Environmental Toxicology and Chemistry—Volume 38, Number 11—pp. 2405–2413, 2019
Received: 12 April 2019 | Revised: 13 May 2019 | Accepted: 19 July 2019

Environmental Toxicology

DNA Double-Strand Breaks in Arctic Char (*Salvelinus alpinus*) from Bjørnøya in the Norwegian Arctic

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Abstract: High levels of organochlorine contaminants (OCs) have been found in arctic char (*Salvelinus alpinus*) from Lake Ellasjøen, Bjørnøya (Norwegian Arctic). The aim of the present study was to investigate the potential genotoxic effect of environmental organochlorine contaminant exposure in arctic char from Ellasjøen compared with arctic char from the low-contaminated Lake Laksvatn nearby. Blood was analyzed using agarose gel electrophoresis and image data analysis to quantify the fraction of total DNA that migrated into the gel (DNA-FTM) as a relative measure of DNA double-strand breaks (DSBs). Analysis by GC-MS of muscle samples showed an average 43 times higher concentration of ΣOCs in arctic char from Ellasjøen (n = 18) compared with Laksvatn char (n = 21). Char from Lake Ellasjøen had a much higher frequency of DSBs, as measured by DNA-FTM, than char from Lake Laksvatn. Principal component analysis and multiple linear regressions show that there was a significant positive relationship between DSBs and levels of organochlorine contaminants in the char. In addition, DSBs were less frequent in reproductively mature char than in immature char. The results suggest that organochlorine contaminants are genotoxic to arctic char. *Environ Toxicol Chem* 2019;38:2405–2413. © 2019 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals, Inc. on behalf of SETAC.

Keywords: Anthropogenic contaminants; Arctic; Fish; Genotoxicity; Organochlorines

INTRODUCTION

Freshwater fish from certain Arctic locations have been reported to contain high levels of organochlorine contaminants (OCs), with Σpolychlorinated biphenyls (PCBs) higher than 1000 ng g^{-1} lipid weight in some cases (Evenset et al. 2004; Christensen and Evenset 2011; Bytingsvik et al. 2015). Although structurally and functionally diverse, many organochlorine contaminants share the common characteristics of being persistent, accumulative in the environment and biota, and toxic (Letcher et al. 2010). Despite international regulations, organochlorine contaminants are still considered a threat to Arctic wildlife, particularly apex predators such as the polar bear (*Ursus maritimus*; Oskam et al. 2003) and glaucous gulls (*Larus hyperboreus*; Verreault et al. 2010). Compared with avian and mammalian top predators, very limited data exist on possible effects of organochlorine contaminants in fish in the Arctic.

Although not that well studied, there are reports showing a clear and significant relationship between DNA damage and exposure to organochlorine contaminants (Binelli et al. 2008; Marabini et al. 2011; Fenstad et al. 2014, 2016). Srinivasan et al. (2001) showed that PCB metabolites can induce breaks in DNA strands in vitro, and Binelli et al. (2008) showed that dichlorodiphenyldichloroethylene, a metabolite of the pesticide dichlorodiphenyltrichloroethane, caused DNA strand breaks in vivo. Genotoxic effects of chemical exposure are of great concern because alteration in the genetic material may have severe consequences for individuals and populations (Friedberg et al. 2006; Brown et al. 2009; Bickham 2011). The DNA double–strand break (DSB) is one of the most severe DNA lesions because it disrupts the continuity of the genetic template, essential for replication and transcription. In somatic cells, DSBs may result in loss of chromosomes, cell death, mutations, chromosomal rearrangements (Jackson 1999; Friedberg et al. 2006), and carcinogenesis (Jeggo 1998; Kanaar et al. 1998). Accumulation of DNA damage and mutations may lead to neurodegenerative diseases, accelerate the aging process, and have negative effects on reproduction

This article contains online-only Supplemental Data.

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Published online 25 July 2019 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.4546

(Friedberg et al. 2006; Devaux et al. 2011). In germ cells DSBs could affect fertility, fecundity, and progeny (Jha 2008). All of the above outcomes will precede potential higher-level effects of genotoxicants (Bickham et al. 2000). One of these higher-level effects is that the selection pressure imposed by the contaminants could lead to a loss of genetic variation, especially in combination with other stressors such as climate change (Bickham 2011; Moe et al. 2013).

Not much is known about the genotoxic effects of organochlorine contaminants in Arctic wildlife. Some studies have been carried out, for example, on glaucous gulls (*L. hyperboreus*) fed environmentally contaminated eggs (Østby et al. 2005; Krøkje et al. 2006) and on fasting common eiders (*Somateria mollissima*; Fenstad et al. 2014, 2016). According to the literature, no studies of genotoxicity in wild Arctic fish have been performed.

Large seabird colonies reside on the steep cliffs of the southern part of Bjørnøya (English, Bear Island), Svalbard, Norway. The birds have a marine diet but use Lake Ellasjøen as a resting area. Over time large quantities of guano, containing organochlorine contaminants, have been deposited in the lake, causing elevated levels of organochlorine contaminants in the water, sediment, zooplankton, and arctic char (*Salvelinus alpinus*, hereafter char) in Lake Ellasjøen (Evenset et al. 2007; Bytingsvik et al. 2015). Arctic char is the only fish species present in the lakes on the island (Klemetsen et al. 1985). Only one study has measured the potential biological responses to elevated organochlorine contaminant levels in arctic char from Ellasjøen. Char from Ellasjøen had 50-fold higher liver *Cyp1A* protein expression compared with char from a low-contaminated lake on Bjørnøya (Lake Øyangen), in addition to lower glucocorticoid receptor protein expression and elevated expression of heat shock proteins (Wiseman et al. 2011).

In the present study, we used gel electrophoresis to examine the integrity of DNA in blood cells of land-locked char from 2 lakes at Bjørnøya, Lake Ellasjøen and Lake Laksvatn—respectively, high- and low-contaminated lakes. By conducting electrophoresis under neutral pH conditions, the detection of relative DNA DSB frequency is possible because the duplex structure of DNA is maintained, and migration of DNA within the gel depends on the release of the duplex fragments produced by DNA DSBs. The fraction of DNA that migrate out of the sample well relative to the total amount of DNA loaded onto the gel, that is, the DNA fraction of total migrating (DNA-FTM), can be used to indicate DSB frequency (Theodorakis et al. 1994; Fenstad et al. 2014, 2016).

