Master's thesis

NTNU
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Expression of DNA repair genes in Arctic char (*Salvelinus alpinus*) from Bjørnøya

Master's thesis in MLREAL
Supervisor: Åse Krøkje
June 2019

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Helene Inderberg
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Abstract

Persistent organic pollutants (POPs) have been detected in relatively high levels in the arctic environment, where they exert toxic effects on the affected organisms. It has been detected that Lake Ellasjøen on Bjørnøya, Norway (74.30°N, 19.01° E), has significantly higher levels of POPs in its sediment and biota compared to other arctic lakes, mainly due to guano depositions from resting seabirds. Arctic char (*Salvelinus alpinus*) is the only fish species resident in Lake Ellasjøen. It has been found that char of Ellasjøen have significantly higher levels of DNA double strand breaks (DSBs) compared to char from the control water Lake Laksvatn, and that the level of DSBs is correlated with the level of organochlorines (OCs) in the respective char. Even though there is increasing evidence of the genotoxic effects of POPs, little is known about the effects of POPs on the DNA repair system. The hypothesis for the present study is that the DNA DSB repair system is affected by the higher OC and DSB level in char of Ellasjøen. This was analysed by comparing the transcript level of 11 genes involved in DNA DSB repair in liver samples from char of Ellasjøen (n=9) with char from Laksvatn (n=12). Six of the investigated genes were significantly upregulated in char of Ellasjøen. This induction was positively correlated with the OC and DNA DSB level, thus supporting the hypothesis. As the expression of DNA DSB repair genes was increased in the contaminant-exposed char, it is likely that the DNA DSB repair capacity is induced in these individuals. However, as char of Ellasjøen still have significantly higher levels of DSBs compared to char of Laksvatn, it is possible that the OC or DSB level is above a certain threshold value, making DNA repair insufficient to prevent DNA breaks.

Sammendrag

Persistente organiske miljøgifter (POPs) har blitt detektert i relativt høye nivåer i arktiske miljøer, hvor de kan utvise toksisk effekt hos eksponerte organismer. Det har blitt funnet at Ellasjøen, lokalisert på Bjørnøya (74.30°N, 19.01° E), har signifikant høyere nivåer av POPs både i sediment og biota sammenlignet med andre arktiske innsjøer. Dette skyldes hovedsakelig tilførsel av guano fra hekkende sjøfugler. Arktisk røye (*Salvelinus alpinus*) er den eneste fiskearten som lever på Bjørnøya. Det er i et tidligere masterprosjekt blitt påvist at Arktisk røye fra Ellasjøen har signifikant høyere nivåer av DNA-dobbeltrådbrudd (DSBs) sammenlignet med røye fra Laksvatn, som har blitt brukt som kontrollvann. Nivået av DNA-DSBs var korrelert med konsentrasjonen av organokloriner (OCs) i vevet til de respektive røyene. Selv om det er økende kunnskap om de genotoksiske effektene til POPs, er det fortsatt mye som er ukjent rundt hvordan POPs påvirker DNA-reparasjonssystemene hos eksponerte individer. Hypotesen for denne studien var at DNA-DSB-reparasjonssystemet blir påvirket av det høye nivået av OC og DSB i røyer fra Ellasjøen. Dette ble analysert ved å sammenligne uttrykkelsesnivået av elleve gener involvert i DNA-DSB-reparasjon i leverprøver fra røyer fra Ellasjøen (n=9) med røyer fra Laksvatn (n=12). Seks av de studerte genene var signifikant oppregulert i røyer fra Ellasjøen. Denne økningen var positivt korrelert med nivået av OCs og DNA-DSB i de respektive fiskene. Hypotesen for denne studien ble dermed bekreftet. Siden uttrykkelsen av gener involvert i DNA-DSB reparasjon var høyere i de eksponerte fiskene, er det sannsynlig at DNA-DSB-reparasjonskapasiteten er høyere i røyer fra Ellasjøen sammenlignet med røyene fra Laksvatn. Røyene fra Ellasjøen hadde likevel høyere nivåer av DSBs enn røyer fra Laksvatn. Det er mulig at nivået av OCs eller DSBs er over en bestemt terskelverdi, som gjør DNA-reparasjonskapasiten utilstrekkelig for å hindre DNA-trådbrudd fra å oppstå.

Abbreviations

ACTB	*β-actin*
Alt-EJ	Alternative end-joining
ATM	Ataxia-telangiectasia mutated
B(a)P	Benzo(a)pyrene
BER	Base excision repair
BRCA1	Breast cancer type 1 susceptibility protein
BRCA2	Breast cancer type 2 susceptibility protein
Cd	Cadmium
cDNA	Complementary DNA
Cf	Condition factor
Co	Cobalt
Cq value	Quantification cycle value
DNA-FTM	DNA fraction of total DNA that migrated into the gel
DNA-PK	DNA dependent kinase complex
DSB	Double strand break
EF1A	Elongation factor 1A
gDNA	Genomic DNA
G2 phase	Gap 2 phase
Hg	Mercury
HPI axis	Hypothalamus-pituitary-interrenal axis
HR	Homologous recombination
HSI	Hepatosomatic index
H_2O_2	Hydrogen peroxide
MeHg	Methyl mercury
MGMT	Methylguanine-DNA methyltransferase
MHEJ	Microhomology-mediated end joining
MML	Median molecular length
MMR	Mismatch repair
MMS	Methyl methanesulphonate
MRN complex	The Mre11-Rad50-Nbs1 complex
NCBI	National Center for Biotechnology Information
NER	Nucleotide excision repair
NHEJ	Non-homologous end-joining
NTC	No template control
NTNU	Norwegian University of Science and Technology
OC	Organochlorine
Ogg1	8-oxoguanine glycosylase
PAH	Polycyclic aromatic hydrocarbons
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
POP	Persistent organic pollutant
qPCR	Quantitative polymerase chain reaction
RPA	Replication protein A
RT	Reverse transcriptase
RT-qPCR	Quantitative reverse transcriptase polymerase chain reaction
S phase	Synthesis phase
SSA	Single-strand annealing

TBA	Tubulin alpha chain
TCBQ	Tetrachlorobenzoquinone
UBIQ	Ubiquitin
W-o-L	Window-of-Linearity
53BP1	p53-binding protein 1

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1 Introduction

1.1 Persistent organic pollutants

Human activities are the source of a wide range of hazardous chemicals, which eventually may end up in the natural environment. Among these chemicals are persistent organic pollutants (POPs). The main sources of POPs are industrial production, by-products from industrial processes and pesticides (UNEP, 2008a). Due to their physical and chemical properties, POPs tend to persist in the natural environment for long periods of time, where they are ubiquitously distributed through long-range transport. POPs accumulate in the fatty tissue of living organisms, and may exert toxic effects to both wildlife and humans (UNEP, 2008a).

Among the largest subgroups of POPs are organochlorines (OCs). Due to the persistency of carbon-chloride bonds toward hydrolysis, this group are relatively resistant to both biological and photolytic degradation (El-Shahawi et al., 2010). Polychlorinated biphenyls (PCBs) are a class of OCs that consists of a biphenyl molecule with various numbers of chlorine atoms attached (El-Shahawi et al., 2010). PCBs were widely used in industrial processes from the 1930s until the late 1970s (El-Shahawi et al., 2010). PCBs were one of the 12 initial POPs that were banned by the Stockholm convention in 2004 (UNEP, 2008b). However, due to PCBs' persistent nature, they are still ubiquitously found in the natural environment and are considered one of the pollutants of most concern (Jørgensen et al., 2006).

One of the regions where POPs are of most concern is the Arctic. The levels of OCs such as PCBs in the Arctic have decreased after their bans and restrictions (AMAP, 2004). However, OCs are still found in relatively high levels in Arctic top predators (Letcher et al., 2010). POPs are mainly transported to the Arctic ecosystems through long-range atmospheric transport, as well as via ocean currents, transpolar ice pack, Arctic rivers and biota (AMAP 2004). Contaminants present in the atmosphere tend to condense onto soil, water, aerosols, snow and ice at low temperatures (Wania and Mackay, 1993). In addition, reaction rates are lower at low temperatures, and low intensity sunlight reduces photolysis (Wania and Mackay, 1993). Together, this makes the Arctic function as a "cold trap" for such contaminants (Wania and Mackay, 1993). As arctic organisms rely on lipids for energy storage, they have the potential of accumulating relatively high levels of lipophilic POPs in their lipid-rich tissues (Borgå et al., 2001). When the lipid rich storages are broken down during times of food shortages, the accumulated contaminants get redistributed (Lassiter and Hallam, 1990). This renders the arctic species especially vulnerable to the potential toxic effects of POPs (Letcher et al., 2010).

There have been demonstrated that OCs cause various adverse effect endpoints in several species in the Arctic, such as Northern fur seals (*Callorhinus ursinus*), polar bears (*Ursus maritimus)* and glaucous gulls (*Larus hyperboreus*) (Jørgensen et al., 2006). The effect endpoints include reproductive, endocrine, immunological and behavioural changes (Jørgensen et al., 2006). The genotoxic properties of OCs are less studied. However, there is increasing evidence that several POPs do elicit genotoxic effects, as shown *in vivo* (Krøkje et al., 2006, Binelli et al., 2008, Fenstad et al., 2014, Fenstad et al., 2016) and *in vitro* (Srinivasan et al., 2001, Østby and Krøkje, 2002). As pollutant-induced DNA

strand breaks are important indications of severe genotoxic effects, most studies have focused on this endpoint (Srinivasan et al., 2001, Krøkje et al., 2006, Fenstad et al., 2014, Fenstad et al., 2016).

1.2 Genetic toxicology

Genetic toxicology is a field within toxicology that assesses the effects of chemicals and physical agents on DNA and on the genetic processes of living cells (Preston and Hoffmann, 2013). The genotoxic agent may cause different types of DNA damage, including DNA strand breaks, cross-links, and addition of chemical groups to DNA bases, known as adducts (Figure 1) (Preston and Hoffmann, 2013).

There are several ways by which genotoxic effects of POPs occur. Several metabolites of POPs are alkylating agents that may react covalently with the bases of DNA, and induce DNA strand breaks (Srinivasan et al., 2001, Binelli et al., 2008). There is also evidence that certain POPs may induce oxidative stress (Fernie et al., 2005, Costantini et al., 2014). Oxidative stress can impair the integrity of cells and tissues by causing oxidative damage to membranes or biomolecules such as DNA, proteins and lipids (Finkel and Holbrook, 2000). Induction of hydroxyl radicals can cause DNA single and double strand breaks by attacking the DNA backbone (Friedberg et al., 2006).

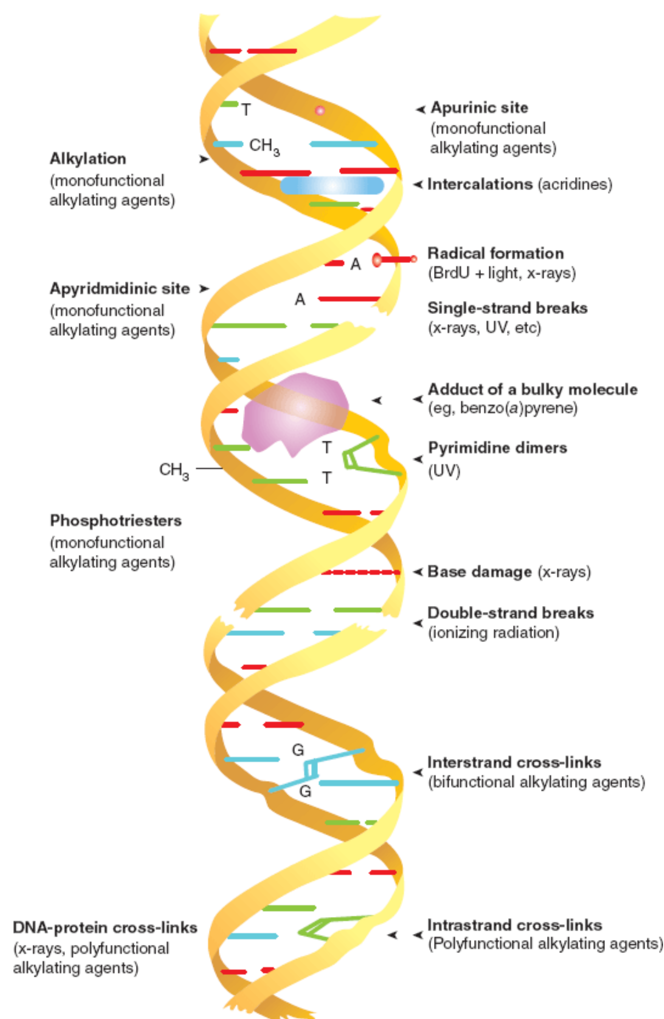


Figure 1: Types of DNA damage caused by physical and chemical agents (Preston and Hoffmann, 2013).

1.2.1 DNA double strand breaks

DNA double strand breaks (DSBs) are the most deleterious form of DNA damage due to the lack of an intact complementary strand that can be used as a template for DNA repair (Polo and Jackson, 2011). They may be produced by ionizing radiation, genotoxic chemicals and free radicals, produced during cellular processes (van Gent et al., 2001). DSBs may also be produced during DNA replication, if a template contains a single-stranded break. This break will end up as a DSB on one of the sister chromatids (Kogoma, 1997).

The unprotected DNA ends in a DSB may be subjected to single- and double-strand exonucleases, which may result in the potential loss of vital genetic information (Cromie et al., 2001). DSBs may also create mutagenic DNA rearrangements, such as translocations and inversions (Cromie et al., 2001). If a DSB remains unrepaired, it can interrupt the coding sequence of a gene, disrupt the linkage between coding and regulatory sequences, alter chromosome organization, and perturb the systems that ensure correct DNA replication, chromosome packaging, and chromosome segregation (Cromie et al., 2001). DSBs can be especially dangerous if they occur during genome replication and during the segregation of sister chromatids into daughter cells (van Gent et al., 2001).

Organisms have evolved several mechanisms in order to prevent DNA damage. This includes detoxification of genotoxicants, DNA repair, apoptosis, DNA redundancy (Jenkins et al., 2010) and antioxidant defence (Hartwig and Schwerdtle, 2002). There are four major DNA repair pathways: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and the double strand break repair systems homologous recombination (HR) and non-homologous end-joining (NHEJ) (Krokan et al., 2004). DNA repair can usually cope with low levels of DNA damage. In cases of excessive damage however, the normal DNA repair systems may be overwhelmed (Jenkins et al., 2010). This may result in an unrepaired or misrepaired DNA, which may be fixed into a mutation during replication. The occurrence of genotoxicants in the natural environment may therefore be detrimental both for the affected organisms and populations (Sarkar et al., 2008).

1.2.2 DNA double-strand break repair

There are two main repair mechanisms that have been developed in order to cope with DSBs: HR, which is mainly error-free, and NHEJ, which is error-prone (van Gent et al., 2001). HR requires a homologous template DNA which usually is a sister chromatid, and is therefore most efficient during the synthesis (S) and gap 2 (G2) phases of the cell cycle (van Gent et al., 2001). NHEJ does not require a template DNA, and can thus operate throughout the cell cycle (van Gent et al., 2001). In addition, two alternative repair pathways have been discovered, which both are intrinsically mutagenic: alternative end-joining (alt-EJ) and single-strand annealing (SSA) (Ceccaldi et al., 2016). Alt-EJ, SSA and HR are end resection-dependent, while NHEJ is not (Ceccaldi et al., 2016). The alternative repair pathways are not only backup repair systems, but can be active even though both NHEJ and HR are available (Truong et al., 2013). Alt-EJ is especially important when proteins responsible for NHEJ, such as the Ku-proteins, are reduced or absent (Truong et al., 2013).

