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Organochlorines, Perfluoroalkyl Substances, Mercury, and Egg Incubation Temperature in an Arctic Seabird: Insights from Data Loggers

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Abstract: In birds, incubation-related behaviors and brood patch formation are influenced by hormonal regulation such as prolactin secretion. Brood patch provides efficient heat transfer between the incubating parent and the developing embryo in the egg. Importantly, several environmental contaminants are already known to have adverse effects on avian reproduction. However, relatively little is known about the effect of contaminants on incubation temperature (T_{inc}) in wild birds. By using temperature thermistors placed into artificial eggs, we investigated whether the most contaminated parent birds are less able to provide appropriate egg warming and thus less committed to incubating their clutch. Specifically, we investigated the relationships among 3 groups of contaminants (organochlorines, perfluoroalkyl substances [PFASs], and mercury [Hg]) with T_{inc} and also with prolactin concentrations and brood patch size in incubating Arctic black-legged kittiwakes (*Rissa tridactyla*). Our results reveal that among the organochlorines considered, only blood levels of oxychlordane, the main metabolite of chlordane, a banned pesticide, were negatively related to the minimum incubation temperature in male kittiwakes. Levels of PFASs and Hg were unrelated to T_{inc} in kittiwakes. Moreover, our study suggests a possible underlying mechanism: since we reported a significant and negative association between blood oxychlordane concentrations and the size of the brood patch in males. Finally, this reduced T_{inc} in the most oxychlordane-contaminated kittiwakes was associated with a lower egg hatching probability. *Environ Toxicol Chem* 2018;37:2881–2894. © 2018 SETAC

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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 of which are considered 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 is critically important for complete embryonic development, improved hatchability, offspring 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 facilitates and maintains incubation-related behaviors (Buntin 1996; Vleck 2002; Sockman et al. 2006; Angelier et al. 2016). Concomitantly,

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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).

The conditions required for optimal incubation behaviors have been largely investigated in the poultry industry, to maximize egg hatchability of domestic fowl (*Gallus gallus domestica;* New 1957; Meijerhof 1992; Tona et al. 2005b; Elibol and Brake 2006a, 2006b). In contrast, the effects of environmental factors such as contaminants (i.e., organic contaminants and trace elements) on incubation behaviors of free-ranging birds *in natura* remain so far poorly investigated. Yet, several environmental contaminants are already known to have adverse effects on avian reproduction (e.g., Fry 1995; Herring et al. 2010; Tartu et al. 2014; Goutte et al. 2015). Through their structural attributes and mode of action potencies, many of these contaminants can disrupt the endocrine system involved in avian reproduction, including prolactin, sex steroid (e.g., testosterone, estradiol, progesterone), and thyroid (e.g., triiodothyronine and thyroxine) secretions (Rattner et al. 1984; Tyler et al. 1998; Dawson 2000; Giesy et al. 2003; Verreault et al. 2004, 2006a, 2007, 2008; Tartu et al. 2015a; Melnes et al. 2017). Organic contaminants and trace elements have the potential to alter parental behaviors, resulting in poor breeding success. For example, different laboratory and field investigations have shown that exposure to organochlorines or mercury (Hg) can be associated with lowered nest or egg temperatures (Peakall and Peakall 1973; Fox et al. 1978; Verboven et al. 2009a), reduced nest attendance (i.e., longer and more frequent absence from the nest site; Fox et al. 1978; Bustnes et al. 2001, 2005; Fisher et al. 2006a; Tartu et al. 2015a), prolonged incubation period (McArthur et al. 1983; Kubiak et al. 1989; Fisher et al. 2006a), and decreased nest defense or increased egg predation (Fox et al. 1978; Fox and Donald 1980; Helberg et al. 2005; Goutte et al. 2018). Such detrimental effects of contaminants on incubation behaviors could induce deleterious effects on hatching success. A previous study conducted on ring doves (*Streptopelia risoria*) reported a lower hatchability of eggs incubated by birds experimentally exposed to high doses of polychlorinated biphenyls (PCBs; Peakall and Peakall 1973). Similarly, Forster terns (*Sterna forsteri)* had a higher hatching success when eggs laid from organochlorine-contaminated birds were incubated by less contaminated surrogate parents (Kubiak et al. 1989).

