Back to the future?
Lakes revisited; Ecological and water chemical impacts of reductions in long-range transported pollutants

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Preface

This study was conducted at the University of Oslo, department of Aquatic Biology, as a part of my Master thesis. First and foremost, I would like to express my gratitude to Dag O. Hessen, my main supervisor. His support and helpful discussion has proven crucial to complete this thesis. Secondly, it is a great privilege to a master student to be connected to the scientific community at NIVA (Norwegian Institute for Water research) and NINA (Norwegian Institute for Nature research). I am in great gratitude to my two co-supervisors Bjørn Walseng and Markus Lindholm. The openness and support has guided a confused and stress student through difficult times. I would also like to thank Brit Lisa Skjelkvåle (UiO), Espen Lydersen (HSN), Heleen de Wit (NIVA), Kari Austnes (NIVA) and Øyvind Garmo (NIVA) for their insights and discussion on water chemistry and catchment processes. It is overdue to thank Tom Andersen with help on statistics and comments on the thesis, Berit Kaasa for analysing parts of the water chemistry and Per-Johan Færøvig for help with logistics concerning the field work and general support. This thesis is funded by the University of Oslo and the County Governor of Buskerud. Lastly, I would like to thank my family, friends and girlfriend for the support during the field work, comments on the script and motivation during the writing of the thesis. I would especially like to thank my grandfather, whom without, this thesis would be literally impossible.

No Water – No Life

Oslo, May 12th 2017

Martin M. Eie

Abstract

Water is a unique substance and a vital part of life. Today, freshwater ecosystems are considered as one of the most vulnerable ecosystems on Earth. Degradation of lakes and rivers caused by water pollution, climate change, eutrophication and habitat destruction and fragmentation is a major concern in a world where fresh and clean water is unevenly and scarcely distributed. Acidification of lakes and rivers caused by long-range transported air pollutants has been considered as one of the biggest threats to biodiversity in freshwater ecosystems in Norway. Although the water quality has significantly improved after decades of legislations and efforts to reduce the emissions of long-range transported air pollutants, acidification is still considered as an environmental concern. The natural recovery of surface water chemistry is a slow process, and further emission reductions is needed to avoid the possible case of long-term acidification of acid-sensitive lakes. Long-term studies of lakes are invaluable, and crucial to understand how freshwater ecosystems are impacted/ respond to changes in the environment. This Master thesis traced the footsteps of a nearly 50-years old regional survey, containing data on the chemical and biological conditions of 100 lakes and ponds in South-East Norway. The present study had the following aims: (1) Conduct a regional survey of the chemical and biological status in Vassfaret, in accordance with the methods and the approach of the regional survey from 1968, (2) Assess water quality changes for selected parameters over these nearly 50-year time span, 3) Assess whether diversity and community compositions have remained the same over this time span, 4) Establish casual links between the eventually observed changes in water chemistry with those of the zooplankton community. Facing multiple stressors, knowledge on the stability of freshwater ecosystems is heavily needed.

Abbreviations

Al = Aluminum

ANC = Acid neutralizing capacity

ANC oaa = Acid neutralizing capacity organic acids adjusted

AOP = Adverse outcome pathway

Ca = Calcium

CCA = Canonical Correspondence Analysis

Cl = Chloride

DCA = Detrended Correspondence Analysis

DOC = Dissolved organic carbon

DOM = Dissolved organic matter

E.D.T.A. titration = Ethylenediaminetetraacetic acid titration

H = Hydrogen

K = Potassium

LAl = Labile aluminum

Mg = Magnesium

Na = Sodium

NMDS = Non-metrical multidimensional scaling

NO_3 = Nitrate

NO_x = Nitrogen oxides

OC = Organic carbon

SO_2= Sulfur dioxide

SO_4 = Sulfate

SSWC = Steady-state water chemistry

TN = Total nitrogen

TOC = Total organic carbon

TP= Total phosphorus

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1   Introduction

The world is in constant change, maybe now more than ever, as we are entering the antropocene – the age of humans. Environmental pollution, climate change, eutrophication, habitat degradation and fragmentation constitutes a potential risk for freshwater ecosystems in all corners of the world (Carpenter *et al*., 2011, Brönmark and Hansson, 2002). Facing multiple stressors, freshwater ecosystems are today considered as one of the most vulnerable ecosystems on Earth (Dudgeon *et al*., 2006) due to water scarcity, pollution and population growth and species loss. Truly pristine lakes are becoming increasingly rare. Not only is freshwater a necessity for life itself, but a source for ecosystem services such as fisheries, flood regulation, recreation, tourism and nutrient cycling (Carpenter *et al*., 1998).

Long-range transported pollution, with the following acidification of rivers and lakes, has been considered as one of the greatest threats to biodiversity in freshwater ecosystems in Norway (Henriksen *et al*., 1989, Rosseland and Henriksen, 1990, Skjelkvåle and Henriksen, 1995). Although national and international efforts to reduce the SO_2 and NO_x emissions clearly has led to improvements in surface water acid-base chemistry (Skjelkvåle *et al*., 2001), acidification is still considered an environmental concern (Garmo *et al.*, 2014). The case of a possibly long-term acidification of Norwegian catchments, is underlined by reduction in runoff sulfate have been offset by equal declines in calcium and magnesium concentrations (Kirchner and Lydersen, 1995). The term critical load is used to describe the exposure limit of a pollutant (or pollutants) that do not cause a significant harmful effect to sensitive elements of the environment (UN/ECE, 1988). Knowledge on the critical loads of long-transported pollutants causing acid rain is an important tool in environmental management and policy-making as it connects water chemistry with a biological response. The load of acid rain cannot exceed the buffer-capacity of a lake, minus a given ANC-limit (Henriksen *et al.,* 1999). The ANC-limit is basically the buffer-capacity needed to prevent harmful effects to a chosen biota (fish populations in Norway). The goal being ultimately biological recovery (*e.g*. return of acid-sensitive species), a crucial first step is restoring surface water chemistry to pre-perturbed/pre-industrial conditions. Leaking of inorganic aluminium (reactive and low molecular mass), due to cation-exchange in the lake catchment, is of great concern under acidification. This, primarily for two reasons: (1) Al and H+ could exert a direct toxic effect on zooplankton, (2) Indirect ecological effects on the zooplankton community through

removal of top-predator (fish kills[1]) and selection of sensitive zooplankton taxa. The zooplankton community can experience significant structural changes as a result of acidification (Mason, 2002, Schartau, 2002). Acidification can be manifested in zooplankton communities through reduction in species richness (complexity of the community) and/or changes in species dominance.

Increased transport of organic carbon (OC) from the lake catchment, with the following browning of surface waters, has emerged as a new environmental concern the two last 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. The most evident being a dramatic decrease in underwater light due to increase in attenuation, which again would reduce primary and secondary production (Karlsson *et al*., 2009, Thrane *et al*., 2014). Additionally, the carbon cycle as well as the temperature regime in lakes might be affected. Even though the causative single-factors are difficult to separate, studies have pointed out reduction in sulfate (Monteith *et al*., 2007), changes in precipitation pattern (*e.g* climate change)(de Wit *et al*., 2016), increasing iron concentrations (Kritzberg and Ekström, 2012) and catchment use and characteristics (vegetation and forest cover, agriculture etc.) as major drivers (Larsen *et al*., 2011). Questions still remain on how freshwater ecosystems will respond and adapt.

Calcium, being an essential element, is crucial to all calcifying organisms. Ambient calcium concentrations have shown to be a limiting factor to certain zooplankton species in lakes (Hessen *et al*., 1995, Hessen *et al*., 2000). Besides being vital to nearly all aquatic organisms (through ion-transport, calcification etc.), Ca is important to the ion balance and contributing to alkalinity. The ecological impacts of the ongoing base-cation depletion (Jeziorski et al., 2008) of Norwegian catchments is still uncertain, and needs further investigation.

Climate change is unequivocal (Pachauri *et al*., 2014, Meehl *et al*., 2007, Vörösmarty et al., 2000). Freshwater ecosystems are considered especially vulnerable, owing this mainly to their patchy distribution and relative isolation, shallow and small size and heavy exploitation by humans. Ecological responses to climate change is documented in an array of different ecosystems - from species to community level (Walther *et al*., 2002, Lindholm *et al*.,

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[1] Salmonids, such as lake trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) are considered to be sensitive to acidification. Inorganic labile aluminium(LAl), which is highly reactive, attaches to the gill-surface and blocks oxygen transport, osmoregulation etc. and can result in both lethal and sublethal effects.

2012). The leading theories on the ecological impact of climate change predicts a poleward and upward expansion/retraction, rendering arctic and alpine ecosystems especially vulnerable (Chen *et al*., 2011, Rogora *et al*., 2003). Additionally, climate change (*e.g.* increasing water temperature, increasing CO_2 concentrations in water) could pose an indirect effect on aquatic organisms through physiological stress – making organisms more vulnerable to other stressors in the environment (Walker *et al*., 2012, Jenssen, 2006).

As simple as a pond might seem, there is a world of complexity beneath the surface. Occupying a central part in the pelagic food web, zooplankton is a vital part of the structure and function of freshwater ecosystems (Pace and Orcutt, 1981). Zooplankton is not only easy to sample, but can be identified with quite ease. This, combined with relatively short generation time (quick community response to ecological and environmental change) and sensitivity to the chemical environment (Hanazato, 2001), makes them suitable in environmental monitoring and, perhaps also, as early warning indicators of ecosystems stress (Marmorek and Korman, 1993). In addition to being relatively homogeneous distributed in the pelagic water column, crustacean zooplankton is widely distributed in all parts of the world, and is (unlike fish) not limited to larger water bodies. This could give zooplankton an advantage over fish in environmental monitoring in smaller lakes and ponds. Also, operating with seed-banks and resting stages, crustacean zooplankton can easily re-colonize after perturbations.

Being semi-closed systems, lakes and ponds could serve as a good opportunity to understand the effects of multiple stressors on natural ecosystems. Also, the water quality of lakes themselves may serve as "sentinels" and early warnings of changes in the catchments (Adrian *et al*., 2009). The surface water chemistry in Norway is mainly determined by bedrock geology, precipitation chemistry, hydrology, soil type and vegetation (Skjelkvåle,1995). With humic rich lakes widely distributed in Norway, knowledge on how these distinct ecosystems will respond to different disturbances, is highly needed.

Long-term studies of lakes are invaluable, and crucial to understand how freshwater ecosystems are impacted/ respond to changes in the environment – short term as well as long term. In 1968, a regional study of the chemical and biological conditions in 100 lakes and ponds were initiated in Southern Norway (Eie, 1974). The goal was primarily to assess the importance of humic influence on aquatic ecosystems, using crustacean zooplankton communities as indicators/biological response. Furthermore, the area investigated (Vassfaret)

was chosen as representative for the central part of Southern Norway in the International Biological program. One of the main conclusions from the study of Eie (1974) challenged the perception that humic influence would have a negative impact on species richness. The study also focused on integrating a biological response (community compositions, species richness and abundance data) to physico-chemical lake conditions, and to better understand the complexity of freshwater ecosystems.

The dataset from 1968 covered zooplankton data as well as a number of water quality parameters, thus offering an ideal opportunity to revisit the same lakes and repeat the survey and analysis to see whether there were any systematic changes in species composition and key parameters related to water quality. The latter issue is highly relevant since long-term changes in surface water chemistry has been manifested in many Boreal areas, included Norway. These changes are primarily related to climate change (temperature and precipitation), reduced acidification and changes in forest density, but the biological impacts of these drivers are not settled. The present study thus had the following aims: (1) Conduct a regional survey of the chemical and biological status in Vassfaret, in accordance with the methods and the approach of the regional survey from 1968, (2) Assess water quality changes for selected parameters over these nearly 50-year time span, 3) Assess whether diversity and community compositions have remained the same over this time span, 4) Establish casual links between the eventually observed changes in water chemistry with those of the zooplankton community.

2   Methods

The study conducted by Eie (1974) lays the foundation for the choice of methods in the present study. While chemical analyses have changed substantially since 1968 (see table 1), in line with the technological development, zooplankton sampling is unchanged. This thesis tried to mimic sampling procedures, parameters and analytical protocols from the previous study to make the data as comparable as possible. Still some key parameters have changed over time both with regard to method and resolution. To minimalize the chance visiting wrong lakes, and to misinterpret techniques and methods, the author of the original study J. A. Eie, was assisting with the field work.

Using a snap-shot approach, 96 of 100 localities were revisited and sampled once between 4. July- 3. August (see appendix for sampling date of the individual waters). Concentrating the sampling period in a highest possible manner as possible, but still ranging within the timespan of the original study was essential. A comparison of the water temperature between the two years (mean 1968= 14,83°C, mean 2016= 14,67°C) indicates that seasonal differences between the two sampling seasons are minimal. The vital role of water as transporting agent, chemical solvent and catalyst, makes high-quality hydrological data crucial to explain the biochemical response of water entering a catchment (Lydersen, 1994). The average daily precipitation in July, 2016, was 1.69 mm (max=6.9, min=0). The average daily precipitation in July, 1968, was comparably 1.59 mm (max=21.8, min=0). The precipitation and temperature records are gathered from Nesbyen-weather station (166 m. a.s.l) and can only be considered a proxy of the climate conditions in the study area (~20 km from the weather- station). Additionally, there could be regional differences in the climate within the study area. But never the less, it gives a very valuable insight to the climate.

Multiple samples in each lake, and multiple year sampling could not be achieved due to economical and time constraints. It should be emphasized that freshwater ecosystems are not under steady-state conditions, and the physical-chemical conditions, along with the zooplankton community, will have natural variations. The present study gives a momentary picture of the summer-composition of pelagic crustacean zooplankton. Long-term monitoring data from Lake Langtjern (500 m.a.s.l and ~15 km from study area) has been used in the discussion.

Study area

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 region (approximately 600-900 m. a.s.l.) and a sub-alpine/alpine region (1000-1250 m. a.s.l.). Of the 96 localities sampled in 2016, 48 are located in the boreal/forest-region, while 48 are located in the alpine region. The bedrock geology in the study area is mainly composed of gneiss and granite – two hard and slowly erodible rock types. The vegetation around the forest localities is dominated by spruce (*Picea abies*), pine (*Pinus silvestris*) and birch (*Betula pubescens*). Also, shrubs, heathers and marshes are strongly represented around the localities – typical of the SE-Norway. The alpine localities, being located above the tree-line (~950 m a.s.l), has a sparse vegetation, mainly of lichens, mosses, heathers and willows. The combination of a thin soil layer and sparse vegetation, renders the localities in the study area low in ions and buffer-capacity. It is important to note that the localities in *Vassfaret* also varies in the degree of human activity the last 50 years. While some ponds have been more or less untouched for decades, the larger lakes are affected by multiple factors such as liming, restocking of fish populations, recreation (bathing, boat activities, fishing etc.) and expansions of cabins. In this study, the main focus has been on liming and restocking of fish populations, as the impacts by recreational activities and physical alterations seems to be limited. Extensive liming has been initiated by Flå Østre Grunneierlag (under supervision of the Buskerud County Governor). Attachment 2 in the appendix contains a list of limed or limed influenced lakes. The high connectivity between ponds and lakes in Vassfaret, makes the distinction between limed/not

limed lakes difficult. The databases *Vann-Nett* and *Vannmiljø* has proven crucial. Calcium concentrations in isolated non-limed lakes/ponds has also been used as a reference to determine the liming influence in other localities. In addition to liming, restocking of fish populations has been initiated in the larger lakes (appendix, attachment 2). Local observations together with the website *Vannmiljø* has been used. It should be stressed that there was not done any fish biological surveys in 1968.
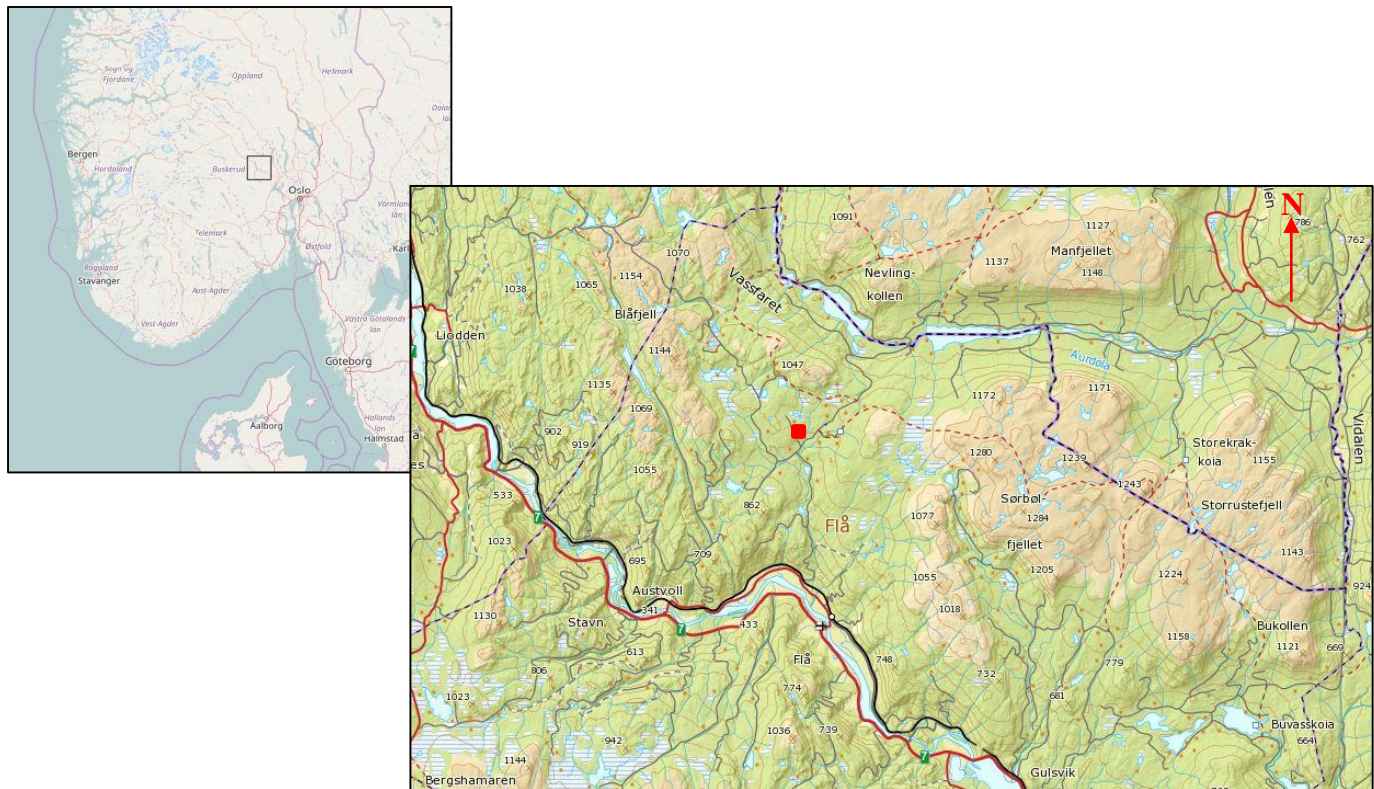
Figure 3. Map of the study area (Vassfaret) situated in the central part of Southern Norway. The field station is marked as a red square on the map. The locations of the specific lakes are given in the appendix, attachment 1.

