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DNA Double-Strand Breaks in Arctic Char, *Salvelinus alpinus*, from Bjørnøya

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Environmental Toxicology and Chemistry
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1. Preface

This thesis is original, unpublished and written wholly by the author, E. D. Neerland.

Fieldwork was performed by Akvaplan-niva, as part of a research project funded by the Research Council of Norway (Norges Forskningsråd), project number 221373: “Forurens: Is the cocktail effect of environmental contaminants a threat for Arctic fish populations?”

Chemical analysis was performed at the Norwegian Institute for Air Research (NILU) in Tromsø.

Analysis of DNA double-strand breaks was performed at the Department of Biology at the Norwegian University of Science and Technology (NTNU), Trondheim.

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2. Acknowledgements

I would like to extend gratitude to my main supervisor Åse Krøkje for the supervision, and practical and theoretical help during the work of this thesis. Further, I am happy to have had Anita Evenset and Jenny Bytingsvik as my co-supervisors in Tromsø – for both letting me in on the project and in answering any questions I've had. Vladimir Nikiforov made sure the chemical analysis were right, and he and the rest of NILU made my stay in Tromsø very enjoyable. Without Chris Bingham, the lab work at NTNU would not have happened – and for that, I am thankful.

I would also like to thank Geir Wing Gabrielsen for helping me get in touch with Anita and Jenny, and for paying for the accommodations for the Tromsø stay. And for the accommodations, I'll have to thank Eldbjørg Heimstad for a good place to stay.

Without family and friends, I would not have had kept my sanity. As far as I know, sanity is a lot better than insanity. Especially would I like to thank my parents for their support, and the social part of the Environmental Toxicology and Chemistry programme. In particular, would I like to thank my friend Randi Rodvelt for laboratory help, and Øystein "Birdmaster" Tennfjord and Tomas "Roggbiff" Taylor for help with statistics. Well … that, and for everything social.

The hivemind of the StackExchange community has been a better teacher than I could have hoped for regarding *R* and *ggplot2*. The serif typeset for used for this body text was *Vollkorn*, designed by Friedrich Althausen. The sans-serif typeface *Roboto*, by Christian Robertson, was used to complement it.

Eirik D. Neerland
15th May 2016

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3. Abstract

Long-range atmospheric transport has led to persistent organic pollutants (POPs) finding their way far from their sources of origin and into the Arctic and are accumulated in biota. The activity of large seabird colonies has caused high levels of biomagnified POPs to be deposited into Lake Ellasjøen of Bjørnøya (74.30° N, 19.01° E). The salmonid fish, Arctic char (*Salvelinus alpinus*), is the only fish species on the island and is also found in Lake Ellasjøen. The aim of the present study was to investigate the potential genotoxic effect of environmental exposure to organochlorines (OCs) in Arctic char from Ellasjøen, as compared to Arctic char from the relative pristine Lake Laksvatn.

A total of 39 individuals, 18 from Ellasjøen and 21 from Laksvatn, were used in the study. Blood of char was analysed by using agarose gel electrophoresis and image data analysis to quantify the DNA-fraction, of total DNA, that migrated into the gel (DNA-FTM) and the median molecular length of the DNA fragments that left the well during gel electrophoresis (MML) as relative measures of DNA double-strand breaks (DSBs). Muscle samples were analysed by GC-MS to quantify the content of organochlorines (OCs) in the fish. Statistical analysis was used to see if there were any associations between DNA-damage, OCs, and biological variables.

Between-lake comparisons showed a 43 times higher concentration of ∑OCs in Arctic char of Ellasjøen compared with Laksvatn char. There was also a significant difference between fish of the lakes regarding DNA DSBs. Char of Lake Ellasjøen had a much higher level of DSBs, as measured by both DNA-FTM and MML, than Lake Laksvatn. The difference in DNA DSBs was significantly correlated with the levels of OCs in the fish. Several other differences were found between the fish of the lakes: Ellasjøen char had smaller relative liver weights and a lower body growth rate.

Further studies should investigate whether this increased level of DNA damage in the Ellasjøen population could impair the health of the fish and by extension the health of the population. The high and low exposure scenario of the two lakes could also provide for powerful studies of evolutionary toxicology of the char stock of Bjørnøya.

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4. Sammendrag

Langtransport av atmosfæriske forbindelser har ført til høye nivåer av persistente organiske miljøgifter (POPs) har blitt transportert fra deres kilder og til Arktis, og akkumuleres i biota. Aktiviteten av store sjøfuglkolonier has ført til at biomagnifiserte POPs blir deponert i Ellasjøen på Bjørnøya (74.30° N, 19.01° E). Laksefisken røye (*Salvelinus alpinus*) er den eneste fiskearten på øya og finnes også i Ellasjøen. Målet for denne oppgaven var å undersøke den potensielle genotoksiske effekten av en eksponering for organokloriner (OCs) i miljøet på røye (*Salvelinus alpinus*) fra Ellasjøen, sammenlignet med røye fra det relativt upåvirkede Laksvatn.

Total ble 39 fisk, 18 fra Ellasjøen og 21 fra Laksvatn, brukt i studiet. Blod fra røya ble analysert ved agarose-gelelektroforese og påfølgende bildedata-behandling for å kvantifisere DNA-fraksjonen, av totalt DNA, som migrerte inn i gelen (DNA-FTM) og median-lengden av DNA-fragmentene som migrete inn i gelen (MML) som relative mål på DNA-dobbeltrådbrudd (DSBs). Nivåer av OCs fra muskelprøver ble bestemt ved GC-MS. Statistiske analyser ble brukt for å se om det var assosiasjoner mellom DNA-skade, OC-nivåer og biologiske variabler.

Forskjeller mellom sjøene viste en 43 ganger høyere konsentrasjon av gjennomsnittet av ∑OCs i røye fra Ellasjøen sammenlignet med Laksvatn. Det var også en signifikant høyere andel DSBs i fisk fra Ellasjøen, målt ved DNA-FTM og MML. Forskjellen var sterkt korrelert med nivåene av OCs i fisken. Andre forskjeller ble også funnet mellom fisken i de to sjøene, blant annet at Ellasjøen-fisk hadde en lavere relativ levervekt og en lavere vekstrate.

Videre studier bør undersøke om disse økte nivåene av DNA-skade hos populasjonen i Ellasjøen vil kunne svekke fiskens helse, og dermed også populasjonens helse. Det høye og lave nivået av miljøgifter i de to sjøene kan også vise seg å være et viktig forsøksoppsett for å studere evolusjonær toksikologi hos røye-bestanden på Bjørnøya.

5.Contents

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6. Abbreviations

AIC	Akaike's information criterion
%lip	percentage lipid in muscle
ΔAIC/c	change in AIC/c to lowest AIC
AICc	AIC corrected
CF	condition factor
DDE	dichlorodiphenyldichlorodiphenyl
DDT	dichlorodiphenyltrichloroethane
DDT	dichlorodiphenyltrichloroethane
DNA-FTM	DNA fraction of total DNA, that migrated
DSB	DNA double-strand breaks
E	lake Ellasjøen
EROD	ethoxyresorufin-O-deethylase
GC	gas chromatography
GCMS	see GC and MS
GSI	gonadosomatic index
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
HR	homologous recombination
HSI	hepatosomatic index
K_{OA}	octanol-air coefficient
K_{OW}	octanol-water coefficient
L	lake Laksvatn
LOD	limit of detection
LOQ	limit of quantification
MML	median molecular length
MS	mass-spectrometry
NC	nonachlor
NER	nucleotide excision repair
NHEJ	non-homologous end-joining
NILU	Norsk Institutt for Luftforskning (Norwegian Institute for Air Research)
NTNU	Norwegian University of Science and Technology
OC	organochlorine
PCA	principal component analysis
PCB	polychlorinated biphenyls
POP	persistent organic pollutant
R^2	coefficient of variance
ROS	radical oxygen species
SD	standard deviation
SE	standard error (of the mean)
∑	sum
t-NC	*trans*-nonachlor

1. Introduction

1.1. Persistent organic pollutants

1.1.1. History

After World War II, technological innovation made for efficient, large-scale production of a wide range of novel chemicals to be used in a myriad of applications. Polychlorinated biphenyls (PCBs) were used as electrical insulators, coolants, plasticizers, and in paints, to name a few (Breivik et al., 2002a, 2002b). The advent of cheap and efficient pesticides, such as dichlorodiphenyltrichloroethane (DDT), led to large amounts being used in agriculture to fight off pests. Agriculture sprays, landfills, demolition of buildings, recycling, evaporation from water and soil, by-products of chemical production, various incineration plants, and household furnaces all emitted, and still emit, what we now know as persistent organic pollutants (POPs) to the environment (AMAP, 2004). The widespread use is the reason as to why POPs are ubiquitously present in urban and rural areas. By wastewater and runoff, many of the compounds eventually find their way to water bodies and the marine environment.

1.1.2. In the Arctic

The major production, emissions, and use of POPs are located far from the Arctic, but they are still ubiquitously present in the region. Long-range atmospheric transport, and to a lesser extent transport by ocean currents, polar ice, Arctic rivers and biota (AMAP, 2004; Blais et al., 2005), is the cause as to why pollutants and their residues manifest themselves in the Arctic environment. There, they are found in air, water, sediment, as well as in wildlife and human populations (AMAP, 2004; Jones and De Voogt, 1999; Letcher et al., 2010).

The reason for the decade-long presence of environmental pollutants in the polar regions is that the Arctic acts as a sink for these anthropogenic chemicals. The propensity of POPs to volatilise at environmental temperatures facilitates atmospheric transport over long distances, either as a gas, an aerosol or adsorbed to particles, before being redeposited at the colder regions of higher latitude (Simonich and Hites, 1995; Wania and Mackay, 1993). This mechanism, repeated over time, is able to transport large amounts of pollutants to the Arctic, due to the resistance to photolytic, biological and chemical degradation (El-Shahawi et al., 2010). This is why there still can be found contaminants in high concentrations in the Arctic environment today (Letcher et al., 2010).

1.1.3. Organochlorine contaminants

Organochlorines (OCs) make up the largest POP subgroup, owing its persistence to the very stable carbon-chloride bond (El-Shahawi et al., 2010). OC pesticides, such as DDT and hexachlorocyclohexanes (HCHs), were used abundantly in the decades following World War II. During the 1960s and 1970s, evidence emerged that these pesticides not only afflicted their targets but had toxic effects in other organisms, such as birds of prey (Gilbertson et al., 1991) and reptiles (Guillette Jr. et al., 1994). Following bans and restrictions, the levels of OCs in the Arctic started to decrease (AMAP, 2004). Mainly first regulated by the Convention on Long-range Transboundary Air Pollution (LRTAP; 1979), then by the Stockholm Convention (UNEP, 2001). But still, levels of OCs can be found in the Arctic that is high enough to cause effects in some species (Letcher et al., 2010).

1.1.4. Bioaccumulation and biomagnification

A hallmark of POPs is their ability to bioaccumulate and biomagnify in the food chain (Jones and De Voogt, 1999; Stockholm Convention, 2001). In aquatic environments, the POPs can be directly taken up by organisms over respiratory surfaces or through the diet. The lipophilic contaminants are distributed and amassed in adipose tissues by passive diffusion. Thus, as organisms at each trophic level must consume a large number of organisms at the preceding level, these contaminants are accumulated in the lipid compartments of the consumers (Borgå et al., 2012). This outlines the mechanism of biomagnification that can result in several orders of difference in contaminant concentration between organisms at the top and bottom of the food chain (AMAP, 2004; Hop et al., 2002).

The potential and variation in biomagnification is greatly impacted by the capacity for metabolizing xenobiotics of each species, in addition to the physicochemical properties of the lipophilic contaminants (Gray, 2002; Hop et al., 2002). The propensity of a compound to bioaccumulate, bioconcentrate and biomagnify are functions of its chemical properties, such as the octanol-water coefficient (K_{OW}) and octanol-air coefficient (K_{OA}). In addition to the mentioned factors, the behaviour and accumulation of a compound rely on the characteristics of the organisms within the food webs – their uptake, absorption, and xenometabolism (Borgå et al., 2012; Hop et al., 2002).

Lipids are very important for Arctic species, compared to more temperate counterparts, as an insulator for homeotherms and widespread use as seasonal energy storage. This seems to exacerbate the bioaccumulation of lipophilic xenobiotics (Dalsgaard et al., 2003). During the productive Arctic summers, species attain energy-rich lipids for the long winters. The lipophilic contaminants associates with these lipid stores, and as the lipids are used during winter and during reproductive events, the contaminants get remobilised. In Arctic char (*Salvelinus alpinus*), winter emaciation redistributed contaminants from muscle (main lipid depot) to brain and liver (Jørgensen et al., 2006). In addition, a majority of the ecosystems of the Arctic are marine – which is important to consider because these food webs generally have one more trophic level (Hairston Jr and Hairston Sr, 1993; Shurin et al., 2006).

There is a great deal of evidence that POPs are able to exert toxic effects in concentrations found in the environment, from endocrine disruption in polar bears (*Ursus maritimus*) (Oskam et al., 2003), neoplasia in beluga whales (*Delphinapterus leucus*) (Martineau et al., 2002), immunotoxic effects in northern fur seal (*Callorhinus ursinus*) (Beckmen et al., 2003), and reproductive toxicity in glaucous gulls (*Larus hyperboreus*) reviewed in Verreault et al. (2010). Genotoxic effects of OCs have been found in glaucous gulls fed environmentally contaminated eggs (Krøkje et al., 2006; Østby et al., 2005) and of POPs in fasting common eider (*Somateria molissima*) (Fenstad et al., 2014).

1.2. Genotoxicity

1.2.1. Effects on the individual

DNA carries all the genetic information of organisms and chemicals that damage these genetic structures or functions are said to be genotoxic. Chemicals can inflict damage by altering the DNA itself in several ways: compounds can form adducts with DNA, modify bases, form abasic sites, and cause strand breaks (Shugart, 2000), (Fig. 1). Several of these DNA-damaging compounds need to go through a metabolic activation before they can cause an effect (Preston and Hoffmann, 2013).