Polar regions are considered a sink for various environmental contaminants due to atmospheric long-range transport and oceanic currents in combination with a cold climate (Burkow and Kallenborn 2000). Given their properties (high volatility and/or persistence), organic contaminants and trace elements such as Hg can reach isolated areas like the Arctic Ocean. Once deposited in the marine ecosystem, contaminants bioaccumulate in living organisms and can biomagnify along the food webs (Borgå et al. 2001; Wania 2003, 2007; Ariya et al. 2004; Tomy et al. 2004; Haukås et al. 2007; Blévin et al. 2013). Long-lived species like many polar seabirds that occupy high trophic levels are exposed to a greater risk of accumulation and sensitivity to high concentrations of contaminants (Letcher et al. 2010; Elliott and Elliott 2013). Consequently, seabirds are considered highly relevant biological models to investigate the influence of sublethal contaminant exposure on reproductive behaviors like incubation temperature (T_{inc}).

In the Norwegian Arctic, black-legged kittiwakes (*Rissa tridactyla*, hereafter termed kittiwakes), are chronically exposed to a complex mixture of harmful organic compounds and trace elements, which have already been linked to disruption of reproductive hormones and impaired reproductive performance (Tartu et al. 2013, 2014, 2015b, 2016; Goutte et al. 2015; Blévin et al. 2017). Among such complex mixture of chemicals are 1) Hg, a toxic trace element originating from both anthropogenic and natural sources able to disrupt hormones involved in incubation behaviors such as prolactin (Arctic Monitoring and Assessment Programme 2007, 2011; Tartu et al. 2016); 2) legacy organochlorines (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; Arctic Monitoring and Assessment Programme 2015; Bustnes et al. 2017); and 3) 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; Arctic Monitoring and Assessment Programme 2015). Despite the few studies that have investigated the effects of organochlorines and Hg on reproductive behaviors, data are still critically lacking, and, importantly, to our knowledge the consequences of PFAS exposure for incubation behaviors in birds are presently unknown.

Using artificial egg loggers, we investigated whether the most contaminated kittiwakes were 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 3 groups of contaminants (organochlorines, 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 are thus tightly linked to T_{inc}, we also investigated relationships among contaminants, plasma prolactin concentrations, and brood patch size as potential underlying mechanisms through which contaminant exposure in kittiwakes may influence T_{inc}. Finally, because T_{inc} is considered key for egg hatchability, we explored potential effects of T_{inc} on hatching probability.

MATERIALS AND METHODS

Fieldwork area and sampling collection

Fieldwork was carried out from 19 June to 12 July 2015, in a colony of black-legged kittiwakes at Kongsfjorden, Svalbard (78°54′N, 12°13′E). We studied 20 incubating pairs because kittiwakes, like other seabirds, share reproduction duties (i.e., incubation and chick rearing) among sexes. A total of 40 individuals (20 males and 20 females) were captured at their nest with a noose fixed at the top of a 6-m fishing rod. We collected

the first blood sample (~0.5 mL) immediately after capture from the alar vein using a heparinized syringe and a 25-gauge needle to assess baseline prolactin concentrations. A second blood sample (~2 mL) was collected to measure the concentrations of contaminants and to determine the sex of individuals using molecular methods. All birds were weighed to the nearest 5 g with a Pesola spring balance to determine the body mass. Finally, a photograph was collected of the whole right brood patch (Figure 1; Canon EOS 1000D, 100 mm; Canon 2018), with a ruler placed next to the bird to calculate its brood patch dimensions using GIMP Ver 2.8 (GIMP Development Team 2018). Brood patch size was determined in duplicates (all coefficients of variation $\leq$ 4.06%). Breast feathers were lightly brushed with a moistened cotton pad to fully expose the brood patch. All study birds exhibited 3 brood patches (right [RBP], left [LBP], and central [CBP]). Thus, to minimize handling time, we measured the RBP of all birds and the LBP and CBP in only 13 individuals, to determine whether the RBP measurement could be used to estimate the size of the other brood patches (LBP, CBP). Before release, each bird was marked with colored spots of a nonpermanent dye on the forehead to distinguish each bird from its mate (also dyed with a different color) during subsequent observations from a distance. Blood samples were stored on ice in the field. Aliquots of whole blood, plasma, and red blood cells were obtained after centrifugation and then kept frozen at –20 °C until subsequent laboratory analysis.

