. Average summer temperature for the sites has not been recorded, but reconstruction of summer temperatures for the periods 1965–1970 and 2005–2010 in a nearby and corresponding subalpine to alpine gradient revealed a thermal increase for the period corresponding to an approximate 200-m upward shift of the local isotherms (Lindholm et al. 2012). By and large, however, the spatial (altitudinal) effect in our study was strong, in contrast to the temporal, suggesting only modest alpine advancement

of species. Ecological responses to climate change include latitudinal and altitudinal range expansions, rendering arctic and alpine ecosystems especially vulnerable to community changes (Rogora et al. 2003, Chen et al. 2011). In addition, increasing water temperature could pose an array of direct (physiological) and indirect effects on aquatic organisms, which would differ among species, potentially causing community shifts. In general, temperature and extension of the ice-free period have increased in temperate lakes (Weyhenmeyer et al. 2011), but our study does not provide direct observation on phenological responses in terms of phytoplankton blooms and microcrustacean responses.

For both years, the decline in microcrustacean richness with altitude was consistent, and altitude (being a proxy of pelagic productivity, temperature, and growing season) explained most of the observed variance in richness and to some extent also the community composition. While altitude per se stands firm of course, a 200-m altitudinal shift in average temperature could potentially have caused a literally upward lift of species richness. This had not happened however, and also with regard to community composition, there was a remarkable stability, both at the beta-diversity level and in the specific localities.

The changes in pH (also upward in the limed lakes), browning, and reduced Ca would be expected to pose a suite of ecological impacts, potentially also causing community changes. Increased transport of dissolved organic carbon (DOC) from the lake catchment, with the following browning of surface waters, has occurred

over the past 3 decades for many boreal areas (Roulet and Moore 2006, Monteith et al. 2007, Finstad et al. 2016). Browning of surface waters is considered to have great impact on the physical, chemical, and biological conditions in lakes and ponds (Ellison et al. 2017). Calcium, being an essential element, is crucial to all calcifying organisms. Ambient calcium concentrations have shown to be a limiting factor to certain microcrustacean species in lakes (Hessen et al. 1995, Hessen et al. 2000).

No doubt, the major stressor has been the acidification, which peaked in the early 1980. 75% of the lakes in this region has been identified as low ANC lakes with high sensitivity to acidification (Skjellkvåle et al. 1996, Henriksen et al. 1999). Further, long-term monitoring has demonstrated that this region was impacted by acid rain, but with strong recovery from the peak period 1978–1982 to present where the region no longer receives S and N above the tolerance levels (Lund et al. 2012). Long-term monitoring in the nearby lake Langtjern revealed a decline in surface water SO_4 from 75 $\mu\text{Eq/L}$ in 1982–1984 to <15 $\mu\text{Eq/L}$ in 2012 (Garmo and Skancke 2018). Long-term monitoring of 80 lakes across the entire mainland also confirms that these boreal areas have experienced consistent declines in Ca and SO_4 , while increased pH and TOC (browning) (Finstad et al. 2014, Thrane et al. 2014). The observed difference between 1968 and 2016 thus reflects real trends; however, they also likely do not encompass the full range of water quality changes in this period, since the peak acidification occurred at least 10 yr after 1968.

As neither the microcrustacean species compositions nor the species richness, was found to be significantly different between the two datasets, this could suggest a high ecological resilience. We do however lack samples from the period in between; hence, this resilience may not necessarily reflect resistance, at least not for all species. Defining resilience as the level of perturbation a community can withstand before being shifted to an alternative state, and recovery as the ability to return to after disturbance (Scheffer and van Nes 2007), both situations may actually apply here. In more severely acidified regions, pronounced losses of microcrustaceans (and other taxa) have been recorded, followed by very slow recovery (Keller and Yan 1991, Keller et al. 2011).