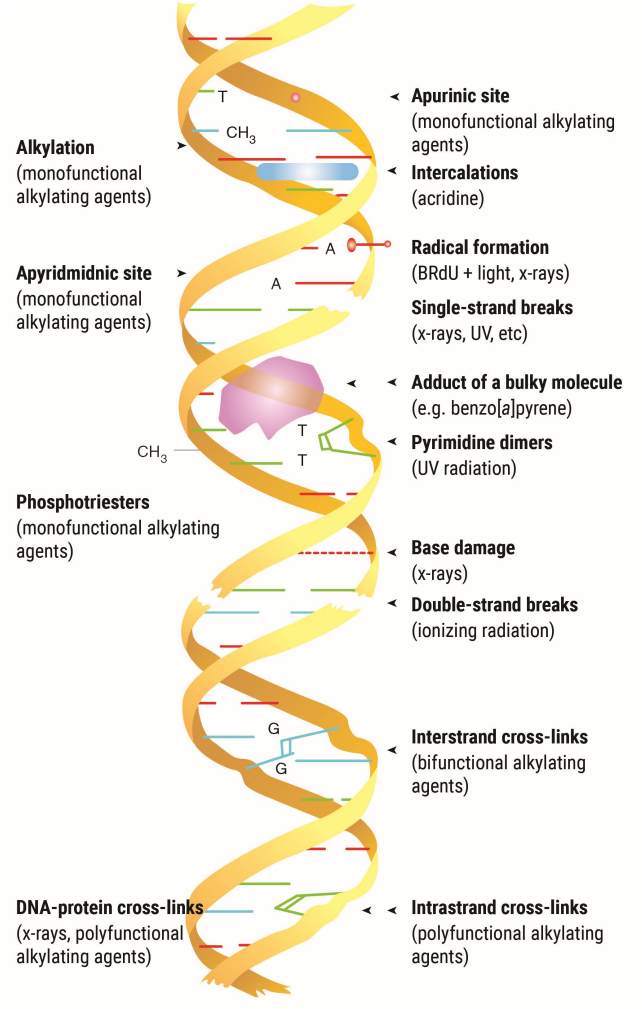

FIGURE 1: Spectrum of DNA damage induced by physical and chemical agents. Modified from Preston and Hoffmann (2013).

Not only exogenous chemicals can damage DNA, but also endogenous factors can have negative effects on the cellular macromolecules. Oxidative damage to DNA alone contributes to more than one 8-hydroxydeoxyguanosine per hundred thousand base pairs, the most common oxidized base (Mitchelmore et al., 1996). Exogenous oxidative stress can also inflict lesions to DNA through production or amplification of (endogenous) reactive oxygen species (ROS). ROS can damage DNA directly (*e.g.* radical formation, Fig. 1) or by reactions with proteins, phospholipids and other vital cell components, which in turn could induce more damage to DNA (Theodorakis, 2008).

The repair systems of the cells are efficient and thorough, and most damage is rapidly undone by repair enzymes (Kienzler et al., 2013). If the rate of DNA damage is greater than the rate of repair, or if the repair capacity is decreased – or in combination – the damage can persist. In the case of double-strand breaks (DSBs), a misrepair or lack of repair can give rise to mutations and chromosomal aberrations. In fish, all basic repair systems, such as homologous recombination (HR), nucleotide excision repair (NER), and non-homologous end-joining (NHEJ), have been proven to exist (Kienzler et al., 2013). If DBSs occur before synthesis (G1 phase), the more error-prone NHEJ repair pathway is more important, whereas the error-free HR is more important for repair in the succeeding stages of interphase (Bignold, 2009; Kienzler et al., 2013; Rothkamm et al., 2003). Mutations and chromosomal aberrations can, in turn, lead to cell death (Jha, 2008; Roos and Kaina, 2006).

DSBs could lead to permanent changes as chromosomal or point mutations. The change could either affect somatic cells, possibly leading to tumorigenesis, or have an impact on germ cells and thus possibly affect fertility, fecundity, and progeny (Jha, 2008). There has also been put forth a theory of the *Genotoxic Disease Syndrome* (Kurelec, 1993), in which of a suite of impairments other than neoplasia manifests themselves as an effect of DNA damage, *e.g.* impaired enzyme function, altered protein turnover, change in general metabolism, aging, and atrophy. DNA damage thus is of concern due to adverse effect that could lead to the reduction of fitness of the individual, which in turn could have multigenerational and population-wide consequences.

1.2.2. Population effects

The possible outcomes outlined above (section 1.2.1) precedes higher-level, or emergent, effects of genotoxicants (Bickham et al., 2000). Endpoints such as neoplasia and reduced fecundity have a detrimental impact on the Darwinian fitness of the individuals (*i.e.* growth and reproduction), which could transcend to the population level (Belfiore and Anderson, 2001). An effect of genotoxicants (and contaminants in general) can be a loss of genetic variation in an exposed population (van Straalen and Timmermans, 2002). Shifts in the genetic patterns of populations could be due to bottlenecks, a selection for certain alleles, or to pleiotropy or linkage related to vital loci (Bickham, 2011; van Straalen and Timmermans, 2002). Noteworthy is the impact that loss of genetic variation could have in a multiple stressor environment, such as contaminant exposure in a changing climate (Moe et al., 2013).

1.3. Arctic char

1.3.1. Distribution

Arctic char (*Salvelinus alpinus*) is a circumpolar fish, but can also be found in cooler waters of the Northern Hemisphere, as in high-alpine lakes of the Alps and Pyrenees and deep Scottish lakes (Klemetsen et al., 2003). For brevity, Arctic char will be referred to as char throughout this thesis. Most char populations are land-locked, but anadromy is more widespread farther North (Klemetsen et al., 2003). If the char is anadromous, the sea-run fish usually spend about 40 days at sea. The global population is considered healthy and is a food source for native populations, as well as a species used in aquaculture (Eriksson et al., 2010).

FIGURE 2: Arctic char, *Salvelinus alpinus*. Photo: Per Harald Olsen

1.3.2. Phenotypic plasticity

Char is dubbed 'a generalist like no other', displaying a spectacular phenotypic plasticity (Klemetsen et al., 2003) – sometimes appearing in two or more morphs in the same lake (Klemetsen, 2010). Body mass of sexually mature individuals has been recorded from as low as 3 g to as heavy as 12 kg (a 4000-fold difference), which also illustrates its variety of life histories and ecology (Klemetsen et al., 2003). Among the morphs is the cannibal. It is common among char populations, and its occurrence seems to increase with latitude (Griffiths, 1994). Slow juvenile growth and food scarcity seems to be drivers of this behaviour, and the cannibal is mainly found in land-locked populations (Hammar, 2000). The juvenile cannibalistic individuals allocate resources into somatic growth at the expense of sexual maturation. Their sympatric conspecifics inhabiting the lower trophic levels seem to invest resources inversely, *i.e.* reproduction trumping somatic growth (Finstad et al., 2006; Klemetsen et al., 2003).

1.3.3. Lifecycle

The life cycle of *S. alpinus* have some interesting toxicological aspects. Analysis of anadromous fish migrating to the sea discovered a five-fold increase in its body lipid content after returning from the sea (Jobling et al., 1998). Land-locked char shows the same pattern, only a smaller increase in the body lipid content during the summers (Klemetsen et al., 2003). This comes after a prolonged stay under the ice and in the dark Arctic winters when primary production is very low. Char is often seen fasting during this period (Gallagher and Dick, 2010; Jørgensen et al., 1997). With seasonal cycles of emaciation and rapid growth comes waves of remobilisation of contaminants as the main lipid depot, muscle, is utilised for metabolism and reproduction (Jørgensen et al., 1997).

1.3.4. Lakes Ellasjøen and Laksvatn

The island of Bjørnøya (74.30° N, 19.01° E) is part of the Svalbard archipelago, found midway between mainland Norway and Spitsbergen. In the flat, northern part of the island is the meteorological station, while the southern part ends in high cliffs diving into the ocean. In these cliffs reside large seabird colonies of mainly kittiwake (*Rissa tridactyla*), little auk (*Alle alle*) and glaucous gull (*Larus hyperboreus*) (Evenset et al., 2007b). These birds rest on Lake Ellasjøen, preens, and deposit large amounts of guano into the catchment area of the lake or directly into it. This guano acts as an effective conveyor of contaminants from the marine to the limnic environment and is the cause of very high levels of POPs in water, sediment, zooplankton and char from Ellasjøen (Evenset et al., 2007a).

Laksvatn is a lake not far from the meteorological station and does not have the same kind of bird activity, nor is it located in a catchment area similar to that of Ellasjøen. This has left the lake free of the large load of contaminants that is present in Lake Ellasjøen, only affected by contaminants transported with atmospheric currents (Bytingsvik et al., 2015). In earlier studies (Evenset et al., 2004; Wiseman et al., 2011), Lake Øyangen has been used as a 'reference' lake, but due to a small population size of Arctic char there, Laksvatn will be used in this project.

1.4. Aim

The aim of the present study was to investigate the potential genotoxic effect, measured as DNA DSB frequency, of environmental exposure to organochlorines in Arctic char (*Salvelinus alpinus*) from the relatively highly polluted population of Lake Ellasjøen, as compared to the population from the relatively pristine Lake Laksvatn.

2. Material and methods

2.1. Sampling and sampling area

2.1.1. Location

Fieldwork was carried out at *Bjørnøya* (en. Bear Island, 74°30' N, 19°00' E, Fig. 1) in August and September 2014. Landlocked Arctic char was collected from two sites: Lake Ellasjøen (E, $n = 18$) and Lake Laksvatn (L, $n = 21$, reference lake). Permissions to conduct the fieldwork on Bjørnøya National Park were obtained from the Governor of Svalbard and The Norwegian Animal Research Authority. The research was carried out according to the established ethics and regulations of the current Norwegian legislation.

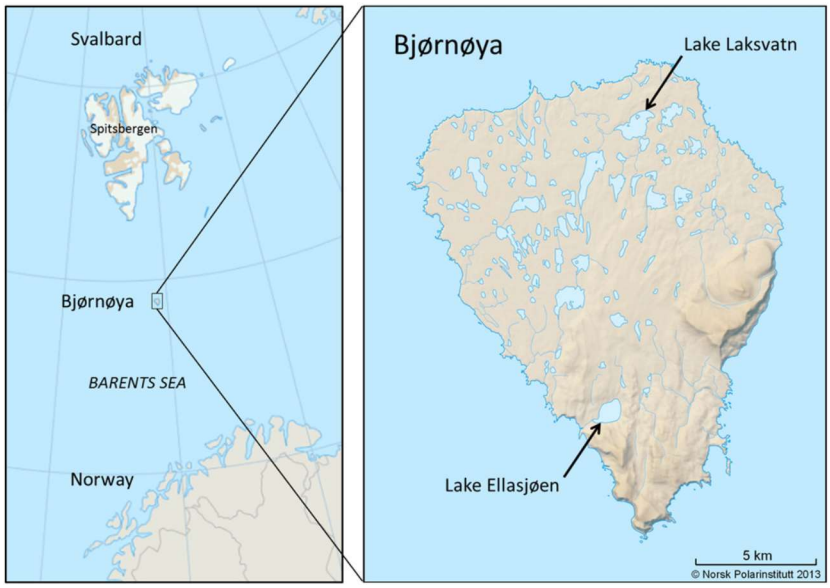

FIGURE 3: Location of Lake Ellasjøen (high levels of contaminants) and Lake Laksvatn (reference lake) on Bjørnøya. From Bytingsvik et al. (2015).

2.1.2. Fieldwork and sampling

Gill nets or fishing rods were used to catch the fish. Muscle samples from the dorsal axial muscle were collected from every fish for chemical analysis, otoliths were collected for age determination, and biological variables were measured. The latter includes fork length (cm), body weight (g), gender, reproductive stage, gonad, and liver weight (Table I). The reproductive stage is classified from 1-7, where 6 is at ovulation, and 7 is just past ovulation/spawning (Sømme, 1941). Samples of whole blood were collected for examining DNA double-strand breaks. To collect blood samples, fish were anaesthetized (benzocaine), and blood was collected from the caudal vein by heparinized vacutainers. Blood samples were frozen in liquid

nitrogen, stored at −80 °C, and transported to NTNU at the end of the field season. Muscle samples were kept in aluminum foil and ziplock bags at −20 °C and transported to Tromsø at the end of the field season.

2.2. Chemical analysis of organochlorines

Extraction, clean-up, and analysis of the contaminants were performed at the Norwegian Institute for Air Research (NILU) in Tromsø. Reference materials were obtained from Cambridge Isotope Laboratories (Woburn, MA, USA), and all solvents used were of pesticide grade (*Suprasolv;* E. Merck, Darmstadt, Germany). The analysis was done as described in Herzke et al. (2003) with modifications from Hallanger et al. (2011).

2.2.1. Principles of gas chromatography & mass spectrometry

The function of gas chromatography (GC) coupled with a mass spectrometer (MS) is to separate complex mixtures and identify its constituents. Gas chromatography functions on the same premise as all chromatography: the migration of a sample by a mobile phase over a stationary phase. The mixture constituents will get separated based on their affinity to the stationary phase – a substance with higher affinity to the stationary phase will adhere more strongly to it, and thus migrate slower. In GC, the mixture is evaporated and an inert gas will act as the mobile phase, while the stationary phase is a thin polymer or liquid layer on an inert support (*e.g.* glass or metal tubing).

The molecules are introduced to the MS via the GC for analysis of the fractions. The evaporated substance is first ionized by a beam of high-energy electrons. Also, this often causes the molecules to break into fragments which in turn can be separated on their mass-to-charge ratio. The separation is done by accelerating these fragments over an electromagnetic field which will deflect the particles differentially by their mass-to-charge properties. Heavier and less charged particles will be deflected less than lighter and higher charged particles. The molecules will travel based on these properties and will create a certain pattern onto a detector. The detector then can produce a mass spectrum to be analyzed, where the ion fragments' abundance to its mass to charge ratio is given.

2.2.2. *Extraction*

A subsample of frozen or partly thawed dorsal axial muscle (0.5 g ± 0.05) was homogenized with a 20-fold amount of preheated sodium sulfate (freshly burnt at 600 °C for 8 h). The homogenate was left in a freezer overnight. Internal standard (^{13}C labelled surrogates; 20 µl) was added after the homogenate had reached room temperature. The internal standard included isotope surrogates for 8 PCBs and 6 additional organochlorines (OCs). The PCB congeners analysed for were: 28, 52, 101, 105, 118, 138, 153, and 180. The additional OCs analysed for were: *trans*-nonachlor (t-NC), *cis*-NC, *trans*-chlordane (t-CD), *cis*-CD, oxy-CD, and Mirex.

