Persistent organic pollutants and organophosphate esters in feathers and blood plasma of adult kittiwakes (*Rissa tridactyla*) from Svalbard – associations with body condition and thyroid hormones

N. B. Svendsen[1*], D. Herzke[2], M. Harju[2], C. Bech[1], G. W. Gabrielsen[3], V. L. B. Jaspers[1]

[1] Department of Biology, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway

[2] Norwegian Institute for Air Research (NILU), FRAM Centre, NO-9296 Tromsø, Norway

[3] Norwegian Polar Institute, FRAM Centre, NO-9296 Tromsø, Norway

*Corresponding author: nbos@niras.dk, +45 6039 4410

Abstract

Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs) and organophosphate esters (OPEs) were assessed in blood plasma and feathers of 19 adult black-legged kittiwakes (*Rissa tridactyla*) breeding in two colonies (Blomstrandhalvøya and Krykkjefjellet) at the Arctic archipelago, Svalbard. Potential associations with body condition index (BCI) and thyroid hormones were investigated. All compound classes were detected in both blood plasma and feathers, but due to low sample size and volumes, OPEs could only be quantified in four individuals, warranting larger follow up studies. Kittiwakes breeding at Blomstrandhalvøya had significantly higher concentrations of organic pollutants in blood plasma than kittiwakes breeding at Krykkjefjellet ($p < 0.001$). Concentrations in blood plasma and feathers did not significantly correlate for any of the investigated compounds, and feather concentrations did not differ significantly between the colonies. This suggests that pollutant levels in adult kittiwake feathers do not reflect local contamination at breeding sites and are as such not useful to monitor local contamination at Svalbard. Significant negative associations between BCI and most pollutants were found in both populations, whereas significant correlations between the BCI, the ratio of total triiodothyronine to free triiodothyronine (TT3:fT3), and several pollutants were only found for kittiwakes from Blomstrandhalvøya (all $r \geq -0.60$ and $p \leq 0.05$). This indicates that higher levels of circulating pollutants during the breeding period covary with the TT3:fT3 ratio, and may act as an additional stressor during this period.

Keywords: Feathers, POPs, organophosphate esters, thyroid hormones, black-legged kittiwakes

40 **Funding sources**

41 N. B. Svendsen and V. L. B. Jaspers received Artic Field Grants (ES520962 and ES520958,
42 respectively) from the Norwegian Research Council to carry out the fieldwork at Svalbard,
43 Norway. V.L.B Jaspers is further supported by the Norwegian University of Science and
44 Technology (NTNU), and the pollutant analyses were funded in a joint collaboration between
45 NTNU and the Norwegian Polar Institute.

46

47 1. **Introduction**

48 The first reports of contaminated Arctic wildlife were published in the early 1970's (AMAP
49 1998), and now the Arctic is considered as an important indicator region for assessing the
50 persistence and bioaccumulative abilities of emerging contaminants (de Wit et al. 2010).
51 Atmospheric transport is the main and most rapid source of semi-volatile persistent organic
52 pollutants (POPs) to the Arctic (Gordeev 2002; AMAP 2015). In the Arctic, POPs enter
53 seabird species, such as the black-legged kittiwake (*Rissa tridactyla*, hereafter just
54 'kittiwake'), mainly through their diet, and are thereafter distributed to lipid rich tissues (AMAP
55 2015). During the reproductive period, when seabirds are believed to function close to their
56 physiological limit (Bech et al. 2002), they rely on energy stored as lipids. Therefore, mass
57 loss during the breeding period is common in birds (Moreno 1989) and kittiwakes are no
58 exception (Henriksen et al. 1996; Bech et al. 2002). This release of lipids to the blood leads to
59 a redistribution of lipophilic contaminants, which increases the concentration of circulating
60 pollutants, and the risk that POPs can reach sites of toxicity (Henriksen et al. 1996). Hence,
61 during the breeding period kittiwakes may be at higher risk of negative effects associated with
62 POPs, than the mean concentration of POPs might suggest (Macdonald and Brewers 1996).

63 In Arctic seabird species, several effects have already been related to POP exposure. These
64 include changed reproductive behavior, reduced adult survival rate, wing feather asymmetry,
65 suppressed immune function, reduced offspring performance, and lowered levels of
66 circulating thyroid hormones (THs) (Grasman et al. 1996; Bustnes et al. 2001; Bustnes et al.
67 2003; Verreault et al. 2004; Verboven et al. 2009; Nøst et al. 2012). In the present study, all
68 investigated legacy POPs, including organochlorine pesticides (OCPs), polybrominated
69 diphenylethers (PBDEs) and polychlorinated biphenyls (PCBs) have the potential properties
70 to be endocrine disrupting chemicals (EDC; Petersen et al. 2007). EDCs may have adverse
71 effects on the TH system, which is vital for seabirds to adapt, reproduce, and survive in the
72 cold Arctic climate (Gabrielsen 2007).

73 In birds, the predominant TH is thyroxine (T4), whereas the biologically active TH is
74 triiodothyronine (T3) (McNabb 1995). T4 is transported in blood mainly by the transport
75 proteins transthyretin and albumin (McNabb 2007; Hill et al. 2008), and mostly converted to

76 the active form T3 by hepatic type 1 deiodinase (Dawson 2000). Active THs exert a wide
77 range of effects and are required for growth, differentiation and maturation of several body
78 systems, central nervous system development, and reproductive activity (Dawson, 2000;
79 McNabb 2007). THs also induce molt and regulate heat production in order to maintain a
80 constant body temperature, which is crucial for Arctic seabirds (McNabb 2007). Since the
81 Arctic summer is short, proper timing of breeding, molting, and migration is essential for
82 survival. Exposure to EDCs could disrupt the ability of the endocrine system to regulate these
83 events as some EDCs have structural resemblance with THs (Verreault et al. 2004) and may
84 cause decreased T3 levels (Blévin et al. 2017). This could lead to less successful breeding
85 and in the worst case reduced survival (Jenssen 2006).

86 Studies, that have investigated the use of feathers for measuring POPs and emerging
87 pollutants, have evaluated feathers as a useful biomonitoring tool for non-destructive
88 detection and quantification of organic pollutants (Dauwe et al. 2005; Jaspers et al. 2006;
89 Jaspers et al. 2007b; van den Steen et al. 2007; Eulaers et al. 2011; García-Fernández et al.
90 2013). (Re-)emerging pollutants, such as organophosphate esters (OPEs), have been
91 detected in the Arctic environment (Salamova et al. 2014), but very few studies have
92 investigated their occurrence in Arctic wildlife (Evenset et al. 2009; Hallanger et al. 2015). The
93 present study further addresses this issue by examining POPs and OPEs in feather and blood
94 samples from kittiwakes breeding at the Arctic archipelago, Svalbard.

95 The main objectives of the present study were to 1) assess plasma and feather
96 concentrations of PCBs, OCPs, PBDEs, and OPEs; 2) examine the relationship between
97 pollutant levels in feathers and blood; 3) evaluate potential correlations between pollutants
98 and thyroid hormones in kittiwakes breeding at Svalbard.

