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1 **Does contaminant exposure disrupt maternal hormones**
2 **deposition? A study on per- and polyfluoroalkyl substances in an**
3 **Arctic seabird**

4

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25 **Abstract**

26 Maternal effects are thought to be essential tools for females to modulate offspring
27 development. The selective deposition of avian maternal hormones could therefore allow
28 females to strategically adjust the phenotype of their offspring to the environmental situation
29 encountered. However, at the time of egg formation, several contaminants are also transferred
30 to the egg, including per- and polyfluoroalkyl substances (PFAS) which are ubiquitous organic
31 contaminants with endocrine disrupting properties. It is, however, unknown if they can disrupt
32 maternal hormone deposition. In this study we explored relationships between female PFAS
33 burden and maternal deposition in the eggs of steroids (dihydrotestosterone, androstenedione
34 and testosterone), glucocorticoids (corticosterone) and thyroid hormones (triiodothyronine and
35 thyroxine) in a population of the Arctic-breeding black-legged kittiwake (*Rissa tridactyla*). Egg
36 yolk hormone levels were unrelated to female hormone plasma levels. Second-laid eggs had
37 significantly lower concentrations of androstenedione than first-laid eggs. Triiodothyronine
38 yolk levels were decreasing with increasing egg mass but increasing with increasing females'
39 body condition. Testosterone was the only transferred yolk hormone correlated to maternal
40 PFAS burden: specifically, we found a positive correlation between testosterone in yolks and
41 circulating maternal perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDCa) and
42 perfluoroundecanoic acid (PFUnA) in first-laid eggs. This correlative study provides a first
43 insight into the potential of some long-chain perfluoroalkyl acids to disrupt maternal hormones
44 deposition in eggs and raises the question about the consequences of increased testosterone
45 deposition on the developing embryo.

46

47 Key-word: Black-legged kittiwake, PFAS, Maternal effect, Corticosterone, Testosterone,
48 Thyroid hormones.

49 **1. Introduction**

50 Freshly laid avian eggs contain, in addition to basic resources, substantial levels of maternal
51 hormones (Groothuis *et al.* 2005; Schwabl 1993). The amount of maternal hormones deposited
52 in the egg yolk is known to be influenced by environmental factors (e.g., abiotic conditions
53 (Lessells *et al.* 2016), food availability (Verboven *et al.* 2003) and social interactions (Pilz and
54 Smith 2004)), the offspring characteristics (e.g., sex of the embryo (Müller *et al.* 2002), laying
55 date (Tomita *et al.* 2011), position of the egg in the laying order (Schwabl 1993), composition
56 of the egg (Groothuis *et al.* 2006)) and the state of the mother bird (Tschirren *et al.* 2009; e.g.,
57 her age, physical condition, past experience, genetic background) or the male quality
58 (Rutkowska *et al.* 2020). Although mainly explored for steroids, experimental prenatal
59 exposure to maternal hormones *in ovo* have shown to impact hatching date and offspring growth
60 (von Engelhardt *et al.* 2006), metabolism (Tobler *et al.* 2007), immune system (Navara *et al.*
61 2005), sex-ratio (Rubolini *et al.* 2006), behavior (Possenti *et al.* 2018) and survival (Saino *et al.*
62 *et al.* 2005). Maternal hormones, in interaction with the offspring and maternal genotype, are
63 believed to behave as messengers enabling adaptive maternal effects to prepare the offspring to
64 prevailing environmental conditions (Mousseau and Fox 1998; von Engelhardt and Groothuis
65 2011). Independent regulation between circulating and transferred maternal hormones is an
66 essential prerequisite for hormones to be adaptive regulators of offspring development.
67 Although very little is known about transfer mechanisms, the literature suggests that such a
68 separate control over deposition and response may at least be possible (Della Costa *et al.* 2020;
69 Groothuis and Schwabl 2008; Kumar *et al.* 2019; Okuliarova *et al.* 2018). Being the results of
70 a coevolutionary process between maternal transfer and offspring responses to these substances,
71 such intergenerational effects may either have beneficial or even detrimental outcomes for the
72 mother and/or the offspring (Hsu *et al.* 2020a; Marshall and Uller 2007). Indeed, maternal
73 effects may increase offspring fitness, but also reduce it to increase maternal survival in

74 unprofitable conditions. Maternal hormones and the offspring's response should therefore
75 match an optimal trade-off for a given situation.