When a DSB is recognized, it activates the MRE11-RAD50-NBS1 (MRN) complex. This complex is involved in all aspects of DSB processing, such as initial detection, triggering of signalling pathways, and by being involved in HR, NHEJ and alt-EJ (Williams et al., 2010). MRN is also present at replication forks and telomeres, where it prevents the formation of DSBs (Williams et al., 2010). In response to DSBs, MRN activates the ataxia-telangiectasia mutated (ATM) protein kinase (Williams et al., 2010), which phosphorylates downstream targets involved in cell cycle arrest, DNA repair and apoptosis (Lee and Paull, 2005). ATM activation is most likely only necessary when there is a lack of efficient DSB repair, such as after high levels of damage (Williams et al., 2010). The tumour suppressor p53-binding protein 1 (53BP1) and the breast cancer type 1 susceptibility protein (BRCA1) are two additional factors that are important for efficient MRN dependent ATM signalling and DSB repair (Williams et al., 2010). 53BP1 interacts with MRN through Rad50, and helps to recruit MRN and ATM to DSBs (Williams et al., 2010). BRCA1 interacts with MRN through CtIP (Williams et al., 2010).

53BP1 and BRCA1 are key regulators of DSB repair pathway, and mutually inhibit each other. During the S and G2-phases, BRCA1 and its interacting partner counteract 53BP1-complexes to promote HR (Panier and Boulton, 2013). 53BP1 antagonizes BRCA1 during the G1-phase, and promotes NHEJ (Panier and Boulton, 2013).

Homologous recombination

HR is mediated through the RAD52 group of proteins, which include RAD50, RAD51, RAD52, RAD54 and MRE11 (Cromie et al., 2001). The repair process consists of three fundamental stages, in a sequence of events that is highly conserved between organisms (Figure 2) (Cromie et al., 2001). In the initiatory stage, presynapsis, the DNA ends are recognized by RAD52 (van Gent et al., 2001) and the blunt or approximately blunt duplex end is converted to a 3' single-strand overhang by exonucleases or by a helicase coupled to an endonuclease (Haber, 2000). This nucleolytic processing require the activity of the MRN complex (van Gent et al., 2001). The single-strand overhang attracts RAD51, which polymerizes onto the single-strand DNA fragment. This process is aided by RAD52 and the breast cancer type 2 susceptibility protein (BRCA2) (Ceccaldi et al., 2016). BRCA2 is recruited by BRCA1 (Zhang and Powell, 2005), and promotes RAD51 loading by displacing the tightly bound replication protein A (RPA) from the single-stranded DNA (Rothkamm et al., 2015).

In the second stage, synapsis, the RAD51 nucleoprotein filament initiates invasion of undamaged homologous DNA. This reaction is stimulated by RAD52 and RAD54 (van Gent et al., 2001). This links the damaged ends to the intact DNA duplex and generates a structure known as D loop, which is a three-way junction (Cromie et al., 2001). The intact homologous DNA duplex is used as a template, while the invading 3' end is used as a primer for DNA synthesis (Cromie et al., 2001). In the third stage, postsynapsis, the cell restores the DNA molecule back into two separate duplexes. This can be conducted in two ways. One option is to convert the D loop to a structure with four double-strand DNA arms, known as Holliday junction. In a process called branch migration, these four DNA arms can be moved along the DNA before it is returned to two duplex DNA strands (Cromie et al., 2001). The alternative way is ejection of the invading 3' strand after DNA synthesis (Cromie et al., 2001).

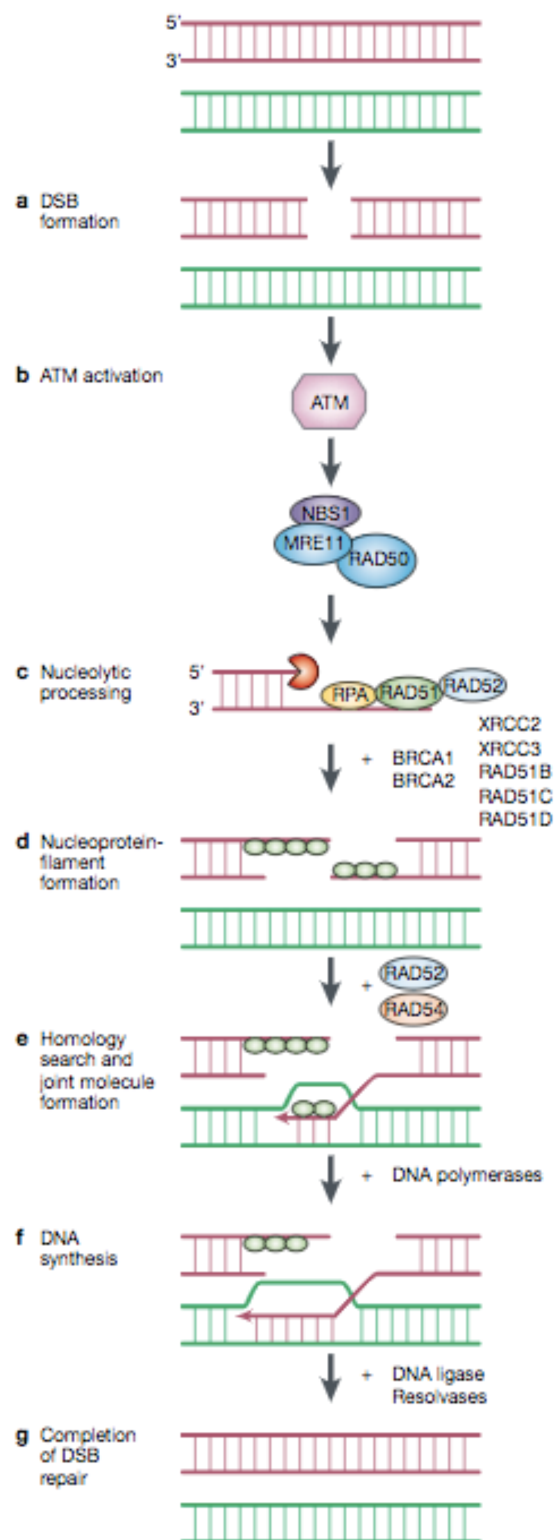


Figure 2: Homologous recombination (van Gent et al., 2001).

Non-homologous end-joining

In NHEJ, the free ends in the DSB are directly religated (Figure 3). The free ends of DSBs pre-ligation may be subjected to DNA nucleases, and the DSB may also have noncomplementary overhanging ends. The DSB are therefore ligated at regions of little or no homology. Since the DSB may have been subjected to degradation, the repaired DNA may lack some of the original DNA, making this approach error-prone (Cromie et al., 2001).

Two protein complexes are required for NHEJ: the DNA dependent kinase complex (DNA-PK), comprising of the two proteins KU70 and KU80 and a DNA-PK catalytic subunit (DNA-PKcs), and a heterodimer-complex, consisting of DNA Ligase IV and XRCC4 (Cromie et al., 2001). The KU proteins recognize the DSB, and bind to the DNA ends as a heterodimer. The KU heterodimer recruit and activate the kinase activity of the DNA-PKcs, and is also responsible for recruiting the XRCC4/Ligase IV complex (Dobbs et al., 2010).

DNA-PKcs have several target proteins, including p53, the KU polypeptides and itself (van Gent et al., 2001). DNA-PK protects the DNA from nonspecific nucleases, and recruits Ligase IV/XRCC4, which accomplishes the final ligation step (Cromie et al., 2001). The role of MRN in NHEJ has been much less clear than its role in HR (Dimitrova and De Lange, 2009). The nuclease activity of MRN is not a key element for NHEJ repair, as it is for HR (Reis et al., 2012). However, MRN is thought to function as a scaffold to support synapsis between the two DNA ends, thereby promoting religation of the DSB (Reis et al., 2012).

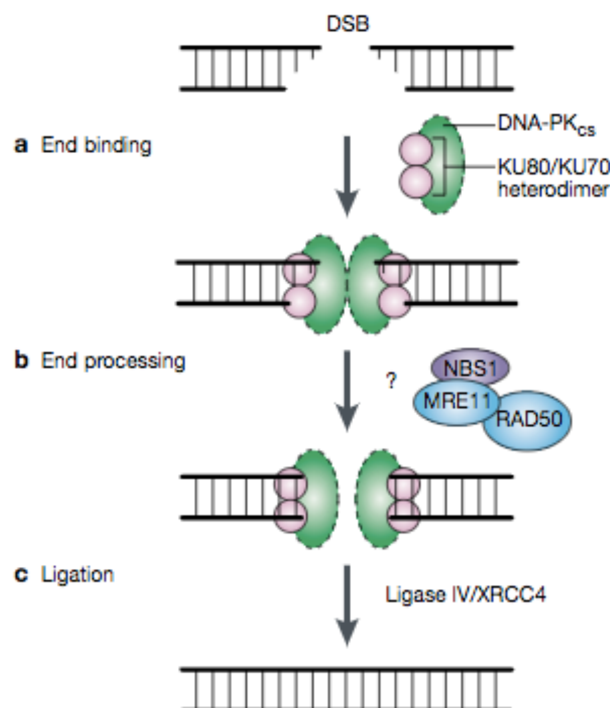


Figure 3: Non-homologous end-joining (van Gent et al., 2001).

1.3 Multidisciplinary studies at Bjørnøya

One of the most recent discoveries of genotoxic effects of POPs in arctic organisms was found by a multidisciplinary study conducted on Bjørnøya, Norway (74°30′ N, 19°00′ E). One of the lakes on the southern part of this island, Lake Ellasjøen, has been found to contain high concentration of POPs both in its sediment and biota. Comparative studies have shown that the POP levels found in Lake Ellasjøen are significantly higher than in the other lakes on Bjørnøya, such as Lake Laksvatn and Lake Øyangen, as well as other arctic lakes not located on Bjørnøya (AMAP, 2004, Evenset et al., 2004). This is mainly due to guano depositions from great populations of seabirds using Lake Ellasjøen as a resting area (Evenset et al., 2007). Arctic char (*Salvelinus alpinus*) is the only fish species inhabiting Lake Ellasjøen, and the levels of PCBs in this population are among the highest reported in both limnic and marine fish species (wet weight concentration) (AMAP, 2004). This fish species is also resident in Lake Laksvatn and Lake Øyangen. Between-lake comparisons of this fish species from Bjørnøya have therefore become of great interest in order to elucidate toxic effects of POPs in the arctic environment.

Neerland (2016) investigated in his master project the potential genotoxic effect of environmental exposure to OCs in Arctic char from Lake Ellasjøen as compared to Arctic char from Lake Laksvatn. This was conducted by measuring the levels of DNA DSBs from blood samples of a total of 39 individuals. He also analysed muscle samples to quantify the content of OCs in the fish, and analysed possible associations between DNA-damage, OC-concentrations and biological variables. His findings showed that char of Ellasjøen had significantly higher levels of DNA DSBs, visualised by significantly higher DNA-fraction of total DNA that migrated into the gel during gel-electrophoresis (DNA-FTM), and significantly lower median molecular length (MML) of the DNA fragments. He found that the level of DNA DSBs in char of Ellasjøen was significantly correlated with its OC levels, which were 43 times higher in the char of Ellasjøen compared to char of Laksvatn. In addition, he found that the Ellasjøen char had lower body growth rate and smaller relative liver weights compared to char of Lake Laksvatn.

1.4 Study species

The salmonid Arctic char is among the most widespread fish species in the world (Klemetsen, 2010). It has a circumpolar distribution, and is the most northernmost of all freshwater and anadromous fish (Klemetsen et al., 2003). Arctic char is a suitable monitoring species for contaminants, first of all due to its wide occurrence. In addition, char are carnivorous, and bioaccumulate OCs in their adipose tissues. They do also exhibit seasonal cycles of fattening and emaciation (Jørgensen et al., 2006).

Arctic char has strong phenotypic, ecological and life history diversity – it has even been found two or more morphs in the same lake (Klemetsen, 2010). There have been found two different morphs of this species from Bjørnøya consisting of small (dwarfs) and large mature fish, respectively (Berg et al., 2010). The small char (<60-150 mm) feed mainly on zooplankton and chironomid larvae and pupae (*Chironomidae* sp.), while the large char (>150 mm) also have cannibalistic tendencies (Berg et al., 2010).

Previous studies have found that Arctic char is susceptible to the toxic effects of OCs. This includes endocrine effects (Jørgensen et al., 2017), immune system effects (Maule et al., 2005), genotoxic effects (Neerland, 2016) and effects on the expression of genes involved in cellular and physiological stress response (Wiseman et al., 2011).

The global population of Arctic char is considered sustainable.

1.5 Objective

Even though the evidence of genotoxic effects of POPs in form of DNA strand breakage is increasing, little is known about the effects of POPs on other aspects of genotoxicity, such as the induction of DNA defence systems, or potential disturbance of DNA synthesis and DNA repair. A broader understanding of the affect on DNA repair capacity is vital for understanding the effects of genotoxicants on organisms. In addition, how DNA repair is regulated gives an insight into the strategies and disease susceptibility for the affected individuals. It is therefore of great interest within the field of genetic toxicology to elucidate potential effects of POPs on the DNA repair system.

Most of the studies on DSB repair have been conducted on mammals, and the knowledge of DSB repair in fish is limited. However, both HR and NHEJ have been identified in fish (Kienzler et al., 2013). As Neerland (2016) found significantly higher levels of DNA DSBs in char of Ellasjøen compared to char of Laksvatn, and that this was correlated with the OC level in the organisms, these two groups of char open up for an excellent opportunity to investigate how DNA repair is affected by such genotoxicants.

The running hypothesis for this master project is that genes involved in DNA DSB repair are differentially regulated in contaminant-exposed and non-contaminant exposed Arctic char, in this case char of Ellasjøen and Laksvatn. It is also hypothesized that a potential induction or decrease in transcript levels is correlated with the level of OCs and/or the level of DNA DSBs that were found by Neerland (2016). This will be analysed on liver samples, using quantitative reverse transcriptase polymerase chain reaction (RT-qPCR).

2 Materials and methods

2.1 Sampling

The fieldwork was conducted by Akvaplan-niva in August and September 2014, for the project named "Forurens: Is the cocktail effect of environmental contaminants a threat for Arctic fish populations?" (project number 221373). The research project was funded by the Research Council of Norway (Norges Forskningsråd), and was led by Dr. Anita Evenset.

Landlocked Arctic char were collected from two sites located at Bjørnøya (74°30' N, 19°00' E, Figure 4): Lake Ellasjøen (n = 18) and Lake Laksvatn (n = 21, reference lake), with the use of gill nets or fishing rods. Permissions to conduct fieldwork in the Bjørnøya National Park were given from the Governor of Svalbard (Sysselmannen) and the Norwegian Animal Research Authority. The fish were handled according to national regulations, and as few fish as possible were collected.

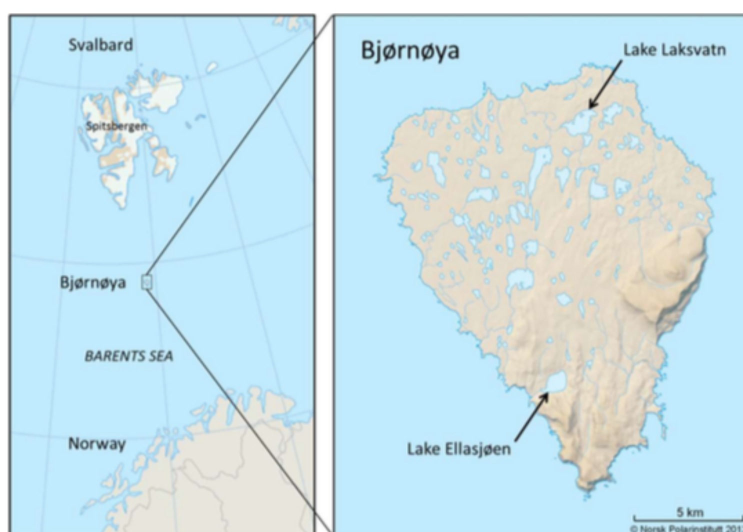


Figure 4: The location of Lake Laksvatn (reference lake) and Lake Ellasjøen (contaminated lake) on Bjørnøya. From Bytingsvik et al. (2015).