In sites with less severe acidification and lack of heavy metal pollution (like in our study), the impacts on community composition during peak acidification have likely been less severe (Walseng et al. 2003). Sites may have local “pockets” with more profitable water quality where species can remain. Also resting stages, at least for cladocera, may survive for extended periods, serving as a seedbank for recolonization. Finally, local connectivity and a regional recolonization may occur at the meta-population level, where high connectivity provides founder populations. Without detailed sampling over the entire period, or sediment cores and paleolimnological examination from each site, we cannot conclude firmly with regard to the causes of the long-term community stability. Moreover, also boreal lakes have a high interannual variability in species composition. A 12-yr study revealed that only one third of the total species pool for each lake was detected every year, and annual data underestimated long-term species pools by 33–50% in Canadian lakes (Arnott et al. 1999). Hence, site-specific divergence in richness and community composition between years should not be inferred as systematic changes. For certain lakes, there are differences in species composition between years, as there clearly would be within years, depending on seasonal dynamics since not all rare species are encountered in a net haul. Still, most sites had fairly similar community composition in 1968 and 2016, but more importantly, the regional community composition and altitudinal distribution was only marginally different. Thus, despite these major changes in water quality parameters and climate over the past 50 yr, there is a striking long-term resilience that suggests microcrustacean communities to possess a buffering capacity toward water quality changes.

LITERATURE CITED

- Arnott, S. E., N. D. Yan, J. J. Magnuson, and T. M. Frost. 1999. Interannual variability and species turnover of crustacean zooplankton in Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 56:162–172.
- Ask, J., J. Karlsson, L. Persson, P. Ask, P. Byström, and M. Jansson. 2009. Terrestrial organic matter and light penetration: effects on bacterial and primary

