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Individual variability in contaminants and physiological status in a resident Arctic seabird species

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Abstract :

While migratory seabirds dominate ecotoxicological studies within the Arctic, there is limited knowledge about exposure and potential effects from circulating legacy and emerging contaminants in species who reside in the high-Arctic all year round. Here, we focus on the case of the Mandt's Black guillemot (*Cepphus grylle mandtii*) breeding at Kongsfjorden, Svalbard (79.00°N, 11.66°E) and investigate exposure to legacy and emerging contaminants in relation to individual physiological status, i.e. body condition, oxidative stress and relative telomere length. Despite its benthic-inshore foraging strategy, the Black guillemot displayed overall similar contaminant concentrations in blood during incubation ($\sum\text{PCB11}$ (15.7 ng/g w.w.) > $\sum\text{PFAS5}$ (9.9 ng/g w.w.) > $\sum\text{Pesticides9}$ (6.7 ng/g w.w.) > $\sum\text{PBDE4}$ (2.7 ng/g w.w.), and Hg (0.3 $\mu\text{g/g}$ d.w.) compared to an Arctic migratory seabird in which several contaminant-related stress responses have been observed. Black guillemots in poorer condition tended to display higher levels of contaminants, higher levels of reactive oxygen metabolites, lower plasmatic antioxidant capacity, and shorter telomere lengths; however the low sample size restrict any strong conclusions. Nevertheless, our data suggests that nonlinear relationships with a threshold may exist between accumulated contaminant concentrations and physiological status of the birds. These findings were used to build a hypothesis to be applied in future modelling for describing how chronic exposure to contaminants may be linked to telomere dynamics.

54 1. Introduction

55 A seabird's contaminant burden at a given time can be viewed as the product of cumulative
56 processes driven by small or large differences in foraging strategies, physiology and life history.
57 As a result, individuals foraging high in the food web, on lipid-rich prey - or in areas with higher
58 inputs of contaminants often present the highest concentrations of contaminants (Muir *et al.*,
59 1992; de March *et al.*, 1998; Dietz *et al.*, 2000). The growing body of research on Arctic seabirds
60 demonstrates that species with higher concentrations of contaminants experience sub-lethal
61 effects, such as lower breeding success, reduced return rate and higher stress responses (e.g.
62 Erikstad *et al.*, 2013; Tartu *et al.*, 2014a; Bustnes *et al.*, 2015). Yet, the fact that the toxicological
63 effects do not depend solely on contaminant exposure, but are also influenced by life history,
64 physiology, and other environmental stressors (Bourgeon *et al.*, 2012; Goutte *et al.*, 2014) often
65 obscure the relationships between contaminant exposure and health impacts. Consequently, two
66 populations - or species - may exhibit different negative effects of contaminants, despite having
67 similar contaminant body burdens (Bustnes *et al.*, 2015). Most seabirds nesting in the Arctic
68 migrate to lower latitudes after breeding and experience different contaminant exposure at their
69 overwintering sites compared to their Arctic breeding grounds (Baert *et al.*, 2013; Fort *et al.*,
70 2014); however species that reside in the high Arctic all year round remain understudied and so
71 does their response to the current multi-stress scenarios in Polar Regions.

72 The Mandt's Black guillemot (*Cephus grylle mandtii*) is known to be one of the few
73 seabird species that overwinter in the Arctic, as it's movements are limited to open water near
74 the sea ice margins close to its breeding site (Brown, 1985; Divoky *et al.*, 2016). While this
75 seabird's life history exposes it to various contaminants that have deposited in the Arctic via
76 long-range transport/deposition mechanisms (Vorkamp *et al.*, 2004 & 2015; Braune *et al.*, 2015
77 & 2016; Peck *et al.*, 2016), the Black guillemot is considered to be sensitive to current and future
78 climate alterations due to its dependency on the sea ice marginal zone in both the breeding
79 (Divoky *et al.*, 2015) and non-breeding seasons (Divoky *et al.*, 2016). Typically, the Black
80 guillemot will alternate between the use of nearshore waters in the breeding season and offshore
81 waters during non-breeding season foraging mostly on Arctic cod (Hobson, 1993), which could
82 differ in contamination and quality of prey (i.e. lipid content and antioxidants). During the
83 breeding season, it rarely feeds more than ten kilometres away from the nest site; while chicks

84 are fed almost exclusively on benthic fish, adults have a mixed diet of both benthic fish and
85 invertebrates (Cairns, 1987a & b; Barrett *et al.*, 2002).

86 The few exposure studies on black guillemots have used invasive and/or lethal sampling
87 matrices or tissues that reflect time-interval of contaminants exposure outside the breeding
88 seasons (e.g. liver, muscles, eggs, feathers) (e.g. Koistinen *et al.*, 1995; Appelquist *et al.*, 1983;
89 Borgå *et al.*, 2001 & 2005 & 2007; Olafsdottir *et al.*, 2005; Vorkamp *et al.*, 2015). Thus, the
90 impact of exposure from blood circulating contaminants on health parameters during sensitive
91 life history events (e.g. breeding season) in this resident, nearshore Arctic species remains
92 unknown.

93 Exposure to contaminants has the potential to cause an imbalance in the anti-oxidant
94 defence system through increased levels of oxidative stress (Constantini *et al.*, 2014; Wielsøe *et al.*,
95 2015). Here contaminants may play two different roles: a) as an actual threat through energy
96 competition resulting from biotransformation and maintenance (e.g. Durant *et al.*, 2007), and/or
97 b) as a “blurring” factor by triggering the hypothalamic-pituitary-adrenalin axis (HPA) (Tartu *et al.*,
98 2014a), *i.e.* due to their endocrine disruptive properties, or by interfering with the antioxidant
99 defence system itself. Both mechanisms may result in increased oxidative stress and ultimately
100 reduced health status over time.

101 A new and promising cellular biomarker in ecotoxicology is telomere dynamics.
102 Telomeres are repetitive, non-coding DNA sequences located at the end of each chromosome,
103 whose length is reduced during cell division (*i.e.* through aging) - and through oxidative stress
104 (see Blackburn, 2000; Von Zglinicki, 2002; Epel *et al.*, 2004; Monaghan & Haussmann, 2006,
105 Reichert & Stier, 2017). Thus, telomere length depends on hereditary factors, early-life
106 conditions and life-history trade-offs (Beaulieu *et al.*, 2011; Schull *et al.*, 2016) and should be
107 informative as to integrated stress during an individual’s lifetime (Horn *et al.*, 2010). In addition,
108 telomere length is a strong predictor of longevity (Barrett *et al.*, 2013). Long-lived species, such
109 as seabirds, are able to decrease the rate of telomere reduction through enzymatic activity
110 (Blackburn, 2005; Haussmann *et al.*, 2007), and telomere length is proposed to be a predictor of
111 intrinsic individual quality (Bauch *et al.*, 2013; Young *et al.*, 2017). Recently, Blévin *et al.* (2016

112 & 2017a) found associations between contaminants (i.e. perfluorinated compounds, oxy-
113 chlordane) and relative telomere length in breeding black-legged kittiwakes (*Rissa tridactyla*).