Following addition of the internal standards, samples were then extracted thrice with an acetone:cyclohexane mixture (1:3; v/v; 50 ml), and placed in an ultrasonic bath after each addition of the solvent for 20, 15 and 15 min. Samples were concentrated by evaporation (Rapidvap) until a volume of 1.5–2.0 ml was reached, transferred to 4-ml screw-cap vials with a hexane:dichloromethane solvent (1:1; v/v), not exceeding a total volume of 3 ml.

2.2.3. *Acid wash and clean-up*

Approximately 1.5 ml of the extract was transferred to glass tubes (15 ml sized), and n-hexane was added until 3.5 ml total volume was reached. Sulfuric acid (2 ml) was added and the extracts were vortexed thoroughly (30 sec) in a screw-cap glass tube. The extracts were subsequently centrifuged (10 min at 3,000 rpm), and the bottom layer was discarded. The addition of sulfuric acid and succeeding steps were repeated until the top layer was light–yellow to clear (1-3 times). Three cycles of washing with water followed: first by the addition of 1 ml ultraclean (MilliQ) water, centrifugation (10 min at 3,000 rpm), and removal of water phase by pipette. Sodium sulfate was added (200 mg) to remove water from the solution, and the subsample extracts were added iso-octane (100 µl; as 'keeper') and evaporated until 0.5 ml total volume.

Further clean-up was performed by first transferring the extract to another tube, evaporated on Rapidvap, and additional clean-up by RapidTrace SPE Workstation (Caliper Life Science, Hopkinton, USA) using florisil columns. The columns were packed with pretreated (heated at 450 °C for 8 h) florisil (1 ± 0.03 g; activated magnesium silicate gel), with particle size of 0.15-0.25 mm, between two glass fiber frits. The extract was eluted using a mobile phase of 10 % dichloromethane (in n-hexane), and the final fractions were evaporated to 0.1 ml (Rapidvap) and transferred to gas chromatography sample vials and the recovery standard $^{13}C_{12}$-PCB159 was added.

2.2.4. *Instrumental analysis (GC-MS)*

The chromatographic separation and detection of PCBs was performed using an Agilent 7890 GC, equipped with a 30 m DB5-MS column (0.25 mm id and 0.25 µm film thickness; J&W, Folsom, USA). Helium (6.0 quality; Hydrogas, Porsgrunn, Norway) was used as carrier gas at a flow rate of 1 ml × min^{-1}. Sample extract (10 ml) was injected with an LVI injector (Agilent Technologies, 7683B) at 250 °C. The temperature programme used is as follows: first 67 °C for 1.5 min followed by a 15 °C × min^{-1} increase until 180 °C reached, then a 5 °C × min^{-1} increase until 280 °C, and a final hold at that temperature for 3 min. The GC instrument was used in electron ionisation mode with an ionisation energy of 70 eV. The detection was performed by multiple reaction monitoring (MRM) measurements using a Quattro MicroTM mass spectrometer (MS; Micromass MS Technologies, Manchester, UK). The transfer line temperature was held at 280 °C, and the source temperature was set to 220 °C.

The analysis of hexachlorobenzene, oxychlordane, *cis*-chlordane, and *trans*-nonachlor was performed with the same GC setup as with the analysis of PCBs, but helium was used as carrier gas with a 1 µl injection volume in splitless mode using a split/splitless injector at 280 °C. The temperature programme used for these contaminants was: 70 °C initially held for 2 min, then 4 °C × min^{-1} to 90 °C (held for 3 min), followed by 15 °C × min^{-1} until a final 320 °C kept for 2 min. Electron capture negative ionization mode was used to determine and quantify the pesticides. Ion source temperature was 160 °C and ionization energy of 70 eV was used.

2.2.5. *Quantification & quality control*

By the addition of internal standard of known volume, the volume of the unknown samples can be determined by the relationship between the analyte and its isotope surrogate. The internal standard solution was provided by NILU ('POP I', batch 41.13). The compounds were measured in pg × g^{-1} wet weight (ww), and converted to pmol × g^{-1} ww. Lipid content of the muscle was determined gravimetrically, and given as a percentage. Equation 1 presents how the concentration of the sample was found, where, C_S is the concentration of a sample, C_{IS} the concentration of known standard, A_S is the known sample's area in the chromatogram, A_{IS} is the internal standard's area, and Rf is the response factor for each factors' calibration curve. The response factor is the ratio between the ^{13}C and the ^{12}C compounds.

$$C_S = Rf \times \frac{C_{IS} \times A_S}{A_{IS}} \quad (1)$$

The performance of the method used was verified by use of standard reference material (SRM) as a control sample with every processed batch (4-12 fish samples) and numerous blank samples during the analysis. The SRM was fish homogenate, "Naturally Contaminated Fish (slurry)" (Cambridge Isotope Laboratories, EDF-2525). These steps ensured a high quality of the analytical results.

2.3. Detection of DNA double-strand breaks

The determination of the DNA double-strand breaks in the blood of Arctic char was carried out by gel electrophoresis at the Department of Biology, Norwegian University of Science and Technology (NTNU).

2.3.1. Principles of electrophoresis

A quantitative measure of DNA double-strand breaks (DSBs) can be detected by gel electrophoresis, as described by Theodorakis et al. (1994). Suspending nucleated cells in agarose gel is key for a careful treatment, as to not introduce excessive damage to the DNA by the method. Cell lysis and enzymatic digestion of nucleases and DNA-associated proteins can then be performed on the cells within the gel. As the cell is disintegrated and auxiliary proteins are dissociated from DNA, the nucleus is now rather a *nucleoid* – tightly wound, spherical DNA, still retaining much of its old topology (Shaposhnikov et al., 2008). DSBs will cause this nucleoid to relax and release DNA fragments: the more DSBs, the more fragments released during the electrophoresis.

During electrophoresis, the DNA fragments migrate through the agarose matrix. The migration is propelled by an electric field, spanning the length of the gel, and is due to the attraction of the negatively charged phosphorous groups of the nucleotides to the anode. By running the electric field over the gel for a given time, the fragments will separate by size – the shorter fragments migrating farther. Double-strand DNA can then be stained within the gel with a specific dye and measured qualitatively by its fluorescence.

2.3.2. Preparation of plugs

Detection of DNA breaks was done as described by Theodorakis et al. (1994) and others (Fenstad et al., 2014; Krøkje et al., 2006) with modifications. Whole blood (2-4 µl) was added to TE buffer (500 µl; Appendix A) at 37 °C. Low melting point agarose (1 %; 37 °C) was added (500 µl) and spun briefly until a speed of ~8,000 rpm was reached, and 50 µl-plugs were cast in molds, and set at 4 °C for 1 h. Blood cells suspended in plugs (then reached room temperature) were lysed overnight at 55 °C in lysis buffer (Appendix A).

2.3.3. *Gel electrophoresis*

Plugs were put in wells of a 0.6 % agarose gel in TBE buffer (Appendix A), and the wells sealed with the low melting point agarose. Electrophoresis was run for 14 h at 2.3 V × cm^{-1} at room temperature. Whole linearized Lambda phage DNA and its Hind III digest fragments were used as molecular ladder and positive control (Appendix A). The gel was then stained with a solution of ethidium bromide in TBE buffer (~0.1 mg × l^{-1}) for 180 min and washed in tap water. Each gel had four samples in triplicates, and three lanes with size markers (15 lanes total). Two gels with the identical setup were run at the same time. Whole blood samples were chosen randomly, but samples from both lakes were to a great extent used in each gel, to counter possible variations on the method over the duration of analysis.

2.3.4. *Quantification of DNA double-strand breaks*

BioRad GelDoc 2000 system was used for gel imaging. The following treatment of densitometric data was done in Excel (2013, Microsoft Corporation) to calculate two measurements. The median molecular length (MML) was calculated by finding the median value of the intensity curve outside the well, and relating this value to a standard curve constructed by a HindIII Lambda phage digest Lambda size marker of known molecular size. Secondly, the fraction of total DNA that migrated into the gel (DNA-FTM) was measured by the amount of DNA in the lane (that had migrated from the well) compared to total DNA (DNA in the gel × (DNA in well + DNA in gel)$^{-1}$ × 100), *cf.* Fenstad et al. (2014).

2.4. Statistical analyses

2.4.1. *Individuals*

A total of 39 individuals were used in the analyses: 7 females and 11 males from Ellasjøen, 9 females and 12 males from Laksvatn (Table 1). Gonadosomatic index (GSI; (gonad weight (g) × body weight (g)$^{-1}$) × 100), hepatosomatic index (HSI; (liver weight (g) × body weight (g)$^{-1}$) × 100) and condition factor (CF; (body weight (g) × body length (cm)$^{-3}$) × 100) were calculated, as presented in Table 1. If the females carried eggs, these were included in the gonad weight.

2.4.2. *Chemical data*

Several organochlorine compounds were omitted from statistical analyses. Compounds measured over the limit of detection (LOD) in more than 50 % of the individuals were included further, which excluded PCB28, PCB52, oxy-chlordane, *cis*-chlordane, and *cis*-nonachlor. Further inclusion was done if the final volume of the SRM was over 20 % of that added. This criterion rejected *trans*-chlordane and Mirex from further analysis. Hexachlorobenzene was excluded due to having only 5 measurements (of 39) above LOQ, albeit none under LOD. The compounds included in the final dataset were: PCB101, -105, -118, -138, -153, -180, and t-NC. Subsequently, measurements below the LOD was replaced by a random integer between 0 and the LOD of that specific compound. The LOD varied between 13 $pg \times g^{-1}$ for t-NC 286 $pg \times g^{-1}$ for PCB138. The analyte concentrations that were over the LOD and under the limit of quantification (LOQ) was noted and used as measured.

2.4.3. *Software*

All statistical procedures were performed in R Studio (Rs. Team, 2015), an integrated development environment for R (R core team, 2015). Principal component analysis (PCA) was carried out with the *FactoMineR* package (Husson et al., 2008), *ggplot2* (Wickham, 2009) was mainly used to plot figures, all other applications used were native functions of the R core package.

2.4.4. *Principal component analysis*

PCA is a powerful tool to unveil patterns in high-dimensional datasets, *i.e.* with many variables (Shlens, 2014). Its goal is to explain the most variance by the fewest dimensions; the emergent new dimensions are called principal components. This is done by deconstructing the correlation or covariance matrix into its eigenvalues and –vectors, which are orthogonally transformed to principal components (PCs). Those variables that have the most variation in the dataset will contribute most to the first principal components. The projection quality can be measured by $cosine^2$ between the individual vector each the principal component: the closer to one the better the projection.

The variables used in the PCA were the individual OC concentrations, age, CF, HSI, reproductive stage, and DNA-FTM. Missing values were imputed from the mean. MML was excluded from the analysis as DNA-FTM and MML are two measures of DNA damage, and DNA-FTM had the lowest coefficient of variance (CV). The OC concentrations were thus ln-transformed to reduce their combined (highly correlated) contribution, and accentuate the other variables included. Fork length, body weight, and liver weight were added as supplementary variables – variables not contributing to the construction of the dimensions, but projected onto them.

2.4.5. Linear regression

Multiple linear regression (*i.e.* linear models) were used to investigate the associations between the biometric variables, the chemical data, and DNA damage. Linear models seek to predict values of a dependent variable by one or several explanatory variables in a linear relationship. Interaction effects can also be accounted for in linear models, which is when one independent variable has different effects on the prediction, depending on the value of another independent variable.

Candidate models were set up based on *a priori* expectations and knowledge, as well as indications from PCA. A stepwise selection process was undertaken to find the best model. Akaike's information criterion (AIC; Akaike 1974), the corrected AIC (AICc) and the coefficient of variance (R^2) of the models were used to select the models that were most likely to fit the data (Burnham and Anderson, 2004). AIC is a measure of the quality of a model, balancing goodness of fit with parsimony. It can be used to compare similar models with the same sample size – only being able to assess the quality within the model set, but not the absolute quality of a model. The lower AIC the better is the model. AICc is better for selecting models than AIC when the n is small ($n < 40$) – penalizing greater than AIC for extra parameters. This is to avoid the possibility of overfitting the model. AICc weights sum to 1 for the model set – and the weight of each model can be interpreted as the weight of evidence for that model being the best in the set (Burnham and Anderson, 2004).

Burnham and Anderson (2004) also suggest as a rule of thumb that models within a model set of a $\Delta AICc \leq 2$ should be considered to have similar weighted support and be compared on equal terms. The assumption of normality was ensured met by diagnostics in R (visual analysis of residuals), as well as Shapiro-Wilk normality test of the model residuals. DNA-FTM was chosen as the one measure of DNA double-strand breaks included in the model. The models were constructed without GSI, gonad weight and MML, as they would have excluded 12 individuals (due to missing values) from the total of 39.

3. Results

3.1. Descriptions

3.1.1. Biological variables

A summary of the biological variables of the fish from Lake Ellasjøen and Lake Laksvatn is presented in Table 1, and all individual measurements are found in Appendix B. Fork length, body weight, liver weight, and HSI were all significantly higher in char from Laksvatn than in Ellasjøen (Mann-Whitney *U*: $p < 0.001$). The Ellasjøen char were significantly older than the fish in Laksvatn (Mann-Whitney *U*: $p < 0.01$). The Ellasjøen individuals were on average 12.7 years ± 2.7 (standard deviation) of age and fish from Lake Laksvatn were 10.4 ± 1.2 years old, and with a wider age range (9-19 vs. 9-12).The collected fish of Laksvatn were significantly longer and heavier than the ones of Ellasjøen, but a difference in CF was not found (Mann-Whitney *U*: $p = 0.14$). There was also a significant difference in GSI of females between the lakes, with Laksvatn having the greater GSI (but not absolute gonad weight; Mann-Whitney *U*: $p < 0.05$).

TABLE 1: Biological variables of Arctic char (*Salvelinus alpinus*) from Lake Laksvatn (n = 21, 9 females, 12 males) and lake Ellasjøen (n = 18, 7 females, 11 males), Bjørnøya (Norway), sampled 2014. Presented as average with standard deviation (SD), median and the range. Significance by p-value of Mann-Whitney *U* test: '***' < 0.001, '**' < 0.01, '*' < 0.05, 'ns' not significant. Length in cm, weights (W) in g; B: body, G: gonads, L:liver, Rs: reproductive stage, lip%: percentage lipid in muscle, GSI: gonadosomatic index, CF: condition factor, HSI: hepatosomatic index, a: females only.