76 In this context, any disruption to the transfer of maternal hormones may be detrimental not
77 only to the offspring but also to the siblings and the parents (Bebbington and Groothuis 2021;
78 Groothuis *et al.* 2019). In birds, endocrine pathways are known to be affected by various
79 environmental contaminants (e.g., trace elements, organic compounds; Metcalfe *et al.* 2022;
80 Tan *et al.* 2009; Tyler *et al.* 1998). These contaminants are transferred to the yolk (Ackerman *et*
81 *al.* 2017; Verreault *et al.* 2006) and may therefore potentially disrupt maternal hormone
82 deposition. The effects of contaminants on maternal hormone deposition has nonetheless been
83 seldom investigated: legacy persistent organic pollutants (POPs: organochlorines, brominated
84 flame retardants) altered maternal steroids deposition in eggs of glaucous gulls (*Larus*
85 *hyperboreus*; Verboven *et al.* 2008), but not in common terns (*Sterna hirundo*; French *et al.*
86 2001) and great tits (*Parus major*; Ruuskanen *et al.* 2019).

87 There is a clear need for further investigations on the effects of contaminants on the
88 transfer of maternal hormones in eggs. Especially since most of the work has focussed on
89 steroids, and not on other deposited hormones (e.g., glucocorticoids and thyroid hormones; von
90 Engelhardt and Groothuis 2011). More specifically, seabirds are exposed to a large variety of
91 deleterious substances (Bianchini *et al.* 2022), but some of them receive little attention. Among
92 them are per- and polyfluoroalkyl substances (PFAS), a family of synthetic compounds used as
93 surface-active agents in a multitude of manufactured products: firefighting foams, waterproof
94 clothing, non-stick cookware, coatings, food packaging; Buck *et al.* 2011). Despite the known
95 PFAS endocrine disrupting capabilities (Chen *et al.* 2019; Coperchini *et al.* 2020; Di Nisio *et*
96 *al.* 2020; Di Nisio *et al.* 2019; Jensen and Leffers 2008; Rickard *et al.* 2022; Shi *et al.* 2019), to
97 date our knowledge on that matter remains extremely limited in wildlife. Indeed, a few
98 correlative studies on birds suggests that PFAS may affect concentrations of thyroid hormones,

99 corticosterone and prolactin, potentially leading to effects on physiology, parental investment
100 and incubation behaviours (Ask *et al.* 2021; Blévin *et al.* 2020; Mortensen *et al.* 2020; Nøst *et*
101 *al.* 2012; Sebastiano *et al.* 2020b; Tartu *et al.* 2014). The disrupting mechanisms are not well
102 identified, but like other endocrine-disrupting compounds, PFAS may cause an alteration of the
103 synthesis and breakdown, the release, transport and binding or elimination of endogenous
104 hormones (Metcalf *et al.* 2022). Specifically, some studies have shown that some PFAS are,
105 for example, particularly prone to bind to hormone transport proteins (Jones *et al.* 2003; Kar *et*
106 *al.* 2017; Mortensen *et al.* 2020). Previous investigations have shown that PFAS may exhibit a
107 dissimilar relationship with circulating hormones than the more widely studied chlorinated
108 POPs (Melnes *et al.* 2017; Tartu *et al.* 2014), which urge for additional studies regarding PFAS
109 endocrine disrupting activities. PFAS can be transferred to the yolk (Bertolero *et al.* 2015;
110 Gebbink and Letcher 2012; Jouanneau *et al.* 2022), but their impact on maternal hormone
111 deposition have not been investigated yet. Nonetheless, any disruption of the formation of
112 maternal hormones or competitive binding by PFAS to transport proteins may lead to the
113 deterioration of maternal hormones transfer and consequently to the transmission of
114 maladaptive information to the offspring.

115 In this context, the aim of the present study is to investigate maternal hormone
116 deposition in eggs of the Arctic-breeding black-legged kittiwake (*Rissa tridactyla*; hereafter
117 “kittiwake”), known to be dietary exposed to PFAS in the wild (Blévin *et al.* 2017a; Jouanneau
118 *et al.* 2022; Tartu *et al.* 2014). Specifically, we studied the relationship between female PFAS
119 burden, and yolk maternal glucocorticoids (corticosterone), steroids (testosterone,
120 dihydrotestosterone and androstenedione), and thyroid hormones (triiodothyronine and
121 thyroxine), all known to be transferred to the eggs and to play a key role in embryo development
122 (Groothuis *et al.* 2005; Groothuis and Schwabl 2002; 2008; Ruuskanen *et al.* 2016; Sarraude *et*
123 *al.* 2020a; Sarraude *et al.* 2020c). By sampling females during the pre-laying stage (i.e., during

124 egg formation), and their eggs as soon as they were laid, we provide valuable insights into the
125 relationship between maternal circulating and maternally deposited hormones, and shed light
126 on the potential impacts of maternal PFAS on maternal transfer. Given the established endocrine
127 disrupting properties of PFAS, we hypothesized a possible alteration of the maternal hormone's
128 deposition, via a disrupted transfer of some hormones in eggs in the most PFAS-contaminated
129 females.