114 The main objective of the present study is to investigate how exposure to both legacy and
115 emerging contaminants may influence physiological status in an understudied resident Arctic
116 seabird species. This is attempted by relating circulating concentrations of both legacy and
117 emerging contaminants in blood to individual physiological status defined by body condition,
118 stress levels and relative telomere length in black guillemots.

119 2. Materials and methods

120 2.1 Sampling

121 Our study was carried out in Kongsfjorden, Svalbard (79.00°N, 11.66°E), on three different
122 black guillemot nest sites (Juttaholmen, Midtholmen, Observasjonsholmen) during the breeding
123 season of 2015 (June-July), with the necessary permissions from the Governor of Svalbard (RIS
124 ID 10290) and Norwegian Animal Welfare authorities (FOTS ID 7666). We used snares to catch
125 black guillemots (n = 15) resting on rocks outside of their nest during incubation phase. Of these,
126 11 birds were successfully sampled for blood (i.e. minimum 2 mL, maximum < 10 % of body
127 mass) from their brachial wing vein, using heparinized syringes. Blood samples were kept cold
128 (< 4°C) and in the dark, before being centrifuged and stored at -20°C. All captured birds were
129 biometrically measured (i.e. body mass, wing length, bill and skull size) and inspected for brood
130 patch.

131 2.2 Contaminant analyses

132 We measured mercury (Hg) in red blood cells (~ 0.5 mL) of 11 black guillemots. Hg was
133 determined using in-house methods: NILU-U-117 “Procedure for sample digestion by use of
134 high performance microwave reactor (UltraClave)” and NILU-U-65 “Procedure for
135 determination of Hg in biological samples using Cold Vapor Atomic Fluorescence
136 Spectrophotometry (CV-AFS)”. Aliquots of 0.20-0.35 g of red blood cells were added 5 mL
137 concentrated supra pure HNO₃ and 3 mL deionized water (MilliQ) and digested at 250°C for 15
138 minutes, using UltraClave (Milestone, Italy). Blank samples and certified reference material
139 (CRM) were digested in the same run as the blood samples. After digestion, samples, blank
140 samples and CRM were diluted to 50 mL. From the extracts, subsamples of 25 mL were further
141 diluted to 50 mL before 5 ml BrCl were added. Determination of Hg was performed using CV-
142 AFS from Tekran, Canada according to method US-EPA-1631.

143 In the statistical analysis, we also included published contaminant data from reported in
144 Haarr *et al.* (2017) on the same black guillemots individual as in present study. These
145 contaminant data include concentrations of polychlorinated biphenyls (PCBs),
146 dichlorodiphenyltrichloroethane (DDTs) and its metabolites, isomers of hexachlorocyclohexane

147 (HCH), hexachlorobenzene (HCB), chlordanes, polybrominated diphenyl ethers (PBDEs),
148 perfluorinated compounds (PFAS) that was extracted from plasma (1 mL) using protocols
149 described in detail in Blévin *et al.* (2017 a & b) and Haarr *et al.* (2017).

150 **2.3 Isotopic signatures in black guillemots**

151 Signatures of stable isotopes are used to estimate individual foraging behaviour in two-
152 dimensions: pelagic-benthic and low-high trophic level feeding. The use of carbon and nitrogen
153 stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in ‰) is an established practice for assessing carbon source
154 (e.g. pelagic vs. benthic) and relative positions in the food web (i.e. high or low trophic levels)
155 (Hobson *et al.*, 1995). Red blood cells (~200 μL) from black guillemots ($n = 11$) were freeze-
156 dried and analysed for heavy and light isotopes of nitrogen and carbon at the University of
157 Windsor, Canada; as described in Fisk *et al.* (2001).

158 **2.4 Oxidative stress, telomere length and body condition in black guillemots**

159 We estimated oxidative stress levels in plasma of black guillemots using well-established
160 methods on derivatives of reactive oxygen metabolites (d-ROM) in plasma and total plasma
161 antioxidant capacity (Oxy), described in detail by Schull *et al.* (2016). In short, we measured the
162 amount of hyperoxides (d-ROM test, Diacron International) in 8 μl of plasma for each bird,
163 (expressed as mg H_2O_2 equivalent dl^{-1}) with duplicates for each sample. We used the Oxy
164 adsorbent test (Diacron International, Grosseto, Italy) to quantify the ability to buffer massive
165 oxidation (i.e. hydroperoxide acid) in 5 μl of 1:100 diluted plasma, for each bird with duplicates
166 for each sample. The variation between individuals was 5.5% and 5.5% for d-ROM and Oxy,
167 respectively (only one plate was run, thus no inter-plate repeatability was assessed).

168 We extracted DNA using Nucleospin Blood QuickPure Kit (Macherey-Nagel, Düren
169 Germany) and estimated the relative length of telomeres by qPCR using a well-established
170 protocol for King penguin (*Aptenodytes patagonicus*) (Stier *et al.*, 2014; Le Vaillant *et al.*, 2015;
171 Reichert *et al.*, 2015). Chromosomal sex of individuals was determined from red blood cells
172 extracted DNA following Sambrook *et al.* (1989) protocol.

173 In order to understand how successfully a bird interacts with its environment, we used a
174 body condition measure to estimate its supply of energy (i.e. fat and protein content) (Labocha &

175 Hayes, 2012). Since larger birds are obviously heavier than smaller birds, the variation in mass
176 generally correlates with size, while departures from this pattern are explained by inter-
177 individual differences in body condition. Therefore, we calculated a Scale Mass Index - SMI,
178 using the biometric measure with the highest correlation coefficient with body mass in our data
179 (i.e. bill length) as length measure to scale the mass accordingly (see Peig & Green 2009):

$$180 \quad \text{SMI}_{\text{individual}} = \text{body mass}_{\text{individual}} \times (\text{bill}_{\text{mean}}/\text{bill}_{\text{individual}})^{\text{coef}_{\text{body mass-beak}}}$$

181 **2.5 Statistical analyses**

182 Statistical analysis was conducted in R 3.4.2 (R Development Core Team, 2016). One black
183 guillemot individual was excluded due to issues during chemical analyses, thus leaving us with a
184 final dataset of ten birds.

185 The Limit of Detection for contaminants (LOD) was set as the mean of the blanks added
186 by three times the standard deviation of the blanks (Armbruster & Pry, 2008). Values below
187 LOD – or non-detects – were treated using distributional methods and imputation by maximum
188 likelihood (Baccarelli *et al.*, 2005). For each contaminant, we assumed a log-normal distribution.
189 Non-detects were treated using three different steps: 1) the most likely distribution was fitted
190 based on both censored and non-censored data using a simple Expectation-Maximisation
191 algorithm; 2) the minimum number of non-censored points (i.e. cut-off) necessary for a robust
192 inference of the log-normal distribution parameters was estimated by simulations, and filtered
193 accordingly; 3) for contaminants with a sufficiently low number of non-detects, multiple
194 imputation by maximum likelihood was performed using a specified interval (i.e. zero to LOD).

