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3 **primary Arctic char hepatocytes**

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25 **Characterizing cytotoxic and estrogenic activity of Arctic char tissue extracts in primary**
26 **Arctic char hepatocytes**

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33

34 **Abstract**

35 Contaminants from various anthropogenic activities find their way to the Arctic through long-
36 range atmospheric transport, ocean currents and living organisms, (e.g. migrating fish or
37 seabirds). Although levels of persistent organic pollutants in arctic fish are generally low, local
38 hot-spots of contamination have been demonstrated in freshwater systems, such as Lake
39 Ellasjøen at Bjørnøya (Bear Island, Norway). Higher concentrations of organic halogenated
40 compounds (OHCs), and higher levels of cytochrome P450 and DNA-double strand breaks
41 have been measured in Arctic char (*Salvelinus alpinus*) from this lake compared to fish from
42 other lakes on Bjørnøya. Although several of the measured contaminants are potential
43 endocrine disrupters, few studies have investigated the potential endocrine disruptive effects of
44 the contaminant cocktail in this fish population. In this study we compared the toxic and
45 estrogenic potency of the cocktail of pollutants in extracts of Arctic char livers from the
46 contaminated Lake Ellasjøen with those from the less contaminated Lake Laksvatn at Bjørnøya
47 to investigate if the contaminant cocktail in these fish populations could have cytotoxic and/or
48 estrogenic effects *in vitro*. This was done by *in situ* sampling and contaminant extraction from

liver tissue, followed by chemical analysis and *in vitro* testing of the following contaminant extracts: F1-nonpolar OHCs, F2-polar pesticides and metabolites of OHCs, and F3-polar OHCs. Contaminant levels were highest in extracts from Ellasjøen fish. The F2 and F3 extracts from Lake Laksvatn and Lake Ellasjøen fish reduced the *in vitro* cell viability at a concentration ratio of 0.03 to 1 relative to tissue concentration in the Arctic char. Only the F3 liver extract from Ellasjøen fish increased the *in vitro* vitellogenin protein expression. Although compounds such as estrogenic OH-PCBs was quantified in the Ellasjøen F3 extracts, it remains to be determined which compounds were causing the estrogenic effect.

Key words: Primary hepatocytes; Arctic char; Vitellogenin; cytotoxicity; chemical analysis

1. Introduction

Organisms in the arctic are exposed to persistent organic pollutants (POPs) from long range-atmospheric transport and deposition, transport via ocean currents, as well as from local hot spots, such as military bases, oil extraction facilities and mining etc. Freshwater fish from certain arctic locations have been reported to contain high levels of POPs, with ∑polychlorinated biphenyls (PCBs) higher than 10 000 ng/g lipid weight in some cases (Bytingsvik et al., 2015; Christensen and Evenset, 2011; Evenset et al., 2004). Populations with higher risk of possible POP-mediated effects have been identified and among these is the Arctic char (*Salvelinus alpinus*) from Bjørnøya (Bear Island) (74°30′ N, 19°00′ E) (Letcher et al., 2010), and especially fish from Lake Ellasjøen. The pollutants generally occurring at high concentrations in these fish are PCBs, 2,2-Bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p*-DDE), chlordanes, chlorobornanes (CHBs), polychlorinated naphthalenes (PCNs) and hexachlorobenzene (HCB) (Bytingsvik et al., 2015; Evenset et al., 2005, 2004). These contaminants are transferred to Lake Ellasjøen by guano from seabirds using the lake as a

74 resting area (Evenset et al., 2007). A between-lake comparison at Bjørnøya performed by
75 Bytingsvik et al. (2015) showed that Arctic char muscle levels (lipid weight) of
76 hexachlorobenzene (HCB), chlordanes (∑CHLs), mirex, dichlorodiphenyltrichloroethanes
77 (∑DDTs) and ∑PCBs were 1.7, 3.1, 13.3, 28.1 and 35.6 times higher in fish from Lake
78 Ellasjøen than in fish from the nearby Lake Laksvatn (12 km north of Ellasjøen), respectively.
79 In Ellasjøen fish, highest concentrations were observed for ∑PCBs with concentrations > 10000
80 ng/g lipid weight in both muscle and ovary tissue (Bytingsvik et al., 2015). The higher
81 contaminant concentrations in Arctic char from Lake Ellasjøen compared to Lake Laksvatn
82 have been linked to higher levels of double DNA-strand breaks (Neerland, 2016), higher hepatic
83 cytochrome P450(CYP)1A enzyme levels, lower glucocorticoid receptor (GR) protein
84 expression, elevated heat shock proteins expression (Wiseman et al., 2011), and altered mRNA
85 abundance of key genes related to the hypothalamic-pituitary-interrenal (HPI) axis functioning
86 suggestive of endocrine disruptive effects (Jørgensen et al., 2017).

87

88 Over the last decade, legacy and emerging compounds have demonstrated a potential for
89 endocrine disruptive effects in *in vitro* and *in vivo* studies on Arctic wildlife and fish (Letcher
90 et al., 2010). Environmentally relevant compounds such as o,p′-DDT (Petersen and Tollefsen,
91 2011; Wojtowicz et al., 2007), *o,p′*-DDE and *p,p′*-DDE (Wojtowicz et al., 2007), and
92 hydroxylated (OH-) PCBs (Andersson et al., 1999; Braathen et al., 2009; Carlson and Williams,
93 2001; Mortensen et al., 2007) display estrogenic effects in fish *in vitro* and/or *in vivo*.
94 Furthermore, there are numerous reports that insecticides such as β-HCH, cis- and trans-
95 chlordane, dieldrin, endosulfan, mirex, oxychlordane, toxaphenes and trans-nonachlor have
96 reproductive and endocrine effects (for full review see Colborn et al., 1993). Several classic
97 industrial chemicals detected in Arctic fish and wildlife, such as polychlorinated dibenzo-*p*-
98 dioxins (PCDDs), have also been reported to have endocrine disruptive properties (Colborn et

al., 1993). Some industrial chemicals including polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA) and perfluorinated chemicals (PFCs) may interact with multiple endocrine piscine targets *in vitro* (Hamers et al., 2006, 2008; Harju et al., 2007; Jensen and Leffers, 2008; Liu et al., 2007; Morgado et al., 2007) and *in vivo* (Kuiper et al., 2007; Oakes et al., 2005).

Endocrine disruptive chemicals (EDCs) with an estrogenic mode of action (MoA) may affect the level of vitellogenin (Vtg) protein and/or messenger RNA (mRNA) in fish (Purdom et al., 1994; Sumpter and Jobling, 1995). Vitellogenin is an egg-yolk precursor protein produced in the liver of female fish during oogenesis, and is induced by increased levels of ovarian estrogens (Sumpter and Jobling, 1995). Once produced, Vtg is transported by the blood to the ovaries of female fish where it is incorporated into the growing oocytes (Mommsen and Walsh, 1988). Male and juvenile fish, which also have the gene coding for Vtg, do not normally express this gene due to low concentrations of circulating endogenous estrogens. Expression of Vtg gene and protein in male and juvenile fish has therefore become a suitable biomarker for (xeno)estrogenic compound exposure (Heppell et al., 1995; Mommsen and Walsh, 1988; Purdom et al., 1994). Synthesis of Vtg has also been used as a biomarker in primary cultures of hepatocytes from temperate fish such as common bream (*Abramis brama*), Siberian sturgeon (*Acipenser baeri*), Japanese eel (*Anguilla japonica*), channel catfish (*Ictalurus punctatus*), common carp (*cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), Mozambique tilapia (*Oreochromis mossambicus*) (reviewed by Navas and Segner, 2006), and Atlantic salmon (*Salmo salar*) (Tollefsen et al., 2003), and in the recently established multi-endpoint and high-throughput *in vitro* bioassay with Arctic char hepatocytes for screening single chemicals, complex mixtures, and environmental extracts (Petersen et al., 2017).