The aim of the present study was to investigate the potential genotoxic effect, measured as DNA DSB frequency, of environmental exposure to organochlorines in char from the relatively highly polluted population of Lake Ellasjøen compared with the population from the low-contaminated Lake Laksvatn. Furthermore, we assessed whether biological factors, such as lipid content or age, affect the potential genotoxic response.

MATERIAL AND METHODS

Field sampling

Blood samples were obtained from char in 2 different lakes at Bjørnøya (74°30′N, 19°00′E), Svalbard: Lake Ellasjøen (n = 18, 11 males and 7 females) and Lake Laksvatn (n = 21, reference lake, 12 males and 9 females) in August to September 2014 (Supplemental Data, Table S3). Whole blood (500 μL) for DNA DSB analysis was frozen in liquid nitrogen, stored at −80 °C, and transported to the Norwegian University of Science and Technology at the end of the field season. Muscle samples for chemical analysis were kept at −20 °C and transported to the laboratory at the Norwegian Institute for Air Research, Tromsø, at the end of the field season. Otoliths were collected for age determination, and biological variables were measured including visual inspections of the fish. This includes fork length (centimeters), body weight (grams), sex, reproductive stage, gonad weight (grams), and liver weight (grams). The following indices were calculated: gonadosomatic index (GSI), (gonad weight × body weight^{-1}) × 100; hepatosomatic index (HSI), (liver weight × body weight^{-1}) × 100; and condition factor, (body weight × fork length^{-3}) × 100. Reproductive stage was determined in the field, where fish in stage 1 show no signs of reproducing in the current season and those in stage 7 (maximal score) are past spawning in the current season (Sømme 1941; see Supplemental Data for details). The present study complies with the Norwegian regulation on animal experimentation, and permissions to conduct the fieldwork in Bjørnøya National Park were obtained from the governor of Svalbard and The Norwegian Animal Research Authority.

Detection of DNA DSBs

The DNA DSB analyses were performed at the Department of Biology, Norwegian University of Science and Technology. Agarose plugs for electrophoresis were prepared according to the procedure described by Theodorakis et al. (1994) and others (Krøkje et al. 2006; Fenstad et al. 2014, 2016). Blood samples of the 39 char were used to determine DNA-FTM. The DNA fragments released from the lysed blood cells and embedded in low–melting point agarose plugs were electrophoretically separated by size. The relative amounts of DNA left in the well and the DNA that had migrated into the gel after electrophoresis were determined by the area under the respective DNA staining intensity curves. The value of DNA-FTM was expressed as a percentage of DNA migrated of the total DNA loaded in the gel and used as a measure of DSB frequency (Fenstad et al. 2014). The median molecular length (MML) of DNA fragments in the gel was calculated by using densitometric data obtained from the gel image analysis. More DSBs result in higher DNA-FTM and a lower MML. A more detailed description is available in the Supplemental Data, including an image of a representative gel.

The gels had 15 lanes: the outermost and the middle lanes were occupied by a DNA size marker and the other by 4 samples in triplicate. Each gel setup was run twice, so in total every sample was run and subsequently measured 6 times. Whole-blood samples were chosen at random, but samples from both lakes were run in each gel.

The dispersion in both MML and DNA-FTM values of the replicates of a sample was generally low, with mean coefficients of variance (±standard deviation [SD]) of 5.6% (±3.9) and 4.2% (±4.0).

Chemical analysis

Analysis of muscle tissue concentrations of organochlorine contaminants was performed at the Norwegian Institute for Air Research, Tromsø, as described by Herzke et al. (2003) with modifications from Hallanger et al. (2011). The final data sets included the following organochlorine contaminants: PCBs 101, 105, 118, 138, 153, and 180 and *trans*-nonachlor (t-NC). The contaminants omitted were PCBs 28 and 52; *oxy*-, *trans*-, and *cis*-chlordane; hexachlorobenzene; *cis*-nonachlor; and Mirex. Measurements below the limit of detection (LOD) were replaced by a random integer between 0 and the LOD of that specific compound. The organochlorine contaminant levels are presented and used in pmol g^{-1} wet weight, unless noted otherwise.

Data analysis

All statistical procedures were performed in R Studio (Ver 1.0.153), an integrated development environment for R (Ver 3.3.0; R Core Development Team 2015). Principal component analysis (PCA) was carried out with the "FactoMineR" package, whereas "ggplot2" was mainly used to plot figures.

The PCA was used to explore the relationships between and covariation of the variables in the data set and as a tool to aid in the construction of linear models. The variables used in PCA were the individual organochlorine contaminant concentrations, age, condition factor, HSI, reproductive stage, percentage of lipids, and DNA-FTM. Only DNA-FTM was chosen to represent DNA damage because it contained a smaller coefficient of variation than MML. The organochlorine contaminant concentrations were $\log_e$-transformed to reduce their impact on the construction of the components. Fork lengths, body weights, and liver weights were added as supplementary variables—not contributing to the construction of the dimensions, only projected onto them—because they were part of the compound variables (i.e., condition factor and HSI).

Linear regression models were used to investigate relationships between DNA-FTM, as the explanatory variable, and ΣOCs and the biological measurements, as response variables. Values of DNA-FTM and ΣOCs were $\log_e$-transformed to be normally distributed. Candidate models were set up based on a priori expectations and indications from the prior PCA. A stepwise selection process was undertaken to find the best model. The corrected Akaike's information criterion (AICc; Akaike 1974) and the coefficient of variance (R^2) of the models were used to select the models that were most likely to fit the data (Burnham and Anderson 2004). Models within the model set with a ΔAICc ≤ 2 were considered to have similar weighted support and were compared on equal terms, as suggested by Burnham and Anderson (2004). The assumption of normality in linear regression was ensured by diagnostics in R (residual inspection by "Q–Q," "residual versus leverage," "residual versus fitted," and "scale-location"). The model set did not include GSI, gonad weight, and MML because they would have excluded 12 individuals for missing data.

The Shapiro-Wilk test was used to verify normality. The Mann-Whitney *U*-test was used for nonnormally distributed data. Pearson's correlation test was used to correlate body weight with fork length. All statistical tests' level of significance was set to $p < 0.05$.