- production in lakes. *Limnology and Oceanography* 54:2034–2040.
- Bledzki, L. A., and J. I. Rybak. 2016. Freshwater Crustacean Zooplankton of Europe: Cladocera & Copepoda (Calanoida, Cyclopoida). Key to species identification, with notes on ecology, distribution, methods and introduction to data analysis. Springer.
- Borcard, D., F. Gillet, and P. Legendre. 2011. Numerical ecology with R. Springer, New York, New York, USA.
- Borcard, D., P. Legendre, and P. Drapeau. 1992. Partialling out the spatial component of ecological variation. *Ecology* 73:1045–1055.
- Chen, I.-C., J. K. Hill, R. Ohlemüller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science* 333:1024–1026.
- Creed, I. F., et al. 2018. Global change-driven effects on dissolved organic matter composition: implications for food webs of northern lakes. *Global Change Biology* 24:3692–3714.
- de Wit, H. A., S. Valinia, G. A. Weyhenmeyer, M. N. Futter, P. Kortelainen, K. Austnes, D. O. Hessen, A. Råike, H. Laudon, and J. Vuorenmaa. 2016. Current browning of surface waters will be further promoted by wetter climate. *Environmental Science & Technology Letters* 3:430–435.
- Eie, J. 1974. A comparative study of the crustacean communities in forest and mountain localities in the Vassfaret area (southern Norway). *Norwegian Journal of Zoology* 22:177–205.
- Einsle, U. 1993. Crustacea: Copepoda, Calanoida and Cyclopoida. Gustav Fischer Verlag.
- Ellison, D., et al. 2017. Trees, forests and water: cool insights for a hot world. *Global Environmental Change* 43:51–61.
- Finstad, A. G., T. Andersen, S. Larsen, K. Tominaga, S. Blumentrath, H. A. de Wit, H. Tømmervik, and D. O. Hessen. 2016. From greening to browning: Catchment vegetation development and reduced S-deposition promote organic carbon load on decadal time scales in Nordic lakes. *Scientific Reports* 6.
- Finstad, A. G., T. Forseth, B. Jonsson, E. Bellier, T. Hesthagen, A. J. Jensen, D. O. Hessen, and A. Foldvik. 2011. Competitive exclusion along climate gradients: energy efficiency influences the distribution of two salmonid fishes. *Global Change Biology* 17:1703–1711.
- Finstad, A. G., I. P. Helland, O. Ugedal, T. Hesthagen, and D. O. Hessen. 2014. Unimodal response of fish yield to dissolved organic carbon. *Ecology Letters* 17:36–43.
- Flössner, D. 2000. Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas. Backhuys.
- Fölster, J., R. K. Johnson, M. N. Futter, and A. Wilander. 2014. The Swedish monitoring of surface waters: 50 years of adaptive monitoring. *Ambio* 43:3–18.
- Garmo, Ø. A., and L. B. Skancke. 2018. Overvåking av langtransportert forurenset luft og nedbør. Årsrapport–Vannkjemiske effekter 2017. NIVA-rapport.
- Garmo, Ø. A., B. L. Skjelkvåle, H. A. de Wit, L. Colombo, C. Curtis, J. Fölster, A. Hoffmann, J. Hruška, T. Høgåsen, and D. S. Jeffries. 2014. Trends in surface water chemistry in acidified areas in Europe and North America from 1990 to 2008. *Water, Air, & Soil Pollution* 225:1880.
- Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4:9.
- Henriksen, A., E. Fjeld, and T. Hesthagen. 1999. Critical load exceedance and damage to fish populations. *Ambio* 28:583–586.
- Henriksen, A., L. Lien, B. O. Rosseland, T. S. Traaen, and I. S. Sevaldrud. 1989. Lake acidification in Norway: present and predicted fish status. *Ambio* 18:314–321.
- Herbst, H. V. 1976. Blattfusskrebse (Phyllopoden: Echte Blattfüsser und Wasserflöhe). Kosmos-Verlag Franckh, Stuttgart, Germany. 130 s.
- Hessen, D. O., N. E. Alstad, and L. Skardal. 2000. Calcium limitation in *Daphnia magna*. *Journal of Plankton Research* 22:553–568.
- Hessen, D. O., B. A. Faafeng, and T. Andersen. 1995. Competition or niche segregation between *Holopedium* and *Daphnia*; empirical light on abiotic key parameters. Pages 253–261 in *Cladocera as model organisms in biology*. Springer.
- Hessen, D. O., J. P. Håll, J. E. Thrane, and T. Andersen. 2017. Coupling dissolved organic carbon, CO₂ and productivity in boreal lakes. *Freshwater Biology* 62:945–953.
- Hill, M. O., and H. G. Gauch. 1980. Detrended correspondence analysis: an improved ordination technique. *Plant Ecology* 43:47–58.
- IBM. 2017. IBM SPSS Statistics for Windows. IBM Corp, Armonk, New York, USA.
- Jeziorski, A., et al. 2008. The widespread threat of calcium decline in fresh waters. *Science* 322:1374–1377.
- Keller, W., and N. Yan. 1991. Recovery of crustacean zooplankton species richness in Sudbury area lakes following water quality improvements. *Canadian Journal of Fisheries and Aquatic Sciences* 48:1635–1644.
- Keller, W., N. Yan, J. Gunn, and J. Heneberry. 2011. Chemical and biological recovery of acidified lakes near Sudbury, Ontario, Canada. In *Abstract the 8th International Conference on Acid Deposition*.