Water chemistry

Water samples for chemical analysis were taken at 1-meter depth (over the approximately greatest depth) with a Ruttner water sampler. Water samples were stored dark and cold until analysed. Prolonged storage of water samples is a problem under longer periods of field work. In this case, it was solved by setting up a field-station in a nearby cabin were parameters such as pH, conductivity and colour (mg Pt/l) could be measured within 4-12 h. after sampling. Less sensitive parameters (TOC, ANC, TP, TN) were analysed at University of Oslo or Norwegian Institute for Water Research, after the field work was finished. Water samples for

pH and conductivity were stored on air-tight Winkler-bottles, while the remaining chemical parameters were stored on 500 mL and 20 mL high density polypropylene bottles. The acid neutralization capacity (ANC) has been calculated as Σ base-cations $-$ Σ strong acids anions. Additionally, ANC was calculated taking TOC (total organic carbon) into account, following the formula ANC-(10.2/3)*TOC (Lydersen et al., 2004). ANC values are not Cl-corrected. NO_3^- concentrations under the analytical detection limit (<2 µg/l) is neglected from ANC calculations.

Water color was previously typically measured against a platinum standard by a comparator, and given as mg Pt/l. Today, TOC and DOC (mg C/L) is commonly used. The measurements of water color (mg Pt/l) has a detection limit at 5 mg Pt/l. All values from 1968 which has the value of 0 mg Pt/l has been sat at 2,5 mg Pt/l. The uncertainties of measurements seem to be highest at very low (0-10 mg Pt/l)) and very high (>120 mg Pt/l) values.

Table 1. A list of chemical parameters, method used in 1968 and 2016, and were the respective parameter were analysed.

Variable	Method 1968	Method 2016	Field/lab 2016
Water temperature	Thermometer(°C)	Thermometer(°C)	*In situ*
pH	Colorimetric, Hellige comparator	Potentiometric, VWR pH 1100H	Field station
mg Pt/l (water color)	B.D.H. Lovinbond Nessleriser color comparator	B.D.H. Lovinbond Nessleriser color comparator	Field station
KMnO4-consumption	Werescagin (1931)		
Lake transparency	Secchi-disc	Secchi-disc	*In situ*
Total organic carbon mg C/l		Oxidation (persulfate) and UV illumination to CO2, measurement with IR-detector	Lab, UiO
Total nitrogen µg N/l		Measured with chemiluminescence after combustion and reaction with ozon	Lab, UiO
Total phosphorus µg P /l		Sample solved in K2S2O8,	Lab, UiO

		Autoanalyzer (G-297-03)	
Acid neutralization capacity(ANC)		Ion chromatography	Lab, NIVA
Alkalinity(buffer-capacity)	Titration (1/10 N HCL/100 ml water sample)		
Total hardness (mg/l CaCO3)	Complex titration with E.D.T.A		
Absorbance (water colour)		Unfiltered water, wavelength: 254, 350, 450 nm	Lab, UiO

Zooplankton sampling and analyzing

Vertical net hauls (26-71 cm, 90µm mesh size) were used to sample pelagic crustacean zooplankton. Net hauls were taken at the approximately greatest depth in each locality. In localities with greatest depth <1m, vertical net hauls were substituted with horizontal net hauls. Generally, 2-3 hauls were conducted. To reduce the possibility of species contamination between different water bodies, the zooplankton net was carefully cleaned between each sampling. Samples used for classification of zooplankton were fixed on lugol's solution.

All cladocerans and copepods were identified to species, with the exception of copepod nauplii, copepodite stages I-III and *Bosmina* spp. Cladocerans were identified in accordance with Flößner *et al*. (1972) and Sars (1895), whereas copepods were identified after Kiefer (1978) and Sars (1895). In high density samples a minimum of 200 individuals were identified and counted (the rest of the sample was looked through for further species). Low density samples (<200 individuals) was counted completely. In the original study, 95% of the pelagic samples were counted completely. In the remaining 5%, ¼ of the sample was counted following a technique by Cushing (1961).

Only species assumed to be pelagic is included in the analysis. This assumption relies on species found in the vertical net hauls by Eie (1974), and the distinction made between pelagic and littoral species in the large survey by (Walseng et al., 2006). Littoral species may outnumber the pelagic ones, and thus would cause trouble for interlake comparisons if they

are included in some localities while not others. Such a differentiation is especially useful in small ponds where there are vague boundaries between a littoral zone and a pelagic zone, and littoral species have a high incidence also in pelagic samples. The geographical distribution of littoral species is known to be in larger degree restricted by vegetation rather than water chemistry. The following species are considered as pelagic in the present study: *Acanthodiaptomus denticornis, Bosmina spp., Bythotrephes longimanus, Cyclops scutifer, Daphnia galeata, Daphnia longispina, Diaphanosoma brachyurum, Hetrocope saliens* and *Holopedium gibberum. Mesocycops leukarti* was considered to be a pelagic specie by Eie (1974), but is today considered as an intermediate (pelagic/littoral) specie and is excluded. Larger species such as *Bythotrephes longimanus* and *Leptodora kindti* are more likely to under-represented due to their ability to avoid the net hauls (*i.e* active movement).

Statistics and data analysis

Detrended Correspondence Analysis and Canonical Correspondence Analysis was carried out in the program CANOCO, while Permutational Multivariate Analysis of Variance using distance matrices, and non-metrical dimensional scaling was conducted in R. Multivariate statistics, such as ordination, is very useful in ecology to present large and complex data. The main idea is that different samples (*e.g* community compositions) will be arranged after their similarity/ dissimilarity. Presence/absence data from 93 localities were used. Lake nr. 25,33 and 39 is excluded due to poor conservation of samples. In the appendix, attachment 4, further statistics can be found.

A Paired-T-test was employed to test if water chemical parameters (assumed to be normal distributed) was significant different between the two datasets. The assumption of normality can however not always be met, and a Wilcoxon signed-rank test was used instead.

Linear regression and multiple regression have been used several times throughout the thesis to explain the variation seen in the dataset (water chemistry, community compositions and species richness). Covariation and multicollinearity has been a notorious problem in the present study, and has carefully been avoided.

3 Lake characteristics and water chemistry: A regional survey

Operating in two fundamentally different regions, a basic understanding of the water chemistry in the studied lakes and ponds is necessary.

Description of lakes and ponds

The localities in the study area, Vassfaret, can generally be characterized as oligotrophic (6.11 ± 5.33 µg P/l), soft (0.63 ±0.5 mg Ca/l), ranging from acidic and dystrophic ponds in the boreal region to neutral and clear water lakes in the alpine and subalpine region. The buffer-capacity in lakes and ponds is naturally low, although considerably higher in limed and limed-influenced localities.

Table 2. Basic water chemistry in lakes and ponds revisited in Vassfaret (N=96).

Class	n	Area ha	Depth m	TP µg/l	TOC mg C/l	Ca mg/l	ANC µeq/l	ANC.oaa µeq/l	pH
A.1	10	8.2±4.1	10.3±6.4	2.4±3.7	1.4±0.5	0.8±0.4	44.05±23.7	39.2±23.6	6.1±0.3
A.2	39	1.3±1.0	2.4±2.2	2.0*	1.8±1.0	0.6±0.7	16.8*	10.8*	6.1±0.5
B.1	11	9.1±5.7	11.1±6.7	7.7±3.8	5.2±2.3	0.9±0.4	63.1±25.0	45.1±23.3	5.7±0.4
B.2	36	0.7±0.8	2.3±1.6	9.0*	8.2±3.5	0.5±0.2	38.9±16.3	11.9±19.2	5.1±0.5

A.1=Alpine lakes, A.2=Alpine ponds, B.1=Boreal lakes, B.2=Boreal ponds. Alpine>950 m.a.s.l, lake>4 ha. Values are presented as mean ± SD. *Median used instead of mean in groups with extreme values.

A great majority (78,1%) of sampled localities are <4 ha. Small and shallow ponds are heavily influenced by rainwater, has a short retention time and are in high connectivity to their catchment. In addition, shallow lakes and ponds are expected to completely bottom freeze and experience significantly oxygen depletion and hypoxic conditions during the winter. Thereby, the presence of fish populations will in large degree be determined by lake depth.
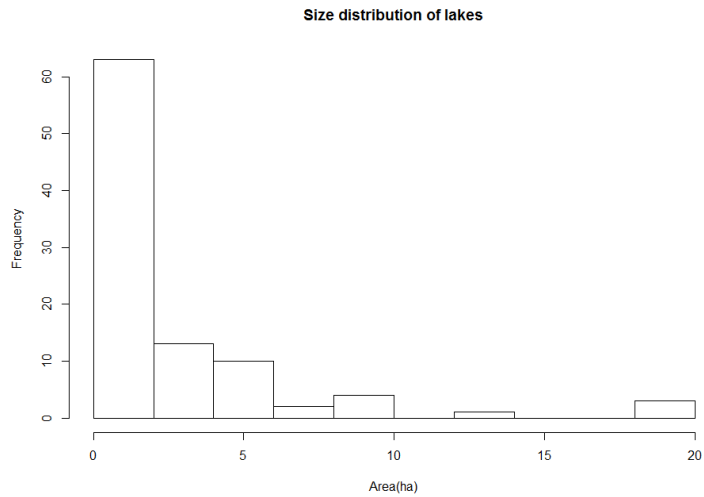
Figure 4. To simplify, the localities in Vassfaret are divided into two size classes; lakes(>4ha) and ponds (<4 ha).

Figure 5, 6, 7 and 8. The different regions and different size classes in the study area, here represented with locality number (left-to-right): 9, 8, 86 and 62 (photo: Martin Eie).

Surface water chemistry

This paragraph approaches different aspects of the surface water chemistry in *Vassfaret*.

Altitude is strongly correlated (-0.78) with total organic carbon(mg/l). The TOC concentrations peak around 800 m a.s.l (max= 17 mg C/l) and then sinks with altitude (min= 0.2 mg C/l).
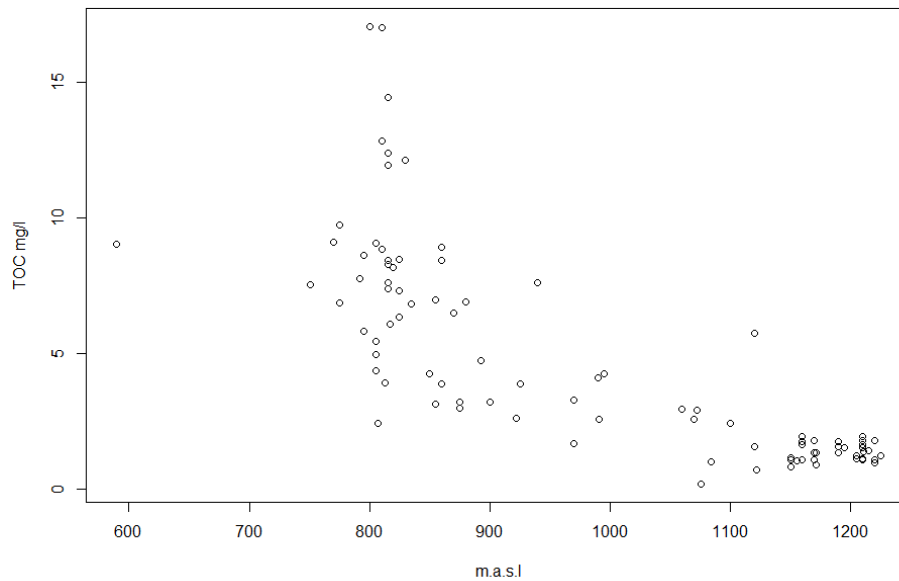
Figure 9. Relationship between altitude (m.a.s.l.) and TOC (total organic carbon).

Sum of calcium and magnesium is used as surrogate for total base cations. By comparing Ca+Mg concentrations to altitude a catchment response can be seen (figure 9). The Ca+Mg concentrations in the Vassfaret localities are in large determined by the catchment characteristics (vegetation and soil), and thus 'depends' on altitude. There is considerable variation within the boreal region, while the Ca+Mg concentrations are generally low in the alpine region. Multiple regression was employed to explain the variation in the dataset (coefficients given in table). Altitude (m.a.s.l.) represents catchment characteristics, sulfate the importance of ion exchange processes in the catchment, and max. depth as an indicator of the retention time. Altitude was the only significant (p-value<0.01) parameter, explaining 31% of the variation in Ca+Mg concentrations. The relatively large unexplained variation point to possibly several factors determining the concentration of base-cations in the study area, that were not captured in this study.
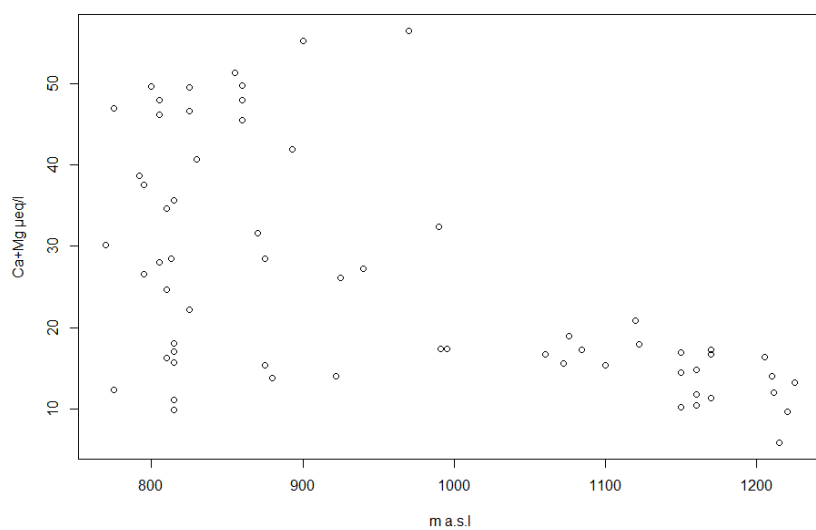
Figure 10. Major base-cations (Ca+Mg) compared to altitude (correlation= -0.57). Limed influenced localities are excluded, N=63.
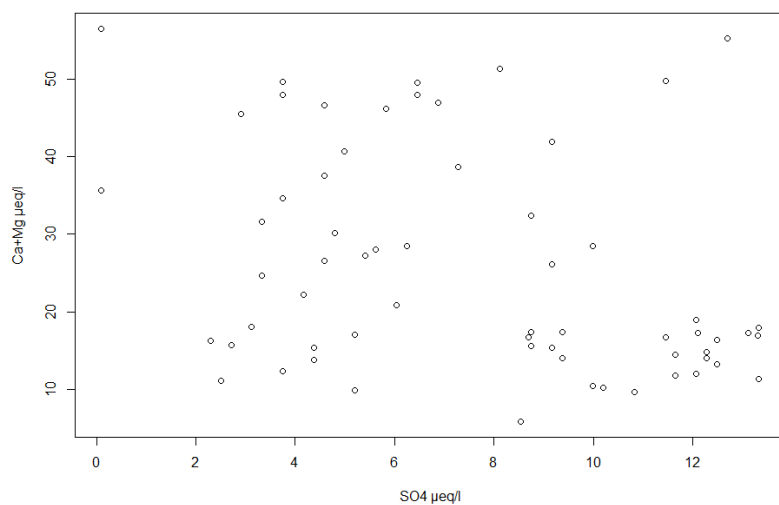
Figure 11. Comparison of Ca+Mg with SO4 (µeql). Cor.= -0.34.

Table 3. Coefficients from multiple regression, R^2=0.31.