RESULTS

DNA DSBs

Blood samples from 39 fish were analyzed for DNA DSBs by the DNA-FTM, shown in Figure 1A. There was a significant difference in DNA-FTM between the lakes. A higher level of DSBs was found in char from the high-contaminated Lake Ellasjøen compared with the reference lake, Laksvatn (Mann-Whitney *U*, $p < 0.001$; Figure 1A). There was no significant difference between the sexes among individuals of Lake Laksvatn (Mann-Whitney *U*, $p = 0.46$), but in Ellasjøen the males had a significantly higher level of DNA-FTM than the females (Mann-Whitney *U*, $p = 0.04$).

Blood was also analyzed for the MML of the DNA fragments that left the well during gel electrophoresis (Figure 1B). There was a significant difference in MML between the lakes (Mann-Whitney *U*, $p < 0.05$), where the individuals from Lake Laksvatn had the largest MML. There was no significant difference in MML between the sexes within any lake.

Organochlorine contaminant levels

Levels of organochlorine contaminants were measured in muscle of 39 individual char from the 2 lakes. Fish from Lake Ellasjøen had much higher levels of organochlorine contaminants than fish from Lake Laksvatn. Average ΣOC concentrations in Ellasjøen char were 43 times higher than for Laksvatn char, 33 739 ± 68 741 and 781 ± 419 pmol g^{-1} wet weight (±SD), respectively. The greatest difference was found for PCB153, which on average was 53 times higher in the Ellasjøen than in the Laksvatn char. On a lipid-normalized scale, PCB153 was measured at 20 147 ± 48 451 ng g^{-1} on average for the Ellasjøen char and 230 ± 128 ng g^{-1} for the Laksvatn char (±SD), an 87-fold difference. Summary statistics and the individual measurements in both pmol g^{-1} wet weight, and in ng g^{-1} in both wet weight and lipid-normalized weight can be found in Supplemental Data, Tables S4, S5, and S6.

The highest concentrations of organochlorine contaminants were measured in 3 old males, ages 15, 17, and 19 yr, from Lake Ellasjøen. The contaminant profiles of the char were similar in the 2 lakes; that is, the individual compounds constituted similar-sized fractions of the measured chemical load (Supplemental Data, Figure S2).

Biological variables

Fish from Laksvatn were significantly longer and heavier than fish from Ellasjøen (Mann-Whitney *U*, $p < 0.001$), but condition factor did not differ in fish from the 2 lakes. A summary of the biological variables of the fish from Lake Ellasjøen and Lake Laksvatn is presented in Table 1, with all measurements in Supplemental Data, Table S3. Notably, the char from Lake Laksvatn were larger than fish at the same age from Lake Ellasjøen: a significant difference between lakes was found by a linear regression of weight explained by age ($F_{[2,36]} = 39.51$,

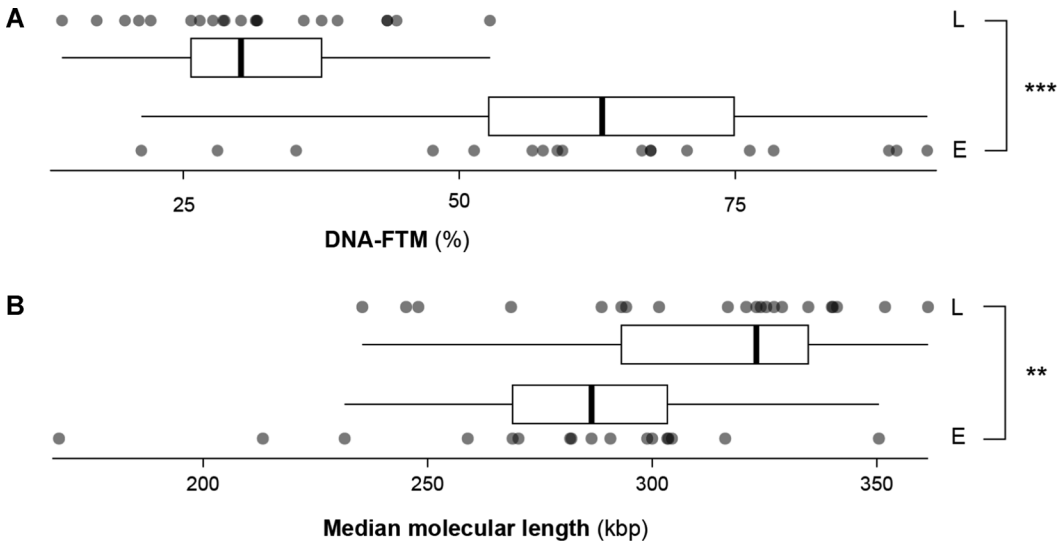

FIGURE 1: DNA double-stranded breaks (DSBs) of arctic char (*Salvelinus alpinus*) from Lake Laksvatn (reference lake) and Lake Ellasjøen (high contaminant load). (**A**) Measure of DSBs by means of DNA-fraction migrated of total DNA loaded from Lake Laksvatn (top; $n = 21$) and Lake Ellasjøen (bottom; $n = 18$). Individual measurements are shown as well as their box plot (where the box boundary is the upper and lower quartile, divided by the median). There was a significant difference between the lakes (Mann-Whitney U ***$p < 0.001$). (**B**) DNA fragment size distributions given as molecular median length, in kilobase pairs, for Lake Laksvatn ($n = 21$) and Lake Ellasjøen ($n = 18$). There was a significant difference between the lakes (Mann-Whitney U **$p < 0.01$). DNA-FTM = fraction of total DNA that migrated into the gel; E = Lake Ellasjøen; L = Lake Laksvatn.

$p < 0.001$). At age 12, the Laksvatn char were almost 2 times heavier than their Ellasjøen conspecifics (1 309.3 g and 712.0 g, respectively), according to the regression.

The reproductive stage of the individuals was determined, and the fish were in stages 1, 2, 6, and 7, including some in 2/7 and 3/7, of the reproductive cycle (see Supplemental Data). No lesions were observed.