130

131 **2. Methods**

132 **2.1 Study area and sample collection**

133 We carried out the field study between May and June 2019 in a kittiwake colony in
134 Kongsfjorden, Svalbard (see Jouanneau et al. 2022 for further information on the field
135 operations). Briefly, adults from 14 nests were captured using a noose at the end of a fishing
136 rod, during the pre-laying period (i.e., at the time of egg formation), and immediately sampled
137 for 2 mL of blood from the alar vein. Skull length (head + bill) was measured with an accuracy
138 of 0.1 mm using a calliper and birds were weighted to the nearest 2 g with a Pesola spring
139 balance. The sex of individuals were identified by molecular sexing from red blood cells
140 following Fridolfsson and Ellegren (1999) and only females ($n = 14$) were kept for further
141 analyses. At the end of each day, we separated plasma and red blood cells via centrifugation
142 and kept both samples frozen ($-20\text{ }^{\circ}\text{C}$) until analyses. In this species, females usually lay a two-
143 egg clutch (Moe *et al.* 2009). We monitored those nests daily and collected first- ($n = 14$) and
144 second-laid eggs ($n = 11$) less than 24h after laying. Hormones measured in freshly laid eggs,
145 as we did in the present study, are entirely synthesised by the female before being transferred
146 to the eggs (von Engelhardt and Groothuis 2011). We weighted the eggs in the laboratory to the
147 nearest 0.01 g, then separated yolk and albumen into microtubes and kept both samples frozen
148 until analyses. Field operations were approved by the Governor of Svalbard and by the

149 Norwegian Animal Research Authority (NARA, permit number 19970).

150

151 **2.2 Per- and polyfluoroalkyl substances analysis**

152 We adapted a method from Powley et al. (2005), contaminants analysis method and
153 concentrations have been extensively described in a previous study on PFAS maternal transfer
154 (Jouanneau *et al.* 2022). Only compounds detected in more than 70 % of the females were kept
155 for further investigations (see supporting information (SI), Table S1 for a list of all measured
156 compounds and detection percentages). Consequently, only the following compounds were kept
157 for statistical analyses: perfluorohexanesulfonic acid (PFHxS), branched
158 perfluorooctanesulfonic acid (brPFOS), linear perfluorooctanesulfonic acid (linPFOS),
159 perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid
160 (PFDcA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFD_oA),
161 perfluorotridecanoic acid (PFTriA) and perfluorotetradecanoic acid (PFTeA). For all included
162 PFAS, values lower than the limit of detection (LOD) were set to half of the LOD of the specific
163 compound. We provided all concentrations in ng g⁻¹ wet weight (ww).

164

165 **2.3 Hormone analyses**

166 We measured the following steroid and thyroid hormones in female plasma and egg
167 yolk: dihydrotestosterone (DHT), androstenedione (AND), testosterone (T), corticosterone
168 (CORT), triiodothyronine (T₃) and thyroxine (T₄). Extraction of the plasma and yolk samples
169 is detailed for all hormones in the SI.

170

171 **2.3.1 Steroid hormones (DHT, AND, T and CORT)**

172 The four hormones were assayed with similar procedures: Radio-immunoassay (RIA)
173 or Enzyme Linked ImmunoSorbent Assay (ELISA) methods. CORT and T were measured with

174 RIA method, 100 μ L of extract were incubated overnight at 4 °C with 4000 cpm of the
175 appropriate H³-steroid (Perkin Elmer, US) and polyclonal antiserum (Ab). Anti-testosterone Ab
176 was provided by Dr. Picaper (Nuclear medicine, CHU La Source, Orléans, France). The two
177 anti-corticosterone Ab (one for plasma and one for yolk) were supplied by Merck, Sigma
178 Aldrich, France. The bound fraction was then separated from the free fraction by addition of
179 dextran-coated charcoal and the activity was counted on a Tri-Carb 2810TR scintillation
180 counter (Perkin Elmer, US). AND and DHT were assayed with commercial ELISA kits supplied
181 by IBL International (AND and DHT in yolk, ref. DB 52161 and DB 52021, respectively),
182 Abnova (AND in plasma, ref. KA 1898) and Demeditec (DHT in plasma, ref. DE 2330).