195 Contaminant profiles are mixtures of many contaminants so to extract robust indicators
196 that reliably compare individuals in regression analyses, we need to reduce variation to a lower
197 number of dimensions. We thus first collapsed contaminants into groups depending on their
198 chemical structure, in order to account for differences in weights (i.e. functional relationships
199 with response variables) between different chemical groups. Secondly, since contaminants within
200 the same chemical group may have different dose-dependency (i.e. toxic potential), we need to
201 scale their relative contribution to focus on the shape and not the size of the data (Greenacre,
202 2017). Hence, we preferred to use an equally weighted index that rank individuals based on their

203 exposure to different chemical groups and total contaminants load. This approach does not
204 account for differences in toxic potency and functional relationships within chemical groupings;
205 however we consider it to address more correctly the underlying theoretical assumptions, and the
206 approach itself produces an index that provide a more transparent interpretation when used as a
207 predictor, compared to principle components for example. Thus, we created a score index for
208 each chemical group (i.e. iPCB, iPesticides, iPBDE, iPFAS and iHg) and one for total
209 contaminant load (iCON), where each individual is given a summed score relative to the mean of
210 the sample population for each contaminant:

$$211 \quad \text{Index Contaminant group } X = \sum(x_i / \bar{x})$$

212 where x_i are the values of each of the individual chemical groups in individual i , \bar{x} represents the
213 mean concentration of the individual chemical over the entire sample population within the given
214 chemical group (X).

215 We applied different generalized linear models (GLMs) to test potential relationships
216 between the contaminant groups and the response variables as well as residual plot analysis to
217 assess the validity of the model. If the residuals showed non-random patterns, we tried different
218 families of transformations, $g(y)$, until the residuals plots of the regression $g(y) = \beta_0 + \sum$
219 βX were consistent with independent Gaussian error.

220 **3. Results**

221 **3.1 Contaminants and isotopic signatures in black guillemots**

222 Concentrations of Hg in red blood cells of black guillemots was measured to 0.32 ± 0.8 ug/g d.w.
223 Treatment of LOD censored values resulted in detection of all 11 targeted PCB congeners, but
224 only 10 of the 17 organochlorine pesticides, 5 of 20 perfluorinated compounds, and 4 of 10
225 PBDEs, in plasma of black guillemots (see Supplementary Table 1). \sum PCBs was the dominating
226 compound group (dominated by PCB153 > PCB138 > PCB180), followed by \sum PFAS (mainly
227 PFOS > PFUnA) > \sum Pesticides (mainly HCB > p,p'-DDE) > \sum PBDEs (mainly PBDE47) (see
228 Fig. 1).

229 The black guillemots show a high dispersion in trophic positions ($\delta^{15}\text{N}$ ranging from 12.0
230 – 13.5‰), while carbon isotopic signatures show a clear benthic signal and overall low
231 dispersion - except one extreme individual ($\delta^{13}\text{C} = -21.5\text{‰}$). This female presented also higher
232 lipid content and was one the two individuals with highest contaminant concentrations, highest
233 stress levels and shortest telomeres. As we did not have the sufficient information (unknown
234 breeding status) to exclude her from the statistical analysis, we kept this individual in further
235 analysis (see Discussion).

236

237 **3.2 Relationships among contaminants, isotopic signatures and physiological status in black** 238 **guillemots**

239 We created four score indices based on the chemical groupings where each bird was given
240 a score relative to the mean of the population per chemical group (i.e. iPCB, iPesticides, iPBDE,
241 iPFAS and iHg) and one for total contaminant load (i.e. iCON). Correlation tests (Spearman's
242 test) between the indices showed that birds with high scores on PCB also had high scores on the
243 other contaminants. However, we found no significant correlations among iPBDE, iPFAS and
244 iHg, or between iHg and iPesticides (see Supplementary Fig 2.). All score indices, except iHg,
245 correlated significantly with iCON.

246 No significant relationship among isotopic signatures, lipid content and physiological
247 status measures (for p-values see results from Spearman's correlation test in Supplementary Fig.
248 1) was detected. We found a significant positive correlation between body condition (SMI) and
249 telomere length (RTL) and a significant negative correlation among iPesticides and SMI and
250 RTL (see p-values from Spearman's correlation test in Supplementary Fig 1).

251 Our low sample size prevented us from conducting a thorough evaluation of the
252 relationship between contaminants and physiological status in black guillemots. However, the
253 data provided insight into these relationships and showed a consistent tendency with several
254 variables driven by the two most contaminated individuals (see Fig 2). It should be noted that
255 one individual (female) obscured this tendency; the individual with the shortest telomeres was in
256 the modal of lower contaminant concentrations.

257 **3.3 From contaminants to telomere length**

258 Initial evaluation of our data through the residual plot analysis revealed a nonlinear relationship
 259 between iCON, SMI, Oxy, d-ROM and RTL (see Fig 2). The observed patterns imply that the
 260 average effect of the predictor on the response variable increases for moderate contaminant
 261 concentrations, but after a certain value is reached, the response approaches a saturation level (K)
 262 that needs to be determined. This suggests that an S-shaped function would be appropriate and
 263 we opted for a regression model of the form:

$$Y_K = K \frac{e^{(\beta_0 + \beta_1 X)}}{1 + e^{(\beta_0 + \beta_1 X)}}$$

264 This function describes all nonlinear effects found in the data better than other simple nonlinear
 265 functions such as quadratic or exponential. This parametric family of functions describes all
 266 nonlinear effects found in our variables and is flexible enough to provide a good fit in all
 267 situations in our dataset. In this equation, Y represents the response (i.e. SMI, d-ROM, Oxy,
 268 RTL), and X represents each of the contaminant score indices (i.e. iCON, iPCB, iPesticides,
 269 iPFAS, iHg) and physiological status variables (i.e. SMI, d-ROM, Oxy) (see Table 1 for
 270 parameter estimates and p-values).

271 K is a constant determined by minimising the mean squared error of the regression fit, that is:

$$272 \quad K = \arg \min \sum (Y_K - Y_K^*)^2$$

273 where Y_K is the value of the function using the measured data, and Y_K^* is the predicted value
 274 under a logistic model fitted with value K as scaling factor, that is:

$$\text{logit}\left(\frac{Y^*}{K}\right) = \beta_0 + \beta_1 X$$

275 We found a significant relationship between RTL and iPesticides (p-value = 0.022) RTL and
 276 iCON (p-value = 0.047), RTL and SMI (p-value = 0.002), and between SMI and iPesticides (p-
 277 value = 0.039). There were no significant relationships between oxidative stress and any of the
 278 other variables.