99

100 2. **Materials and methods**

101 *2.1 Study area and sample*

102 *collection*

103 Sampling was conducted during the
104 kittiwake breeding season in July and
105 August 2014. Two colonies located
106 close to Ny-Ålesund, Kongsfjorden,
107 Svalbard (78°55’N, 11°55’E), Norway,
108 were studied – the ‘Krykkjefjellet’
109 colony approximately 7 km southeast
110 of Ny-Ålesund, and the
111 ‘Blomstrandhalvøya’ colony on the
112 northeast side of Blomstrandhalvøya
113 (Fig. 1). Eight birds (5 males, 3
114 females) from Krykkjefjellet were
115 sampled mid-July to early-August, and
116 eleven birds (6 males, 5 females) from
117 Blomstrandhalvøya were sampled in
118 early-August. All sampled kittiwakes
119 were adult and caught on their nest or adjacent cliffs with a noose at the end of a 5 m long
120 fishing rod. Biometric measurements of weight, skull-, tarsus- and wing length, as well as
121 blood and feather sampling were carried out immediately after capture. Feathers from the
122 back, the head, and the sixth primary feather (both wings) were sampled and pooled for
123 analysis. Approximately 2 mL of blood was drawn from the alar vein with a 2 mL heparinized
124 syringe (25 G) and stored on ice until samples were centrifuged at 4000 rpm and then frozen
125 (-20 °C) until analysis. All handling and sampling of the birds occurred by trained personnel

Figure 1. An overview of Kongsfjorden situated on the west side of the Arctic archipelago Svalbard, Norway. The two colonies are marked with an asterisk. All map data are from the Norwegian Polar Institute. Map design: Niels Borup Svendsen.

126 and was in accordance with ethical guidelines and approval by the Norwegian Animal
127 Research Authority (FDU permission number 2014/59453-2).

128 *2.2 Sex determination*

129 All birds were sexed at the Norwegian University of Science and Technology (NTNU) in
130 Trondheim, Norway, following methods described by Griffiths et al. (1998). In short, DNA was
131 isolated from blood samples by using the Chelex method as described by Walsh et al. (1991),
132 and Chromobox-helicase-DNA-binding genes (CHD-W and CHD-Z) were amplified by PCR.
133 The avian sex chromosome CHD is widely used for sexing purposes, and as CHD-W only
134 occurs in females (ZW) and not in males (ZZ), PCR products separated by electrophoresis
135 result in one band for males and two bands for females.

136 *2.3 Thyroid hormone analysis*

137 Total triiodothyronine (TT3) and free triiodothyronine (fT3) were quantified in plasma by a
138 competitive enzyme immunoassay human kit (MP Biomedicals, Ohio, USA) at NTNU,
139 Trondheim. Two blank samples and a human T3 standard reference set were used as quality
140 assurance of the quantification. The mean of two replicates was calculated for both TT3 and
141 fT3 with an average intra-assay coefficient of variation (CV) of 10 % for fT3 and 6 % for TT3.
142 Levels of T4 and glandular hormones could not be investigated due to limited plasma
143 amounts.

144 *2.4 Contaminant analysis*

145 Contaminant analyses were conducted at the Norwegian Institute for Air Research (NILU) in
146 Tromsø, Norway. In all samples, 8 PBDE congeners (28, 47, 99, 100, 138, 153, 154 and
147 184), 12 PCB congeners (28, 52, 99, 101, 105, 118, 138, 153, 180, 183, 187 and 194),
148 hexachlorobenzene (HCB), *oxy*-, *cis*- and *trans*-chlordane (OxC, CC, and TC), *cis*- and *trans*-
149 nonachlor (CN and TN), mirex, *α*-, *β*-, and *γ*-hexachlorocyclohexane (HCH), *o,p'*-DDT and
150 *p,p'*-DDT and transformation products (*p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDE and *o,p'*-DDE) were
151 analyzed. In four individuals, the following 13 organophosphate esters were analyzed in both
152 feathers and blood as well: tris(2-chloroethyl) phosphate (TCEP), tripropyl phosphate (TnPP),

153 tris(2-chloroisopropyl) phosphate (TCIPP), tri isobutyl phosphate (TIBP), tri-*n*-butyl phosphate
154 (TNBP), butyl diphenyl phosphate (BdPhP), triphenyl phosphate (TPHP), dibutyl phenyl
155 phosphate (DBPhP), tris(1,3-dichloro-2-propyl)phosphate (TDCIPP), tris(2-
156 butoxyethyl)phosphate (TBOEP), 2-ethylhexyl diphenyl phosphate (EHDP), sum of tricresyl
157 phosphates (sum of TMPP isomers), and tris(2-ethyl hexyl) phosphate (TEHP).

158 *2.4.1 POP extraction and clean up*

159 Approximately 0.5-1.1 g of plasma was spiked with an internal standard containing labeled
160 standards of PCBs, PBDEs, HCB, chlordane, nonachlor, mirex, HCHs, and DDTs. The
161 plasma samples were subsequently denaturated with ethanol and ammonium sulphate in
162 deionized water. Samples were extracted thrice with *n*-hexane, and cleaned up on Florisil®
163 (Fisher Scientific, Pittsburgh, USA) solid phase extraction (SPE) cartridges as described by
164 Sandanger et al. (2007).

165 Approximately 500 mg of feathers were washed thoroughly with Milli-Q water and dried
166 overnight at ambient temperature (Jaspers et al. 2007b; Jaspers et al. 2008). Thereafter they
167 were cut into 1 mm pieces, spiked with internal standards (same standard as above), and
168 covered with cyclohexane/acetone 3:1 (v:v) and sonicated for 15 min. Lastly, feather extracts
169 were fractionated with gel permeation chromatography (GPC - Waters Corporation, Milford,
170 Massachusetts, USA) and cleaned up on Florisil® SPE cartridges. Procedures were modified
171 from Dauwe et al. (2005) and Eulaers et al. (2011, 2014).

172 *2.4.2 OPE extraction and clean up*

173 Due to insufficient sample volume from the remaining individuals, only four individuals (two
174 males and two females) were used in the OPE determination. Approximately 1 mL of plasma
175 was spiked with 20 ng of an internal standard consisting of deuterated D21-TPHP and D27-
176 TNBP (Chiron AS, Trondheim, Norway) before denaturation with acetonitrile and ammonium
177 sulphate in Oasis® HLB cleaned Milli-Q water (Waters Corporation). Samples were
178 centrifuged and the upper acetonitrile phase was transferred to new 15 mL glass centrifuge
179 tubes with 0.5 g of Supelclean™ PSA (primary-secondary amine bonded silica) and 0.2 g
180 magnesium sulphate (Sigma-Aldrich Inc., St. Louis, Missouri, USA). Samples were then

181 centrifuged and supernatant was transferred to new glass tubes and evaporated to 0.2 mL.
182 Lastly, samples were transferred to 2 mL glass vials and 20 ng of deuterated Tris(propyl)
183 phosphate (D21-TPrP) was added as recovery standard (Chiron AS, Trondheim, Norway).

184 Clean up procedures for feather samples were adapted from the protocol described by
185 Eulaers et al. (2014). Briefly, feather samples were washed thoroughly with Milli-Q water and
186 dried overnight at ambient temperature. Hereafter cut into 1mm pieces, spiked with internal
187 standards consisting of deuterated D21-TPHP and D27-TNBP, and incubated for 5 h at 45 °C
188 with hydrogen chloride (HCl, 1 M) and 6 mL of hexane:dichloromethane (4:1; v:v). After
189 liquid/liquid extraction using hexane:dichloromethane (4:1; v:v), extracts were cleaned up on
190 glass SPE columns with primary-secondary amine (PSA) and eluted with methyl tert-butyl-
191 ether (MTBE).

