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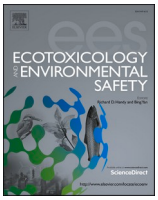

Expression of DNA repair genes in arctic char (*Salvelinus alpinus*) from Bjørnøya in the Norwegian Arctic

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ABSTRACT

High levels of organochlorines (OCs) have been measured in arctic char (*Salvelinus alpinus*) from Lake Ellasjøen on Bjørnøya, Norway (74.30°N, 19.0°E). In a nearby lake, Laksvatn, the OC-levels in arctic char were low. A previous study has shown that char from Ellasjøen had significantly higher levels of DNA double strand breaks (DSBs) than char from Lake Laksvatn. Even though there is increasing evidence of the genotoxic effects of OCs, little is known about the effects of OCs on the DNA repair system. The aim of the present study was to determine if the two main DNA DSB repair mechanisms, homologous recombination (HR) and non-homologous end-joining (NHEJ), are affected by the higher OC and DSB level in char from Ellasjøen. This was analysed by comparing the transcript level of 11 genes involved in DNA DSB repair in char liver samples from Ellasjøen (n = 9) with char from Laksvatn (n = 12). Six of the investigated genes were significantly upregulated in char from Ellasjøen. 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. This induction was positively correlated with the DNA DSB and negatively correlated with one or several OCs for four of these genes. However, the strongest predictor variable for DNA repair genes was habitat, indicating genetic differences in repair capacity between populations. As char from Ellasjøen still had significantly higher levels of DSBs compared to char from Laksvatn, it is possible that chronic exposure to OCs and continued production of DSB has caused selective pressure within the population for fixation of adaptive alleles. It is also possible that DSB production was exceeding the repair capacity given the prevailing conditions, or that the OC or DSB level was above the threshold value of inhibition of the DNA repair system resulting in the rate of DNA damage exceeding the rate of repair.

1. Introduction

High concentrations of organochlorines (OCs) have been measured in sediment and biota from Lake Ellasjøen at Bjørnøya (Svalbard, Norway) (74°30' N, 19°00' E), compared to Lake Øyangen, another lake on Bjørnøya, and other Arctic lakes (Evenset et al., 2004, 2007a). More recently Bytingsvik et al. (2015) showed that arctic char (*Salvelinus alpinus*, hereafter char) from lake Ellasjøen had up to 36 times higher levels of OCs than char from another lake on Bjørnøya, Laksvatn. The elevated levels in Ellasjøen are mainly due to guano depositions from large populations of seabirds using the lake as a resting area (Evenset et al., 2007a). The birds feed in the marine environment and when they rest in the lake they drop guano, containing low levels of organic contaminants, into the water. Over time guano deposition, as well as feathers and residues of dead birds, have caused accumulation of contaminants in Lake Ellasjøen (Evenset et al., 2007a). Studies carried out over the past 15 years have documented elevated levels of OCs, brominated compounds, polychlorinated naphthalenes, chlorinated paraffines and mercury in sediment and biota from Lake Ellasjøen (Evenset et al., 2004, 2005, 2007a, 2007b; Bytingsvik et al., 2015). Resident arctic char is the only fish species inhabiting Lake Ellasjøen, and the levels of polychlorinated biphenyls (PCBs) in this population are among the highest reported in both limnic and marine fish species from the Arctic region (wet weight concentration) (AMAP, 2004). This fish

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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 the elucidation of toxic effects of OCs in the arctic environment.

A few recent studies have documented biological responses to elevated organochlorine contaminant levels in arctic char from Ellasjøen (Wiseman et al., 2011; Jørgensen et al., 2017; Gauthier et al., 2018; Neerland et al., 2019). Further, previous studies have found that arctic char are susceptible to the toxic effects of OCs. These include endocrine effects (Jørgensen et al., 2017), immune system effects (Maule et al., 2005), effects on expression of genes involved in cellular and physiological stress response (Wiseman et al., 2011), metabolomic disruption (Gauthier et al., 2018) and most recently genotoxic effects (Neerland et al., 2019).

Several studies of other species have documented that there is a clear and significant relationship between DNA damage and exposure to OCs (Binelli et al., 2008; Marabini et al., 2011; Fenstad et al., 2014, 2016). PCB metabolites have caused DNA strand breaks in vitro (Srinivasan et al., 2001), while dichlorodiphenyldichlorethylene (p,p'-DDE), a metabolite of the pesticide dichloro-diphenyl-trichloroethane (DDT), has been shown to produce DNA strand breaks in vivo (Binelli et al., 2008). DNA 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). The two main repair mechanisms that have evolved in order to cope with DSBs are: (i) homologous recombination (HR), which is mainly error free, and (ii) non-homologous end-joining (NHEJ), which is error prone (van Gent et al., 2001). Previous studies on DSB repair have been mainly conducted on mammals, and the knowledge of DSB repair in fish is limited. However, both HR and NHEJ have been observed in fish; in early embryonic cells and adult medaka cells (Kienzler et al., 2013).

HR is mediated through a number of proteins that include RAD50, RAD51, RAD52, RAD54, MRE11 (Cromie et al., 2001) and BRCA2 (Ceccaldi et al., 2016). Two protein complexes are required for NHEJ: the DNA dependent kinase complex (DNA-PK), consisting of the 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). Collectively these genes can be quantified to determine the activity of the differing DNA repair systems.