	Estimate	Std. error	t-value	p-value
Intercept	82.768	11.331	7.304	0.01
m.a.s.l.	-0.066	0.015	-4.450	0.01
SO4 (µeq/l)	0.841	0.638	1.318	0.193
Max. depth	0.169	0.362	0.468	0.641

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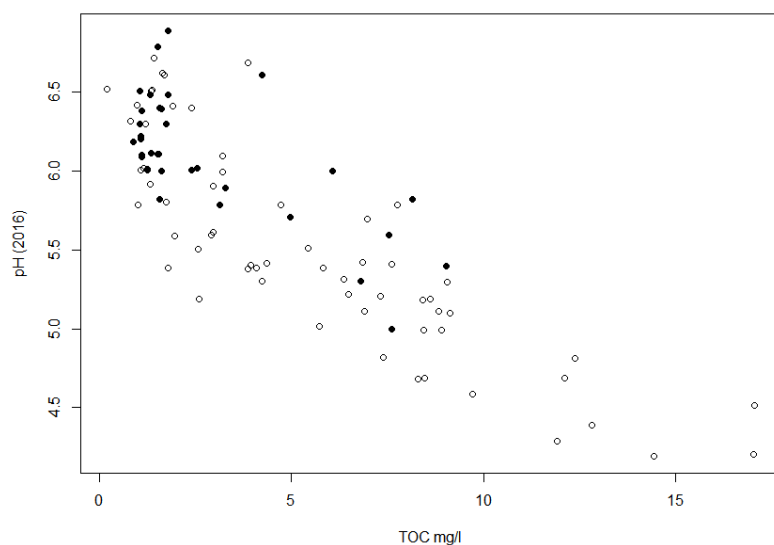
Figure 12. Relationship between TOC (mg C/l) and pH. Black dots represent limed localities.

Table 4. Multiple regression. How can the variation in pH be explained? $R^2 = 0.77$, p-value $<<0.01$. The coefficients are presented in the table. Limed localities are excluded.

	Estimate	Standard error	t-value	p-value
Intercept	6.157	0.065	93.96	<0.01
TOC (mg C/l)	-0.144	0.008	-17.413	<0.01
ANC µeq/l	0.005	0.001	4.881	<0.01

The acid neutralizing capacity has been heavily linked to the water chemical and biological status of lakes. ANCoaa is significant lower than ANC (p-value<0.01, paired t-test), indicating major importance of organic acids. The average ANCoaa for the study area is 10.8 µeq/l (sd=16.9). 74.6% of natural localities in Vassfaret are below the ANC critical limit (20µeq/l), when ANC is adjusted for organic acids. Comparably 47.65% of localities are below the 20 µeq/l limit with ANC not adjusted.
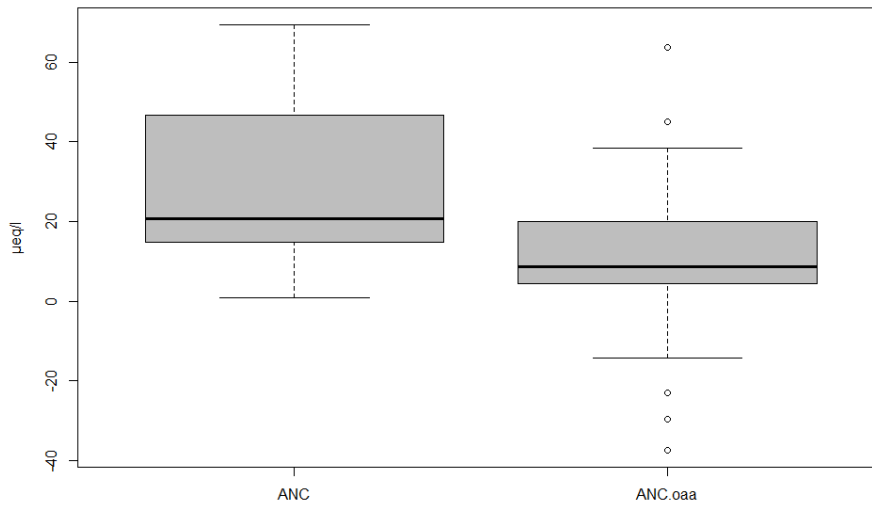
Figure 13. The acid-neutralization capacity in natural localities (n=63=).

Linking environmental chemistry to biology

This paragraph looks at regional surface water chemistry in the context of biology. How can the study area be defined in terms of pelagic zooplankton species, and can different species be related to specific physico-chemical parameters?

In total, ten different pelagic species were observed in Vassfaret: *Acanthodiaptomus denticornis*, *Bosmina* spp., *Bythotrephes longimanus*, *Cyclops scutifer*, *Daphnia galeata*, *Daphnia longispina*, *Diaphanosoma brachyurum*, *Hetrocope saliens*, *Holopedium gibberum* and *Mixodiaptomus laciniatus* (see appendix, attachment 4 for complete species lists). Only one species, *M laciniatus*, was found exclusively above the tree-line (~950 m. a.s.l.) in 59.2% of alpine localities(n=49). While on the contrary, five species were exclusive to the boreal region(n=37) (*A. denticornis* (31,9%), *B. longimanus* (8.5%), *D. galeata* (2.1%), *D. longispina* (10.6%) and *D. brachyurum* (55.3%). The most common species in the study area (n=93) were *Bosmina* spp. (95.3%), *H. saliens* (73.1%), *H. gibberum* (59.1%) and *C. scutifer* (47.3%). In an effort to connect basic physico-chemical water parameters to species compositions, a Canonical Correspondence Analysis (CCA) was conducted (figure 14). CCA is a tool designed to reduce the dataset complexity and identify major patterns and putative casual factors. The first axis corresponds to altitude (m. a.s.l), while the second axis corresponds to greatest depth.

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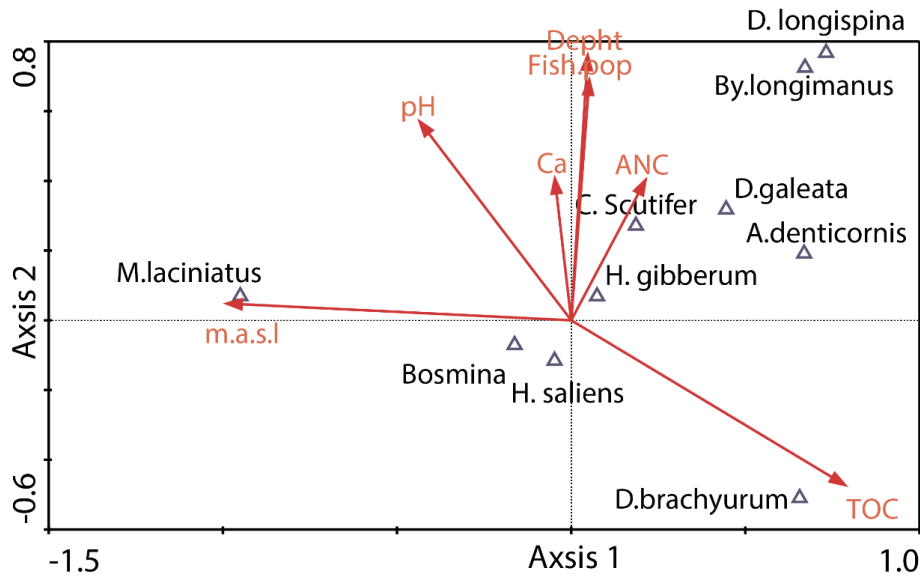
Figure 14. CCA (Canonical Correspondence Analysis) of 93 species compositions (presence/absence) in 2016.

Table 5. Summary of CCA (Canonical Correspondence Analysis) output.

Axes	1	2	3	4	Total inertia
Eigenvalues:	0.253	0.084	0.036	0.013	1.374
Species-environment correlations:	0.887	0.639	0.521	0.302	
Cumulative percentage variance of species data:	18.4	24.5	27.2	28.1	
of species-environment relation:	63.4	84.4	93.5	96.8	
Sum of all eigenvalues					1.374
Sum of all canonical eigenvalues					0.399

Species richness has been closely linked to ecosystem stability, resilience and services, and represents a typical community end-point. Table 1 gives the coefficient of a simple multiple regression model aiming at predicting species richness. The model is kept simple in an effort to explain as much variation, with as few parameters, as possible. Altitude (m. a.s.l) represent the main physical factor (incorporating other factors such as water temperature, vegetation, geographical barrier, UV-radiation) important to species richness and pH representing acidity of the locality. Different models were tested with parameters such as ANC, Ca and TP, max depth without being significant ($p > 0.05$). Ecological parameters such

as presence of fish populations and Chaoborus could not be used as their presence will in large will be determined (collinearity/covariance) by other physical and chemical factors (max. depth, altitude). TOC is highly correlated with altitude and although a possibly important parameter, could not be used. The average pelagic species richness in Vassfaret (2016) is 3.6 species (max=7, min=1), and is not significant different from 1968 (p-value= 0.133, Wilcoxon signed rank).

Table 6. Coefficients from multiple regression, $R^2=0.33$, p-value<0.01.

	Estimate	Std. error	t-value	p-value
Intercept	3.860	1.129	3.418	<<0.01
m a.s.l.	-0.006	0.001	-6.810	<<0.01
pH	1.049	0.266	3.937	<<0.01

4 Changes in surface water chemistry

This chapter looks at changes in surface water chemistry related to acidification. In regards to this, the focus will be centered around four different parameters: pH, base-cations(Ca+Mg) and TOC/ mg Pt/l (here used as an measurement of water color).

pH

pH, being one of the key parameters in studies of acidification, is compared between 1968 and 2016 (figure 1). The pH is significant different between the two years (p<<0.01, paired T-test) when all localities are included. When only natural (not limed) localities are compared, the pH is still significantly different (p<<0.01, paired T-test). An increase in pH from 1968 is observed in 85/95 (89,5%), while 6/95 are unchanged (6,3%) and 4/95 have decreased (5,2%). Despite the increase in pH in the majority of localities, 38 % are still acidic (pH<5.5).
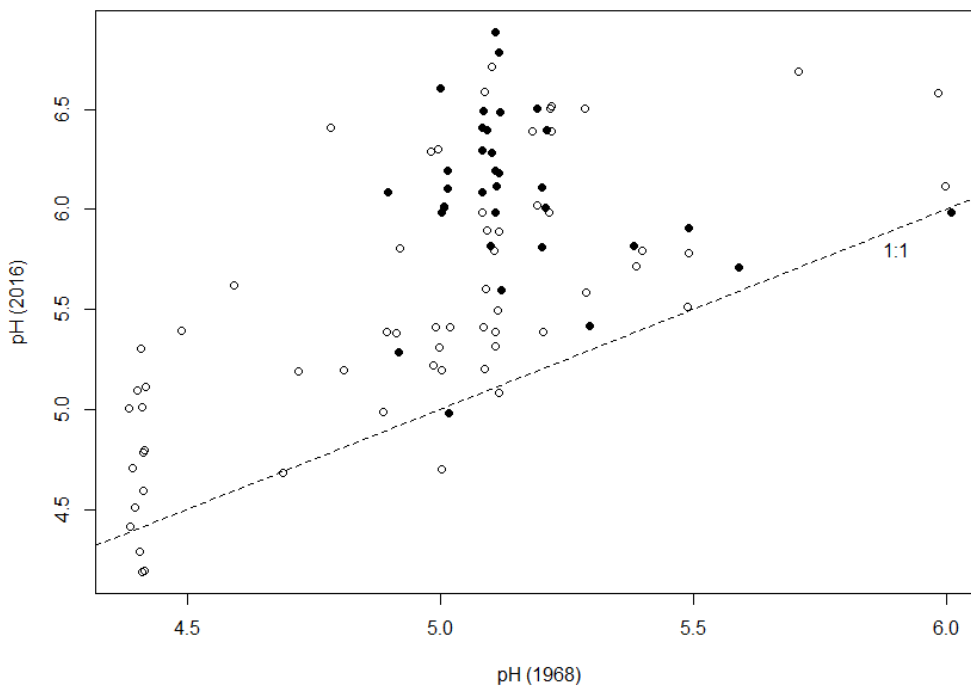
Figure 15. A comparison of the pH measured in 1968 and 2016. Black points represent limed/ limed influenced localities.

Base-cations

41 out of 63 (65%) natural localities revisited in Vassfaret show a reduction in Ca+Mg since 1968. The largest decrease can be observed in the boreal region (average change in Ca+Mg= -5.22 μM, max.= 4.0 μM, min=-14.6 μM), while the Ca+Mg concentrations slow little or no change in the alpine region (average change in Ca+Mg= 0.75 μM, max=8.6 μM, min=-2.1 μM)). Locality nr. 37 is excluded from statistical analysis due to extreme value.
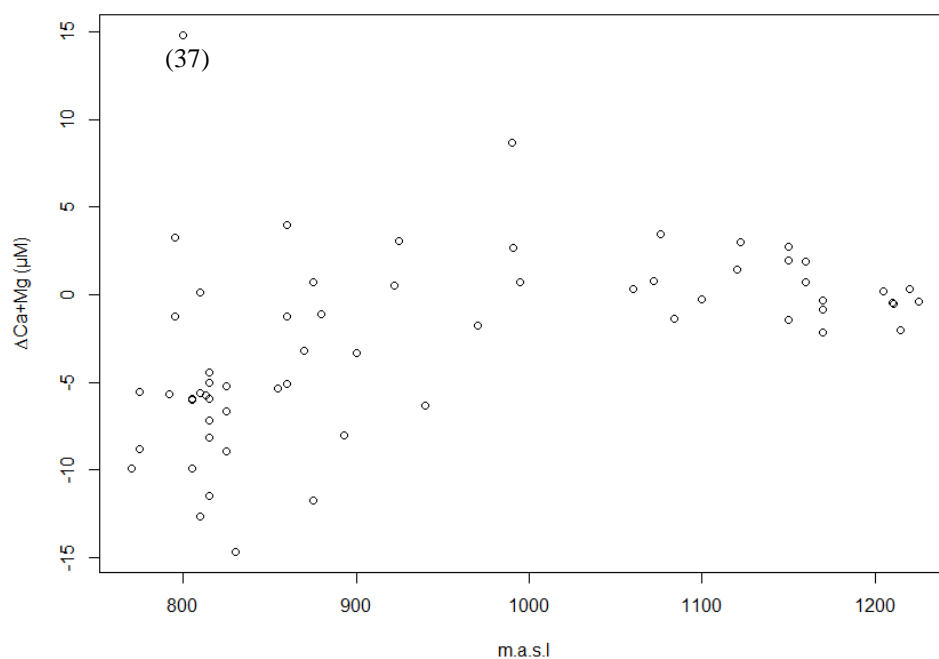
Figure 16. Ca+Mg 2016(μM)÷Ca+Mg 1968(μM). Tree-line at ~950 m. a.s.l. N=63 (limed influenced localities excluded). Note extreme value in locality number 37 (a small and shallow pond not influenced by liming).

Browning of surface waters

The water color (mg Pt/l) is significantly different between 1968 and 2016($p \ll 0.01$, Wilcoxon signed rank). Of 96 revisited localities, there is an increase in (mg Pt/l) in 50 localities, 40 are unchanged and 6 have declined (figure 1). The average increase in the boreal region is 20 mg Pt/l, while the alpine region shows a more modest increase (mean= 6 mg Pt/l.). Figure 2 compares one old (mg Pt/l) and one new method (TOC). The linear regression ($R^2=0.95$, p-value<0.05) indicates a tight relationship between the two parameters, and that they are compatible.

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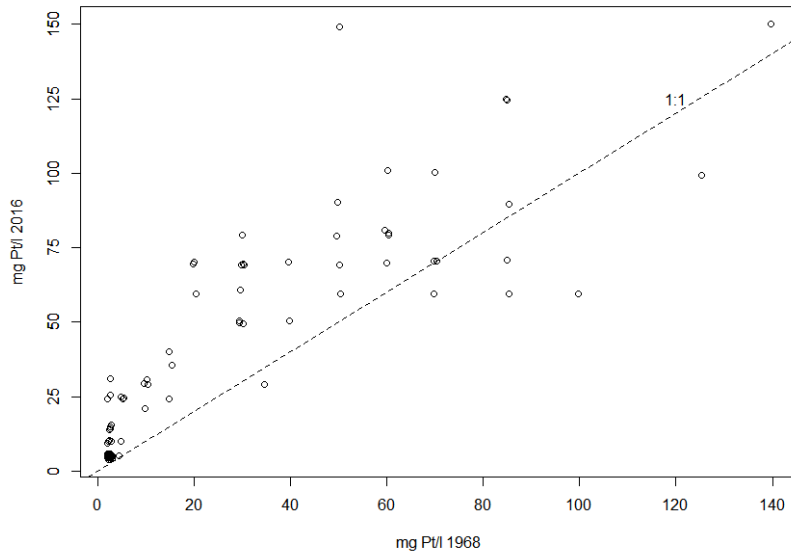
Figure 17. Comparison of color (mg Pt/l) between 1968 and 2016. The 1:1 line is based on values from 1968. The dots above the 1:1 line indicates an increase in color, while dots below indicates a decrease. Note that the detection limit is 5 mg Pt/l.
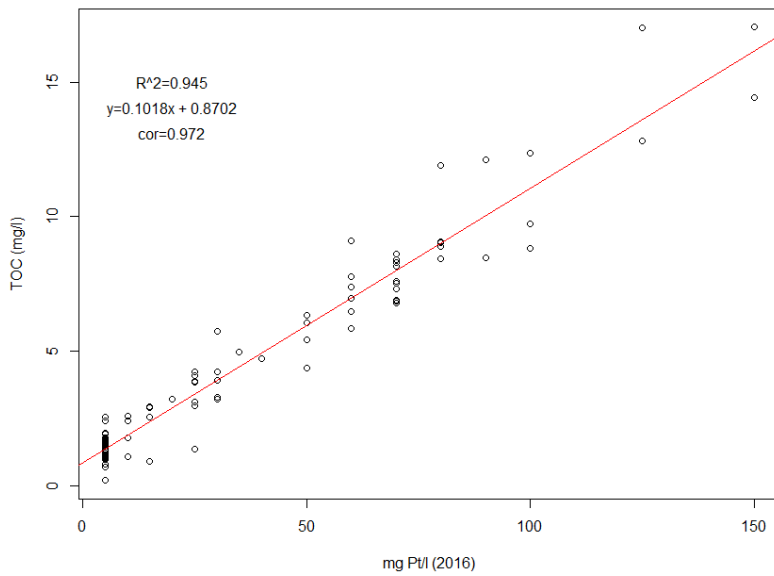
Figure 18. A comparison (linear regression) between TOC (mg C/l) and mg Pt/l.

