Organochlorines, perfluoroalkyl substances, mercury and egg incubation temperature in an Arctic seabird: insight from data loggers

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25   **Abstract**

26

27   In birds, incubation-related behaviors and brood patch formation are influenced by

28   hormonal regulation like prolactin secretion. Brood patch provides efficient heat transfer

29   between the incubating parent and the developing embryo in the egg. Importantly, several

30   environmental contaminants are already known to have adverse effects on avian reproduction.

31   However, relatively little is known about the effect of contaminants on incubation temperature

32   (T_{inc}) for wild birds. By using temperature thermistors placed into artificial eggs, we

33   investigated whether the most contaminated parent birds are less able to provide appropriate

34   egg warming and thus less committed in incubating their clutch. Specifically, we investigated

35   the relationships between three groups of contaminants (organochlorines (OCs), perfluoroalkyl

36   substances (PFASs), and mercury (Hg)) with T_{inc} and also with prolactin concentrations and

37   brood patch size in incubating Arctic black-legged kittiwakes (*Rissa tridactyla*). Our results

38   reveal that among the considered OCs, only blood levels of oxychlordane, the main metabolite

39   of "chlordane", a banned pesticide, were negatively related to the minimum incubation

40   temperature in male kittiwakes. PFAS and Hg levels were unrelated to T_{inc} in kittiwakes.

41   Moreover, our study suggests a possible underlying mechanism since we reported a significant

42   and negative association between blood oxychlordane concentrations and the size of the brood

43   patch in males. Finally, this reduced T_{inc} in the most oxychlordane-contaminated kittiwakes was

44   associated with a lower egg hatching probability.

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1. Introduction

Egg incubation is an essential stage in the life history of most bird species because developmental conditions for embryos can have long-term fitness consequences (Lindström 1999; Deeming 2002; Berntsen and Bech 2016). Generally, egg attendance patterns involve different parental behaviors such as egg turning and active egg warming; both being considered as key determinants for embryo viability and egg hatchability (Funk and Forward 1953; Decuypere and Michels 1992; Tona et al. 2005a; Elibol and Brake 2006a). Indeed, maintaining eggs at an optimal temperature during incubation is a complex process (Turner 2002) and critically important for complete embryonic development, improved hatchability, offspring's phenotype, and overall survival (Webb 1987; Feast et al. 1998; Olson et al. 2006; Nilsson et al. 2008; Ardia et al. 2010; Nord and Nilsson 2011, 2016; DuRant et al. 2013; Hepp et al. 2015). In birds, incubation behaviors are strongly influenced by hormonal regulation (Vleck and Vleck 2011). Accordingly, a rise in the secretion of the pituitary hormone, prolactin, during egg-laying in combination with a decrease in sex steroid levels facilitate and maintain incubation-related behaviors (Buntin 1996; Vleck 2002; Sockman et al. 2006; Angelier et al. 2016). Concomitantly, the pectoral skin of incubating birds can become a fleshy, edematous and well-vascularized brood patch, devoid of feathers (Jones 1971; Lea and Klandhorf 2002). During incubation, the brood patch comes into direct contact with the egg to ensure proper heat transfer between a parent and the developing embryo in the egg (Jones 1971).

Conditions required for optimal incubation behaviors have been largely investigated in the poultry industry to maximize egg hatchability of domestic fowl (*Gallus gallus domestica;*

71 New 1957; Meijerhof 1992; Tona et al. 2005b; Elibol and Brake 2006a, 2006b). In contrast, the

72 effects of environmental factors like contaminants (i.e. organic contaminants and trace

73 elements) on incubation behaviors of free-ranging birds *in natura* remain so far poorly

74 investigated. Yet, several environmental contaminants are already known to have adverse

75 effects on avian reproduction (e.g. Fry 1995; Herring et al. 2010; Tartu et al. 2014; Goutte et

76 al. 2015). Through their structural attributes and mode of action potencies, many of these

77 contaminants can disrupt the endocrine system involved in avian reproduction, including

78 prolactin, sex steroid (e.g. testosterone, estradiol, progesterone) and thyroid (e.g. T3, T4)

79 secretions (Rattner et al. 1984; Tyler et al. 1998; Dawson 2000; Giesy et al. 2003; Verreault et

80 al. 2004, 2006a, 2007, 2008; Tartu et al. 2015a; Melnes et al. 2017). Organic contaminants and

81 trace elements have the potential to alter parental behaviors resulting in poor breeding success.

82 For example, different laboratory and field investigations have shown that exposure to

83 organochlorines or mercury (Hg) can be associated with lowered nest or egg temperatures

84 (Peakall and Peakall 1973; Fox et al. 1978; Verboven et al. 2009a), reduced nest attendance

85 (i.e. longer and more frequent absence from the nest site) (Fox et al. 1978; Bustnes et al. 2001,

86 2005; Fisher et al. 2006a; Tartu et al. 2015a), prolonged incubation period (McArthur et al.

87 1983; Kubiak et al. 1989; Fisher et al. 2006a) and decreased nest defense /or increased egg

88 predation (Fox et al. 1978; Fox and Donald 1980; Helberg et al. 2005; Goutte et al. 2018). Such

89 detrimental effects of contaminants on incubation behaviors could induce deleterious effects on

90 hatching success. A previous study conducted on ring doves (*Streptopelia risoria*) reported a

91 lower hatchability of eggs incubated by birds experimentally exposed to high doses of

92 polychlorinated biphenyls (PCBs) (Peakall and Peakall 1973). Similarly, Forster terns (*Sterna*

93   *forsteri)* had a higher hatching success when eggs laid from organochlorine contaminated birds

94   were incubated by less contaminated surrogate parents (Kubiak et al. 1989).

95   Polar regions are considered a sink for various environmental contaminants due to

96   atmospheric long-range transport and oceanic currents in combination with a cold climate

97   (Kurkow and Kallenborn 2000). Given their properties (i.e. high volatility and/or persistence),

98   organic contaminants and trace elements such as Hg can reach isolated areas like the Arctic

99   Ocean. Once deposited in the marine ecosystem, contaminants bioaccumulate in living

100   organisms and can biomagnify along the food webs (Borgå et al. 2001; Wania 2003, 2007;

101   Ariya et al. 2004; Tomy et al. 2004; Haukås et al. 2007; Blévin et al. 2013). Long-lived species

102   like many polar seabirds that occupy high trophic levels are exposed to a greater risk of

103   accumulation and sensitivity to high concentrations of contaminants (Letcher et al. 2010; Elliott

104   and Elliott 2013). Consequently, seabirds are considered as highly relevant biological models

105   to investigate the influence of sub-lethal contaminant exposure on reproductive behaviors like

106   incubation temperature (T_{inc}).

107   In the Norwegian Arctic, black-legged kittiwakes (*Rissa tridactyla*, hereafter

108   'kittiwakes'), are chronically exposed to a complex mixture of harmful organic compounds and

109   trace elements, which have already been linked to disruption of reproductive hormones and

110   impaired reproductive performance (Tartu et al. 2013, 2014, 2015b, 2016; Goutte et al. 2015;

111   Blévin et al. 2017). Among such complex mixture of chemicals are (i) Hg, a toxic trace element

112   originating from both anthropogenic and natural sources able to disrupt hormones involved in

113   incubation behaviors such as prolactin (AMAP 2007, 2011; Tartu et al. 2016); (ii) legacy

114   organochlorines (OCs; chlorinated pesticides and PCBs), showing decreasing trends in the

Arctic, which have been associated with lower incubation temperatures in an Arctic seabird (Helgason et al. 2008; Verboven et al. 2009a; AMAP 2015; Bustnes et al. 2017); and (iii) the globally increasing poly- and perfluoroalkyl substances (PFASs), widely used as surface-active agents (Kissa 2001), especially the perfluoroalkyl carboxylic acids (PFCAs; Braune and Letcher 2013; AMAP 2015). Despite the few studies that have investigated the effects of OCs and Hg on reproductive behaviors, data are still critically lacking and importantly, to our knowledge, the consequences of PFASs exposure on incubation behaviours in birds are presently unknown.