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, 2016). However, little is known about the effects of OCs on other aspects of genotoxicity, such as the induction of DNA defence systems, or potential disturbances of DNA synthesis and repair. DNA repair genes merit scrutiny as a biomarker for genotoxic stress as they can provide insight into the physiological and disease susceptibility for affected populations. Further, investigations into the genomics of defence systems has shown that OCs can act as a selective pressure causing contemporary evolution and fixation of adaptive alleles within a population (Whitehead et al., 2017). While evolutionary adaptations may rescue a population it also causes decreased genetic diversity (Matson et al., 2006), bottle necks and fitness cost (Whitehead, 2014).

The objective of the present study was to investigate if the higher contaminant levels in fish from Ellasjøen affected the DNA-repair system, and if a potential up- or downregulation would be correlated with the DSB and/or OC level. The genes investigated in the present study included five genes specific for NHEJ (*XRCC4, KU80, KU70, DNA-PKcs* and *Ligase IV*), and two genes specific for HR (*RAD51* and *BRCA2*), in order to investigate whether one of the two pathways were more affected than the other. Morphological data, OC content and levels of DNA DSBs in the individuals in the present study were compiled from Neerland et al. (2019).

2. Materials and methods

Additional details concerning the materials and methods are included in Supplementary information (SI).

2.1. Field sampling

Landlocked arctic char were collected from two sites at Bjørnøya (74°30' N, 19°00' E, Fig. 1): Lake Ellasjøen and Lake Laksvatn (reference lake), with the use of gill nets or fishing rods in August to September 2014. Blood samples were obtained from char in the two lakes. Whole blood (500 μl) for DNA DSB analyses was frozen in liquid nitrogen and stored at −80 °C. Muscle samples for chemical analysis were kept at −20 °C. Whole liver was dissected from 21 individuals (n = 9 from Lake Ellasjøen; n = 12 from Lake Laksvatn), flash frozen in liquid nitrogen and stored at −80 °C for further gene expression analysis. The 21 fishes in the present study were also included in the study by Neerland et al. (2019). Otoliths were collected for determination of age, biological variables were measured and a visual inspection of the fish was conducted (SI, Table S1). At the end of the field season the muscle samples were transported to the laboratory of the Norwegian Institute for Air Research (NILU), Tromsø, while the blood samples and livers were transported to the Norwegian University of Science and Technology (NTNU). Permissions to conduct fieldwork in the Bjørnøya National Park were given by the Governor of Svalbard and the Norwegian Animal Research Authority. The fish were handled according to national regulations, and as few fish as experimentally feasible were collected.

2.2. Sample preparation

The whole frozen liver samples were homogenised in 10 ml grinding jars, with 20 mm Teflon grinding balls (Qiagen, Hilden Germany), all precooled to −80 °C. The grinding jars were immediately placed in a Tissue Lyser II (Qiagen, Hilden Germany) for 120 s x 25 Hz. The homogenised samples were stored at −80 °C until RNA extraction.

2.3. RNA isolation and cDNA synthesis

Aliquots of the homogenised liver tissue (30 mg) were lysed and disrupted in 2 ml microcentrifuge tubes with a 5 mm steel bead using Tissue Lyser II (QIAGEN, Hilden, Germany). RNA was extracted by RNeasy Plus Universal Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The quantity of total RNA was measured by NanoDrop spectrophotometry (ND1000, v3.7, ThermoFisher Scientific, USA), and RNA integrity was analysed with Agilent 2100 Bioanalyzer, and Agilent RNA 6000 nano kit (all from Agilent Technologies). The analysis showed no signs of RNA degradation and RNA integrity numbers (RIN) were typically higher than eight.

Complementary DNA (cDNA) was synthesized from 1 μg of total RNA using QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

2.3.1. Primer design

We selected 11 genes involved in DNA DSB damage response and along the two potential main repair mechanisms, HR and NHEJ (Table 1). The primers (Sigma-Aldrich, Germany) were designed using Primer-BLAST by National Center for Biotechnology Information (NCBI), using *Salvelinus alpinus* genomic sequences. The HR genes *RAD52*, *RAD54* and *BRCA1* were originally included, but were excluded from the final analysis due to dysfunctional primer pairs.

2.3.2. Quantitative (real-time) PCR analysis

cDNA corresponding to 25 ng total RNA was analysed using the 480 SYBR Green I Master kit (Roche Applied Science, Germany) in 20 μl reactions and final primer concentration 500 nM. The qPCR reactions were run on a LightCycler® 96 (Roche, Switzerland) with initial