183

184 **2.3.2 Thyroid hormones (T₃ and T₄)**

185 Total T₃ and T₄ were measured with RIA method, 25 μ L of yolk extract or plasma were
186 incubated overnight at 4 °C with 10 000 cpm of the appropriate I¹²⁵-steroid (Perkin Elmer, US)
187 and polyclonal rabbit antiserum (Sigma Aldrich, France). The bound fraction was then
188 separated from free fraction by addition of a polyclonal sheep antiserum against rabbit
189 antiserum and the activity was counted on a Gamma Wizard 2470 counter (Perkin Elmer, US).
190 We also calculated the T₃/T₄ ratio, often used when investigating thyroid hormones.

191

192 **2.3.3 Quality control**

193 We previously validated RIA and ELISA hormones assays on the kittiwake plasma and
194 yolk. We diluted all extracts in the appropriate assay buffer, their displacement regression lines
195 were all parallel to the standard ones. To guarantee the quality and control for reproducibility
196 and precision of the RIA, hormone standards, blanks, total activity and non-specific activity
197 were concurrently analysed. We ran all samples in duplicate in one or two runs. Additional
198 information on the hormone analyses quality control can be found in SI, minimal detectable

199 and intra-assay variation for plasma and yolk can be found in SI, Table S2. Samples below the
200 LOD ($n = 6$) were replaced with a value equal to half of the LOD of the specific hormone. We
201 provided all plasma concentrations in ng mL^{-1} , and yolk concentrations in ng g^{-1} ww. Bleeding
202 time was kept as short as possible (mean \pm SD: 02 min 09 sec \pm 36 sec) and was not related to
203 baseline CORT levels in plasma (linear model: $F_{1, 12} = 0.15, p = 0.70$).

204

205 **2.4 Statistical analyses**

206 **2.4.1 Preliminary analyses**

207 All statistical analyses were performed using R (v. 4.0.0; R Core Team, 2020). We tested
208 the normality of residuals and visually inspected diagnostic plots for each models, to check
209 whether the data met the linear model assumptions (Zuur *et al.* 2009). First, we investigated the
210 relationship between each hormone or PFAS in females' plasma and the sampling date using
211 linear models (LMs). If the relationship was significant ($p < 0.05$) we used the residuals of the
212 LM instead of the specific hormone or PFAS in the following analyses. This enabled us to
213 obtain comparable individuals by minimizing the variance due to the sampling time. T_3 and
214 PFTeA were significantly decreasing with time in females (LM: $F_{1, 12} = 7.06, p = 0.02$ and $F_{1, 12} = 6.56, p = 0.03$ respectively) and residuals of the LM were consequently used in subsequent
215 analyses.

217

218 **2.4.2 Factors influencing the maternal transfer of hormones**

219 We used linear mixed-effect models (LMMs, package "nlme") to investigate the effect
220 of female and egg characteristics on the concentrations of each transferred hormone
221 independently (i.e., one model per hormone). The full models included the maternal circulating
222 concentration for the specific hormone, the female's scaled mass index (a proxy of the body
223 condition of the female calculated following Peig and Green (2009)), the egg mass (used as a

224 proxy of egg quality), the rank of the egg in the clutch by laying order (egg rank: 1 or 2) and
225 the laying date of the first-laid eggs as fixed factors (see models formula in the SI). For all full
226 LMMs, we built a set of models ranging from the full to the null model with all possible
227 combination of predictors and we selected the best model according to the Akaike's Information
228 Criterion for small sample size (AICc; i.e., the most parsimonious one among those with a
229 $\Delta AICc < 2$; package "AICcmodavg"). In all LMMs, the nest ID was used as a random variable
230 to account for the relation between both eggs of a clutch and therefore avoid pseudo-replication.

231

232 **2.4.3 Relationship between PFAS concentration in females and the maternal** 233 **hormones in their eggs**

234 We assessed the effect of each maternal circulating PFAS on the transfer of each maternal
235 hormone in eggs using LMs. As females were sampled before they laid their first egg, and since
236 females may excrete a significant amount of PFAS during the laying process, we only included
237 first-laid eggs in these analyses to avoid biased results. We built one model per hormone. For
238 each PFAS independently, the full model included the specific PFAS as fixed factor as well as
239 any variables identified as having a significant impact on the specific maternal hormone
240 concentrations in the yolk in section 2.4.2 (except "egg rank" since only first-laid eggs were
241 used here; see models formula in the SI). AICc selection was also performed on the full models,
242 although only two models were compared: the full model and the full model without the PFAS
243 variable.