124 The population of Arctic char in lake Ellasjøen at Bjørnøya is known to be exposed to several
125 different POPs, and endocrine disruptive effects have been suggested to occur in this population
126 based on observations of altered mRNA abundance of key genes related to the HPI axis
127 functioning (Jørgensen et al., 2017). It is therefore of interest to investigate whether the
128 contaminant cocktail in the livers of spawning Arctic char can affect reproductive processes
129 and cell viability. In this study the contaminant cocktail in the livers of spawning Arctic char
130 from two different lakes (Ellasjøen and Laksvatn) on Bjørnøya were extracted and fractionated
131 into three extracts; F1 - non-polar OHCs (PCBs, PBDEs and most of the nonpolar pesticides),
132 F2 - polar pesticides and metabolites of OHCs ($MeSO_2$-PCBs/DDE), and F3 - polar OHCs
133 (phenolics such as chlorinated phenols and hydroxylated metabolites of PCBs and PBDEs). The
134 effect of these extracts on the *in vitro* cell viability and Vtg protein expression in primary Arctic
135 char hepatocytes was investigated. The observed effects were compared to the detected
136 chemicals present in the individual extracts to potentially identify candidate compounds
137 contributing to the observed effects.
138
139

140 # 2. Materials and Methods

141 ## 2.1. Chemicals

142 The chemicals used as positive controls, 17β-estradiol (E2, CAS 50-28-2, purity of ≥ 98%,
143 positive control for Vtg protein expression), and copper sulphate ($CuSO_4 \times 5H_2O$, CAS 7758-
144 99-8, positive control for cell viability), were obtained from Sigma-Aldrich (St. Lois, MI, US).
145 $CuSO_4 \times 5H_2O$ was dissolved in fresh culture media on the day of exposure, whereas E2 was
146 dissolved in dimethylsulfoxide (DMSO) and stored at -20°C when not in use. The solvents used
147 for chemical analysis (n-hexane, dichloromethane, acetone, cyclohexane, methanol) were all

148 Suprasolv quality for Gas chromatography MS, and obtained from Merck (Darmstadt,
149 Germany). All ^{13}C standards were obtained from Cambridge Isotop Laboratory, Inc.
150 (Tewksbury, MA, US).
151

152 ## 2.2. Sampling and extraction of Arctic char tissue

153 Female and male Arctic char was sampled from Lake Ellasjøen (n = 20) and Lake Laksvatn (n
154 = 22) on Bjørnøya (74°30′ N, 19°00′ E), Svalbard (Norway) during the first two weeks of
155 September 2012. Most individuals were maturing or mature at the time of sampling. Biometric
156 data were recorded (supplementary table 1). Length (cm), body weight (g) and liver weight (g)
157 were measured, and condition factor (CF: [body weight (g)/body length (cm)^3]x100) and liver-
158 somatic index (LSI: [liver weight (g)/body weight (g)]x100) were calculated. Otoliths were
159 collected for age determination. Liver tissue was weighed, wrapped in aluminum foil, and
160 frozen at -20°C. Approximately 0.75 –5.9 g of liver from each fish were pooled and used for
161 preparation of extracts. The extracts were produced from fish of both sexes and variable
162 maturation status. All males were mature (running milt). The females reproductive stage was
163 determined based on Sømme (1941) where fish in reproductive stages 1 to 3 are non-spawning
164 fish (will not spawn during the year of sampling), fish in stages 4, 5 and 6 are spawning fish
165 (will spawn during the year of sampling), and fish in stage 7 have recently spawned
166 (supplementary table 1). The females used for liver extractions represented reproductive stages
167 from 1 to 7.
168

169 All glass equipment was rinsed with solvents and burned for 8 h at 450°C before use. A total
170 of 55 to 86 g of liver was homogenized in dry Na_2SO_4 in a glass kitchen mixer with stainless
171 steel blades. The homogenate was placed in 1000 mL glass flasks and extracted with 200 mL
172 of acetone/cyclohexane (two times with 1/3 v/v and once with 1/1 v/v) using shaking for 30

173 min on a shaking table and ultra-sonication for 10 min. Solvent extracts were decanted through
174 a paper filter (Whatman Grade 1, diameter 185mm, Sigma-Aldrich, Darmstadt, Germany)
175 placed in a glass funnel into a turbovap glass and concentrated in a Turbovap 500 (Biotage,
176 Uppsala, Sweden) to a volume of 1 mL at 35°C, transferred to a 4 mL glass vial and further
177 evaporated until dryness under a gentle stream of nitrogen until constant weight to determine
178 lipid content. Sample cleanup was performed by adding the lipid extracts (diluted in n-Hexane)
179 into a 30 cm long semipermeable membrane device (SPMD, EST-Lab, MO, USA) inside of
180 100 mL Pyrex cylindrical separator funnel with a PTFE stopcock (Sigma-Aldrich, Darmstadt,
181 Germany) and collecting the acetone/n-Hexane extracts (two times with 1/3 v/v and two times
182 with 1/1 v/v) every 24 h for 4 days. Solvent was evaporated in the Turbovap and further cleanup
183 was achieved using a high performance liquid chromatography (HPLC) system utilizing gel
184 permeation chromatography (Waters Envirogel GPC cleanup) using dichloromethane as eluent
185 at a flow of 5 mL/min and collecting the fraction between 12.5 min and 25 min, which removes
186 additional lipids from the samples. A final cleanup step was performed using a 300 mm x 20
187 mm ID glass column packed with activated florisil (450°C, 8h) and 2 g of Na_2SO_4 on top,
188 fractionating the sample into three extracts (F1-F3). The fractionation of the samples was
189 performed with an up scaled version of that used by Nøst et al., (2012) and Sandanger et al.,
190 (2004). The columns were washed with dichloromethane and conditioned with n-hexane and
191 then 200 mL of 10% dichloromethane/n-hexane (v/v) was added to obtain the first fraction (F1)
192 which contain neutral compounds such as PCBs, PBDEs and organochlorine pesticides, 250
193 mL of 10% aceton/n-hexane to obtain the second fraction (F2) containing polar pesticides and
194 metabolites of OHCs like $MeSO_2$-PCBs/DDE, and 300 mL of 20% methanol/dichloromethane
195 to obtain the third fraction (F3) containing polar OHCs like hydroxylated OH-PCBs/phenols.
196 The second fraction was later cleaned from lipid residues on a column (same as above) packed
197 with 2 g of Na_2SO_4, 10 g of 25% w/w sulfuric acid silica (silica activated at 600°C for 8 h) on

198 bottom, and 2 g of neutral active silica on top. The column was washed with dichloromethane
199 and conditioned with n-hexane. Sample was diluted with a small amount of n-hexane and added
200 on top of the column and eluted with 350 mL of dichloromethane. The third fraction was
201 cleaned three times using liquid-liquid extraction with 50 mL concentrated sulphuric acid. All
202 extracts (12 in total) were evaporated to 0.5 ml and transferred to the solvent dimethyl sulfoxide
203 (DMSO) for *in vitro* testing. A procedural blank sample was fractionated in parallel to the tissue
204 samples (producing the three procedural blanks F1 – F3) to correct for effects by
205 methodologically introduced compounds.

206

207 ## 2.3. Chemical analysis

208 An equivalent of 6 g liver of each extract was used for chemical analysis. ^{13}C-labeled standards
209 of PCBs, PBDEs, new BFRs and pesticides (F1), surrogate standard $MeSO_2$-4Me-PCB120 (F2)
210 and ^{13}C-OH-PCBs/PCP (F3) were added before chemical analysis. Further, extract F3 was
211 derivatized using diazomethane before final cleanup on a solid phase extraction (SPE)-column
212 with 25% sulfuric acid silica, concentrated and transferred to an analytical vial GC/MS vial
213 with a 200 µL insert.