		Laksvatn			Ellasjøen		
		Average ± SD	Median	Range	Average ± SD	Median	Range
Length	***	48.8 ± 3.1	48.7	43.5 - 56.1	43.5 ± 6.7	41.9	36.2 - 62.4
W_B	***	1092.7 ± 143.6	1052.4	845.4 - 1433.9	808.6 ± 476.7	636.4	436.3 - 2372.6
Age	**	10.4 ± 1.2	10	9 - 12	12.7 ± 2.7	12	9 - 19
Rs		4.8 ± 2.1	6	1 - 7	4.0 ± 2.1	5	1 - 6
W_G [a]		16.1 ± 12.0	17.3	0.7 - 37.8	7.6 ± 7.9	6.6	0.4 - 20.7
Lip%	***	0.5 ± 0.0	0.5	0.5 - 0.6	0.4 ± 0.2	0.4	0.2 - 1.0
W_L	***	11.23 ± 2.31	11.15	7.67 - 16.60	6.16 ± 2.40	5.38	3.52 - 12.72
GSI [a]	*	7.05 ± 7.91	1.82	0.08 - 18.41	4.12 ± 5.57	1.22	0.07 - 14.18
CF		0.94 ± 0.11	0.99	0.69 - 1.07	0.91 ± 0.06	0.91	0.82 - 1.04
HSI	**	1.05 ± 0.27	1.02	0.66 - 1.70	0.83 ± 0.23	0.77	0.54 - 1.47

The percentage muscle lipid content (lip%) was significantly higher in Laksvatn than in Ellasjøen char (Mann-Whitney *U*: $p < 0.01$). The percentage of lipid in the muscles of Ellasjøen char had a very large coefficient of variance of 42.3 % compared with Laksvatn with a 3.3 % score.

Char from Laksvatn had a higher growth rate, shown in Figure 4. There was a significant difference in the relationship between (ln transformed) weight by age ($F_{2, 33} = 37.07$, $p < 0.001$) of the two lakes. Note that the growth rate of Laksvatn char is inferred from a smaller span in age.

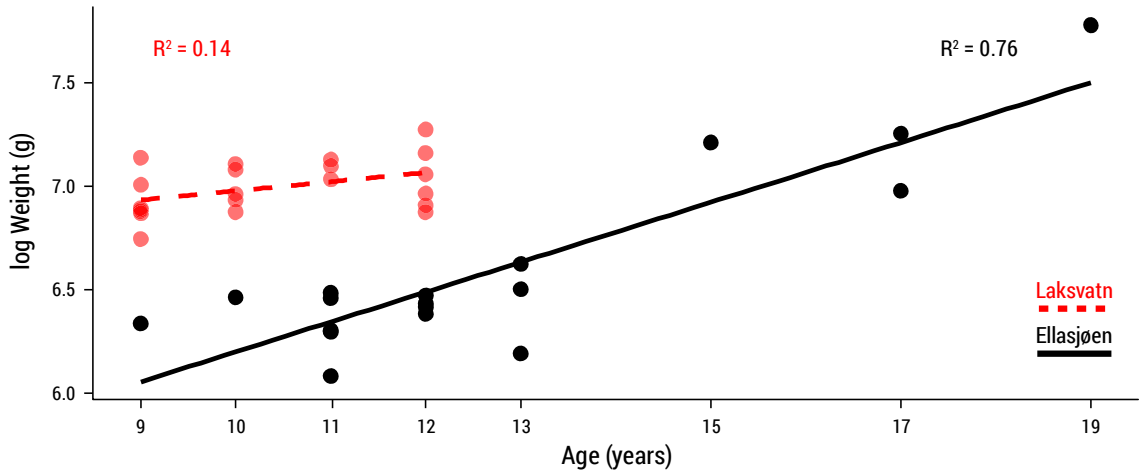

FIGURE 4: Weight (g, log transformed) by age (years) of Arctic char (*Salvelinus alpinus*) from lake Ellasjøen (black) and lake Laksvatn (red). A linear regression line for each of the populations shows the significant difference (ANOVA: $F_{2, 33} = 37.07$, $p < 0.0001$).

The reproductive stage of the different fish was determined, and the fish were mainly found in stage 2 and 6 of the reproductive cycle (out of 7 total stages). The complete distribution of fish in reproductive stages by sex and lake is shown in Table 2.

TABLE 2: Number of female (F) and male (M) Arctic char (*Salvelinus alpinus*) from in Ellasjøen (E) and Laksvatn (L; Bjørnøya, Svalbard) in different reproductive stages (Sømme, 1941). T: total

	Stage 1			Stage 2			Stage 3			Stage 6			Stage 7		
Lake	E	L	T	E	L	T	E	L	T	E	L	T	E	L	T
F	0	0	0	2	3	5	1	0	1	4	5	9	0	1	1
M	1	3	4	5	0	5	0	0	0	5	9	14	0	0	0
All			4			10			1			23			1

3.1.2. Contaminant load

OC levels were measured in muscle of 39 individual char from the two lakes on Bjørnøya (Table 3). The individual measurements in both pmol × g^{-1} ww and ng × g^{-1} in both ww and lipid-normalized weight, can be found in Appendix D. Fish from Lake Ellasjøen had much higher levels of OCs than Lake Laksvatn. The concentrations differed greatly: average ∑OC concentrations in Ellasjøen char were 43 times higher than for Laksvatn char. The greatest difference was found for PCB153, which on average was 53 times higher in Ellasjøen than in Laksvatn char.

TABLE 3: The concentration of organochlorine compounds measured in muscle of Arctic char (*Salvelinus alpinus*) from lake Laksvatn and lake Ellasjøen, Bjørnøya, Svalbard (2014), in pmol × g^{-1} wet weight (N = 39). All compounds differed significantly between lakes (Mann-Whitney *U*: $p < 0.01$).

	Laksvatn			**Ellasjøen**		
	Average ± st.d	Median	Range	Average ± st.d	Median	Range
PCB101	33.2 ± 15.3	30.0	11.0 - 67.1	231.6 ± 368.6	104.8	37.7 - 1472.6
PCB105	18.6 ± 9.6	16.8	4.0 - 43.2	592.4 ± 1044.7	229.6	88.2 - 4237.0
PCB118	64.3 ± 31.6	53.0	20.2 - 152.3	2622.8 ± 4761.3	927.8	309.1 - 19539.4
PCB138	216.0 ± 120.4	195.9	57.1 - 550.6	9432.0 ± 19070.1	2814.0	952.7 - 76625.3
PCB153	328.2 ± 190.3	272.9	98.9 - 856.0	17607.0 ± 38234.3	4836.7	1370.5 - 156362.7
PCB180	102.7 ± 66.9	78.7	29.3 - 283.6	3194.6 ± 7219.0	784.5	211.0 - 29871.5
t-NC[1]	17.9 ± 8.2	14.9	7.2 - 37.4	58.7 ± 83.6	28.5	11.7 - 326.6
ΣOC[2]	781.0 ± 429.1	664.8	227.8 - 1971.6	33739.1 ± 70734.4	9741.1	2980.9 - 288435.2

1: *trans*-nonachlor, 2: ΣOCs includes all organochlorines above

On a lipid-normalized scale, PCB153 was measured at 20147 ng × g^{-1} on average for Ellasjøen char, and 230 ng × g^{-1} for Laksvatn char – an 87-fold difference.

The order of OCs by median concentration differed somewhat between the lakes. The median concentration of PCB153 was highest in both lakes, followed by PCB138, and in both lakes, t-NC displayed the lowest median concentration. The descending order for Ellasjøen was: PCB153, PCB138, PCB118, PCB180, PCB105, PCB101, and t-NC. For Lake Laksvatn, the order of OCs by their median concentration was: PCB153, PCB138, PCB180, PCB118, PCB101, PCB105, and t-NC. The individual OC measurements are shown in Figure 5, which reiterates the relationship between contaminants as well as their lake-specific levels.

Notably for Ellasjøen is the recurring three individuals with high concentrations of OCs: which are three old males (id: 73, 74, and 75, ages: 19, 17, and 15, respectively). The contaminant profile between the lakes was similar, as can be seen in Figure 5 and Appendix D. The individual compounds constituted similar shares of the total chemical load between the two lakes.

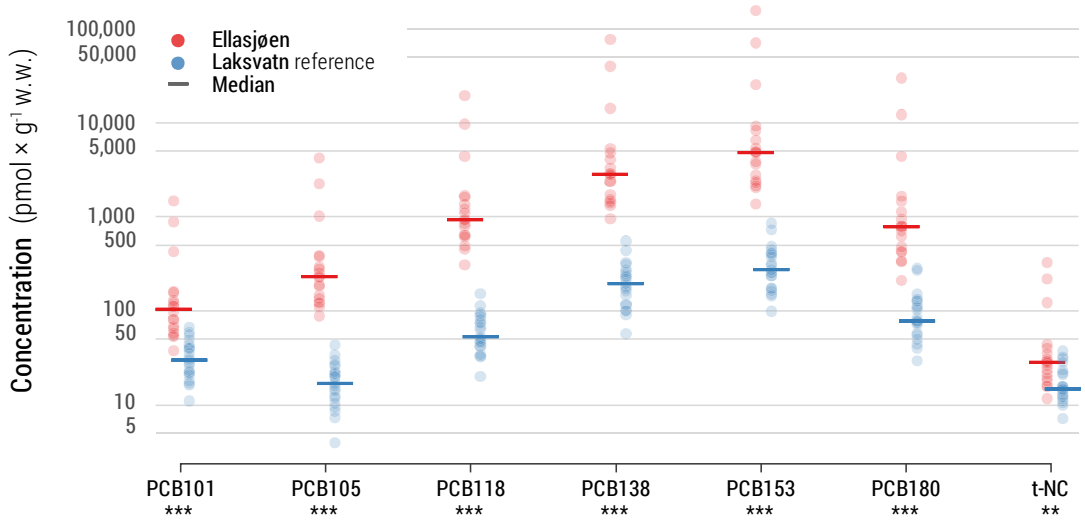

FIGURE 5: Organochlorine compound concentrations in pmol × g^{-1} wet weight in Arctic char (*Salvelinus alpinus*; muscle) from Lake Ellasjøen (red, $n = 18$) and Lake Laksvatn (blue, $n = 21$). Lake median is marked by a horizontal line. t-NC: *trans*-nonachlor. All compounds had a significantly higher concentration in Lake Ellasjøen (Mann-Whitney *U*-test. *p* value: '***' < 0.001, '**' < 0.01)

3.2. DNA double-strand breaks

3.2.1. Level of DNA double-strand breaks

Blood samples from 39 fish were analyzed for DNA double-strand breaks (DSBs) by the fraction of total DNA that migrated into the gel (DNA-FTM), *cf.* Fenstad et al. (2014). There was a significant difference in DNA-FTM between the lakes of which a higher level of DSBs was found in char from the high-contaminated lake Ellasjøen compared with the reference lake, Laksvatn (Mann-Whitney *U*, $p < 0.001$; Figure 6). There was no significant difference between the sexes within individuals of Lake Laksvatn ($F_{1,\ 18} = 0.23$, $p = 0.64$), but there was a significant difference between the sexes in Ellasjøen ($F_{1,14} = 8.496$, $p = 0.01$). The males of Ellasjøen had significantly higher DNA-FTM than the females of Ellasjøen. Measurements of DNA-FTM per individual are provided in Appendix B.

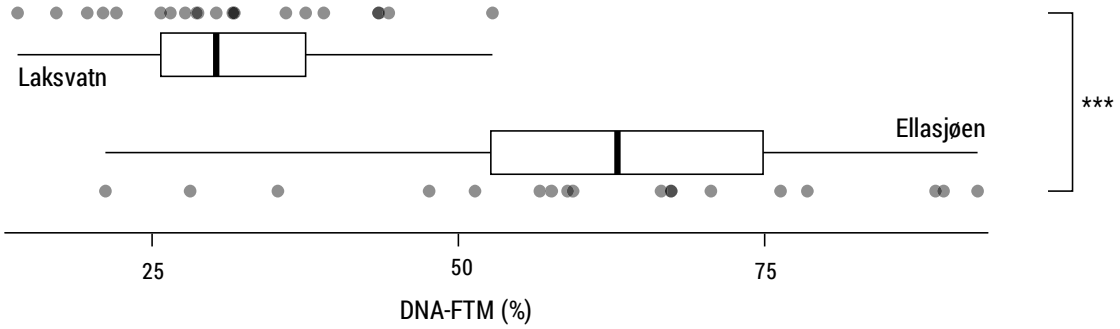

FIGURE 6: Measure of double-strand breaks by means of DNA-fraction of total DNA, that migrated into the gel (DNA-FTM) in Arctic Char (*Salvelinus alpinus*) from Lake Laksvatn (reference; $n = 21$) and Lake Ellasjøen (high contaminant load; $n = 18$). Individual measurements are shown, as well as their box plot where the box boundary is the the upper and lower quartile, divided by the median. There was a significant difference between the lakes (Mann-Whitney *U*: $p < 0.001$).

3.2.2. DNA median molecular length

Blood samples from 39 fish were also analyzed for the median molecular length of the DNA fragments that left the well during gel electrophoresis (MML; *cf.* Haldsrud and Krøkje [2009]). There was a significant difference in MML between the lakes (Mann-Whitney *U*, $p < 0.05$), as illustrated in Fig. 7, where the individuals of Lake Laksvatn had the larger MML. There was no significant difference between the sexes within any of the lakes. Individual measurements of MML are provided in Appendix B.

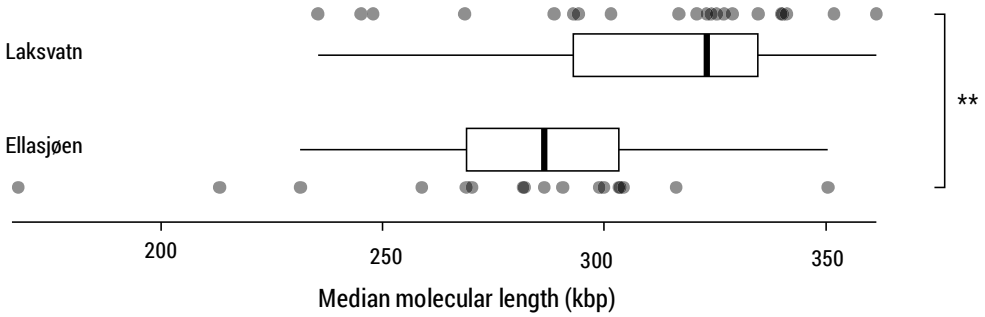

FIGURE 7: DNA fragment size distributions given as molecular median length, in kilobase pairs (kbp), for lake Laksvatn (reference lake; top; $n = 21$) and lake Ellasjøen (high contaminant load; $n = 18$). Individual measurements are shown, as well as their box plot where the box boundary is the the upper and lower quartile, divided by the median. There was a significant difference between the lakes (Mann-Whitney U: $p < 0.01$).