279 4. Discussion

280 4.1 Contaminant concentrations and profiles in black guillemots during the breeding 281 season

282 To our knowledge, the present study is the first to report concentrations of circulating
283 contaminants in blood, a less-invasive matrix, in breeding Mandt's Black guillemot. Blood
284 reflect contaminant concentrations from recently consumed prey at a relatively short time-frame
285 (< 10 hours, Drouillard & Norstrom, 2000) and thus our study is restricted to reflect the
286 contamination situation for black guillemots during incubation period. Our results shed light on
287 different aspect of the black guillemots interactions with contaminants: i) black guillemots seem
288 to experience different contaminant exposure depending on the Arctic region and
289 season/foraging areas, and ii) black guillemots during breeding season appear to experience
290 similar exposure to circulating contaminants compared to another seabird species in which
291 several studies have reported increased stress-response, decreased physiological condition and
292 adult survival related to contaminants (e.g. Tartu *et al.* 2013; Goutte *et al.*, 2015; Blevin *et al.*,
293 2017 a & b).

294 Black guillemots are diving, subsurface feeders that switch from offshore Arctic cod
295 (*Boreogadus saida*) to nearshore and near-nest-site benthic prey such as sculpin (*Myoxocephalus*
296 *scorpioides*) or different invertebrates during their breeding season (Cairns, 1987a & b; Mehlum
297 & Gabrielsen, 1993; Byers *et al.*, 2010). While non-breeding guillemots and guillemots from
298 other Arctic regions share similar contaminant patterns, black guillemots present during the
299 breeding season in Kongsfjorden different patterns of POPs in the plasma (\sum DDTs > \sum PCBs >
300 HCB > \sum CHL > Mirex > \sum HCHs) compared to what has been previously reported for liver and
301 muscle of black guillemots from the Barents Sea and in eggs from the Canadian Arctic (\sum PCBs
302 > \sum DDTs > HCB > \sum CHL > \sum HCHs > Mirex) (Borgå *et al.*, 2001; Braune *et al.*, 2018). We also
303 found a higher number of congeners of PFASs than earlier studies investigating liver and eggs of
304 black guillemots in the West Arctic (Martin *et al.*, 2004; Peck *et al.*, 2016).

305 The black guillemots in our study show similar mean $\delta^{13}\text{C}$ signal as earlier studies on non-
306 breeding black guillemots from Greenland which indicate that black guillemots breeding in
307 Kongsfjorden may forage less benthic at least during incubation (Linnebjerg *et al.*, 2016). One

308 female, however, has a more pelagic $\delta^{13}\text{C}$ signal similar to juvenile black guillemots during non-
309 breeding season in the central Barents Sea (Borgå *et al.*, 2005). This specific female also displays
310 higher contaminant concentrations, especially HCB, *trans*-Nonachlor, PBDE-47, Hg and certain
311 PFASs which support a different exposure scenario depending on either breeding state or
312 foraging grounds. Unfortunately, although there was no apparent brood patch, we do not have
313 sufficient information to determine the breeding status (e.g. juvenile or inexperienced/late/failed
314 breeder). Implications of differences in breeding status on physiological status is further
315 discussed in chap. 4.2. $\delta^{15}\text{N}$ signals range from 11.9 to 13.5‰, which is similar to the isotopic
316 range of juveniles reported by Borgå *et al.* (2005). At best, our results suggest that black
317 guillemots experience different contaminant situation at different foraging grounds (offshore-
318 nearshore/benthic-pelagic). Whether foraging offshore or pelagic lead to higher exposure
319 remains unclear as the most contaminated bird (a male with especially high level of PFASs)
320 displayed a $\delta^{13}\text{C}$ signal similar to the rest of the sample population.

321 Compared to other seabird species breeding in the same fjord, black guillemots i) have
322 lower Hg concentrations (0.32 ug/g d.w.) than previously reported for black-legged kittiwakes
323 (*Rissa tridactyla*; 1.8 ug/g d.w. red blood cells, Tartu *et al.*, 2013) and ii) show similar wet
324 weight plasma of ΣPCBs , $\Sigma\text{Pesticides}$, ΣPBDEs and ΣPFAS compared to black-legged
325 kittiwake sampled the same year (Haarr *et al.*, 2017). However, plasma concentrations are often
326 used as proxy for body burden, expressed as lipid weight concentrations to control for the
327 varying lipid content in blood within and between species (Bustnes *et al.*, 2001; Hebert &
328 Keenleyside, 1995). The two species differed in lipid weight contaminant concentrations, thus,
329 we cannot exclude that the two species may still differ in total body burden, i.e. stored
330 contaminants in other organs and tissues accumulated outside breeding season.

331 Seabirds can go through drastic changes in body mass during breeding season, resulting in
332 lipid-mobilisation and subsequently re-mobilise of contaminants into the bloodstream (Henriksen
333 *et al.*, 1998; Bustnes *et al.*, 2012 & 2017; Fenstad *et al.*, 2016). This seems unrealistic as black
334 guillemots appear rather to increase their body mass during incubation (Aevar Petersen,
335 *pers.com.*) and we find no relationship between body condition and blood lipid content. It is
336 more plausible that black guillemots have a higher lipid content than other seabird species as
337 reported in Harr *et al.* (2017) due to frequent inputs from more lipid-rich prey during incubation.

338 Black-legged kittiwakes are subsurface feeders foraging on fish, invertebrates and
339 zooplankton in the pelagic part of the ecosystem, with an offshore foraging distribution during
340 winter. During the breeding season, the black-legged kittiwakes of Kongsfjorden forage within
341 the same fjord system as the black guillemots in this study (nearshore) but in different parts
342 (benthic vs. pelagic) (Vihtakari *et al.*, 2018). Our results also support that auks have a lower
343 biotransformation capacity compared to other gulls (Borgå *et al.*, 2005); as plasma
344 concentrations mirror the same high proportion of cis-Chlordane in Σ CHL as found in internal-
345 tissue samples of non-breeders and black guillemot eggs (Borgå *et al.*, 2001; Braune *et al.*,
346 2018). Thus, it may seem that even though these two seabirds species have different foraging
347 strategies and biotransformation capacities, they end up with the same exposure to circulating
348 contaminants (of the ones we targeted in this study, except Hg). Furthermore, even though blood
349 lipid content and isotopic signatures fall short to explain contaminants concentrations in the
350 Black guillemot of our study, foraging in less contaminated areas but on prey with higher lipid
351 could lead to the same exposure as the opposite. This aspect may only be understood through
352 integrative methods combined with individual tracking data, as demonstrated by Fort *et al.*
353 (2014).