214

215 PCBs, DDTs and PBDEs were analyzed by GC-MS (Waters Quattro micro) in EI mode in
216 single ion monitoring using a 30m x 0.25mmID DB5-MS column (Agilent) for PCBs and DDTs
217 and a 15m x 0.18mmID RTx1-MS (Restek) for PBDEs. The pesticides, $MeSO_2$-PCBs/DDE
218 and derivatized OH-PCBs/PCP were analyzed by GC/MS (Agilent 7890A/5975B MSD) in NCI
219 mode in single ion monitoring, with methane as CI gas using a 30m x 0.25mmID DB5-MS
220 column. OPFRs were analysed by LC–Q-TOF–MS after having spiked samples with internal
221 standards, extracted in acetonitrile (1 ml) by repeated sonication and vortexing and cleaned up

222 the supernatant using ENVI-Carb graphitized carbon absorbent and glacial acetic acid. For
223 further information on analysis see Nøst et al. (2012) and Sandanger et al. (2004).
224

225 ### 2.4. Rearing of Arctic char for *in vitro* study

226 Fertilized roe of Arctic char (*Salvelinus alpinus*) (300-500g) were obtained from Tydalfisk
227 (Løvøya, Tydal, Norway), transported to the animal facilities at the Norwegian University of
228 Life Sciences, NMBU (Ås, Norway), where they were hatched and reared (< 100g) before they
229 were transferred to the animal facilities at the University of Oslo. The fish were kept at the
230 animal facilities at the University of Oslo in 1250 L circular, flow-through tanks, with a water
231 temperature of 8 ± 3°C, 100% oxygen saturation, pH 6.6 and a 12 h light / 12 h dark cycle. The
232 fish were fed daily with pellets (Skretting, Stavanger, Norway) corresponding to approximately
233 0.5% of the total biomass.
234

235 ### 2.5. Isolation of primary hepatocytes

236 Arctic char were collected and terminated in November 2015 with a blow to the head and
237 subjected to a two-step liver perfusion as described in Tollefsen et al. (2003) with minor
238 modifications for Arctic char as described in Petersen et al. (2017). Blood was removed from
239 the liver by perfusion with a calcium free buffer (NaCl 122 mM, KCl 4.8 mM, $MgSO_4$ 1.2 mM,
240 Na_2HPO_4 11mM, NaH_2PO_4 3.3 mM, $NaHCO_3$ 3.7 mM, EGTA 26 µM, 0°C) at 5 ml/min for
241 10-15 min. The liver tissue was perfused with the same buffer (37°C) now without EGTA and
242 with added $CaCl_2$ (1.5 mM) and collagenase (0.3 mg/ml) type VIII at 5 ml/min for 10-15 min.
243 The liver was excised and transferred to a glass beaker on ice containing calcium free buffer
244 with 0.1% w/v bovine serum albumin (BSA) and gently stirred. The resulting cell suspension
245 was filtered twice (250 µm and 100 µm sterile nylon mesh) before it was centrifuged three times

246 (4 min, 3 min, 3 min) at 500 rpm. Following the first centrifugation, the supernatant was
247 removed and the cells re-suspended in calcium free buffer with 0.1% w/v BSA on ice. After the
248 second and third centrifugation the cells were re-suspended in serum-free, refrigerated L-15
249 medium containing L-glutamin (0.29 mg/ml), $NaHCO_3$ (4.5 mM), penicillin (100 units/ml),
250 streptomycin (100 µg/L) and amphotericin (0.25 µg/ml). After the last centrifugation, the cell
251 suspension was filtered through a 100 µm nylon mesh, diluted to 250 000 cells per ml, plated
252 (200 µl per well) in 96-well primaria^{TM} plates (Falcon, Becton Dickinson Labware, Oxnard,
253 CA, USA) and left to acclimatize for 24 h at 10˚C in an incubator. Only cell isolations with ≥
254 80% viable cells determined by the trypan blue method were used in the experiments. All
255 extracts, blanks and controls were tested with cells from 3 to 4 independent cell isolations.
256

257 ## 2.6. Exposure of primary hepatocytes

258 After 24 h of acclimatization, cells were exposed to positive controls and the different liver
259 extracts from Arctic char from Ellasjøen and Laksvatn. A total of 12 liver extracts and three
260 procedural blanks were produced (see Table 1). A dilution series was prepared for each extract
261 resulting in 6 stock solutions. All stocks were diluted 1000 times in exposure media (DMSO
262 concentration = 0.1%). In addition, the two highest stock concentrations were diluted 100 times
263 in exposure media (DMSO = 1%) to obtain a total of 8 exposure concentrations of each extract)
264 (Table 1). The exposure concentration is given as concentration ratio (CR), referring to the
265 extracted tissue to exposure concentration. A CR of 1 indicate that the nominal concentrations
266 in exposure media corresponds to the concentrations in the liver tissue. Contaminant
267 concentrations in the extracts at CRs above or below 1 are concentrated (CR>1) and diluted
268 (CR<1) compared to the original tissue concentrations. The procedural blanks were tested at
269 the highest CR (1% DMSO) and 10 times diluted (0.1% DMSO) (Table 1). The hepatocytes
270 were exposed for a total of 96 h with re-exposure after 48 h. At the end of the exposure period,

271 100 µl cell culture media from each well was transferred to 96-well Maxisorp Nunc-
272 immunoplates (Nunc, Roskilde, Denmark) and stored at -80˚C for subsequent Vtg analysis,
273 whereas the cells were subjected to analysis for cell viability without further delay.
274

275 2.7. Cell viability

276 Cell viability was measured at the end of the exposure period essentially as described by Schreer
277 et al. (2005) by use of the two probes Alamar blue (AB) and 5-Carboxyfluorescein Diacetate,
278 Acetoxymethyl Ester (CFDA-AM) for measuring the metabolic activity (AB) and membrane
279 integrity (CFDA-AM). The probes are commonly used in combination to assess cytotoxicity.
280 CFDA-AM is hydrolysed to the fluorescent 5-carboxyfluorescein (CF) by unspecific esterases
281 (Schreer et al 2005) which is negatively correlated with cellular damage (Schirmer et al., 1997).
282 After 96 h of exposure, exposure media was removed and cells were incubated in tris buffer (50
283 mM, pH 7.5, 100 µl per well) containing 5% AB and 4 µM CFDA-AM. After 30 min incubation
284 in the dark on an orbital shaker (100 rpm), the fluorescence was read using Victor V^3 multilabel
285 counter (Perkin Elmer, Waltham, MA, USA) with wavelength pairs of excitation and emission
286 of 530-590 (AB) and 485-530 (CFDA-AM). The results were normalised between the negative
287 control (solvent, DMSO = 100% viability) and positive control ($CuSO_4$ 10 mM = 0% viability).
288

289 2.8. Vitellogenin protein expression

290 Analysis of Vtg secreted to the growth media was performed with a capture ELISA essentially
291 as described in Tollefsen et al. (2003). Plates containing 100 µl growth media sampled at the
292 end of the exposure period were thawed for minimum 4 h in a refrigerator before 100 µl of
293 standards were applied to assigned empty wells. The plates were left overnight (16 h) in the
294 dark at 4˚C. The following day, the plates were washed three times with 250 µl washing buffer

295 (PBS with 0.05% Tween-20) and incubated with 200 µl blocking buffer (PBS with 2% BSA)
296 in the dark at 20˚C for 1 h. After three washes with 250 µl washing buffer, 100 µl monoclonal
297 mouse anti-salmon Vtg (BN-5, Biosense Laboratories, Bergen, Norway) diluted 1:6000 in
298 incubation buffer (PBS with 1% BSA) was added to each well and the plates were incubated 2
299 h at 37˚C. The BN-5 antibody cross-reacts with Vtg in plasma from Arctic char Vtg (Biosence
300 product no. V01402101, product description sheet). The plates were washed three times and
301 100 µl of the secondary antibody goat anti-mouse IgG (Bio-Rad, Hercules, CA, USA) diluted
302 1:6000 in incubation buffer was added to each well before the plates were incubated in the dark
303 at 37˚C. After 1 h the plates were washed five times before 100 µl of enzyme substrate (TMB
304 plus, KEMENTEC diagnostics, Taastrup, Denmark) was added to each well. After 15 min of
305 incubation, the enzymatic reaction was stopped by adding 50 µl 1M H_2SO_4. The absorbance
306 was measured at 450 nm using a VersaMax microplate reader (Molecular Devices LLC.,
307 Sunnyvale, CA, USA). The relative expression of Vtg was normalized between the negative
308 (DMSO, 0%) and positive control (30 nM 17β-estradiol, 100% Vtg protein expression).
309

310 ## 2.9. Data analysis

311 All data analyses were performed with Graphpad prism v6.01 software (GraphPad Software
312 Inc., San Diego, CA, USA). The measured data for cell viability and Vtg were expressed as
313 percent of induction/reduction between the solvent control (DMSO) and their individual
314 positive controls. Data were fitted with non-linear sigmoidal concentration-response curve with
315 variable slope and with constraints for bottom (0) and top (100). The data were checked for
316 homogeneity of variance and normality, and statistics were performed using a one-way
317 ANOVA with a Tukey post hoc test ($p<0.05$). Statistical differences in responses observed after
318 exposure to the extracts and their corresponding procedural blanks were investigated to detect

319 responses caused by the contaminant load in the liver extracts. Statistical differences in the
320 responses after exposure to extracts from the two different fish populations were also
321 investigated.
322

323 3. Results

324 Liver extracts from Arctic char from a high- and low-contaminated lake at Bjørnøya were
325 assessed for their contaminant contents, and for their ability to reduce cell viability and increase
326 Vtg protein expression in Arctic char primary hepatocytes in 3-4 individual exposure
327 experiments (Table 3).