3.3. Associations

3.3.1. Principal component analysis

A PCA was performed as described in section 2.4.4 to examine the association between DNA-damage, OCs, and biometrics in char from Laksvatn and Ellasjøen. The loading and scores plots are provided in Figure 8. Individual eigenvalues and –vectors, PC contributions, cosine² values and more is provided in Appendix E.

Principal component 1 and 2 accounted for 74.16 % of the total variance (62.45 % and 11.70 %, respectively). Nearly all variance of PC1 was accounted for by the OCs: they contributed for 80.4 % of its variation combined. Age and DNA-FTM contributed with additional 8.6 % and 5.2 %, respectively. The OCs contributed nearly 0 % of variance to the remaining dimensions. The main contributors of the construction of PC2 were reproductive stage (48.7 %), CF (15.6 %), %lip (12.9 %), and DNA-FTM (10.9 %). There was a strong positive correlation between the OCs by their clustering. The eigenvalues of the PCBs were very similar – the PCBs having nearly identical eigenvalues. The OCs were also positively associated with age. The third dimension, accounting for only 7.4 % of the total variance, was constructed by mainly CF, % lipids, and DNA-FTM (65.3, 17.6, and 10.6 %, respectively).

The PCA plot of PC1 and PC2 indicates a negative association between DNA-FTM and HSI, and to a lesser degree with %lip and CF. The CF and %lip, in turn, also seemed to correlate positively with each other. DNA-FTM associated somewhat with the OCs on PC1 and is the only variable with a noteworthy negative PC2 score. The supplementary variables of (body) weight, (fork) length, and liver weight did not indicate any strong associations to any of the axes.

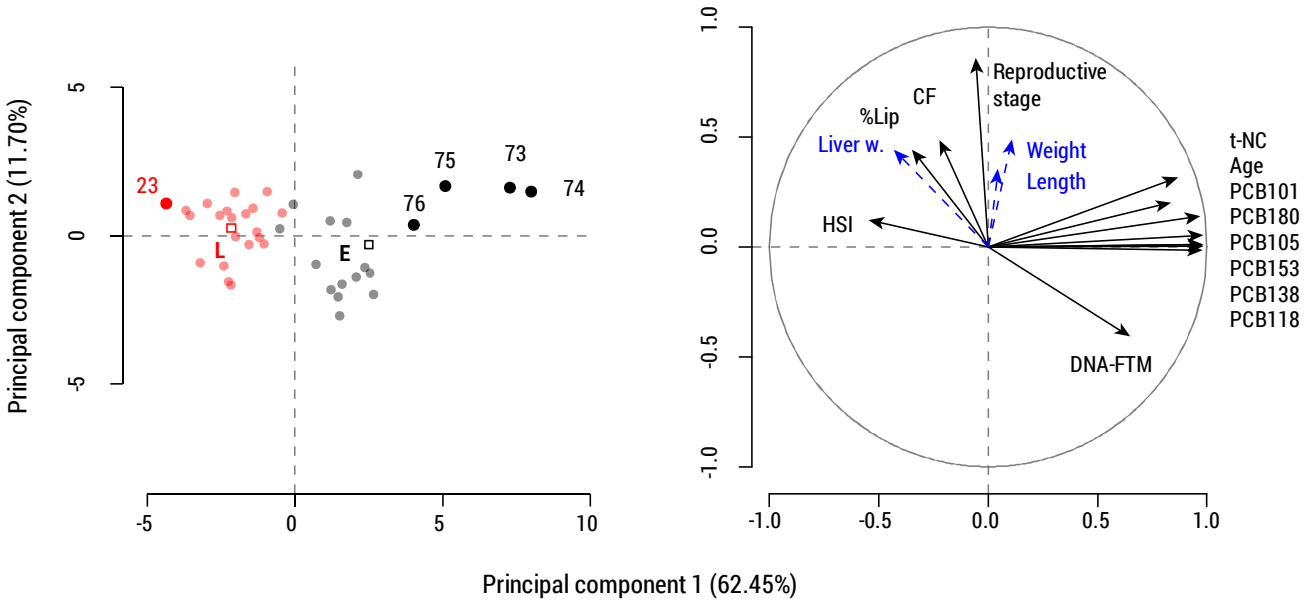

FIGURE 8: Scores plot (left) and loadings plot (right) from a principal component analysis of Arctic char (*Salvelinus alpinus*) from lake Laskvatn and lake Ellasjøen, in red and black of the scores plot, respectively (barycenters marked by squares). The 5 individuals that contributed with the most variation in the construction of the dimensions are marked. HSI: hepatosomatic index, CF: condition factor, DNA-FTM: fraction of total DNA that migrated into the gel, t-NC: *trans*-nonachlor, PCB: polychlorinated biphenyl, %lip. Blue (dashed line) represent supplementary variables.

There is an apparent division of the individuals from the two lakes in the scores plot by PC1. Generally, Ellasjøen individuals had positive PC1 values, while Laksvatn char had negative loadings. Noteworthy is the three recurring old males with the highest OC levels measured (see section 3.1.2) from Ellasjøen (id: 73, 74, and 75); they also score the highest on PC1. Of the Laksvatn char, the opposite picture emerges: young individuals with low concentrations of OCs stand out to the far left (with a negative PC1 score). Individual 23 has the lowest ∑OC level of the 39 individuals (227.8 pmol × g^{-1}). Of the clustered Ellasjøen individuals within the fourth quadrant (positive PC1 values, and negative PC2 values), all were in reproductive stage 1-3 with relatively low DNA-FTM scores.

3.3.2. Multiple regression models

A model selection (process discussed in section 2.4.5) was performed to examine which factors best could explain the level of DNA double-strand breaks. DNA-FTM was ln-transformed to meet normality. The four best candidate models, determined by their AICc score (Table 4), all included the reproductive stage (besides ∑OC). Liver weight and relative liver weight (*i.e.* HSI) was also seen in the top tier models.

The models with a $\Delta AICc \leq 2$ are presented in their entirety in Table 5. Generally, all the models show a significant decrease in DNA-FTM with an a higher reproductive stage. The coefficient estimates for both ∑OC and reproductive stage were similar between the four best models: ∑OC ranged from 0.151 (standard error of the mean [SE] = 0.039) to 0.171 (SE = 0.035), reproductive stage ranged from -0.073 (SE = 0.029) to -0.096 (SE = 0.032). Liver weight, absolute and by HSI, was included in model 2 and 3, but had no significant impact on the regression model ($p = 0.26$, $p = 0.31$, respectively).

TABLE 4: Selection table for models constructed to explain the level of DNA double-strand breaks, measured by fraction of total DNA that migrated into the gel (DNA-FTM, ln transformed), given in descending order of value of corrected Akaike's information criterion (AICc). AICc weight, cumulative AICc weight, and log likehood is also provided. K: number of parameters, ∑OC: ln ∑organochlorines, Rs: reproductive stage, WG: gonad weight, WL: liver weight, WB: body weight, HSI: hepatosomatic index, %lip: muscle lipid concentration. A colon indicates an interaction effect.

Explanatory var	ID	K	AICc	ΔAICc	AICc wt.	Cum. wt.	LL
∑OC + Rs	1	4	38.91	0.00	0.30	0.30	-14.86
∑OC + Rs + W_L	2	5	40.15	1.24	0.16	0.46	-14.16
∑OC + Rs + HSI	3	5	40.41	1.50	0.14	0.60	-14.29
∑OC + Rs:Sex	4	5	40.65	1.74	0.12	0.72	-14.41
∑OC + Rs + Age	5	5	41.06	2.15	0.10	0.82	-14.62
∑OC + Rs + %lip	6	5	41.41	2.51	0.08	0.90	-14.80
∑OC + Rs:Sex + HSI	7	6	43.12	4.22	0.04	0.94	-14.25
∑OC + W_L	8	4	43.83	4.92	0.03	0.97	-17.33
∑OC	9	3	44.87	5.97	0.01	0.98	-19.09
∑OC + HSI	10	4	46.23	7.33	0.01	0.99	-18.53
∑OC + CF	11	4	46.71	7.80	0.01	0.99	-18.77
∑OC + %lip	12	4	47.26	8.35	0.00	1.00	-19.04
W_L	13	3	51.95	13.04	0.00	1.00	-22.63
Age	14	3	52.77	13.87	0.00	1.00	-23.04
HSI	15	3	55.46	16.55	0.00	1.00	-24.39
Rs	16	3	56.37	17.46	0.00	1.00	-24.84
Sex	17	3	59.47	20.56	0.00	1.00	-26.39
%lip	18	3	60.09	21.19	0.00	1.00	-26.7
CF	19	3	62.15	23.24	0.00	1.00	-27.73

Model 4 showed a significant difference in the impact of reproductive stage on DNA-FTM by sex, but the coefficient estimates were of similar magnitude. The model shows that there seems to be a slightly smaller increase in DNA-FTM for females over males with increasing ∑OC and reproductive stage.

Model 1 is to be considered the best model, following the principles of parsimony. The model predicted ln-transformed DNA-FTM values by ln ∑OC and reproductive stage. A significant regression coefficient (adjusted) of 0.455 was found ($F_{2, 36} = 16.89$, $p < 0.001$) for the entire model. The ∑OC coefficient (± SEM) was 0.171 (± 0.035) while tshe reproductive stage had a negative coefficient in the model of -0.083 (± 0.028). Both estimates were significant: ∑OC $p < 0.001$, gonad weight $p = 0.006$. The model shows a clear increase in DNA-FTM when ∑OC increases and the char is in the earlier reproductive stages. Late reproductive stages and low ∑OC concentrations are associated with lower DNA-FTM.

TABLE 5: Top candidate models to explain the level of DNA double-strand breaks, measured by the fraction of total DNA that migrated into the gel (DNA-FTM). Both DNA-FTM and ∑organochlorines (∑OCs) were ln-transformed. AICc: corrected Akaike's information criterion. Rs: reproductive stage, W_L: liver weight, HSI: hepatosomatic index. Annotation of *p*-value: '***' < 0.001, '**' < 0.01, '*' < 0.05, 'ns' not significant.

Model ID	ΔAICc	Adj. R^2	Resp. vars.	Estimate	Std.E	t-value	*p*	
1	0.00	0.455	(Intercept)	2.715	0.311	8.740	0.000	***
			∑OC	0.171	0.035	4.904	0.000	***
			Rs	-0.083	0.028	-2.953	0.006	**
2	1.24	0.459	(Intercept)	3.036	0.420	7.233	0.000	***
			∑OC	0.151	0.039	3.842	0.000	***
			Rs	-0.073	0.029	-2.482	0.018	*
			W_L	-0.023	0.020	-1.132	0.265	ns
3	1.50	0.456	(Intercept)	3.096	0.486	6.371	0.000	***
			∑OC	0.152	0.039	3.867	0.000	***
			Rs	-0.082	0.028	-2.914	0.006	**
			HSI	-0.253	0.248	-1.019	0.315	ns
4	1.74	0.452	(Intercept)	2.778	0.319	8.702	0.000	***
			∑OC	0.163	0.036	4.513	0.000	***
			Rs males	-0.096	0.032	-3.035	0.005	**
			Rs females	-0.073	0.030	-2.422	0.021	*

4. Discussion

4.1. Biological variation

Several differences were found in fish between the two lakes, presented in Table 1. The differences found mainly describe the differences in the fish sampled, not necessarily those of the lake population as a whole. But some will be highlighted.

4.1.1. Liver weight

The larger liver and higher HSI observed in low contaminated char from Lake Laksvatn compared to the high-exposed char from Ellasjøen contradicts other findings. Several studies have found that both chronic and transient exposure induces an increase in liver size (hepatomegaly): in flatfish from a PCB-contaminated site (*Glyptocephalus cynoglossus*, *Limanda ferruginea*, and *Purenonectes americanus*; Khan, 2003), and in rainbow trout (*Oncorhynchus mykiss*) exposed to wastewater effluent (Höger et al., 2006). Brown bullheads (*Ameiurus nebulosus*) from three contaminated sites in the lower Great Lakes (USA) also had an increased HSI compared to fish from reference sites (Arcand-Hoy and Metcalfe, 1999). Contrary, common carps (*Cyprinus carpio*) from some PCB and petroleum contaminated sites in Karakaya Dam Lake (Turkey) showed a decrease in relative liver size (Ozmen et al., 2006), and the same did rainbow trout (*O. mykiss*) when exposed to Cadmium for 30 days (Richard et al., 1998). Unfortunately, elements were not analyzed in the fish in this project. Another cause for the lower HSI can be that the liver can act as a lipid storage and that the Ellasjøen individuals were in a 'worse' condition, although this is not supported in a difference of the CF.

4.1.2. Growth

On a weight by year basis, the Laksvatn individuals were significantly heavier, as can be seen in Figure 4. Not that this was inferred from a differnce in range, the age of Ellasjøen individuals ranged from 9-19 whereas those fish of Laksvatn only were 9-12 years of age. The lower growth rate could be due to the high burden of OCs found in the Ellasjøen char. It is believed that there is a certain energetic cost to detoxification, and some studies have examined the relationship between contaminant exposure in fish and metabolism and energy allocation, but no clear picture emerges (Beyers et al., 1999; Nault et al., 2012; Smolders et al., 2003). But in an exposure experiment with Arctic char, it was found that a high dose of PCBs (Aroclor 1254) reduced the growth rate compared to a control (Jørgensen et al., 2004). The study administered PCBs orally, which resulted in a final liver PCB concentration of up to 42 $mg \times kg^{-1}$ ww, and a significantly lowered growth rate compared to a control. The average ∑PCB measured in muscle from char of Ellasjøen in the present study was six orders lower than that.

The Laksvatn char population is believed to be smaller than the one of Ellasjøen. In addition, there is a relative abundance of tadpole shrimp (*Lepidurus arcticus*) in Laksvatn, a nutritious prey of char (Klemetsen et al., 1985). The tadpole shrimp is overgrazed in Ellasjøen, and nearly absent. The increased resource competition of Ellasjøen is the probable cause of the higher growth rate of the Ellasjøen char.

4.1.3. Reproductive stage

The female char of Lake Laksvatn had a significantly higher gonad weight and GSI than those in Lake Ellasjøen (Table 1). The gonad weight inherently increases during sexual maturation, and given that the sampling period spanned about two weeks, the significant difference in gonad weight and GSI between the lakes will not be emphasized *per se*.