192 *2.4.3 Analyte identification and quantification*

193 The analysis of PCBs, PBDEs, and OCPs by high-resolution gas chromatography (HRGC) on
194 an Agilent 7890A gas chromatograph equipped with an Agilent 7683B automatic injector and
195 an Agilent 5975C mass spectrometer (Agilent, Folsom, USA), was performed as described by
196 Herzke et al. (2009). Analysis of OPEs using liquid chromatography on a UPLC column (BEH
197 Phenyl, 100 mm x 2.1 mm ID, 1.8 µm particles, Waters Corp., Milford, USA) on an Accella
198 1250 quaternary pump fitted to a Vantage triple quadrupole mass spectrometer was run in the
199 ESI mode (Thermo Fisher Scientific, Waltham, USA). Injections were 10 µL with a mobile
200 phase gradient of 80 % to 0 % of HLB-cleaned Milli-Q water with 0.1 % formic acid and
201 methanol with 0.1 % formic acid and a column flow of 0.3 mL/min to 0.4 mL/min. Limit of
202 detection (LOD) was defined as three times the signal to noise ratio. For validation of results,
203 one blank sample was included for every tenth sample. Four blanks were included in OPE
204 analyses due to very fluctuating background levels. The standard reference material (SRM)
205 used for plasma samples was SRM 1958 human serum from the National Institute of
206 Standards and Technology (NIST), Gaithersburg, Maryland, USA, with an added OPE
207 standard (d21-TPrP) for quality assurance. No SRM was available for feather samples.
208 However, recoveries of the internal standards in feathers were used to assess the analytical

209 quality of the applied method for POPs (65-75%). Recovery of OPEs was from 42 to 128 %
210 with an average recovery of 75 %.

211 *2.5 Statistics*

212 For statistical analyses, JMP® from SAS Institute Inc., Microsoft Excel® 2013, SigmaPlot
213 13.0, and the free statistical software R (version 3.1.2) (R Core Team, 2015) were used. To
214 investigate the data including compounds with a high percentage of data below LOQ (limit of
215 quantification; LOD times three), we used methods of survival analysis for left-censored data
216 (Gillespie et al. 2010; Helsel 2005, 2006). The distributions of concentrations in feathers and
217 blood were estimated using the reverse Kaplan–Meier (KM) method (Gillespie et al. 2010;
218 Jaspers et al. 2013) for all PBDEs, PCBs, and OCPs where at least one value above the LOD
219 was available. The reverse KM method is non-parametric and presents the distribution
220 without substituting values below LOD (Jaspers et al. 2013). The "survival failure" procedure
221 in JMP 12 (SAS Institute Inc., Cary, NC, USA) was used to estimate the cumulative
222 distribution of each pollutant concentration level. The cumulative distributions can be found in
223 supplementary information. Due to the low number of samples (n=4) for OPEs, they were not
224 included in further statistics. Further statistics on POPs were performed on compounds with
225 more than 50 % of the measurements above LOQ. Levels below LOQ were assigned a value
226 of p × LOQ, were 'p' is the proportion of measurements with a value above LOQ (Voorspoels
227 et al., 2002; Jaspers et al., 2007a).

228 The concentrations of the majority of the pollutants were not normally distributed according to
229 the Shapiro-Wilk test of normality. Common logarithmic (base 10) transformations of all POP
230 concentrations were performed in order to approximate normal distribution. Data were
231 checked for homogeneity of variances using Bartlett's test. ΣPOPs was calculated as the sum
232 of all PCB, PBDE, and OCP levels in each sample (feather and plasma separately).
233 Differences in mean contributions of pollutants to ΣPOPs between colonies were separately
234 investigated for both colonies using one-way ANOVA. A body condition index (BCI) was
235 calculated in order to investigate how the kittiwake body condition correlates with pollutant
236 levels in blood plasma. BCI was expressed as residual mass from the linear regression
237 relating body mass to skull length (r^2=0.65, n=19, p<0.001) as described by Chastel et al.

238 (2005). Skull length was used due to its high correlation with body mass (*r*=0.82, *p*<0.001).
239 The linear regressions did not vary between sexes(ANCOVA *p*=0.46).

240 Pearson product-moment coefficients were carried out to evaluate correlations between levels
241 in feathers and blood. Univariate general linear models (GLMs) were performed for ∑OCPs,
242 ∑PCBs, ∑PBDEs, and ∑POPs to investigate relations between the pollutant groups, sex,
243 colonies, thyroid hormones, and BCI. Univariate GLMs were performed separately for
244 individual PCB congeners to investigate a possible OH-PCB mediated interference with THs
245 because of their structural resemblance with thyroid hormones. The best models were
246 selected based on stepwise Akaike's Information Criterion adjusted for low sample sizes
247 (AICc).

248 ## 3. **Results**

249 The following compounds were detected in plasma in more than 50 % of the 19 samples: *p,p'*-
250 DDE, HCB, *β*-HCB, *oxy*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, mirex, CB -28, -99, -
251 105, -118, -138, -153, -180, -183, -187, and BDE 47. In feathers, *p,p'*-DDE, HCB, *oxy*-
252 chlordane, *trans*-nonachlor, and CB 153 were detected in more than 50 % of the 19 samples.
253 Of the thirteen investigated OPEs, seven were detected in feathers (TCEP, TNBP, TPHP,
254 TBOEP, sum of TMPP isomers, EHDP, and TEHP) and one in plasma (TCIPP).

255 ### *3.1 Levels of pollutants*

256 Sexes were pooled since no significant differences were found between sexes for the
257 different pollutant groups in either Blomstrandhalvøya or Krykkjefjellet (*p*>0.05 in all cases).
258 The mean concentrations of ΣPOPs for Blomstrandhalvøya and Krykkjefjellet were
259 respectively 72.9 ± 8.63 ng/g ww (wet weight) and 29.6 ± 1.67 ng/g ww in plasma, and 13.4 ±
260 3.63 ng/g and 7.08 ± 1.58 ng/g in feathers. The mean concentration of ΣPOPs in plasma for
261 kittiwakes from Blomstrandhalvøya was more than twice as high as the mean concentration of
262 ΣPOPs for kittiwakes breeding in Krykkjefjellet (Fig. 2).

263 No significant differences were found between the colonies in the mean contribution of CB
264 153, -138, -180, and *p,p'*-DDE to ΣPOPs. These were the major contaminants in plasma for

265 kittiwakes from both Blomstrandhalvøya and Krykkjefjellet constituting 68.5 % and 66.8 % of
266 the total POP load, respectively (figure SI 7 in supplementary information).