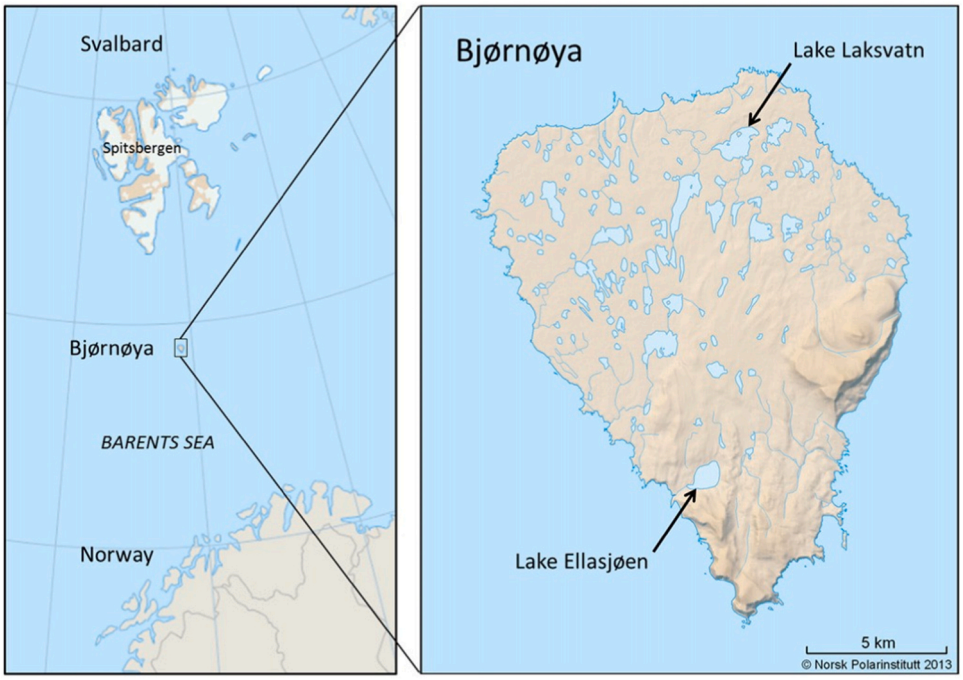

Fig. 1. The location of Lake Laksvatn (reference lake) and Lake Ellasjøen (contaminated lake) on Bjørnøya.

Table 1
Nucleotide sequences used for RT-qPCR.

Gene		Sequence (5'->3')	Amplicon length	Access code	Source
EF1A	Forward	AGGCATTGACAAGAGAACCATT	119	AF498320.1	Jørgensen et al. (2017)
	Reverse	TGATACCACGCTCCCTCTC			
ACTB	Forward	GAAGATCAAGATCATCGCCC	122	NW_019949595	Ahi et al. (2013)
	Reverse	CAGACTCGTCGTACTCCTGCT			
UBiQ	Forward	GACTACAACATCCAGAAAGAGTCCA	120	NC_036844.1	Ahi et al. (2013)
	Reverse	GCGGCAGATCATTTTGTC			
XRCC4	Forward	AGGGACATCTCGTTCTGCCTG	117	NC_036860.1	
	Reverse	GTGGTTCTGCAGTGCGGTTC			
RAD50	Forward	CGTTGAGGAAGCGCATCGAG	175	NC_036860.1	
	Reverse	AAGCCCTTCTGTCGACCCAG			
RAD51	Forward	AGGCTAGCAGACGAGTTTGG	182	NC_036868.1	
	Reverse	ATTTTGCAGATCCTCGTTTCGC			
KU80	Forward	AAGAGTTTTGCTGTGCTGGGC	70	NW_019945760.1	
	Reverse	CCTGAGTCCCCACGAACTGG			
Ligase IV	Forward	GGTTCGTCAGGAAATGTATGATGC	196	NC_036867.1	
	Reverse	GGAACTTGAGCAGCGACAGAG			
MRE11	Forward	CGTTCTCGGAAGGCCTCAGT	198	NW_019942973.1	
	Reverse	TGGTCGTGATGCAGGAGTCA			
ATM	Forward	CCGCCGCAAGTTACTACTGC	158	NC_036841.1	
	Reverse	AGCAAAGATCCGCCAGAGGT			
53BP1	Forward	GCAGGCTTCATCCTCCACGA	91	NW_019957590.1	
	Reverse	TCCTGGTTCGGCTATGCTGG			
DNA-PKcs	Forward	TTGGCGCATTCCGCAAGAAG	82	NC_036854.1	
	Reverse	AAAAGCTTGGCACCACCACG			
BRCA2	Forward	CTATTGCAGGTCAGCTAGCCCC	70	NC_036841.1	
	Reverse	AGCTGCAAGCACTCTGCATCATA			
KU70	Forward	ATGGCCCCAGAGCACATAGAG	183	NC_036858.1	
	Reverse	CTTGGGCTTCTTTTCTGTGCC			

denaturation for 600 s at 95 °C, followed by 45 cycles with 95 °C for 10 s, 55 °C for 10 s, 72 °C for 15 s

The qPCR data was analysed with qbase + 3.2 (Biogazelle, Zwijnaarde, Belgium - www.qbaseplus.com), using Cq values determined by the LightCycler software, and the mean PCR efficiency for each primer set calculated using the LinRegPCR software (Ruijter et al., 2009). The expression of the genes of interest was normalized against the reference genes *EFIA*, as recommended by Olsvik et al. (2005), and *ACTB* and *UBIQ*, found to be among the most stably expressed genes in arctic char by Ahi et al. (2013).

2.4. OC analysis

The analysis of organochlorine concentration in muscle tissue was performed at NILU, Tromsø, as described by Herzke et al. (2003), with modifications from Hallanger et al. (2011). The OCs included in the present study were: PCBs 101, 105, 118, 138, 153 and 180 and trans-nonachlor (t-NC). In statistical analyses measurements below limit of detection (LOD) were assigned a random value between zero and the LOD of that specific compound. The measured OC concentrations per individual char in pmol g^{-1} wet weight is presented in SI, Table S2.

2.5. Data analysis

All statistical analyses were performed in R studio (version 3.6.1). Packages 'pls' and 'ggplot2' were used for partial least squared regressions and graphical representations, respectively.