354

355 **4.2 The relationships between contaminants and physiological status**

356 Telomere length is a time-integrated measure of life history processes, including age and
357 experienced stress (Young *et al.*, 2013). It has also been proposed as a predictor for *intrinsic*
358 quality (see discussions on different definitions and use of quality in Wilson & Nussey (2010)
359 and Bergeron *et al.* (2011)). In this case, lower quality individuals are expected to present a
360 lower physiological status (i.e. poorer condition) experience more stress and thus greater
361 telomere attrition. Our results show that birds in poor condition had shorter telomeres than birds
362 in better condition, which is consistent with findings in other seabird species showing that
363 relative telomere length and body condition are positively related (Le Vaillant *et al.*, 2015). In
364 addition, birds with higher contaminant scores also tended to be in poorer condition and have
365 shorter telomeres (only significant for total contaminant load and pesticides). This is consistent

366 with what has been observed for oxychlorane and telomere length in the chick-rearing black-
367 legged kittiwakes by Blévin *et al.* (2016), despite that concentrations were lower in our study.

368 The two most contaminated individuals (one female and one male) separating the dataset
369 into two groups dictated the significance in the correlations observed in our data. Although
370 observed at the nest site together with other breeding individuals, we were not able to determine
371 breeding status for the female (i.e. non-breeder or failed or late breeder). Moreover, we lack
372 information about the age of these birds, thus it is difficult to conclude whether or not this female
373 was a juvenile visitor that did not try to breed at all or an unexperienced breeder attempting and
374 failing to reproduce, returning to off-shore foraging ground to eat and coming back to the colony.
375 This bird could also have been a late breeder that recently arrived from off-shore foraging
376 grounds: in the closely related Pigeon guillemot (*Cephus columba*), experienced breeders arrive
377 earlier than unexperienced, and during egg-formation, females have shown reduced nest
378 attendance compared to males (Nelson, 1987). This female may also have failed because of a
379 poor condition as indicated, by her low body condition, high stress levels and short telomeres.
380 Only in the first situation (juvenile), would it be statistically sound to exclude the individual from
381 the analysis as an outlier; however immature individuals tend to have smaller body size measures
382 (i.e. wing length, bill depth) and this was not the case for this specific individual compared to the
383 rest of the sample population. Thus, with noted caution, we consider it more plausible that the
384 female reflect natural variation of contaminant concentrations and physiological condition
385 related to individual foraging tactics within breeding black guillemots.

386 Another aspect is the sample size: an increase of sample size could well have distinguished
387 these two individuals as clear outliers; on the other hand, one should also take caution being-
388 blinded by the mean-response of the population (Bennett, 1987) as toxic effects can also
389 influence the variance of the physiological response (Forbes & Depledge, 1999). The distribution
390 in our contaminant data may also be the product of cumulative processes: some birds end up as
391 extremes due to individual strategies over time (e.g. individual foraging specialisation as
392 observed for diving seabirds by Bearhop *et al.* (2006)). Such extreme values induce non-linearity
393 in the data (i.e. S-shaped function) with 1) lower contamination score with little or no effect
394 observed on response variables measured, and 2) high contamination score and higher effect on
395 response variables measured. This is also what we see as the main tendency in our data. Yet iHg

396 did not correlate with the other score indices but showed a more linear functional relationship
397 with the physiological status responses.

398 The same tendency of relationship with contaminants was also observed in respect to
399 stress: although insignificant, the reactive oxygen metabolites (d-ROM) levels seemed to
400 increase with increasing contaminant score, while for the variance, observations indicate a
401 decrease. The individuals with higher d-ROM had also lower global plasmatic antioxidant
402 defence (Oxy). This is consistent with previous findings in birds (Hegseth *et al.*, 2011; Bourgeon
403 *et al.*, 2012; Sletten *et al.*, 2016; Abbasi *et al.*, 2017) that support the hypothesis that contaminant
404 exposure may contribute to increased oxidative stress (Isaksson *et al.*, 2010; Constantini *et al.*,
405 2014). The lack of relationship between d-ROM and telomeres may be due to a mismatch of
406 tissue (Constantini *et al.*, 2014) or time scales. Telomere length is commonly understood as an
407 integrative measure of accumulated oxidative stress, while d-ROM is an indicator of
408 instantaneous oxidative stress (Urvik *et al.*, 2016).

409 **4.3 Theoretical response pathway for contaminant exposure and telomere length**

410 The low sample size restricted us from evaluating our proposed response pathway in regards to
411 contaminant loading and telomere length. However, the tendency in the data allowed us to build
412 a hypothetical scenario for future investigation in which oxidative stress and telomere length will
413 be dictated by accumulated contaminant burden and body condition above and below certain
414 thresholds.

415 The threshold idea is based on inter-individual physiological variability (Depledge, 1990)
416 resulting in different abilities to perform across different environmental conditions (e.g., low and
417 high food availability, low and high contamination), without increasing the cost (i.e. stress,
418 condition) – or *intrinsic quality* (Bergeron *et al.*, 2011). Furthermore, at some point, contaminant
419 exposure may start influencing these abilities. A central question is then if birds with higher
420 contaminant concentrations are a result of lower body condition, as observed in Wayland *et al.*
421 (2002) – or vice versa – if higher concentrations of contaminants have led to lower body
422 condition. In the former, body condition may determine physiological status while the correlating
423 contaminants play a co-founding role; the effect of weight loss is more important for the overall
424 stress situation compared to the contribution from contaminants (Fenstad *et al.*, 2014).

425 However, black guillemots do not experience significant weight loss during incubation due to
426 shared parental effort and strategies of short-distance trips (both partners forage every day)
427 (Aevar Petersen & George Divoky, *pers.com.*). This is supported by our findings, where no
428 relationship between lipid content, body condition and contaminants was observed. An
429 alternative explanation is that the most contaminated birds may be in poorer condition due to the
430 presence of high contaminant concentrations over time (i.e. strategy driven cumulative
431 processes). In this case, we may expect a shift between physiological states where an individual
432 with contaminant concentrations above a certain threshold value is bound to have a lower
433 physiological status over time.

434 **5. Conclusions**

435 In conclusion, our results demonstrate that, during the breeding season, black guillemots
436 experience the same circulating contaminant concentrations as a pelagic migratory seabirds, the
437 Black-legged kittiwake, for which several clear associations between contaminants, stress
438 response and breeding success have been reported. Our study also emphasize the importance of
439 individual tracking datasets and information on breeding and foraging status in wildlife
440 ecotoxicology. Despite non-significant relationships, our data shows a consistent tendency that
441 black guillemots in poorer condition had shorter telomeres, higher concentrations of
442 contaminants and higher levels of oxidative stress. Our threshold hypothesis should be tested
443 with a larger dataset, coupled with life history data, in the future: if a threshold for effects exists,
444 it will likely both depend on breeding behaviour (e.g. fasting during incubation shift) and
445 environmental stressors (e.g. food availability, habitat quality acting on body condition), and a
446 species' behavioural and physiological traits (e.g. life history, biotransformation). Thus, we
447 should expect the same non-linear relationship between telomere length and contaminants and
448 body condition, with species-specific thresholds, in other seabirds as well.