Using artificial egg loggers, we investigated whether the most contaminated kittiwakes are less committed in incubating their clutch and less able to provide appropriate egg warming. Embedded in artificial eggs, these loggers can provide almost continuous (every second) and precise recording of incubation behaviors (Shaffer et al. 2014; Kelsey et al. 2016; Clatterbuck et al. 2017; Taylor et al. 2018). Specifically, we examined the relationships between blood levels of three groups of contaminants (OCs, PFASs and Hg) and T_{inc} in a kittiwake population from Svalbard in the Norwegian Arctic. Because prolactin secretion and brood patch formation are involved in the onset and maintenance of avian incubation behaviors and thus, tightly linked to T_{inc}, we also investigated relationships between contaminants, plasma prolactin concentrations and brood patch size as potential underlying mechanisms through which contaminant exposure in kittiwakes may influence T_{inc}. Finally, since T_{inc} is considered as a key for egg hatchability, we explored potential effects of T_{inc} on hatching probability.

2. Material and methods

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2.1. Fieldwork area and sampling collection

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140   Fieldwork was carried out from 19 June to 12 July 2015, in a colony of black-legged

141   kittiwakes at Kongsfjorden, Svalbard (78°54'N; 12°13'E). We studied 20 incubating pairs

142   because kittiwakes, like other seabirds, share reproduction duties (i.e. incubation and chick

143   rearing) among sexes. A total of 40 individuals (20 males, 20 females) were captured at their

144   nest with a noose fixed at the top of a 6 m fishing rod. We collected the first blood sample (~0.5

145   mL) immediately after capture from the alar vein using a heparinized syringe and a 25-gauge

146   needle to assess baseline prolactin concentrations. A second blood sample (~2 mL) was

147   collected to measure the concentrations of contaminants and to determine the sex of individuals

148   using molecular methods. All birds were weighed to the nearest 5 g with a Pesola spring balance

149   to determine the body mass. Finally, a photograph was collected of the whole right brood patch

150   (Fig.1; Canon EOS 1000D, 100 mm, Canon 2018), with a ruler placed next to the bird in order

151   to calculate its brood patch dimensions using Gimp 2.8 (Gimp 2018). Brood patch size was

152   determined in duplicates (all coefficients of variation ≤ 4.06%). Breast feathers were lightly

153   brushed with moistened cotton pad to fully expose the brood patch. All study birds exhibited

154   three brood patches (right: RBP, left: LBP and central: CBP). Thus, to minimize handling time,

155   we only measured the RBP of each bird. The size of the LBP and CBP were measured in 13

156   individuals in order to check whether the RBP measurement can be used to estimate the size of

157   the others brood patches (LBP, CBP). Before release, each bird was marked with colored spots

158   of a non-permanent dye on the forehead to distinguish each bird from its mate (also dyed with

159   a different color) during subsequent observations from a distance. Blood samples were stored

160   on ice in the field. Aliquots of whole blood, plasma and red blood cells were obtained after

161   centrifugation and then kept frozen at -20°C until subsequent laboratory analyses were

162   performed.

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164   **2.2. Egg logger experiment and data processing**

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166   All study nests initially contained two natural eggs. However, one of these two egg

167   was collected and replaced by an artificial egg containing a temperature thermistor (as described

168   in Shaffer et al. 2014). Artificial eggs were designed and painted to mimic as much as possible

169   the real egg morphology (similar size and shape, approximate mass; Table 1 in Supplementary

170   Materials) and coloration pattern of kittiwakes using a non-toxic water-based paint (Fig.1). Data

171   loggers recorded core egg temperature every second with a manufacturer-reported accuracy <

172   2°C (but testing in the lab in a controlled environment showed the accuracy to be approximately

173   0.5°C) and precision of 0.125°C based on thermistor component specifications (Shaffer et al.

174   2014). Subsequent tests were also conducted to verify these parameters using a standard poultry

175   incubator with automatic egg turner (Top hatch Incubator, Brower Equipment, Houghton, IA,

176   USA). Study nests were selected according to their accessibility and to minimize disturbance

177   to the rest of the colony. Collected eggs were candled and all were determined to be fertile.

178   Eggs were further dissected to assess the age of the embryo and for use in other contaminants

179   studies (n = 12). To control for potential changes in incubation behaviour that may have

180   occurred across the incubation period, we used the embryo age as a proxy of incubation stage.

181   However, we do not report here any suggested effects of the age of embryo on T_{inc} parameters

182   (Linear mixed effect models (LMMs); T_{min}: $F_{1,10} = 0.14$, p = 0.72; T_{max}: $F_{1,10} = 1.13$, p = 0.31;

183   T_{mean}: $F_{1,10} = 0.61$, p = 0.45; T_{modal}: $F_{1,10} = 1.03$, p = 0.33).

184   Artificial eggs were deployed for 7 and 10 days during the incubation period and all

185   birds readily accepted the artificial egg and exhibited no abnormal incubation behaviours. All

186   loggers recorded data for the entire duration of deployment in the nest. Because each partner of

187   a pair was dye marked on the forehead, we could determine some incubation bouts of each

188   partner at a nest using a spotting scope. Thus, we recorded and kept for further statistical

189   analyses all incubation bouts when we knew which bird was incubating (excluding data

190   recorded at night because checks were not conducted at night). The day of egg deployments

191   and all records during our presence in the colony (i.e. for blood sampling) were also excluded

192   from the data set in order to avoid any biased data. Recording duration (19.83 ± 9.38 (SD)

193   hours, ranging from 4.64 to 43.07 hours) did not influence T_{inc} parameters (LMMs, all p $\geq$

194   0.169). Upon completion of each deployment, artificial eggs were removed and only one egg

195   was left in the nest. Using a mirror at the end a long pole, we then regularly checked the

196   experimental nest contents to monitor hatching success of the remaining egg until the end of

197   the field season (i.e. 12th July; laying peak from 6th to 9th July).

198   Logger temperature data were processed using purpose-built routines in MATLAB

199   (The Mathworks, Natick, MA, USA) following methods of Shaffer et al. (2014). Overall, we

200   processed T_{inc} profiles of 40 individuals (Fig.1. in Supplementary Materials) and determine

201   extreme temperature values (minimum temperature: T_{min}; maximum temperature: T_{max}), mean

202 temperature (T_{mean}) and the most frequent incubation temperature within the record period

203 (T_{modal}).

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205 **2.3. Contaminant analyses**

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207 OCs were analyzed from whole blood at the Norwegian Institute for Air Research

208 (NILU) in Tromsø, Norway. We scanned for the following compounds: the organochlorine

209 pesticides (*o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, HCB, α-, β-, γ-

210 HCH, *trans*-, *cis*-chlordane, oxychlordane, *trans*-, *cis*-nonachlor and mirex) and the

211 polychlorinated biphenyls (CB−28, −52, −99, −101, −105, −118, −138, −153, −180, −183, −187

212 and −194). Concentrations below the limit of detection (LoD) were assigned by ½ LoD value

213 but only compounds detected in at least 70% of the data set were kept for further statistical

214 analyses. Consequently, compounds remaining for further investigations were the

215 organochlorine pesticides (oxychlordane, *trans*-, *cis*-nonachlor, mirex, HCB and *p,p'*-DDE) and

216 the PCBs (CB−28, −99, −105, −118, −138, −153, −180, −187; expressed here as the ΣPCBs).

217 It is worth noting that *p,p'*-DDE concentrations of 3 males are missing because of injection

218 issues into the GC/ MS. To a whole blood sample of 0.70-1.13 mL, a 100 µL of an internal

219 standard solution was added (^{13}C-labeled compounds from Cambridge Isotope Laboratories:

220 Woburn, MA, USA). We first proceeded to the sample denaturation using a mix of ethanol and

221 saturated solution of ammonium sulphate in water. We then ran extraction twice with 6 mL of

222 n-hexane. Matrix removal on florisil columns, separation on an Agilent Technology 7890 GC

223 and detection on an Agilent Technology 5975 CMSD were performed following Herzke et al.