Principal component analysis (PCA) was used to explore patterns and covariation of the variables in the data set. The variables used in the PCA were the relative transcript level per individual for the 11 genes, as found in the present study; and sum of OC concentrations; the amount of DNA migrating out of the sample well relative to the total amount of DNA loaded (DNA-FTM level); age; condition factor (CF; (body weight (g) x body length $(cm)^{-3}$) x 100); hepatosomatic index (HSI; liver weight (g) x body weight $(g)^{-1} \times 100$); %lip and reproductive stage, obtained by Neerland et al. (2019). Partial least-squares (PLS) analysis was used to model gene expression (*XRCC4, RAD50, RAD51, KU80, Ligase IV, MRE11, ATM, 53BP1, DNA-PKcs, BRCA2* and *KU70*) as a function of lake, DNA-FTM, age, *trans*-nonachlor (t-NC), and PCB congeners; 101, 105, 118, 138, 153 and 180. Lake was included in the analysis to account for differences in habitat that could not be sampled for or included in this study. PLS modelling was chosen over linear regressions to account for the small sample sizes and issues of multicollinearity between predictor variables. Furthermore, PLS enables analysis of variance in the dependent variable (expression of DNA repair genes) without penalization of increasing parameters (Carrascal et al., 2009). All data was scaled to unit variance (mean=0, SD=1) and centred before analysis. Coefficient plots were used to determine significant variables affecting gene expression. Variables were considered significant if their 95% confidence intervals did not include zero.

Based on the coefficient plots, linear models were run to confirm the relationship and effect size between independent variables and gene expression. All significant factors from the PLS were included in the linear model. However, in situations where multiple PCBs were significant (*ATM, MRE11*), PCBs were grouped together in ΣPCBs which was then used in the linear model to avoid issues of overfitting. A backward stepwise procedure was then used to simplify the model by excluding non-significant variables until the most parsimonious model was achieved, while still including all significant factors and interactions. Histograms, Q-Q plots, leverage plots, fitted plots and scale-location plots of the residuals were checked for normality and assurance that all assumptions of linear models were met.

All gene expression and OC data were log-transformed to create normal distribution of residuals. The statistical level of significance was set at $p < 0.05$.

3. Results

3.1. Relative transcript levels

There were significantly higher transcript levels for 6 of the 11 genes analysed in char from.

Ellasjøen than in char from the control lake Laksvatn (Fig. 2), 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 0.0416, respectively). The variation of relative quantity of transcripts was quite high in both comparison groups. The clear differences between the two sample groups regarding these six genes were further visualised by the PLS analysis (Fig. 3). 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 from Ellasjøen. Neither *RAD51* nor *BRCA2* from HR were significantly differently regulated in the char of Ellasjøen. However, *BRCA2* showed trends of being slightly induced.

3.2. OC contaminant levels

The levels of seven OCs (PCBs 101, 105, 118, 138, 153 and 180 and trans-nonachlor (t-NC)) were measured in muscle of the individuals that were analysed for DNA damage response. Char from Lake Ellasjøen had significantly higher levels of OC contaminants than fish from Lake Laksvatn. Average ∑OC concentrations in char from Ellasjøen were 94 times higher than in char from Laksvatn, 59,367.9 ± 95,625 and 630.6 ± 280 pmol g^{-1} wet weight (±SD), respectively. The greatest difference was found for PCB 153, which on average was 129 times higher in the char from Ellasjøen than in char from Laksvatn. The individual measurements for OC compounds can be found in SI (Table S2).

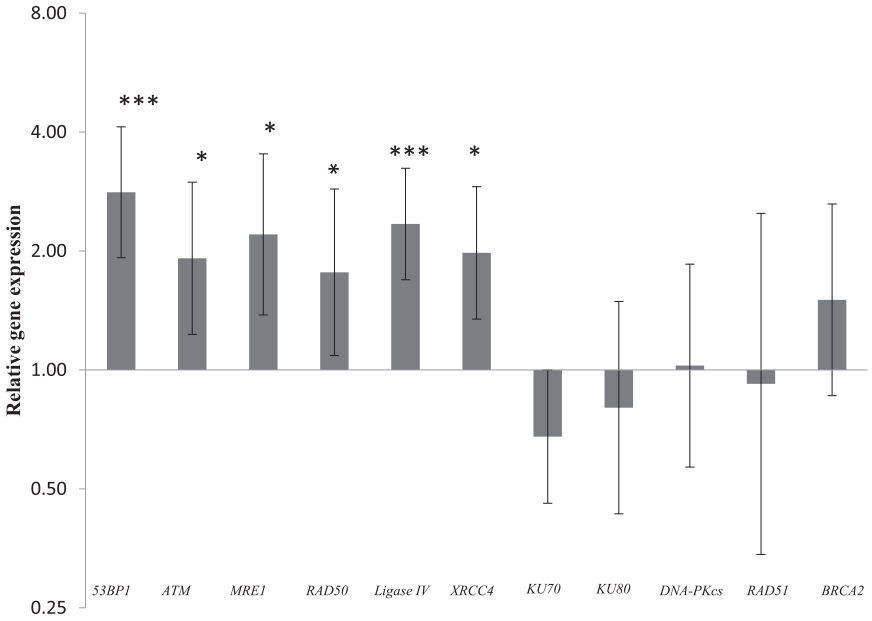

Fig. 2. Bars show relative gene expression of 11 genes involved in DNA DSB repair in Arctic char (*Salvelinus alpinus*) from Lake Ellasjøen (n = 9) compared to Lake Laksvatn (n = 12; n = 11 for *DNA-PKcs*). A relative gene expression higher than 1 indicate higher gene expression in sample from Lake Ellasjøen compared to Lake Laksvatn. Error bars represent 95% confidence interval. * = p-value from 0,01 to 0,05; *** = p-value from 0,0001 to 0001.

