High levels of organochlorine contaminants (OCs) have been found in arctic char (Salvelinus alpinus) from Bjørnøya in the Norwegian Arctic. Anthropogenic contaminants; Arctic; Fish; Genotoxicity; Organochlorines

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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.
(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 (Østbye 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 in the gel and used as a measure of DSB frequency (Fenstad et al. 1994). 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 fragmentation (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).

**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 (Semme 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 gel 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.
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, percent 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 log₂-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 log₂-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 (R²) 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,
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*

|               | 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*** |
| **Wb (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 |
| **Wg** | 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*** |
| **Wl (g)** | 11.23 ± 2.31 | 11.15 | 7.67–16.60 | 6.16 ± 2.40 | 5.38 | 3.52–12.72*** |
| **GSI** | 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** |

*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.

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| **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** |

*aPresented as average with standard deviation, median, and range.

*bFemales 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.
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 \( \Sigma \text{OC} \) level of the 39 individuals (227.8 pmol g\(^{-1}\)). Of the clustered Ellasjøen individuals within the fourth quadrant (positive PC1 values and negative PC2 values), all were in reproductive 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 \( \Sigma \text{OC} \) (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 \( \Sigma \text{OC} \) and reproductive stage were similar between the 4 best models: \( \Sigma \text{OC} \) 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 = 0.028) 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 | \( \Delta \text{AICc} \) | Adjusted \( R^2 \) | Resp. vars. | Estimate | SE  | \( t \) value | \( p \) |
|----------|-----------------|-----------------|------------|----------|-----|--------------|------|
| 1        | 0.00            | 0.455           | (Intercept) | 2.715    | 0.311| 8.740        | 0.000*** |
|          |                 |                 | \( \Sigma \text{OC} \) | 0.171    | 0.035| 4.904        | 0.000*** |
|          |                 |                 | \( \text{Rs} \) | -0.083   | 0.028| -2.953       | 0.006**  |
| 2        | 1.24            | 0.459           | (Intercept) | 3.036    | 0.420| 7.233        | 0.000*** |
|          |                 |                 | \( \Sigma \text{OC} \) | 0.151    | 0.039| 3.842        | 0.000*** |
|          |                 |                 | \( \text{Rs} \) | -0.073   | 0.029| -2.482       | 0.018*   |
|          |                 |                 | \( \text{WL} \) | -0.023   | 0.020| -1.132       | 0.265ns  |
| 3        | 1.50            | 0.456           | (Intercept) | 3.096    | 0.486| 6.371        | 0.000*** |
|          |                 |                 | \( \Sigma \text{OC} \) | 0.152    | 0.039| 3.867        | 0.000*** |
|          |                 |                 | \( \text{Rs} \) | -0.082   | 0.028| -2.914       | 0.006**  |
|          |                 |                 | \( \text{HSI} \) | -0.253   | 0.248| -1.019       | 0.315ns  |
| 4        | 1.74            | 0.452           | (Intercept) | 2.778    | 0.319| 8.702        | 0.000*** |
|          |                 |                 | \( \Sigma \text{OC} \) | 0.163    | 0.036| 4.513        | 0.000*** |
|          |                 |                 | \( \text{Rs} \) males | -0.096   | 0.032| -3.035       | 0.005**  |
|          |                 |                 | \( \text{Rs} \) females | -0.073   | 0.030| -2.422       | 0.021*   |

\(^{a}\)Both DNA-FTM and \( \Sigma \text{organochlorines} \) were ln-transformed.

AICc = corrected Akaike’s information criterion; DNA-FTM = total DNA that migrated into the gel; HSI = hepatosomatic index; ns = not significant; \( \Sigma \text{OCs} = \Sigma \text{organochlorines}; \text{Resp. vars.} = \text{response variables}; \text{Rs} = \text{reproductive stage}; \text{WL} = \text{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 ($F_{1,234} = 16.89$, $p < 0.001$). 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.

**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 (wt wt) of ΣOCs 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, 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 ($F_{1,234} = 19.13$, $p < 0.001$), with an adjusted $R^2 = 0.488$. DNA-FTM = fraction of total DNA that migrated into the gel; OC = organochlorine.

**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 Ameer 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 deethylylase) than sexually immature salmon. Similarly, low CYP1A activity has been measured in liver samples from sexually mature fish 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 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 fish 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 Ameer et al. 2012; Simon and Burskey 2016). These lesions can conflict 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.

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**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).

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