Egg logger experiment and data processing

All study nests initially contained 2 natural eggs. However, one of these 2 eggs was collected and replaced by an artificial egg containing a temperature thermistor (as described in Shaffer et al. 2014). Artificial eggs were designed and painted to mimic as much as possible the real egg morphology (similar size and shape, approximate mass; Supplemental Data, Table S1) and coloration pattern of kittiwakes using a nontoxic water-based paint (Figure 1). Data loggers recorded core egg temperature every second with a manufacturer-reported accuracy of <2 °C (but testing in the laboratory in a controlled environment showed the accuracy to be ~0.5 °C) and precision of 0.125 °C based on thermistor component specifications (Shaffer et al. 2014). Subsequent tests were also conducted to verify these parameters using a standard poultry incubator with an automatic egg turner (Top Hatch Incubator; Brower Equipment). Study nests were selected according to their accessibility and to minimize disturbance to the rest of the colony. Collected eggs were candled, and all were determined to be fertile. Eggs were further dissected to assess the age of the embryo and for use in studies of other contaminants ($n = 12$). To control for potential changes in incubation behavior that may have occurred across the incubation period, we used the embryo age as a proxy of incubation stage. However, we do not report any suggested effects of age of embryo on T_{inc} parameters (linear mixed effect models [LMMs]; T_{min}: $F_{1,10} = 0.14$, $p = 0.72$; T_{max}: $F_{1,10} = 1.13$, $p = 0.31$; T_{mean}: $F_{1,10} = 0.61$, $p = 0.45$; T_{modal}: $F_{1,10} = 1.03$, $p = 0.33$).

Artificial eggs were deployed for 7 and 10 d during the incubation period; all birds readily accepted the artificial egg and exhibited no abnormal incubation behaviors. All loggers recorded data for the entire duration of deployment in the nest. Because each partner of a pair was dye-marked on the forehead, we could determine some of the incubation bouts for each partner at a nest using a spotting scope. Thus, we recorded and kept for further statistical analyses all incubation bouts when we knew which bird was incubating (excluding data recorded at night because checks were not conducted at night). The day of egg deployments and all records during our presence in the colony (i.e., for blood sampling) were also excluded from the data set to avoid any biased data. Recording duration (19.83 ± 9.38 h standard deviation [(SD]; range = 4.64–43.07 h) did not influence T_{inc} parameters (LMMs, all $p \geq 0.169$). After completion of each deployment, artificial eggs were removed, and only 1 egg was left in the nest. Using a mirror at the end of a long pole, we then regularly checked the experimental nest contents to monitor hatching success of the remaining egg until the end of the field season (i.e., 12 July; laying peak = 6–9 July).

Logger temperature data were processed using purpose-built routines in MATLAB (The Mathworks) following the methods of Shaffer et al. (2014). Overall, we processed T_{inc} profiles of 40 individuals (Supplemental Data, Figure S1) and determined extreme temperature values (minimum temperature: T_{min}; maximum temperature: T_{max}), mean temperature (T_{mean}), and the most frequent incubation temperature within the record period (T_{modal}).

Contaminant analyses

Organochlorines were analyzed from whole blood at the Norwegian Institute for Air Research in Tromsø, Norway.

FIGURE 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, Norway.