Principal component analysis

A PCA was performed to explore the association between DNA damage, organochlorine contaminants, and biological variables in char from the 2 lakes; loading and score plots are provided in Figure 2. Principal component 1 (PC1) and PC2 accounted for 62.5 and 11.7% of the total variance, respectively. Nearly all of the variance of PC1 was accounted for by the organochlorine contaminants: combined, they contributed 80.4% of the variation within PC1. Age and DNA-FTM contributed an additional 8.6 and 5.2%, respectively. The contribution of variation of the organochlorine contaminants to the remaining PCs was minimal. The main contributors of the construction of PC2 were reproductive stage (48.7%), condition factor (15.6%), percentage of lipids (12.9%), and DNA-FTM (10.9%). The organochlorine contaminants were also positively associated with age.

The PCA plot indicates a negative association between DNA-FTM and HSI and, to a lesser degree, with percentage of lipids and condition factor. The supplementary variables body

TABLE 1: Biometric data of Arctic char (*Salvelinus alpinus*) from Lake Laksvatn ($n = 21$, 9 females, 12 males) and Lake Ellasjøen ($n = 18$, 7 females, 11 males), Bjørnøya (Norway), sampled 2014[a]

	Laksvatn			Ellasjøen		
	Average ± SD	Median	Range	Average ± SD	Median	Range
Length (cm)	48.8 ± 3.1	48.7	43.5–56.1	43.5 ± 6.7	41.9	36.2–62.4***
W_B (g)	1092.7 ± 143.6	1052.4	845.–1433.0	808.6 ± 476.7	636.4	436.3–2372.6***
Age	10.4 ± 1.2	10	9–12	12.7 ± 2.7	12	9–19**
Rs	4.8 ± 2.1	6	1–7	4.0 ± 2.1	5	1–6
W_G[b]	16.1 ± 12.0	17.3	0.7–37.8	7.6 ± 7.9	6.6	0.4–20.7
Lip%	0.5 ± 0.0	0.5	0.5–0.6	0.4 ± 0.2	0.4	0.2–1.0***
W_L (g)	11.23 ± 2.31	11.15	7.67–16.60	6.16 ± 2.40	5.38	3.52–12.72***
GSI[b]	7.05 ± 7.91	1.82	0.08–18.41	4.12 ± 5.57	1.22	0.07–14.18*
CF	0.94 ± 0.11	0.99	0.69–1.07	0.91 ± 0.06	0.91	0.82–1.04
HSI	1.05 ± 0.27	1.02	0.66–1.70	0.83 ± 0.23	0.77	0.54–1.47**

[a]Presented as average with standard deviation, median, and range.
[b]Females only.
B = body; CF = condition factor; G = gonads; GSI = gonadosomatic index; HSI = hepatosomatic index; L = liver; Lip% = percentage lipid in muscle; Rs = reproductive stage.
Significance by p value of Mann-Whitney U test: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

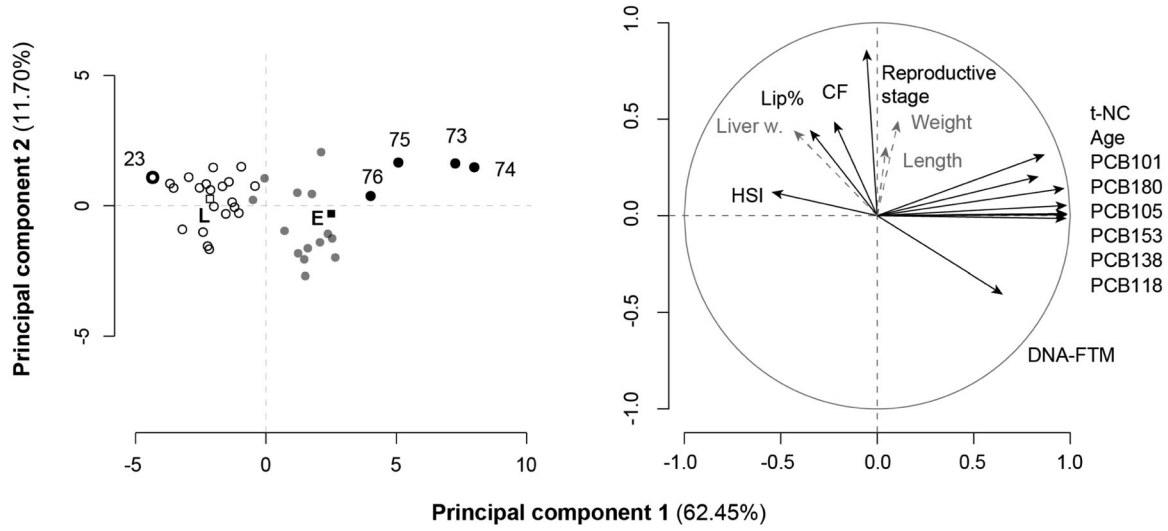

FIGURE 2: Scores plot (left) and loadings plot (right) from a principal component analysis of arctic char (*Salvelinus alpinus*) from Lake Laksvatn (open circles) and Lake Ellasjøen (solid circles), respectively (barycenters marked by squares). The 5 individuals that contributed the most variation in the construction of the dimensions are marked with their identification number. Gray, dashed lines represent supplementary variables. CF = condition factor; DNA-FTM = fraction of total DNA that migrated into the gel; HSI = hepatosomatic index; Lip% = percentage of lipids in muscle; PCB = polychlorinated biphenyl; t-NC = *trans*-nonachlor.

weight, fork length, and liver weight did not indicate any strong associations to any of the axes.

The organochlorine contaminant concentrations in char cause the PCA to order individuals with organochlorine contaminant concentrations in the 3 oldest males from Ellasjøen (positive PC1 values) to low organochlorine contaminant concentrations in char from Laksvatn (negative PC1 values). Individual 23 had the lowest ΣOC level of the 39 individuals (227.8 pmol g^{-1}). Of the clustered Ellasjøen individuals within the fourth quadrant (positive PC1 values and negative PC2 values), all were in reproductive stages 1, 2, or 3 with relatively low DNA-FTM scores. Inversely, individuals in reproductive stages 4, 5, or 6 had positive PC2 values.