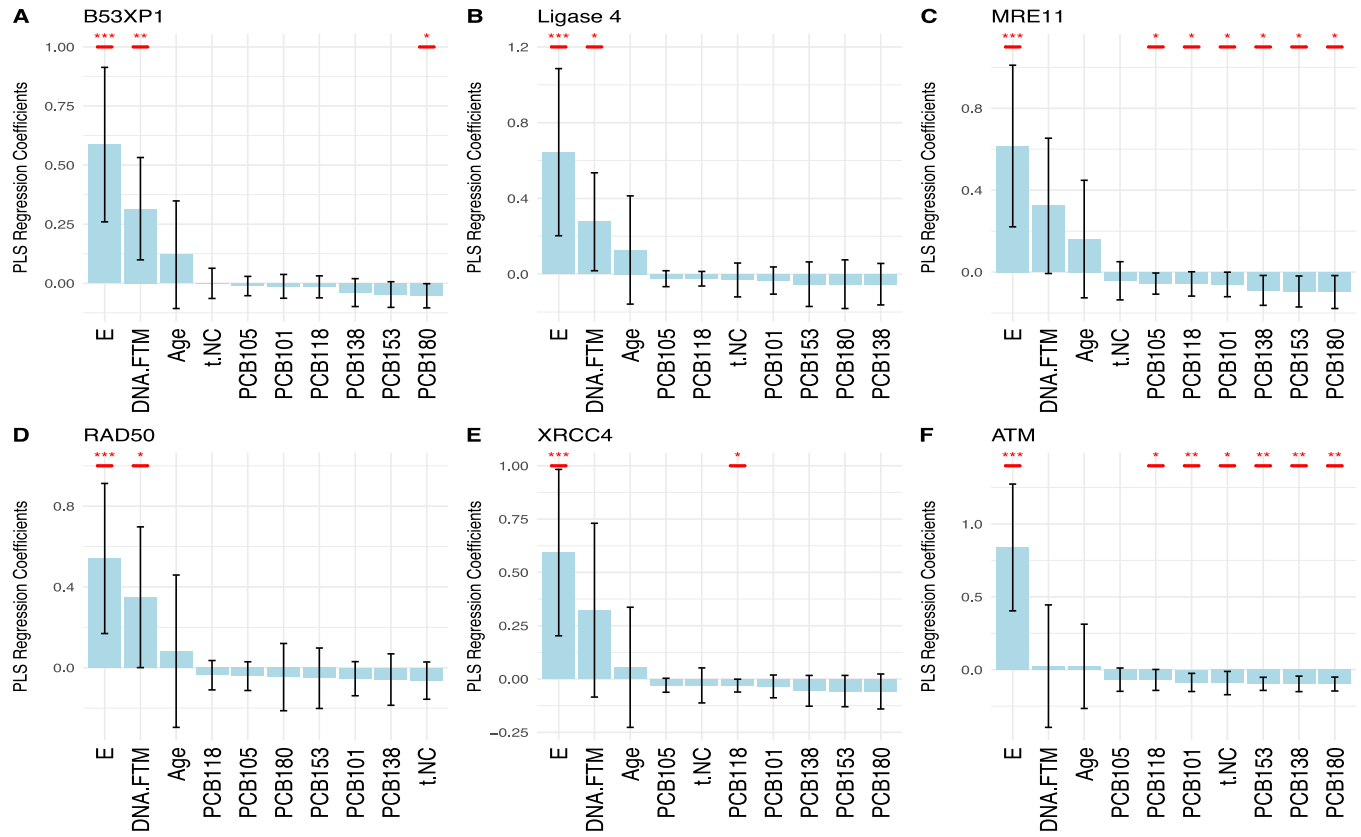

Fig. 3. Coefficient plots from the PLS models with DNA-repair gene expression in Arctic charr (*Salvelinus alpinus*) from Lake Ellasjøen (n = 9) and Lake Laksvatn (n = 11). Gene expression was a response variable and Lake, DNA-FTM, Age, t.NC, PCB congers; 101, 105, 118, 153, 138 and 180 were all predictor variables. A positive relationship to gene expression is depicted by boxes above zero while negative relationships are represented by boxes below zero. When the 95% confidence intervals do not cross zero the coefficients are significant, as denoted by asterisks (*,**,**) representing a p-value of <0.05, <0.01, and <0.001, respectively.

3.3. Multivariate analyses

A PCA was conducted in order to visualise potential correlations between the transcript levels and the level of OCs, DNA DSBs and biological parameters in the individuals (Fig. 4). PC1 explains 30.33%, while PC2 explains 15.88% of the variability in the data set. The individuals from the two populations clustered on opposite sides of the plot. Sum OCs, DNA-FTM and age were positively correlated. The biological variables: reproductive stage, CF, %lip and HSI were not correlated with the expression of the upregulated genes.

The coefficient plots from the PLS models showed that transcript levels of *53BP1, Ligase IV* and *RAD50* were best explained by levels of DNA DSBs and the lake of origin (Fig. 3). None of the OCs, or age, could significantly explain transcript levels of *Ligase IV* or *RAD50*. However, PCB180 explained some of the variation in transcript levels of *53BP1*. Transcript levels of *MRE11, XRCC4* and *ATM* were best explained by lake of origin as well as by one or several OCs. However, neither DNA-FTM nor age could significantly explain any variation in the transcript levels of *MRE11, XRCC4* and *ATM*. These results were only partly confirmed by linear modelling and reflected in the low R^2Y and Q^2 of the PLS models. Lake of origin was still highly significant for transcript levels of all six genes (*53BP1, Ligase IV, RAD50*, *MRE11, XRCC4* and *ATM*), however DNA-FTM and OCs were no longer significant for any gene except *ATM*. There was a negative association between *ATM* gene expression and increasing PCB concentrations in both lakes (Fig. 5). DNA-FTM and OCs have likely lost significance once modelled linearly due to issues of overfitting as the number of parameters approached the number of observations (Carrascal et al., 2009). In summary, *53BP1, Ligase IV, RAD50*, *MRE11,* and *XRCC4* transcript levels were affected only by their lake of origin, while *ATM* transcript levels showed a significant decrease based on increasing PCB180 concentrations.