5 Species compositions

The species compositions in 1968 is not significantly different from 2016 (p-value= 0.221). Using non-metical multitalented scaling (NMDS) as an ordination technique, distance matrices with two dimensions were created to compare the species compositions between the two datasets (figure 19 and 20). In the appendix (attachment 5) further data analysis of the species compositions can be found.

Table 7. Comparison of the species compositions with Permutational Multivariate Analysis of Variance, using distance matrices. Coefficients are given in the table.

	Df	Sums of Sqs	Mean Sqs	F-model	R^2	p-value
Year	1	0.153	0.153	1.619	0.008	0.221
Residuals	183	17.358	0.094		0.991	
Total	184	17.511			1.0	

A linear model was employed to explain the variation in NMDS 1 and 2 scores in 2016 using NMDS 1 and 2 scores from 1968 as an explanatory variable. The variation in species compositions in 2016 cannot significantly (p-value>0.05) be explained by the species compositions of 1968, explaining less than 1% of the variation.
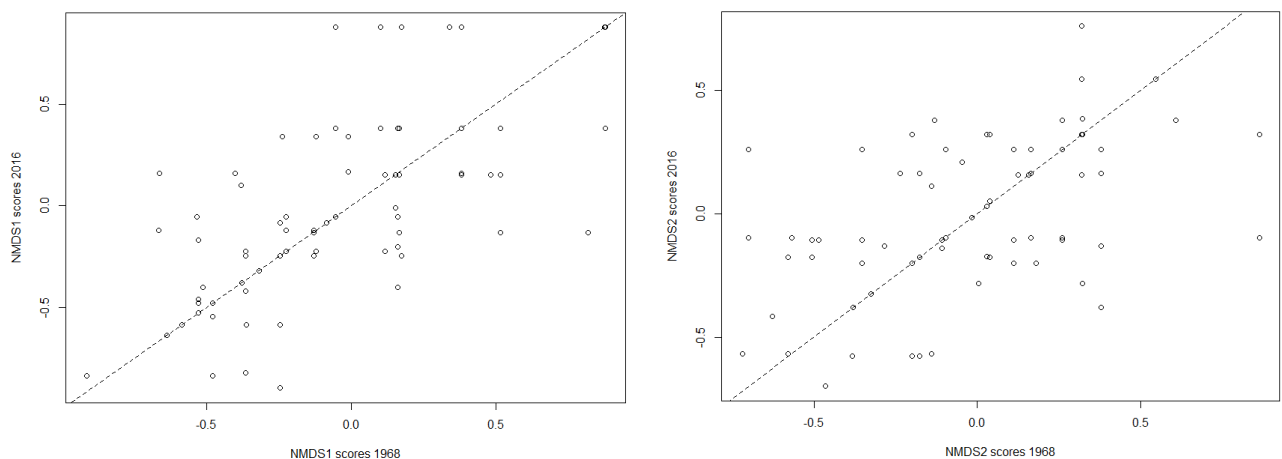
Figure 19 and 20. A comparison of NMDS1 and NMDS2 scores in 1968 and 2016.

6   Discussion

This thesis aimed to trace the footsteps for a nearly 50 years old regional study – looking at essential water chemical parameters in the context of biology. By integrating a biological response to basic surface water chemistry, questions have been asked on the stability of freshwater ecosystems in the light of old and new environmental concerns. The following chapter will discuss the reliability and different aspects of the results in chapter 3 and 4 and 5.

In the light of the discussion on the surface water chemistry in *Vassfaret*, it should be emphasized that water chemistry within a lake fluxes naturally – both spatially and temporally. Multiple measurements throughout the season and at multiple depths would give a more complete understanding of the chemical and physical parameters working in concert, forming the abiotic frame. For practical reasons this could not be achieved, and hence the values should be seen more as an indicator of the chemical condition at the respective lake at the respective time. It should be further noted that autumn sampling, after circulation, is preferred when looking and trends in water chemistry.

Acidification

The natural recovery of surface water chemistry in Norway after acidification is a slow process. The surface water chemistry in Norway is mainly determined precipitation chemistry, hydrology, bedrock geology, soil type and vegetation (Skjelkvåle *et al.*, 1996). Norway is generally comprised by a thin soil layer and weather resistant bedrock, making lakes acid sensitive. Although a clear increase in pH is observed in the study area, the acid neutralization capacity is still low, suggesting that lakes and ponds are still vulnerable to acidification. In addition, 74.6% of the localities in Vassfaret have an ANC_{oaa} value below the $20\mu eq/l$ critical limit, indicating possibly harmful effects of acidification to sensitive organisms (Lien *et al.*, 1996, Henriksen *et al.*, 1999). The natural high concentration of organic acids in the lakes in the study area, greatly affects the pH – making lakes and ponds natural acidic. It is vital to emphasize that the regional survey conducted in 1968 was before the strongest effects of acidification was observed, and could represent an early stage of acidification. Still, reports as early as 1968 recorded snow containing long-transported pollutants such as lead, zinc and acidifying compounds (Elgmork, 1983). Since there are no sulfate measurements from Vassfaret in 1968, it is difficult to estimate the acid loading in

1968 and its effect on biota. But with the acid deposition reduced the last two decades, we are seemingly approaching the natural conditions (pre-perturbed conditions) in lakes and ponds in Vassfaret.
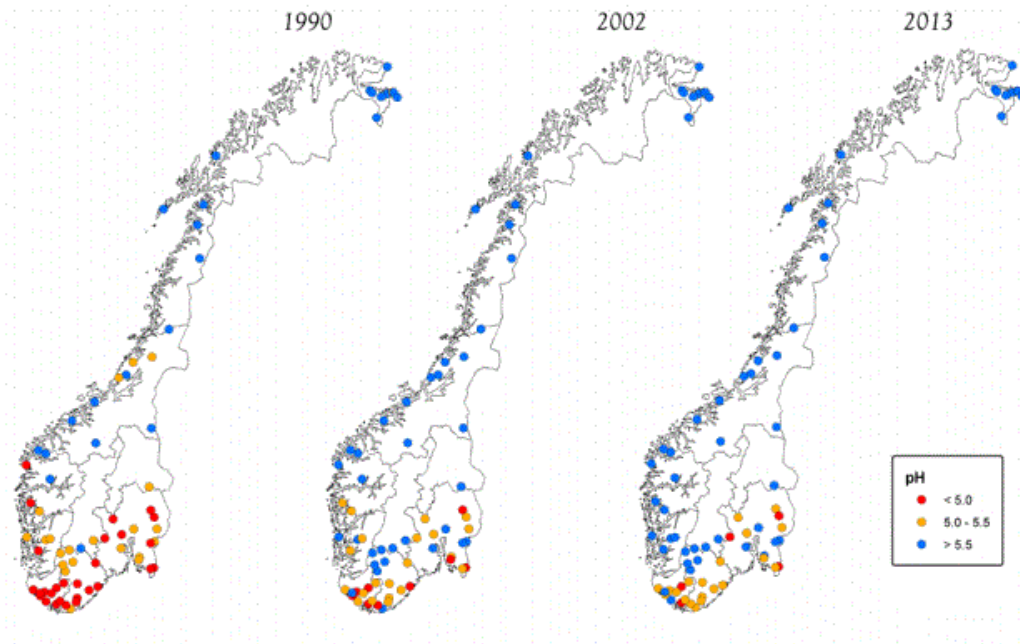
Figure 21. The pH in 78 lakes in Norway in 1990, 2002 and 2013 (Garmo et al., 2013).

Following the calculations by Lund *et al*. (2012) 8% of Norway's area exceeded the critical loads of acidic components to surface waters between 2007 and 2011. Comparatively, 29 % of Norway's area in the period 1978-1982 and 16% in 1992-1996. Further emission reductions in anthropogenic long-transported S is needed to match the depletion of base-cations in Norwegian catchments.
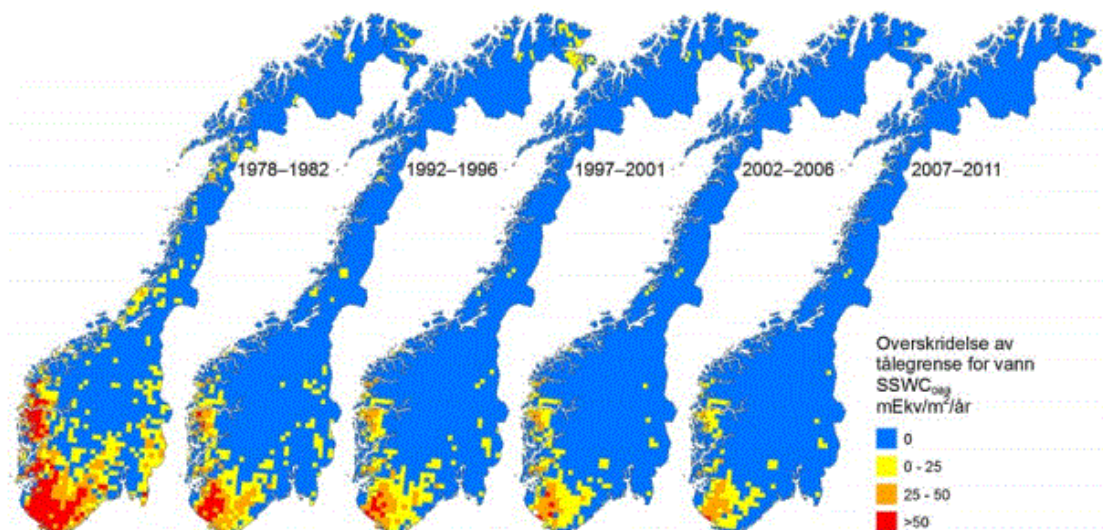
Figure 22. Exceedance of critical loads in surface waters in Norway, calculated with *The Stady-State Water chemistry* (SSWC) (Lund et al., 2012).

There is considerable variation in measured pH in 2016 (max= 6.7, min= 4.2). This variation could be the result of natural variation and/or methodical uncertainty. pH is a parameter notoriously difficult to measure, as it could be influenced by storage time, light and temperature and fluxes with time of day and with season. It is generally recommended to either measure pH *in situ* or in lab following accredited protocols. Although the expected influence of storage time, light exposure etc., is considered minimal in the present study, it cannot be disregarded. Also, field pH-meters (hand-held) is considered to be less precise than stationary lab equipment. Measuring pH in lakes with low ion content (such as high alpine ponds and lakes) is additionally more difficult. Although, some of the variation seen in the measured pH could potentially be methodical, the instrument was properly calibrated before use, and the effect should be minimal.
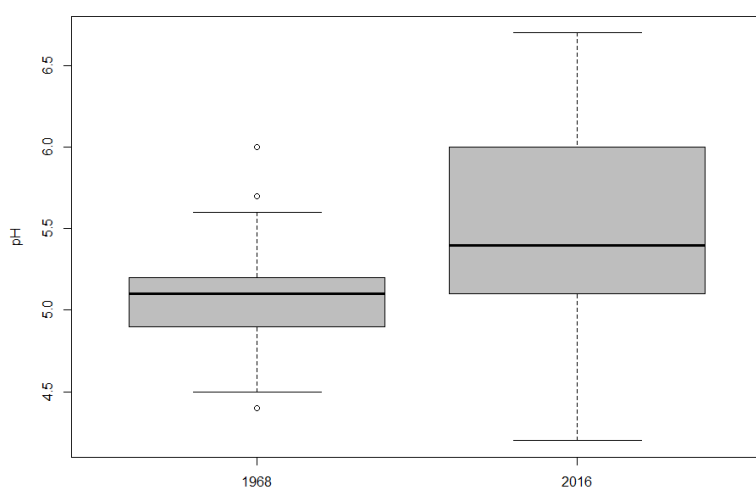
Figure 23. Boxplot of measured pH in 1968 and 2016. All limed/ limed influenced lakes in 2016 are excluded.

It should further be noted that colorimetric measurements of pH in 2016 should have been conducted. This, would have excluded the possibility of measuring uncertainty (methodology, protocols) between the years. It is thereby, highly recommended that this will be followed up in further investigations will be proceeded.

Decline in base-cations

The link between the ongoing decline of base-cations in lake catchments and the reduction of acid loading the last twenty years is well documented. Even though a decline in base-cations from 1968 can be observed in the study area, there are still several important questions unanswered. Firstly, there is a considerable uncertainty around the natural variation in Ca+Mg concentrations, as the study only include data from two seasons. Differences in hydrological conditions could explain the change in Ca+Mg between the two datasets, as precipitation can both have a diluting and a mobilizing effect on base-cations in catchments. Trends on precipitation patterns should hence, also be analysed to get a more complete understanding for biochemical response of water entering a catchment (Lydersen, 1994). Secondly, the weak relationship between SO4 and Ca+Mg (figure 11) indicates little effect of sulfate on base-cations in this dataset. Sulfate is not and mobile anion, and could be retained in the boreal catchments, while washed out in the alpine region. In addition, there could be regional differences in precipitation between the boreal region and the alpine region, and hence the difference in SO4-concentrations. Snow-smelt (which is later in the alpine region) could also contribute to higher sulfate concentrations in the alpine region. Lastly, there are uncertainties regarding the compatibility of results from Ca+Mg complex titrations (using E.D.T.A.) from 1968 and ion chromatography derived Ca and Mg concentrations in 2016. Both methods considered reliable, although question can be asked about the reliability of E.D.T.A.-titration at low concentrations. Implementing E.D.T.A. titration in 2016 would have minimized the uncertainties regarding the difference in methodology.
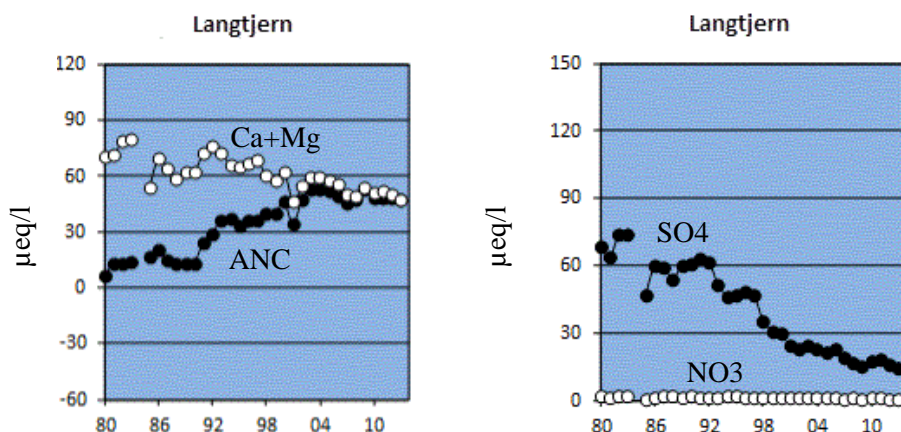
Figure 24 and 25. Trends in surface water chemistry from 1980 to 2013 in Lake Langtjern (Garmo et al., 2013). The reduced acid loading (mainly SO4) has been matched by an equal decline in base cations.

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Browning of surface waters

Changes in the atmospheric deposition chemistry, with the following catchment response, is the main factor linked to the increasing concentrations of DOC/TOC. The browning of surface waters in Vassfaret can be linked to the trends recorded in Lake Langtjern (figure 26). The transport of allochthonous material, which is the main source of organic carbon in lakes, from catchment to lake could also be influenced by the hydrological conditions and will variate naturally. The increase in water color seen in the alpine region could be partially caused by climate warming and the upwards expansion of vegetation. The degree and rate of browning caused by different factors should hence be investigated. The increasing transport of organic material form catchments is predicted to have a double role in lakes. In oligotrophic nutrient limited waters, increased transport of allochthonous material could facilitate primary production through increased concentrations of carbon and phosphorus in runoff. While on the other hand, as we have seen in the present study, lakes and ponds are browning and organic acids affecting the pH and water quality.

Using the same method in 2016 as in 1968, has proven crucial to show the browning of surface waters in Vassfaret. The colour readings (Lovinbond Nessleriser color comparator) from 2016 seems to comply well with modern techniques such as TOC (mg/l). The largest uncertainties in colour readings seems to occur at low (<10 mg Pt/l) and at high (>120 mg Pt/l) values.
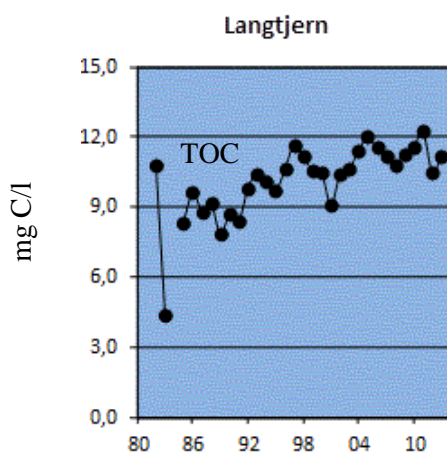
Figure 26. Trend in total organic carbon (TOC) in Lake Langtjern from 1980 to 2013.

Disturbance ecology

Can the observed environmental changes be connected to a community response (*i.e.* changes in species compositions and species richness)? The preceding paragraph aims to ask questions on how stabile freshwater ecosystems are, and to look at impacts of nearly 50-years of changes in water chemistry on pelagic zooplankton communities.

First of all, comparing species compositions (presence/absence data) is a relative robust way to look at community responses. Micro-crustaceans are considered to be quick in re-colonization, as many taxa operate with resting-stages/resting eggs. Abundance data, on the contrary, is more sensitive to natural variation and time-trends in water chemistry. Still, abiotic factors are considered in ecology to frame the biotic interactions, meaning that the presence of species within a locality will be a reflection of the physico-chemical environment.