224   (2009). Recovery of the internal standards ranged between 52% and 60%. Results were

225   validated with blanks (clean and empty glass tubes treated like a sample) and standard reference

226   material (1958 human serum from NIST) run every 10 samples. The deviation of the target

227   concentrations in the SRMs were within the laboratory's accepted range (75‑111%). All blanks

228   contained concentrations below the instrument detection limits except for HCB (525 pg/ g),

229   PCB-28 (81.8 pg/ g) and -105 (60.8 pg/g).

230   PFASs were analysed from plasma at NILU. The following compounds were scanned

231   for presence and concentration: perfluorooctanesulfonamide (PFOSA),

232   perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), linear

233   perfluorooctanesulfonate (PFOSlin), branched perfluorooctanesulfonate (PFOSbr),

234   perfluorodecanesulfonate (PFDcS), perfluorohexanoate (PFHxA), perfluoroheptanoate

235   (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate

236   (PFDcA), perfluoroundecanoate (PFUnA), perfluorododecanoate (PFDoA),

237   perfluorotridecanoate (PFTrA), and perfluorotetradecanoate (PFTeA). Concentrations below

238   LoD were assigned by ½ LoD value but only compounds detected in at least 70% of the data

239   set were kept for further statistical analyses. In short, a sample (0.2 mL) spiked with internal

240   standards (carbon labeled PFAS, Hanssen et al. 2013) was extracted in methanol (1 mL) by

241   repeated sonication and vortexing. The supernatant was cleaned-up using ENVICarb

242   graphitized carbon absorbent and glacial acetic acid. Extracts were analysed by UPLC/MS/MS.

243   Recovery of the internal standards ranged between 74% and 128%. Results were validated with

244   blanks (clean and empty glass tubes treated like a sample) and standard reference material (1957

245   human serum from NIST) run every 10 samples. The deviation of the target concentrations in

246   the SRMs were within the laboratory's accepted range (69‑130%). All blanks contained

247   concentrations below the instrument detection limits, except for PFCAs, ranging between 5 and

248   30 pg/ mL.

249   Total Hg was analyzed at the Littoral Environment et Sociétés laboratory (LIENSs) in

250   La Rochelle, France from freeze-dried and powdered red blood cells placed in an Advanced Hg

251   Analyzer Spectrophotometer (ALTEC AMA 254) as described in Bustamante et al. (2006).

252   Aliquots ranging from 0.44 to 8.59 mg were analysed for each individual, in duplicates (all

253   coefficients of variation ≤ 5.42%). Blanks were run at the beginning of each set of samples and

254   certified reference material (CRM; Tort-2 Lobster Hepatopancreas, NRC, Canada; certified

255   value 0.27 ± 0.06 (SD) µg/g dw) were used to validate the accuracy of the analyses. Measured

256   values of the CRM were 0.25 ± 0.01 (SD) µg/g dw, n = 11. All blanks contained concentrations

257   below the instrument detection limit (0.005 µg/g dw).

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259   **2.4. Molecular sexing and prolactin assays**

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261   Molecular sexing and prolactin assays were conducted at the Centre d'Etudes

262   Biologiques de Chizé (CEBC), France. Kittiwakes were sexed from red blood cells by

263   polymerase chain reaction amplification as part of two highly conserved genes (CHD) present

264   on sexual chromosomes as described in Fridolfsson and Ellegren (1999). Plasma prolactin

265   concentrations were determined by radioimmunoassay as previously described and validated

266   for this kittiwake population (Chastel et al. 2005). Intra-assay (within assay) variation was

267   estimated by including internal standards to the assay. Both samples and internal standards were

268   run in duplicates. CV was 7.13%. Blood collection time (i.e. time elapsed from capture to the

269   end of the first blood sampling: 2.48 ± 0.52 min (SD), on average) did not affect baseline

270   prolactin concentrations (LMM, $F_{1,19} = 0.606$, p = 0.446).

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272   **2.5. Statistical analyses**

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274   All statistical analyses were performed using R 3.2.3. Linear mixed effect models

275   (LMMs) with the nest identity as a random factor were used to test whether contaminant

276   concentrations, baseline prolactin levels, brood patch size, body mass and T_{inc} differed between

277   sexes. As suggested in Zuur et al. (2009), we used the restricted maximum likelihood estimation

278   (REML) method to avoid any potential biased statistic estimations. Second, we tested the

279   influence of each contaminant concentration on incubation temperatures (T_{min}, T_{max}, T_{mean} and

280   T_{modal}) using linear models (LMs) for each sex separately as males were determined to be more

281   contaminated than females (see Results). Moreover, it is now well established that males and

282   females can react in very different ways to environmental stressors like OCs, PFASs and Hg

283   contamination. Specifically, previous studies conducted on kittiwakes from the same colony

284   reported sex differences regarding effects of contaminants on hormone levels, body condition,

285   breeding decisions, metabolic activity, telomere length and even survival rate (Tartu et al. 2013,

286   2014, 2016; Goutte et al. 2015; Blévin et al. 2016, 2017). Influence of body mass was also

287   tested since egg temperature is likely warmer as the mass of the incubating bird increases. The

288   best models were selected based on the bias-adjusted Akaïke's Information Criterion (AICc),

289   which is a small sample size adjustment (Burnham and Anderson 2003). As a general guideline,

290 if AICc values differ by more than 2, the lowest AICc is the most accurate, whereas models

291 with AICc values differing by less than 2 have a similar level of support in their ability to

292 describe the data. Additionally, the Akaike weight (*Wi*) was estimated and can be interpreted

293 as the approximate probability that the model *i* is the best one for the observed data, given the

294 candidate set of models (Burnham and Anderson 2003; Johnson and Omland 2004). Since the

295 concentration of *p,p'-DDE* was missing for 3 males (see Methods) and because model selection

296 based on AICc requires the same number of observations among models, we performed a

297 second run of model selection with removing these 3 individuals from the data set and we found

298 no change in the results. Third, we investigated the relationships between contaminant

299 concentrations, baseline prolactin, brood patch size and body mass with LMs. Finally, we tested

300 whether T_{inc} can affect hatching probability using generalized linear model (GLM) constructed

301 with a "binomial" family and a "cloglog" link function which is consistent with the use of an

302 asymmetric data set (hatched: n = 15; not hatched: n = 5; Zuur et al. 2009). Diagnostic plots

303 and Shapiro normality tests were finally performed on residuals to test whether the data

304 sufficiently met the assumption of the models (i.e. LMM, LM, GLM) and data were log-10

305 transformed when necessary (Zuur et al. 2009). All data are presented as mean ± SD and we

306 used a significance level of $\alpha < 0.05$.

307

308 **3. Results**

309

310 **3.1. Sex-related differences**

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312   OCs, PFASs and Hg mean concentrations and LODs in female and male incubating

313   adult kittiwakes are listed in Table 1. LMMs with nest identity as a random factor to test sex-

314   related differences indicated that all OCs except *trans-* and *cis*-nonachlor, all PFASs except

315   PFTeA, and Hg concentrations significantly differed between sexes, where males had higher

316   contamination levels than females. LMMS indicated that males incubated the egg at a higher

317   T_{mean} compared to their female partner (LMM, $F_{1,19}$ = 9.518, p = 0.006; Fig.2). Mean plasma

318   prolactin concentrations, brood patch size and body mass of female and male incubating adult

319   kittiwakes are given in Table 2. LMMs with nest identity as a random factor to test sex-related

320   differences indicated no significant differences between sexes for baseline prolactin

321   concentrations, or brood patch size (Table 2). However, as expected, males were significantly

322   heavier than their female partners (Table 2).

323

324   **3.2. Incubation temperatures and contaminants**

325

326   According to the model selection, the model including oxychlordane was the best fit

327   model in males (ΔAICc = 5.77; Table 3). Specifically, we observed a negative and highly

328   significant relationship between oxychlordane concentrations in blood and T_{min} in males (LM,

329   slope = $-3*10^{-3}$; p = 0.001; R^2 = 0.45; Fig.3), indicating a lower T_{min} with increasing

330   oxychlordane concentrations. To a lesser extent, both models with HCB or mirex as explanatory

331   variables were also better than the null model (ΔAICc from null model > 2; Table 3).