2.2 Gene expression analysis

Liver samples from 21 individuals (n = 9 from Lake Ellasjøen; n = 12 from Lake Laksvatn) were frozen in liquid nitrogen, stored at -80 °C and transported to the Norwegian University of Science and Technology (NTNU) in autumn 2014. The data on biological variances, OC content and levels of DNA DSBs on these individuals obtained from the master project conducted by Neerland (2016) are presented Appendix A.

The 21 liver samples were investigated for the relative expression of 14 genes involved in DNA DSB repair using RT-qPCR.

2.2.1 Principles of RT-qPCR

Polymerase chain reaction (PCR) is a widely used method to amplify a specific DNA segment into thousands to millions of copies. The relative quantity of mRNA in a sample can be measured using RT-qPCR. The mRNA transcripts are first converted to complementary DNA (cDNA) by reverse transcriptase (RT). The cDNA is then amplified using quantitative PCR (qPCR). This method measures the quantity of amplified template during the exponential phase of amplification in each cycle. This is achieved by measuring of the fluorescent signal from a fluorescent reporter molecule, such as the double stranded DNA-binding dye SYBR-GREEN. The increase in fluorescence intensity is proportional to the increase of amplicon concentration for each amplification cycle, and is measured by the qPCR instrument system. The relative quantity is set by a quantification cycle (Cq) value, which is directly correlated to the amount of starting template. The lower the Cq value, the higher quantity of RNA in the sample (Agilent Technologies, 2012).

A melting curve analysis can be performed after PCR to make sure that only the desired PCR product has been amplified. The melting curve analysis is based on the fact that SYBR-GREEN only sends fluorescent signals when bonded to a double stranded DNA. The reaction mixture is slowly heated to 97 °C to denature the amplified PCR product, which results in a decrease of SYBR-GREEN fluorescence. This decrease is what is displayed as melting peaks, and represents the melting temperature of a DNA product when 50 % are double-stranded and 50 % single-stranded. A particular DNA product has a characteristic melting peak, which means that if PCR only generated one amplicon, there should be only one melting peak. Additional peaks would mean that there are also non-specific products such as primer-dimers present (Agilent Technologies, 2012).

2.2.2 Primer design

14 genes were selected based on their important role in different aspects of DNA DSB repair. This involves genes in initial detection of DSBs, genes involved in both NHEJ and HR, and genes exclusive for one of these pathways. The selected genes were *53BP1*, *ATM*, *MRE11*, *RAD50, RAD51, RAD52, RAD54, BRCA1, BRCA2, Ligase IV, XRCC4, KU70, KU80* and *DNA-PKcs,*

Two primer pairs were designed for each gene, in case one of the pairs was malfunctional. Primers were designed using Primer-BLAST by National Center for Biotechnology Information (NCBI). In order to prevent amplification of remnants of genomic DNA, all primer pairs were designed so that they either were located on different exons or span an exon-exon junction. They were as far as possible chosen to be located toward the 3' end of the mRNA, as this is the location of the polyA-tail. The oligo-dt primers used for cDNA synthesis attach to the polyA-tail (Nikiforova and Nikiforov, 2011), and it is therefore more likely a higher abundance of cDNA from this part of the mRNAs. The primers were checked to avoid secondary structure by the RNAfold web server by the Institute for Theoretical Chemistry, University of Vienna.

Four reference genes were ordered, with primer sequences copied from published studies conducted on Arctic char: elongation factor 1A (*EF1A*), as recommended by Olsvik et al. (2005), with nucleotide sequence obtained from Jørgensen et al. (2017), and β-actin (*ACTB*), ubiquitin (*UBIQ*) and Tubulin alpha chain (*TBA)*, obtained from Ahi et al. (2013).

The primers were ordered from Sigma-Aldrich (Germany). Primer details are listed in Table 1.

Table 1: Nucleotide sequences for the ordered primer pairs

Gene	**Sequence**	**Amplicon length**	**Access code**
EF1A FW	AGGCATTGACAAGAGAACCATT		AF498320.1
EF1A RW	TGATACCACGCTCCCTCTC	119	
ACTB FW	GAAGATCAAGATCATCGCCC		NW_019949595
ACTB RW	CAGACTCGTCGTACTCCTGCT	122	
TBA FW	GTCACTACACCATTGGCAAAGA		NC_036866.1
TBA RW	GCTGTGGAAGATGAGGAATCC	104	
UBiQ FW	GACTACAACATCCAGAAAGAGTCCA		NC_036844.1
UBiQ RW	GCGGCAGATCATTTTGTC	120	
XRCC4 A FW	AGGGACATCTCGTTCTGCCTG		
XRCC4 A RW	GTGGTTCTGCAGTGCGGTTC	117	NC_036860.1
XRCC4 B FW	AGCCCATTGGACGACAGTCT		
XRCC4 B RW	GGTCCCTCCTCGGTTTCTGTG	136	
RAD50 A FW	CGTTGAGGAAGCGCATCGAG		
RAD50 A RW	AAGCCCTTCTGTCGACCCAG	175	NC_036860.1
RAD50 B FW	GATGGAACGAGCCGGGAGAG		
RAD50 B RW	CTCACCTGGGAGTCACGAGC	132	
RAD51 A FW	AGGCTAGCAGACGAGTTTGG		
RAD51 A RW	ATTTTGCAGATCCTCGTTTCGC	182	
RAD51 B FW	ACGCTAGGGCCTTCAACAC		
RAD51 B RW	AGTCGGTTCTGTAGAGGGCTG	123	NC_036868.1
KU80 A FW	AAGAGTTTTGCTGTGCTGGGC		
KU80 A RW	CCTGAGTCCCCACGAACTGG	70	
KU80 B FW	GGCAGTGACATCGTCCCCTT		
KU80 B RW	GGTGTCTGTTGATCATGCTCTGT	115	NW_019945760.1
Ligase IV A FW	TGGTTTGTTTCAGGTTCGTCAGG		
Ligase IV A RW	CGCCTTTTGAGGTTCCCTCC	168	
Ligase IV B FW	GGTTCGTCAGGAAATGTATGATGC		
Ligase IV B RW	GGAACTTGAGCAGCGACAGAG	196	NC_036867.1
MRE11 A FW	CGTTCTCGGAAGGCCTCAGT		
MRE11 A RW	TGGTCGTGATGCAGGAGTCA	198	
MRE11 B FW	TGACCAGGAGATTCGCCGTT		
MRE11 B RW	ATGCGGTGAGCTTTAGCCCT	99	NW_019942973.1

Gene	**Sequence**	**Amplicon length**	**Access code**
ATM A FW	TGTGGACCCCAATGACACCC		
ATM A RW	GCAGTAGTAACTTGCGGCGG	182	
ATM B FW	CCGCCGCAAGTTACTACTGC		
ATM B RW	AGCAAAGATCCGCCAGAGGT	158	NC_036841.1
BRCA1 A FW	CAACGGTGGTCCAAGAGCCT		
BRCA1 A RW	ATTGGTCCGAGCCTGGTTGT	84	
BRCA1 B FW	TTCCAAGGCCCTTTCACCGA		
BRCA1 B RW	TGGTGATTGGTCCGAGCCTG	153	NC_036858.1
53BP1 A FW	AGTGGCGTTTCAGCAGACCA		
53BP1 A RW	GTCTCTGGCTCGCCTAAGGA	85	
53BP1 B FW	GCAGGCTTCATCCTCCACGA		
53BP1 B RW	TCCTGGTTCGGCTATGCTGG	91	NW_019957590.1
RAD52 A FW	GGGCTTCACCATCCCTACGA		
RAD52 A RW	CTGAATGCCTCACCCAGTCTCA	137	
RAD52 B FW	TCTGACAGGGCAGGTGAGACTG		
RAD52 B RW	ACAGCCTTTTCTCAAGTGTCCAG	96	NW_019942714.1
DNA-PKcs A FW	CACTGCTCTGCAAGCCGAAG		
DNA-PKcs A RW	CCGTCACTCCACTCCGTGTT	87	
DNA-PKcs B FW	TTGGCGCATTCCGCAAGAAG		
DNA-PKcs B RW	AAAAGCTTGGCACCACCACG	82	NC_036854.1
BRCA2 A FW	CTGACGGTACCCTTCTAGACGTT		
BRCA2 A RW	AGGTCTAATGGTCTGCCGCTT	107	
BRCA2 B FW	CTATTGCAGGTCAGCTAGCCCC		
BRCA2 B RW	AGCTGCAAGCACTCTGCATCATA	70	NC_036841.1
RAD54 A FW	TCAACAGCCCATCTAGCCCA		
RAD54 A RW	AGATTCAGGCCACAACCCCC	70	
RAD54 B FW	AACTGTTCCTGAGGCAGGCT		
RAD54 B RW	GCCGGGTGGTTACAGAGCTT	112	NC_036856.1
KU70 A FW	AGCCTCAGGTGGACAAGATGAA		
KU70 A RW	CCAGGGAACCAAGACGCTGG	198	
KU70 B FW	ATGGCCCCAGAGCACATAGAG		
KU70 B RW	CTTGGGCTTCTTTTCTGTGCC	183	NC_036858.1

2.2.3 Homogenizing of liver samples

The liver samples were taken directly out of a -80 °C freezer into modules for homogenization using Tissue Lyser II (QIAGEN, Hilden, Germany), with the frequency of 25 turns per second for two minutes. The modules in which the tissue were homogenized were cooled down in a -80 °C freezer for minimum 10 minutes, or flash frozen in liquid nitrogen between each homogenization. The homogenized livers were quickly transferred to 15 ml tubes placed in liquid nitrogen, and stored at -80 °C.

2.2.4 Extraction of RNA

About 0.3 g of the homogenized livers was transferred to 2 ml microcentrifuge tubes placed on dry ice for RNA extraction. RNA extraction was conducted using RNeasy Plus Universal Mini Kit (QIAGEN, Hilden, Germany), as described by the manufacturer's protocol. First, the tissue samples were homogenized in QIAzol Lysis Reagent. Genomic DNA (gDNA) was removed by addition of gDNA Eliminator Solution, and chloroform was added for separation of the homogenate into aqueous and organic phases by centrifugation. The aquatic phase was collected, and mixed with ethanol to ensure appropriate binding conditions before RNA purification using RNeasy spin columns. Phenol and other contaminants were washed away from the RNA-binding membrane using washing buffers, and RNA was then eluted in RNase-free water.

The concentration and purity of the RNA extract were measured in duplicates using NanoDrop ND1000 (Thermo Scientific, MA USA). As the initial RNA concentrations were too high for reliable detection by NanoDrop, the samples were first diluted 1:2 or 1:4 with RNase free water. The resulting spectrophotometric curves had distinct tops at 260 nm, and 260/280 ratios and 260/230 ratios above or close to 2, reflecting pure samples. A representative figure is shown in Appendix B.

As the RNA concentrations were still too high for cDNA synthesis, a second dilution step was conducted. 10 µl of each extract were transferred to a 96 wells plate and diluted 1:5 with autoclaved Milli-Q water for the concentrations to get below 500 ng/µl. The concentration and purity were again measured in duplicates using NanoDrop ND1000, and is presented in Appendix B. The 260/280 ratios dropped to approximately 1.8 for all samples after the second dilution. This is most probable not caused by protein contamination, but rather due to the alteration of laboratory water, as this may alter the 260/280 ratio of the same RNA preparation due to alterations of pH and salt content (Wilfinger et al., 1997).

The plate was stored at -80 °C until cDNA synthesis.

2.2.5 cDNA synthesis

Template RNA was thawed on ice, and gDNA Wipeout buffer, Quantiscript RT, Quantiscript RT buffer, RT Primer Mix and RNase free water were thawed at room temperature and kept on ice.

gDNA elimination reaction was set up on ice in a 96 wells plate, with template concentrations calibrated by the initial concentration of RNA found using NanoDrop ND1000 spectrophotometer, for a total of 1,5 µg template. In addition to template RNA, 3 µl gDNA wipeout buffer and RNase free water were added to the gDNA elimination

reaction, for a total volume of 21 µl per sample. The plate was incubated at 42 °C for 2 minutes for the gDNA elimination reaction to occur, and was then put back on ice.

14 µl of the DNA elimination reaction was transferred to another row in the 96 wells plate for cDNA synthesis, leaving 7 µl for reverse transcriptase control (-RT control).

"Reverse-transcription master mix" consisting of 1 µl Quantiscript RT, 4 µl RT buffer and 1 µl RT-Primer mix per reaction was added to each of the wells intended for cDNA synthesis, to a total volume of 20 µl. "–RT control master mix", consisting of 0.5 µl RNase free water, 2 µl RT-buffer and 0.5 µl RT-primer mix (final volume 3 µl) were added to each of the –RT reactions, to a total volume of 10 µl. The plate was incubated at 42 °C for 15 minutes for cDNA synthesis, followed by 3 minutes at 95 °C for the inactivation of the Quantiscript reverse transcriptase.

The cDNA and –RT controls were diluted 1:10 using autoclaved Milli-Q water, and stored at -20 °C until analysis of relative gene transcription using qPCR.

2.2.6 Quantitative PCR

A qRT-PCR master mix was prepared for each gene, containing 3 µl autoclaved Milli-Q water, 2 µl 10x PCR primer mix (containing forward and reverse primers, both at 5 µM), and 10 µl LightCycler 480 SYBR Green I Master mix per reaction. 5 µl cDNA was added to each of the reactions. Three no-template controls (NTC) were included per gene, where 5 µl autoclaved Milli-Q water was added instead of cDNA. –RT control reactions were conducted once, using the primer mix for the reference gene *Ef1a*.

The qPCR reactions were conducted in white 96 well qPCR plates (LightCycler ® 480 Multiwell Plate 96, Roche, Switzerland).
The general setup for the qPCR plates is presented in Appendix C.

The PCR plates were spun at 1500 g for 2 minutes prior to amplification in LightCycler ® 96 (Roche, Switzerland). The PCR products were subjected to a melting curve analysis to verify product specificity. The prepared program was run as shown in Table 2.

Table 2: Program for quantitative PCR

Process	Temperature	Time
Denaturation	95 °C	10 minutes
Amplification	95 °C	10 seconds
(45 cycles)	55 °C	10 seconds
	72 °C	15 seconds
Melting curve	95 °C	5 seconds
	65 °C	60 seconds
	97 °C	1 second
Cooling	37 °C	30 seconds

qPCR was conducted once per gene of interest, provided that the first tested primer pair gave reliable results. For some genes, the second primer pair had to be used.

The quality of the qPCR data from each gene was determined by the specificity of the melting curve analysis, as well as by the absence of PCR product in the non-template control wells.

For the genes *RAD52, RAD54* and *BRCA1*, none of the primer pairs gave reliable results. They were therefore not included in the analyses of the qPCR data. The primer pairs that gave reliable results were *XRCC4* A, *RAD50* A, *RAD51* A, *KU80* A, *Ligase IV* B, *MRE11* A, *ATM* B, *53BP1* B, *DNA-PKcs* B, *BRCA2* B and *KU70* B.