We scanned for the following compounds: the organochlorine pesticides (*o,p′*-dichlorodiphenyltrichloroethane [DDT], *p,p′*-DDT, *p,p′*-dichlorodiphenyldichloroethylene [DDE], *o,p′*-DDE, *o,p′*-dichlororodiphenyldichlorethane [DDD], *p,p′*-DDD, hexachlorobenzene [HCB], α-, β-, γ-hexachlorocyclohexane, *trans*-, *cis*-chlordane, oxychlordane, *trans*-, *cis*-nonachlor, and mirex) and the PCBs (-28, -52, -99, -101, -105, -118, -138, -153, -180, -183, -187, and -194). Concentrations below the limit of detection (LOD) were assigned by one-half the LOD value, but only compounds detected in at least 70% of the data set were kept for further statistical analyses. Consequently, the compounds remaining for further investigation were the organochlorine pesticides (oxychlordane, *trans*-, *cis*-nonachlor, mirex, HCB, and *p,p′*-DDE) and the PCBs (-28, -99, -105, -118, -138, -153, -180, and -187; termed ΣPCBs in the present study). It is worth noting that the *p,p′*-DDE concentrations of 3 males are missing because of injection issues during the gas chromatography–mass spectrometry procedure. To a whole blood sample of 0.70 to 1.13 mL, 100 μL of an internal standard solution was added (^{13}C-labeled compounds from Cambridge Isotope Laboratories). We first proceeded to sample denaturation using a mix of ethanol and a saturated solution of ammonium sulfate in water. We then ran extraction twice with 6 mL of *n*-hexane. Matrix removal on Florisil columns, separation on an Agilent Technology 7890 gas chromatograph, and detection on an Agilent Technology 5975 gas chromatography–mass selective detector were performed following Herzke et al. (2009). Recovery of the internal standards ranged between 52 and 60%. Results were validated with blanks (clean and empty glass tubes treated like a sample) and standard reference material (SRM; 1958 human serum from the US National Institute of Standards and Technology [NIST]) run every 10 samples. The deviation of the target concentrations in the SRMs were within the laboratory's accepted range (75–111%). All blanks contained concentrations below the instrument detection limits except for HCB (525 pg/g), PCB-28 (81.8 pg/g), and PCB-105 (60.8 pg/g).

The PFASs were analyzed from plasma at the Norwegian Institute for Air Research. The following compounds were scanned for presence and concentration: perfluorooctanesulfonamide, perfluorobutanesulfonate, perfluorohexanesulfonate, linear perfluorooctanesulfonate, branched perfluorooctanesulfonate, perfluorodecanesulfonate, perfluorohexanoate, perfluoroheptanoate, perfluorooctanoate, perfluorononanoate, perfluorodecanoate, perfluoroundecanoate, perfluorododecanoate, perfluorotridecanoate, and perfluorotetradecanoate (PFTeA). Concentrations below the LOD were assigned by one-half of the LOD value, but only compounds detected in at least 70% of the data set were kept for further statistical analyses. In short, a sample (0.2 mL) spiked with internal standards (carbon-labeled PFAS; Hanssen et al. 2013) was extracted in methanol (1 mL) by repeated sonication and vortexing. The supernatant was cleaned up using ENVI-Carb (Sigma-Aldrich) graphitized carbon absorbent and glacial acetic acid. Extracts were analyzed by ultra-performance liquid chromatography–MS/MS. Recovery of the internal standards ranged between 74 and 128%. Results were validated with blanks (clean and empty glass tubes treated like a sample) and SRM (1957 human serum from NIST) run every 10 samples. The deviation of the target concentrations in the SRMs were within the laboratory's accepted range (69–130%). All blanks contained concentrations below the instrument detection limits, except for PFCAs, which ranged between 5 and 30 pg/mL.

Total Hg was analyzed at the Littoral Environment et Sociétés laboratory (La Rochelle, France) from freeze-dried and powdered red blood cells placed in an Advanced Hg Analyzer Spectrophotometer (AMA 254; Altec) as described in Bustamante et al. (2006). Aliquots ranging from 0.44 to 8.59 mg were analyzed for each individual, in duplicates (all coefficients of variation $\leq$ 5.42%). Blanks were run at the beginning of each set of samples and certified reference material (CRM; Tort-2 Lobster Hepatopancreas, National Research Council, Canada; certified value 0.27 ± 0.06 [SD] μg/g dry wt) were used to validate the accuracy of the analyses. Measured values of the CRM were 0.25 ± 0.01 (SD) μg/g dry weight, $n = 11$. All blanks contained concentrations below the instrument detection limit (0.005 μg/g dry wt).