4.1.4. Muscle lipid content

There was a significant difference in the lipid content of muscle of the char between the lakes. Lipid content was somewhat higher in char from Laksvatn, indicative of a better nutritional status. But maybe more interesting than the averages, is the vast difference in variance of the lipid content. The Laksvatn individuals had a very narrow distribution, with an average of 0.5 ± 0.02 % lipid content (± SD), while Ellasjøen had an average of 0.41 ± 0.18. The lower lipid content with a greater variation in Lake Ellasjøen char could be due to the increased resource competition of Ellasjøen.

4.2. Organochlorines

4.2.1. Concentrations

There was a large difference in the level of contaminants in fish between the two lakes – a 43 times higher average concentration of ∑OCs in Lake Ellasjøen than Lake Laksvatn. The PCB levels are similar to those reported in earlier studies from both lakes when the number of congeners is taken into account. The average $\sum_6$PCB concentration of Ellasjøen in the current study was 121.5 ng × g^{-1} (ww), and previous studies have reported ∑PCB concentrations of 165.3, 695, and 686 ng × g^{-1} (ww; Bytingsvik et al. (2015), Evenset et al. (2004), and Skotvold et al. (1998), respectively). Lake Laksvatn char is also comparable between studies. In this study $\sum_6$PCB concentration of 2.7 ng × g^{-1} (ww) was found – similarly, a $\sum_9$PCB concentration of 3.48 ng × g^{-1} (ww) has been found previously (Bytingsvik et al., 2015). In a study by Evenset et al. (2004), a $\sum_7$PCB concentration of 49 ng × g^{-1} (ww) was found in Arctic char of Øyangen on Bjørnøya, earlier used as a reference lake.

Arctic char of Laksvatn is similar in summed PCB concentrations to char from lakes in East Canada as reported in Braune et al. (2005), ranging from 10.3 ng × g^{-1} (ww) to 30.8 ng × g^{-1} (ww), as well as a North Greenland location with char ∑PCB levels of 9 ng × g^{-1} (ww). The pesticide, t-NC, is reported in similar concentrations as in both lakes in land-locked Arctic char from South-West Greenland (Rigét et al., 2010).

4.2.2. Profiles

Prior studies of biota of Ellasjøen and Øyangen (a lake similar to Laksvatn), have revealed that PCB-congener 153, 138, and 180 are dominating in biota of both lakes (Evenset et al., 2007a), and the pattern is reflected in the levels of PCB153, -138 and -180 found in sediments of Ellajsøen (Evenset et al., 2007b). The same congener patterns were discovered in both lakes in the present study as well. Heavy metals have previously not been found in similarly high concentrations in Ellasjøen as OCs, evident of the biovector of biomagnified POPs (Evenset et al., 2007b).

4.3. DNA-damage

4.3.1. Level of DNA double-strand breaks

There was a clear and significantly higher level of DNA double-strand breaks in blood cells of Arctic char in Ellasjøen compared with Laksvatn. A difference in the levels of DSBs could be attributed to a host of factors, but the most prevailing difference between the lakes, from a genotoxicological viewpoint, is the large input of contaminants into Lake Ellasjøen. Several studies have shown that some of the same contaminants found at high levels in the fish can harm the integrity of DNA (González-Mille et al., 2010; Marabini et al., 2011; Winter et al., 2004). The mechanism of action is outlined in the introduction, but it may be noted that the effect of genotoxicants could either be direct (such as in association with the DNA molecule itself) or indirect (*e.g.* formation of ROS).

No difference in DNA-FTM was found between the sexes in Laksvatn, but in Ellasjøen, there was a significant difference between males and females. The males had higher levels of DBSs than the females. One factor that could explain this observed difference is that the female deposits OCs with the lipid-rich eggs (Bytingsvik et al., 2015). For anadromous char, the lipid content of the gonads can account for up to 25 % of the total lipid content in females, whereas less than 3 % in males (Jørgensen et al., 1997b). The toxicokinetics was investigated in land-locked char from Ellasjøen, where this additional route of elimination was found to be substantial (Bytingsvik et al., 2015). This process could lead to a lower body burden of OCs in females, subsequently leading to lower levels of DNA damage.

The median DNA fragment length of DNA that migrated into the gel showed a clear difference, albeit of a lesser significance than of DNA-FTM, between the lakes. The same factors that govern the level of DNA double-strand break will cause a decrease in the MML. There was not seen a significant difference in the MML between the sexes within the lakes, and the pooled population samples had a smaller difference between the lakes, compared to the DNA-FTM measurements. This could indicate that the MML is a less sensitive measure for DSBs than DNA-FTM.

4.3.2. Organochlorines

There was a positive association between the OCs and DNA-FTM. The pattern was present in the PCA, and made clear in the regression models: the higher the levels of OCs, the higher the levels of DNA DSBs. The causality of the association is not given from the models, but it is presumed that an increase in the concentration of OCs is causing the observed increase in DNA damage.

In the linear models, the individual OCs were summed together, and could thus mask effects of singular compounds. From the PCA, it can be seen that there is a correlation between the PCB-congeners. However, t-NC differs somewhat, as can be seen from the partly deviation from PC1. This is probably attributed to the structural-chemical difference of t-NC from the PCBs.

4.3.3. Reproductive stage

A clear relationship was also found between levels of DSBs and reproductive stage (see Table 2 and 5). The fish in the later stages of the reproductive cycle had lower levels of DSBs. The finding is hard to explain from existing literature.

During the later stages of the reproductive cycle, lipids are transferred from (mainly) muscle and to the gonads (Jørgensen et al., 1997). But reproduction has a considerable cost, and it is such an energetic constraints that they will not reproduce every year (Dutil, 1986). Char in reproductive stage 1 to 3 in September do not spawn that particular year, but so do those who has reached stage 4 to 6 in September (Sømme, 1941). That means that those individuals in the later stages would be remobilising lipids from their reserves into the development of the gonads the current season. With the systemic transfer of lipids from storage compartments (*e.g.* muscle) to the gonads, it is assumed that OCs could remobilise associated with the transferring lipids (Debier et al., 2006; Fenstad et al., 2014). This was the expected scenario, and that this possible increase in systemic OC concentrations could lead to elevated levels of DNA damage. However, in this study, this was not the case, as the outcome was that the immature char had the highest levels of DNA-damage.

One explanation for the higher level of DNA-damage in immature char versus mature char could be the differences in physiological processes associated with lipid dynamics in mature char the last months before sampling. The sampling time of the current study was close to, or at the point of, spawning for the char of Bjørnøya. This means that during the late summer, the fish that would go on to spawn had gone through a reproductive maturation process. This may have led to a physiological situation where the blood lipid levels are depressed, which, in turn, could lead to lower levels of OCs, and subsequently lower the levels of DNA damage.

A second explanation could be linked to the need for some genotoxicants to be metabolised before they exert their toxic effects. The metabolic transformation of contaminants has a certain energetic cost (Bains and Kennedy, 2004), and it could be that a reduced nutritional state could reduce the biotransformation of OCs into the ultimate genotoxicants. More probable is that this is due to a lowered metabolisation of OCs into these ultimate genotoxicants. The different PCBs can be metabolised to different degrees, such as by cytochrome P450 1A (Grimm et al., 2015). Some of these metabolites are known to induce ROS and DNA-damage (Dong et al., 2015; Song et al., 2015). Mature Atlantic salmon (*Salmo salar*) was found to have a lower CYP1A activity (EROD) than immature *S. salar* (Goksøyr and Larsen, 1991). Similarly low EROD activity has been measured in mature char from Bjørnøya (Akvaplan-niva, unpublished data). This could indicate that there is a lowered xenometabolism of mature char, which subsequently lowers the levels of genotoxic compounds.

4.4. Potential pollutant-induced genotoxicity

4.4.1. Oxidative damage

The observed increased level of DNA damage in the blood cells could be the consequence of the OCs interacting with the cellular environment and its molecular constituents. OCs, such as PCBs, is believed to be able to cause oxidative damage in the cell (Schlezinger et al., 2006; Theodorakis, 2008). This oxidative damage could be ROS directly interacting with the DNA, or through a cascade of cellular effects, and inflict lesions (illustrated in Fig. 1). Effects such as oxidative stress are believed to be parts of the mechanisms that induce DNA damage. By overwhelming the defense and repair systems, permanent DNA damage can occur.

4.4.2. Other factors

As seen in Figure 4, Ellasjøen individuals exhibited a slower growth rate, and the lipid content of the muscle had a greater variation. Together with the different ecological factors, it may be indications of a higher degree of resource competition within Lake Ellasjøen. This might subsequently have a suite of effects. One effect could be a low nutritional status, which could affect allocation from the energy budget to functions such as self-maintenance, of which DNA repair is part of. Although this is not confirmed in a difference in CF of the char between the lakes. Other effects of a low nutritional status could be an increase in endogenous ROS formation, as during an increased metabolism or reduced antioxidant capability. These factors can likely impact the level of DNA damage in themselves, and in combination with contaminants.

The levels of DNA damage was measured in blood cells, primarily erythrocytes. Albeit a lack of metabolic capacity, it is believed that damage to these cells can be reflective of the rest of the organism (Mitchelmore and Chipman, 1998). But it is noteworthy that a study has found a difference in the senescence of the blood cells of char during the course of a year (Hofer et al., 2000). DNA damage could be influenced by this differential aging of the cell. But nonetheless, the level of DNA damage in blood cells could be indicative of DNA damage in other tissues, which in turn could lead to the formation of lesions, which is an effect associated with PCBs (Ben Ameur et al., 2012; Simon and Burskey, 2016). These lesions when formed in the proper tissues, can inflict neurological, endocrine, and reproductive effects. These effects can, in turn, reduce the fitness of the individuals and cause population-wide effects. The worst case scenario would be the collapse of the population.

4.5. Further prospects

Little can be inferred from this study on the health of the populations in itself, but the results will fit into the bigger context of studies performed on the same populations. Subparts of the overarching project of the current study are concerned with the impact of the complex mixture of POPs on reproduction, immunity, and stress response in Arctic char.

According to Bickham (2011), one outcome of a chronic exposure of contaminants to a population could be a selection for resistance associated alleles. This could cause a loss of genetic variation as a whole in the population, a phenomenon that has been called “the genetic erosion” hypothesis (van Straalen and Timmermans, 2002). Comparing the Ellasjøen population to other char populated lakes on the island would be very interesting in that respect. The two populations are both landlocked, and probably were landlocked at the same time, evolutionary speaking, during the retreat of the last ice cap. Additionally, contaminants only start to show up in the sediments during the 1940s (Evenset et al., 2007b). This could prove a very interesting site to study in this regard, to see if there have been any changes in the genetic make-up between the lakes. Despite a significant difference in DNA damage between the lakes, and high levels of OCs in Ellasjøen, Arctic char still persevere. This could provide some insight to possible adaptations to the exposure, and evidence for the genetic erosion hypothesis, or even indications of an evolutionary adaptation.

5.Conclusion

Arctic char from Lake Ellasjøen was found to have high levels of OCs, and PCBs in particular. The OCs measured in the muscle of the char of Ellasjøen was an order higher than those of Laksvatn. The analysis of the frequency of DNA DSBs showed a parallel pattern: blood from char of Ellasjøen had a much higher frequency of breaks than their conspecifics of Laksvatn. There are indications that the DNA damage was caused by the OCs. Still, there seem to be indications of other underlying differences between the two populations, as for instance by the lower growth rate found in the Ellasjøen char. To which degree this could influence the level of DNA damage, alone or in combination with contaminants, is not known. This thesis contributes with new information about potential genotoxic responses in Arctic char of Ellasjøen, Svalbard.

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7.Appendices

A. List of chemicals used in DNA-DSB analysis

TE-buffer (500 ml)

Tris 0.6057 g (10 mM)	*Sigma*
EDTA 1 ml 0.5-M (1 mM)	*Sigma*

pH = 8

Lambda-ladder (200 µl)

100 ul λ-HindII-digest (50 µg)
37 ul λ-DNA

Digestion buffer (200 ml)

NaCl 1.1688 g (100 mM)	*Sigma*
Tris 0.2423 (10 mM)	*Sigma*
0.5 M-EDTA 10 ml (25 mM)	*Sigma*
10 %-SDS 10 ml (0.5 %)	*Bio-Rad*
proteinase K (1 mg per ml)	*Sigma-Aldrich*

pH = 8

TBE/running buffer (1000 ml)

Trisbase 54 g (45 mM)	*Sigma*
H_3BO_3 27.5 g	*Sigma*
EDTA 20 ml 0.5-M (1 mM)	*Sigma*

pH = 8

LMA (1 %, in TE)

20 ml TE	
200 mg low melting point agaorose	*Sigma*

B. Biological variables and DNA damage

TABLE A: Biological variables of Arctic char (*Salvelinus alpinus*) measured from lake Laksvatn and lake Ellasjøen, Bjørnøya, Svalbard. GSI: gonadosomatic index, CF: condition factor, HSI: hepatosomatic index, FTM: DNA-fraction of total DNA, that migrated into the gel, MML: median molecular length (kbp) of DNA fragments in the gel , %Lip: percentage of lipids in muscle, Rs: reproductive stage.