Multiple regression models

The 4 best candidate models, determined by AICc score, all included reproductive stage in addition to ΣOC (Table 2). Generally, all of the models showed a significant increase in DNA-FTM with higher organochlorine contaminant levels and a decrease in DNA-FTM at later reproductive stages. The coefficient estimates for both ΣOC and reproductive stage were similar between the 4 best models: ΣOC ranged from 0.151 (standard error of the mean [SEM] = 0.039) to 0.171 (SEM = 0.035), and reproductive stage ranged from −0.073 (SEM = 0.029) to −0.096 (SEM = 0.032). The complete set of models from the model selection can be found in Supplemental Data, Table S2.

TABLE 2: Top candidate models to explain the level of DNA double-strand breaks, measured by the fraction of total DNA that migrated into the gel (DNA-FTM)[a]

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

[a]Both DNA-FTM and Σorganochlorines were ln-transformed.
AICc = corrected Akaike's information criterion; DNA-FTM = total DNA that migrated into the gel; HSI = hepatosomatic index; ns = not significant; ΣOCs = Σorganochlorines; Resp. vars. = response variables; Rs = reproductive stage; W_L = liver weight.
Annotation of *p*-value: ***<0.001, **<0.01, *<0.05, ns, not significant.

The model considered to be the best was DNA-FTM, explained only by ΣOC and the reproductive stage (model 1). Selection was done on the principles of AICc model selection and parsimony. The model shows a clear increase in DNA-FTM when ΣOC increases and the char are in the earlier reproductive stages. Late reproductive stages and low ΣOC concentrations are associated with lower DNA-FTM. A significant regression coefficient (adjusted) of 0.455 was found for the entire model ($F_{[2,36]} = 16.89$, $p < 0.001$). The ΣOC coefficient (± SEM) was 0.171 (±0.035), whereas the reproductive stage had a negative coefficient in the model of −0.083 (±0.028). Both estimates were significant: ΣOC $p < 0.001$, reproductive stage $p = 0.006$. The best model is illustrated in Figure 3, and in the model, the individuals are pooled into the 2 groups representing the earlier reproductive stages (stages 1–3), that is, immature char and the mature char that are about to spawn (stages 6 and 7).

Liver weight, absolute and by HSI, was included in models 2 and 3 but had no significant impact on the regression model ($p = 0.26$, $p = 0.31$, respectively). Model 4 showed a significant difference in the impact of reproductive stage on DNA-FTM by sex, but the coefficient estimates were of similar magnitude. That is, the sex difference was significant but small. The model shows that there seems to be a slightly smaller increase in DNA-FTM for females than for males, with both increasing ΣOC and reproductive stage. A selection table of the models and the top-tier model makeup are given in the Supplemental Data, Table S2.

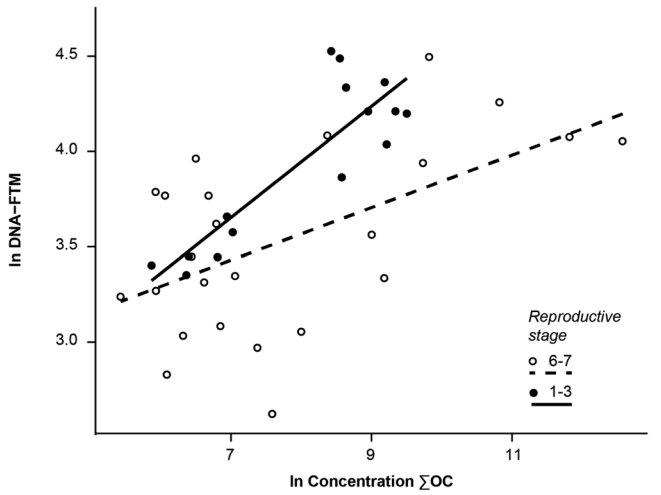

FIGURE 3: Linear regression model of frequency of DNA double-strand breaks by measure of DNA-fraction of total DNA that migrated into the gel, explained by concentration of $\Sigma_7$organochlorines (pmol g^{-1} wet wt) and reproductive stage in arctic char (*Salvelinus alpinus*) from 2 lakes on Bjørnøya, Svalbard. The dashed line (individuals as open circles) represents char that likely will reproduce in the current year (reproductive stages 6 and 7). The solid line (individuals as solid circles) represents immature char that will not reproduce in the current year (reproductive stages 1–3). The model was statistically significant ($F_{[2,36]} = 19.13$, $p < 0.001$), with an adjusted $R^2 = 0.488$. DNA-FTM = fraction of total DNA that migrated into the gel; OC = organochlorine.

DISCUSSION

Biological variation

Individuals of similar age were significantly heavier in Lake Laksvatn than in Lake Ellasjøen. This is in line with previous findings from the same study population (Jørgensen et al. 2017; Gauthier et al. 2018), which include materials from the same sampling as the present study. The 2 studies propose that the high levels of organochlorine contaminants of Ellasjøen char could contribute to the lower body mass found in this population compared with that in Lake Laksvatn because of a certain metabolic cost of activating the xenobiotic defense and detoxification system. Others have examined this possible relationship between contaminant exposure in fish and metabolism and energy allocation further (Smolders et al. 2003; Nault et al. 2012). An exposure experiment with arctic char found that a high dose of PCBs reduced the specific growth rate compared to a control (Jørgensen et al. 2004). However, there are other differences between the 2 lakes that could have contributed to growth differences, such as food availability and quality, population density (the fish density is much higher in Lake Ellasjøen than in Lake Laksvatn), parasite load (more parasites in Ellasjøen because of the presence of seabirds), and ectomorphs (Hawley et al. 2016). The factors could also account for the difference in muscle lipid content, which was somewhat higher in char from Laksvatn.

Organochlorines

There was a large difference in the level of contaminants in char between the 2 lakes—a 43 times higher average molar concentration (wet wt) of Σ_7OCs in Lake Ellasjøen than Lake Laksvatn char. However, PCB levels of Ellasjøen char are similar to or a bit lower than those reported in earlier studies (Evenset et al. 2004; Bytingsvik et al. 2015; Jørgensen et al. 2017). The contaminant concentration of Lake Laksvatn char is also comparable between studies (Bytingsvik et al. 2015) as well as to char of Øyangen on Bjørnøya, used earlier as the reference lake (Evenset et al. 2004; Wiseman et al. 2011). Laksvatn char are also similar in summed PCB concentrations to char from lakes in eastern and northern Canada as reported by Braune et al. (2005). The only non-PCB component, t-NC, is reported in similar concentrations in both lakes as in land-locked arctic char from southwest Greenland (Rigét et al. 2010).