328

329 3.1. Contaminants in liver extracts from Arctic char

330 The chemical analysis revealed that fish from Lake Ellasjøen contain higher levels of
331 contaminants than fish from Lake Laksvatn (Table 2). The largest difference in contaminant
332 concentrations was observed in the F1 extract. Highest concentrations were observed for the
333 PCBs in the Ellasjøen F1 extract with ΣPCBs (709 000 pg/g ww) 108 times higher than in the
334 Laksvatn F1 extract. A similar pattern was observed for ΣPBDEs (6580 pg/g ww) where the
335 Ellasjøen extracts contained 120 times higher levels than the Laksvatn extracts. In this extract,
336 only two compounds were found in higher concentrations in the Laksvatn extract; o,p,'-DDD
337 and heptachlor, than the Ellasjøen extract. However, the concentration of heptachlor was lower
338 in the Laksvatn extract than the procedural blank and the concentration of o,p,'-DDD was only
339 two times higher in the Laksvatn than the Ellasjøen F1 extract, and ΣDDT was 32 times higher
340 in extracts from Ellasjøen fish (27 900 pg/g ww) than Laksvatn fish (864 pg/g ww).

341

342 $MESO_2$-PCB concentrations above LOD were only detected in the F2 extract from Ellasjøen
343 fish. Organophosphorus flame retardants (OPFRs), OH-PCBs and OH-PBDEs were detected in
344 the F3 extracts. The concentration of TBEP, and the OH-PCBs were higher in the Ellasjøen
345 liver extract than the Laksvatn liver extract, whereas the concentrations of the OH-BDEs (OH-
346 BDE-68, OH-BDE-47/75 and OH-BDE-101 (<LOQ)) were higher in the Laksvatn liver extract
347 than the Ellasjøen liver extract (Table 2). For several compounds, the concentration in the
348 Laksvatn extracts (and in some cases in the Ellasjøen extracts) were below or similar to
349 concentrations measured in the blank. The chemical results for these compounds should
350 therefore be interpreted with caution.

351

352 3.2. In vitro effects of Arctic char liver extracts

353 Cell viability, measured as membrane integrity and metabolic activity, of the Arctic char
354 hepatocytes was only slightly affected (>85% of DMSO control) at the highest tested CR by
355 extract F1 of Arctic char liver from both fish populations of Ellasjøen and Laksvatn (Figure 1,
356 Table 3). The slight decrease in cell viability was not significantly different from the F1
357 procedural blank.

358

359 Only the highest tested CR of the Ellasjøen F2-liver extract significantly reduced the metabolic
360 activity compared to the F2 procedural blank. No significant reduction in the membrane
361 integrity was observed for the F2-liver extracts. The F2 liver extracts reduced the metabolic
362 activity in a concentration dependent manner with 50% reduction at a CR of 0.5 (Ellasjøen F2)
363 and at a CR of 1.0 (Laksvatn F2). The Ellasjøen F2 had a significantly higher effect on the
364 metabolic activity than the Laksvatn F2 in the CR range 0.3 – 1 (Figure 1, Table 3).

365

366 The F3-liver extract reduced both the membrane integrity and metabolic activity in a
367 concentration-dependent manner with a reduction to less than 10% of DMSO control at the
368 highest tested CR (CR=0.3). The 50% effect concentration (EC_{50}) for metabolic activity and
369 membrane integrity was a CR of 0.11 (metabolic activity) and of 0.15 (membrane integrity) for
370 the Ellasjøen extract, and a CR of 0.06 (metabolic activity) and 0.09 (membrane integrity) for
371 the Laksvatn extract. The F3 procedural blank did not reduce the metabolic activity or
372 membrane integrity compared to the DMSO control. A significant reduction in metabolic
373 activity was observed at a CR of 0.03 in Ellasjøen F3- and at a CR of 0.1 in Laksvatn F3-liver
374 extract when compared to the procedural blank F3. The Laksvatn F3-liver extract was
375 significantly more effective in reducing the metabolic activity of the primary Arctic char
376 hepatocytes than the Ellasjøen F3-liver extract, but only at a CR of 0.1 (Figure 1, Table 3).
377

378 The F3-liver extract from Ellasjøen fish significantly increased the production of Vtg at a CR
379 of 0.03 (Figure 1), with a maximum Vtg protein expression of 10% of positive control at a CR
380 of 0.1. The response of the Ellasjøen F3-liver extract on the Vtg protein expression was
381 significantly higher than the response of the Laksvatn F3-liver extract. No increased *in vitro*
382 Vtg protein expression was observed after exposure to any of the other liver extracts.
383

384 4. Discussion

385 Liver extracts from Arctic char from two different lakes at Bjørnøya were subjected to chemical
386 analysis and *in vitro* effect assessment using primary hepatocytes from Arctic char. Not
387 surprisingly, the highest levels of contaminants were found in liver extracts from Ellasjøen fish,
388 a population where high OHC concentrations have previously been reported (Bytingsvik et al.,
389 2015). The F2 and F3 extracts from both fish populations reduced the *in vitro* cell viability.

390 There was no clear difference between the lakes in terms of effects of the liver extracts on cell
391 viability, but only the Ellasjøen F3-extract affected the Vtg protein expression.

392

393 The results from the chemical analyses were consistent with previously published studies on
394 fish from these locations (Bytingsvik et al., 2015; Evenset et al., 2005, 2004). The chemical
395 analysis indicates that the fish population in Ellasjøen is subjected to higher risk of biological
396 effects than the Laksvatn fish population. A statement supported by previous observation of
397 approximately 50 fold higher hepatic cytochrome P450(CYP)1A enzyme levels in Arctic char
398 from Ellasjøen compared to those from another low-contaminated lake on Bjørnøya (Lake
399 Øyangen) in addition to lower glucocorticoid receptor (GR) protein expression, elevated heat
400 shock proteins expression (Wiseman et al. 2011), higher level of DNA double strand breaks in
401 Lake Ellasjøen char (Neerland., 2016), higher liver *cyp1a* mRNA abundance, and altered
402 mRNA abundance of key genes related to HPI axis functioning in the Lake Ellasjøen char
403 compared to the Lake Laksvatn char (Jørgensen et al., 2017).

404

405 ## 4.1. Effects on *in vitro* cell viability of Arctic char liver extracts

406 The lowest effect on cell viability was observed after exposure to the F1 extract from Ellasjøen
407 and Laksvatn fish. This is interesting as these extracts (and especially F1 from Ellasjøen fish)
408 contain high levels of OHCs like PCBs, PBDEs, DDTs and mirex. Moreover, there were large
409 differences in concentration between the two lakes. The octanol-water partition coefficient
410 (logKow) for several of these compounds are high (e.g. calculated LogKow for PCBs range
411 from 3.76 for biphenyl to 8.26 for decachlorobiphenyl). This could have implications on the
412 bioavailability of these contaminants in the *in vitro* assay due to potential compartmentalization

of highly hydrophobic contaminants to passive lipid reservoirs (lipid vacuoles) inside the isolated char hepatocytes.

Both F2 and F3 extracts reduced the cell viability at concentrations below and around original tissue concentrations, indicating that both fish populations are at risk for hepatotoxic effects. None of the analyzed compounds were detected in the Laksvatn F2 extract (all values < LOD). Since the F2 extract from both Ellasjøen and Laksvatn fish reduced the cell viability, endogenous compounds and/or environmental contaminants not included in the targeted chemical analysis likely mediate the effect.

The F3 extract from Laksvatn fish affected the cell viability at lower concentrations than the F3 extract from Ellasjøen. Of the contaminants measured in F3, concentrations of certain OH-BDEs (OH-BDE-68, OH-BDE-47/75 and OH-BDE-101) were higher in the Laksvatn than the Ellasjøen extract. Some of these compounds are known to have toxic effects. For example, OH-BDE-47/75 is cytotoxic in primary adult neural stem/progenitor cells from adult mice and is also more toxic than the parent compound BDE-47 (Li et al., 2013). Even though the concentrations measured in the extracts were lower than those known to cause an effect, the compounds might contribute to the observed effects on metabolic activity together with the other measured contaminants as well as contaminants not included in the targeted chemical analysis.