Secondly, community-studies are an increasingly popular biological level of organization to investigate effect of disturbances (Clements and Rohr, 2009). Communities offer insight into the mechanistic effect of chemicals on species level, and at the same time are closely linked to end-points such as ecosystem health and ecosystem services.

In the present study, two different community end-points were used to assert the biological effect of changes in water chemistry on freshwater ecosystems; Species compositions and species richness. As neither the species compositions or the species richness was found to be significantly different between the two datasets, the pelagic zooplankton communities in lakes and ponds in Vassfaret shows high stability. What are the mechanisms behind this stability? The stability could be caused by high ecological resilience, quick biological recovery and, or, high community resistance (figure 27). In this thesis, ecological resilience is defined as the level of perturbation a community can withstand before it is pushed to an alternative state, and recovery is defined as the process at which a community returns to equilibrium after disturbance (Van Nes and Scheffer, 2007). In Vassfaret, the main core of the zooplankton community is composed by tolerant taxa such as *Bosmina* spp., *H. gibberum* and *H. saliens* – species with high threshold levels to environmental stress – making the communities resistant to environmental changes. This resistant could be a pollution induced community tolerance (Blanck *et al*., 1988), through selection towards increased resistance due to elimination of sensitive species. Another explanation to the large similarity in species compositions, could be a result of high connectivity between ponds and lakes within each region and possibly creating meta-populations. The lack for response to

observed environmental changes could also be caused by a delayed biological response (Patten, 2013), and that we might see a community response in the future, with further improvements of water quality (pH, acid neutralizing capacity). Unexpectedly, no species has shown a tendency of range expansion/ range retraction in the study area that can be connected to climate change. While *M. laciniatus* is exclusively found in the alpine region, this seems more an effect of competition with *A. denticornis*, as it is found below the tree-line in other studies (Hessen and Walseng, 2008). In fact, of the five species only found in the boreal region, only *D. brachyurum* is exclusively found below 1000 m.a.s.l (on a national scale), but has shown to tendency of range expansion. *Daphnia galeata* is new to the study area, but was only represented by one individual in a single locality and thereby have little ecological relevance.
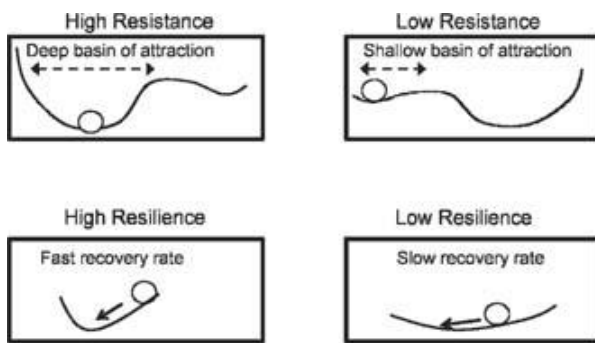
Figure 27. The concept of community resistance and resilience (Clements and Rohr, 2009).

A multiple linear regression model was conducted to predict the pelagic species richness (PSR) in the study area. Using two independent parameters (m.a.s.l. and pH), 33 % of the variation could be explained. One of the major factors determining the species richness in Vassfaret is altitude – a parameter representing the combined effect of several other biological important factors such as climate and water temperature, catchment characteristics (vegetation, nutrients, ions) and primary production. In addition, the altitude gradient could serve as a barrier to geographical distribution of species between the boreal region and the alpine region. The second parameter, acidity (pH), proved also to be linked to species richness. The impact of acidification on species richness is well documented (Walseng and Schartau, 2001), and is taken to have a negative effect on zooplankton communities and species richness (Havens and Hanazato, 1993). Lake area/ greatest depth could possibly also be a driver for species richness (Dodson, 1992), although the large contribution of intermediate species (operating both in the pelagic and the littoral zone) in small ponds could

mask this pattern. The considerable low species richness in the study area could be an indication of ecosystems experiencing stress (Odum, 1985). The relatively large unexplained variation (67%) shows that there are still important factors that needs to be assessed.

Biological indicators (bioindicators) have popularly been used to assess the biological impact of specific pollutions/perturbations in environmental monitoring (Zhou *et al.*, 2008, Bauer, 1988) . The presence and relative abundance of the acid-sensitive species *Daphnia longispina* has been used to indicate non-acidic conditions in lakes and ponds, and as an indication of biological recovery after acidification (Hesthagen *et al.*, 2011, Schartau *et al.*, 2001). Can basic ecological and toxicological principles explain why acid-sensitive species are scares in the study area, using *Daphnia longispina* as an example? Figure 29 is a simplification of a chosen direct and indirect threats, possibly supressing the *D. longispina* populations in the study area. Although the effect of long-range transported air pollutants is decreasing, the *Daphnia longispina* populations could be affected by several other environmental stressors causing an adverse outcome pathway (the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment (Kramer *et al.*, 2011)). This adverse outcome pathway seems to be induced by combined effect of acidic conditions (pH<5.5), low Ca-concentrations (<1.0 mg Ca/l), high competition (tolerant taxa such as *H. gibberum*) and possibly also heavy predation pressure by Salmonids. The resistance threshold (concentration/level at which an abrupt ecological change can be observed) could be significantly lowered by interactive effect of multiple stressors (Walker *et al.*, 2012, Newman, 2009), and also delaying the biological recovery (figure 28). The current and future impact of climate change and browning of surface waters is uncertain, but cannot be ruled out as possible indirect stressors.
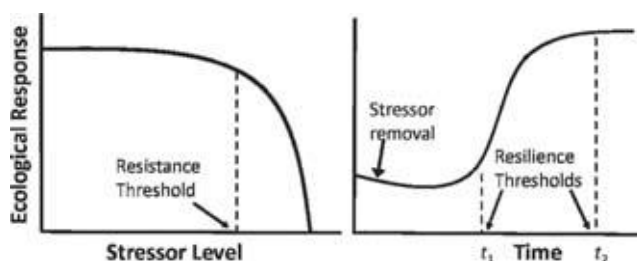
Figure 28. An ecological response to a stress level, t1- t2= time from recovery is initiated to completed (Clements and Rohr, 2009). This concept can be employed at several levels of biological organization.

Daphnia longispina is found in nearly all corners of Norway – from the harsh environments in rock- pools in the alpine areas, to eutrophic lakes in the lowlands (Hessen and Walseng, 2008). Considering this, it is therefore a pressing question why it is not recorded in the alpine localities in Vassfaret. Are the populations of Daphnia in Vassfaret not adapted to the harsh environment in the alpine region (nutrient poor lakes, DNA damage by UV-light, low Ca-concentrations), or does the altitude gradient 'simply' serve as a geographical barrier?
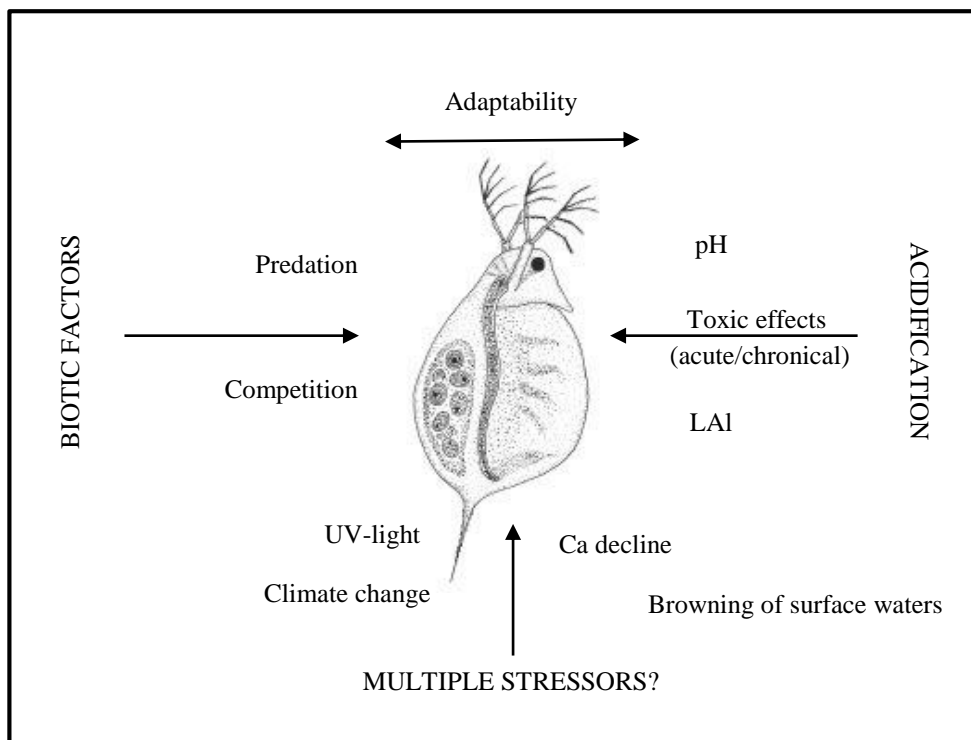
Figure 29. The curious case of *Daphnia longispina*. Multiple factors limiting the geographical distribution of *Daphnia longispina*? (Daphnia sp. illustration by Brage Førland).

Further studies should be aimed connecting basic water chemistry data to a biological response, and identifying threshold levels of possible environmental stressors to sensitive species. Also, it is crucial to identify early warning signs of future regime shifts and ecosystem deterioration. As community compositions seems to be fairly rigid to environmental change, this thesis propose to integrate abundance data in further monitoring.

7   Conclusion

This Master thesis aimed to revisit lakes and replicate the study by Eie (1974). By tracing the footsteps of a nearly 50-years old study, important questions have been asked on the resistance and resilience of freshwater ecosystems, facing abrupt chemical changes. Keeping the authenticity regarding the procedures and methodology has proven crucial in doing so. With continuously technological advances, new and old methods need to be compared as they might not be compatible. Singling out natural variation from long-term trends in water chemistry has been difficult in the present study, as it only contains two datasets. The long-term monitoring of Lake Langtjern has hence been crucial to explain the results seen in this study.

The study area, Vassfaret, can neatly be divided in an alpine a boreal region – creating two fundamentally different ecosystems. This makes them both interesting and difficult to compare. The changes observed in pH, Ca, and water color is heavily linked to catchment responses of the decline in long-transported pollutants such as $SO4$ and NO_x. The lack of a biological response on a community level in this study seems to be caused by high ecological resistance and, or, resilience.

Future focus should be aimed at identifying threshold levels of multiples stressors on sensitive organisms and early warning signs of ecosystem instability. As community compositions seems to be fairly rigid to environmental change, this thesis propose to integrate abundance data in further monitoring. Also, focusing on studies from early- and pre-acidic conditions in lakes, could be important to predict future chemical and biological recovery.

References

ADRIAN, R., O'REILLY, C. M., ZAGARESE, H., BAINES, S. B., HESSEN, D. O., KELLER, W., LIVINGSTONE, D. M., SOMMARUGA, R., STRAILE, D. & VAN DONK, E. 2009. Lakes as sentinels of climate change. *Limnology and oceanography,* 54**,** 2283.

BAUER, G. 1988. Threats to the freshwater pearl mussel Margaritifera margaritifera L. in central Europe. *Biological Conservation,* 45**,** 239-253.

BLANCK, H., WÄNGBERG, S.-Å. & MOLANDER, S. 1988. Pollution-induced community tolerance—a new ecotoxicological tool. *Functional testing of aquatic biota for estimating hazards of chemicals.* ASTM International.

BRÖNMARK, C. & HANSSON, L.-A. 2002. Environmental issues in lakes and ponds: current state and perspectives. *Environmental Conservation,* 29**,** 290-307.

CARPENTER, S. R., CARACO, N. F., CORRELL, D. L., HOWARTH, R. W., SHARPLEY, A. N. & SMITH, V. H. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological applications,* 8**,** 559-568.

CARPENTER, S. R., STANLEY, E. H. & VANDER ZANDEN, M. J. 2011. State of the world's freshwater ecosystems: physical, chemical, and biological changes. *Annual Review of Environment and Resources,* 36**,** 75-99.

CHEN, I.-C., HILL, J. K., OHLEMÜLLER, R., ROY, D. B. & THOMAS, C. D. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science,* 333**,** 1024-1026.

CLEMENTS, W. H. & ROHR, J. R. 2009. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. *Environmental Toxicology and Chemistry,* 28**,** 1789-1800.

CUSHING, C. E. 1961. A plankton sub sampler. *Limnology and Oceanography***,** 489-490.

DE WIT, H. A., VALINIA, S., WEYHENMEYER, G. A., FUTTER, M. N., KORTELAINEN, P., AUSTNES, K., HESSEN, D. O., RAIKE, A., LAUDON, H. & VUORENMAA, J. 2016. Current Browning of Surface Waters Will Be Further Promoted by Wetter Climate. *Environmental Science & Technology Letters,* 3**,** 430-435.

DODSON, S. 1992. Predicting crustacean zooplankton species richness. *Limnology and Oceanography,* 37**,** 848-856.

DUDGEON, D., ARTHINGTON, A. H., GESSNER, M. O., KAWABATA, Z.-I., KNOWLER, D. J., LÉVÊQUE, C., NAIMAN, R. J., PRIEUR-RICHARD, A.-H., SOTO, D. & STIASSNY, M. L. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological reviews,* 81**,** 163-182.

EIE, J. 1974. A comparative study of the crustacean communities in forest and mountain localities in the Vassfaret area (southern Norway). *Norw. J. Zool,* 22**,** 177-205.

ELGMORK, K. 1983. Natur og menneske i Vassfaret. *Universitetsforlaget, Oslo***,** 136-138.

FINSTAD, A. G., ANDERSEN, T., LARSEN, S., TOMINAGA, K., BLUMENTRATH, S., DE WIT, H. A., TØMMERVIK, H. & HESSEN, D. O. 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.

FLÖßNER, D., DAHL, F., DAHL, M. & BISCHOFF, H. 1972. *Krebstiere oder Crustacea: Kiemen-und Blattfüßer, Branchiopoda Fischläuse, Branchiura*, Veb Gustav Fischer.

GARMO, Ø., SKANCKE, L. & HØGÅSEN, T. 2013. Overvåking av langtransportert forurenset luft og nedbør. Årsrapport–vannkjemiske effekter 2012. *Miljødirektoratet, Rapport M-173/2014*.

GARMO, Ø. A., SKJELKVÅLE, B. L., DE WIT, H. A., COLOMBO, L., CURTIS, C., FÖLSTER, J., HOFFMANN, A., HRUŠKA, J., HØGÅSEN, T. & JEFFRIES, D. S. 2014. Trends in surface water chemistry in acidified areas in Europe and North America from 1990 to 2008. *Water, Air, & Soil Pollution,* 225**,** 1880.

HANAZATO, T. 2001. Pesticide effects on freshwater zooplankton: an ecological perspective. *Environmental Pollution,* 112**,** 1-10.

HAVENS, K. E. & HANAZATO, T. 1993. Zooplankton community responses to chemical stressors: a comparison of results from acidification and pesticide contamination research. *Environmental pollution,* 82**,** 277-288.

HENRIKSEN, A., FJELD, E. & HESTHAGEN, T. 1999. Critical load exceedance and damage to fish populations. *Ambio (Sweden)*.

HENRIKSEN, A., LIEN, L., ROSSELAND, B. O., TRAAEN, T. S. & SEVALDRUD, I. S. 1989. Lake acidification in Norway: present and predicted fish status. *Ambio***,** 314-321.

HESSEN, D. O., ALSTAD, N. E. & SKARDAL, L. 2000. Calcium limitation in Daphnia magna. *Journal of Plankton Research,* 22**,** 553-568.

HESSEN, D. O., FAAFENG, B. A. & ANDERSEN, T. 1995. Competition or niche segregation between Holopedium and Daphnia; empirical light on abiotic key parameters. *Cladocera as Model Organisms in Biology.* Springer.

HESSEN, D. O. & WALSENG, B. 2008. The rarity concept and the commonness of rarity in freshwater zooplankton. *Freshwater Biology,* 53**,** 2026-2035.

HESTHAGEN, T., FJELLHEIM, A., SCHARTAU, A. K., WRIGHT, R. F., SAKSGARD, R. & ROSSELAND, B. O. 2011. Chemical and biological recovery of Lake Saudlandsvatn, a formerly highly acidified lake in southernmost Norway, in response to decreased acid deposition. *Sci Total Environ,* 409**,** 2908-16.

JENSSEN, B. M. 2006. Endocrine-disrupting chemicals and climate change: a worst-case combination for arctic marine mammals and seabirds? *Environmental health perspectives,* 114**,** 76.

JEZIORSKI, A., YAN, N. D., PATERSON, A. M., DESELLAS, A. M., TURNER, M. A., JEFFRIES, D. S., KELLER, B., WEEBER, R. C., MCNICOL, D. K. & PALMER, M. E. 2008. The widespread threat of calcium decline in fresh waters. *science,* 322**,** 1374-1377.

KARLSSON, J., BYSTRÖM, P., ASK, J., ASK, P., PERSSON, L. & JANSSON, M. 2009. Light limitation of nutrient-poor lake ecosystems. *Nature,* 460**,** 506-509.

KIEFER, F. 1978. Freilebende Copepoda. *Die Binnengewässer,* 26**,** 1-343.

KIRCHNER, J. W. & LYDERSEN, E. 1995. Base cation depletion and potential long-term acidification of Norwegian catchments. *Environmental science & technology,* 29**,** 1953-1960.

KRAMER, V. J., ETTERSON, M. A., HECKER, M., MURPHY, C. A., ROESIJADI, G., SPADE, D. J., SPROMBERG, J. A., WANG, M. & ANKLEY, G. T. 2011. Adverse outcome pathways and ecological risk assessment: Bridging to population-level effects. *Environmental Toxicology and Chemistry,* 30**,** 64-76.