332   Specifically, we observed a significant negative relationship between blood HCB and mirex

333   concentrations and T_{min} in males (LM, slope = $-1*10^{-3}$; p = 0.023; R^2 = 0.26 for HCB; LM,

334   slope = -5*10^{-3}; p = 0.029; R^2 = 0.24 for mirex). PFASs and Hg concentrations were not related

335   to T_{min} in males (Table 3). Finally, model selection also indicated a significant effect of body

336   mass on T_{min} (ΔAICc from null model > 2; Table 3), where heavier males had a higher T_{min}

337   (LM, slope = 0.109; p = 0.021; R^2 = 0.26). It is worth noting that oxychlordane concentrations

338   and body mass were significantly and negatively correlated in males ($r_{pearson}$ = -0.62; p = 0.004;

339   n = 20). Running an additive model including oxychlordane and body mass simultaneously did

340   not improve predictions of T_{min} compared to the model with oxychlordane only (AICc

341   (oxychlordane): 107.08/ AICc (oxychlordane + body mass): 109.67). We found no significant relationships

342   between contaminants and body mass on T_{min} in females (Table 3; Fig.3).

343   The AICc model selection that explained T_{mean} variations based on contaminant

344   concentrations and body mass is presented in Table 4. We found no significant relationships

345   between contaminant concentrations and T_{mean}, in either males, or females. However, the model

346   including body mass was considered as the best predictor in males among the set of candidate

347   models (ΔAICc = 3.65; Table 4), whereas for females no relationship was found. Indeed, there

348   is a significant positive relationship between body mass and T_{mean} in males (LM, slope = 0.049;

349   p = 0.018; R^2 = 0.28).

350   The AICc model selection that explained T_{max} variations based on contaminant

351   concentrations and body mass is presented in Table 5. There was no significant relationship

352   between contaminant concentrations and T_{max}, either in males, or females. However, the model

353   including body mass was considered as the best predictor in males (ΔAICc = 5.97; Table 5),

354   whereas for females, there was no relationship. There was a significant positive relationship

355   between body mass and T_{max} in males (LM, slope = 0.056; p = 0.006; R^2 = 0.36).

356   The AICc model selection that explained T_{modal} variations based on contaminant

357   concentrations and body mass is presented in Table 6. There was no significant effect of

358   contaminant concentrations and body mass on T_{modal}, either in males, or females.

359

360   **3.3. Baseline prolactin, brood patch and contaminants**

361

362   We observed a relationship between oxychlordane concentrations and T_{min} in males

363   but not in females. Consequently, we examined relationships between oxychlordane

364   concentrations and baseline prolactin levels, and the size of the brood patch to evaluate potential

365   underlying mechanisms. Baseline prolactin levels in males were not significantly related to

366   oxychlordane concentrations (log-10 transformed; LM, slope = -16.21; p = 0.47; Fig.4), to

367   brood patch size (LM, slope = 0.039; p = 0.15), nor to body mass (LM, slope = 0.475; p = 0.07).

368   Baseline prolactin levels in females were not significantly related to oxychlordane

369   concentrations (LM, slope = -6.10^{-3}; p = 0.50), to brood patch size (LM, slope = 0.042; p =

370   0.23), nor to body mass (LM, slope = 0.044; p = 0.67). However, we found a highly significant

371   negative relationship between oxychlordane concentrations and the size of the brood patch in

372   males but not in females (log-10 transformed; LM, slope = -5.10^{-5}; p = 0.16). Thus, the most

373   oxychlordane contaminated males had the smallest brood patch (LM, slope = $-2*10^{-3}$; p = $2*10^{-4}$

374    $^{-4}$; R^2 = 0.53; Fig.4). Body mass and the size of the brood patch were also positively related in

375   males (LM, slope = 0.067; p = 0.029; R^2 = 0.24) but not in females (log-10 transformed; LM,

376   slope = 4.10^{-4}; p = 0.404). Importantly, the size of the brood patch was positively and

377   significantly related to T_{min} in males (LM, slope = 1.178; p = $1*10^{-4}$; R^2 = 0.56; Fig.5).

378   The size of the LBP and CBP were marginally correlated to the size of the RBP (LBP,

379   $r_{spearman} = 0.45$; p = 0.13; n = 13 and CBP, $r_{spearman} = 0.51$; p = 0.078; n =13). We assume that

380   results presented here regarding the RBP could also be relevant for the LBP and CBP.

381

382   **3.3. Consequences on hatching success**

383

384   Because there was a relationship between oxychlordane concentrations and T_{min} in

385   males, we evaluated the consequences of T_{min} variations on hatching success. There was a

386   positive and marginally significant relationship between T_{min} and the probability that the

387   remaining egg in the experimental nests successfully hatched (GLM, $Z = 1.932$; p = 0.053;

388   Fig.6). As a result, the lower T_{min} was, the lower was the hatching success.

389

390   **4. Discussion**

391

392   Using temperature thermistors placed into artificial eggs, our results reveal that among

393   the considered OCs, only blood levels of oxychlordane, the main metabolite of the chlorinated

394   pesticides "chlordanes", were negatively related to T_{min} in male kittiwakes. PFAS and Hg levels

395   were unrelated to T_{inc} in kittiwakes. Moreover, our study suggests a possible underlying

396   mechanism between T_{inc} and contaminants since we reported a highly significant and negative

397   association between blood oxychlordane concentrations and the size of the brood patch in

398   males. Such effects on T_{inc} could induce deleterious consequences on egg hatchability.

399

4.1. Incubation temperature and contaminants

Contaminants such as OCs, PFASs and Hg are ubiquitous and toxic for wildlife. There is now clear evidence about their detrimental effects on the reproductive ecology of birds (e.g. Fry 1995; Herring et al. 2010; Tartu et al. 2014; Goutte et al. 2015). However, little is documented, especially for PFASs, about their potential influence on incubation behaviors and especially on T_{inc}. In the glaucous gull (*Larus hyperboreus*), another polar seabird, a study conducted in Svalbard (Bjørnøya island) showed that $\sum$PCBs, $\sum$DDTs and a number of quantitatively minor persistent organic pollutant (POP) classes (total-(α)-HBCD, $\sum$PBDE, $\sum$MeO-PBDE, mirex and 3-MeSO$_2$-*p,p'*-DDE) in plasma of incubating birds were negatively correlated with mean nest temperature (Verboven et al. 2009a). Additionally, exposure to $\sum$PCBs and oxychlordane was found to be associated with reduced nest attendance (i.e. longer and/ or more frequent absences from the nest site during incubation period) in the same species (Bustnes et al. 2001, 2005). Therefore, our results on Svalbard kittiwakes consistently with previous studies seem to highlight some potential associations between some OCs and their metabolites on T_{inc} in seabird species. However, our results do not report any relationships between PFASs, Hg and T_{inc}. This is supported by a recent investigation by Taylor et al. (2018) where no relationship was found between egg Hg contamination and T_{inc} of Forster terns. Thus, our research contributes to filling the gap in knowledge but additional studies are needed to confirm the generality of our findings in other bird species and importantly, targeting the specific chemicals involved in avian T_{inc} variations.

4.2. What are the possible mechanisms of this relationship?

Incubation is an energy-consuming phase of the avian reproductive cycle (Tinbergen and Williams 2002; Nord et al. 2010; Nord and Nilsson 2012; Nord and Williams 2015) and the efficiency with which heat is transferred from an incubating bird to its egg is related to the energy expenditure of the parent (Gabrielsen and Steen 1979; Gabrielsen and Unander 1987). In other words, a higher metabolic rate increases heat production thereby increasing heat transfer from the parent to embryo, and conversely. Interestingly, lowered thyroid hormone levels and reduced basal metabolic activity have already been observed in the most chlordane-contaminated individuals, including kittiwakes from the same population and glaucous gulls (Verreault et al. 2004, 2007; Blévin et al. 2017; Melnes et al. 2017). In this context, the quantity of heat transferred from parent to eggs might be reduced in the most contaminated birds thus explaining why we observed a negative relationship between oxychlordane concentrations and T_{inc} of male kittiwakes.

Another non-mutually exclusive hypothesis could rely directly on the manner in which heat is transferred. Indeed, because contact between the brood patch and egg ensures heat transfer from parents to embryo (Jones 1971), investigating relationships between contaminants and the size of the brood patch is relevant. In this context, a reduction in size of the brood patch in the most oxychlordane-contaminated male kittiwakes logically decrease the amount of heat transferred to their eggs. This reasoning is consistent with an experimental study on American kestrels (*Falco sparverius)* where smaller brood patches were observed in males exposed to PCBs compared to controls (Fisher et al. 2006b).