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458 **Data availability**

459 Data is publicly available on FigShare (DOI: 10.6084/m9.figshare.6225071). [During the review
460 process these data can be accessed using this temporary URL:
461 <https://figshare.com/s/8d10a917a3274aba1d6b>].

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695 **Figure legends**

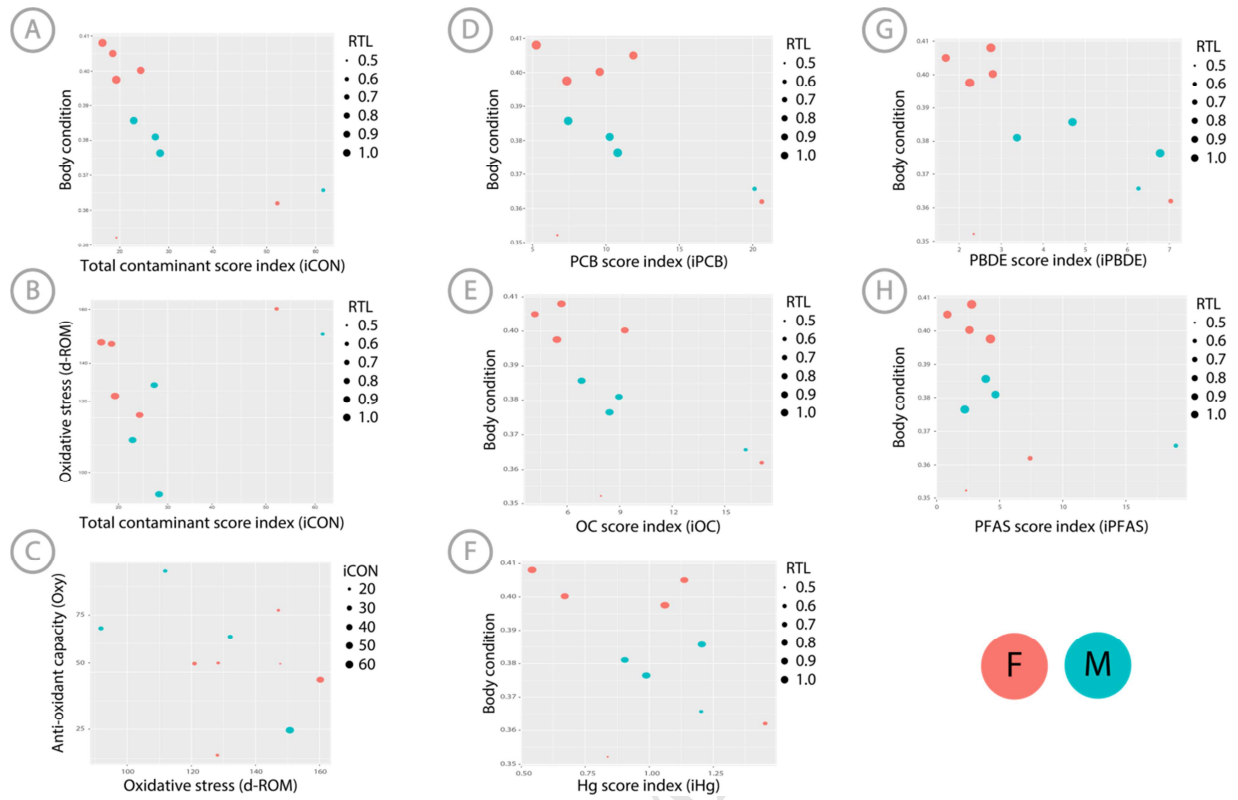
696 **Figure 1. Contaminant profiles in the Black guillemot.** Radar plot showing concentrations
697 (ng/g w.w.) and profile signatures of PCB, pesticides, PBDE and PFAS congeners detected in
698 incubating black guillemots (points, with 'extreme' male marked in red colour) and line (black)
699 indicating the mean.

700 **Figure 2. Scatterplots of relations between contaminant score indices, body condition,**
701 **oxidative stress, anti-oxidant capacity, and relative telomere length, in females (red) and**
702 **males (blue).** (a) iCON and SMI with RTL indicated by the size of the points. (b) iCON and d-
703 ROM with RTL indicated by the size of the points. (c) d-ROM and Oxy with iCON indicated by
704 the size of the points. (d) iPCB and SMI with RTL indicated by the size of the points. (e)
705 iPesticides and SMI with RLT indicated by the size of the points. (f) iHg and body SMI with
706 RTL indicated by the size of the points. (g) iPBDE and body SMI with RTL indicated by the size
707 of the points. (h) iPFAS and SMI with RTL indicated by the size of the points.

708 **Table 1: Parameter estimates for modelling the relationships among contaminants (iCON)**
709 **and physiological status (SMI, d-ROM, RTL) in incubating black guillemots.** Bold
710 characters refer to significant relationships under $\alpha < 0.05$.

711

712



718

719 **FIGURE 2**

720

721 **Table 1.**

722

Dependent variable	Independent variable	K	Beta0	Beta1	Df	t-value	P-value
RTL	iCON	1.5	0.77	-0.02	8	-2.34	0.047
	iPCB	1.5	0.77	-0.06	8	-2.12	0.067
	iPesticides	1.5	0.88	-0.08	8	-2.85	0.022
	iPBDE	1.5	0.48	-0.09	8	-1.04	0.328
	iPFAS	1.54	0.33	-0.05	8	-1.79	0.112
	iHg	1.5	0.85	-0.73	8	-1.16	0.279
	SMI	1.5	-8.70	23.00	8	4.69	0.002
SMI	d-ROM	1.5	1.55	-0.01	8	-1.34	0.218
	Oxy	1.64	-0.31	0.004	8	0.64	0.538
	iCON	2.04	-1.40	-0.002	8	-1.95	0.086
	iPCB	0.61	0.65	-0.012	8	-1.51	0.168
	iPesticides	2.04	-1.38	-0.009	8	-2.47	0.039
	iPBDE	0.63	0.57	-0.04	8	-1.90	0.094
	iPFAS	2.04	-1.44	-0.005	8	-1.35	0.216
d-ROM	iHg	2.04	-1.37	-0.098	8	-1.33	0.219
	iCON	800.95	-1.80	0.005	8	1.17	0.277
Oxy	SMI	257.19	0.27	-0.557	8	-0.09	0.928
	iCON	141.27	0.01	-0.018	8	-0.98	0.355
SMI	iCON	141.27	-1.91	3.616	8	0.236	0.819

723

724 **SUPPLEMENTARY MATERIAL**

725 **Supplementary Table 1.** Concentrations of contaminants (pg/g w.w., Hg given as ng/g d.w.),
726 lipid content, dietary signatures (%), oxidative stress, and relative telomere length in blood from
727 black guillemots. Values are given as detection frequency, mean, standard deviation, and range
728 (min-max).