For the 11 genes with reliable data and the three reference genes, all replicates were included in the analyses (n = 12 from Lake Laksvatn and n = 9 from Lake Ellasjøen), except for *DNA-PKcs*, in which the well representing individual 76 from Lake Laksvatn gave non-specific melting curves, possibly due to contamination. This individual was therefore not included in the final analyses for this gene.

2.3 Analyses of qPCR data

The theoretical PCR efficiency for each amplicon is 2, but the real PCR efficiency can vary over a range from 1.8 to 2.0 (Ramakers et al., 2003). This is because the applied baseline fluorescence also includes fluorescence of unbound fluorochrome, and of fluorochrome bound to e.g. double strand cDNA and to primers annealed to non-target DNA (Ruijter et al., 2009). This variation may result in quite different calculated values of initial amplicon quantity, and it is therefore important to find the real PCR efficiency for each amplicon to obtain the most accurate results. This was calculated using the LinRegPCR software. This software first subtracts the baseline fluorescence. Then it finds the data points in the log-linear phase with the minimum coefficient of variation of efficiency values, to minimize the residual measurement noise. These data points are referred to as the Window-of-Linearity (W-o-L). The real PCR efficiency is then calculated (Ruijter et al., 2009).

The software qBASE^{+} was then used for relative quantification of the selected genes. Integrated in the qBASE^{+} software is the geNORM technology, which is used for determining the quality of and the optimal number of candidate reference genes for most reliable normalization (Vandesompele et al., 2002). In the present study, geNORM recommended normalization against the reference genes *EF1A, ACTB* and *UBIQ*. Relative quantity was calculated based the delta-delta-Ct approach, as explained in Hellemans et al. (2007). Statistical differences between the two groups were analysed by the statistical wizard integrated in qBASE$^{+,}$ which recommended the Mann Whitney *U*-test due to lack of normal distributions in the data set. P-values below 0.05 were considered statistically significant.

Individual relative quantity of transcript from the respective genes after normalization against *EF1A*, *ACTB* and *UBIQ* is presented in Appendix D.

2.4 Principal component analysis

A principal component analysis (PCA) is used to reveal simple underlying structures in complex data sets. This is conducted by creating new variables called principal components (PCs), which are constructed as linear combinations or mixtures of the initial variables. There are equal numbers of PCs as there are variables in the data. The PCs are made in such a way that as much as possible of the initial information is compressed into the first component – in other words, it accounts for the largest possible variance in the data set. The second component accounts for maximum possible remaining information, with the condition that it is uncorrelated with, and thus orthogonal to the first PC. The other components are computed in the same way (Abdi and Williams, 2010).

Useful information from a PCA is the scores and the loadings. Scores are the coordinates of an object in the new PCs. Loadings inform about how much a variable contributes to each PC. Clustering of variables means that they are correlated, while negatively correlated variables are at opposite positions along a PC. Variables at 90° are not correlated. A plot that combines a scores and loadings plot is called biplot. Objects in close proximity to a variable have high values for that variable (Alsberg).

The relative transcript level per individual for the 11 genes were plotted in the PCA, along with individual OC concentrations and DNA-FTM level, to investigate potential correlations. As all of the individual OCs were strongly correlated, total OCs were used as the variable for clarity. DNA-FTM was used as the variable for DNA DSBs, as FTM had lower coefficient of variance than MML (Neerland, 2016). The variables age, condition factor (CF; body weight (g) x body length $(cm)^{-3}$ x 100), hepatosomatic index (HSI; liver weight (g) x body weight $(g)^{-1}$ x 100) and reproductive stage obtained by Neerland (2016) were also included into the PCA plot, to check for potential associations.

3 Results

3.1 Relative transcript levels

There were significant higher transcript levels for 6 of the 11 genes analysed in char of Ellasjøen than in char of Laksvatn (Figure 5), indicated by the Mann Whitney *U*-test. This includes two genes involved in NHEJ (*XRCC4* and *Ligase IV*; p-value = 0.0158 and 0.0004, respectively), and four genes involved in DNA damage response (*53BP1*, *ATM*, *MRE11* and *RAD50*; p-value = 0.0001, 0.0324, 0.0199 and 0.04164, respectively). None of the genes involved in the DNA-PK complex were induced, although *KU70* and *KU80* showed trends of having lower expression rate in char of Ellasjøen. Neither *RAD51* nor *BRCA2* from HR were significantly differently regulated in the char of Ellasjøen. However, *BRCA2* showed trends of being slightly induced.

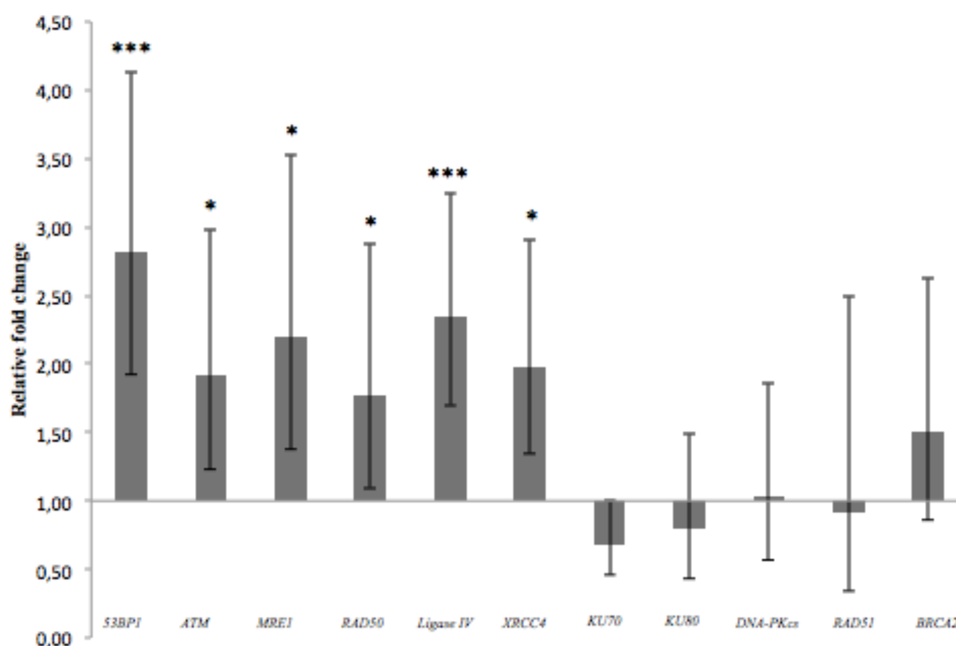


Figure 5: Expression of genes (relative fold change, $2^{-\Delta\Delta Ct}$) involved in DNA DSB repair in liver samples of Arctic char (*Salvelinus alpinus*) from the contaminated Lake Ellasjøen (n = 9). The values are relative to the control group from Lake Laksvatn (n = 12; n = 11 for *DNA-PKcs*). Error bars represent 95 % confidence interval. Asterisks represent significant differences between the two groups, using Mann Whitney *U*-test. * = p-value < 0.05; *** = p-value < 0.001.

3.2 Correlation analysis

Potential correlations between the transcript levels and the level of OC and/or DNA DSBs in the individuals were analysed using PCA (Figure 6). PC1 and PC2 explain 46.2 % of the variability in the data set. The individuals from the two populations clustered on opposite sides of the plot. Sum OCs and DNA-FTM were positively correlated. All of the induced genes were positively correlated with OC and DNA-FTM level, and this was also found for *Rad51.* The biological variables reproductive stage, CF, %lip and HSI were not correlated with the expression of the upregulated genes. The variable "age" contributed so much to the creation of the PCs that the correlation between OCs, DNA-FTM and transcript levels did not appear. This variable is therefore excluded in the present biplot. The biplot with all variables, including age, can be found in Appendix E.

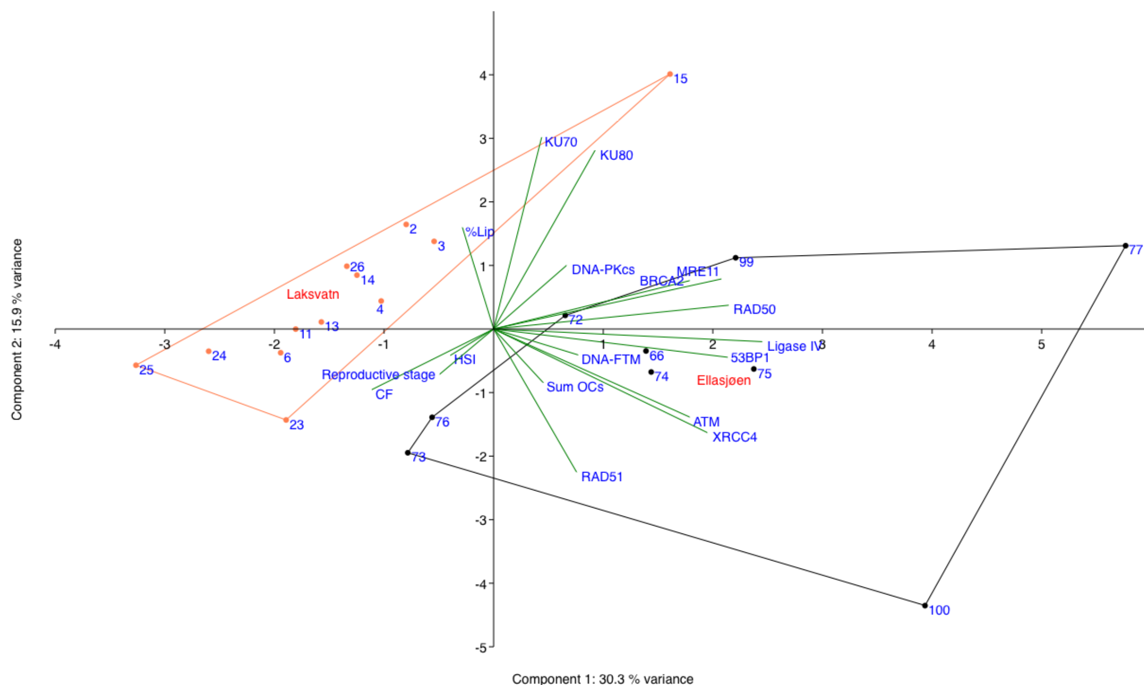


Figure 6: Principal component analysis biplot of the relative transcript level of 11 genes, OC content, DNA-FTM values and biological variables for 9 individuals of Arctic char (*Salvelinus alpinus*) from the contaminated Lake Ellasjøen (black dots) and 12 individuals from Lake Laksvatn (red dots). Sum OCs = total OC concentration in muscle sample; CF = condition factor; %lip: percentage of lipids in muscle samples; HSI: hepatosomatic index; DNA-FTM: fraction of total DNA that migrated into the gel.

4 Discussion

4.1 Induction of DNA DSB repair genes

Liver samples from Arctic char resident in the contaminated Lake Ellasjøen were investigated for the transcript level of genes involved in DNA DSB repair, and compared to char from the control water Lake Laksvatn. As Neerland (2016) found correlations between the levels of DNA DSBs and OCs in the individuals from Lake Ellasjøen, it was hypothesised that the higher contaminant levels in these individuals also affected the DNA DSB repair system, and that a potential up- or down-regulation would be correlated with the DSB and/or OC level.

As expected, there were significant differences between the two groups. Six of the investigated genes were significantly upregulated in the contaminated group compared to the control group. There were also found correlations between the transcript levels of the upregulated genes and DSB and OC level, thus supporting the hypothesis.

The genes that had significantly higher expression rate in char of Ellasjøen was *RAD50, MRE11, ATM* and *53BP1,* which are involved in initial detection and DSB damage response, and *Ligase IV* and *XRCC4*, which cooperate in the final ligation step in NHEJ. Although a significant increase was observed in the contaminated char, this increase was relatively low – about a doubling for all upregulated genes. As far as we know, no studies have been performed that investigated the expression rate of these specific genes in fish after contaminant exposure, which means that no direct comparison can be made. However, DNA repair genes are generally stably expressed, and have even been used as housekeeping genes (Iwanaga et al., 2004). Thus, it is likely that even small changes in expression may be indicative of induced DNA-repair activity.

There is increasing evidence of the genotoxic effects of OCs, mostly in form of the endpoint DNA breaks (Srinivasan et al., 2001, Krøkje et al., 2006a, Fenstad et al., 2014, Fenstad et al., 2016). The present study gives further insight into the molecular effects of OCs in chronically exposed fish. As the transcript level of the induced DNA repair genes were positively correlated with OC levels, it may seem like this group of contaminants induces DNA repair. However, this upregulation could be more directly attributed to the level of DNA DSBs caused by the OCs rather than a direct cause of OCs itself, as a defence mechanism for the increased level of DNA damage (Jenkins et al., 2010).

Little is known about the effect of organic contaminants on DNA repair, especially in fish. Coal-tar based sealcoat, containing elevated concentrations of polycyclic aromatic hydrocarbons (PAHs) and related compounds have been found to impair base excision repair in RTL-W1 fish liver cell line, even a month after application (Kienzler et al., 2015). The DNA repair machinery in hepatic cells of the fish *Anguilla anguilla* slowed down during exposure to the glycophase-based pesticide Roundup® in environmentally realistic concentrations (Marques et al., 2014). No other studies have been found addressing this connection in fish. However, several studies have been conducted on mammals. Tung et al. (2014) found induction of HR and NHEJ *in vitro* and *in vivo* in the CHO 3–6 cell line and pKZ1 mouse model after exposure to benzo(a)pyrene (B(a)P) (10 μM for *in vitro* exposure; 2 injections of 100 mg/kg for *in vivo* exposure). They also found significantly decreased expression of *ATM* and *KU70* in liver and lung, and of *DNA-*

PKcs and *KU80* in lung of mouse after *in vivo* B(a)P exposure. The base excision repair gene 8-oxoguanine glycosylase (*Ogg1*) was significantly decreased in cultured CD-1 mouse liver cells exposed to benzoquinone (25 µM), but no change in expression level was found for *BRCA1, BRCA2* and *XRCC4* (Philbrook and Winn, 2016). Organic contaminants from electric waste have been found to induce DSB repair genes in exposed humans (He et al., 2015a). PCB quinone induced the level of DNA DSB repair proteins in human HepG2 cells in a concentration-dependent manner (Dong et al., 2015). Increased levels of the KU80 protein were found in human lymphocyte cultures exposed to the pesticides glyphosate and paraoxon (Suárez-Larios et al., 2017). Pinto et al. (2015) found that estuarine contaminants both directly and indirectly induce DNA strand breakage in human HepG2 cells, the latter through impairment of DNA repair. Impairment of DNA repair after POP exposure have also been found in non-mammalian species: Qiao et al. (2007) found that earthworms exposed to soils contaminated with PAHs had slower DNA repair and had significantly more DNA damages after a 14 days exposure period, compared to the control.

All of the mentioned studies found that the organic contaminant caused an effect on the DNA repair system. This is in concert with the current finding that there are correlations between OC content and the DNA repair system. However, there are no clear trends in the mentioned studies for how organic contaminant exposure affects the DNA repair system: some found impaired DNA repair capacity or decreased expression of DNA repair genes, while other found induced DNA repair activity or induced DNA repair gene expression. As different types of organic pollutants may exert different types of effect in organisms, the results from the mentioned studies may not necessarily be comparable to the present study. Even though some organic pollutants affect DNA repair systems in a specific way, it is not certain that e.g. PCBs would give the same effects. In addition, the char of Ellasjøen is exposed to a complex mixture of both organic and inorganic substances, which may exert additive, antagonistic or synergistic effect in the affected organisms (Groten et al., 2001). Only two of the mentioned studies were conducted on fish, and they did not look at DSB repair or transcript level of DSB repair genes. As there is still limited knowledge of the effects of OCs or other POPs on the DNA repair system in fish, it remains for future studies to confirm the effect observed in the present study.