444   Since incubation behaviors (including brood patch formation) are triggered by an array

445   of different hormones (Buntin 1996; Lea and Klandorf 2002; Vleck 2002; Sockman et al. 2006;

446   Angelier and Chastel 2009; Vleck and Vleck 2011; Lynn 2016) and because of the potential

447   endocrine-disrupting properties of some OCs, reproductive hormones like prolactin could have

448   a key role in explaining why the most oxychlordane-contaminated male kittiwakes exhibited a

449   reduced brood patch and a lowered T_{inc}. However, we did not observe a relationship between

450   prolactin levels and brood patch size, nor to oxychlordane concentrations in male kittiwakes.

451   Several explanations could explain this discrepancy. Firstly, relationships between prolactin

452   and contaminants could be dose-dependent. A previous study on glaucous gulls revealed some

453   negative relationships, although only marginally significant, between blood concentrations of

454   several OCs and plasma prolactin secretions (Verreault et al. 2008). However, levels of

455   chlordanes in glaucous gulls (44.0 ± 7.0 ng/g ww; reported as the sum of heptachlor epoxide,

456   oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor) were around

457   28 times higher than those of our kittiwakes (1.569 ± 0.908 ng/g ww; reported as the sum of

458   oxychlordane, *trans*-nonachlor and *cis*-nonachlor). Secondly, the establishment and

459   maintenance of incubation behaviors (including brood patch formation) is orchestrated by a

460   complex cocktail of different reproductive hormones acting synergistically (Buntin 1996; Lea

461   and Klandorf 2002; Vleck 2002; Sockman et al. 2006; Vleck and Vleck 2011; Angelier et al.

462   2016; Lynn 2016) and further studies focusing on sex steroids (e.g. testosterone, estradiol,

463   progesterone) may provide greater clarity about which endocrine mechanisms are involved in

464   a reduced brood patch size and lowered T_{inc} in response to oxychlordane contamination. Finally,

465   the timing of blood sampling for prolactin assays could have been conducted too late in the

466   season for comparison to the timing of brood patch formation or the maximum of prolactin

467   secretion. Although brood patch formation is initiated only a few days before egg-laying (Lea

468   and Klandorf 2002), our sampling for prolactin assessment was performed several days after

469   egg-laying. Moreover, it has been suggested that prolactin levels in altricial pelagic seabird

470   species remains high in a relatively steady state throughout incubation and sometimes even

471   during the chick-rearing period, as a strategy to achieve parental care despite parents

472   undertaking prolonged foraging trips at sea (Vleck 1998, 2002; Lormée et al. 2000; Angelier et

473   al. 2016), thus partly excluding this scenario.

474

475   **4.3. Sex-related differences**

476

477   Considering each nest separately, our study indicates that male parents generally

478   incubate their eggs at a higher temperature (T_{mean}) compared to their female partners. This

479   observation could rely on a potential difference between sexes regarding energetic expenditure

480   and thus, heat production because males are heavier than females (~8% in the present study).

481   Furthermore, both basal and field metabolic rates have been shown to scale with body mass in

482   kittiwakes from the same colony (Elliott et al. 2013; Welcker et al. 2013; Blévin et al. 2017)

483   and Arctic glaucous gulls (Verreault et al. 2007). Finally, results from the model selection

484   presented in this study indicate a significant contribution of the body mass to several T_{inc}

485   parameters (T_{min}, T_{mean}, T_{max}) in male kittiwakes. Hence, males incubate at a higher temperature

486   than their female partners likely related to difference in body mass.

The relationship between oxychlordane and T_{inc} was sex-dependent and a significant relationship was found in male kittiwakes, but not in females. Interestingly, a previous study conducted on the glaucous gull showed that males were less able to maintain an optimal nest temperature than females during a costly reproductive event (i.e. induced by clutch enlargement) (Verboven et al. 2009a). This is similar to what was reported in American kestrels, where incubation behaviors of males experimentally exposed to PCBs were more disrupted than that of females of the same treatment (Fisher et al. 2006a). Furthermore, several studies conducted on kittiwakes, snow petrels (*Pagodroma nivea*) and glaucous gulls also reveal a higher susceptibility of males to the effects of contaminant exposure on incubation-related endocrine mechanisms (Verreault et al. 2004, 2006a, 2008; Tartu et al. 2015a, 2016). So, why there is a difference between sexes? Unlike females, males do not have a mechanism to reduce the body burden of contaminants compared to females who can excrete contaminants into their eggs. Indeed, several correlational and experimental studies have shown that females can lower a significant part of their contaminant body burden into their eggs (Becker 1992; Bargar et al. 2001; Drouillard and Nostrom 2001; Verreault et al. 2006b; Verboven et al. 2009b; Gebbink and Letcher 2012; Bustnes et al. 2017). Contaminant levels of incubating males are higher than those reported in females and thus posing a greater challenge for males to cope with costly reproductive tasks.

4.4. What consequences on hatching success?

508   T_{inc} is critically important for egg hatchability (Funk and Forward 1953; Decuypere and

509   Michels 1992) and several studies have reported a reduced hatching success of eggs incubated

510   at suboptimal temperatures (Webb 1987; Feast et al. 1998; Deeming and Ferguson 1991; French

511   2000; Moraes et al. 2004; Mortola 2006; Nord and Nilsson 2011, 2012; DuRant et al. 2013).

512   The reduced T_{inc} reported here in the most contaminated kittiwakes could impair hatchability

513   by decreasing hatching probability. However, we cannot completely rule-out another possible

514   non-mutually exclusive hypothesis which relies on a delay of hatching in response to low T_{inc}

515   events. Although kittiwakes displayed a high synchrony in the date of hatching (Mehlum 2006),

516   our fieldwork was completed within a few days after the peak lay date (around 5 days) so it is

517   conceivable that some eggs we considered to be non-viable in fact hatched soon after we

518   stopped monitoring nest contents. This is entirely consistent with previous investigations

519   showing an extended incubation period in eggs incubated below the optimal temperature range

520   (Webb 1987; Deeming and Ferguson 1991; Feast et al. 1998; Martin 2002; Mortola 2006;

521   Martin et al. 2007; Ardia et al. 2010; Nord and Nilsson 2011, 2012; DuRant et al. 2013). An

522   experimental study on wood ducks (*Aix sponsa*) revealed that low T_{inc} resulted in prolonged

523   incubation periods and lower hatching success (Hepp et al. 2006). Even though further

524   investigations are needed, we assume that a reduced T_{min} in the most oxychlordane-

525   contaminated kittiwakes could *in fine*, impair egg hatchability, either by lengthening incubation

526   period and/ or reducing hatching success.

527

528   **4.5. Limitations of the study and other potential confounding factors**

529

530   Our study was conducted on a limited sample size and the reported relationships,

531   although statistically significant, appear to be partly influenced by one individual with a fairly

532   strong relative statistical power (Cook's distance > 1; indicated with an arrow in Fig. 3, 4, 5

533   and further discussed in Supplementary Materials). However, after removing this bird from the

534   data set, we found similar results (see Supplementary Materials). In addition, there was no valid

535   reason to discard this bird from the data set. Hence, this male kittiwake was the most

536   oxychlordane-contaminated bird of our study. It had the smallest brood patch, exhibited the

537   lowest T_{inc}, failed at hatching, and was observed several times standing on the nest instead of

538   incubating its eggs. Finally, when applying the outlier test of Bonferroni (Hay-Jahans 2011;

539   Fox 2016), this individual was not considered as an outlier in our data set. Nevertheless, we

540   have to be cautious with our findings and further investigations using a larger sample size of

541   individuals will yield a wider range of contamination levels and thus, will certainly help to

542   confirm or refute the reported relationships.

543   Among the different T_{inc} parameters considered in this study, only T_{min} was related to

544   contaminant levels. One possible explanation is about the duration of recording periods (19.83

545   ± 9.38 (SD) hours, ranging from 4.64 to 43.07 hours). A longer duration for each record would

546   ultimately result in more extreme temperature variations including low T_{inc} events that have a

547   stronger impact on $T_{mean.}$ In this case, it would be possible, *in fine*, to find relationships between

548   contaminants and T_{mean}. Nevertheless, our study highlights the importance of focusing on

549   several T_{inc} parameters (such as extreme values) for detecting any subtle effects.