244

245 **3. Results**

246 We provide the descriptive statistics for hormone concentration in females' plasma and egg
247 yolk as well as PFAS concentrations in females' plasma in Table 1 & Table 2.

248

3.1 Factors influencing the maternal transfer of hormones

Egg yolk hormone levels were unrelated to female hormone plasma levels (SI Table S3). First-laid eggs had significantly lower AND concentrations than second-laid eggs (SI Table S3 & Table S4; mean of first- and second-laid eggs respectively: 855 and 1030 ng g⁻¹; confidence interval (CI) of the difference: [93.1 – 218]). We also found decreasing T₃ and T₃/T₄ ratio with egg mass (CI of the slope: [-0.021 – -0.001] and [-0.282 – 0.079] respectively; SI Table S4 & Figure 1), but increasing T₃ and T₃/T₄ ratio with female's scaled mass index (CI of the slope: [0.000 – 0.002] and [0.001 – 0.019] respectively; SI Table S4 & Figure 1). No other investigated egg or female characteristics affected maternal hormones deposition (SI Table S4).

3.2 Female PFAS contamination and maternal hormone deposition

Yolk concentration of CORT, AND, DHT, T₃ and T₄, as well as T₃/T₄ ratios were not related to maternal PFAS burden in first-laid eggs (SI Table S5). The model selection showed that T was the only maternally transferred hormone related to certain female PFAS, PFNA, PFDcA and PFUnA (SI Table S5). Specifically, we found increasing T in eggs with increasing plasma circulating maternal concentrations of these three compounds with comparable strength even with different range of concentration found (PFNA: $F_{1, 12} = 5.88$; $p = 0.032$; CI of the slope: [0.41 – 7.75], PFDcA: $F_{1, 12} = 5.87$; $p = 0.032$; CI of the slope: [0.28 – 5.27], and PFUnA: $F_{1, 12} = 6.27$; $p = 0.028$; CI of the slope: [0.10 – 1.47]; SI Table S6 and Figure 2).

4. Discussion

We investigated maternally deposited concentrations of androgens, glucocorticoids and thyroid hormones in egg yolk of black-legged kittiwake in relation with the female's and eggs characteristics, and the potential disrupting effect of PFAS on maternal hormone deposition. We found that egg yolk hormone levels were unrelated to female hormone plasma levels at the

274 time of sampling, but that laying order, egg mass and the female's scaled mass index appeared
275 to influence yolk concentration of some of the studied hormones. We observed an increased
276 maternal T deposition in the first-laid egg in females bearing high levels of PFNA, PFDcA or
277 PFUnA.

278

279 **4.1 Factors influencing the maternal transfer of hormones**

280 Differential deposition of androgens along the laying sequence – as observed for AND
281 in the present study – have been described as a mean to influence the outcome of sibling rivalry
282 in various avian species implementing brood reduction, ultimately maximising females'
283 reproductive outputs (Eising *et al.* 2001; Muriel *et al.* 2019; Poisbleau *et al.* 2011). Studies on
284 black-legged kittiwakes have reported higher T and AND in second-laid eggs, the amount
285 deposited likely being determined by a complex trade-off between offspring and mother fitness
286 and the outcomes of such a differential deposition largely depends on females and
287 environmental conditions (Benowitz-Fredericks *et al.* 2013; Gasparini *et al.* 2007; Müller *et al.*
288 2012; Vallarino *et al.* 2011). Higher androgen concentrations enhance embryo growth and
289 therefore reduce the detrimental effect of hatching asynchrony for the junior egg, however, at
290 the expense of potential long-term costs for the offspring (Groothuis *et al.* 2005). Interestingly,
291 contrary to AND, we did not observe any difference in the deposition of T, DHT or CORT in
292 first- and second-laid eggs in the present study, despite the fact that they are all products of the
293 same metabolic pathway.

294 Recent studies showed that maternal deposited thyroid hormones were associated with
295 environmental factors including food availability and temperature experienced during the pre-
296 laying stage (Hsu *et al.* 2022; Ruuskanen and Hsu 2018). Their effects are still unclear but seem
297 to occur mainly during the embryological development period and largely act on offspring
298 growth and positively impact hatching success and telomere length during early postnatal

299 period (Hsu *et al.* 2019; Ruuskanen *et al.* 2016; Sarraude *et al.* 2020a; Sarraude *et al.* 2020b;
300 Sarraude *et al.* 2020c; Stier *et al.* 2020). The increasing concentration of maternal T₃ (but not
301 T₄) deposited with increasing females scaled mass index found in the present study may
302 illustrate that females in good condition can invest more in their offspring than those in lower
303 condition. However, heavier eggs – which have been identified in many species as eggs of
304 higher quality, leading to a higher survival probability of the offspring (Williams 1994) –
305 received lower T₃ concentrations (but not T₄) than lighter eggs, although we investigated
306 concentrations and not total deposited amount in eggs. Data on yolk thyroid hormones are very
307 limited for birds at the moment and the factors driving thyroid hormone deposition in eggs are
308 still unclear and need further clarifications (Hsu *et al.* 2020b).