4. Discussion

There is increasing evidence of the genotoxic effects of OCs, mostly in the form of DNA strand breaks (Srinivasan et al., 2001; Krøkje et al., 2006; Fenstad et al., 2014, 2016; Neerland et al., 2019). The present study gives further insight into the molecular effects of OCs on DNA stability in chronically exposed fish. The results showed that there were significant differences in the gene expression of DNA repair systems between fish from the two lakes. The expression levels of six of the investigated genes were approximately two-fold higher in the char from Ellasjøen compared to char from Laksvatn. The genes induced in the Ellasjøen char were two genes specific for NHEJ (*XRCC4* and *Ligase IV*) and three genes important in several aspects of DNA DSB repair (*53BPI, ATM* and *MRE11*). None of the investigated genes specific for HR were significantly upregulated. However, NHEJ and HR are not mutually exclusive (Takata et al., 1998; Rapp and Greulich, 2004) and both groups have shown to be upregulated after exposure to different stressors (Reinardy et al., 2013a, 2013b; Rhee et al., 2013). It is therefore possible that both pathways are induced, despite the lack of upregulation of *RAD51* and *BRCA2* in the present study. Further, HR is mainly active during the S and G2 phases of the cell cycle, while NHEJ is active throughout the whole cell cycle. However, our results cannot determine the variations in expression levels during the cell cycle because they reflect the situation in the cells at the exact time point of freezing. It is also noteworthy that for NHEJ, only genes from XRCC4/Ligase IV complex were upregulated in the char from Ellasjøen, indicating repair complexes may be sensitive to the level of contaminants and/or level of DNA damage. There were significant correlations