DNA damage

There was a significantly higher level of DNA DSBs in blood cells of arctic char in Ellasjøen compared with those from Laksvatn. This difference could be attributable to the higher level of organochlorine contaminants in Lake Ellasjøen because a strong positive relationship between organochlorine contaminant concentration and increasing DNA DSBs was found in both the PCA and regression models. The causality of the association is not given, but it is presumed that the higher concentration of organochlorine contaminants could explain parts of the observed difference in DNA damage. Several studies have indicated that some of the contaminants found at high levels in the Lake Ellasjøen fish can damage DNA (Winter et al. 2004; González-Mille

et al. 2010; Marabini et al. 2011). Damage to DNA can in turn, if not repaired or repaired inadequately, affect the health of the organism, such as the formation of lesions, an effect associated with PCBs (Ben Ameur et al. 2012; Simon and Burskey 2016). Such damages could thus impair reproduction and, as such, higher organizational levels (Jha 2008).

In addition to differences in DNA damage in fish from the 2 lakes, a relationship was found between DNA DSBs and reproductive stage. The fish in the later stages of the reproductive cycle had lower levels of DNA DSBs. In a study by Goksøyr and Larsen (1991) it was found that sexually mature Atlantic salmon (*Salmo salar*) had a lower hepatic CYP1A activity (ethoxyresorufin *O* deethylase) than sexually immature salmon. Similarly, low CYP1A activity has been measured in liver samples from sexually mature char from Bjørnøya (Akvaplan-niva AS, unpublished data), although it was reported to be generally higher in Ellasjøen compared to Laksvatn (Jørgensen et al. 2017). It is known that different PCBs can be metabolized to different degrees by CYP1A (Grimm et al. 2015), and some of the metabolites are known to be able to induce reactive oxygen species and DNA damage (Song et al. 2015). This suggests that the lower levels of DNA damage observed in mature char are attributable to a lowered biotransformation capacity, which consequently may result in less DNA damage. The pattern observed could also be explained by energy budget strategies: energy is invested most in reproductive organs rather than biotransformation.

Another reason for the difference in DNA damage between fish in different reproductive stages could be that the DNA repair capacity differs with the reproductive stages. If fish in the earlier stages of the reproductive cycle have a greater ability to repair DNA damage, this could explain the disparity in DNA damage between the stages. And such a difference between young and adult fish has been found in medaka (*Oryzias latipes*), where the adult has decreased DNA alkylation repair (Kienzler et al. 2013). But, conversely, in *Kryptolebias marmoratus* the pattern was observed to be the opposite (Kienzler et al. 2013). The 2 main pathways for DSB repair (homologous recombination and nonhomologous end-joining), which are most relevant in the present study, have so far gained much less attention in fish than in mammals. Both homologous recombination and nonhomologous end-joining have been registered in early embryonic cells and adult medaka cells (Kienzler et al. 2013)—yet another reason which could be linked to the energetic cost of detoxification.

The DNA-FTM was higher in males than in females from Ellasjøen. This could be attributable to a biased sample size of each sex: the 4 oldest individuals that also had the highest concentrations of organochlorine contaminants were male. Another explanation for higher DNA-FTM in males of Ellasjøen could be that the female deposits organochlorine contaminants into the lipid-rich eggs. For anadromous char, the lipid content of the gonads can account for up to 25% of the total lipid content in females but <3% in males (Jørgensen et al. 1997). The toxicokinetics of spawning was investigated in the landlocked char from Ellasjøen, where this additional route of elimination was found to be substantial (Bytingsvik et al. 2015). The mechanism may lead to lowering the body burden of organochlorine contaminants in females only, subsequently leading to lower levels of observed DNA damage compared with males. The effect might also suggest that the reproductive stage leads to a better explanation of DNA damage in the models than does age (see Supplemental Data, Table S2).

The damage to DNA was measured in blood cells, primarily erythrocytes. Albeit lacking metabolic capacity, it is believed that damage to these cells can be reflective of the status of the organism (Mitchelmore and Chipman 1998). The level of DNA damage in blood cells could be indicative of DNA damage in other tissues, which, for instance, could lead to the formation of lesions, an effect associated with PCBs (Ben Ameur et al. 2012; Simon and Burskey 2016). These lesions can inflict neurological, endocrine, and reproductive effects and further lead to a reduction of fitness unless repaired.

Other parts of the overarching project of the present study have found metabolomic (Gauthier et al. 2018) and endocrine disruption (Jørgensen et al. 2017) of the char from Ellasjøen, which points to adverse effects of the pollutant load. According to Bickham (2011), one outcome of chronic exposure of contaminants to a population could be selection for resistance-associated alleles. This could cause a loss of genetic variation as a whole in the population, referred to as the "genetic erosion hypothesis" (van Straalen and Timmermans 2002). Despite a significant difference in DNA damage between the lakes and high levels of organochlorine contaminants in Ellasjøen, adult arctic char continue to reproduce in the lake. Further studies of the genetics of these populations could provide some insight into possible adaptations to the exposure and evidence for the genetic erosion hypothesis or indications of an evolutionary adaptation.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4546.

Acknowledgment—The present study was financed by the Research Council of Norway (project no. 221371/E40), the Norwegian Ministry of Environment through the Fram Centre flagship (Tromsø, Norway; Hazardous substances—Effects on ecosystems and human health), and the Norwegian University of Science and Technology. The authors also thank C. Bingham and R. Rodvelt for aid in laboratory work, and M. Frantzen and G. N. Christensen for taking part in the fieldwork.

Disclaimer—The manuscript is original, unpublished, and written wholly by the authors.

Data Accessibility—Data, associated metadata, and calculation tools are available from the corresponding author (science@eirikdn.com or ase.krokje@ntnu.no).

REFERENCES

Akaike H. 1974. A new look at the statistical model identification. *IEEE Trans Automat Contr* 19:716–723.