729 * Due to poor extraction recoveries with two of the three method blanks of the internal standard for
730 oxychlorane, we calculated a conservative detection limit by multiplying the blank response in the blank sample
731 which acceptable extraction recoveries were obtained by a factor of three to avoid the potential of reporting false
732 positives. In addition, a minor isobaric interference coeluted with oxychlorane. As blank subtraction was not
733 performed, concentrations approaching detection limits will be heavily influenced by co-eluting interference and
734 should be treated with caution. We decided on a conservative, cautionary approach and excluded oxychlorane from
735 further analyses.

	Detection frequency (%)	Mean (median)	Standard deviation	Min	Max	
Σ Pesticides ₁₇	PeCB	100	109 (92.8)	60.5	45.1	274.5
	aHCH	60	14.8 (15.5)	3.7	9.1	19.2
	bHCH	100	186.2 (180.6)	94.8	59.0	382
	γ HCH	10	<LOD			
	HCB	100	2227 (1900)	925	929	4300
	Oxychlordane*	50	900 (778)	248	699	1246
	Trans-chlordane	0	<LOD			
	Cis-chlordane	100	16.1 (16.5)	2.4	12.1	19.5
	Trans-nonachlor	100	85.0 (61.3)	81.6	24.7	297
	Cis-nonachlor	100	605 (452)	456	278	1491
	Mirex	100	352 (327)	214	131	877
	o,p DDE	0	<LOD			
	p,pDDE	100	3188 (2259)	2235	1338	8653
	o,p DDD	0	<LOD			
	p,p DDD	0	<LOD			
	o,p DDT	0	<LOD			
	p,p DDT	0	<LOD			
Σ PCB ₁₁	PCB28	60	118 (90.6)	69.6	74.2	258
	PCB47	100	173 (147)	83.8	101	328
	PCB52	100	184 (145)	128	86.0	442
	PCB66	100	337 (302)	191	111	612
	PCB71	100	231 (196)	115	82.0	478
	PCB99	100	1390 (1179)	757	625	3033
	PCB101	100	479 (360)	349	147	1277
	PCB118	100	1977 (1779)	1145	577	4529

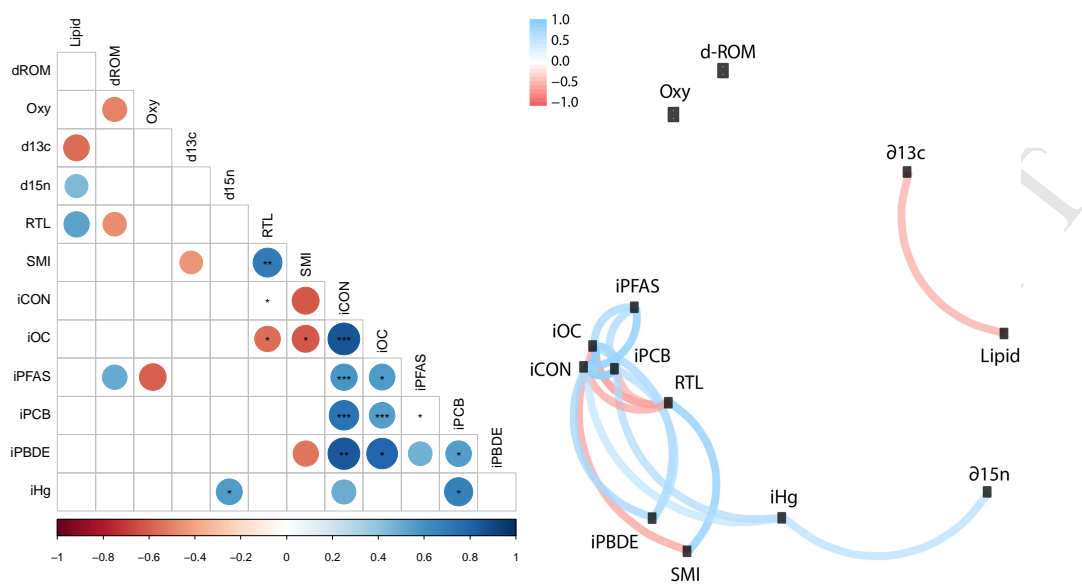
	PCB138	100	4689 (3793)	2646	2378	11156
	PCB153	100	6897	4143	3169	17303
	PCB180	100	2474 (2076)	1710	1034	6922
Σ PBDE ₁₀	PBDE28	20	<LOD			
	PBDE71	0	<LOD			
	PBDE47	100	145 (127)	80.4	72.0	322
	PBDE66	0	<LOD			
	PBDE100	100	48.1 (37.0)	24.2	24.0	88.0
	PBDE99	90	35.9 (32.2)	20.2	12.0	79.0
	PBDE154	90	43.1 (38.0)	19.4	24.0	70.0
	PBDE153	20	<LOD			
	PBDE183	10	<LOD			
	PBDE209	0	<LOD			
Σ PFAS ₂₀	6:2FTS	0	<LOD			
	8:2FTS	0	<LOD			
	PFOSA	0	<LOD			
	PFBS	0	<LOD			
	PFPS	0	<LOD			
	PFHxS	40	<LOD			
	PFHpS	0	<LOD			
	brPFOS	0	<LOD			
	linPFOS	100	3892 (1984)	4923	630	17162
	PFNS	0	<LOD			
	PFDCs	0	<LOD			

	PFHxA	0	<LOD			
	PFHpA	0	<LOD			
	PFOA	20	<LOD			
	PFNA	100	1067 (807)	876	139	3247
	PFDCa	100	568 (393)	581	44.4	2048
	PFUnA	100	2718 (2086)	2033	926	7450
	PFDoA	20	<LOD			
	PFTriA	90	1781 (872)	2457	577	8286
	PFTeA	20	<LOD			
Σ Hg	totalHg	100	323 (330)	83.2	175	469
%Lipid	EOM	100	1.2 (1.34)	0.5	0.5	1.9
Trophic position	$\delta^{15}\text{N}$ (‰)	100	12.7 (12.9)	0.6	11.9	13.5
Carbon source	$\delta^{13}\text{C}$ (‰)	100	-19.9 (-19.7)	0.7	-21.5	-19.2
Baseline-corrected trophic level	TL	100	4.5	0.2	5.0	4.3
Derivatives of reactive oxygen metabolites	d-ROM	100	132 (130.2)	20.5	91.8	160
Plasmatic antioxidant barrier	Oxy	100	55.3 (53.9)	23.7	13.9	94.2
Relative telomere length	RTL	100	0.80 (0.87)	0.2	0.5	1.0

736

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741 **Supplementary Figure 1.** Left: Correlations among the contaminant score indices (iCON, iPCB,
 742 iPesticides, iHg, iPBDE, iPFAS), dietary descriptors ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, lipid content) and
 743 physiological status measures (SMI, d-ROM, Oxy, RTL) in the Black guillemot. Significant
 744 relations (Spearman's test) indicated by *** (p<0.001), ** (p<0.05) and * (p<0.1). Right:
 745 Correlation network graph between the contaminant score indices, dietary descriptors and
 746 physiological status.