- Kritzberg, E. S. 2017. Centennial-long trends of lake browning show major effect of afforestation. *Limnology and Oceanography Letters* 2:105–112.
- Larsen, S., T. Andersen, and D. O. Hessen. 2011. Climate change predicted to cause severe increase of organic carbon in lakes. *Global Change Biology* 17:1186–1192.
- Likens, G. E., C. T. Driscoll, and D. C. Buso. 1996. Long-term effects of acid rain: response and recovery of a forest ecosystem. *Science* 272:244–246.
- Lindholm, M., F. Stordal, S. J. Moe, D. O. Hessen, and P. Aass. 2012. Climate-driven range retraction of an Arctic freshwater crustacean. *Freshwater Biology* 57:2591–2601.
- Liu, Q. 1997. Variation partitioning by partial redundancy analysis (RDA). *Environmetrics* 8:75–85.
- Löfgren, S., A. Ågren, J. P. Gustafsson, B. A. Olsson, and T. Zetterberg. 2017. Impact of whole-tree harvest on soil and stream water acidity in southern Sweden based on HD-MINTEQ simulations and pH-sensitivity. *Forest Ecology and Management* 383:49–60.
- Lund, M., T. R. Christensen, A. Lindroth, and P. Schubert. 2012. Effects of drought conditions on the carbon dioxide dynamics in a temperate peatland. *Environmental Research Letters* 7:045704.
- Monteith, D. T., et al. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450:537–540.
- Muniz, I. P. 1990. Freshwater acidification: its effects on species and communities of freshwater microbes, plants and animals. *Proceedings of the Royal Society of Edinburgh. Section B. Biological Sciences* 97:227–254.
- Økland, R. H. 1990. Vegetation ecology: theory, methods and applications with reference to Fennoscandia. *Sommerfeltia* 1:1–172.
- O'Reilly, C. M., S. Sharma, D. K. Gray, S. E. Hampton, J. S. Read, R. J. Rowley, P. Schneider, J. D. Lenters, P. B. McIntyre, and B. M. Kraemer. 2015. Rapid and highly variable warming of lake surface waters around the globe. *Geophysical Research Letters* 42:10773–10781.
- Rogora, M., R. Mosello, and S. Arisci. 2003. The effect of climate warming on the hydrochemistry of alpine lakes. *Water, Air, & Soil Pollution* 148:347–361.
- Roulet, N., and T. R. Moore. 2006. Environmental chemistry: browning the waters. *Nature* 444:283–284.
- Sars, G. O. 1903. An account of the Crustacea of Norway: with short descriptions and figures of all the species. *Bergen Museum*.
- Sars, G. O. 1918. An account of the Crustacea of Norway. VI Copepoda, Cyclopoida. *Bergen Museum*.
- Scheffer, M., and E. H. van Nes. 2007. Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. Pages 455–466 *in* *Shallow lakes in a changing world*. Springer.
- Shapiera, M., A. Jeziorski, N. D. Yan, and J. P. Smol. 2011. Calcium content of littoral Cladocera in three softwater lakes of the Canadian Shield. *Hydrobiologia* 678:77–83.
- Skjelkvåle, B., and A. Henriksen. 1995. Acidification in Norway—Status and trends. *Water, Air, and Soil Pollution* 85:629–634.
- Skjelkvåle, B., A. Henriksen, B. Faafeng, E. Fjeld, T. Traaen, L. Lien, E. Lydersen, and A. Buan. 1996. Regional innsjøundersøkelse 1995. En vannkjemisk undersøkelse av 1500 norske innsjøer.
- Smirnov, N. N. 1971. Chydoridae of the world's fauna. *ter Braak, C. J. F., and P. Šmilauer. 2012. CANOCO Reference Manual and User's Guide: Software for Ordination (version 5.0). Microcomputer Power, Ithaca, New York, USA.*
- Thrane, J.-E., D. O. Hessen, and T. Andersen. 2014. The absorption of light in lakes: negative impact of dissolved organic carbon on primary productivity. *Ecosystems* 17:1040–1052.
- Walseng, B. 2002. Plankton versus littoral microcrustaceans (copepods and cladocerans) as indicators of recovery from acidification. *Verhandlungen International Vereinigung Limnologie* 28:140–143.
- Walseng, B., D. O. Hessen, G. Halvorsen, and A. K. Schartau. 2006. Major contribution from littoral crustaceans to zooplankton species richness in lakes. *Limnology and Oceanography* 51:2600–2606.
- Walseng, B., N. D. Yan, and A. K. Schartau. 2003. Littoral microcrustacean (Cladocera and Copepoda) indicators of acidification in Canadian Shield lakes. *AMBIO: A Journal of the Human Environment* 32:208–213.
- Weyhenmeyer, G. A., D. M. Livingstone, M. Meili, O. Jensen, B. Benson, and J. J. Magnuson. 2011. Large geographical differences in the sensitivity of ice-covered lakes and rivers in the Northern Hemisphere to temperature changes. *Global Change Biology* 17:268–275.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3676/full>