Ben Ameur W, de Lapuente J, El Megdiche Y, Barhoumi B, Trabelsi S, Camps L, Serret J, Ramos-López D, Gonzalez-Linares J, Driss MR, Borras

M. 2012. Oxidative stress, genotoxicity and histopathology biomarker responses in mullet (*Mugil cephalus*) and sea bass (*Dicentrarchus labrax*) liver from Bizerte Lagoon (Tunisia). *Mar Pollut Bull* 64:241–251.

Bickham JW. 2011. The four cornerstones of evolutionary toxicology. *Ecotoxicology* 20:497–502.

Bickham JW, Sandhu S, Hebert PDN, Chikhi L, Athwal R. 2000. Effects of chemical contaminants on genetic diversity in natural populations: Implications for biomonitoring and ecotoxicology. *Mutat Res* 463:33–51.

Binelli A, Riva C, Cogni D, Provini A. 2008. Genotoxic effects of p,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. *Environ Mol Mutagen* 49:406–415.

Braune BM, Outridge PM, Fisk AT, Muir DCG, Helm PA, Hobbs K, Hoekstra PF, Kuzyk ZA, Kwan M, Letcher RJ. 2005. Persistent organic pollutants and mercury in marine biota of the Canadian Arctic: An overview of spatial and temporal trends. *Sci Total Environ* 351:4–56.

Brown AR, Hosken DJ, Balloux F, Bickley LK, LePage G, Owen SF, Hetheridge MJ, Tyler CR. 2009. Genetic variation, inbreeding and chemical exposure—Combined effects in wildlife and critical considerations for ecotoxicology. *Philos Trans R Soc B Biol Sci* 364:3377–3390.

Burnham KP, Anderson DR. 2004. Multimodel inference: Understanding AIC and BIC in model selection. *Sociol Methods Res* 33:261–304.

Bytingsvik J, Frantzen M, Götsch A, Heimstad ESS, Christensen G, Evenset A. 2015. Current status, between-year comparisons and maternal transfer of organohalogenated compounds (OHCs) in Arctic char (*Salvelinus alpinus*) from Bjørnøya, Svalbard (Norway). *Sci Total Environ* 521:421–430.

Christensen GN, Evenset A. 2011. Miljøgifter i røye fra innsjøer på Svalbard, Akvaplan-niva-rapport. Akvaplan-niva AS Rapp 4232-1. Tromsø, Norway.

Devaux A, Fiat L, Gillet C, Bony S. 2011. Reproduction impairment following paternal genotoxin exposure in brown trout (*Salmo trutta*) and arctic char (*Salvelinus alpinus*). *Aquat Toxicol* 101:405–411.

Evenset A, Christensen GN, Carroll J, Zaborska A, Berger U, Herzke D, Gregor D. 2007. Historical trends in persistent organic pollutants and metals recorded in sediment from Lake Ellasjøen, Bjørnøya, Norwegian Arctic. *Environ Pollut* 146:196–205.

Evenset A, Christensen GN, 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. *Sci Total Environ* 318:125–141.

Fenstad AA, Bustnes JO, Bingham CG, Öst M, Jaatinen K, Moe B, Hanssen SA, Moody AJ, Gabrielsen KM, Herzke D, Lierhagen S, Jenssen BM, Krøkje A. 2016. DNA double-strand breaks in incubating female common eiders (*Somateria mollissima*): Comparison between a low and a high polluted area. *Environ Res* 151:297–303.

Fenstad AA, Jenssen BM, Moe B, Hanssen S, Bingham C, Herzke D, Bustnes JO, Krøkje Å. 2014. DNA double-strand breaks in relation to persistent organic pollutants in a fasting seabird. *Ecotoxicol Environ Saf* 106:68–75.

Friedberg EC, Walker GC, Wood RD, Siede W, Schultz RA, Ellenberger T. 2006. *DNA Repair and Mutagenesis*, 2nd ed. ASM Press, Washington, DC.

Gauthier PT, Evenset A, Christensen GN, Jorgensen EH, Vijayan MM. 2018. Lifelong exposure to PCBs in the remote Norwegian Arctic disrupts the plasma stress metabolome in arctic charr. *Environ Sci Technol* 52:868–876.

Goksøyr A, Larsen HE. 1991. The cytochrome P450 system of Atlantic salmon (*Salmo salar*): I. Basal properties and induction of P450 1A1 in liver of immature and mature fish. *Fish Physiol Biochem* 9:339–349.

González-Mille DJ, Ilizaliturri-Hernández CA, Espinosa-Reyes G, Costilla-Salazar R, Díaz-Barriga F, Ize-Lema I, Mejía-Saavedra J. 2010. Exposure to persistent organic pollutants (POPs) and DNA damage as an indicator of environmental stress in fish of different feeding habits of Coatzacoalcos, Veracruz, Mexico. *Ecotoxicology* 19:1238–1248.

Grimm FA, Hu D, Kania-Korwel I, Lehmler H-J, Ludewig G, Hornbuckle KC, Duffel MW, Bergman Å, Robertson LW. 2015. Metabolism and metabolites of polychlorinated biphenyls. *Crit Rev Toxicol* 45:245–272.

Hallanger IG, Warner NA, Ruus A, Evenset A, Christensen G, Herzke D, Gabrielsen GW, Borgå K. 2011. Seasonality in contaminant accumulation in Arctic marine pelagic food webs using trophic magnification factor as a measure of bioaccumulation. *Environ Toxicol Chem* 30:1026–1035.

Hawley KL, Rosten CM, Christensen G, Lucas MC. 2016. Fine-scale behavioural differences distinguish resource use by ecomorphs in a closed ecosystem. *Sci Rep* 6:24369.

Herzke D, Gabrielsen GW, Evenset A, Burkow IC. 2003. Polychlorinated camphenes (toxaphenes), polybrominated diphenylethers and other halogenated organic pollutants in glaucous gull (*Larus hyperboreus*) from Svalbard and Bjørnøya (Bear Island). *Environ Pollut* 121:293–300.

Jackson SP. 1999. Colworth Medal lecture. Detection, repair and signalling of DNA double-strand breaks. *Biochem Soc Trans* 27:1–13.

Jeggo PA. 1998. Identification of genes involved in repair of DNA double-strand breaks in mammalian cells. *Radiat Res* 150(5 Suppl.):S80–S91.

Jha AN. 2008. Ecotoxicological applications and significance of the comet assay. *Mutagenesis* 23:207–221.