ID	Lake	Length (cm)	Weight (g)	Age	Rs	Gonad w. (g)	Liver w. (g)	GSI	CF	HSI	FTM	MML	%Lip
1	L	50.2	1252.0	9	1	1.52	11.06	0.12	0.99	0.88	28.54	340.17	0.50
2	L	49.4	1052.4	12	6	NA	14.01	13.25	0.87	1.33	20.76	361.37	0.52
3	L	48.0	1155.9	12	6	NA	7.67	NA	1.05	0.66	43.27	245.18	0.54
4	L	50.8	1050.0	10	6	NA	8.55	NA	0.80	0.81	31.43	323.16	0.53
5	L	52.5	1202.4	11	6	25.30	10.43	2.10	0.83	0.87	13.80	247.89	0.51
6	L	51.2	1433.9	12	6	NA	11.20	NA	1.07	0.78	28.41	320.91	0.51
7	L	43.5	845.4	9	1	0.68	8.66	0.08	1.03	1.02	30.02	327.07	0.49
8	L	56.1	1213.3	10	6	22.11	11.50	1.82	0.69	0.95	19.50	324.12	0.56
10	L	47.0	962.4	12	2, 7	13.80	13.04	1.43	0.93	1.35	35.71	339.96	0.50
11	L	45.0	963.0	10	6	26.18	13.18	15.99	1.06	1.37	21.84	294.17	0.49
13	L	48.7	1181.1	10	6	NA	9.09	NA	1.02	0.77	37.32	268.56	0.50
14	L	51.6	1279.5	12	6	NA	10.99	NA	0.93	0.86	27.47	328.88	0.52
15	L	52.7	1239.3	11	6	NA	9.53	NA	0.85	0.77	43.26	316.82	0.51
17	L	49.1	994.6	12	1	1.12	10.60	0.11	0.84	1.07	31.49	325.34	0.49
18	L	50.5	966.5	9	2, 7	10.14	11.53	1.05	0.75	1.19	31.35	293.11	0.51
19	L	46.6	1020.0	10	6	0.85	11.15	0.08	1.01	1.09	52.56	351.81	0.53
20	L	48.5	1127.4	11	2, 7	14.10	15.00	1.25	0.99	1.33	38.76	301.51	0.52
23	L	46.8	1097.7	9	7	20.45	11.27	18.23	1.07	1.03	25.49	288.71	0.51
24	L	46.5	980.8	9	6	37.80	12.90	15.48	0.98	1.32	26.28	341.09	0.51
25	L	45.0	956.2	9	6	24.35	7.94	18.41	1.05	0.83	16.95	235.45	0.52
26	L	45.6	973.7	9	6	27.63	16.60	16.36	1.03	1.70	44.11	334.73	0.53
36	E	41.9	635.5	11	2	0.42	5.08	0.07	0.86	0.80	78.47	316.23	0.51
38	E	39.6	540.0	11	3, 7	6.58	5.29	1.22	0.87	0.98	47.62	299.97	0.42
39	E	42.3	663.8	13	2	0.75	3.77	0.11	0.88	0.57	56.62	281.69	0.45
40	E	42.0	651.4	11	2	0.56	4.75	0.09	0.88	0.73	76.31	290.66	0.30
41	E	37.6	486.2	13	2, 7	11.84	4.24	2.44	0.91	0.87	67.36	350.45	0.35
42	E	43.4	749.6	13	1	0.65	5.45	0.09	0.92	0.73	88.92	231.50	0.52
43	E	41.9	643.8	12	2	0.75	4.56	0.12	0.88	0.71	92.39	77.92	0.18
44	E	39.5	562.1	9	2, 7	7.53	5.31	1.34	0.91	0.94	67.34	303.33	0.32
45	E	42.0	607.9	12	2	0.72	4.82	0.12	0.82	0.79	66.55	303.59	0.27
66	E	39.4	637.3	10	6	19.42	6.91	11.75	1.04	1.08	59.34	286.47	0.63
72	E	38.3	543.6	11	6	10.64	5.69	14.18	0.97	1.05	21.20	282.00	0.26
73	E	62.4	2372.6	19	6	NA	12.72	NA	0.98	0.54	58.88	270.21	0.29
74	E	55.1	1405.2	17	6	NA	9.72	NA	0.84	0.69	57.58	268.90	0.40
75	E	50.8	1346.3	15	6	NA	9.99	NA	1.03	0.74	70.63	213.29	0.48
76	E	48.3	1065.8	17	6	NA	6.82	NA	0.95	0.64	89.62	167.90	0.38
77	E	40.9	618.7	12	6	NA	3.52	NA	0.90	0.57	35.23	298.87	0.39
99	E	40.5	588.4	12	6	18.32	5.85	10.07	0.89	0.99	51.34	304.37	0.97
100	E	36.2	436.3	11	6	20.68	6.43	11.92	0.92	1.47	28.10	258.90	0.25

C. Organochlorine concentrations

TABLE C.1: Individual molar concentrations of organochlorines in muscle of Arctic char (*Salvelinus alpinus*) in pmol × g^{-1}. Average (Avg) and standard deviation (SD) is given. t-NC: *trans*-nonachlor.

ID	PCB101	PCB105	PCB118	PCB138	PCB153	PCB180	∑PCB	t-NC
Laksvatn								
1	31.2	15.9	48.7	162.7	236.9	71.6	567.1	14.0
2	22.1	12.3	45.6	147.7	235.5	75.4	538.6	15.5
3	22.1	14.1	42.0	115.6	173.2	49.8	416.7	12.2
4	35.5	14.7	50.9	180.4	253.5	75.4	610.4	15.5
5	67.1	43.2	152.3	550.6	856.0	271.2	1940.3	31.3
6	43.5	34.0	113.0	325.6	485.2	132.5	1133.9	28.4
7	16.2	10.4	32.8	91.4	144.1	44.3	339.2	14.9
8	30.0	21.4	80.9	440.3	730.2	283.6	1586.4	9.9
10	59.1	26.3	92.5	315.9	447.0	151.3	1092.1	32.4
11	40.7	27.0	87.9	261.3	395.7	108.5	921.1	21.6
13	49.6	21.4	73.5	233.6	375.8	111.6	865.5	23.4
14	39.5	19.9	63.4	218.1	306.5	84.7	732.1	15.8
15	40.1	19.9	67.1	228.1	317.3	102.7	775.2	21.2
17	27.9	8.6	46.6	172.9	256.9	77.9	590.7	10.6
18	20.8	16.8	75.4	245.0	407.3	127.2	892.6	12.8
19	27.0	22.7	53.0	195.9	272.9	78.7	650.2	14.6
20	55.8	29.7	95.3	276.0	413.7	128.2	998.7	37.4
23	11.0	4.0	20.2	57.1	98.9	29.3	220.6	7.2
24	18.1	9.5	34.3	100.3	163.5	40.2	365.9	11.3
25	23.3	11.9	42.3	116.9	173.2	57.7	425.3	14.6
26	16.8	7.4	33.1	100.3	149.6	55.4	362.6	12.4
Avg	33.2	18.6	64.3	216.0	328.2	102.7	763.1	17.9
SD	15.0	9.4	30.8	117.5	185.7	65.3	414.0	8.0
Ellasjøen								
36	111.8	226.7	934.0	2885.2	4795.2	793.8	9746.8	27.9
38	54.8	122.8	640.3	1720.8	2318.2	429.5	5286.5	19.4
39	111.8	255.2	1101.6	2865.5	4903.0	784.4	10021.6	29.0
40	65.3	146.1	613.3	1537.9	2775.5	478.3	5616.4	21.4
41	129.3	279.4	1364.8	3299.2	5343.1	940.2	11355.9	29.3
42	68.0	135.7	632.9	1464.5	2417.4	422.2	5140.7	23.4
43	57.9	121.0	490.1	1403.0	2137.6	338.5	4548.0	16.0
44	81.5	188.7	795.6	2367.0	3651.6	615.7	7700.1	18.0
45	121.9	293.5	1208.2	4083.6	6531.8	1126.9	13366.0	26.3
66	53.3	110.0	456.1	1311.5	2035.6	332.4	4298.9	15.8
72	37.7	88.2	309.1	952.7	1370.5	211.0	2969.2	11.7
73	880.7	2257.1	9657.7	39804.0	70585.6	12138.2	135323.4	219.0
74	1472.6	4237.0	19539.4	76625.3	156362.7	29871.5	288108.5	326.6
75	426.4	1018.6	4402.4	14198.1	25421.6	4395.6	49862.8	122.5
76	160.5	377.1	1626.4	5313.2	9197.0	1658.4	18332.5	44.1
77	80.9	184.1	850.7	2389.2	3874.4	710.1	8089.4	35.1
99	156.2	390.0	1666.8	4793.0	8327.2	1471.2	16804.4	40.1
100	97.7	232.5	921.5	2762.4	4878.1	784.7	9676.9	30.6
Avg	231.6	592.4	2622.8	9432.0	17607.0	3194.6	33680.4	58.7
SD	358.2	1015.3	4627.1	18532.8	37157.0	7015.6	68661.9	81.3

TABLE C.2: Individual lipid normalized concentrations of organochlorines in muscle of Arctic char (*Salvelinus alpinus*) in ng × g^{-1}. Average (Avg) and standard deviation (SD) is given. t-NC: *trans*-nonachlor.

ID	PCB101	PCB105	PCB118	PCB138	PCB153	PCB180	∑PCB	t-NC
Laksvatn								
1	20.0	10.2	31.2	115.1	167.6	55.5	399.6	12.2
2	14.7	8.2	30.4	108.8	173.5	60.8	396.3	14.1
3	13.6	8.7	25.8	78.7	117.9	37.2	281.9	10.2
4	22.7	9.4	32.5	127.6	179.4	58.4	430.2	13.5
5	40.6	26.1	92.0	368.0	572.0	198.5	1297.2	25.7
6	27.3	21.3	71.0	226.0	336.7	100.8	783.1	24.2
7	10.2	6.5	20.6	63.5	100.0	33.7	234.4	12.7
8	19.6	14.0	52.8	317.8	527.0	224.2	1155.4	8.8
10	39.4	17.6	61.6	232.7	329.2	122.0	802.4	29.4
11	25.1	16.6	54.2	177.9	269.4	80.9	624.2	18.1
13	30.6	13.2	45.3	159.1	255.8	83.2	587.2	19.6
14	24.8	12.5	39.8	151.3	212.7	64.4	505.6	13.5
15	25.7	12.7	42.9	161.4	224.5	79.6	546.9	18.4
17	18.2	5.6	30.4	124.8	185.4	61.6	426.0	9.4
18	13.3	10.8	48.2	173.3	288.2	98.6	632.5	11.2
19	18.0	15.1	35.3	144.3	201.0	63.5	477.1	13.3
20	35.0	18.7	59.8	191.5	287.1	97.5	689.6	31.9
23	7.2	2.6	13.2	41.2	71.4	23.2	158.8	6.4
24	11.6	6.1	22.0	71.0	115.7	31.2	257.5	9.8
25	13.6	7.0	24.6	75.4	111.6	40.7	272.9	11.6
26	10.8	4.7	21.2	71.0	105.9	42.9	256.5	10.8
Avg	21.0	11.8	40.7	151.4	230.1	79.0	534.1	15.5
SD	9.3	5.8	18.9	81.1	128.2	50.0	286.8	6.9
Ellasjøen								
36	140.4	284.6	1172.7	4004.6	6655.8	1206.9	13465.0	47.7
38	28.4	63.7	331.7	985.7	1327.9	269.5	3007.0	13.7
39	202.8	462.8	1997.8	5745.0	9830.0	1722.8	19961.1	71.7
40	41.0	91.7	385.0	1067.3	1926.2	363.7	3874.8	18.3
41	100.5	217.1	1060.7	2834.8	4591.0	885.0	9689.0	31.0
42	74.0	147.7	688.7	1761.7	2908.0	556.3	6136.3	34.7
43	59.1	123.4	500.0	1582.2	2410.6	418.1	5093.4	22.2
44	68.2	157.9	665.9	2190.3	3379.0	624.1	7085.4	20.5
45	159.2	383.2	1577.6	5894.8	9428.8	1782.0	19225.6	46.8
66	34.1	70.4	292.0	928.0	1440.4	257.6	3022.5	13.7
72	27.3	64.0	224.2	764.0	1099.1	185.3	2364.0	11.6
73	821.4	2105.1	9007.4	41041.1	72779.4	13710.0	139464.6	278.0
74	1780.4	5122.6	23623.3	102416.3	208992.2	43736.7	385671.5	537.4
75	143.5	342.8	1481.5	5282.3	9457.8	1791.4	18499.4	56.1
76	137.9	323.9	1397.1	5045.8	8734.2	1725.3	17364.2	51.6
77	55.0	125.2	578.5	1796.3	2912.9	584.8	6052.7	32.5
99	175.9	439.0	1876.2	5964.5	10362.4	2005.5	20823.4	61.4
100	79.8	189.8	752.0	2492.3	4401.0	775.5	8690.3	34.0
Avg	229.4	595.3	2645.1	10655.4	20146.5	4033.4	38305.0	76.8
SD	414.4	1186.4	5438.4	23960.6	48451.0	10079.5	89494.7	125.9

TABLE C.3: Individual wet weight concentrations of organochlorines in muscle of Arctic char (*Salvelinus alpinus*) ng × g^{-1}. Average (Avg) and standard deviation (SD) is given. t-NC: *trans*-nonachlor.

ID	PCB101	PCB105	PCB118	PCB138	PCB153	PCB180	∑PCB	t-NC
Laksvatn								
1	0.102	0.052	0.159	0.587	0.855	0.283	2.000	0.062
2	0.072	0.040	0.149	0.533	0.850	0.298	1.942	0.069
3	0.072	0.046	0.137	0.417	0.625	0.197	1.494	0.054
4	0.116	0.048	0.166	0.651	0.915	0.298	2.194	0.069
5	0.219	0.141	0.497	1.987	3.089	1.072	7.005	0.139
6	0.142	0.111	0.369	1.175	1.751	0.524	4.072	0.126
7	0.053	0.034	0.107	0.330	0.520	0.175	1.219	0.066
8	0.098	0.070	0.264	1.589	2.635	1.121	5.777	0.044
10	0.193	0.086	0.302	1.140	1.613	0.598	3.932	0.144
11	0.133	0.088	0.287	0.943	1.428	0.429	3.308	0.096
13	0.162	0.070	0.240	0.843	1.356	0.441	3.112	0.104
14	0.129	0.065	0.207	0.787	1.106	0.335	2.629	0.070
15	0.131	0.065	0.219	0.823	1.145	0.406	2.789	0.094
17	0.091	0.028	0.152	0.624	0.927	0.308	2.130	0.047
18	0.068	0.055	0.246	0.884	1.470	0.503	3.226	0.057
19	0.088	0.074	0.173	0.707	0.985	0.311	2.338	0.065
20	0.182	0.097	0.311	0.996	1.493	0.507	3.586	0.166
23	0.036	0.013	0.066	0.206	0.357	0.116	0.794	0.032
24	0.059	0.031	0.112	0.362	0.590	0.159	1.313	0.050
25	0.076	0.039	0.138	0.422	0.625	0.228	1.528	0.065
26	0.055	0.024	0.108	0.362	0.540	0.219	1.308	0.055
Avg	0.108	0.061	0.210	0.779	1.185	0.406	2.749	0.080
SD	0.049	0.031	0.101	0.424	0.670	0.258	1.499	0.036
Ellasjøen								
36	0.365	0.740	3.049	10.412	17.305	3.138	35.009	0.124
38	0.179	0.401	2.090	6.210	8.366	1.698	18.944	0.086
39	0.365	0.833	3.596	10.341	17.694	3.101	35.930	0.129
40	0.213	0.477	2.002	5.550	10.016	1.891	20.149	0.095
41	0.422	0.912	4.455	11.906	19.282	3.717	40.694	0.130
42	0.222	0.443	2.066	5.285	8.724	1.669	18.409	0.104
43	0.189	0.395	1.600	5.063	7.714	1.338	16.299	0.071
44	0.266	0.616	2.597	8.542	13.178	2.434	27.633	0.080
45	0.398	0.958	3.944	14.737	23.572	4.455	48.064	0.117
66	0.174	0.359	1.489	4.733	7.346	1.314	15.415	0.070
72	0.123	0.288	1.009	3.438	4.946	0.834	10.638	0.052
73	2.875	7.368	31.526	143.644	254.728	47.985	488.126	0.973
74	4.807	13.831	63.783	276.524	564.279	118.089	1041.313	1.451
75	1.392	3.325	14.371	51.238	91.741	17.377	179.444	0.544
76	0.524	1.231	5.309	19.174	33.190	6.556	65.984	0.196
77	0.264	0.601	2.777	8.622	13.982	2.807	29.053	0.156
99	0.510	1.273	5.441	17.297	30.051	5.816	60.388	0.178
100	0.319	0.759	3.008	9.969	17.604	3.102	34.761	0.136
Avg	0.756	1.934	8.562	34.038	63.540	12.629	121.459	0.261
SD	1.169	3.314	15.104	66.881	134.092	27.734	248.135	0.361