550   One aspect that is a potential confounding effect is that of body mass which is suggested

551   to positively affect several T_{inc} parameters in males. Body mass and oxychlordane

552 concentrations are negatively related in male kittiwakes. Previous research shows that birds

553 (including kittiwakes), with high OC burdens generally have poor body condition and are

554 lighter in mass than birds with low OC levels (Henriksen 1995; Henriksen et al. 1998, 2000;

555 Helberg et al. 2005; Bustnes et al. 2017). When body mass decreases, the lipophilic OCs such

556 as oxychlordane, previously stored in adipose tissues, are released into the blood circulation

557 and become very toxic to the whole organism (Henriksen 1995; Borgå et al. 2007; Nøst et al.

558 2012; Routti et al. 2013). It is thus difficult to disentangle a potential confounding effect of

559 body mass or a real impact of contaminants on T_{inc}.

560 Finally, being a metabolite itself, oxychlordane might not be the direct link in the

561 mechanistic processes, rather than the parent compounds ("chlordanes") which cannot be

562 measured with our sampling design, since they would be metabolized at time of sampling. Also,

563 the metabolization process itself might be playing a role explaining our observations. However,

564 a causal order of the mechanistic relationships cannot be established here.

565

566 **5. Conclusion**

567

568 Chlordane has been listed as a legacy POP by the Stockholm convention since 2004. Its

569 usage as a pesticide was extensive for more than 35 years but decreased in the 1980's (US

570 Department of Health and Human Services 1994). Oxychlordane (primary metabolite of

571 "chlordanes") is considered extremely toxic for wildlife (Wiemeyer 1996; Bondy et al. 2003;

572 Bustnes 2006; Erikstad et al. 2013). Indeed, recent studies reported potential adverse effects of

573 this chemical on thyroid hormones, energy expenditure, nest attendance, reproductive outputs,

574   immune function, morphological traits, telomere length and even survival rate in different

575   seabird species (Bustnes et al. 2002, 2003, 2004, 2005; Verreault et al. 2004, 2007, 2010;

576   Bustnes 2006; Blévin et al. 2016, 2017; Erikstad et al. 2013; Goutte et al. 2015). Our study in

577   combination with previous findings highlights the high toxicity of this compound on wildlife

578   despite its relatively small proportion compared to other OCs (< 5% of ΣOCs considered in this

579   study).

580

581   **Conflict of interest**

582

583   The authors declare no conflicts of interest.

584

585   **Acknowledgments**

586

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Table 1

OCs, PFASs (ng/g ww) and Hg (µg/g dw) mean concentrations ± standard deviation and limits of detection (LODs) for male and female incubating kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. Sex-related differences have been tested using linear mixed effects models with nest identity as a random factor. OCs have been measured in whole blood, PFASs in plasma and Hg in red blood cells.

		Males (n = 20)	Females (n = 20)		
	LODs	**Mean ± SD**	**Mean ± SD**	$F_{1,19}$	**P-value**
Organochlorines					
oxychlordane[*]	286×10^{-3}	1.431 ± 0.864	0.983 ± 0.318	5.552	**0.029**
trans-nonachlor[*]	18.4×10^{-3}	0.078 ± 0.048	0.079 ± 0.069	0.308	0.585
cis-nonachlor[*]	17.6×10^{-3}	0.059 ± 0.033	0.03 ± 0.050	0.032	0.861
mirex[*]	31.4×10^{-3}	0.790 ± 0.398	0.491 ± 0.219	12.836	**0.002**
HCB[a][*]	525×10^{-3}	3.230 ± 1.486	2.083 ± 0.610	9.629	**0.006**
p,p'-DDE[*]	47×10^{-3}	3.781 ± 1.858	2.122 ± 1.272	10.157	**0.006**
∑PCBs[b][*]	166×10^{-3}	25.179 ± 14.725	15.485 ± 6.345	7.451	**0.013**
PFASs					
PFOSlin[c][*]	270.5×10^{-3}	7.330 ± 3.338	2.102 ± 1.028	100.094	**<0.001**
PFNA[d][*]	20.5×10^{-3}	0.949 ± 0.450	0.511 ± 0.233	18.21	**<0.001**
PFDcA[e]	36.9×10^{-3}	1.207 ± 0.507	0.489 ± 0.228	42.608	**<0.001**
PFUnA[f][*]	88.5×10^{-3}	5.783 ± 1.933	2.911 ± 0.882	58.694	**<0.001**
PFTrA[g][*]	133.1×10^{-3}	7.367 ± 2.197	2.779 ± 1.200	101.031	**<0.001**
PFTeA[h]	24.8×10^{-3}	0.497 ± 0.399	0.370 ± 0.305	2.021	0.171
Trace element					
Hg[i][*]	5×10^{-3}	2.004 ± 0.591	1.426 ± 0.377	20.325	**<0.001**

Significant p-values are in bold. (*) indicates a log-10 transformation.
[a] HCB: Hexachlorobenzene
[b] *p,p'*-DDE: Dichlorodiphenyldichloroethylene (17 males)
[b] ∑PCBs (∑Polychlorinated biphenyls): CB-28, -99, -105, -118, -138, -153, -180, -183, -187
[c] PFOSlin: Perfluorooctane sulfonate
[d] PFNA: Perfluorononanoate
[e] PFDcA: Perfluorodecanoate
[f] PFUnA: Perfluoroundecanoate
[g] PFTrA: Perfluorotridecanoate
[h] PFTeA: Perfluorotetradecanoate
[i] Hg: Mercury

969 **Table 2**

970 Plasma baseline prolactin concentrations (ng/ mL)**,** brood patch size (cm²) and body mass (g)

971 for male and female incubating kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. Sex-

972 related differences have been tested using linear mixed effects models with nest identity as a

973 random factor.

	Males (n = 20)	Females (n = 20)		
	Mean ± SD	Mean ± SD	$F_{1,19}$	P-value
Prolactin	94.726 ± 21.915	93.181 ± 10.830	0.084	0.775
Brood patch	12.267 ± 2.565	12.646 ± 1.624	0.313	0.583
Body mass	407.25 ± 18.812	375.75 ± 25.146	34.735	**<0.001**

974 Significant p-values are in bold.

975 **Table 3**

976 AICc model selection to explain minimum incubation temperature (T_{min}) variations based on

977 OCs, PFASs, Hg concentrations and body mass in male and female kittiwakes *Rissa tridactyla*

978 from Kongsfjorden, Svalbard. Effects of contaminants and body mass on T_{min} have been tested

979 using linear models. OCs have been measured in whole blood, PFASs in plasma and Hg in red

980 blood cells.

LMs (T_{min}~)	AICc	ΔAICc[a]	Wi[b]
Males (n = 20)			
oxychlordane	107.08	0.00	0.83
body mass	112.85	5.77	0.05
HCB	113.00	5.92	0.04
mirex	113.42	6.35	0.03
cis-nonachlor	115.57	8.49	0.01
null	116.10	9.02	0.01
Females (n = 20)			
Hg	93.22	0.00	0.16
null	93.47	0.25	0.14
p,p'-DDE	94.47	1.25	0.09
trans-nonachlor	95.03	1.81	0.06
PFTrA	95.18	1.96	0.06
oxychlordane	95.48	2.26	0.05

981 Abbreviations: AICc, bias-adjusted Akaike's Information Criteria values; *Wi,* AICc weights.
982 Only the five best ranked and the null models are presented.
983 (a) Scaled ΔAICc; ΔAICc = 0 is interpreted as the best fit to the data among the models.
984 (b) Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

Table 4

986 AICc model selection to explain mean incubation temperature (T_{mean}) variations based on OCs,

987 PFASs, Hg concentrations and body mass in male and female kittiwakes *Rissa tridactyla* from

988 Kongsfjorden, Svalbard. Effects of contaminants and body mass on T_{mean} have been tested using

989 linear models. OCs have been measured in whole blood, PFASs in plasma and Hg in red blood

990 cells.