KRITZBERG, E. & EKSTRÖM, S. 2012. Increasing iron concentrations in surface waters–a factor behind brownification? *Biogeosciences,* 9**,** 1465-1478.

LARSEN, S., ANDERSEN, T. & HESSEN, D. O. 2011. Climate change predicted to cause severe increase of organic carbon in lakes. *Global Change Biology,* 17**,** 1186-1192.

LIEN, L., RADDUM, G. G., FJELLHEIM, A. & HENRIKSEN, A. 1996. A critical limit for acid neutralizing capacity in Norwegian surface waters, based on new analyses of fish and invertebrate responses. *Science of the total Environment,* 177**,** 173-193.

LINDHOLM, M., STORDAL, F., MOE, S. J., HESSEN, D. O. & AASS, P. 2012. Climate-driven range retraction of an Arctic freshwater crustacean. *Freshwater Biology,* 57**,** 2591-2601.

LUND, E., AAS, W., HØGÅSEN, T. & LARSSEN, T. 2012. Overskridelser av tålegrenser for forsuring og nitrogen for Norge–oppdatering med perioden 2007–2011.

LYDERSEN, E. 1994. Long-term monitoring catchments in Norway-a hydrologic and chemical evaluation-summary report.

LYDERSEN, E., LARSSEN, T. & FJELD, E. 2004. The influence of total organic carbon (TOC) on the relationship between acid neutralizing capacity (ANC) and fish status in Norwegian lakes. *Sci Total Environ,* 326**,** 63-9.

MARMOREK, D. R. & KORMAN, J. 1993. The use of zooplankton in a biomonitoring program to detect lake acidification and recovery. *Water, Air, and Soil Pollution,* 69**,** 223-241.

MASON, C. 2002. Biology of freshwater pollution. *Pearson Education Limited* 4 edition.

MEEHL, G. A., STOCKER, T. F., COLLINS, W. D., FRIEDLINGSTEIN, A., GAYE, A. T., GREGORY, J. M., KITOH, A., KNUTTI, R., MURPHY, J. M. & NODA, A. 2007. Global climate projections.

MONTEITH, D. T., STODDARD, J. L., EVANS, C. D., DE WIT, H. A., FORSIUS, M., HØGÅSEN, T., WILANDER, A., SKJELKVÅLE, B. L., JEFFRIES, D. S. & VUORENMAA, J. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature,* 450**,** 537-540.

NEWMAN, M. C. 2009. *Fundamentals of ecotoxicology*, CRC press.

ODUM, E. P. 1985. Trends expected in stressed ecosystems. *Bioscience,* 35**,** 419-422.

PACE, M. L. & ORCUTT, J. D. 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. *Limnology and Oceanography,* 26**,** 822-830.

PACHAURI, R. K., ALLEN, M. R., BARROS, V. R., BROOME, J., CRAMER, W., CHRIST, R., CHURCH, J. A., CLARKE, L., DAHE, Q. & DASGUPTA, P. 2014. *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change*, IPCC.

PATTEN, B. G. 2013. Sublethal biological effects of petroleum hydrocarbon exposures: Fish. *In:* MALINS, D. C. (ed.) *Biological effects.* Elsevier.

ROGORA, M., MOSELLO, R. & ARISCI, S. 2003. The effect of climate warming on the hydrochemistry of alpine lakes. *Water, Air, & Soil Pollution,* 148**,** 347-361.

ROSSELAND, B. & HENRIKSEN, A. 1990. Acidification in Norway—loss of fish populations and the 1000-lake survey 1986. *Science of the Total Environment,* 96**,** 45-56.

ROULET, N. & MOORE, T. R. 2006. Environmental chemistry: browning the waters. *Nature,* 444**,** 283-284.

SARS, G. O. 1895. *An account of the Crustacea of Norway: with short descriptions and figures of all the species*, A. Cammermeyer.

SCHARTAU, A., WALSENG, B., NØST, T. & HALVORSEN, G. Freshwater crustaceans as monitors of long-range transported air pollutants. Verh. Int. Ver. Theor. Angew. Limnol./Proc. Int. Assoc. Theor. Appl. Limnol./Trav. Assoc. Int. Limnol. Theor. Appl., 2001. 2484-2487.

SCHARTAU, A. K. 2002. Zooplankton. *In:* ELIASSEN, A. (ed.) *Sur nedbør - tilførsel og virkning.* Oslo: Landbruksforlaget.

SKJELKVÅLE, B. & HENRIKSEN, A. 1995. Acidification in Norway—Status and trends. *Water, Air, and Soil Pollution,* 85**,** 629-634.

SKJELKVÅLE, B., HENRIKSEN, A., FAAFENG, B., FJELD, E., TRAAEN, T., LIEN, L., LYDERSEN, E. & BUAN, A. 1996. Regional innsjøundersøkelse 1995. En vannkjemisk undersøkelse av 1500 norske innsjøer.

SKJELKVÅLE, B., MANNIO, J., WILANDER, A. & ANDERSEN, T. 2001. Recovery from acidification of lakes in Finland, Norway and Sweden 1990? 1999. *Hydrology and Earth System Sciences Discussions,* 5**,** 327-338.

THRANE, J.-E., HESSEN, D. O. & ANDERSEN, T. 2014. The absorption of light in lakes: negative impact of dissolved organic carbon on primary productivity. *Ecosystems,* 17**,** 1040-1052.

UN/ECE 1988. Report of the sixth session of the Executive Body. *ECE/EB.AIR/18,Geneve*.

VAN NES, E. H. & SCHEFFER, M. 2007. Slow recovery from perturbations as a generic indicator of a nearby catastrophic shift. *The American Naturalist,* 169**,** 738-747.

VÖRÖSMARTY, C. J., GREEN, P., SALISBURY, J. & LAMMERS, R. B. 2000. Global water resources: vulnerability from climate change and population growth. *science,* 289**,** 284-288.

WALKER, C. H., SIBLY, R., HOPKIN, S. & PEAKALL, D. B. 2012. *Principles of ecotoxicology*, CRC press.

WALSENG, B., HESSEN, D. O., HALVORSEN, G. & SCHARTAU, A. K. 2006. Major contribution from littoral crustaceans to zooplankton species richness in lakes. *Limnology and Oceanography,* 51**,** 2600-2606.

WALSENG, B. & SCHARTAU, A. K. L. 2001. Crustacean communities in Canada and Norway: comparison of species along a pH gradient. *Acid rain 2000.* Springer.

WALTHER, G.-R., POST, E., CONVEY, P., MENZEL, A., PARMESAN, C., BEEBEE, T. J., FROMENTIN, J.-M., HOEGH-GULDBERG, O. & BAIRLEIN, F. 2002. Ecological responses to recent climate change. *Nature,* 416**,** 389-395.

ZHOU, Q., ZHANG, J., FU, J., SHI, J. & JIANG, G. 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. *Analytica chimica acta,* 606**,** 135-150.

Appendix

Attachment 1: Overview of localities sampled in 1968 and 2016
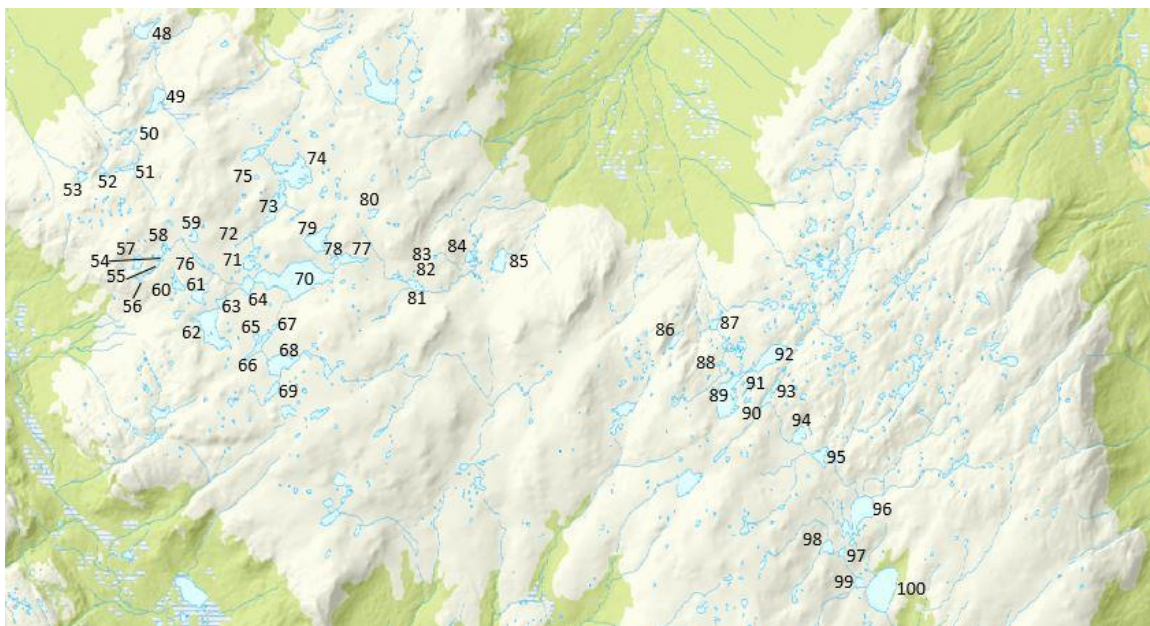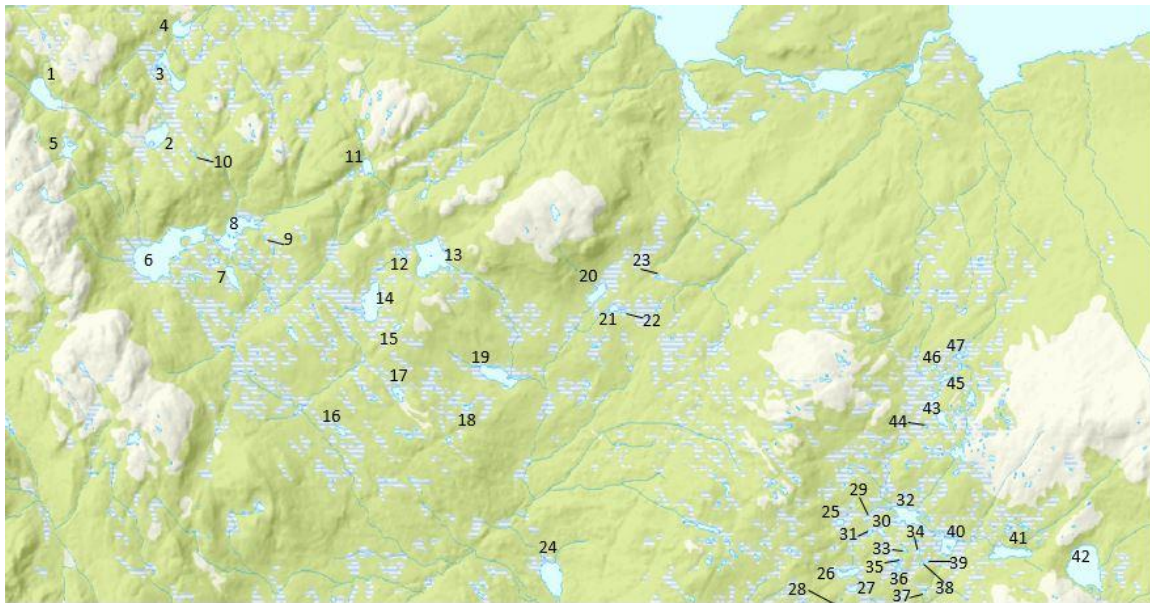
Attachment 2: Table with lake and pond characteristics

Lake.ID	Sampling date 2016	Sampling date 1968	Coordinates (UTM33)	m.a.s.l	Max depth (m)	Area. ha	Limed influenced
SV1-1	06.07.2016	18.07.1968	190224, 6723351	970	12,6	6	
SV2-2	06.07.2016	17.07.1968	191351, 6722833	900	9,0	4	
SD1-3	06.07.2016	17.07.1968	191515, 6722833	893	3,1	3	
SV3-4	06.07.2016	17.07.1968	191684, 6723896	860	5,9	2,6	
SD2-5	06.07.2016	18.07.1968	190415, 6722770	970	1,5	1,4	X
SV4-6	05.07.2016	18.07.1968	191647, 6721791	807	11,6	20	X
SD3-7	05.07.2016	18.07.1968	191980, 6721379	805	4,6	2	
SV5-8	05.07.2016	18.07.1968	192091, 6721833	805	7,0	6	X
SD4-9	05.07.2016	20.07.1968	192382, 6721680	815	1,7	0,1	
SD5-10	06.07.2016	17.07.1968	192165, 6723182	940	5,5	0,48	
SD6-11	07.07.2016	20.07.1968	193482, 6722320	875	3,0	0,12	
SD7-12	07.07.2016	20.07.1968	193794, 6721379	855	4,0	0,48	
SV6-13	07.07.2016	20.07.1968	194016, 6721299	855	8,5	9,8	X
SV7-14	05.07.2016	21.07.1968	193419, 6720881	817	5,5	6,2	X
SP1-15	05.07.2016	21.07.1968	193673, 6720659	825	1,3	0,28	
SD8-16	04.07.2016	21.07.1968	193017, 6719633	775	2,5	0,25	
SD9-17	04.07.2016	28.07.1968	193630, 6719998	810	3,1	1,5	
SP2-18	04.07.2016	09.07.1968	194323, 6719781	775	1,0	0,4	
SV8-19	04.07.2016	09.07.1968	194566, 6720104	751	4,8	5,6	X
SD10-20	04.07.2016	04.07.1968	195778, 6720829	792	1,5	2	

SD11-21	04.07.2016	08.07.1968	195915, 6720659	795	1,9	0,6	
SD12-22	04.07.2016	08.07.1968	196010, 6720622	795	2,8	0,16	
SD13-23	04.07.2016	04.07.1968	196402, 6720934	770	2,0	0,12	
SV9-24	07.07.2016	28.07.1968	195048, 6718004	590	15,2	5,9	X
SP3-25	09.07.2016	06.07.1968	197343, 6718591	830	1,3	0,5	
SD14-26	08.07.2016	06.07.1968	197396, 6718073	805	2,0	1,6	
SD15-27	08.07.2016	06.07.1968	197428, 6717904	805	1,5	0,4	
SV10-28	08.07.2016	07.07.1968	197169, 6717697	813	4,9	1,2	
SD16-29	08.07.2016	06.07.1968	198363, 6718143	825	3,4	0,1	
SD17-30	08.07.2016	05.07.1968	198478, 6718158	825	4,5	0,4	
SD18-31	08.07.2016	10.07.1968	198480, 6717995	815	6,0	0,1	
SV11-32	09.07.2016	05.07.1968	197983, 6718660	820	7,5	5,4	X
SP4-33	09.07.2016	10.07.1968	198680, 6717998	815	0,3	0,1	
SD19-34	09.07.2016	10.07.1968	198779, 6717988	815	2,3	0,1	
SP5-35	09.07.2016	10.07.1968	198705, 6717941	815	0,4	0,2	
SP6-36	08.07.2016	10.07.1968	198583, 6717810	815	0,3	0,3	
SP7-37	08.07.2016	07.07.1968	198152, 6717803	800	0,8	0,1	
SP8-38	09.07.2016	11.07.1968	199279, 6718179	810	0,8	0,1	
SP9-39	09.07.2016	11.07.1968	199538, 6718237	810	1,0	0,1	
SP10-40	09.07.2016	11.07.1968	198433, 6718306	815	0,5	2,6	X
SV12-41	09.07.2016	11.07.1968	198999, 6718152	835	8,0	5,6	X
SV13-42	26.07.2016	11.07.1968	199882, 6717830	850	18,4	12,2	X

SP11-43	10.07.2016	23.07.1968	199321, 6718898	875	1,1	1,5	
SP12-44	10.07.2016	23.07.1968	199078, 6718909	880	1,4	0,1	
SD20-45	10.07.2016	23.07.1968	199189, 6719274	870	2,7	1,6	
SP13-46	10.07.2016	23.07.1968	199268, 6719670	860	1,0	0,72	
SP14-47	10.07.2016	23.07.1968	199443, 6719877	860	0,7	0,44	
FD1-48	27.07.2016	26.07.1968	202807, 6721098	1076	1,9	2,5	
FD2-49	27.07.2016	26.07.1968	202913, 6720252	1122	2,6	3,7	
FD3-50	27.07.2016	26.07.1968	202500, 6719702	1150	1,5	0,7	
FD4-51	27.07.2016	26.07.1968	202458, 6719353	1160	2,8	2	
FD5-52	27.07.2016	27.07.1968	202130, 6719332	1160	1,8	0,6	
FD6-53	27.07.2016	27.07.1968	201887, 6719268	1160	1,8	1,1	
FP1-54	28.07.2016	29.07.1968	202804, 6718163	1210	1,0	0,3	X
FP2-55	28.07.2016	30.07.1968	202730, 6718094	1210	0,9	0,4	X
FD7-56	28.07.2016	30.07.1968	202667, 6718015	1215	2,0	0,1	
FD8-57	28.07.2016	30.07.1968	202513, 6718110	1220	2,3	1	X
FP3-58	28.07.2016	30.07.1968	202846, 6718285	1210	0,8	0,8	X
FD9-59	27.07.2016	30.07.1968	203227, 6718390	1220	1,6	1	
FD10-60	28.07.2016	31.07.1968	203000, 6717793	1211	1,4	1,7	
FV1-61	28.07.2016	31.07.1968	203238, 6717650	1210	9,3	2	X
FV2-62	28.07.2016	31.07.1968	203336, 6717306	1205	15,9	9	X
FD11-63	01.08.2016	31.07.1968	203510, 6717539	1205	1,7	0,8	X
FP4-64	01.08.2016	31.07.1968	203875, 6717713	1171	0,6	2,1	X