D. Organochlorine profile

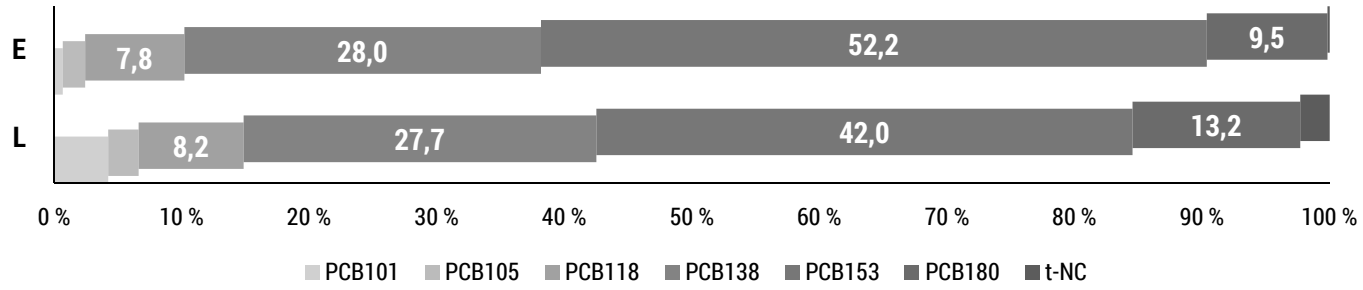

FIGURE D: The relative share of organochlorines of total load in muscle of Arctic char (*Salvelinus alpinus*), by the average of Lake Ellasjøen (E) and Lake Laksvatn (L), Bjørnøya, Norway (2014). Those compounds contributing >5 % is labelled by their percentage score. t-NC: *trans*-nonachlor.

E. Principal component analysis

TABLE E.1: Accountance of PC variation.

Dimensions	1	2	3	4	5	6	7	8	9	10	11	12	13
Variance	8.119	1.521	0.961	0.789	0.744	0.423	0.301	0.109	0.015	0.012	0.003	0.001	0.001
% of variance	62.45	11.70	7.394	6.069	5.726	3.256	2.317	0.840	0.118	0.094	0.022	0.005	0.004
Cumulative % of variance	62.5	74.2	81.6	87.6	93.3	96.6	98.9	99.8	99.9	100.0	100.0	100.0	100.0

TABLE E.2: Eigenvalues of individuals from dimensions 1-5. Provided is: individuals' distance from center of gravity, contribution to the dimensional construction, and quality of projection (cosine^2).

ID	Lake	Dist	Dim. 1	Ctr.	Cos^2	Dim. 2	Ctr.	Cos^2	Dim. 3	Ctr.	Cos^2	Dim. 4	Ctr.	Cos^2	Dim. 5	Ctr.	Cos^2
1	L	3.096	-2.413	1.839	0.607	-1.014	1.733	0.107	0.497	0.659	0.026	-0.493	0.790	0.025	0.345	0.409	0.012
2	L	3.158	-2.546	2.048	0.650	0.686	0.794	0.047	-1.024	2.798	0.105	0.732	1.742	0.054	-0.229	0.180	0.005
3	L	3.272	-2.289	1.654	0.489	0.831	1.163	0.064	1.292	4.450	0.156	-1.487	7.191	0.207	-0.820	2.318	0.063
4	L	2.837	-1.996	1.258	0.495	-0.030	0.002	0.000	-1.312	4.591	0.214	-0.661	1.418	0.054	-1.207	5.019	0.181
5	L	2.110	-0.419	0.056	0.040	0.754	0.958	0.128	-1.502	6.018	0.507	0.123	0.049	0.003	-1.000	3.446	0.225
6	L	2.273	-0.946	0.283	0.173	1.496	3.773	0.433	1.045	2.911	0.211	-0.528	0.906	0.054	-0.460	0.729	0.041
7	L	3.768	-3.205	3.243	0.723	-0.910	1.396	0.058	0.937	2.345	0.062	-0.237	0.182	0.004	0.624	1.342	0.027
8	L	3.513	-1.538	0.747	0.192	-0.314	0.166	0.008	-2.804	20.975	0.637	-0.119	0.046	0.001	-1.185	4.835	0.114
10	L	2.308	-1.040	0.342	0.203	-0.284	0.136	0.015	-0.289	0.223	0.016	0.753	1.845	0.107	1.161	4.647	0.253
11	L	2.906	-2.023	1.292	0.485	1.467	3.629	0.255	0.540	0.777	0.034	1.164	4.402	0.160	0.694	1.657	0.057
13	L	2.153	-1.420	0.637	0.435	0.915	1.411	0.181	0.742	1.469	0.119	-0.628	1.282	0.085	-0.506	0.881	0.055
14	L	2.158	-1.667	0.877	0.596	0.745	0.935	0.119	-0.186	0.092	0.007	-0.483	0.759	0.050	-0.875	2.639	0.164
15	L	2.116	-1.295	0.530	0.375	0.133	0.030	0.004	-0.628	1.051	0.088	-0.800	2.080	0.143	-1.030	3.652	0.237
17	L	3.208	-2.236	1.579	0.486	-1.552	4.058	0.234	-0.744	1.478	0.054	-0.129	0.054	0.002	0.013	0.001	0.000
18	L	3.367	-2.173	1.492	0.417	-1.671	4.707	0.246	-1.878	9.405	0.311	0.352	0.402	0.011	0.219	0.165	0.004
19	L	2.516	-2.126	1.428	0.714	0.605	0.617	0.058	0.690	1.269	0.075	-0.291	0.275	0.013	0.350	0.421	0.019
20	L	2.418	-1.203	0.457	0.248	-0.060	0.006	0.001	0.215	0.123	0.008	0.585	1.114	0.059	1.504	7.791	0.387
23	L	4.695	-4.344	5.960	0.856	1.086	1.986	0.053	1.083	3.127	0.053	-0.225	0.164	0.002	-0.550	1.043	0.014
24	L	3.698	-3.544	3.966	0.918	0.679	0.777	0.034	0.051	0.007	0.000	0.648	1.364	0.031	0.210	0.152	0.003
25	L	3.456	-2.961	2.769	0.734	1.096	2.024	0.101	0.727	1.411	0.044	-0.446	0.646	0.017	-0.642	1.418	0.034
26	L	4.455	-3.680	4.277	0.682	0.847	1.210	0.036	0.449	0.537	0.010	1.250	5.082	0.079	1.516	7.917	0.116
36	E	2.870	2.072	1.356	0.521	-1.406	3.333	0.240	-0.367	0.358	0.016	-0.810	2.134	0.080	0.905	2.821	0.099
38	E	1.461	0.714	0.161	0.239	-0.965	1.571	0.437	-0.444	0.525	0.092	0.386	0.485	0.070	0.170	0.099	0.013
39	E	2.852	2.375	1.781	0.693	-1.080	1.967	0.144	-0.118	0.037	0.002	-0.851	2.352	0.089	-0.191	0.125	0.004
40	E	2.677	1.470	0.683	0.302	-2.059	7.145	0.591	0.619	1.022	0.053	-0.099	0.032	0.001	-0.223	0.171	0.007
41	E	2.917	2.542	2.040	0.759	-1.258	2.665	0.186	0.416	0.462	0.020	0.262	0.223	0.008	0.347	0.414	0.014
42	E	3.004	1.605	0.814	0.286	-1.632	4.491	0.295	0.569	0.864	0.036	-1.463	6.959	0.237	0.988	3.360	0.108
43	E	3.590	1.509	0.719	0.177	-2.693	12.219	0.563	1.353	4.881	0.142	0.080	0.021	0.000	-0.690	1.642	0.037
44	E	2.680	1.234	0.481	0.212	-1.828	5.632	0.465	0.424	0.480	0.025	0.591	1.136	0.049	0.489	0.823	0.033
45	E	3.399	2.660	2.235	0.613	-1.988	6.661	0.342	-0.142	0.054	0.002	0.524	0.892	0.024	-0.366	0.461	0.012
66	E	2.329	-0.050	0.001	0.000	1.050	1.859	0.203	0.412	0.452	0.031	-0.498	0.806	0.046	1.353	6.309	0.338
72	E	2.402	-0.498	0.078	0.043	0.221	0.082	0.008	0.560	0.835	0.054	1.625	8.580	0.457	-1.115	4.285	0.216
73	E	7.631	7.262	16.653	0.906	1.622	4.436	0.045	0.832	1.846	0.012	0.463	0.696	0.004	-0.830	2.374	0.012
74	E	8.267	7.992	20.170	0.935	1.476	3.669	0.032	-1.107	3.268	0.018	0.641	1.337	0.006	-0.137	0.065	0.000
75	E	5.454	5.079	8.147	0.867	1.658	4.635	0.092	0.789	1.661	0.021	-0.245	0.195	0.002	0.655	1.476	0.014
76	E	4.549	4.016	5.093	0.779	0.372	0.233	0.007	1.069	3.050	0.055	-0.678	1.493	0.022	-0.496	0.848	0.012
77	E	2.440	1.764	0.983	0.523	0.450	0.341	0.034	-0.185	0.092	0.006	-0.118	0.045	0.002	-1.366	6.430	0.313
99	E	4.849	2.117	1.415	0.191	2.057	7.133	0.180	-2.307	14.198	0.226	-1.890	11.611	0.152	2.260	17.587	0.217
100	E	3.345	1.204	0.458	0.130	0.498	0.418	0.022	-0.273	0.199	0.007	2.998	29.209	0.803	0.118	0.048	0.001

TABLE E.3: Individual wet weight concentrations of organochlorines in muscle of Arctic char (*Salvelinus alpinus*). Average (Avg) and standard deviation (SD) is given. t-NC: *trans*-nonachlor.

Variables	Dim. 1	Ctr.	Cos^2	Dim. 2	Ctr.	Cos^2	Dim. 3	Ctr.	Cos^2	Dim. 4	Ctr.	Cos^2	Dim. 5	Ctr.	Cos^2
PCB138	0.985	11.951	0.970	0.004	0.001	0.000	-0.064	0.426	0.004	0.068	0.579	0.005	0.053	0.379	0.003
PCB153	0.984	11.938	0.969	0.010	0.007	0.000	-0.067	0.466	0.004	0.067	0.573	0.005	0.055	0.407	0.003
PCB180	0.983	11.900	0.966	0.053	0.184	0.003	-0.120	1.490	0.014	0.066	0.560	0.004	0.045	0.275	0.002
PCB105	0.982	11.870	0.964	0.011	0.008	0.000	-0.002	0.001	0.000	0.051	0.333	0.003	0.075	0.763	0.006
PCB118	0.981	11.843	0.961	-0.015	0.014	0.000	-0.019	0.037	0.000	0.056	0.395	0.003	0.082	0.914	0.007
PCB101	0.970	11.594	0.941	0.141	1.313	0.020	-0.055	0.310	0.003	0.031	0.122	0.001	0.053	0.383	0.003
t-NC	0.868	9.271	0.753	0.317	6.598	0.100	-0.016	0.026	0.000	0.057	0.414	0.003	0.080	0.854	0.006
Age	0.835	8.595	0.698	0.202	2.691	0.041	0.083	0.723	0.007	-0.076	0.727	0.006	-0.141	2.665	0.020
DNA-FTM	0.651	5.212	0.423	-0.407	10.905	0.166	0.320	10.624	0.102	-0.280	9.943	0.078	0.249	8.329	0.062
HSI	-0.547	3.684	0.299	0.122	0.983	0.015	-0.158	2.590	0.025	0.606	46.535	0.367	0.510	34.945	0.260
%lipids	-0.348	1.494	0.121	0.444	12.944	0.197	-0.411	17.596	0.169	-0.557	39.258	0.310	0.448	26.982	0.201
CF	-0.223	0.611	0.050	0.488	15.638	0.238	0.792	65.336	0.628	-0.016	0.034	0.000	0.246	8.160	0.061
Rs	-0.056	0.038	0.003	0.861	48.716	0.741	-0.060	0.376	0.004	0.064	0.526	0.004	-0.334	14.942	0.111

TABLE E.4: Eigenvalues of supplementary continous (top) and categorical (bottom) variables of the PCA, with distance to center of gravity and quality of projections ($cosine^2$)

Variables	Dist.	Dim.1	Cos^2	Dim.2	Cos^2	Dim.3	Cos^2	Dim.4	Cos^2	Dim.5	Cos^2
Length	-	0.045	0.002	0.357	0.128	-0.211	0.045	-0.170	0.029	-0.342	0.117
Weight	-	0.109	0.012	0.491	0.241	0.051	0.003	-0.123	0.015	-0.278	0.077
Liver Wt.	-	-0.432	0.186	0.442	0.195	-0.098	0.010	0.250	0.063	0.155	0.024
Ellasj.	2.544	2.504	0.969	-0.306	0.014	0.117	0.002	0.051	0.000	0.104	0.002
Laksvt.	2.180	-2.146	0.969	0.262	0.014	-0.100	0.002	-0.044	0.000	-0.089	0.002