309 Other factors including adult features (e.g., age, past experience, phenotype and genetic
310 (Tschirren *et al.* 2009; von Engelhardt and Groothuis 2011)), and the environment experienced
311 before or during egg laying by females (e.g., food quality and availability (Gasparini *et al.* 2007;
312 Verboven *et al.* 2003) or social interactions (Bentz *et al.* 2016)) were also proven important
313 predictors of maternal hormones deposition in eggs. These data were not available in the present
314 study and would be interesting to include in further integrative studies. That being said,
315 breeding occurred extremely late in 2019 (laying date median in the whole colony in 2019: 25th
316 of June, 10 days later than usual average median laying date for the same population; Keogan
317 *et al.*, 2022). It is likely that this late breeding was a consequence of delayed food supply which
318 may have affected hormone levels (e.g., corticosterone; Goutte *et al.*, 2014, Riechert *et al.*,
319 2014). The maternal hormones deposition may thus have been largely impacted by these
320 particular conditions. Therefore, we do recommend additional investigations in various
321 environmental conditions, as well as experimental studies, to better identify the drivers of
322 maternal hormone deposition in eggs.

323

4.2 Female PFAS contamination and maternal hormone deposition

Very few studies explored the relationship between endocrine-disrupting chemicals (EDCs) and maternal transferred hormones in avian or non-avian oviparous species, and to the best of our knowledge, only two studies found a correlation (Hamlin *et al.* 2010; Verboven *et al.* 2008), when others could not demonstrate any clear tendency (French *et al.* 2001; Johnston *et al.* 2005; Ruuskanen *et al.* 2019). Several emerging and legacy PFAS are suspected or identified EDCs (Coperchini *et al.* 2020; Johnson *et al.* 2021), and recent studies on seabirds and raptors have reported association between glucocorticoids, thyroid hormones and some long-chain perfluoroalkyl acids (Ask *et al.* 2021; Choy *et al.* 2022; Melnes *et al.* 2017; Sebastiano *et al.* 2020a; Sun *et al.* 2021; Tartu *et al.* 2014). Regarding maternal hormones transfer to the eggs, our study shows that PFNA (C₉), PFDCa (C₁₀) and PFUnA (C₁₁) concentrations in females are positively correlated with maternal T concentration in eggs. No significant correlations could be found for the perfluoroalkyl carboxylic acids (PFCAs) with longer carbon chains (C₁₂ – C₁₄), even though they were present at comparable concentrations. Long-chain PFCAs (\geq C₈) have very similar physico-chemical characteristics (Thackray *et al.* 2020), we could therefore expect a similar mode of action for them, and so, an additive effect when adding up the concentrations. However, no correlation was found between the sum of long-chain PFCAs (C₈ – C₁₄) and T (LM: $F_{1, 12} = 3.24$; $p = 0.097$; CI of the slope: [-0.05 – 0.50]). There was also no significant relationship between maternal T deposited in eggs and females PFOS (brPFOS or linPFOS) although it is the predominant compound in females' plasma (Table 2). Perfluoroalkyl sulfonic acids (PFSAs) including PFHxS and PFOS have different physicochemical properties than PFCAs. Despite high PFOS concentrations and that this compound was previously found to downregulate the production of T in humans and rats (Tarapore and Ouyang 2021), PFOS may be less prone to affect T transfer than long-chain PFCAs. Although we cannot exclude that the observed relationships for C₉ – C₁₁ PFCAs may

349 have been induced by unidentified, correlated factors, our results suggest that at least some
350 long-chain PFCAs may cause a disruption of the maternal hormones concentrations in eggs.