434 ## 4.2. Effects on *in vitro* Vitellogenin protein expression of Arctic char extracts

435 Only the F3 extract of liver from Ellasjøen fish significantly increased *in vitro* Vtg protein
436 expression compared to the procedural blank, suggesting that one or more compound(s) in this
437 extract is estrogenic and may cause changes in the biological activity related to the estrogenic
438 hormone system. A significant effect was observed at a CR of 0.03, which is well below the
439 original tissue concentration (CR=1). It should also be noted that having a CR of 1 in the
440 exposure media does not necessarily mean that the internal cell concentration will be the same.
441 The internal cell concentration depends on the uptake from exposure media, and binding of
442 compounds to the plastic wells, cell surface or other media components. Thus the actual internal
443 exposure concentrations will most likely be lower than the original tissue concentration at a CR
444 of 1.
445
446 The F3 extracts originates from tissue from female and male fish in different reproductive
447 stages. High concentrations of the endogenous estrogen E2 were present in the chars' plasma
448 (supplementary table 1), with higher concentrations measured in females from Ellasjøen than
449 Laksvatn (Bytingsvik et al., in prep). Although the estrogen levels in the liver extracts were not
450 measured, endogenous estrogens present in the liver samples will likely end up in F3 during the
451 fractionation. Hence, endogenous estrogens may have contributed to the effect on the Vtg
452 protein expression after exposure to the F3 liver extract from Ellasjøen.
453
454 The F3 extract also contains hydroxylated metabolites of PCBs, several of which are known to
455 exert estrogenic (Braathen et al., 2009) and/or anti-estrogenic effects (Gustayson et al., 2015;
456 Oh et al., 2007). Of the analysed compounds in the F3 extracts, concentrations of OH-PCBs (4-
457 OH-PCB-107, 4-OH-PCB-146, 4-OH-PCB-163, and 4-OH-PCB-187) were found to be higher
458 in the Ellasjøen liver extract than Laksvatn liver extract. Primary Atlantic salmon and rainbow

459 trout hepatocytes have previously shown a concentration-specific induction of *Vtg* mRNA when
460 exposed to 4-OH-CB107, 4-OH-CB146, 4-OH-CB187, and 3-OH-CB138 (Braathen et al.,
461 2009) and Vtg protein when exposed to 4-OH-PCB50, 4-OH-PCB30, 4-OH-PCB 72 and 4-OH-
462 PCB 112 (Andersson et al., 1999). Increased plasma Vtg concentrations were also observed in
463 rainbow trout dietary exposed to 4-hydroxy-2',4',6'-trichlorobiphenyl (OH-PCB30) and 4-
464 hydroxy-2',3',4',5'-tetrachlorobipheny (OH-PCB-61) (Carlson and Williams, 2001). Although
465 effects of endogenous estrogens cannot be ruled out, contribution to the observed increased Vtg
466 protein expression by OH-PCBs detected in the F3-liver extract from Ellasjøen fish is likely as
467 OH-PCBs are known to be estrogenic (Andersson et al., 1999; Braathen et al., 2009; Carlsson
468 and Williams, 2001) and effects of estrogenic compounds in mixtures are known to be additive
469 both *in vitro* and *in vivo* (Brian et al., 2005; Correia et al., 2007; Petersen and Tollefsen, 2011;
470 Thorpe et al., 2001).

471

472 ### 4.3. Environmental implications

473 Arctic char from Ellasjøen might be subjected to a total load of pollutants with a potential to
474 disrupt estrogenic processes, ultimately leading to effects on reproduction if exposure occurs
475 during sensitive developing stages (Bytingsvik et al., 2015; Letcher et al, 2010). This is further
476 supported by altered mRNA abundances of key genes related to HPI axis functioning in the
477 Ellasjøen char, suggestive of endocrine disruptive effects in this char population (Jørgensen et
478 al., 2017). Dose-response relationships from *in vitro* and *in vivo* studies with temperate and
479 Arctic species in a steady (high) nutritional state might not be relevant for Arctic animals that
480 have fasting periods. Several studies have shown that fasting fish mobilize PCBs bound to lipid
481 storages, potentially causing a larger effect at lower concentrations than in PCB-exposed fish
482 that are still fed (Maule et al., 2005; Jørgensen et al. 2006). Fertilized fish eggs (Atlantic
483 croacker, *Micropogonias undulates*) from PCB dosed adults (0.4 mg Aroclor 1254/ kg fish)

484 contained 0.66 µg/g egg (660 ng/g egg) PCB and showed reduced growth rate and impaired
485 startle response (McCarthy et al., 2003). From previous studies it seems that low PCB levels in
486 fish eggs from temperate species (<500 ng/g ww) affected embryonic and larval survival and
487 caused reproductive dysfunctions later in life at levels as low as 10–30 ng/g ww (reviewed by
488 Letcher et al., 2010). A negative correlation between lake trout (*S. namaycush*) egg PCB
489 concentration (124–314 ng/g w.w.) and egg and fry survival have also been reported (Mac and
490 Edsall, 1991). Interestingly, it was the extract containing OH-PCBs that showed estrogenic
491 effects in the present study. High levels of PCBs might lead to higher formation of OH-PCBs
492 through biotransformation, resulting in effects on the endocrine system as observed in the
493 present study, potentially affecting reproduction. Previous studies also support that early life
494 stages of fish are more vulnerable to pollutants than adult stages, which complicates the
495 determination of acceptable threshold concentration for contaminants in the environment
496 (reviewed by Letcher et al., 2010).

497

498 ## 5. Conclusion

499 Arctic char from Lake Laksvatn and Lake Ellasjøen are exposed to a cocktail of pollutants, and
500 highest concentrations were generally found in liver extracts from Ellasjøen. The extracts
501 affected *in vitro* cell viability and Vtg protein expression. Cell viability was affected by F2 and
502 F3 from both fish populations. Potential contributors to the effects on cell viability of the F2
503 liver extracts could not be determined, whereas OH-BDEs may be partly responsible for the
504 observed effect on cell viability of the F3 liver extracts. Only the F3 liver extract from Ellasjøen
505 fish affected the Vtg protein expression, and although compounds such as estrogenic OH-PCBs
506 was quantified in the F3 extracts, it remains to be determined which compounds were causing
507 the estrogenic effect.

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518

519 ## References

520 Andersson, P.L., Blom, A., Johannisson, A., Pesonen, M., Tysklind, M., Berg, A.H., Olsson, 521 P.E., and Norrgren, L. 1999. Assessment of PCBs and hydroxylated PCBs as potential 522 xenoestrogens: *In vitro* studies based on MCF-7 cell proliferation and induction of 523 vitellogenin in primary culture of rainbow trout hepatocytes. Arch. Environ. Contam. 524 Toxicol. 37: 145–150.

525 Braathen, M., Mortensen, A.S., Sandvik, M., Skare, J.U., and Arukwe, A. 2009. Estrogenic 526 Effects of Selected Hydroxy Polychlorinated Biphenyl Congeners in Primary Culture 527 of Atlantic Salmon (*Salmo salar*) Hepatocytes. Arch. Environ. Contam. Toxicol. 56: 528 111–122.

529 Brian, J.V., Harris, C.A., Scholze, M., Backhaus, T., Booy, P., Lamoree, M., Pojana, G., 530 Jonkers, N., Runnalls, T., Bonfa, A., Marcomini, A., Sumpter, J.P., 2005. Accu-rate

531 prediction of the response of freshwater fish to a mixture of estrogenic chemicals.
532 Environmental Health Perspectives 113 (6): 721–728.

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

537 Bytingsvik, J., Frantzen, M., Christensen, G., Bender, M.L., Hestdahl, H.I., Johnsen, H.K.,
538 Neerland, E., Nikiforov, V., Evenset A. In prep. Combined effects of persistent organic
539 pollutants (POPs) on reproduction in Arctic char (*Salvelinus alpinus*) from Bjørnøya,
540 Svalbard (Norway)

541 Carlson, D.B., and Williams, D.E. 2001. 4-hydroxy-2 ',4 ',6 '-trichlorobiphenyl and 4-hydroxy-
542 2 ',3 ',4 ',5 '-tetrachlorobiphenyl are estrogenic in rainbow trout. Environ. Toxicol.
543 Chem. 20: 351–358.

544 Christensen, G., and Evenset, A. 2011. Miljøgifter i røye fra innsjøer på Svalbard, Akvaplan-
545 niva rapport. Akvaplan-Niva Tromsø, 30p.