It is interesting that *RAD51* expression is correlated with OC content and DSB level, even though this gene was not significantly differentially regulated in the char of Ellasjøen. Several studies have found induction of the transcript level of *RAD51* in zebrafish (*Danio rerio)* after exposure to heavy metals (Gonzalez et al., 2005, Gonzalez et al., 2006, Lerebours et al., 2009, Reinardy et al., 2013b) or through the induction of oxidative stress (Reinardy et al., 2013a). Thus, *RAD51* expression in fish is sensitive to the presence of these types of genotoxicants, and it would therefore not be surprising that it is also affected by the presence of organic genotoxicants. Why there was not observed a significant difference between the two groups, even though the expression level was correlated with OC and DSB level, is not easy to explain, but it could be accounted for by the relatively large variation in transcript level seen for this gene.

Even though the DNA repair capacity seems to be induced in char of Ellasjøen, they still had significantly higher levels of DSBs compared to char of Laksvatn (Neerland, 2016). Cells may tolerate low doses of genotoxicants through homeostatic mechanisms by DNA repair. However, the DNA repair becomes saturated at high levels of DNA damage (Jenkins et al., 2010). Song et al. (2016) found an upregulation of *Rad51* in the early

phase of tetrachlorobenzoquinone (TCBQ) exposure, suggesting an attempted repair of the TCBQ induced DNA damage. However, *RAD51* was downregulated in the late phase, possibly due to a too high level of DNA damage. Similarly, treatment with the alkylating agent methyl methanesulphonate (MMS) have been found to upregulate the DNA repair protein methylguanine-DNA methyltransferase (MGMT), which removes the O^6G alkyl lesion from DNA, but only at doses below the threshold - indicating that MGMT was saturated or repressed at higher doses (Doak et al., 2008). The same could be true for the genes investigated in the present study, in that they may have been repressed or saturated due to contaminant loads or DNA damage above a certain threshold value, making DNA repair insufficient to prevent DNA breaks. Other fish species could have more or less efficient repair systems, and thus have other threshold values than the Arctic char.

Gene expression changes over time in response to environmental perturbations, in a manner that is coordinated in magnitude and time. The time curve of gene expression responses is often initiated with an abrupt response that saturates and is then followed by a relaxation to a new steady state (Chechik and Koller, 2009). The char of Ellasjøen have been chronically exposed to high levels of contaminants throughout their life, and it is thus likely that the expression level of the investigated genes is at relaxed steady state. However, Arctic char is a species with high phenotypic diversity, and it is likely that DNA repair gene polymorphism exists in these populations, as has been found in other organisms (Jenkins et al., 2010). This could lead to some individuals having a DNA repair variant protein with lower or higher than average efficiency (Jenkins et al., 2010). This would result in varying threshold levels among individuals, rendering some individuals more susceptible to the genotoxic effects of OCs and other toxicants. A high diversity within the populations could also partly explain the relatively high variation of transcript levels that were found for most of the genes in the present study, visualised by the 95 % confidence intervals.

The genes investigated in the present study included originally five genes specific for NHEJ, and four genes specific for HR, in order to investigate whether one of the two pathways is more affected than the other. Unfortunately, only two genes specific for HR were mapped due to dysfunctional primer pairs for *RAD52* and *RAD54*. The two genes were *RAD51* and *BRCA2*, none of which were significantly upregulated. For NHEJ, two of the genes were induced in the char of Ellasjøen, namely *XRCC4* and *Ligase IV*. As most DSB repair events occur by NHEJ (Thompson, 2012), it is not surprising that it is found induction of genes involved in NHEJ in the present study. It is noteworthy that for NHEJ, only genes from one complex were upregulated in the char of Ellasjøen. The genes from the DNA-PK complex were not significantly differentially regulated in char of Ellasjøen compared to char of Laksvatn. As this shows that genes involved in the same repair system can be differentially regulated, this can also be the case for HR. Based on the present data, it is hard to make clear assumptions for which of these two repair systems are most affected. However, NHEJ and HR are not mutually exclusive (Takata et al., 1998, Rapp and Greulich, 2004). Several studies have found upregulation of genes from both HR and NHEJ after exposure to different stressors (Reinardy et al., 2013a, Reinardy et al., 2013b, Rhee et al., 2013). It is therefore possible that both pathways are induced. This is underscored by the fact that *BRCA2* showed trends of being induced in the Ellasjøen char, in addition to *MRE11, RAD50* and *ATM* being upregulated – genes that can be involved in both pathways.

As mentioned, the genes of the two complexes of NHEJ were differentially regulated: *Ligase IV* and *XRCC4* were significantly induced, while this was not the case for any of the genes from the DNA-PK complex. *KU70* and *KU80* did in fact seem to be slightly downregulated. This is in contrast to a study conducted by Rhee et al. (2013), which found induction of *XRCC4*, *KU70*, *KU80* and *DNA-PKcs* in the hermaphroditic fish *Kryptolebias marmoratus* larvae – but only after exposure to high levels of gamma rays (6 Gy). *KU70* and *KU80* have also been upregulated in zebrafish testes after exposure to 25 mg/l of cobalt (Co) (Reinardy et al., 2013b), and in zebrafish larvae after a brief exposure to 100 mM hydrogen peroxide (H_2O_2) (Reinardy et al., 2013a). No studies have been found on the effect of organic substances on the NHEJ genes in fish, but such studies have been conducted in mammals: Philbrook and Winn (2016) did not find changes in expression level of *XRCC4* in mouse liver exposed to 25 µM benzoquinone, while Tung et al. (2014) found significant decreases of *KU70, KU80* and *DNA-PKcs* in different organs of mouse after B(a)P exposure. The results obtained in the study by Tung et al. (2014) could be regarded as more in accordance with the present results, in that *KU70* and *KU80* had lower expression rate. However, none of the mentioned studies investigated exposure to OCs or contaminants in complex mixtures in environmentally relevant concentrations. In any case, these studies show that the regulation of DNA-PK do respond to genotoxic stressors. It is possible that the levels in char of Ellasjøen are too low for significant differences to occur.

Since genes from the XRCC4/Ligase IV complex were induced, but not those from the DNA-PK-complex, this indicates that the promoters for these two complexes are not necessarily coordinately regulated. That genes in the same repair system can be differentially regulated has also been found by Iwanaga et al. (2004). They investigated the effect of growth stimulation on expression of *RAD50* and *RAD51*, as well as the *MSH2*, *MSH3* and *MLH1* genes involved in DNA mismatch repair, and found that *RAD50* and *MSH3* reacted differently to this stimulation than *RAD51* and *MSH2* and *MLH1*. They suggested that this difference could be because the expression of DNA repair genes is regulated according to the need of the gene products in the cellular circumstances. The average distance between two KU molecules in a typical nucleus is only 4-6 times the KU diameter (Lieber et al., 2003). The high abundance, in addition to their high affinity to DNA ends, makes it unlikely that KU level is rate-limiting for NHEJ (Lieber et al., 2003). Thus, an increase of Ligase IV and XRCC4 proteins could be enough to induce the repair capacity. Since genes of the same repair system can be differentially regulated, it is important for future studies investigating expression rates for DNA repair genes to include genes from several complexes, to get an overall idea of the situation of that specific repair system.

As the Ku-proteins seem to be slightly downregulated, it is also possible that a KU-independent alternative repair pathway is induced as compensation (Truong et al., 2013). Of the alternative NHEJ pathways, the microhomology-mediated end joining (MHEJ) is the most important, and is used in the levels of 10-20 % of HR in mammalian cells, even when both HR and NHEJ are available (Truong et al., 2013). This pathway is independent of KU and Ligase IV, but require the nuclease activity of the MRN complex in the initial end resection step (Truong et al., 2013). MMEJ has been found in fish (He et al., 2015b, Thyme and Schier, 2016), but how they respond to increased levels of contaminants or DSBs remains to be learned.

An important note to make is that an upregulation at the transcript level does not necessarily mean that there is an equal induction at the protein level, and thus increased DNA repair capacity. Yuan et al. (1999) did not find correlation between the decrease of mRNA and protein levels in prostate cancer cells after exposure to DNA damaging agents and a reducing agent. In contrast, Iwanaga et al. (2004) found that the transcriptional regulation of the DNA repair genes *MLH1, MSH2* and *Rad51* was reflected at the protein level in peripheral blood lymphocytes treated with growth stimulation. To be certain that DNA repair capacity indeed has increased, the level of DNA repair protein should also be considered. It is also possible that DNA repair is in a significant extent regulated at the protein level in addition to at the transcription level. Thus, it is possible that some of the proteins involved in HR or NHEJ is positively or negatively regulated at the protein level through e.g. phosphorylation, acetylation or ubiquitylation (Thompson, 2012).

It is also noteworthy that the situation that is recorded in the present study is simply a "snap shot" of the situation in the cells at the exact time point of freezing, and does not tell anything about variations in expression levels during e.g. the cell cycle. As HR is mainly productive during the S and G2 phases of the cell cycle, while NHEJ is active throughout the whole cell cycle, it is possible that other results would be obtained at another time point.

Arctic organisms are facing multiple threats, from climate change and landscape alterations to pollution (ACIA, 2004). Increasing loads of contaminants in aquatic environments impairs the health, fitness, growth and fecundity of aquatic organisms (Mitra et al., 2018). Thus, genotoxicants are only one of many environmental stressors that the affected organisms must endure, all of which require different types of defence mechanisms. As a concrete example, Jørgensen et al. (2017) found endocrine disruption of the hypothalamus-pituitary-interrenal (HPI) axis in char of Ellasjøen, which may have long-term implications for their stress coping ability and general fitness. As an individual does not have infinite amounts of energy, they must allocate their energy to the most vital processes, and make compromises to maximize survival and reproductive success. Even though DSBs are the most severe type of DNA lesion, it is possible that the energy requirements for repairing the total amount of DSBs found in char of Ellasjøen is so high that it will severely reduce the overall fitness of the individuals, and that the observed levels are the optimal compromise.

Even though DSB repair is induced in char of Ellasjøen, DSB repair is not error free. An induction of NHEJ is accompanied by an increase in the frequency of misrepair, as has been found when the repair efficiency of NHEJ increased in myeloid malignancies (Gaymes et al., 2002, Nowicki, 2004). Induction of this defence mechanism can thus result in an accumulation of DNA mutations, however less severe than a DSB. If the obtained mutations, whether they are a result of a DNA lesion such as a DSB or the repair process, affect the meiotic cells, they can be transferred to subsequent generations. Thus, induced levels of DNA damage can negatively affect the individual itself as well as the whole population, even when the repair capacity is induced. This potential mutagenic effect may contribute to the mechanism of toxicity and carcinogenesis of OCs.

4.2 Possible confounding factors

An important factor that could have influenced the present result is that the measurements of the three different parameters were conducted in different tissues. The transcript levels were measured in liver samples, while the DSB level was measured in blood samples, and the OC level was measured in muscle samples. It has been found that zebrafish regulate genes involved in DNA repair differently in different types of organs. Cadmium (Cd) exposure resulted in induction of the genes *mt1*, *cyt*, *bax*, *gad* and *RAD51* in skeletal muscles, but only two of these genes were upregulated in liver, even though the liver had the highest concentration of Cd (Gonzalez et al., 2006). Methylmercury (MeHg) exposure resulted in upregulation of *RAD51* in liver, but downregulation in skeletal muscles, and no changes in the transcript level were observed in the brain (Gonzalez et al., 2005). Different transcript levels of specific genes in different organs have also been found in Arctic char: the transcript level of genes in the HPI-axis was found to be higher in the brain and pituitary in char of Ellasjøen compared to char of Laksvatn, but were lower in the head and kidney (Jørgensen et al., 2017). It is therefore likely that RNA extracts from other tissues would give other results than the ones obtained in this study. For more valid conclusions, the transcriptomal analysis should be conducted in the same biological sample as the DSBs or OC levels were measured in.

The relationship between gene expression and POP concentration in wild fish can be influenced by biotic factors, such as fat status and maturity stage, and by abiotic factors, such as season and temperature (Jørgensen et al., 2017). As most POPs are lipophilic, high body fat content protects against impact from lipophilic chemicals (Lassiter and Hallam, 1990). Low tissue body fat content will therefore render the fish more susceptible to the toxic effects of lipophilic chemicals. Jørgensen et al. (2017) found high sensitivity to aryl hydrocarbon receptor-agonists in Ellasjøen char, and linked this to enhanced sensitivity due to very low tissue fat contents. Thus, body fat content should be taken into account when data on wild fish are interpreted. However, the fat content of the liver of the chars investigated in the present study has not been measured.

It is possible that contaminants such as heavy metals contribute to the observed endpoints such as induced DNA DSBs and upregulated DNA repair genes in the char of Ellasjøen. However, the project that was led by Akvaplan-niva only investigated the levels of organic pollutants. The levels of e.g. heavy metals in the lakes and biota of Lake Laksvatn and Ellasjøen are therefore unknown. There are unpublished data from 2001-2003 that show that char of Ellasjøen had higher levels of both mercury (Hg) and MeHg in muscle tissues than in char of Øyangen (n=22 and 21, respectively; mean values were 0,218 ug/g ww Hg and 0,171 ug/g ww MeHg in char of Ellasjøen, and 0,109 ug/g ww Hg and 0,09 ug/g ww MeHg in char of Øyangen) (Christiensen, G., unpubl. data, 2004, from AMAP, 2005). Both Lake Øyangen and Lake Laksvatn have been used as reference lakes, and it is thus likely that they have comparable levels of Hg. No data on other inorganic contaminants have been found on these specific lakes. However, heavy metals such as cadmium, lead, selenium, copper, zinc and mercury have been detected in freshwater sediments, freshwater or freshwater fish of a number of other arctic lakes (AMAP 2005). The possibility that there are other types of heavy metals in Lake Ellasjøen and Lake Laksvatn is therefore high, and could contribute to the cocktail effect the chars are exposed to.

No correlation was found between the transcript levels of the upregulated DNA repair genes and the OC and DNA DSB level when age was included as one of the variables. This could be attributed to four old males from Lake Ellasjøen (id: 73, 74, 75 and 76), which also had the highest OC and DNA DSB levels measured. These individuals had the lowest scores on PC2 when age was included as a variable. Neerland (2016) found that these individuals scored highest on PC1 on his scores plot. Thus, the age of these individuals probably contribute so much in the creation of the PCs that the correlation between OCs, DNA-FTM and transcript levels do not appear.

5 Conclusion

In the present master project, six of the eleven DNA DSB repair genes were significantly upregulated in char from the contaminated Lake Ellasjøen compared to char from the control water Lake Laksvatn. The transcript level of these genes was positively correlated with the OC and DNA DSB level in the respective individuals. Thus, both parts of the hypothesis were confirmed.

As several of the DNA DSB repair genes were upregulated in the char of Ellasjøen, it is likely that the DNA DSB repair capacity is induced in the contaminant-exposed fish. However, these individuals do still have significantly higher levels of DSBs compared to char of Laksvatn. This could be due to OC or DSB level above a certain threshold value, making DNA repair insufficient to prevent DNA breaks.

Genes from the Ligase IV/XRCC4 complex were significantly induced, but the sames was not found for genes involved in the DNA-PK complex. Thus, it may seem like these two complexes are differentially regulated in fish in response to genotoxic stress. None of the investigated genes specific for HR were significantly induced. However, the expression level for only two genes specific for HR were mapped. It is therefore not possible based on the present data to make clear conclusions for which of these two repair systems are most affected.