351 A hypothesis behind this relationship may include disturbance of T biosynthesis or
352 breakdown mechanisms by PFAS. Among hormones, if androgens are mainly formed in the
353 theca interna cells of the follicles wall, thyroid hormones and corticosterone are synthesized by
354 distant glands (thyroid and adrenal glands, respectively) of the mother bird (Groothuis and
355 Schwabl 2008). All hormones are then transferred to the oocyte via blood, although the
356 mechanisms are still largely unexplored (von Engelhardt and Groothuis 2011). For instance, a
357 disrupting effect of PFAS on the steroidogenesis may happen as steroids are produced from
358 cholesterol, whose concentration in blood is known to be affected by PFAS in vertebrates
359 (Geiger *et al.* 2014; Geng *et al.* 2019; Jacobsen *et al.* 2018; Lin *et al.* 2019; Roth *et al.* 2021).
360 However, we would expect a similar disruption in females' plasma, which was not the case
361 (LM for PFNA: $F_{1, 12} = 1.79$; $p = 0.206$; CI of the slope: $[-0.67 - 0.16]$, PFDcA: $F_{1, 12} = 1.17$; p
362 $= 0.30$; CI of the slope: $[-0.43 - 0.15]$, and PFUnA: $F_{1, 12} = 0.73$; $p = 0.41$; CI of the slope: $[-$
363 $0.11 - 0.05]$). A disruption of the steroidogenesis may also happen at the enzymatic level. AND
364 is metabolized to T by the 17β -hydroxysteroid dehydrogenase (17β -HSD) which is known to
365 be inhibited by PFAS in humans and rats (Zhu *et al.* 2020). However, this reduced the
366 production of T and thus cannot explain the higher levels of T observed in eggs of females with
367 higher PFAS concentration. Another hypothesis could involve gonadotrophic hormones such as
368 luteinizing hormone (LH). PFAS are known to affect LH in humans (Raymer *et al.* 2012), but
369 this hormone was found essential to control T deposition in Japanese quails (*Coturnix japonica*;
370 Okuliarova *et al.* 2018). We did not measure LH in females and thus cannot explore this
371 assumption, but this may be a valuable lead to explore in further studies.

372 Maternal hormone transfer mechanisms may also be affected. Unlike other vertebrates,
373 birds do not produce any transport protein with high affinity to steroids as the sex hormone-

374 binding globulin (Wingfield *et al.* 1984). Instead, the corticosteroid-binding globulin which has
375 a high affinity for CORT, may also transport androgens (Lin *et al.* 2021; Vashchenko *et al.*
376 2016). Lipoproteins and albumin may bind to all steroids as well, with a low affinity (Malisch
377 and Breuner 2010; McNabb and Morgan 1997). PFAS in blood are bound to albumin, fatty-acid
378 binding proteins and organic anion transporters (Ng and Hungerbuhler 2014). Therefore, a
379 competition for blood transporters, including albumin, between PFAS and maternal T during
380 the process of maternal transfer to egg should result in a negative relationship between PFAS
381 in females and deposited T in eggs, but we observe an opposite trend. Also, a displacement of
382 hormones fixed to albumin by PFAS have previously been described as unlikely at
383 environmental concentrations (Jones *et al.* 2003). An alternative explanation can be offered on
384 the basis of the molecular structure of the observed PFCAs. In kittiwakes, PFAS are linearly
385 positively correlated between females and their eggs for most compounds, with the transfer
386 efficiency depending on their physico-chemical characteristics caused by the increasing
387 perfluorinated carbon chain length (Jouanneau *et al.* 2022). Therefore, we can predict that the
388 disrupting activity of PFAS in general and PFCAs with medium long chain length (C₉ – C₁₁) on
389 T will vary according to the PFAS carbon chain length. In our study, the sample size was
390 relatively low, which may limit the relationship between T and some of the PFAS. Further
391 studies with a larger sample size would be essential to investigate the aforementioned
392 hypotheses.

393 Overall, our study provides further evidence that certain long-chain PFCAs are the most
394 associated with effects in this kittiwakes' population (Blévin *et al.* 2017a; Blévin *et al.* 2020;
395 Blévin *et al.* 2017b; Costantini *et al.* 2019; Tartu *et al.* 2014), and that it is essential to
396 investigate how single compounds affect physiological markers, in addition to how they act
397 together (Sebastiano *et al.* 2020a). However, correlative studies in wild populations need to be
398 interpreted with caution as the observed relationship between C₉ to C₁₁ PFCAs and T may be

399 the consequence of alternative and unknown correlated contextual cues. Maternal yolk hormone
400 concentrations are related to egg quality in some species, therefore in this wild population,
401 females feeding on higher trophic level could accumulate more PFAS and invest more in
402 reproduction by laying eggs with higher T concentrations.