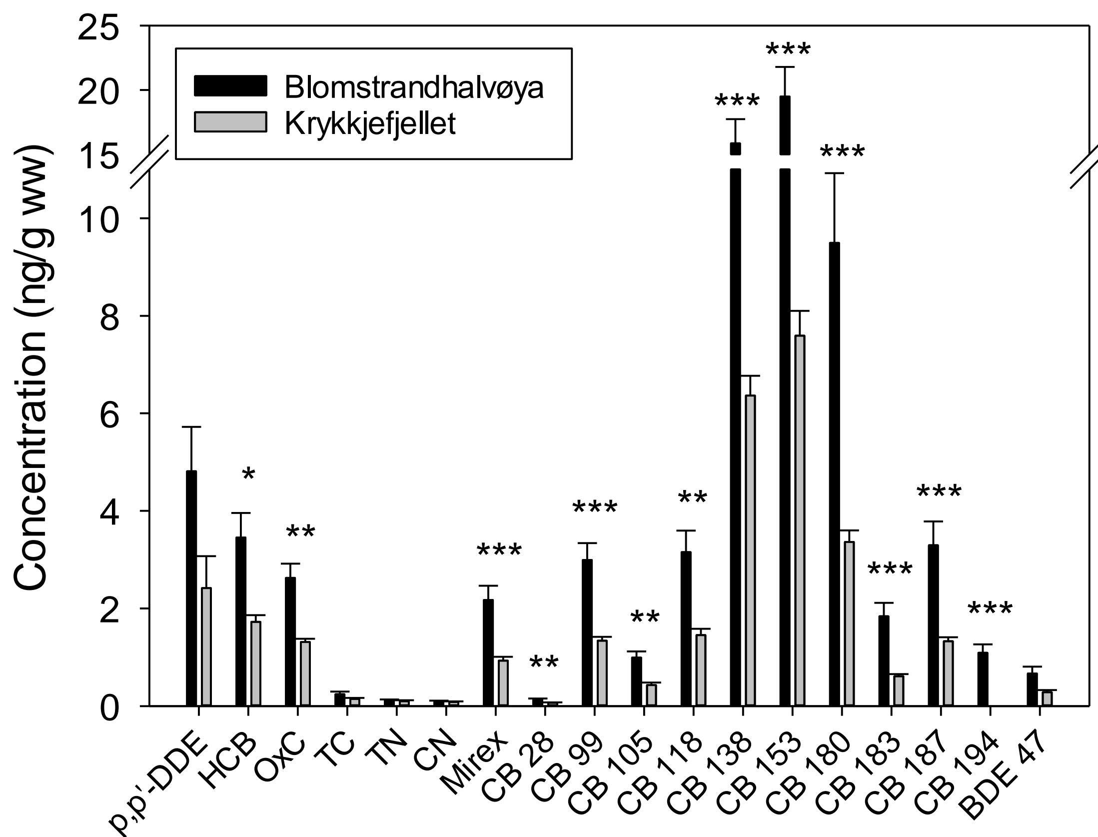

Figure 2. Comparison of mean concentration of POPs in ng/g ww in plasma ± SE between Blomstrandhalvøya (n=11) and Krykkjefjellet (n=8). Significant differences between the two colonies: *: $p<0.05$. **: $p<0.01$. ***: $p<0.001$.

267 Pollutant levels in feathers did not differ significantly between colonies for ΣPCBs, ΣPBDEs,
268 and ΣOCPs. CB 153, *p,p'*-DDE, HCB, OxC, and TN were the only compounds that were
269 detected in more than 50 % of the feather samples, and constituted 33.0 %, 23.0 %, 10.7 %,
270 7.2 %, and 1.8 % of the total POP load in feathers, respectively. The biggest contributor to
271 mean ΣPOPs was ΣPCBs (Blomstrandhalvøya 80.1 % and 51.1 %; Krykkjefjellet 75.7 % and
272 41.8 %, for plasma and feathers, respectively) (Table SI 1).

273 Levels of OPEs were only investigated in four individuals from Krykkjefjellet, since only their
274 sample amounts of plasma and feathers were sufficient for OPE analyses. Two of the feather

275 samples had no detectable levels of any of the investigated OPEs after blank correction. The
276 main contributors to ΣOPEs in the other two feather samples were EHDP and TPHP, with
277 TPHP detected in both feather samples. Only one plasma sample showed OPE levels
278 (TCIPP) above LOQ after blank correction.

279 ### *3.2 Correlations between pollutants in feathers and plasma*

280 The mean contribution of ΣPCBs and ΣOCPs to the total contaminant load differed
281 significantly between plasma and feather samples (p=0.002 and p=0.009, respectively) (figure
282 3). Levels of ΣPCBs contributed significantly more to the total contaminant load in plasma,
283 whereas the mean contribution of ΣOCPs in feathers was more than twice as high as in
284 plasma (41.4 % vs 20.4 %, respectively). Pearson correlations between log transformed
285 concentrations of pollutants in plasma and feather samples for the colonies combined
286 revealed no significant correlations,
287 except for a negative relationship for *oxy*-
288 chlordane (r=-0.58, p=0.008). Due to
289 high differences in plasma contaminant
290 levels between colonies, correlations
291 were also investigated for each colony
292 separately. The only significant
293 relationship between feather and plasma
294 concentrations was for CB 153 in
295 Krykkjefjellet (r=0.81, p=0.02).

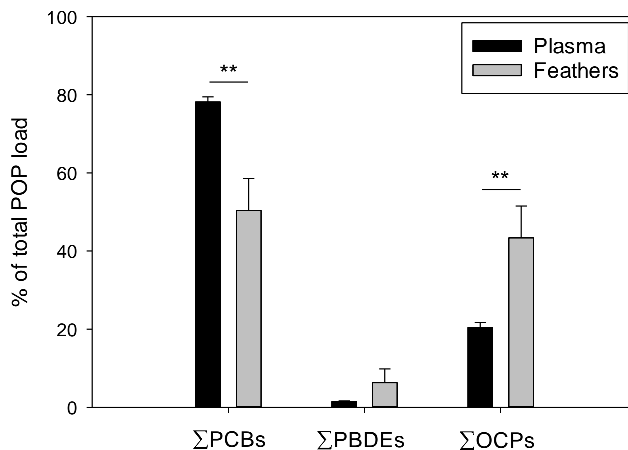

Figure 3. Sum (Σ) of PCBs, PBDEs, and OCPs expressed as mean percentage (%) of total POP load ± SE in plasma and feathers for 19 kittiwakes from Svalbard. **: significant difference between plasma and feather samples, p<0.001.

296

297 ### *3.3 TH levels*

298 fT3 levels differed significantly between sexes (p=0.007), also when body mass was
299 considered (p=0.003), with a range from 2.45 to 6.11 pg/mL for males and 1.25 to 3.48 pg/mL
300 for females. No significant differences in fT3 levels were found between the colonies. TT3
301 levels ranged from 1.68 to 5.12 ng/mL for males and from 0.70 to 3.52 ng/mL for females, but
302 no significant differences were found between sexes nor colonies. The ratio between fT3 and

303 TT3 ranged from 0.47 to 1.78 for males and from 0.56 to 1.53 for females and did not differ
304 significantly between neither colonies nor sexes.

305 *3.4 Associations between contaminants, thyroid hormones, and physiological parameters*

306 Body mass of the 19 studied kittiwakes ranged from 300 to 433 g, and an overall significant
307 difference was found between the sexes (p<0.001), with lower body mass in females, as
308 expected. The BCI did not differ significantly between sexes for Krykkjefjellet (p=0.609), but a
309 trend was found for Blomstrandhalvøya (p=0.057), with female kittiwakes from
310 Blomstrandhalvøya having the lowest BCI. Breeding status did not affect body mass or BCI
311 (p=0.25 and p=0.33, respectively). By inspecting GLM regression analyses for the pollutant
312 groups in plasma, the best models comprised BCI and colony for ΣPCBs ($F_{2,16}$=25.01,
313 p=0.00001, r^2=0.73) and for ΣOCPs ($F_{2,16}$
314 =8.41, p=0.003, r^2=0.45). These findings
315 are supported by GLM regression analyses
316 for ΣPOPs, as the best significant
317 regression analysis comprised both colony
318 and BCI ($F_{2,16}$=21.82, p=0.00003, r^2=0.70).
319 No significant results were found for
320 explaining the level of ΣPBDEs.

321 CB -28, -138, -187 (all 2, 4, 4' or 2, 2', 4
322 substituted), and ΣPBDE were negatively
323 correlated with TT3:fT3 ratio (all r≥-0.60
324 and all p≤0.05) for kittiwakes from
325 Blomstrandhalvøya (CB 187 as example in
326 Fig. 4), but not for Krykkjefjellet. All
327 pollutant groups had a positive, but not
328 significantly, correlation with fT3 levels.