References

ABDI, H. & WILLIAMS, L. J. 2010. Principal component analysis. *Wiley Interdisciplinary Reviews: Computational Statistics,* 2**,** 433-459.

ACIA, 2004. *Arctic Climate Impact Assessment.* Cambridge University Press

AGILENT TECHNOLOGIES. 2012. *Introduction to Quantitative PCR: Methods and Applications Guide.* US and Canada: Agilent Technologies, p. 2-11.

AHI, E. P., GUÐBRANDSSON, J., KAPRALOVA, K. H., FRANZDÓTTIR, S. R., SNORRASON, S. S., MAIER, V. H. & JÓNSSON, Z. O. 2013. Validation of Reference Genes for Expression Studies during Craniofacial Development in Arctic Charr. *PLoS ONE,* 8**,** e66389.

Alsberg, B. K. Latent variable analysis. *In: Chemometrics.* Compendium for TKJ4175/KJ8175, NTNU, version 0,50, p. 197-230.

AMAP. 2004. AMAP Assessment 2002: Persistent organic pollutants in the Arctic. Oslo, Norway: Arctic Monitoring and Assessment Programme (AMAP).

AMAP. 2005. AMAP Assessment 2002: Heavy Metals in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xvi + 265 pp.

BERG, O. K., FINSTAD, A. G., OLSEN, P. H., ARNEKLEIV, J. V. & NILSSEN, K. 2010. Dwarfs and cannibals in the Arctic: production of Arctic char (*Salvelinus alpinus* (L.)) at two trophic levels. *Hydrobiologia*, 652**,** 337-347.

BINELLI, A., RIVA, C., COGNI, D. & PROVINI, A. 2008. Genotoxic effects ofp,p′-DDT (1,1,1-trichloro-2,2-bis-(chlorophenyl)ethane) and its metabolites in Zebra mussel (*D. polymorpha*) by SCGE assay and micronucleus test. *Environmental and Molecular Mutagenesis,* 49**,** 406-415.

BORGÅ, K., GABRIELSEN, G. W. & SKAARE, J. U. 2001. Biomagnification of organochlorines along a Barents Sea food chain. *Environmental Pollution,* 113**,** 187-198.

BYTINGSVIK, J., FRANTZEN, M., GOTSCH, A., HEIMSTAD, E. S., CHRISTENSEN, G. & EVENSET, A. 2015. Current status, between-year comparisons and maternal transfer of organohalogenated compounds (OHCs) in Arctic char (*Salvelinus alpinus*) from Bjornoya, Svalbard (Norway). *Science of the Total Environment,* 521-522**,** 421-430.

CECCALDI, R., RONDINELLI, B. & D'ANDREA, A. D. 2016. Repair Pathway Choices and Consequences at the Double-Strand Break. *Trends in Cell Biology,* 26**,** 52-64.

CHECHIK, G. & KOLLER, D. 2009. Timing of Gene Expression Responses to Environmental Changes. *Journal of Computational Biology*, 16**,** 279-290.

COSTANTINI, D., MEILLERE, A., CARRAVIERI, A., LECOMTE, V., SORCI, G., FAIVRE, B., WEIMERSKIRCH, H., BUSTAMANTE, P., LABADIE, P., BUDZINSKI, H. & CHASTEL, O. 2014. Oxidative stress in relation to reproduction, contaminants, gender and age in a long-lived seabird. *Oecologia,* 175**,** 1107-16.

CROMIE, G. A., CONNELLY, J. C. & LEACH, D. R. F. 2001. Recombination at Double-Strand Breaks and DNA Ends: Conserved Mechanisms from Phage to Humans. *Molecular Cell,* 8**,** 1163-1174.

DIMITROVA, N. & DE LANGE, T. 2009. Cell Cycle-Dependent Role of MRN at Dysfunctional Telomeres: ATM Signaling-Dependent Induction of Nonhomologous End Joining (NHEJ) in G1 and Resection-Mediated Inhibition of NHEJ in G2. *Molecular and Cellular Biology,* 29**,** 5552-5563.

DOAK, S. H., BRÜSEHAFER, K., DUDLEY, E., QUICK, E., JOHNSON, G., NEWTON, R. P. & JENKINS, G. J. S. 2008. No-observed effect levels are associated with up-regulation of MGMT following MMS exposure. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 648**,** 9-14.

DOBBS, T. A., TAINER, J. A. & LEES-MILLER, S. P. 2010. A structural model for regulation of NHEJ by DNA-PKcs autophosphorylation. *DNA Repair,* 9**,** 1307-1314.

DONG, H., SHI, Q., SONG, X., FU, J., HU, L., XU, D., SU, C., XIA, X., SONG, E. & SONG, Y. 2015. Polychlorinated biphenyl quinone induces oxidative DNA damage and repair responses: The activations of NHEJ, BER and NER via ATM-p53 signaling axis. *Toxicology and Applied Pharmacology*, 286, 10-16.

EL-SHAHAWI, M. S., HAMZA, A., BASHAMMAKH, A. S. & AL-SAGGAF, W. T. 2010. An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. *Talanta,* 80**,** 1587-1597.

EVENSET, A., CARROLL, J., CHRISTENSEN, G. N., KALLENBORN, R., GREGOR, D. & GABRIELSEN, G. W. 2007. Seabird Guano Is an Efficient Conveyer of Persistent Organic Pollutants (POPs) to Arctic Lake Ecosystems. *Environmental Science & Technology*, 41**,** 1173-1179.

EVENSET, A., CHRISTENSEN, G. N., SKOTVOLD, T., FJELD, E., SCHLABACH, M., WARTENA, E. & GREGOR, D. 2004. A comparison of organic contaminants in two high Arctic lake ecosystems, Bjørnøya (Bear Island), Norway. *Science of The Total Environment,* 318**,** 125-141.

FENSTAD, A. A., BUSTNES, J. O., BINGHAM, C. G., ÖST, M., JAATINEN, K., MOE, B., HANSSEN, S. A., MOODY, A. J., GABRIELSEN, K. M., HERZKE, D., LIERHAGEN, S., JENSSEN, B. M. & KRØKJE, Å. 2016. DNA double-strand breaks in incubating female common eiders (*Somateria mollissima*): Comparison between a low and a high polluted area. *Environmental Research,* 151**,** 297-303.

FENSTAD, A. A., JENSSEN, B. M., MOE, B., HANSSEN, S. A., BINGHAM, C., HERZKE, D., BUSTNES, J. O. & KRØKJE, Å. 2014. DNA double-strand breaks in relation to persistent organic pollutants in a fasting seabird. *Ecotoxicology and Environmental Safety,* 106**,** 68-75.

FERNIE, K. J., SHUTT, J. L., MAYNE, G., HOFFMAN, D., LETCHER, R. J., DROUILLARD, K. G. & RITCHIE, I. J. 2005. Exposure to polybrominated diphenyl ethers (PBDEs): changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). *Toxicological Sciences,* 88**,** 375-83.

FINKEL, T. & HOLBROOK, N. J. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature,* 408**,** 239-47.

FRIEDBERG, E. C., WALKER, G. C., SIEDE, W., WOOD, R. D., SCHULTZ, R. A. & ELLENBERGER, T. 2006. DNA damage. *In*: *DNA Repair and Mutagenesis.* 2nd ed. Washington DC: ASM Press, p. 9-69.

GAYMES, T. J., MUFTI, G. J. & RASSOOL, F. V. 2002. Myeloid Leukemias Have Increased Activity of the Nonhomologous End-Joining Pathway and Concomitant DNA Misrepair that Is Dependent on the Ku70/86 Heterodimer. *Cancer Research,* 62**,** 2791.

GONZALEZ, P., BAUDRIMONT, M., BOUDOU, A. & BOURDINEAUD, J.-P. 2006. Comparative Effects of Direct Cadmium Contamination on Gene Expression in Gills, Liver, Skeletal Muscles and Brain of the Zebrafish (*Danio rerio*). *BioMetals,* 19**,** 225-235.

GONZALEZ, P., DOMINIQUE, Y., MASSABUAU, J. C., BOUDOU, A. & BOURDINEAUD, J. P. 2005. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (*Danio rerio*). *Environtal Science & Technology,* 39**,** 3972-80.

GROTEN, J. P., FERON, V. J. & SÜHNEL, J. 2001. Toxicology of simple and complex mixtures. *Trends in pharmacological sciences,* 22**,** 316-322.

HABER, J. E. 2000. Partners and pathways: repairing a double-strand break. *Trends in Genetics,* 16**,** 259-264.

HARTWIG, A. & SCHWERDTLE, T. 2002. Interactions by carcinogenic metal compounds with DNA repair processes: toxicological implications. *Toxicology Letters,* 127**,** 47-54.

HE, X., JING, Y., WANG, J., LI, K., YANG, Q., ZHAO, Y., LI, R., GE, J., QIU, X. & LI, G. 2015a. Significant accumulation of persistent organic pollutants and dysregulation in multiple DNA damage repair pathways in the electronic-waste-exposed populations. *Environmental Research*, 137, 458-466.

HE, M.-D., ZHANG, F.-H., WANG, H.-L., WANG, H.-P., ZHU, Z.-Y. & SUN, Y.-H. 2015b. Efficient ligase 3-dependent microhomology-mediated end joining repair of DNA double-strand breaks in zebrafish embryos. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 780**,** 86-96.

HELLEMANS, J., MORTIER, G., DE PAEPE, A., SPELEMAN, F. & VANDESOMPELE, J. 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology,* 8**,** R19-R19.

IWANAGA, R., KOMORI, H. & OHTANI, K. 2004. Differential regulation of expression of the mammalian DNA repair genes by growth stimulation. *Oncogene*, 23**,** 8581-8590.

JENKINS, G. J. S., ZAÏR, Z., JOHNSON, G. E. & DOAK, S. H. 2010. Genotoxic thresholds, DNA repair, and susceptibility in human populations. *Toxicology,* 278**,** 305-310.

JØRGENSEN, E. H., MAULE, A. G., EVENSET, A., CHRISTENSEN, G., BYTNINGSVIK, J., FRANTZEN, M., NIKIFOROV, V., FAUGHT, E. & VIJAYAN, M. M. 2017. Biomarker response and hypothalamus-pituitary-interrenal axis functioning in Arctic charr from Bjørnøya (74 degrees 30' N), Norway, with high levels of organohalogenated compounds. *Aquatic Toxicology,* 187**,** 64-71.

JØRGENSEN, E. H., VIJAYAN, M. M., KILLIE, J.-E. A., ALURU, N., AAS-HANSEN, Ø. & MAULE, A. 2006. Toxicokinetics and Effects of PCBs in Arctic Fish: A Review of Studies on Arctic Charr. *Journal of Toxicology and Environmental Health, Part A,* 69**,** 37-52.

KIENZLER, A., BONY, S. & DEVAUX, A. 2013. DNA repair activity in fish and interest in ecotoxicology: A review. *Aquatic Toxicology,* 134-135**,** 47-56.

KIENZLER, A., MAHLER, B. J., VAN METRE, P. C., SCHWEIGERT, N., DEVAUX, A. & BONY, S. 2015. Exposure to runoff from coal-tar-sealed pavement induces genotoxicity and impairment of DNA repair capacity in the RTL-W1 fish liver cell line. *Science of The Total Environment,* 520**,** 73-80.

KLEMETSEN, A. 2010. The Charr Problem Revisited: Exceptional Phenotypic Plasticity Promotes Ecological Speciation in Postglacial Lakes. *Freshwater Reviews,* 3**,** 49-74.

KLEMETSEN, A., AMUNDSEN, P. A., DEMPSON, J. B., JONSSON, B., JONSSON, N., O'CONNELL, M. F. & MORTENSEN, E. 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish,* 12**,** 1-59.

KOGOMA, T. 1997. Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. *Microbiology and Molecular Biology Reviews,* 61**,** 212.

KROKAN, H. E., KAVLI, B. & SLUPPHAUG, G. 2004. Novel aspects of macromolecular repair and relationship to human disease. *Journal of Molecular Medicine*, 82**,** 280-297.

KRØKJE, A., BINGHAM, C., HUSMO TUVEN, R. & GABRIELSEN, G. 2006a. Chromosome Aberrations and DNA Strand Breaks in Glaucous Gull (*Larus Hyperboreus*) Chicks Fed Environmentally Contaminated Gull Eggs. *Journal of Toxicology and Environmental Health*, *Part A,* 69, 159-174.

LASSITER, R. & HALLAM, T. 1990. Survival of the fattest: Implications for acute effects of lipophilic chemicals on aquatic populations. *Environmental Toxicology and Chemistry*, 9, 585-595.

LEE, J.-H. & PAULL, T. T. 2005. ATM Activation by DNA Double-Strand Breaks Through the Mre11-Rad50-Nbs1 Complex. *Science,* 308**,** 551.

LEREBOURS, A., GONZALEZ, P., ADAM, C., CAMILLERI, V., BOURDINEAUD, J.-P. & GARNIER-LAPLACE, J. 2009. Comparative analysis of gene expression in brain, liver, skeletal muscles, and gills of zebrafish (*Danio rerio*) exposed to environmentally relevant waterborne uranium concentrations. *Environmental Toxicology and Chemistry,* 28**,** 1271.

LETCHER, R. J., BUSTNES, J. O., DIETZ, R., JENSSEN, B. M., JØRGENSEN, E. H., SONNE, C., VERREAULT, J., VIJAYAN, M. M. & GABRIELSEN, G. W. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Science of The Total Environment,* 408**,** 2995-3043.

LIEBER, M. R., MA, Y., PANNICKE, U. & SCHWARZ, K. 2003. Mechanism and regulation of human non-homologous DNA end-joining. *Nature Reviews Molecular Cell Biology,* 4**,** 712-720.

MARQUES, A., GUILHERME, S., GAIVÃO, I., SANTOS, M. A. & PACHECO, M. 2014. Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods — Insights into the mechanisms of genotoxicity and DNA repair. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 166**,** 126-133.

MAULE, A. G., JØRGENSEN, E. H., VIJAYAN, M. M. & KILLIE, J.-E. A. 2005. Aroclor 1254 exposure reduces disease resistance and innate immune responses in fasted Arctic charr. *Environmental Toxicology and Chemistry,* 24**,** 117-124.

MITRA, T., MOHANTY, B. P., MOHANTY, S., PUROHIT, G. K. & DAS, B. K. 2018. Expression patterns and mutation analysis of p53 in fish *Rita rita* from polluted riverine environment. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 832-833**,** 41-51.

NEERLAND, E. D. 2016. *DNA Double-Strand Breaks in Arctic Char (Salvelinus alpinus) from Bjørnøya.* Master thesis, NTNU.

NIKIFOROVA, M. N. & NIKIFOROV, Y. E. 2011. Molecular Anatomic Pathology: Principles, Techniques, and Application to Immunohistologic Diagnosis. *In:* DABBS, D. J. (ed.) *Diagnostic Immunohistochemistry.* 3rd edition. Philadelphia: W.B. Saunders, p. 42-57.

NOWICKI, M. O. 2004. BCR/ABL oncogenic kinase promotes unfaithful repair of the reactive oxygen species-dependent DNA double-strand breaks. *Blood*, 104**,** 3746-3753.

OLSVIK, P. A., LIE, K. K., JORDAL, A.-E. O., NILSEN, T. O. & HORDVIK, I. 2005. Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. *BMC Molecular Biology,* 6**,** 21.

PANIER, S. & BOULTON, S. J. 2013. Double-strand break repair: 53BP1 comes into focus. *Nature Reviews Molecular Cell Biology,* 15**,** 7.