546 Colborn, T., vom Saal, F.S., and Soto, A.M. 1993. Developmental effects of endocrine-
547 disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101: 378.

548 Correia, A.D., Freitas, S., Scholze, M., Goncalves,J.F., Booij, P.,Lamoree,M.H.,Ma˜ nanós, E.,
549 Reis-Henriques, M.A., 2007. Mixtures of estrogenic chemicals enhance vitellogenic
550 response in sea bass. Environmental Health Perspectives 115 (Suppl. 1): 115–121.

551 Evenset, A., Christensen, G.N., Skotvold, T., Fjeld, E., Schlabach, M., Wartena, E., and Gregor,
552 D. 2004. A comparison of organic contaminants in two high Arctic lake ecosystems,
553 Bjørnøya (Bear Island), Norway. Sci. Total Environ. 318: 125–141.

554 Evenset, A., Christensen, G.N., and Kallenborn, R. 2005. Selected chlorobornanes,
555 polychlorinated naphthalenes and brominated flame retardants in Bjørnøya (Bear
556 Island) freshwater biota. Environ. Pollut. 136: 419–430.

557 Evenset, A., Carroll, J., Christensen, G.N., Kallenborn, R., Gregor, D., Gabrielsen, G.W. 2007.
558 Seabird Guano Is an Efficient Conveyer of Persistent Organic Pollutants (POPs) to
559 Arctic Lake Ecosystems. Environ. Sci. Technol. 41 (4): 1173–1179.

560 Gustayson, L., Ciesielski, T.M., Bytingsvik, J., Styrishave, B., Hansen, M., Lie, E., Aars, J.,
561 and Jenssen, B.M. 2015. Hydroxylated polychlorinated biphenyls decrease circulating
562 steroids in female polar bears (*Ursus maritimus*). Environ. Res. 138: 191–201.

563 Hamers, T., Kamstra, J.H., Sonneveld, E., Murk, A.J., Kester, M.H.A., Andersson, P.L., Legler,
564 J., Brouwer, A. 2006. *In vitro* profiling of the endocrine-disrupting potency of
565 brominated flame retardants. Toxicol Sci 92: 157–73.

566 Hamers, T., Kamstra, J.H., Sonneveld, E., Murk, A.J., Visser, T.J., Van Velzen, M.J.M.,
567 Brouwer, A., Bergman, Å. 2008. Biotransformation of brominated flame retardants into
568 potentially endocrine-disrupting metabolites, with special attention to 2, 2′,4,4′-
569 tetrabromodiphenylether (BDE-47). Mol Nut Food Res 52: 284–98.

570 Harju, M., Hamers, T., Kamstra, J.H., Sonneveld, E., Boon, J.P., Tysklind, M., Andersson, P.L.
571 2007. Quantitative structure–activity relationship modeling on *in vitro* endocrine effects
572 and metabolic stability involving 26 selected brominated flame retardants. Environ
573 Toxicol Chem 26: 816–26.

574 Heppell, S.A., Denslow, N.D., Folmar, L.C., and Sullivan, C.V. 1995. Universal assay of
575 vitellogenin as a biomarker for environmental estrogens. Environ. Health Perspect. 103:
576 9.

577 Jensen, A.A., Leffers, H. 2008. Emerging endocrine disrupters: perfluoroalkylated substances.
578 Internat J Androl 31: 161–9.

579 Jørgensen, E.H., Maule, A.G., Evenset, A., Christensen, G., Bytningsvik, J., Frantzen, M.,
580 Nikiforov, V., Faught, E., Vijayan, M.M., 2017. Biomarker response and hypothalamus-
581 pituitary-interrenal axis functioning in Arctic charr from Bjørnøya (74°30′ N), Norway,
582 with high levels of organohalogenated compounds. Aquat Toxicol 187: 64-71.

583 Jørgensen, E.H., Vijayan, M.M., Killie, J.-E.A., Aluru, N., Aas-Hansen, Ø., Maule, A., 2006.
584 Toxicokinetics and Effects of PCBs in Arctic Fish: A Review of Studies on Arctic
585 Charr. J. Toxicol. Environ. Heal. Part A 69,:37–52.

586 Kuiper, R.V., Canton, R.F., Leonards, P.E.G., Jenssen, B.M., Dubbeldam, M., Wester, P.W.,
587 van den Berg, M., Vos, J.G., Vethaak, A.D. 2007. Long-term exposure of European
588 flounder (*Platichthys flesus*) to the flame-retardants tetrabromobisphenol A (TBBPA)
589 and hexabromocyclododecane (HBCD). Ecotoxicol Environ Safety 67: 349–60.

590 Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jorgensen, E.H., Sonne, C., Verreault, J.,
591 Vijayan, M.M., and Gabrielsen, G.W. 2010. Exposure and effects assessment of
592 persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ.
593 408: 2995–3043.

594 Li, T., Wang, W., Pan, Y.-W., Xu, L., & Xia, Z. 2013. A Hydroxylated Metabolite of Flame-
595 Retardant PBDE-47 Decreases the Survival, Proliferation, and Neuronal Differentiation
596 of Primary Cultured Adult Neural Stem Cells and Interferes with Signaling of ERK5
597 MAP Kinase and Neurotrophin 3. Toxicological Sciences, 134(1): 111–124.

598 Liu, C.S., Du, Y.B., Zhou, B.S. 2007. Evaluation of estrogenic activities and mechanism of
599 action of perfluorinated chemicals determined by vitellogenin induction in primary
600 cultured tilapia hepatocytes. Aquat Toxicol 85: 267–77.

601 Mac, M.J., Edsall, C.C. 1991. Environmental contaminants and the reproductive success of lake
602 trout in the Great Lakes: an epidemiological approach. J Toxicol Environ Health 33:
603 375–94

604 McCarthy, I., Fuiman, L., Alvarez, M., 2003. Aroclor 1254 affects growth and survival skills
605 of Atlantic croaker *Micropogonias undulatus* larvae. Mar. Ecol. Prog. Ser. 252: 295–
606 301.

607 Maule, A.G., Jørgensen, E.H., Vijayan, M.M., Killie, J.-E.A. 2005. Aroclor 1254 exposure
608 reduces disease resistance and innate immune responses in fasted Arctic charr. Environ.
609 Toxicol. Chem. 24: 117–124.

610 Mommsen, T.P., and Walsh, P.J. 1988. Vitellogenesis and oocyte assembly. Fish Physiol. 11:
611 347–406.

612 Morgado, I., Hamers, T., van der Ven, L., Power, D.M. 2007. Disruption of thyroid hormone
613 binding to sea bream recombinant transthyretin by ioxinyl and polybrominated diphenyl
614 ethers. Chemosphere 69:155–63.

615 Mortensen, A.S., Braathen, M., Sandvik, M., and Arukwe, A. 2007. Effects of hydroxy-
616 polychlorinated biphenyl (OH-PCB) congeners on the xenobiotic biotransformation
617 gene expression patterns in primary culture of Atlantic salmon (*Salmo salar*)
618 hepatocytes. Ecotoxicol. Environ. Saf. 68: 351–360.

619 Navas, J.M., and Segner, H. 2006. Vitellogenin synthesis in primary cultures of fish liver cells
620 as endpoint for in vitro screening of the (anti)estrogenic activity of chemical substances.
621 Aquat. Toxicol. 80: 1–22.

622 Neerland, E.D., 2016. DNA Double-Strand Breaks in Arctic Char, *Salvelinus alpinus*, from
623 Bjørnøya. Master Thesis. Norwegian University of Science and Technology,
624 Environmental Toxicology & Chemistry, 64p.

625 Nøst, T.H., Helgason, L.B., Harju, M., Heimstad, E.S., Gabrielsen, G.W., and Jenssen, B.M.
626 2012. Halogenated organic contaminants and their correlations with circulating thyroid
627 hormones in developing Arctic seabirds. Sci. Total Environ. 414: 248–256.

628 Oakes, K.D., Sibley, P.K., Martin, J.W., MacLean, D.D., Solomon, K.R., Mabury, S.A., Van
629 der Kraak, G.J. 2005. Short-term exposures of fish to perfluorooctane sulfonate: acute
630 effects on fatty acyl-CoA oxidase activity, oxidative stress, and circulating sex steroids.
631 Environ Toxicol Chem 24: 1172–81.