FD12-65	01.08.2016	31.07.1968	203933, 6717042	1205	1,5	0,3	
FD13-66	01.08.2016	31.07.1968	203944, 6716909	1210	2,7	2,2	X
FD14-67	01.08.2016	31.07.1968	204177, 6717163	1210	2,6	0,6	X
FV3-68	01.08.2016	31.07.1968	204108, 6716640	1220	9,5	6	X
FD15-69	01.08.2016	31.07.1968	204076, 6716338	1225	1,7	1,1	
FV4-70	28.07.2016	01.08.1968	204489, 6717740	1171	3,9	19	X
FD16-71	29.07.2016	01.08.1968	203896, 6717999	1170	1,7	3,5	X
FP5-72	29.07.2016	01.08.1968	203783, 6718232	1170	0,5	0,1	
FV5-73	29.07.2016	01.08.1968	204288, 6718618	1160	5,3	5	X
FD17-74	29.07.2016	01.08.1968	204563, 6719073	1155	2,0	8,8	X
FP6-75	29.07.2016	01.08.1968	204113, 6719104	1150	1,4	0,2	
FP7-76	27.07.2016	04.08.1968	203275, 6718094	1190	0,6	1	X
FV6-77	29.07.2016	04.08.1968	205176, 6717967	1190	7,5	3,1	X
FD18-78	29.07.2016	04.08.1968	204991, 6717957	1190	1,9	0,5	X
FD19-79	29.07.2016	04.08.1968	204764, 6718221	1195	3,0	5,2	X
FD20-80	29.07.2016	03.08.1968	205541, 6718496	1210	1,8	1,5	
FP8-81	30.07.2016	05.08.1968	206012, 6717581	1170	0,9	1,1	
FD21-82	30.07.2016	05.08.1968	206023, 6717724	1170	1,7	0,1	
FD22-83	na		na				
			na				
FD23-84	na						
FD24-85	30.07.2016	05.08.1968	207133, 6717713	1084	3,9	2,6	
FV7-86	30.07.2016	11.08.1968	209281, 6716529	1150	10,8	1,2	

FD25-87	30.07.2016	11.08.1968	209836, 6716735	1070	1,5	0,9	X
FP9-88	30.07.2016	11.08.1968	209926, 6716190	1120	0,9	0,2	
FV8-89	30.07.2016	11.08.1968	209894, 6715746	1120	18,5	9,2	X
FD26-90	na		na				
FD27-91	na		na				
FV9-92	30.07.2016	12.08.1968	210476, 6716243	1120	18,0	5,8	X
FD28-93	31.07.2016	12.08.1968	210444, 6715735	1100	3,0	3	
FD29-94	31.07.2016	12.08.1968	210772, 6715185	1072	2,0	1,8	
FD30-95	31.07.2016	12.08.1968	211037, 6714952	1060	1,7	3	
FV10-96	31.07.2016	12.08.1968	211491, 6714170	991	14,6	8	
FP10-97	31.07.2016	13.08.1968	211248, 6713672	990	5,8	0,6	
FD31-98	31.07.2016	13.08.1968	211058, 6713757	995	2,6	1,8	
FD32-99	31.07.2016	13.08.1968	211439, 6713281	925	1,8	1	
FV11-100	31.07.2016	13.08.1968	211608, 6713080	922	27,5	19,5	

Attachment 3: Water chemistry in 96 Vassfaret localities

Lake ID	pH	K (mg/l)	Ca (mg/l)	Cl (mg/l)	Mg (mg/l)	Na (mg/l)	NO3 (µg/l)	SO4 (mg/l)	ANC µeql	ANC.oaa µeql	mg Pt/l	TOC (mg/l)	Abs. 254	TN (µg/l)	TP (µg/l)
SV1-1	6,6	0,09	0,90	0,30	0,14	0,44	1,00	0,005	69,352684	63,627084	5	1,684	0,062	140	13
SV2-2	6,1	0,074	0,86	0,25	0,15	0,43	1,00	0,61	56,079387	45,1381869	20	3,218	0,157	137	9
SD1-3	5,8	0,069	0,61	0,26	0,14	0,43	1,00	0,44	45,91553	29,8743303	40	4,718	0,241	180	10
SV3-4	6,7	0,065	0,75	0,25	0,15	0,43	1,00	0,55	51,611623	38,4570229	25	3,869	0,196	148	5
SD2-5	5,9	0,54	1,41	0,22	0,15	0,42	1,00	0,55	97,089146	85,923546	30	3,284	0,159	131	6
SV4-6	6,0	0,11	0,98	0,34	0,17	0,49	1,00	0,73	62,203741	54,0029411	10	2,412	0,185	109	9
SD3-7	5,4	0,07	0,43	0,27	0,08	0,36	1,00	0,27	32,237694	17,4034936	50	4,363	0,223	134	11
SV5-8	5,7	0,08	0,81	0,34	0,15	0,55	1,00	0,005	69,085456	52,2180557	35	4,961	0,262	169	8
SD4-9	4,8	0,024	0,50	0,22	0,13	0,44	1,00	0,005	49,071508	6,97950775	100	12,38	0,609	282	7
SD5-10	5,4	0,05	0,41	0,15	0,083	0,36	1,00	0,26	34,568758	8,72195793	70	7,602	0,409	225	8
SD6-11	6,0	0,11	0,41	0,32	0,098	0,48	1,00	0,48	33,181193	22,2501934	30	3,215	0,199	139	9
SD7-12	5,7	0,092	0,78	0,39	0,15	0,55	1,00	0,39	58,396451	34,7120511	60	6,966	0,334	207	16
SV6-13	5,8	0,094	1,69	0,37	0,120	0,53	1,00	0,49	98,978444	88,3296441	25	3,132	0,128	137	13
SV7-14	6,0	0,14	1,15	0,36	0,120	0,49	3	0,005	81,64457	61,0099701	50	6,069	0,306	172	10
SP1-15	5,3	0,082	0,35	0,28	0,057	0,37	1,00	0,20	28,271081	6,65728093	50	6,357	0,321	185	7
SD8-16	5,4	0,11	0,66	0,36	0,170	0,50	1,00	0,33	54,438911	31,1081109	70	6,862	0,311	181	8
SD9-17	5,1	0,13	0,48	0,32	0,130	0,46	1,00	0,18	45,19165	15,1798502	100	8,827	0,433	246	10
SP2-18	4,6	0,024	0,12	0,21	0,077	0,37	1,00	0,18	19,353319	-13,701481	100	9,722	0,442	202	16
SV8-19	5,6	0,055	1,04	0,33	0,120	0,55	1,00	0,42	69,01678	43,4147798	70	7,53	0,383	182	14
SD10-20	5,8	0,058	0,61	0,27	0,10	0,51	1,00	0,35	47,411242	21,0170417	60	7,763	0,355	233	7
SD11-21	5,4	0,18	0,43	0,30	0,062	0,40	1,00	0,22	35,502962	15,6673622	60	5,834	0,252	194	17
SD12-22	5,2	0,15	0,60	0,27	0,092	0,50	1,00	0,22	50,877726	21,590126	70	8,614	0,396	203	10
SD13-23	5,1	0,05	0,44	0,25	0,100	0,46	1,00	0,23	8,7233973	8,72339728	60	9,116	0,417	235	9
SV9-24	5,4	0,059	1,02	0,30	0,16	0,62	1,00	0,46	74,471031	43,7928309	80	9,023	0,494	188	5
SP3-25	4,7	0,03	0,60	0,22	0,130	0,56	1,00	0,24	54,539144	13,3311441	90	12,12	0,62	232	8
SD14-26	5,3	0,031	0,71	0,22	0,13	0,53	1,00	0,28	57,91419	27,0965898	80	9,064	0,444	214	7
SD15-27	5,5	0,081	0,78	0,26	0,11	0,47	1,00	0,31	56,676748	38,2147484	50	5,43	0,232	184	7
SV10-28	5,4	0,092	0,44	0,32	0,079	0,41	1,00	0,30	33,355928	19,9905282	30	3,931	0,145	151	6
SD16-29	5,2	0,09	0,84	0,28	0,093	0,41	1,00	0,31	55,327399	30,4291986	70	7,323	0,339	221	12
SD17-30	4,7	0,068	0,80	0,21	0,081	0,34	1,00	0,22	52,586255	23,7712548	90	8,475	0,419	237	7
SD18-31	5,0	0,061	0,26	0,26	0,049	0,34	1,00	0,25	20,806027	-7,9035734	80	8,444	0,388	218	10
SV11-32	5,8	0,048	1,25	0,22	0,120	0,50	1,00	0,28	83,154248	55,4068477	70	8,161	0,405	218	7
SP4-33	4,2	0,020	0,21	0,086	0,063	0,19	1,00	0,13	19,299912	-29,762088	150	14,43	0,682	338	16
SD19-34	4,3	0,022	0,14	0,15	0,050	0,29	1,00	0,12	17,540415	-22,9875848	80	11,92	0,491	320	13
SP5-35	4,8	0,020	0,12	0,25	0,048	0,29	1,00	0,25	10,800759	-14,3422405	60	7,395	0,299	270	10

SP6-36	4,7	0,026	0,29	0,16	0,044	0,30	1,00	0,15	24,158532	-4,01726786	70	8,287	0,375	236	10
SP7-37	4,5	0,032	0,73	0,18	0,160	0,51	1,00	0,18	63,746357	5,81035734	150	17,04	0,826	298	8
SP8-38	4,4	0,034	0,34	0,17	0,094	0,40	1,00	0,16	34,82975	-8,79225026	125	12,83	0,604	316	12
SP9-39	4,2	0,020	0,22	0,048	0,064	0,17	1,00	0,11	20,49957	-37,3684302	125	17,02	0,8	320	12
SP10-40	5,0	0,046	0,78	0,21	0,10	0,48	1,00	0,27	57,636249	31,7962485	70	7,6	0,347	173	5
SV12-41	5,3	0,043	0,56	0,21	0,096	0,47	1,00	0,29	45,40672	22,2527198	70	6,81	0,321	174	5
SV13-42	6,6	0,10	0,77	0,34	0,120	0,48	2,00	0,54	50,735519	36,3229194	30	4,239	0,226	178	4
SP11-43	5,6	0,098	0,25	0,27	0,036	0,29	1,00	0,21	18,560072	8,46547188	25	2,969	0,175	137	9
SP12-44	5,1	0,035	0,19	0,26	0,053	0,43	1,00	0,21	21,724521	-1,75587909	70	6,906	0,277	216	7
SD20-45	5,2	0,15	0,52	0,25	0,069	0,39	1,00	0,16	42,024962	19,9895624	60	6,481	0,273	201	9
SP13-46	5,2	0,034	0,77	0,15	0,086	0,34	1,00	0,14	53,98941	25,3750099	70	8,416	0,401	236	25
SP14-47	5,0	0,31	0,78	0,52	0,11	0,61	1,00	0,18	63,991724	33,7453239	80	8,896	0,403	236	8
FD1-48	6,5	0,095	0,28	0,30	0,060	0,37	1,00	0,58	16,8866	16,2099999	5	0,199	0,091	125	4
FD2-49	NA	0,032	0,26	0,25	0,061	0,46	1,00	0,64	18,434926	16,0957264	5	0,688	0,143	100	2
FD3-50	6,3	0,028	0,19	0,24	0,061	0,42	1,00	0,56	15,049242	12,2714422	5	0,817	0,093	107	1
FD4-51	6,6	0,026	0,20	0,25	0,059	0,44	1,00	0,59	15,295363	9,70916349	5	1,643	0,124	89	6
FD5-52	5,8	0,035	0,16	0,25	0,046	0,40	1,00	0,56	11,34579	5,40939041	5	1,746	0,104	86	6
FD6-53	6,4	0,052	0,13	0,27	0,048	0,34	1,00	0,48	8,9409845	2,40618453	5	1,922	0,157	114	28
FP1-54	6,0	0,046	0,81	0,26	0,068	0,38	8,00	0,55	44,341808	38,8372078	5	1,619	0,079	111	3
FP2-55	6,4	0,043	1,19	0,26	0,070	0,37	9,00	0,54	63,084214	57,5728144	5	1,621	0,065	137	3
FD7-56	6,7	0,034	0,084	0,23	0,021	0,22	6,00	0,41	0,9033428	-3,93145721	5	1,422	0,071	95	8
FD8-57	6,5	0,030	2,26	0,25	0,071	0,31	3,00	0,45	116,17563	110,059034	10	1,799	0,116	142	4
FP3-58	6,9	0,041	1,83	0,25	0,096	0,40	5,00	0,54	98,96533	92,8725302	5	1,792	0,094	107	3
FD9-59	6,4	0,038	0,14	0,26	0,032	0,28	3,00	0,52	4,3914489	1,04924886	5	0,983	0,051	80	2
FD10-60	6,5	0,045	0,15	0,25	0,055	0,36	4,00	0,58	9,4024547	4,70025469	25	1,383	0,09	85	2
FV1-61	6,8	0,049	2,69	0,26	0,087	0,29	1,00	0,53	136,82252	131,668124	5	1,516	0,061	105	3
FV2-62	6,1	0,053	0,95	0,26	0,061	0,28	5,00	0,55	46,793708	43,0469076	5	1,102	0,078	23	3
FD11-63	6,0	0,049	0,90	0,26	0,061	0,29	3,00	0,55	44,77528	40,5490799	5	1,243	0,071	79	2
FP4-64	6,1	0,037	0,92	0,25	0,063	0,33	2,00	0,56	47,51502	42,9012198	5	1,357	0,067	4	3
FD12-65	6,0	0,027	0,26	0,25	0,042	0,33	2,00	0,60	11,780698	7,55109831	5	1,244	0,065	70	1
FD13-66	6,2	0,031	0,43	0,26	0,049	0,30	2,00	0,53	20,808315	17,1057153	5	1,089	0,077	67	1
FD14-67	6,1	0,033	0,45	0,26	0,045	0,30	1,00	0,53	21,670611	17,8694115	5	1,118	0,065	80	1
FV3-68	6,5	0,033	0,48	0,26	0,044	0,29	1,00	0,51	23,066017	19,4552168	5	1,062	0,08	4	1
FD15-69	6,3	0,059	0,20	0,27	0,040	0,33	2,00	0,60	5,47781	4,76040998	5	1,211	0,094	88	2
FV4-70	6,2	0,047	0,55	0,26	0,050	0,28	10,00	0,53	25,843871	22,8042706	15	0,894	0,057	4	1
FD16-71	6,4	0,045	0,73	0,25	0,057	0,30	7,00	0,55	36,296113	32,5731128	10	1,095	0,063	4	1

|															
FP5-72	5,4	0,017	0,29	0,24	0,027	0,37	2,00	0,55	14,848436	8,79303589	10	1,781	0,109	74	1
FV5-73	6,2	0,041	0,67	0,26	0,055	0,31	7,00	0,56	32,981262	29,2786619	5	1,089	0,086	93	1
FD17-74	6,3	0,040	0,63	0,25	0,058	0,32	5,00	0,57	31,859198	28,2755982	5	1,054	0,064	69	1
FP6-75	6,0	0,028	0,27	0,25	0,042	0,38	5,00	0,6	10,032188	9,49838802	5	1,157	0,071	72	1
FP7-76	6,4	0,036	1,42	0,26	0,097	0,42	3,00	0,59	78,160632	72,8124319	5	1,573	0,105	97	1
FV6-77	6,5	0,061	2,54	0,26	0,100	0,30	4,00	0,55	130,45093	125,905127	5	1,337	0,07	117	2
FD18-78	6,3	0,047	1,26	0,24	0,066	0,28	3,00	0,44	65,509438	59,6240382	5	1,731	0,084	150	1
FD19-79	6,1	0,054	1,56	0,24	0,076	0,27	1,00	0,42	81,669841	76,5256413	5	1,513	0,06	92	1
FD20-80	5,6	0,032	0,18	0,23	0,062	0,40	1,00	0,59	13,523268	6,88306798	5	1,953	0,082	116	1
FP8-81	6,5	0,032	0,26	0,24	0,053	0,40	3,00	0,63	15,443713	10,8605128	5	1,348	0,09	4	1
FD21-82	5,9	0,043	0,16	0,26	0,041	0,41	3,00	0,64	9,4123484	4,85634844	5	1,34	0,071	87	1
FD24-85	5,8	0,054	0,26	0,26	0,052	0,39	4,00	0,6	11,618709	8,19490925	5	1,007	0,067	4	1
FV7-86	6,0	0,063	0,13	0,27	0,045	0,32	1,00	0,49	7,8977191	4,19851911	5	1,088	0,056	71	1
FD25-87	6,0	0,045	0,57	0,22	0,064	0,31	6,00	0,42	32,950183	24,2699832	5	2,553	0,141	112	1
FP9-88	5,0	0,024	0,30	0,16	0,072	0,28	1,00	0,29	23,127464	3,63186426	30	5,734	0,303	215	11
FV8-89	5,8	0,059	0,67	0,26	0,068	0,29	24,00	0,46	34,508893	29,1776933	5	1,568	0,101	115	1
FV9-92	6,1	0,063	1,49	0,26	0,093	0,27	16,00	0,44	77,68466	72,4214603	5	1,548	0,093	69	1
FD28-93	6,4	0,034	0,23	0,22	0,048	0,33	2,00	0,44	15,133421	6,95302131	5	2,406	0,125	147	2
FD29-94	5,6	0,025	0,24	0,20	0,044	0,30	1,00	0,42	14,891695	4,9840946	15	2,914	0,144	138	4
FD30-95	5,9	0,027	0,26	0,20	0,045	0,30	1,00	0,4	16,022656	5,98585625	15	2,952	0,186	144	1
FV10-96	5,5	0,026	0,28	0,22	0,041	0,33	1,00	0,45	16,781192	8,05679223	15	2,566	0,126	111	1
FP10-97	5,4	0,028	0,54	0,21	0,066	0,33	1,00	0,42	32,763974	18,8307744	25	4,098	0,116	160	3
FD31-98	5,3	0,021	0,26	0,20	0,054	0,33	1,00	0,42	17,914292	3,51529213	25	4,235	0,228	172	1
FD32-99	5,4	0,003	0,43	0,21	0,057	0,36	1,00	0,44	26,785678	13,6038779	25	3,877	0,221	140	1
FV11-100	5,2	0,034	0,21	0,23	0,04	0,34	4,00	0,45	4,1007258	-4,73247418	10	2,598	0,133	110	1

Attachment 4: Species composition in 93 localities in Vassfaret (83,84,90,91 not sampled, 25,33,39 not included due to poor conservation of samples).