PHILBROOK, N. A. & WINN, L. M. 2016. Benzoquinone toxicity is not prevented by sulforaphane in CD-1 mouse fetal liver cells. *Journal of Applied Toxicology,* 36**,** 1015-1024.

PINTO, M. F., LOURO, H., COSTA, P. M., CAEIRO, S. & SILVA, M. J. 2015. Exploring the Potential Interference of Estuarine Sediment Contaminants with the DNA Repair Capacity of Human Hepatoma Cells. *Journal of Toxicology and Environmental Health, Part A,* 78**,** 559-570.

POLO, S. E. & JACKSON, S. P. 2011. Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. *Genes & Development,* 25**,** 409-433.

PRESTON, R. J. AND HOFFMANN, G. R. 2013. Genetic Toxicology. *In*: KLAASSEN, C.D. (ed.) *Casarett and Doull's Toxicology: The Basic Science of Poisons.* 8th ed. New York (NY): McGraw-Hill Education, p. 445–480.

QIAO, M., CHEN, Y., WANG, C.-X., WANG, Z. & ZHU, Y.-G. 2007. DNA damage and repair process in earthworm after in-vivo and in vitro exposure to soils irrigated by wastewaters. *Environmental Pollution,* 148**,** 141-147.

RAMAKERS, C., RUIJTER, J. M., DEPREZ, R. H. & MOORMAN, A. F. 2003. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters,* 339**,** 62-6.

RAPP, A. & GREULICH, K. O. 2004. After double-strand break induction by UV-A, homologous recombination and nonhomologous end joining cooperate at the same DSB if both systems are available. *Journal of Cell Science,* 117**,** 4935.

REINARDY, H. C., DHARAMSHI, J., JHA, A. N. & HENRY, T. B. 2013a. Changes in expression profiles of genes associated with DNA repair following induction of DNA damage in larval zebrafish *Danio rerio*. *Mutagenesis,* 28**,** 601-608.

REINARDY, H. C., SYRETT, J. R., JEFFREE, R. A., HENRY, T. B. & JHA, A. N. 2013b. Cobalt-induced genotoxicity in male zebrafish (*Danio rerio*), with implications for reproduction and expression of DNA repair genes. *Aquatic Toxicology,* 126**,** 224-230.

REIS, C. C., BATISTA, S. & FERREIRA, M. G. 2012. The fission yeast MRN complex tethers dysfunctional telomeres for NHEJ repair. *The EMBO Journal,* 31**,** 4576.

RHEE, J.-S., KIM, B.-M., KIM, R.-O., SEO, J. S., KIM, I.-C., LEE, Y.-M. & LEE, J.-S. 2013. Co-expression of antioxidant enzymes with expression of p53, DNA repair, and heat shock protein genes in the gamma ray-irradiated hermaphroditic fish *Kryptolebias marmoratus* larvae. *Aquatic Toxicology,* 140-141**,** 58-67.

ROTHKAMM, K., BARNARD, S., MOQUET, J., ELLENDER, M., RANA, Z. & BURDAK-ROTHKAMM, S. 2015. DNA damage foci: Meaning and significance. *Environmental and Molecular Mutagenesis,* 56**,** 491-504.

RUIJTER, J. M., RAMAKERS, C., HOOGAARS, W. M. H., KARLEN, Y., BAKKER, O., VAN DEN HOFF, M. J. B. & MOORMAN, A. F. M. 2009. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research*, 37**,** e45.

SARKAR, A., GAITONDE, D. C. S., SARKAR, A., VASHISTHA, D., D'SILVA, C. & DALAL, S. G. 2008. Evaluation of impairment of DNA integrity in marine gastropods (*Cronia contracta*) as a biomarker of genotoxic contaminants in coastal water around Goa, West coast of India. *Ecotoxicology and Environmental Safety,* 71**,** 473-482.

SONG, X., SHI, Q., LIU, Z., WANG, Y., WANG, Y., SONG, E. & SONG, Y. 2016. Unpredicted Downregulation of RAD51 Suggests Genome Instability Induced by Tetrachlorobenzoquinone. *Chemical Research in Toxicology,* 29**,** 2184-2193.

SRINIVASAN, A., LEHMLER, H.-J., ROBERTSON, L. W. & LUDEWIG, G. 2001. Production of DNA Strand Breaks in Vitro and Reactive Oxygen Species in Vitro and in HL-60 Cells by PCB Metabolites. *Toxicological Sciences,* 60**,** 92-102.

SUÁREZ-LARIOS, K., SALAZAR-MARTÍNEZ, A.-M. & MONTERO-MONTOYA, R. 2017. Screening of Pesticides with the Potential of Inducing DSB and Successive Recombinational Repair. *Journal of Toxicology,* 2017**,** 1-9.

TAKATA, M., SASAKI, M. S., SONODA, E., MORRISON, C., HASHIMOTO, M., UTSUMI, H., YAMAGUCHI-IWAI, Y., SHINOHARA, A. & TAKEDA, S. 1998. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *The EMBO Journal,* 17**,** 5497.

THOMPSON, L. H. 2012. Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: The molecular choreography. *Mutation Research/Reviews in Mutation Research,* 751**,** 158-246.

THYME, SUMMER B. & SCHIER, ALEXANDER F. 2016. Polq-Mediated End Joining Is Essential for Surviving DNA Double-Strand Breaks during Early Zebrafish Development. *Cell Reports,* 15**,** 707-714.

TRUONG, L. N., LI, Y., SHI, L. Z., HWANG, P. Y.-H., HE, J., WANG, H., RAZAVIAN, N., BERNS, M. W. & WU, X. 2013. Microhomology-mediated End Joining and Homologous Recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. *Proceedings of the National Academy of Sciences,* 110**,** 7720.

TUNG, E. W. Y., PHILBROOK, N. A., BELANGER, C. L., ANSARI, S. & WINN, L. M. 2014. Benzo[a]pyrene increases DNA double strand break repair in vitro and in vivo: A possible mechanism for benzo[a]pyrene-induced toxicity. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 760**,** 64-69.

UNEP. 2008a. *What are POPs?* [Online]. Available: http://chm.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/673/Default.aspx [Accessed 17.04.2019].

UNEP. 2008b. *The 12 initial POPs under the Stockholm Convention* [Online]. Available: http://chm.pops.int/TheConvention/ThePOPs/The12InitialPOPs/tabid/296/Default.aspx [Accessed 17.04.2019].

VAN GENT, D. C., HOEIJMAKERS, J. H. J. & KANAAR, R. 2001. Chromosomal stability and the DNA double-stranded break connection. *Nature Reviews Genetics,* 2**,** 196.

VANDESOMPELE, J., DE PRETER, K., PATTYN, F., POPPE, B., VAN ROY, N., DE PAEPE, A. & SPELEMAN, F. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology,* 3**,** Research0034.

WANIA, F. & MACKAY, D. 1993. Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. *AMBIO. A Journal of the Human Environment*, 2, 10-18.

WILFINGER, W. W., MACKEY, K. & CHOMCZYNSKI, P. 1997. Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity. *BioTechniques,* 22**,** 474-481.

WILLIAMS, G. J., LEES-MILLER, S. P. & TAINER, J. A. 2010. Mre11–Rad50–Nbs1 conformations and the control of sensing, signaling, and effector responses at DNA double-strand breaks. *DNA Repair,* 9**,** 1299-1306.

WISEMAN, S., JØRGENSEN, E. H., MAULE, A. G. & VIJAYAN, M. M. 2011. Contaminant loading in remote Arctic lakes affects cellular stress-related proteins expression in feral charr. *Polar Biology,* 34**,** 933-937.
YUAN, R., FAN, S., WANG, J.-A., MENG, Q., MA, Y., SCHREIBER, D., GOLDBERG, I. D. & ROSEN, E. M. 1999. Coordinate alterations in the expression of BRCA1, BRCA2, p300, and Rad51 in response to genotoxic and other stresses in human prostate cancer cells. *The Prostate,* 40**,** 37-49.
ZHANG, J. & POWELL, S. N. 2005. The Role of the BRCA1 Tumor Suppressor in DNA Double-Strand Break Repair. *Molecular Cancer Research,* 3**,** 531.
ØSTBY, L. & KRØKJE, Å. 2002. Cytochrome P450 (CYP1A) induction and DNA adducts in a rat hepatoma cell line (Fao), exposed to environmentally relevant concentrations of organic compounds, singly and in combinations, *Environmental Toxicology Pharmacology*, 12**,** 15-26.

Appendices

Appendix A

Table A: Experimental data obtained from Neerland (2016) for the individuals of Arctic char investigated in the present study. FTM and MML values indicate levels of DNA double strand breaks. CF = condition factor; HSI = hepatosomatic index; DNA-FTM = DNA fraction of total DNA migrated into the gel; MML = median molecular length of DNA fragments that migrated into the gel; PCB = polychlorinated biphenyls; t-NC = trans-nonachlor.

	ID	Sex	Length (cm)	Weight (g)	Age	Liver w. (g)	CF	HSI	DNA-FTM	MML	ΣPCB (pmol * g^{-1})	t-NC (pmol * g^{-1})
Laksvatn	2	F	49.4	1052.4	12	14.01	0.87	1.33	20.76	361.37	538.6	15.5
	3	M	48.0	1155.9	12	7.67	1.05	0.66	43.27	245.18	416.7	12.2
	4	M	50.8	1050.0	10	8.55	0.80	0.81	31.43	323.16	610.4	15.5
	6	M	51.2	1433.9	12	11.20	1.07	0.78	28.41	320.91	1133.9	28.4
	11	F	45.0	963.0	10	13.18	1.06	1.37	21.84	294.17	921.1	21.6
	13	M	48.7	1181.1	10	9.09	1.02	0.77	37.32	268.56	865.5	23.4
	14	M	51.6	1279.5	12	10.99	0.93	0.86	27.47	328.88	732.1	15.8
	15	M	52.7	1239.3	11	9.53	0.85	0.77	43.26	316.82	775.2	21.2
	23	F	46.8	1097.7	9	11.27	1.07	1.03	25.49	288.71	220.6	7.2
	24	F	46.5	980.8	9	12.90	0.98	1.32	26.28	341.09	365.9	11.3
	25	F	45.0	956.2	9	7.94	1.05	0.83	16.95	235.45	425.3	14.6
	26	F	45.6	973.7	9	16.60	1.03	1.70	44.11	334.73	362.6	12.4
Ellasjøen	66	F	39.4	637.3	10	6.91	1.04	1.08	59.34	286.47	4298.9	15.8
	72	F	38.3	543.6	11	5.69	0.97	1.05	21.2	282.00	2969.2	11.7
	73	M	62.4	2372.6	19	12.72	0.98	0.54	58.88	270.21	135323.4	219
	74	M	55.1	1405.2	17	9.72	0.84	0.69	57.58	268.00	288108.5	326.6
	75	M	50.8	1346.3	15	9.99	1.03	0.74	70.63	213.29	49862.8	122.5
	76	M	48.3	1065.8	17	6.82	0.95	0.64	89.62	167.90	18332.5	44.1
	77	M	40.9	618.7	12	3.52	0.90	0.57	35.23	298.87	8089.4	35.1
	99	F	40.5	588.4	12	5.85	0.89	0.99	51.34	304.37	16804.4	40.1
	100	F	36.2	436.3	11	6.43	0.92	1.47	28.1	258.90	9676.9	30.6

Appendix B

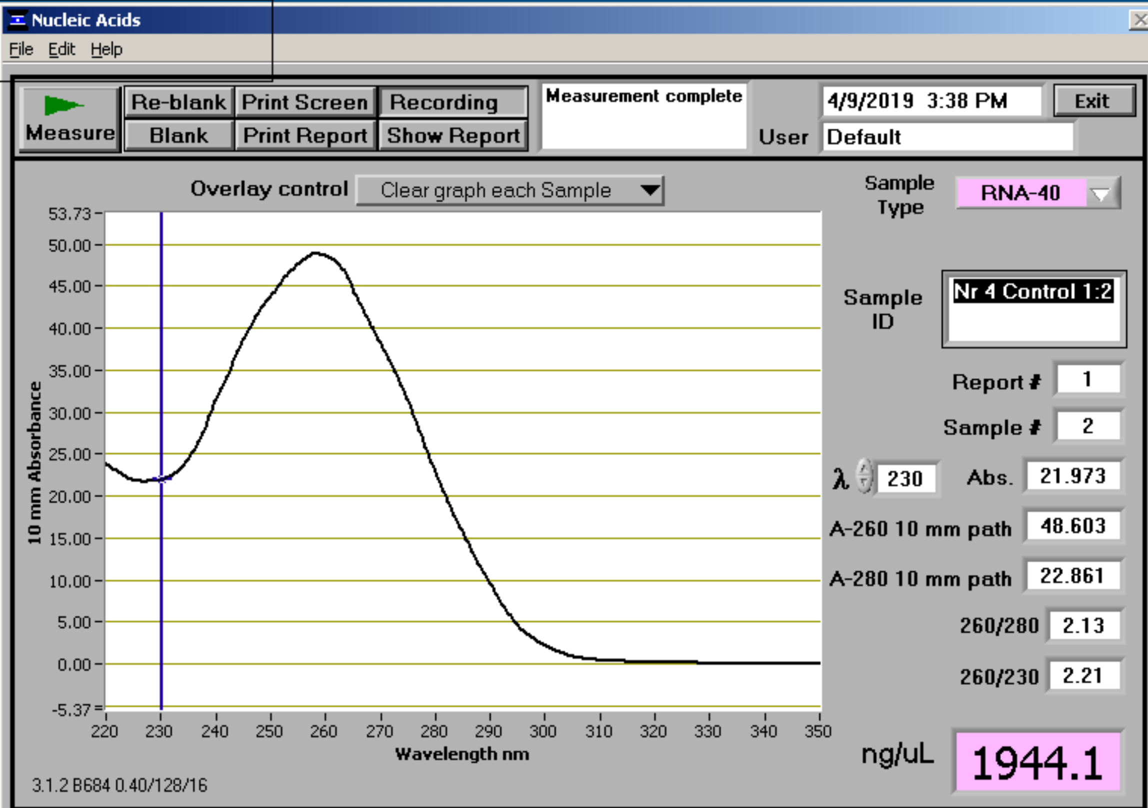


Figure B: Representative curve of the RNA extracts after spectrophotometric measuring using NanoDrop ND1000.

Table B: RNA concentrations after two dilution steps in RNA extracts from liver samples from 21 individuals of Arctic char, measured spectrophotometric using NanoDrop ND1000. The 260/280 and 260/230 ratios indicate purity of the samples.