632 Oh, S.M., Ryu, B.T., Lee, S.K., and Chung, K.H. 2007. Antiestrogenic potentials of ortho-PCB
633 congeners by single or complex exposure. Arch. Pharm. Res. 30: 199–209.

634 Petersen, K., and Tollefsen, K.E. 2011. Assessing combined toxicity of estrogen receptor
635 agonists in a primary culture of rainbow trout (*Oncorhynchus mykiss*) hepatocytes.
636 Aquat. Toxicol. 101: 186–195.

637 Petersen, K., Hultman, M.T., Tollefsen, K.E., (2017). Primary hepatocytes from Arctic char
638 (*Salvelinus alpinus*) as a relevant Arctic *in vitro* model for screening contaminants and
639 environmental extracts. Aquat. Toxicol. 187, 141–152.

640 Purdom, C., Hardiman, P., Bye, V., Eno, N., Tyler, C., and Sumpter, J. 1994. Estrogenic effects
641 of effluents from sewage treatment works. Chem. Ecol. 8: 275–285.

642 Sandanger, T.M., Dumas, P., Berger, U., and Burkow, I.C. 2004. Analysis of HO-PCBs and
643 PCP in blood plasma from individuals with high PCB exposure living on the Chukotka
644 Peninsula in the Russian Arctic. J. Environ. Monit. 6: 758.

645 Schirmer, K., Chan, A.G.J., Greenberg, B.M., Dixon, D.G., Bols, N.C., 1997. Methodology for
646 demonstrating and measuring the phototoxicity of fluoranthene to fish cells in culture.
647 Toxicology In Vitro 11: 107-119.

648 Schreer, A., Tinson, C., Sherry, J.P., and Schirmer, K. 2005. Application of Alamar blue/5-
649 carboxyfluorescein diacetate acetoxymethyl ester as a noninvasive cell viability assay
650 in primary hepatocytes from rainbow trout. Anal. Biochem. 344: 76–85.

651 Sumpter, J., and Jobling, S. 1995. Vitellogenesis as a Biomarker for Estrogenic Contamination
652 of the Aquatic Environment. Environ. Health Perspect. 103: 173–178.

653 Sømme, I. D. 1941. Ørretboka. Jacob Dybwads forlag. Oslo. 222-224.

654 Thorpe, K.L., Hutchinson, T.H., Hetheridge, M.J., Scholze, M., Sumpter, J.P., Tyler, C.R.,
655 2001. Assessing the biological potency of binary mixtures of environmental estrogens
656 using vitellogenin induction in juvenile rainbow trout (*Oncorhynchus mykiss*).
657 Environmental Science and Technology 35 (12): 2476–2481.

658 Tollefsen, K.E., Mathisen, R., and Stenersen, J. 2003. Induction of vitellogenin synthesis in an
659 Atlantic salmon (*Salmo salar*) hepatocyte culture: a sensitive *in vitro* bioassay for the
660 oestrogenic and anti-oestrogenic activity of chemicals. Biomarkers 8: 394–407.

661 Wiseman, S., Jørgensen, E.H., Maule, A.G., Vijayan, M.M. 2011. Contaminant Loading in
662 Remote Arctic Lakes Affects Cellular Stress-Related Proteins Expression in Feral
663 Charr. Polar Biol 34: 933–37.

664 Wojtowicz, A.K., Kajta, M., and Gregoraszczuk, E.L. 2007. DDT- and DDE-induced
665 disruption of ovarian steroidogenesis in prepubertal porcine ovarian follicles: A possible
666 interaction with the main steroidogenic enzymes and estrogen receptor beta. J. Physiol.
667 Pharmacol. 58: 873–885.

Figure legends

Figure 1. Effects of Arctic char (*Salvelinus alpinus*) liver extracts F1-F3 on the cell viability (membrane integrity (□) and metabolic activity (○)) and Vitellogenin production (●) in Arctic char hepatocytes. N=3-4. The asterisk (*) indicate results significantly different ($p<0.05$) from procedural blanks (membrane integrity (□) and metabolic activity (○)). F1 contained neutral compounds such as PCBs, PBDEs and organochlorine pesticides, F2 contained $MeSO_2$-PCBs/DDE, and F3 contained hydroxylated (OH) PCBs/phenols.

Table 1. Overview of the tested liver extracts and the concentration ratio (CR) range used in the exposure studies with Arctic char hepatocytes.

Fish population	Extract	CR range
Ellasjøen	F1	0.0003-1
	F2	0.0003-1
	F3	0.0001-0.3
Laksvatn	F1	0.0003-1
	F2	0.0003-1
	F3	0.0001-0.3
Procedural blank	F1	0.1, 1
	F2	0.1, 1
	F3	0.03, 0.3

Table 2. Measured concentrations of different chemicals in the three extracts. Only compounds measured in concentrations above LOD are included and values are given in pg/g ww.

Compound	Abbreviation	Procedural blank	Ellasjøen	Laksvatn
Liver extract F1		pg/g ww	pg/g ww	pg/g ww
Hexachlorobenzene	HCB	9.3	1 084.7	415.8
Heptachlor		397.0	330.0	395.8
Oxychlordane	Oxy-CD	119.9	137.3	110.1
Cis-chlordane	c-CD	26.1	191.9	50.0
Trans-nonachlor	t-NC	<1.0	577.1	51.5
Cis-nonachlor	c-NC	10.9	229.4	36.9
Endosulfan I		11.3	125.7	26.3
Endosulfan sulfate		4.2	<0.4	<0.4
Mirex		8.2	1 545.0	28.0
1.1-Dichloro-2.2-bis(4-chlorophenyl)ethene	p.p-DDE	29.3	27 655.0	377.0
2-(2-Chlorophenyl)-2-(4-chlorophenyl)-1.1-dichloroethene	o.p-DDE	<2.2	39.1	<2.2
Mitotane	o.p-DDD	<61.0	253.0	487.0
Sum DDTs		**29.3**	**27 947.4**	**863.7**
2.2'.4-tribromodiphenylether	PBDE 17	<6.7	63.8	<6.7
2.4.4'-Tribromodiphenyl ether	PBDE 28	<7.6	139.3	<7.6
2.2'.4.5'-Tetrabromodiphenyl ether	PBDE 49	<10.7	98.3	<10.7
2.2'4.4'-Tetrabromodiphenyl ether	PBDE 47	<8.5	5 008.0	26.5
2.3'.4.4'-Tetrabromodiphenyl ether	PBDE 66	<13.6	39.4	<13.6
2.2'.4.4'.6-Pentabromodiphenyl ether	PBDE 100	<15.3	570.8	9.1
2.3'.4.4'.6-Pentabromodiphenyl ether	PBDE 119	<20.8	34.9	<20.8
2.2'.4.4'.5-Pentabromodiphenyl ether	PBDE 99	<19.5	386.8	19.1
2.2'.4.4'.5.6'-Hexabromodiphenyl ether	PBDE 154	<12.5	84.9	<12.5
2.2'.4.4'.5.5'-Hexabromodiphenyl ether	PBDE 153	<17.8	152.4	<17.8
sum PBDEs		**0.0**	**6 578.5**	**54.7**
BATE	BATE	<3.1	2.0 (<LOQ)	1.9 (<LOQ)
PBT	PBT	1.9	3.3 (<LOQ)	3.5 (<LOQ)
PBEB	PBEB	0.7	0.9 (<LOQ)	0.4 (<LOQ)
DPTE	DPTE	0.8	<0.5	1.3 (<LOQ)
BTBPE	BTBPE	2.8	<0.3	2.5 (<LOQ)
BEHTBP	BEHTBP	2.0	<1.3	2.3 (<LOQ)
2.2'.5-trichlorobiphenyl	PCB 18	<5.0	25.0	<5.0
2.4.4'- trichlorobiphenyl /2.4'.5- trichlorobiphenyl	PCB 28/31	3.7	640.5	47.9
2.2'.4.4'-tetrachlorobiphenyl/ 2.2'.4.5'-tetrachlorobiphenyl	PCB 47/49	<3.0	1 022.1	18.8
2.2'.5.5'- tetrachlorobiphenyl	PCB 52	16.4	158.1	41.0
2.3'.4.4'- tetrachlorobiphenyl	PCB 66	81.7	5 639.8	87.2
2.4.4'.5- tetrachlorobiphenyl	PCB 74	<2.0	3 538.3	<2.0
2.2'.4.4'.5-pentachlorobiphenyl	PCB 99	147.8	35 028.2	267.3
2.2'.4.5.5'- pentachlorobiphenyl	PCB 101	172.5	4276.2	245.1
2.3.3'.4.4'-Pentachlorobiphenyl	PCB 105	303.1	9854.0	168.7