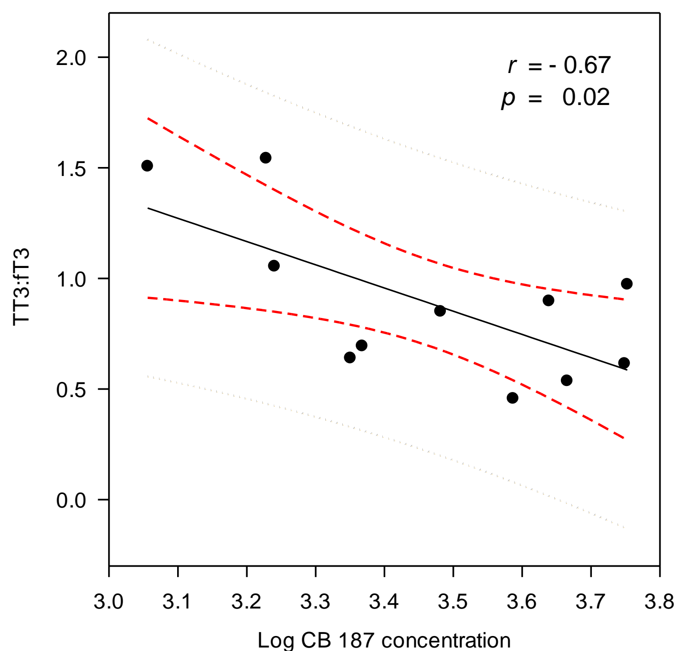

Figure 4. Correlation plot between TT3:fT3 ratio and the log concentration of CB 187 in 11 kittiwakes from Blomstrandhalvøya, Kongsfjorden. The p- and r-values are displayed in the upper right corner. The unbroken line is the regression, the red dashed line is the 95 % confidence interval for the regression, and the dotted line is the 95 % confidence interval for the samples.

329 4. **Discussion**

330 *4.1 Pollutant levels*

331 To the knowledge of the authors, this is the first study to investigate OPEs in feathers and
332 plasma from kittiwakes from Svalbard. Due to elevated levels of OPEs in the blank samples
333 (ranging between 0.03 to 4.47 ng/g ww and 0.02 to 26.5 ng/g feather for plasma and feather
334 blanks, respectively) indicating possible external contamination, most OPE levels in the
335 samples were lower than blank sample concentrations. Therefore, the OPE results should be
336 interpreted with caution. Nevertheless, OPEs show long-range atmospheric transport (OPEs
337 in the Arctic atmospheric are now exceeding both contemporary and historical levels of
338 PBDEs) and bioaccumulative abilities (Salamova et al. 2014). Detections of OPEs in Arctic
339 wildlife are increasing (Hallanger et al. 2015), although most OPEs are readily metabolized
340 (Greaves and Letcher 2014). This warrants a further investigation of OPEs in Arctic wildlife
341 with larger sample sizes.

342 The lack of difference in pollutant load between sexes has been reported previously in liver
343 samples from adult Arctic seabirds as glaucous gulls (*Larus hyberboreus*; Sagerup et al.
344 2009) and kittiwakes (Buckman et al. 2004; Borgå et al. 2005; Bustnes et al. 2017). Plasma
345 levels of pollutants are variable and highly dependent on the diet (Borgå et al. 2005), and as
346 both female and male kittiwakes nurture nestlings (Coulson 2011), both sexes are supposed
347 to have similar diet and energy expenditure during the feeding period (Barrett et al. 1985).
348 This could partly explain why we found no significant differences in plasma levels of pollutants
349 between sexes.

350 In kittiwakes from the Krykkjefjellet colony, POP levels were lower in feathers but similar in
351 plasma compared to previously reported levels for the same colony (Johnsen 2011; Nordstad
352 et al. 2012; Solheim et al. 2016), independent of sex. However, in kittiwakes from
353 Blomstrandhalvøya, plasma levels of ΣPCBs, HCB, OxC, and *p,p'*-DDE were more than twice
354 as high than previously reported levels for kittiwakes from Krykkjefjellet (Johnsen 2011;
355 Nordstad et al. 2012). The higher levels of almost all halogenated pollutants found at the
356 Blomstrandhalvøya colony may be caused by several factors. This includes individual

357 variations in breeding status, body size, sex, feeding ecology, and area, which may affect the
358 trophic transfer of pollutants (Henriksen et al. 1996; Borgå et al. 2004). However, similar POP
359 profiles were found for the two colonies, suggesting that their feeding ecology may be similar,
360 but time and energy spent on searching for food may differ.

361 Females from Blomstrandhalvøya were sampled late in the breeding season, and had lower
362 BCI than the rest of the kittiwakes from both colonies. This suggests a higher redistribution of
363 stored lipids, and thereby release of pollutants. As a result, female kittiwakes from
364 Blomstrandhalvøya may experience higher levels of circulating pollutants. Body mass and
365 BCI did not differ significantly between the colonies for male kittiwakes, but males from
366 Blomstrandhalvøya, sampled late in the breeding season still had significantly higher levels of
367 POPs than males from Krykkjefjellet. No differences were, however, found in body condition
368 between breeding and non-breeding kittiwakes in the present study. To further investigate this
369 difference between the two colonies, blood samples from adult breeding female kittiwakes
370 were sampled mid-July 2015 at both colonies. No significant differences between the colonies
371 were found in 2015 (unpublished data, see figure SI 8 in supplementary information),
372 indicating that timing of sampling is of utmost importance when investigating levels and
373 potential effect of POPs in Arctic seabirds.

374 *4.2 Correlations*

375 In general, only low correlations between feathers and internal levels have previously been
376 reported for aquatic birds (Jaspers et al. 2007a), and correlations between feathers and preen
377 oil have mostly been absent (Solheim et al. 2016).

378 The kittiwake is a migratory bird, and its overwintering areas throughout the North Atlantic
379 differ from its breeding grounds (Strøm 2006; González-Solís et al. 2011; Frederiksen et al.
380 2012). As the sampled primary feathers in kittiwakes grow between September to May, when
381 kittiwakes primarily reside at their overwintering areas (Baird 1994; González-Solís et al.
382 2011), they will not reflect contamination at the Arctic breeding grounds, as opposed to
383 plasma, since most of the kittiwakes do not arrive at Kongsfjorden, Svalbard before April

384 (Strøm 2006). This is illustrated by the different PCB and OCP composition in the reported
385 plasma and feather samples.

386 Although feathers have proven to be good biomarkers for pollution in terrestrial and resident
387 bird species (Dauwe et al. 2005; Jaspers et al. 2007a) kittiwakes are not resident, and
388 feathers sampled from adult migratory birds may not be a good biomarker for pollution at the
389 breeding grounds. Nestling feathers, grown at the breeding ground, would presumably act as
390 better biomarkers for pollution levels. It is important to take these considerations into account
391 to improve future studies on migratory marine bird species, like the kittiwake.