Lokalitet (ID)	A.denticornis	Bosmina spp.	B. longiremis	C. scutifer	D.brachyurum	D.galeata	D. longispina	H. saliens	H. gibberum	M.laciniatus	Total
SV1-1		19		82				3	230		334
SV2-2		10	1	83				1	102		197
SD1-3	12	1	1	83				10	160		267
SV3-4	93	1		3	1			2	3		103
SD2-5		19		202				1	2		224
SV4-6	1	15		121					38		175
SD3-7	100	9			94			10	102		315
SV5-8		9		59			1	3	98		170
SD4-9		52		3	65			18	135		273
SD5-10				111				8	143		262
SD6-11		68	1	58		1		18	253		399
SD7-12	134	1		10	152		1	32	2		332
SV6-13		29		10				1	166		206
SV7-14		1		8			41	1	9		60
SP1-15	6	3			156			44	2		211
SD8-16	104	4		64	19			12	1		204
SD9-17		6			42			52	67		167
SP2-18		31			104			33	68		236
SV8-19		167		149	9			2	235		562
SP10-20	1	116		1				157			275
SD11-21	151	6			28			7	24		216
SD12-22	37	21		8	26			9	3		104
SD13-23		1		2	154			16	59		232
SV9-24		32	1	70					167		270
SD14-26		227		1	26			6			260
SD15-27	262	13		19	60			53			407
SV10-28	16	2		19	12			11	241		301
SD16-29	57	1			6			30	61		155
SD17-30	89			37	15			27	198		366
SD18-31		110		69	1			2	123		305
SV11-32		7		10	1		1	8	201		228
SD19-34		274		1				9			284
SP5-35					24			50	172		246
SP6-36		37						73			110
SP7-37		9			15						24
SP8-38		58			74			4	2		138
SP10-40		25		1					5		31
SV12-41		65		18				8	94		185
SV13-42	41	27		25			1	33	153		280
SP11-43		207						19			226
SP12-44		1			162			14			177

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SD20-45	183		10	10	1		204
SP13-46	178		61	62			301
SP14-47	96		32				128
FD1-48	43			23		336	402
FD2-49	50			35			85
FD3-50	200						200
FD4-51	200	3		1			204
FD5-52	200			5			205
FD6-53	200			2		1	203
FP1-54	41						41
FP2-55	44			1		11	56
FD7-56	169			1		218	388
FD8-57	2			2		200	204
FP3-58	200			1			201
FD9-59	154			7		114	275
FD10-60	112			2	76	94	284
FV1-61	1	51		7	12	127	198
FV2-62	160	13		5		60	238
FD11-63	60	2		5		82	149
FP4-64	300				8	8	316
FD12-65	1			3		200	204
FD13-66	4			1	2	49	56
FD14-67	1				2	4	7
FV3-68	1	19		21		45	86
FD15-69	118				70	2	190
FV4-70	42			8		29	79
FD16-71	12			2	2	10	26
FD5-72					1		1
FV5-73	84				31	25	140
FD17-74	19			1	8		28
FP6-75	216						216
FP7-76	191	2					193
FV6-77	5	8			1	162	176
FD18-78	57			1	14	53	125
FD19-79	2			2	18	181	203
FD20-80	217	1				2	220
FP8-81	6						6
FD21-82	32			9	58	146	245
FD24-85	13			1		176	190
FV7-86	1			20			21
FD25-87	200					7	207
FP9-88	339			4	107		450
FV8-89	145	13		1	102	13	274
FV9-92	181	20			22	2	225
FD28-93	5	6					11

FD29-94	45				1		46
FD30-95	90						90
FV10-96	29	4			38	1	72
FP10-97	36	2		1			39
FD31-98	14			2	135		151
FD32-99	137	2					139
FV11-100	36	39			130		205
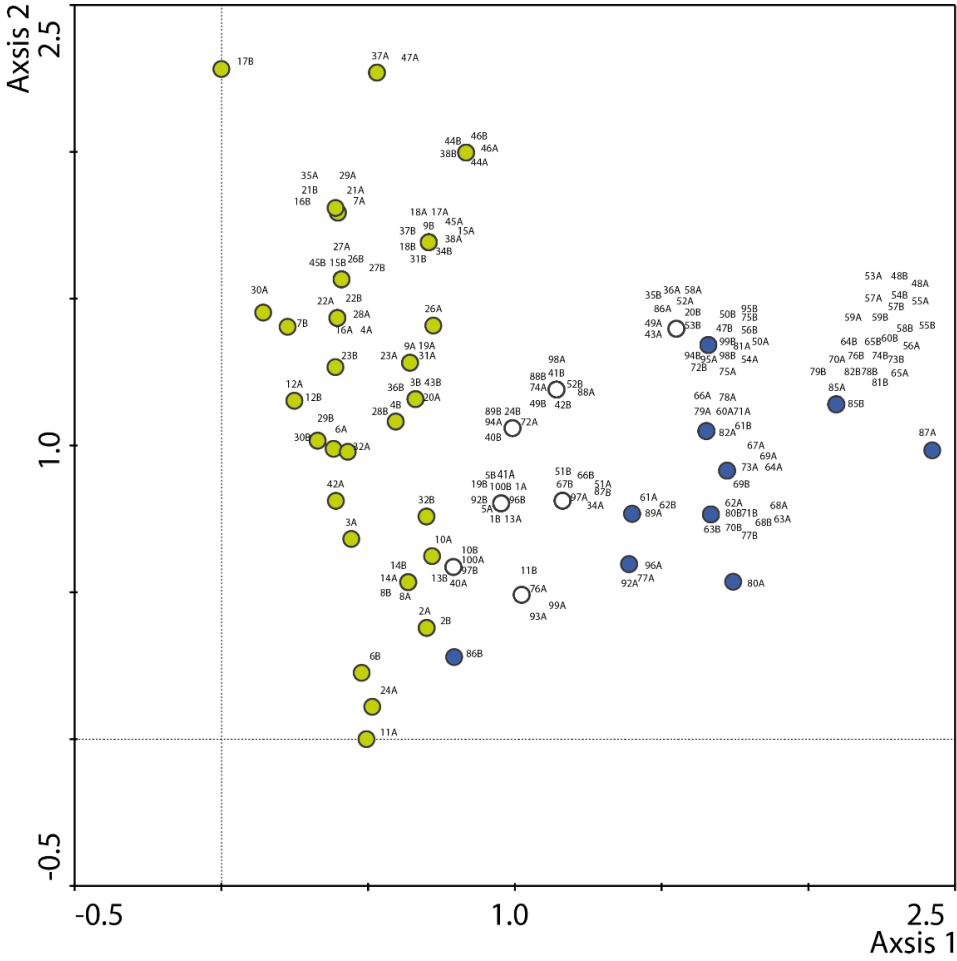
DCA of presence/absence data of 93 localities in 1968(B) and 2016(A). The DCA arrange and compare the species composition (species list) between localities. Localities with identical specie composition will overlap.
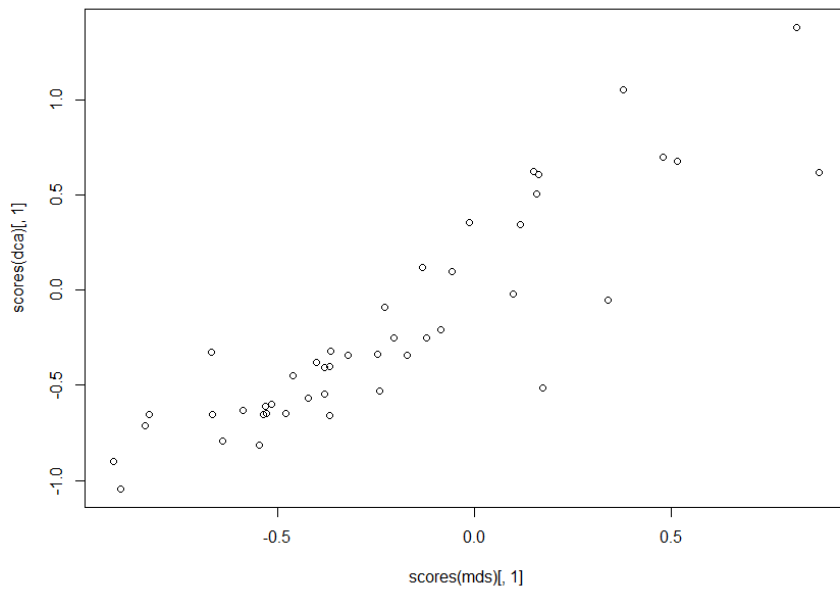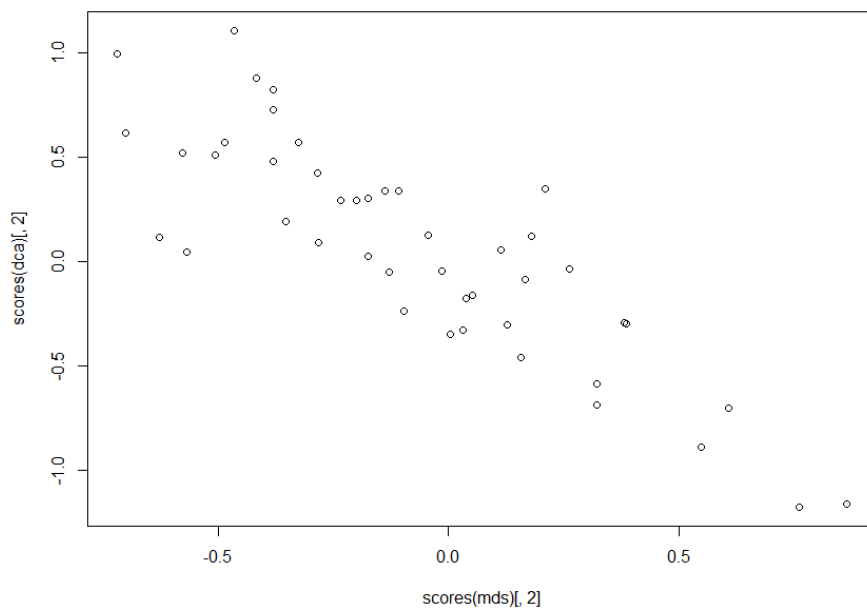
Kendall's rank correlation tau

data: scores(mds)[, 1] and scores(dca)[, 1]
z = 14.705, p-value < 2.2e-16
alternative hypothesis: true tau is not equal to 0
sample estimates:
      tau
0.7533515

Kendall's rank correlation tau
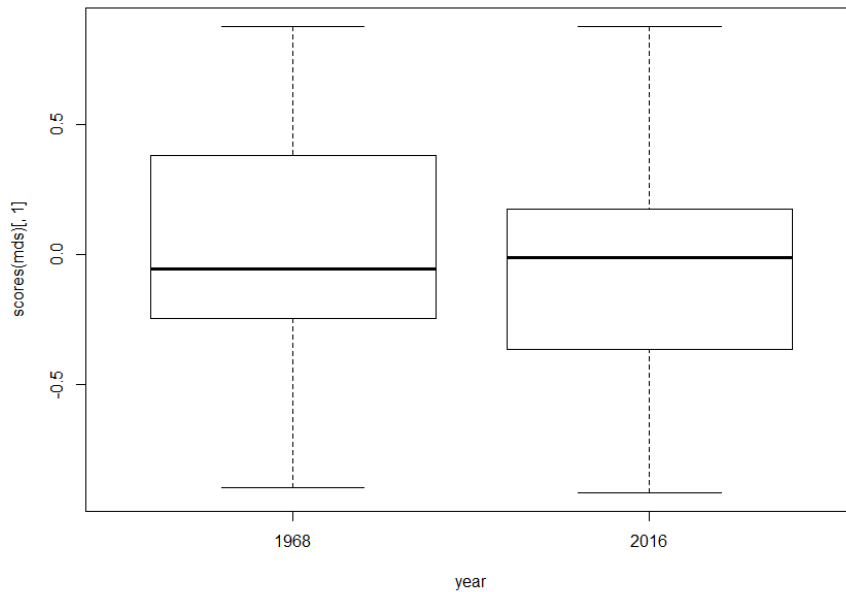
data: scores(mds)[, 2] and scores(dca)[, 2]
z = -11.396, p-value < 2.2e-16
alternative hypothesis: true tau is not equal to 0
sample estimates:
    tau
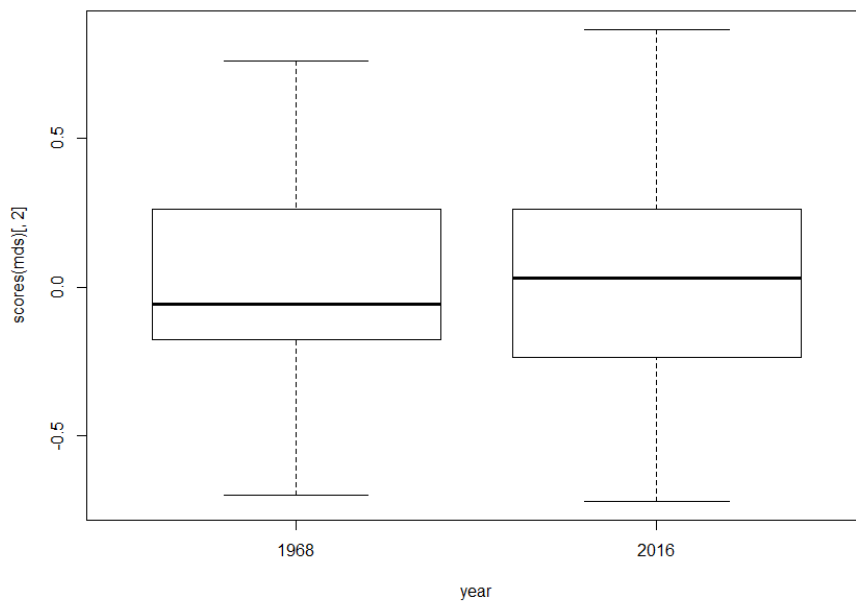-0.5839864

lm(formula = scores(mds)[, 1] ~ year)

	Estimate	Standard error	T-value	P-value
Intercept	0.008	0.044	0.187	0.852
Year 2016	-0.016	0.062	-0.264	0.792

lm(formula = scores(mds)[, 2] ~ year)

	Estimate	Standard error	T-value	P-value
Intercept	0.017	0.034	0.503	0.616
Year 2016	-0.034	0.048	-0.709	0.479

Permutational Multivariate Analysis of Variance Using Distance Matrices
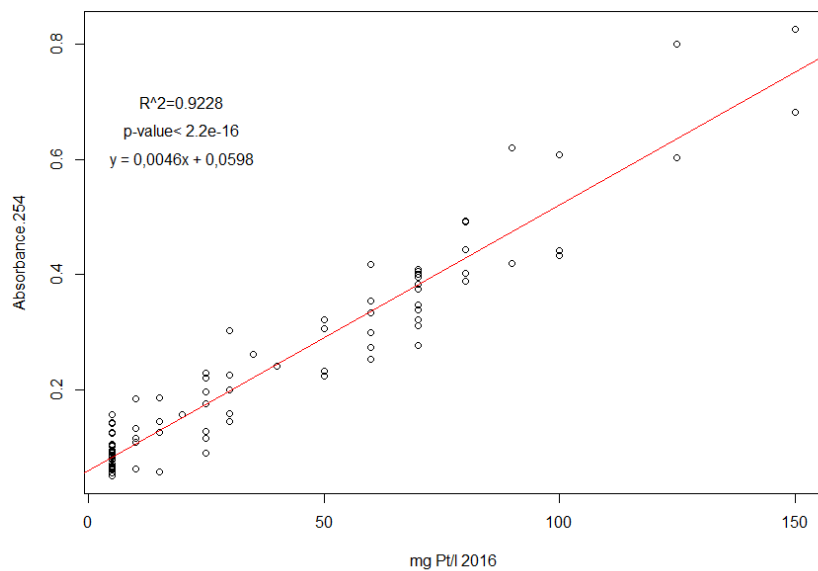adonis(formula = d ~ year)
Permutation: free

Number of permutations: 999
Terms added sequentially (first to last)

```
      Df SumsOfSqs  MeanSqs F.Model     R2 Pr(>F)
year     1   0.1536 0.153639  1.6198 0.00877  0.221
Residuals 183   17.3581 0.094853         0.99123
Total    184   17.5117              1.00000
```

Spektrophometric absorbance at λ254 compared to mg Pt/l.

Adjusted R-squared: 0.9228

p-value: < 2.2e-16

Localities with presence of *D. longispina*

Loc. ID	max depth (m)	Area (ha)	pH	ANCoaa (µeq/l)	Ca (mg/l)	Dominating species
8	7,0	6,0	5,7	52,21	0,81	*H. gibberum*
12	4,0	0,48	5,7	34,71	0,78	*D. brachyurum*
14	5,5	6,2	6,0	61,00	1,15	*D.longispina*
32	7,5	5,4	5,8	55,40	1,25	*H.gibberum*
42	18,4	12,2	6,6	36,22	0,77	*H.gibberum*