LMs (T_{mean}~)	AICc	ΔAICc[a]	Wi[b]
Males (n = 20)			
body mass	79.15	0.00	0.56
null	82.80	3.65	0.09
Hg	84.17	5.02	0.05
HCB	84.63	5.48	0.04
trans-nonachlor	84.92	5.77	0.03
oxychlordane	84.93	5.78	0.03
Females (n = 20)			
null	79.23	0.00	0.18
PFOSlin	80.62	1.39	0.09
Hg	81.22	1.99	0.07
PFNA	81.25	2.02	0.07
PFTrA	81.27	2.04	0.06
p,p'-DDE	81.28	2.05	0.06

991 Abbreviations: AICc, bias-adjusted Akaike's Information Criteria values; *Wi*, AICc weights.
992 Only the five best ranked and the null models are presented.
993 (a) Scaled ΔAICc; ΔAICc = 0 is interpreted as the best fit to the data among the models.
994 (b) Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

995 **Table 5**

996 AICc model selection to explain maximum incubation temperature (T_{max}) variations based on

997 OCs, PFASs, Hg concentrations and body mass in male and female incubating kittiwakes *Rissa*

998 *tridactyla* from Kongsfjorden, Svalbard. Effects of contaminants and body mass on T_{max} have

999 been tested using linear models. OCs have been measured in whole blood, PFASs in plasma

1000 and Hg in red blood cells.

LMs (T_{max}~)	AICc	ΔAICc[a]	*Wi*[b]
Males (n = 20)			
body mass	76.78	0.00	0.75
null	82.95	5.97	0.04
ΣPCBs	83.53	6.55	0.03
HCB	83.66	6.68	0.03
trans-nonachlor	83.79	6.81	0.03
mirex	83.85	6.87	0.02
Females (n = 20)			
null	83.69	0.00	0.17
cis-nonachlor	85.24	1.56	0.08
PFOSlin	85.34	1.65	0.08
mirex	85.38	1.69	0.07
PFTrA	85.48	1.79	0.07
PFDcA	86.11	2.42	0.05

1001 Abbreviations: AICc, bias-adjusted Akaike's Information Criteria values; *Wi*, AICc weights.
1002 Only the five best ranked and the null models are presented.
1003 (a) Scaled ΔAICc; ΔAICc = 0 is interpreted as the best fit to the data among the models.
1004 (b) Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

1005 **Table 6**

1006 AICc model selection to explain modal incubation temperature (T_{modal}) variations based on

1007 OCs, PFASs, Hg concentrations and body mass in male and female incubating kittiwakes *Rissa*

1008 *tridactyla* from Kongsfjorden, Svalbard. Effects of contaminants and body mass on T_{modal} have

1009 been tested using linear models. OCs have been measured in whole blood, PFASs in plasma

1010 and Hg in red blood cells.

LMs ($T_{modal}\sim$)	AICc	ΔAICc[a]	Wi[b]
Males (n = 20)			
mass	84.20	0.00	0.25
null	85.62	1.42	0.12
Hg	85.80	1.60	0.11
trans-nonachlor	86.74	2.54	0.07
oxychlordane	87.10	2.90	0.06
PFTeA	87.23	3.04	0.05
Females (n = 20)			
null	82.78	0.00	0.17
PFNA	84.07	1.29	0.09
PFOSlin	84.20	1.42	0.08
p,p'-DDE	84.47	1.69	0.07
oxychlordane	84.73	1.95	0.06
HCB	84.85	2.07	0.06

1011 Abbreviations: AICc, bias-adjusted Akaike's Information Criteria values; *Wi,* AICc weights.
1012 Only the five best ranked and the null models are presented.
1013 (a) Scaled ΔAICc; ΔAICc = 0 is interpreted as the best fit to the data among the models.
1014 (b) Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

Fig.1.

Photograph of the whole right brood patch of an incubating kittiwake (on the left) and

deployments of one artificial egg (indicated with an arrow) containing a temperature sensor in

a nest of incubating kittiwakes (on the right) *Rissa tridactyla* from Kongsfjorden, Svalbard.

1020   **Fig.2.**

1021   Mean incubation temperature (T_{mean}) of both partners of adult kittiwakes *Rissa tridactyla* from

1022   Kongsfjorden, Svalbard in each investigated nest. Solid red lines indicated pairs with males

1023   incubating at a higher temperature than females. Dashed black lines indicated pairs with females

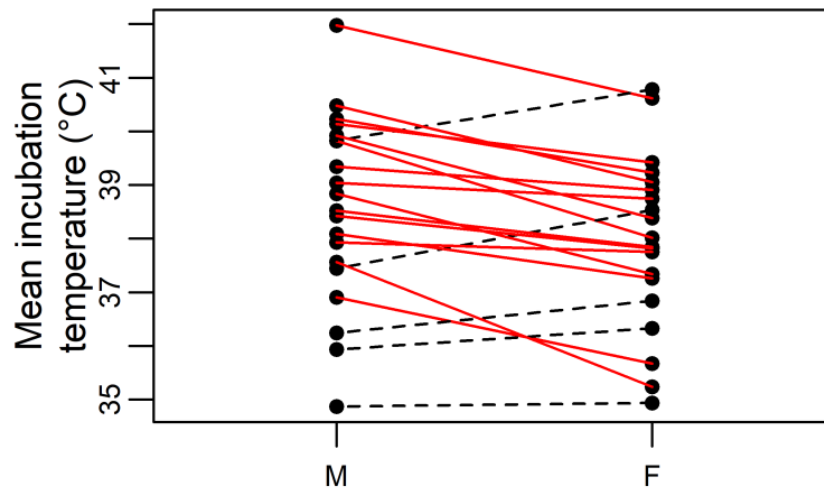1024   incubating at a higher than males.

Fig.3.

1026 Relationships between oxychlordane concentrations and the minimum incubation temperature

1027 in male and female adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard.

1028 Oxychlordane concentrations have been measured in whole blood. The arrow indicates one

1029 individual with a fairly strong relative statistical power (see section 4.5 for more details).
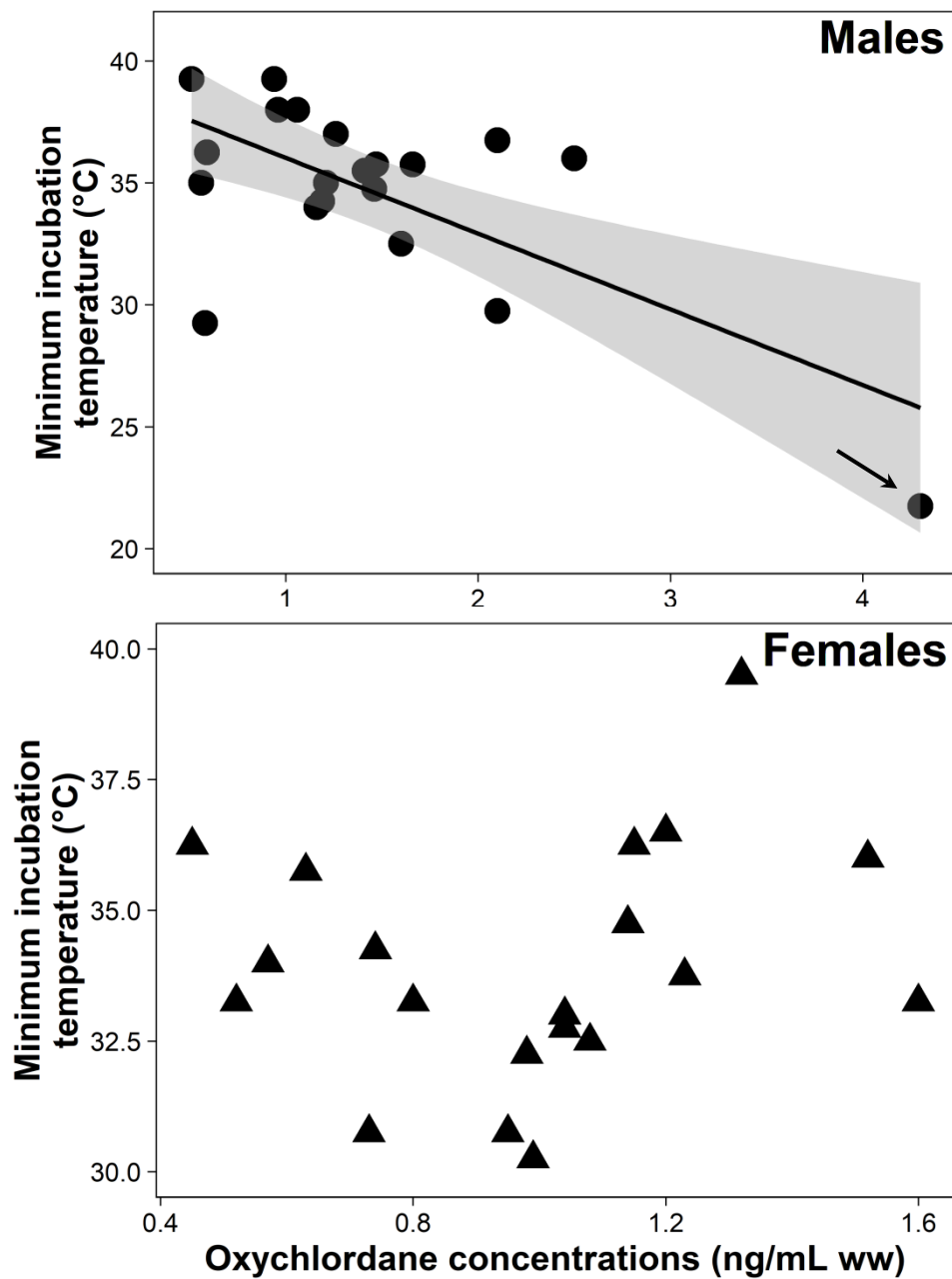
1030