392 *4.3 Thyroid hormones and pollution*

393 Plasma levels of TT3 were similar to previously reported TT3 levels in kittiwakes (Rønning et
394 al. 2008; Johnsen 2011). However, mean fT3 were lower than previously reported levels for
395 both male and female kittiwakes (Welcker et al. 2013). Rønning et al. (2008), Johnsen (2011),
396 and Welcker et al. (2013) all determined fT3 levels by radioimmunoassay (RIA), whereas the
397 current study used an enzyme-linked immunosorbent assay (ELISA). Maybe the use of
398 different assays could explain the reported difference in fT3 levels, although TT3 levels
399 reported were found similar. Male kittiwakes had significantly higher levels of fT3 than
400 females in the current study. Similar results for kittiwakes have been reported (Welcker et al.
401 2013) although these were not significant. Further, in a study by Verreault et al. (2004),
402 reported levels of fT3 in male glaucous gull were 28 % higher than in females. The latter
403 study also found decreasing levels of T4 and T4:T3 ratio with increasing pollutant load, but
404 only for male glaucous gulls, indicating a sex-specific thyrotoxicity. The ratios between THs
405 have previously been described as sensitive indicators of revealing contaminant exposure
406 (Peakall, 1992).

407 No sex differences were found in kittiwakes from Blomstrandhalvøya, yet overall they had
408 significantly higher levels of pollutants than kittiwakes from Krykkjefjellet. Higher levels of
409 circulating contaminants were associated with lower TT3:fT3 levels in Blomstrandhalvøya
410 kittiwakes. As most of the pollutants had a positive, but not statistically significant, correlation
411 with fT3 levels, increased levels of fT3 might be a possible explanation for the decreased

412 TT3:fT3 ratio. Positive correlations between fT3 levels and pollutant levels have previously
413 been reported in glaucous gulls (Verreault et al. 2004). It has been speculated that
414 thyrotoxicity is sex-specific, but both males and females have been reported as seemingly
415 more susceptible to thyrotoxicity (Verreault et al. 2004; Melnes et al. 2017). The positive
416 correlations between fT3 levels and pollutant levels reported in the current study, although not
417 significant, might partly explain the significantly higher levels of fT3 found in males from both
418 colonies. Pollutant mediated interference with TH plasma carrier proteins has been
419 suggested, as some OH-PCBs have structural resemblance with THs (Verreault et al. 2004).
420 As avian transthyretin has higher affinity for T3 than T4 (Chang et al. 1999), it is possible that
421 most transthyretin will be saturated with T3. The displacement of T3 from transthyretin by
422 organic contaminants could facilitate excretion of T3, thereby reducing levels of TT3 in
423 plasma and cause the TT3:fT3 ratio to decrease with increasing levels of pollutants (Blévin et
424 al. 2017).

425 The significant correlations reported in the current study may possibly be representing a
426 potential pollutant mediated influence on the thyroid system, as high levels of circulating
427 contaminants were associated with a lower TT3:fT3 ratio. However, adaptive responses to
428 food availability and fasting during the breeding period may also cause a decrease in T3
429 levels, especially in birds (McNabb 2007), resulting in a possible covariation between
430 increasing levels of circulating pollutants and a lower TT3:fT3 ratio. Further studies including
431 a larger sample size, histology, T4 levels, and glandular hormones would be necessary to
432 draw definite conclusions regarding the observed relations.

433 ## 5. Conclusion

434 This study is the first to report detection and quantification of OPEs in kittiwake feathers from
435 Svalbard and emphasize their occurrence in Arctic wildlife. Further studies with a larger
436 sample size are required to conclude on trends and population levels. This study provides
437 new insights into the applicability of using feathers as biomonitors of exposure for emerging
438 and legacy pollutants. Our results suggest low usability of adult kittiwake feathers when
439 investigating contamination at the local breeding colony, in contrast to plasma levels.
440 Therefore, adult migratory bird feathers are not recommended for biomonitoring pollutants at

441 breeding grounds, while nestling feathers, or feathers grown at the breeding grounds, may
442 serve as a more reliable biomonitor. Moreover, the significant correlations found in this study
443 between the BCI, TT3:fT3 ratio and several POPs, warrants further investigation of the
444 observed relations during the breeding season. Our study further underpins that timing of
445 sampling is of utmost importance when investigating levels and potential effects of organic
446 pollutants in Arctic seabirds.

447 **Acknowledgments**

448 We thank Pierre-Axel Monternier for assistance during fieldwork, the staff of Sverdrup
449 Research Station in Ny-Ålesund for logistic support, and Arntraut Götsch from NILU for
450 support during the chemical analysis of the samples.

451 **Compliance with ethical standards**

452 N. B. Svendsen and V. L. B. Jaspers received Artic Field Grants (ES520962 and ES520958,
453 respectively) from the Norwegian Research Council to carry out the fieldwork at Svalbard,
454 Norway. V.L.B Jaspers is further supported by the Norwegian University of Science and
455 Technology (NTNU), and the pollutant analyses were funded in a joint collaboration between
456 NTNU and the Norwegian Polar Institute. All applicable international, national, and/or
457 institutional guidelines for the care and use of animals were followed. The sampling from
458 kittiwakes at Svalbard occurred in accordance with approval from the Norwegian Animal
459 Research Authority (FDU permission number 2014/59453-2). The authors confirm that no
460 competing personal or financial interests exist regarding the submitted manuscript.

461 **Supplementary information**

462 Supplementary information (SI) includes cumulative probability plots for PCBs, PBDEs and
463 OCPs in blood and feathers, levels of OPEs in blood and feathers, and comparisons between
464 POP levels at the two colonies. SI is available online.

465 **References**

466 AMAP. 1998. Assessment report: Arctic pollution issues. Arctic Monitoring and Assessment
467 Programme (AMAP). Oslo, Norway: 859 pp.

468 AMAP. 2015. Assessment report: temporal trends in persistent organic pollutants in the
469 Arctic. Arctic Monitoring and Assessment Programme (AMAP). Oslo, Norway: vi+71
470 pp.
471 Baird, P. H. 1994. Black-legged Kittiwake (*Rissa tridactyla*), The Birds of North America
472 Online (eds. Poole, A). Ithaca, Cornell Lab of Ornithology.
473 Barrett, R. T., Fieler, R., Anker-Nielsen, T. and Rikardsen, F., 1985. Measurements and
474 weight changes of Norwegian adult Puffins *Fratercula arctica* and Kitiwakes *Rissa*
475 *tridactyla* during the breeding season. Ringing and Migration 6:102-112.
476 Bech, C., Langseth, I., Moe, B., Fyhn, M. and Gabrielsen, G. W., 2002. The energy economy
477 of the Arctic-breeding kittiwake *(Rissa tridactyla*): a review. Comparative Biochemistry
478 and Physiology Part A: Molecular and Integrative Physiology 133: 765-770.
479 Blévin, P., Tartu, S., Ellis, H. I., , O., Bustamante, P., Parenteau, C., Herzke, D., Angelier, F.
480 and Gabrielsen, G. W., 2017. Contaminants and energy expenditure in an Arctic
481 seabird: Organochlorine pesticides and perfluoroalkyl substances are associated with
482 metabolic rate in a contrasted manner. Environmental Research, 157: 118-126.
483 Borgå, K., Fisk, A. T., Hoekstra, P. F. and Muir, D. C., 2004. Biological and chemical factors
484 of importance in the bioaccumulation and trophic transfer of persistent organochlorine
485 contaminants in Arctic marine food webs. Environmental Toxicology and Chemistry 23:
486 2367-2385.
487 Borgå, K., Wolkers, H., Skaare, J. U., Hop, H., Muir, D. C. and Gabrielsen, G. W., 2005.
488 Bioaccumulation of PCBs in Arctic seabirds: influence of dietary exposure and
489 congener biotransformation. Environmental Pollution 134: 397-409.
490 Buckman, A. H., Norstrom, R. J., Hobson, K. A., Karnovsky, N. J., Duffe, J. and Fisk, A. T.,
491 2004. Organochlorine contaminants in seven species of Arctic seabirds from northern
492 Baffin Bay. Environmental Pollution 128: 327-338.
493 Bustnes, J. O., Bakken, V., Erikstad, K. E., Mehlum, F. and Skaare, J. U., 2001. Patterns of
494 incubation and nest-site attentiveness in relation to organochlorine (PCB)
495 contamination in glaucous gulls. Journal of Applied Ecology 38: 791-801.
496 Bustnes, J. O., Erikstad, K. E., Skaare, J. U., Bakken, V. and Mehlum, F., 2003. Ecological
497 effects of organochlorine pollutants in the Arctic: a study of the glaucous gull.
498 Ecological Applications 13: 504-515.
499 Bustnes, J. O., Bårdsen, B.-J., Moe, B., Herzke, D., Hanssen, S. A., Sagerup, K., Bech, C.,
500 Norstad, T., Chastel, O., Tartu, S. and Gabrielsen, G. W. 2017. Temporal variation in
501 circulating concentrations of organochlorine pollutants in a pelagic seabird breeding in
502 the high Arctic. Environmental Toxicology and Chemistry 36-2: 442-448.
503 Chang, L., Munro, S. L., Richardson, S. J. and Schreiber, G., 1999. Evolution of thyroid
504 hormone binding by transthyretins in birds and mammals. European Journal of
505 Biochemistry 259: 534-542.
506 Chastel, O., Lacroix, A., Weimerskirch, H. and Gabrielsen, G. W., 2005. Modulation of
507 prolactin but not corticosterone responses to stress in relation to parental effort in a
508 long-lived bird. Hormones and Behavior 47: 459-466.
509 Coulson, J. C. 2011. The Kittiwake. London, England. T & AD Poyser.
510 Dauwe, T., Jaspers, V. L. B., Covaci, A., Schepens, P. and Eens, M., 2005. Feathers as a
511 nondestructive biomonitor for persistent organic pollutants. Environmental Toxicology
512 and Chemistry 24: 442-449.