Jørgensen EH, Aas-Hansen Ø, Maule AG, Strand JET, Vijayan MM. 2004. PCB impairs smoltification and seawater performance in anadromous arctic char (*Salvelinus alpinus*). *Comp Biochem Physiol Part C Toxicol Pharmacol* 138:203–212.

Jørgensen EH, Johansen SJS, Jobling M. 1997. Seasonal patterns of growth, lipid deposition and lipid depletion in anadromous arctic char. *J Fish Biol* 51:312–326.

Jørgensen EH, Maule AG, Evenset A, Christensen G, Bytningsvik J, Frantzen M, Nikiforov V, Faught E, Vijayan MM. 2017. Biomarker response and hypothalamus–pituitary–interrenal axis functioning in arctic char from Bjørnøya (74°30′N), Norway, with high levels of organohalogenated compounds. *Aquat Toxicol* 187:64–71.

Kanaar R, Hoeijmakers JH, van Gent DC. 1998. Molecular mechanisms of DNA double-strand break repair. *Trends Cell Biol* 8:483–489.

Kienzler A, Bony S, Devaux A. 2013. DNA repair activity in fish and interest in ecotoxicology: A review. *Aquat Toxicol* 134–135:47–56.

Klemetsen A, Grotnes PE, Holthe H, Kristoffersen K. 1985. Bear Island Barents Sea charr. *Report: Institute of Freshwater Research, Drottningholm* 62:98–119.

Krøkje Å, Bingham C, Husmo Tuven R, Wing Gabrielsen G. 2006. Chromosome aberrations and DNA strand breaks in glaucous gull (*Larus hyperboreus*) chicks fed environmentally contaminated gull eggs. *J Toxicol Environ Health A* 69:159–174.

Letcher RJ, Bustnes JO, Dietz R, Jenssen BM, Jørgensen EH, Sonne C, Verreault J, Vijayan MM, Gabrielsen GW. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Sci Total Environ* 408:2995–3043.

Marabini L, Calò R, Fucile S. 2011. Genotoxic effects of polychlorinated biphenyls (PCB 153, 138, 101, 118) in a fish cell line (RTG-2). *Toxicol In Vitro* 25:1045–1052.

Mitchelmore C, Chipman JK. 1998. Detection of DNA strand breaks in brown trout (*Salmo trutta*) hepatocytes and blood cells using the single cell gel electrophoresis (comet) assay. *Aquat Toxicol* 41:161–182.

Moe SJ, De Schamphelaere K, Clements WH, Sorensen MT, Van den Brink PJ, Liess M. 2013. Combined and interactive effects of global climate change and toxicants on populations and communities. *Environ Toxicol Chem* 32:49–61.

Nault R, Al-Hameedi S, Moon TW. 2012. Effects of polychlorinated biphenyls on whole animal energy mobilization and hepatic cellular respiration in rainbow trout, *Oncorhynchus mykiss*. *Chemosphere* 87:1057–1062.

Oskam IC, Ropstad E, Dahl E, Lie E, Derocher AE, Wiig Ø, Larsen S, Wiger R, Skaare JU. 2003. Organochlorines affect the major androgenic hormone, testosterone, in male polar bears (*Ursus maritimus*) at Svalbard. *J Toxicol Environ Health A* 66:2119–2139.

Østby L, Wing Gabrielsen G, Krøkje Å. 2005. Cytochrome P4501A induction and DNA adduct formation in glaucous gulls (*Larus hyperboreus*), fed with environmentally contaminated gull eggs. *Ecotoxicol Environ Saf* 62:363–375.

R Core Development Team. 2015. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rigét F, Vorkamp K, Muir D. 2010. Temporal trends of contaminants in arctic char (*Salvelinus alpinus*) from a small lake, southwest Greenland during a warming climate. *J Environ Monit* 12:2252–2258.

Simon TP, Burskey JL. 2016. Deformity, erosion, lesion, and tumor occurrence, fluctuating asymmetry, and population parameters for bluntnose minnow (*Pimephales notatus*) as indicators of recovering water quality in a Great Lakes Area of concern, USA. *Arch Environ Contam Toxicol* 70:181–191.

Smolders R, De Boeck G, Blust R. 2003. Changes in cellular energy budget as a measure of whole effluent toxicity in zebrafish (*Danio rerio*). *Environ Toxicol Chem* 22:890–899.

Sømme I. 1941. *Ørretboka*. Jakob Dybwads Forlag, Oslo, Norway.

Song X, Li L, Shi Q, Lehmler H-J, Fu J, Su C, Xia X, Song E, Song Y. 2015. Polychlorinated biphenyl quinone metabolite promotes p53-dependent DNA damage checkpoint activation, S-phase cycle arrest and extrinsic

apoptosis in human liver hepatocellular carcinoma HepG2 cells. *Chem Res Toxicol* 28:2160–2169.

Srinivasan A, Lehmler HJ, Robertson LW, Ludewig G. 2001. Production of DNA strand breaks in vitro and reactive oxygen species in vitro and in HL-60 cells by PCB metabolites. *Toxicol Sci* 60:92–102.

Theodorakis CW, D'Surney SJ, Shugart LR. 1994. Detection of genotoxic insult as DNA strand breaks in fish blood cells by agarose gel electrophoresis. *Environ Toxicol Chem* 13:1023–1031.

van Straalen N, Timmermans M. 2002. Genetic variation in toxicant-stressed populations: An evaluation of the "genetic erosion" hypothesis. *Hum Ecol Risk Assess* 8:983–1002.

Verreault J, Gabrielsen GW, Bustnes JO. 2010. The Svalbard glaucous gull as bioindicator species in the European Arctic: Insight from 35 years of contaminants research. *Rev Environ Contam Toxicol* 205: 77–116.

Winter MJ, Day N, Hayes RA, Taylor EW, Butler PJ, Chipman JK. 2004. DNA strand breaks and adducts determined in feral and caged chub (*Leuciscus cephalus*) exposed to rivers exhibiting variable water quality around Birmingham, UK. *Mutat Res* 552:163–175.

Wiseman S, Jørgensen EH, Maule AG, Vijayan MM. 2011. Contaminant loading in remote Arctic lakes affects cellular stress-related proteins expression in feral charr. *Polar Biol* 34:933–937.