2.3.4.4'.5- pentachlorobiphenyl / 2.3.3'.4'.5'-Pentachlorobiphenyl	PCB 114/122	14.2	1 189.2	<12.0
2.3'.4.4'.5- pentachlorobiphenyl	PCB 118	513.1	46 037.6	533.5
2.3'.4.4'.5'-Pentachlorobiphenyl	PCB 123	23.1	4 107.3	50.8
2.2'.3.3'.4.4'-Heachlorobiphenyl	PCB 128	105.3	10 106.8	126.7
2.2'.3.4.4'.5'- Heachlorobiphenyl	PCB 138	451.4	175 561.7	1 399.2
2.2'.3.4.5.5'- Heachlorobiphenyl	PCB 141	41.9	443.7	41.7
2.2'.3.4'.5'.6- Heachlorobiphenyl	PCB 149	95.5	1 384.8	112.7
2.2'.4.4'.5.5'-Hexachlorobiphenyl	PCB 153	271.8	268 701.7	1 791.1
2.3.3'.4.4'.5- Heachlorobiphenyl	PCB 156	82.4	8 551.9	107.3
2.3.3'.4.4'.5'- Heachlorobiphenyl	PCB 157	18.7	1 648.3	28.2
2.3'.4.4'.5.5'-Hexachlorobiphenyl	PCB 167	21.5	5 795.1	60.3
2.2'.3.3'.4.4'.5-Heptachlorobiphenyl	PCB 170	37.2	22 122.6	274.5
2.2'.3.4.4'.5.5'- Heptachlorobiphenyl	PCB 180	49.3	66 243.3	665.8
2.2'.3.4.4'.5'.6- Heptachlorobiphenyl	PCB 183	<4.0	12 154.5	123.7
2.2'.3.4'.5.5'.6- Heptachlorobiphenyl	PCB 187	<5.0	21 275.6	299.5
2.3.3'.4.4'.5.5'- Heptachlorobiphenyl	PCB 189	<5.0	612.3	8.9
2.2'.3.3'.4.4'.5.5'-Octachlorobiphenyl	PCB 194	<10.0	3 097.4	46.8
Sum PCBs		**2 450. 5**	**709 215.5**	**6 546.3**
Octachlorostyrene	OCS	<26.0	125.2	26.0
6-methoxy-2.2'.4.4'-tetrabromodiphenyl ether/6'-methoxy-2.4.4′.6-tetrabromodiphenyl ether	6-MeOBDE47/75	<9.0	330.0	<9.0
4'-methoxy-2.2'.4.5'-Tetrabromodiphenyl ether	4-MeOBDE49	20.0	54.0 (<LOQ)	<18.0
Liver extract F2		pg/g ww	pg/g ww	pg/g ww
4'-Methylsulfonyl-2.4.5.2'.5'-pentachlorobiphenyl	4MeSOPCB101	0.8	5.5	<0.8
4-MeSO2-2.2′.3.4′.5′.6-hexachlorobiphenyl	4MeSOPCB149	<0.9	13.8	<0.9
4-Methylsulfonyl-2.2'.3'.4'.5.5'-hexachlorobiphenyl	4MeSOPCB141	<0.8	3.2	<0.8
Liver extract F3		pg/g ww	pg/g ww	pg/g ww
triisobutyl phosphate	TiBP	420.0	200.0 (<LOQ)	300.0 (<LOQ)
tributyl phosphate	TBP	500.0	200.0 (< LOQ)	200.0 (< LOQ)
tris(2-chloroethyl)phosphate	TCEP	4280	4 260.0 (<LOQ)	4 380.0 (<LOQ)
tri(1-chloro-2-propyl)phosphate	TCPP	1760	1 500.0 (<LOQ)	1 720.0 (<LOQ)
tris(2-butoxyethyl)phosphate	TBEP	<87.0	420.0	240.0
Pentachlorophenol	PCP	5.9	53.3 (<LOQ)	53.3 (<LOQ)
4-Hydroxy-2'.3.3'.4'.5'-pentachlorobiphenyl	4-OH-PCB 107	0.9	7.8 (LOQ)	<2.6
4-Hydroxy-2.2'.3.4'.5.5'-hexachlorobiphenyl	4-OH-PCB 146	<0.1	4.4	<0.1
3-Hydroxy-2.2'.3.4.4'.5'-hexachlorobiphenyl	3-OH-PCB 138	0.1	1.3 (<LOQ)	<0.4
4-hydroxy- 2.2'.3.3'.4.5'-Hexachlorobiphenyl	4-OH-PCB 130	0.3	2.7 (<LOQ)	<0.9
4-Hydroxy-2.3.3'.4'.5.6-hexachlorobiphenyl	4-OH-PCB 163	0.1	1.1	<0.4
4-Hydroxy-2.2’.3.4’.5.5’.6-heptachlorobiphenyl	4-OH-PCB 187	0.1	5.6	<0.4
sum OH-PCB		**1.6**	**11.1**	**0.0**
2'-Hydroxy-2.3'.4.5'-tetrabromodiphenylether	2-OH-BDE68	<0.1	1.4	7.2
6-hydroxy-2.2'.4.4'-tetrabromodiphenyl ether /6'-hydroxy-2.4.4′.6-tetrabromodiphenyl ether	6-OH-BDE47/75	<0.1	1.6	3.7
4'-Hydroxy-2.2'.4.5.5'-pentabromodiphenyl ether	4-OH-BDE101	<0.1	<0.1	0.3 (<LOQ)

1 Table 3. Summary of results from the Arctic char (*Salvelinus alpinus*) hepatocytes exposed to extracts (F1-F3) of liver from Arctic char sampled

2 in Laksvatn (low-contaminated lake) and Ellasjøen (high-contaminated lake) at Bjørnøya (Bear Island) in 2012. The table presents the no observed

3 effect concentration (NOEC), lowest observed effect concentration (LOEC), 10% and 50% effect concentration (EC_{10} and EC_{50}) given as the

4 extracts concentration ratio (CR, ratio between concentration in the sampled tissue and exposure media) for the endpoints metabolic activity (Met.

5 Act.) and vitellogenin protein expression (Vtg). The LOEC is the lowest CR causing a significant effect compared to the procedural blank ($p <$

6 0.05). F1 contained neutral compounds such as PCBs, PBDEs and organochlorine pesticides, F2 contained $MeSO_2$-PCBs/DDE, and F3 contained

7 hydroxylated (OH) PCBs/phenols. Significant differences between corresponding extracts from the two fish populations are given by the CR at

8 which the significant differences were observed with the lake from which the most effective extract was obtained in parenthesis.

Extract	**Endpoint**	**Ellasjøen**					**Laksvatn**					**Between lake comparison**
		NOEC CR	**LOEC CR**	**EC_{10} CR**	**EC_{50} (R^2)**	**Max effect (CR)**	**NOEC CR**	**LOEC CR**	**EC_{10} CR**	**EC_{50} (R^2)**	**Max effect (CR)**	
F1 liver	Met. Act.	1	na	na	na	90% (1)	1	na	0.030	na	na	ns
F2 liver	Met. Act.	0.3	1	0.074	0.499 (0.926)	35% (1)	1	na	0.14	0.985 (0.937)	51 % (1)	CR 0.3 and 1, (Ellasjøen)
F3 liver	Met. Act.	0.01	0.03	0.036	0.111 (0.927)	6% (0.3)	0.03	0.1	0.027	0.0599 (0.973)	3 % (0.3)	CR 0.1 (Laksvatn)
F1 liver	Vtg	1	na	na	na	0.3% (0.1)	1	na	na	na	0.1 (0.01)	ns
F2 liver	Vtg	1	na	na	na	0.6% (1)	1	na	na	na	0.2 % (1)	na
F3 liver	Vtg	0.01	0.03	na	na	10% (0.1)	0.3	na	na	na	0.15 % (0.003)	CR 0.03 (Ellasjøen)

Max effect: the lowest obtained metabolic activity or the highest obtained Vtg protein expression (% of control). The corresponding CR is given in parenthesis.
na: not applicable, no measurable effect observed, not possible to fit a concentration response curve
ns: not significant (one-way ANOVA with a Tukey post hoc test, $p > 0.05$)

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