513 Dawson, A., 2000. Mechanisms of endocrine disruption with particular reference to
514 occurrence in avian wildlife: a review. Ecotoxicology 9: 59-69.
515 de Wit, C. A., Herzke, D. and Vorkamp, K., 2010. Brominated flame retardants in the Arctic
516 environment – trends and new candidates. Science of the Total Environment 408:
517 2885-2918.
518 Eulaers, I., Covaci, A., Herzke, D., Eens, M., Sonne, C., Moum, T., Schnug, L., Hanssen, S.
519 A., Johnsen, T. V., Bustnes, J. O. and Jaspers, V. L. B., 2011. A first evaluation of the
520 usefulness of feathers of nestling predatory birds for non-destructive biomonitoring of
521 persistent organic pollutants. Environment International 37: 622-630.
522 Eulaers, I., Jaspers, V. L. B., Halley, D. J., Lepoint, G., Nygård, T., Pinxten, R., Covaci, A. and
523 Eens, M., 2014. Brominated and phosphorus flame retardants in white-tailed eagle
524 *Haliaeetus albicilla* nestlings: bioaccumulation and associations with dietary proxies
525 ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$). Science of the Total Environment 478: 48-57.
526 Evenset, A., Leknes, H., Christensen, G. N., Warner, N., Remberger, M. and Gabrielsen, G.
527 W., 2009. Screening of new contaminants in samples from the Norwegian Arctic.
528 Norwegian Pollution Control Authority. NIVA report 4351-1: 1-43.
529 Frederiksen, M., Moe, B., Daunt, F., Phillips, R. A., Barrett, R. T., Bogdanova, M. I., Boulinier,
530 T., Chardine, J. W., Chastel, O., Chivers, L. S., Christensen-Dalsgaard, S., Clément-
531 Chastel, C., Colhoun, K., Freeman, R., Gaston, A. J., González-Solís, J., Goutte, A.,
532 Grémillet, D., Guilford, T., Jensen, G. H., Krasnov, Y., Lorentsen, S.-H., Mallory, M. L.,
533 Newell, M., Olsen, B., Shaw, D., Steen, H., Strøm, H., Systad, G. H., Thórarinsson, T.
534 L. and Anker-Nilssen, T., 2012. Multicolony tracking reveals the winter distribution of a
535 pelagic seabird on an ocean basin scale. Diversity and Distributions 18-6: 530-542.
536 Gabrielsen, G. W., 2007. Levels and effects of persistent organic pollutants in arctic animals.
537 In: Arctic alpine ecosystems and people in a changing environment (eds. Ørbæk J. B.,
538 Kallenborn, R., Tombre, I., Hegseth, E. N., Falk-Petersen, S. and Hoel, A. H.).
539 Springer, New York, USA. pp. 390-412.
540 García-Fernández, A. J., Espín, S. and Martínez-López, E., 2013. Feathers as a
541 biomonitoring tool of polyhalogenated compounds: a review. Environmental Science
542 and Technology 47: 3028-3043.
543 Gillespie, B. W., Chen, Q., Reichert, H., Franzblau, A., Hedgeman, E., Lepkowski, J.,
544 Adriaens, P., Demond, A., Luksemburg, W. and Garabrant, D. H., 2010. Estimating
545 population distributions when some data are below a limit of detection by using a
546 reverse Kaplan-Meier estimator. Epidemiology 21: S64-S70.
547 González-Solís, J., Smyrli, M., Militão, T., Gremillet, D., Tveraa, T., Phillips, R. A. and
548 Boulinier, T., 2011. Combining stable isotope analyses and geolocation to reveal
549 kittiwake migration. Marine Ecology Progress Series 435: 251-261.
550 Gordeev, V., 2002. Pollution of the Arctic. Regional Environmental Change 3: 88-98.
551 Grasman, K. A., Fox, G. A., Scanlon, P. F. and Ludwig, J. P., 1996. Organochlorine-
552 associated immunosuppression in prefledgling caspian terns and herring gulls from the
553 Great Lakes: an ecoepidemiological study. Environmental Health Perspectives 104:
554 829-842.
555 Greaves, A. K. and Letcher, R. J., 2014. Comparative body compartment composition and *in*
556 *ovo* transfer of organophosphate flame retardants in North American Great Lakes
557 herring gulls. Environmental Science and Technology 48: 7942-7950.