403 A large body of literature describes the consequences of high T concentrations in eggs.
404 In semi-precocial seabirds, elevated yolk T was related to various development factors on
405 offspring of seabirds from the same family (impaired sex-ratio (Rubolini *et al.* 2006), growth
406 and body mass (Eising *et al.* 2001; Parolini *et al.* 2017; Rubolini *et al.* 2006), increased begging
407 (Boncoraglio *et al.* 2006), competitiveness (Müller *et al.* 2009) and survival (Eising and
408 Groothuis 2003), decreased hatching time (Eising *et al.* 2001), immune response (Muller *et al.*
409 2005) and telomere length (Parolini *et al.* 2019)). In kittiwakes more specifically,
410 experimentally elevated T in eggs led to an increased aggressiveness and dominance in the
411 clutch (Müller *et al.* 2013; Müller *et al.* 2012). Most of the studies cited above investigate the
412 effects of experimentally elevated T, which often exceed T concentrations observed in wild
413 populations. Embryos *in natura* may buffer maternal T through inactivation pathways, which
414 could at least reduce the impact of an elevated deposition of maternal T (Campbell *et al.* 2020).
415 A disruption of maternal deposited T may impact the embryo at different levels, inducing a
416 mismatch between the environment experienced early and later in life by the offspring and its
417 phenotype, which may ultimately affect its survival and fitness. Moreover, in kittiwakes, the
418 most PFAS contaminated females laid the most contaminated eggs (Jouanneau *et al.* 2022),
419 therefore the disrupted deposition of maternal T and the high concentrations of PFAS in females
420 and eggs may have additive consequences on the embryo (known to be particularly sensitive to
421 EDCs; Hamlin and Guillette 2011) through disruptions of the maternal adaptive effect, of
422 embryo development and of the female's behavior during incubation and chick-rearing periods.

423 In the present study, we found no effect of PFAS on CORT and thyroid hormones (THs)

424 deposition in eggs, despite these contaminants being known to negatively correlate with
425 circulating CORT, and positively correlate with circulating THs in seabirds including kittiwakes
426 and some birds of prey (Ask *et al.* 2021; Choy *et al.* 2022; Sebastiano *et al.* 2020b; Sun *et al.*
427 2021; Tartu *et al.* 2014). CORT is known to be affected by food availability (Goutte *et al.* 2014;
428 Riechert *et al.* 2014). It is likely that the late breeding in 2019 was a consequence of delayed
429 food supply which may have affected CORT levels. This may have, in turn, affected the
430 maternal deposition of CORT in the yolk and possibly affected the relationship between PFAS
431 and CORT deposition. Investigating of this relationship in different environmental contexts
432 (“good” and “poor” conditions), may help in concluding on how PFAS may affect the maternal
433 transfer of CORT. Concerning THs disruption by PFAS, mechanisms affected were explored in
434 humans and birds and may include interaction with THs receptors and binding proteins, with
435 the thyroid peroxidase enzyme activity or with thyroid-stimulating hormone (Fenton *et al.*
436 2021; Kar *et al.* 2017; Mortensen *et al.* 2020). In birds, maternal THs were found to impact
437 several parameters in embryogenesis development and in post-hatch performance and fitness
438 (Darras 2019; Hsu *et al.* 2019; Sarraude *et al.* 2020a; Sarraude *et al.* 2020c; Stier *et al.* 2020).
439 Therefore, the impact of PFAS may be limited on these endpoints during kittiwake embryo
440 development, at least at the concentrations we measured in the present study.

441

442 **5. Conclusion**

443 This study identified some of the main factors driving the deposition of several major
444 maternal hormones in eggs of the black-legged kittiwake. It also produced a first insight into
445 the relationship between females PFAS burden in plasma and maternal transferred hormones in
446 egg yolk. None of the investigated thyroid (T₃ and T₄) and glucocorticoid (CORT) hormones
447 deposition seemed to be affected by female contamination. Similar results were observed for
448 steroid hormones including AND and DHT, but we observed a positive relationship between

449 some long-chain PFAS and T deposited in eggs. Although our results do not provide evidence
450 for a causal relationship between PFAS contamination in females and maternal T in eggs, we
451 suggested that the transfer mechanism may be similar for some PFAS and T, which may
452 eventually affect maternal adaptive effects driven by T deposition.

453 Nonetheless, maternal hormones deposition depends on a complex combination of
454 various intrinsic and extrinsic factors, including environmental conditions. The specific context
455 in 2019, leading to a very late breeding may thus have affected maternal hormones transfer in
456 a specific manner. We strongly recommend additional investigations among species and time,
457 as well as experimental studies to draw strong conclusions on the main effects of PFAS on
458 hormone deposition in avian eggs.

459

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