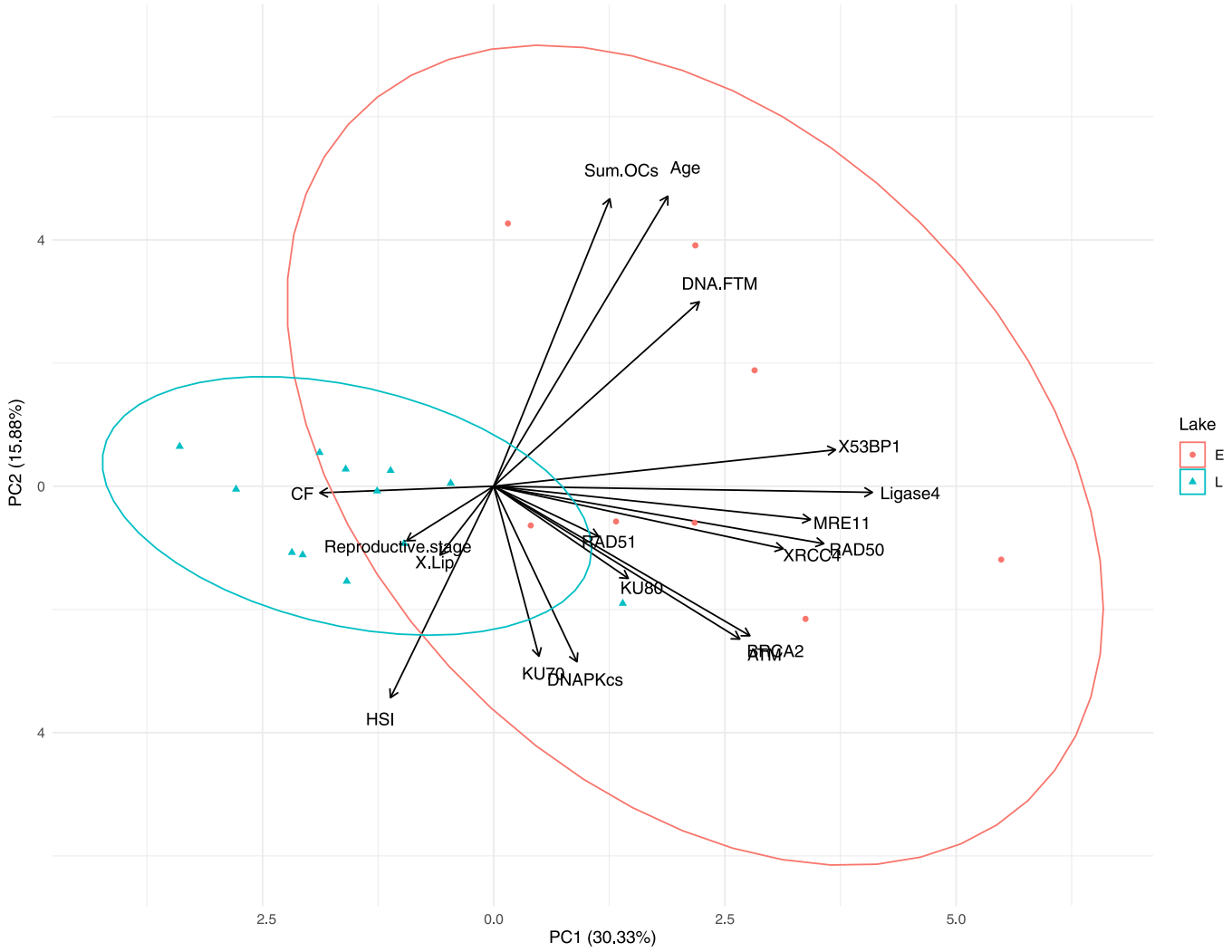

Fig. 4. Principal component analysis biplot of the relative transcript level of 11 genes, OC content, DNA-FTM values and biological variables for 9 individuals of Arctic char (*Salvelinus alpinus*) from the contaminated Lake Ellasjøen (orange circles) and 12 individuals from Lake Laksvatn (blue triangles). 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.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between OC concentrations and DNA repair gene expression with a decrease in DNA repair activity due to increasing levels of contamination in the lake Ellasjøen. The relationship between gene expression and OC 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 OCs are lipophilic, high body fat content protects against impact from lipophilic chemicals (Lassiter and Hallam, 1990). Low tissue body fat content will 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 enhanced sensitivity to very low tissue fat contents. The fat content of the liver of the char investigated in the present study has not been measured. However, Neerland et al. (2019) reported low lipid levels (range 0.2–1%) in muscle, and it is therefore likely that lipid levels were also low in liver, rendering the char more susceptible to negative effects of OCs.

To the best of our knowledge, no other studies concerning the effect of OCs on the DNA repair system in fish have been performed. Effects of other organic contaminants have been studied in fish (Marques et al., 2014; Kienzler et al., 2015), mammals (Tung et al., 2014; Dong et al., 2015; He et al., 2015; Pinto et al., 2015; Philbrook and Winn, 2016; Suárez-Larios et al., 2017) and earthworms (Qiao et al., 2007). However, there were no clear trends for the effects of organic contaminant exposure on DNA repair systems: both increases and decreases of DNA repair capacity and expression of DNA repair genes have been observed. Furthermore, as different types of organic pollutants may exert different effects in organisms, across species extrapolation may not be valid. Lastly, the char in Ellasjøen are exposed to a complex mixture of both organic and inorganic substances. For example, unpublished data from 2001 to 2003 show that char of Ellasjøen had higher levels of both mercury (Hg) and MeHg in muscle tissues than in char of Øyangen (Christensen, 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. Heavy metals could contribute to a potential cocktail effect and potential additive, antagonistic or synergistic effects in the affected organism. As there is still limited knowledge of the effects of OCs or other persistent organic pollutants (POPs) on the DNA repair system in fish, it remains for future studies to confirm the effects observed in the present study.

DNA damage also showed positive correlations to DNA repair gene expression. Increased levels of DNA repair systems do not necessarily equate maintenance of the genome. 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

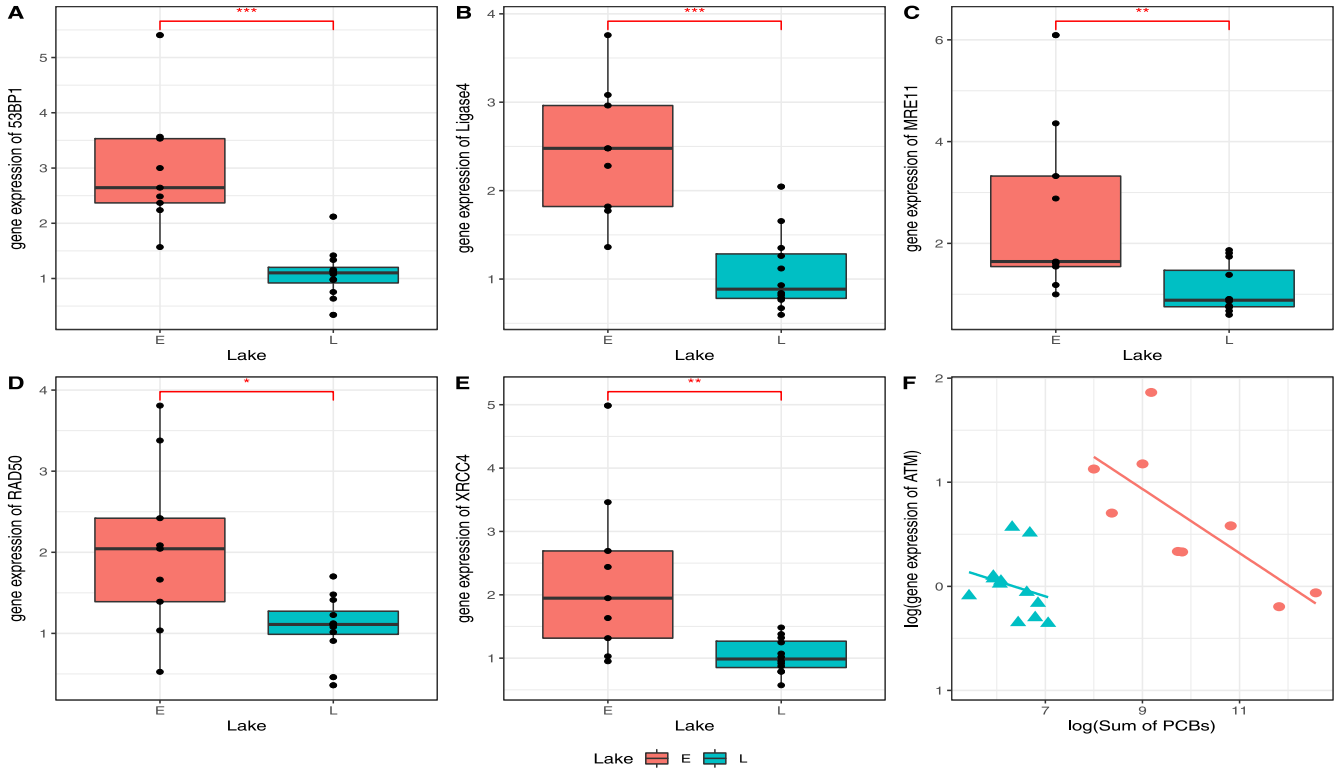

Fig. 5. Relationships between gene expression and significant predictor variables from the linear model analysis (Fig. 2). Linear models were run with the significant variables, denoted by red dots, then simplified by excluding non-significant variables from the linear models. The results presented here represent only the significant predictor variables for gene expressions.