558 Griffiths, R., Double, M. C., Orr, K. and Dawson, R. J., 1998. A DNA test to sex most birds.
559 Molecular Ecology 7: 1071-1075.
560 Hallanger, I. G., Sagerup, K., Evenset, A., Kovacs, K. M., Leonards, P., Fuglei, E., Routti, H.,
561 Aars, J., Strøm, H., Lydersen, C. and Gabrielsen, G. W., 2015. Organophosphorous
562 flame retardants in biota from Svalbard, Norway. Marine Pollution Bulletin 101: 442-
563 447.
564 Helsel, D. R., 2005. Nondetects and data analysis: statistics for censored environmental data.
565 John Wiley, New York, USA.
566 Helsel, D. R., 2006. Fabricating data: how substituting values for nondetects can ruin results,
567 and what can be done about it. Chemosphere 65: 2434–2439.
568 Henriksen, E. O., Gabrielsen, G. W. and Skaare, J. U., 1996. Levels and congener pattern of
569 polychlorinated biphenyls in kittiwakes (*Rissa tridactyla*), in relation to mobilization of
570 body-lipids associated with reproduction. Environmental Pollution 92: 27-37.
571 Herzke, D., Nygård, T., Berger, U., Huber, S. and Røv, N., 2009. Perfluorinated and other
572 persistent halogenated organic compounds in European shag (*Phalacrocorax*
573 *aristotelis*) and common eider (*Somateria mollissima*) from Norway: A suburban to
574 remote pollutant gradient. Science of The Total Environment 408: 340-348.
575 Hill, R. W., Wyse, G. A. and Anderson, M. 2008. Animal Physiology. Sunderland, USA.
576 Sinauer Associates.
577 Jaspers, V. L. B., Voorspoels, S., Covaci, A. and Eens, M., 2006. Can predatory bird feathers
578 be used as a non-destructive biomonitoring tool of organic pollutants? Biology Letters
579 2: 283-285.
580 Jaspers, V. L. B., Voorspoels, S., Covaci, A., Lepoint, G. and Eens, M., 2007a. Evaluation of
581 the usefulness of bird feathers as a non-destructive biomonitoring tool for organic
582 pollutants: a comparative and meta-analytical approach. Environment International 33:
583 328-337.
584 Jaspers, V. L. B., Covaci, A., Van den Steen, E. and Eens, M., 2007b. Is external
585 contamination with organic pollutants important for concentrations measured in bird
586 feathers? Environment International 33: 766-772.
587 Jaspers, V. L. B., Covaci A., Deleu P., Neels H. And Eens M., 2008. Preen oil as the main
588 source of external contamination with organic pollutants onto feathers of the common
589 magpie (*Pica pica*). Environment International 34: 741-748.
590 Jaspers, V. L. B., Herzke, D., Eulaers, I., Gillespie, B. W. and Eens, M., 2013. Perfluoroalkyl
591 substances in tissues and feathers of Belgian barn owls using methods for left-
592 censored data to handle non-detects. Environment International 52: 9-16.
593 Jenssen, B. M., 2006. Endocrine-disrupting chemicals and climate change: A worst-case
594 combination for arctic marine mammals and seabirds? Environmental Health
595 Perspectives 114: 76-80.
596 Johnsen, I. E., 2011. The effect of persistent organic pollutants on thyroid hormone levels in
597 Arctic breeding kittiwake (*Rissa tridactyla*). Master's thesis, Dept. of Biology, NTNU.
598 Macdonald, R. and Bewers, J., 1996. Contaminants in the arctic marine environment:
599 priorities for protection. ICES Journal of Marine Science: Journal du Conseil 53: 537-
600 563.
601 McNabb, F. A., 2007. The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird
602 development and reproduction. Critical Reviews in Toxicology 37: 163-193.

603 McNabb, F. A., 1995. Thyroid hormones, their activation, degradation and effects on
604 metabolism. The Journal of Nutrition 125: 1773S-1776S.
605 Melnes, M., Gabrielsen, G. W., Herzke, D., Sagerup, K. and Jenssen, B. M., 2017. Dissimilar
606 effects of organohalogenated compounds on thyroid hormones in
607 glaucous gulls. Environmental Research 158: 350-357.
608 Nordstad, T., Moe, B., Bustnes, J. O., Bech, C., Chastel, O., Goutte, A., Sagerup, K., Trouvé,
609 C., Herzke, D. and Gabrielsen, G. W., 2012. Relationships between POPs and
610 baseline corticosterone levels in black-legged kittiwakes *(Rissa tridactyla*) across their
611 breeding cycle. Environmental Pollution 164: 219-226.
612 Nøst, T. H., Helgason, L. B., Harju, M., Heimstad, E. S., Gabrielsen, G. W. and Jenssen, B.
613 M., 2012. Halogenated organic contaminants and their correlations with circulating
614 thyroid hormones in developing Arctic seabirds. Science of the Total Environment 414:
615 248-256.
616 Peakall, D., 1992. Thyroid function, retinols, haem and regulatory enzymes. Animal
617 Biomarkers as Pollution Indicators: 108-154.
618 Petersen, G., Rasmussen, D. and Gustavson, K., 2007. Study on enhancing the endocrine
619 disrupter priority list with a focus on low production volume chemicals. European
620 Commission.
621 R Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation
622 for Statistical Computing, Vienna, Austria.
623 Rønning, B., Moe, B., Chastel, O., Broggi, J., Langset, M. and Bech, C., 2008. Metabolic
624 adjustments in breeding female kittiwakes (*Rissa tridactyla*) include changes in kidney
625 metabolic intensity. Journal of Comparative Physiology B 178: 779-784.
626 Sagerup, K., Savinov, V., Savinova, T., Kuklin, V., Muir, D. C. and Gabrielsen, G. W., 2009.
627 Persistent organic pollutants, heavy metals and parasites in the glaucous gull (*Larus*
628 *hyperboreus*) on Spitsbergen. Environmental Pollution 157: 2282-2290.
629 Salamova, A., Hermanson, M. H. and Hites, R. A., 2014. Organophosphate and halogenated
630 flame retardants in atmospheric particles from a European Arctic site. Environmental
631 Science and Technology 48: 6133-6140.
632 Sandanger, T. M., Sinotte, M., Dumas, P., Marchand, M., Sandau, C. D., Pereg, D., Bérubé,
633 S., Brisson, J. and Ayotte, P., 2007. Plasma concentrations of selected organobromine
634 compounds and polychlorinated biphenyls in postmenopausal women of Quebec,
635 Canada. Environmental Health Perspectives 115: 1429-1434.
636 Solheim, S. A., Sagerup, K., Huber, S., Byrkjedal, I. and Gabrielsen, G. W., 2016. The black-
637 legged kittiwake preen gland – an overlooked organ for depuration of fat soluble
638 contaminants? Polar Research 35: 29651.
639 Strøm, H., 2006. Krykkje, *Rissa tridactyla*. In: Svalbards fugler og pattedyr (eds. Kovacs, K.
640 M. and Lydersen C.). Norsk polarinstitutt, Tromsø. pp. 147-150.
641 van den Steen, E., Covaci, A., Jaspers, V. L. B., Dauwe, T., Voorspoels, S., Eens, M. and
642 Pinxten, R., 2007. Experimental evaluation of the usefulness of feathers as a non-
643 destructive biomonitor for polychlorinated biphenyls (PCBs) using silastic implants as a
644 novel method of exposure. Environment International 33: 257-264.
645 Verboven, N., Verreault, J., Letcher, R. J., Gabrielsen, G. W. and Evans, N. P., 2009.
646 Differential investment in eggs by Arctic-breeding glaucous gulls (*Larus hyperboreus*)
647 exposed to persistent organic pollutants. The Auk 126: 123-133.

648 Verreault, J., Skaare, J. U., Jenssen, B. M. and Gabrielsen, G. W., 2004. Effects of
649 organochlorine contaminants on thyroid hormone levels in Arctic breeding glaucous
650 gulls, *Larus hyperboreus*. Environmental Health Perspectives 112: 532-537.
651 Voorspoels, S., Covaci, A., Maervoet, J. and Schepens, P., 2002. Relationship between age
652 and levels of organochlorine contaminants in human serum of a Belgian population.
653 Bulletin of Environmental Contamination and Toxicology 69: 22-29.
654 Walsh, P. S., Metzger, D. A. and Higuchi, R., 1991. Chelex 100 as a medium for simple
655 extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506-
656 513.
657 Welcker, J., Chastel, O., Gabrielsen, G. W., Guillaumin, J., Kitaysky, A. S., Speakman, J. R.,
658 Tremblay, Y. and Bech, C., 2013. Thyroid hormones correlate with basal metabolic
659 rate but not field metabolic rate in a wild bird species. PloS one 8-2: e56229.