Sample ID	ng/µl	260/280	260/230	Mean cons. (ng/µl)
2	199.13	1.77	2.24	
2	199.97	1.76	2.23	**199.550**
3	376.02	1.78	2.23	
3	376.94	1.78	2.23	**376.480**
4	491.27	1.73	2.20	
4	492.15	1.73	2.20	**491.710**
6	438.01	1.76	2.21	
6	433.32	1.76	2.21	**435.665**
11	310.87	1.78	2.11	
11	313.73	1.79	2.12	**312.300**
13	341.84	1.80	2.10	
13	337.89	1.80	2.10	**339.865**
14	286.87	1.79	2.22	
14	285.10	1.79	2.24	**285.985**
15	314.77	1.81	2.03	
15	312.87	1.80	2.03	**313.820**
23	344.09	1.78	2.14	
23	343.07	1.79	2.14	**343.580**
24	475.35	1.76	2.17	
24	472.13	1.76	2.17	**473.740**
25	331.05	1.77	2.30	
25	331.77	1.77	2.30	**331.410**
26	454.29	1.75	2.28	
26	452.74	1.75	2.28	**453.515**
66	390.02	1.77	2.08	
66	386.15	1.78	2.08	**388.085**
72	323.67	1.77	2.24	
72	323.63	1.77	2.26	**323.650**
73	415.09	1.77	2.14	
73	415.61	1.77	2.14	**415.350**
74	344.31	1.78	2.30	
74	344.49	1.78	2.30	**344.400**
75	355.63	1.79	2.36	
75	356.60	1.78	2.36	**356.115**
76	282.28	1.80	2.18	
76	279.00	1.80	2.18	**280.640**
77	304.02	1.77	2.40	
77	298.61	1.77	2.40	**301.315**
99	358.91	1.77	2.26	
99	360.70	1.76	2.26	**359.805**
100	373.31	1.77	2.21	
100	371.74	1.77	2.21	**372.525**

Appendix C

Table C1: Set up of the PCR plate with –RT control.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Gene 1, Ind. 2	Gene 1, Ind. 3	Gene 1, Ind. 4	Gene 1, Ind. 6	Gene 1, Ind. 11	Gene 1, Ind. 13	Gene 1, Ind. 14	Gene 1, Ind. 15	Gene 1, Ind. 23	Gene 1, Ind. 24	Gene 1, Ind. 25	Gene 1, Ind. 26
B	Gene 1, Ind 66	Gene 1, Ind 72	Gene 1, Ind 73	Gene 1, Ind 74	Gene 1, Ind 75	Gene 1, Ind 76	Gene 1, Ind 77	Gene 1, Ind 99	Gene 1, Ind 100	Gene 1, NTC	Gene 1, NTC	Gene 1, NTC
C	-RT Gene 1, Ind. 2	-RT Gene 1, Ind. 3	-RT Gene 1, Ind. 4	-RT Gene 1, Ind. 6	-RT Gene 1, Ind. 11	-RT Gene 1, Ind. 13	-RT Gene 1, Ind. 14	-RT Gene 1, Ind. 15	-RT Gene 1, Ind. 23	-RT Gene 1, Ind. 24	-RT Gene 1, Ind. 25	-RT Gene 1, Ind. 26
D	-RT Gene 1, Ind 66	-RT Gene 1, Ind 72	-RT Gene 1, Ind 73	-RT Gene 1, Ind 74	-RT Gene 1, Ind 75	-RT Gene 1, Ind 76	-RT Gene 1, Ind 77	-RT Gene 1, Ind 99	-RT Gene 1, Ind 100	-RT Gene 1, NTC	-RT Gene 1, NTC	-RT Gene 1, NTC
E	Gene 2, Ind. 2	Gene 2, Ind. 3	Gene 2, Ind. 4	Gene 2, Ind. 6	Gene 2, Ind. 11	Gene 2, Ind. 13	Gene 2, Ind. 14	Gene 2, Ind. 15	Gene 2, Ind. 23	Gene 2, Ind. 24	Gene 2, Ind. 25	Gene 2, Ind. 26
F	Gene 2, Ind 66	Gene 2, Ind 72	Gene 2, Ind 73	Gene 2, Ind 74	Gene 2, Ind 75	Gene 2, Ind 76	Gene 2, Ind 77	Gene 2, Ind 99	Gene 2, Ind 100	Gene 2, NTC	Gene 2, NTC	Gene 2, NTC
G	Gene 3, Ind. 2	Gene 3, Ind. 3	Gene 3, Ind. 4	Gene 3, Ind. 6	Gene 3, Ind. 11	Gene 3, Ind. 13	Gene 3, Ind. 14	Gene 3, Ind. 15	Gene 3, Ind. 23	Gene 3, Ind. 24	Gene 3, Ind. 25	Gene 3, Ind. 26
H	Gene 3, Ind 66	Gene 3, Ind 72	Gene 3, Ind 73	Gene 3, Ind 74	Gene 3, Ind 75	Gene 3, Ind 76	Gene 3, Ind 77	Gene 3, Ind 99	Gene 3, Ind 100	Gene 3, NTC	Gene 3, NTC	Gene 3, NTC

Table C2: General set up for the qPCR plates.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Gene 1, Ind. 2	Gene 1, Ind. 3	Gene 1, Ind. 4	Gene 1, Ind. 6	Gene 1, Ind. 11	Gene 1, Ind. 13	Gene 1, Ind. 14	Gene 1, Ind. 15	Gene 1, Ind. 23	Gene 1, Ind. 24	Gene 1, Ind. 25	Gene 1, Ind. 26
B	Gene 1, Ind 66	Gene 1, Ind 72	Gene 1, Ind 73	Gene 1, Ind 74	Gene 1, Ind 75	Gene 1, Ind 76	Gene 1, Ind 77	Gene 1, Ind 99	Gene 1, Ind 100	Gene 1, NTC	Gene 1, NTC	Gene 1, NTC
C	Gene 2, Ind. 2	Gene 2, Ind. 3	Gene 2, Ind. 4	Gene 2, Ind. 6	Gene 2, Ind. 11	Gene 2, Ind. 13	Gene 2, Ind. 14	Gene 2, Ind. 15	Gene 2, Ind. 23	Gene 2, Ind. 24	Gene 2, Ind. 25	Gene 2, Ind. 26
D	Gene 2, Ind 66	Gene 2, Ind 72	Gene 2, Ind 73	Gene 2, Ind 74	Gene 2, Ind 75	Gene 2, Ind 76	Gene 2, Ind 77	Gene 2, Ind 99	Gene 2, Ind 100	Gene 2, NTC	Gene 2, NTC	Gene 2, NTC
E	Gene 3, Ind. 2	Gene 3, Ind. 3	Gene 3, Ind. 4	Gene 3, Ind. 6	Gene 3, Ind. 11	Gene 3, Ind. 13	Gene 3, Ind. 14	Gene 3, Ind. 15	Gene 3, Ind. 23	Gene 3, Ind. 24	Gene 3, Ind. 25	Gene 3, Ind. 26
F	Gene 3, Ind 66	Gene 3, Ind 72	Gene 3, Ind 73	Gene 3, Ind 74	Gene 3, Ind 75	Gene 3, Ind 76	Gene 3, Ind 77	Gene 3, Ind 99	Gene 3, Ind 100	Gene 3, NTC	Gene 3, NTC	Gene 3, NTC
G	Gene 4, Ind. 2	Gene 4, Ind. 3	Gene 4, Ind. 4	Gene 4, Ind. 6	Gene 4, Ind. 11	Gene 4, Ind. 13	Gene 4, Ind. 14	Gene 4, Ind. 15	Gene 4, Ind. 23	Gene 4, Ind. 24	Gene 4, Ind. 25	Gene 4, Ind. 26
H	Gene 4, Ind 66	Gene 4, Ind 72	Gene 4, Ind 73	Gene 4, Ind 74	Gene 4, Ind 75	Gene 4, Ind 76	Gene 4, Ind 77	Gene 4, Ind 99	Gene 4, Ind 100	Gene 4, NTC	Gene 4, NTC	Gene 4, NTC

Appendix D

Table D1: Relative quantity of transcripts for 11 genes involved in DNA DSB repair in liver samples from Arctic char, analysed using quantitative PCR.

Individual	*53BP1*	*ATM*	*BRCA2*	*DNA-PKcs*	*KU80*	*KU70*	*Ligase IV*	*MRE11*	*RAD50*	*RAD51*	*XRCC4*
2	1.4178	1.7639	0.7887	1.3043	1.3356	1.4404	1.3519	1.7385	1.1071	0.1381	1.0722
3	0.7559	1.0217	0.5096	1.1410	2.0696	1.5217	1.6562	1.8097	1.7021	1.5356	1.4840
4	1.0787	0.7054	1.4597	0.6807	0.9702	0.8521	1.2620	0.9052	1.1135	1.4925	0.8729
6	1.1237	0.7005	1.3684	0.8365	0.6893	0.8636	0.8185	0.7720	0.9073	1.8538	0.9175
11	1.1327	0.8497	1.2049	1.8475	0.7815	0.8526	0.5960	0.9042	1.1242	1.3093	1.2488
13	0.9826	0.7400	1.4172	0.7205	1.5694	0.8296	0.7867	0.7567	1.0794	1.7813	1.0188
14	0.9738	0.9433	1.3301	1.3347	1.4813	0.9533	0.8424	0.6732	1.4131	1.2601	0.9541
15	1.1566	1.6686	3.1331	1.9602	3.9247	1.7964	2.0458	1.8677	1.4807	0.6047	1.3219
23	2.1192	0.9126	0.5751	1.9755	0.4021	0.7265	0.6711	0.7446	1.2272	2.3389	1.3804
24	0.6341	1.0745	0.5460	0.7065	0.4972	0.6852	1.1201	0.8622	0.3604	0.5383	0.7863
25	0.3422	1.0508	0.4154	0.1814	0.3327	0.6815	0.7652	0.5959	0.4606	0.5956	0.5716
26	1.3354	1.1014	1.3419	1.3384	1.1406	1.5087	0.9303	1.3819	1.0154	1.2786	0.7882
66	2.6441	2.0212	1.1096	1.6888	0.5711	0.8546	1.7723	1.6064	3.3780	0.4818	3.4615
72	1.5680	3.0872	2.3552	0.7410	0.9645	0.9725	2.2807	3.3227	1.3908	0.0330	0.9502
73	2.2367	0.8224	0.5880	0.9513	0.4037	0.3545	1.3617	1.1816	1.0365	1.6527	1.3152
74	2.9995	0.9394	0.9314	1.1302	1.1773	0.7868	2.4781	1.5429	2.0436	1.1322	1.9472
75	3.5662	1.7883	2.1456	0.3320	1.1845	0.8212	2.9630	4.3585	1.6635	1.2685	2.4394
76	2.4853	1.3916	0.6995		0.6513	0.2938	1.8201	0.9974	0.5267	1.1738	1.0293
77	5.4057	3.2412	2.1383	1.5584	2.4344	1.3770	3.7586	6.0924	3.8086	1.5533	2.6928
99	3.5317	1.3973	3.0488	1.4832	0.8361	0.8336	2.4780	2.8817	2.4211	1.4588	1.6331
100	2.3683	6.4415	2.8205	1.1860	0.3357	0.4709	3.0827	1.6430	2.0864	4.7897	4.9860

Table D2: Ratio of mRNA transcripts in char of Ellasjøen compared to char of Laksvatn for 11 DNA DSB repair genes, with 95 % confidence intervals (ci) and p-values.

Gene	Ratio Ellasjøen/Laksvatn	95% ci low	95% ci high	p-value (Mann Whitney *U*-test)
53BP1	2.82	1.92	4.12	0.0001
ATM	1.92	1.23	2.99	0.0325
MRE11	2.20	1.38	3.52	0.0199
RAD50	1.77	1.09	2.87	0.0416
Ligase IV	2.34	1.69	3.24	0.0004
XRCC4	1.98	1.34	2.91	0.0158
KU70	0.68	0.46	1.00	0.2020
KU80	0.80	0.43	1.50	0.6207
DNA-PKcs	1.03	0.57	1.85	1.0000
RAD51	0.92	0.34	2.50	0.9483
BRCA2	1.50	0.86	2.63	0.2654

Appendix E

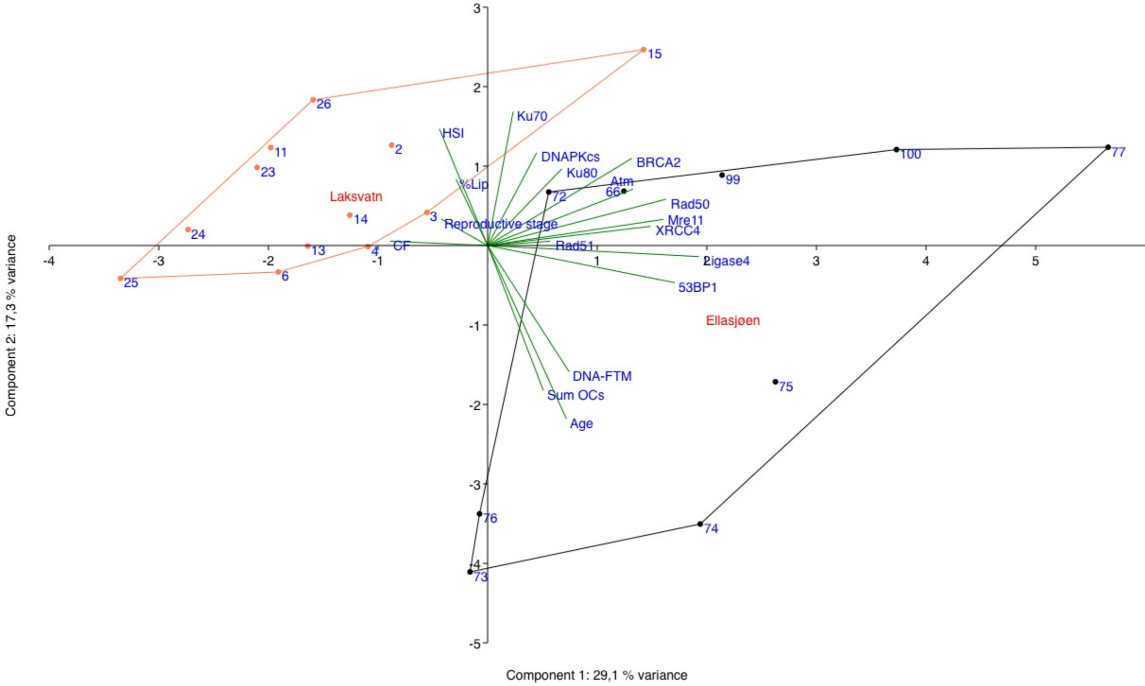
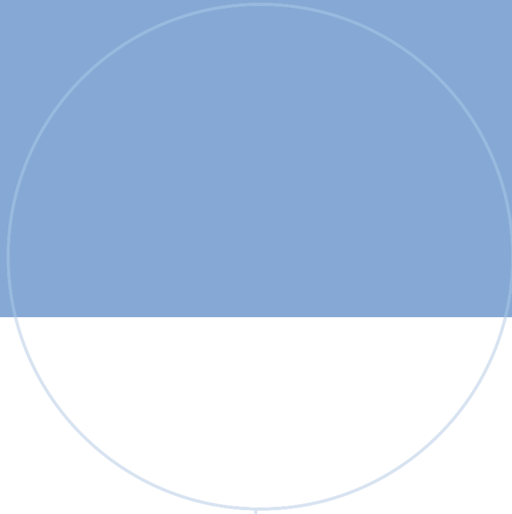
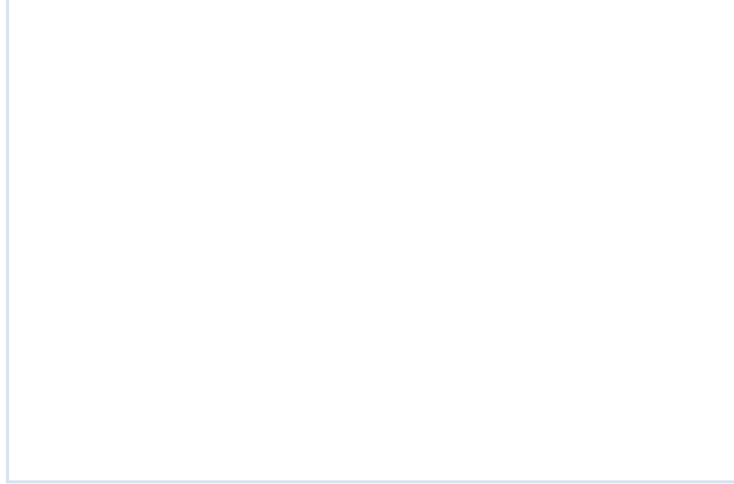


Figure E: PCA biplot with all variables, including age.

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