747

748 **Chemical analysis: Details of instrument**

749

750 **Analysis of PCBs**

751

752 Analysis of PCBs was performed by gas chromatography mass spectrometry (GC/MS) using an
 753 Agilent 7890A gas chromatograph (Agilent Technologies, Böblingen, Germany) coupled to a
 754 Quattro Micro triple quadrupole mass spectrometer (Waters Corporation, Manchester, United
 755 Kingdom). Separation was carried out using a DB-5 MS column (30 m x 250 μm inner diameter
 756 (id), 0.25 μm film thickness, J&W, Folsom, USA) equipped with a 5 m guard column (Restek

757 Corporation, Bellefonte, PA, USA) at 1ml/min with Helium as a carrier gas. Samples were
758 injected at 250°C using a split/splitless injector equipped with a 4 mm id packed (wool) glass
759 liner operated in splitless overflow mode with an injection of 5 µL injection volume. Oven
760 temperature program started at an initial temperature of 67°C (1.5 min hold), ramped at
761 15°C/min to 180°C, followed by a second temperature ramp of 5°C/min to 280°C (hold 3 min).
762 Single ion monitoring was used for the detection of PCB congeners using mass spectrometry in
763 electron impact mode (70 eV) with the ion source temperature of 250°C. Ions monitored for both
764 parent and ¹³C₁₂ mass-labelled internal standards are outlined in Supplementary Table 2.

765

766 **Analysis of DDT/DDE**

767

768 Analysis of DDT and DDE isomers was performed by GC/MS) using an Agilent 7890A gas
769 chromatograph (Agilent Technologies, Böblingen, Germany) coupled to a Quattro Micro triple
770 quadrupole mass spectrometer (Waters Corporation, Manchester, United Kingdom). Separation
771 was performed using a Zebron multi-residue1 column (30 m x 250 µm id, 0.25µm film
772 thickness, Phenomenex, Denmark) equipped with a 5 m guard column (Restek Corporation,
773 Bellefonte, PA, USA) at 1ml/min with Helium as a carrier gas. Similar to PCBs, samples
774 injected using a split/splitless injector equipped with a 4 mm id packed (wool) Sky liner (Restek
775 Corporation, Bellefonte, PA, USA) in splitless overflow mode using a 5 µL injection volume at
776 220°C. Oven temperature program started at an initial temperature of 85°C (1.0 min hold),
777 ramped at 25°C/min to 280°C (hold 2 min), followed by a second temperature ramp of 40°C/min
778 to 300°C (hold 5 min). Detection of DDT and DDE isomers my mass spectrometry used
779 conditions as described for PCB analysis. Parent and ¹³C₁₂ mass-labelled internal standards are
780 outlined in Supplementary Table 2.

781

782 **Analysis of organochlorine pesticides**

783

784 Analysis of organochlorine pesticides was performed by GC/MS using an Agilent 7890A gas
785 chromatograph equipped with a 5975C inert XL mass spectrometer (Agilent Technologies,
786 Böblingen, Germany). Separation was carried out using a DB-Ultra2 column (25 m x 200 µm id,
787 0.11µm film thickness, J&W, Folsom, USA) equipped with a 5 m guard column (Restek

788 Corporation, Bellefonte, PA, USA) at 1ml/min with Helium as a carrier gas. Injection of samples
 789 was carried out under similar conditions as described for DDT/DDE isomers. Oven temperature
 790 program started at an initial temperature of 80°C (2 min hold), ramped at 20°C/min to 100°C
 791 (hold 5 min), 20°C/min to 170°C (hold 3 min), 5°C/min to 200°C, and a final ramp of 20°C/min
 792 to 300 (hold 2 min). Detection of organochlorine pesticides was performed using chemical
 793 ionization with methane as a reagent gas at a flow rate of 0.4 ml/min with a source temperature
 794 of 160°C. Parent and ¹³C₁₂ mass-labelled internal standards are outlined in Supplementary
 795 Table 2.

796

797 **Supplementary Table 2.** Quantification and qualifier ions for targeted compounds and their
 798 ¹³C-mass labelled internal standards.

Compound	Quantification/Qualifier ions
PCBs	
Tri-chlorinated PCB	256/258
¹³ C ₁₂ - Tri-chlorinated PCB	268
Tetra-chlorinated PCB	290/292
¹³ C ₁₂ - Tetra-chlorinated PCB	302
Penta-chlorinated PCB	324/326
¹³ C ₁₂ - Penta-chlorinated PCB	336
Hexa-chlorinated PCB	360/362
¹³ C ₁₂ - Hexa-chlorinated PCB	370
Hepta-chlorinated PCB	392,394
¹³ C ₁₂ - Hepta-chlorinated PCB	404
DDT/DDE isomers	
o,p-DDE	246/248
p,p-DDE	246/248
¹³ C ₁₂ -p,p-DDE	258
o,p-DDD	235/237
p,p-DDD	235/237
o,p-DDT	235/237
p,p-DDT	235/237
¹³ C ₁₂ p,p-DDT	247
Organochlorine pesticides	
HCH isomers	253/255

13C6-HCH isomers	260	799
HCB	284/286	
13C6 -HCB	290	
Oxychlordane	350/352	
trans-chlordane	408/410	
cis-chlordane	408/410	
13C12 –cis-chlordane	418	
trans-nonachlor	442/444	
cis-nonachlor	442/444	
13C12-cis-nonachlor	452	
Mirex	441/439	
13C12-Mirex	449	

800

801 **Quality Assurance**

802

803 To avoid the reporting of false positives, concentrations were only reported for targeted analytes
 804 in which both quantification and qualifier ions were detected. In addition, target compounds
 805 detected in sample extracts must possess ion ratio between quantification and qualifier within
 806 20% to the ion ratio observed within the quantification standard. A minimum of three method
 807 blanks were extracted with each sample batch to calculate limits of detection per extraction
 808 batch. Extraction recoveries for internal standards from sample extracts are listed in
 809 Supplementary Table 3.

810

811 **Supplementary Table 3.** Internal standard recoveries (%) and variation within sample extracts.

Compound	Average Recovery (%) and variation
13C12-PCB 28	94 ± 19
13C12-PCB 52	93 ± 16
13C12-PCB 101	90 ± 16
13C12-PCB 118	90 ± 15
13C12-PCB 138	86 ± 16
13C12-PCB 153	88 ± 17
13C12-PCB 180	90 ± 18
13C12-p,p'-DDE	92 ± 14
13C12-p,p'-DDT	59 ± 23

13C6- α HCH	81 \pm 17
13C6- β HCH	75 \pm 24
13C6- γ HCH	85 \pm 19
13C6-HCB	80 \pm 19
13C12-trans-chlordane	99 \pm 22
13C12-cis-chlordane	96 \pm 21
13C12-trans-nonachlor	101 \pm 24
13C12-cis-nonachlor	84 \pm 14
13C12-trans-nonachlor	94 \pm 20

812