Fig.4.

Relationships between oxychlordane concentrations, baseline prolactin levels and brood patch

size in male incubating adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard.

Oxychlordane concentrations have been measured in whole blood and baseline prolactin in

plasma. Brood patch size here reflects the size of the right brood patch. The arrow indicates one

individual with a fairly strong relative statistical power (see section 4.5 for more details).
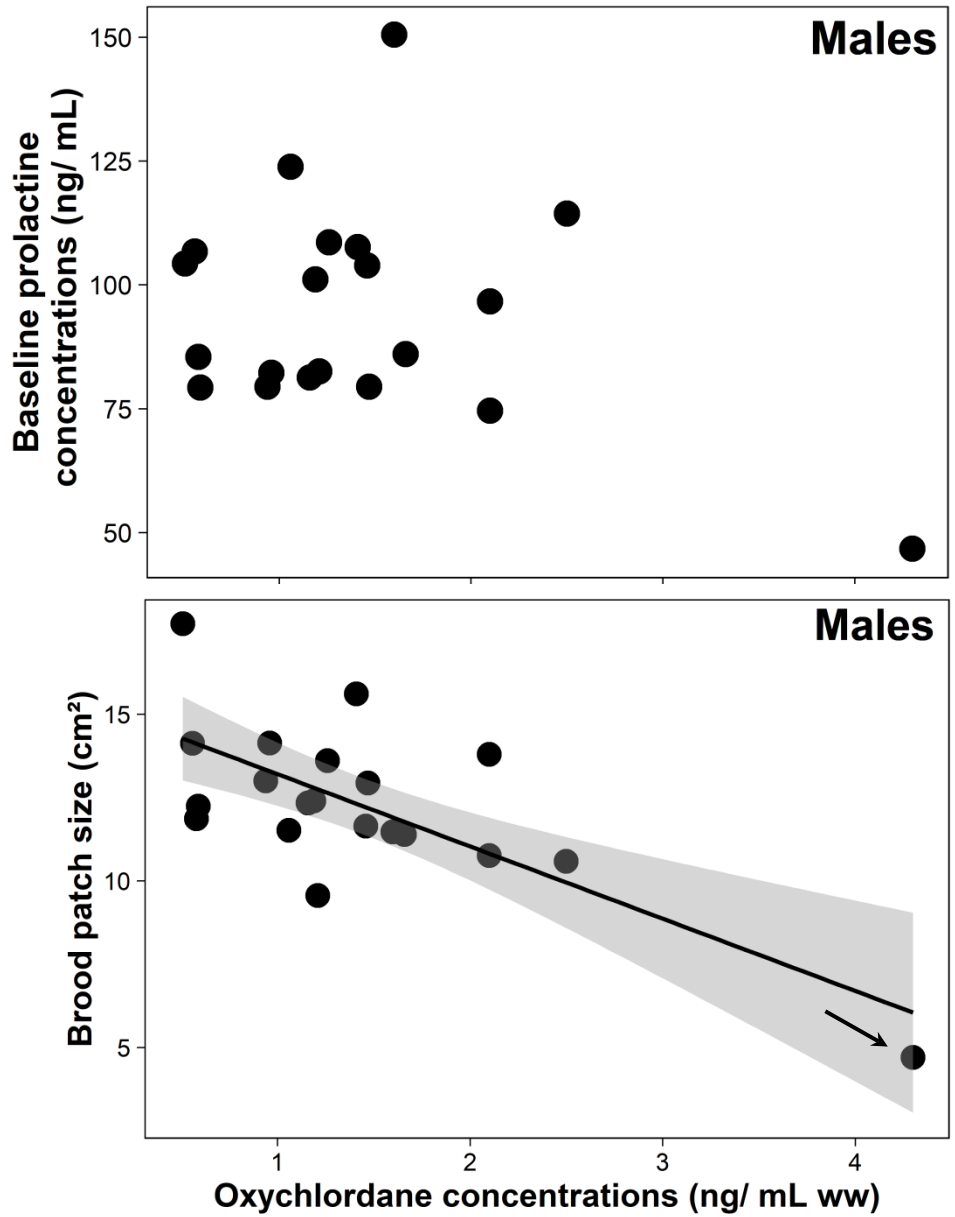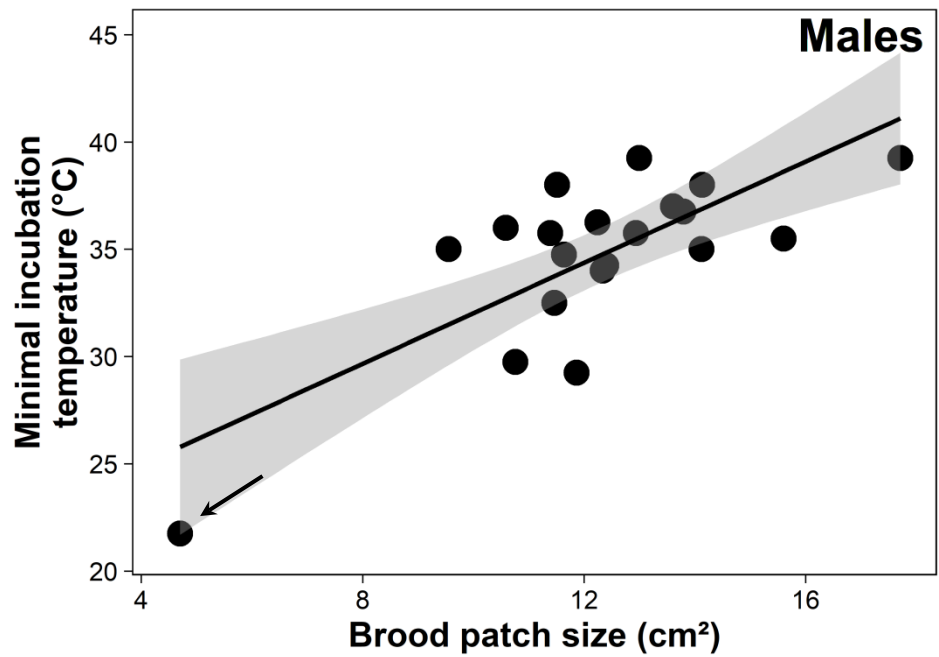
Fig.5.

Relationships between brood patch size and minimum incubation temperature in male

incubating adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. Brood patch size

here reflects the size of the right brood patch. The arrow indicates one individual with a fairly

strong relative statistical power (see section 4.5 for more details).

1043 **Fig.6.**

1044 Hatching probability (0 = not hatched; 1 = hatched) of the remaining egg in the experimental

1045 nests in relation to the minimal incubation temperature (T_{min}). T_{min} has been calculated by

1046 meaning the minimal incubation temperature of both partners in each nest.