(Gaymes et al., 2002; Nowicki, 2004). Induction of this defence mechanism can thus result in an accumulation of DNA mutations. If the obtained mutations affect the meiotic cells, they can be transferred to subsequent generations contributing to the mechanism of toxicity and carcinogenesis.

Although the induced DNA repair capacity is increased in char of Ellasjøen, they still had significantly higher levels of DSBs compared to char of Laksvatn (Neerland et al., 2019). DNA repair mechanisms allow cells to tolerate low doses of genotoxicants, but these repair system may become saturated at high levels of DNA damage (Doak et al., 2008; Jenkins et al., 2010; Song et al., 2016). If the DNA damage surpasses the capacity of the DNA repair system it would be impossible to prevent the accumulation of DNA damage (Jenkins et al., 2010). This would explain why, in the present study, we do not observe inverse correlations between DNA repair and damage. Furthermore, upregulation at the transcript level does not necessarily mean there is an equal increase at the protein level, and thus increased DNA repair capacity (Yuan et al., 1999). It is possible that some of the proteins involved in HR or NHEJ are positively or negatively regulated at the protein level through e.g. phosphorylation, acetylation or ubiquitylation (Thompson, 2012). To be certain that DNA repair capacity indeed has increased, the level of DNA repair protein should also be considered in future studies (Iwanaga et al., 2004).

The most significant predictor variable for gene expression of DNA repair genes in the present study was habitat. Lake of origin was most strongly correlated to the expression of 6 of the 11 genes included in this study. This is an indication of different genomics between the populations irrespective of DNA damage or OC levels. Previous studies have shown that chronic exposure to stressors can cause contemporary evolution and the subsequent fixation of adaptive alleles into a population (Turcotte and Talbot, 2013). Low doses of radiation have shown to cause adaptive DNA damage and oxidative stress responses in birds living around Chernobyl (Galvan, 2014). Additionally, fish populations chronically exposed to high levels of POPs have shown selective duplication and deletions in their genomes to favour desensitization to pollution (Reid et al., 2016). Therefore, the increased levels of DNA repair gene expression observed in lake Ellasjøen could be an adaptive response of chronic exposure to OCs. Furthermore, these adaptations often result in the desensitization or reverse effect than what is observed in control populations upon exposure to stressors. For example, CYP1A activation also decreases in the presence of PCBs in the fish populations mentioned above (Reid et al., 2016). This would explain the negative correlation between OCs and gene expression observed in our study and be further evidence of adaptation occurring in the char residing in the highly contaminated Ellasjøen. However, while these adaptive responses enable populations to survive in contaminated environments, they are not without long-term adverse effects (Matson et al., 2006). Decreased genetic diversity, bottle necks, and fitness costs are all related to adaptation and pose serious threats to entire populations (Matson et al., 2006; Reid et al., 2016). This is the first study indicating contemporary evolution, a mechanism that may have severe population wide effects, and that should be the subject of further studies.

Arctic organisms are facing multiple threats, from climate change and landscape alterations to pollution (Usher et al., 2005). Increasing loads of contaminants in aquatic environments impair the health, fitness, growth and fecundity of aquatic organisms (Mitra et al., 2018). Thus, genotoxicants are only one of many environmental stressors that affected organisms must endure, all of which require different types of defence mechanisms. As an individual has limited amounts of energy, it must allocate its energy to the most vital processes and make compromises to maximize survival and reproductive success. It is possible that chronic exposure to OCs has already altered the genomic landscape of the char found in Ellasjøen, making them less equipped to handle future stressors and reducing the overall fitness of the individuals.

5. Conclusion

In the present study, six of eleven investigated DNA DSB repair genes were significantly upregulated in char from Lake Ellasjøen compared to char from the reference Lake Laksvatn. Genes from the Ligase IV/XRCC4 complex were significantly induced, but the same was not found for genes involved in the DNA-PK complex, indicating that these two complexes are differentially regulated in response to genotoxic stress.

Individuals with upregulated DNA DSB repair genes still have significantly higher levels of DSBs compared to char of Laksvatn. This could be due to DSB levels exceeding the threshold value for DNA repair, i.e. the rate of repair is in-sufficient to keep up with the rate of damage. Only the expression of *ATM* was significantly negatively correlated to any of the OCs. Habitat was most strongly correlated to expression level indicating genomic differences between populations. Further, taken together with the negative correlation between gene expression and OCs it is likely that contemporary evolution is occurring as a means of adaptation to high levels of contaminants in the char in Ellasjøen.

CRediT authorship contribution statement

Helene Inderberg: Writing - original draft, Writing - review & editing, Methodology, Data curation. **Eirik Neerland:** Writing - original draft, Methodology, Software, Data curation. **Molly McPartland**: Writing - original draft, Writing - review & editing, Methodology, Visualization, Software. **Torfinn Sparstad**: Resources, Formal analysis, Validation, Methodology. **Jenny Bytingsvik**: Methodology, Data curation. **Vladimir A Nikiforov:** Resources, Formal analysis, Validation, Methodology. **Anita Evenset**: Project administration, Methodology, Funding acquisition, Writing - original draft, Writing - review & editing. **Åse Krøkje**: Project administration, Conceptualization, Methodology, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111846.

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