Molecular sexing and prolactin assays

Molecular sexing and prolactin assays were conducted at the Centre d'Etudes Biologiques de Chizé (France). Kittiwakes were sexed from red blood cells by polymerase chain reaction amplification as part of 2 highly conserved genes (CHD) present on sexual chromosomes as described in Fridolfsson and Ellegren (1999). Plasma prolactin concentrations were determined by radioimmunoassay as previously described and validated for this kittiwake population (Chastel et al. 2005). Intra-assay variation was estimated by including internal standards to the assay. Both samples and internal standards were run in duplicates. The coefficient of variation was 7.13%. Blood collection time (i.e., time elapsed from capture to the end of the first blood sampling: 2.48 ± 0.52 min [SD], on average) did not affect baseline prolactin concentrations (LMM, $F_{1,19} = 0.606$, $p = 0.446$).

Statistical analyses

All statistical analyses were performed using R Ver 3.2.3. The LMMs with the nest identity as a random factor were used to test whether contaminant concentrations, baseline prolactin levels, brood patch size, body mass, or T_{inc} differed between sexes. As suggested in Zuur et al. (2009), we used the restricted maximum likelihood estimation method to avoid any potential biased statistic estimations. Second, we tested the influence of each contaminant concentration on incubation temperatures (T_{min}, T_{max}, T_{mean}, and T_{modal}) using linear models for each sex separately, because males were determined to be more contaminated than females (see the *Results* section). Moreover, it is now well established that males and females can react in very different ways to environmental stressors such as organochlorines, PFASs, and Hg contamination. Specifically, previous studies conducted on kittiwakes from the same colony reported sex differences regarding effects of contaminants on hormone levels, body condition, breeding decisions, metabolic activity, telomere length, and even survival rate (Tartu et al. 2013, 2014, 2016; Goutte et al. 2015; Blévin et al. 2016, 2017). Influence of

body mass was also tested because egg temperature likely becomes warmer as the mass of the incubating bird increases. The best models were selected based on the bias-adjusted second-order Akaike's information criterion (AICc), which is a small sample size adjustment (Burnham and Anderson 2003). As a general guideline, if AICc values differ by >2, the lowest AICc is the most accurate, whereas models with AICc values differing by <2 have a similar level of support in their ability to describe the data. In addition, the Akaike weight (*Wi*) was estimated and can be interpreted as the approximate probability that the model *i* is the best one for the observed data, given the candidate set of models (Burnham and Anderson 2003; Johnson and Omland 2004). Because the concentration of *p*,*p*′-DDE was missing for 3 males (see the *Materials and Methods* section) and because model selection based on AICc requires the same number of observations among models, we performed a second run of model selection with these 3 individuals removed from the data set and we found no change in the results. Third, we investigated the relationships between contaminant concentrations, baseline prolactin, brood patch size, and body mass with linear models. Finally, we tested whether T_{inc} can affect hatching probability using a generalized linear model (GLM) constructed with a binomial family and a cloglog link function, which is consistent with the use of an asymmetric data set (hatched: $n = 15$; not hatched: $n = 5$; Zuur et al. 2009). Diagnostic plots and Shapiro normality tests were finally performed on residuals to test whether the data sufficiently met the assumption of the models (i.e., LMM, linear model, and GLM), and data were log-10 transformed when necessary (Zuur et al. 2009). All data are presented as mean ± SD, and we used a significance level of $\alpha < 0.05$.

RESULTS

Sex-related differences

Organochlorines, PFASs, and Hg mean concentrations and LODs in female and male incubating adult kittiwakes are listed in Table 1. The LMMs with nest identity as a random factor to test sex-related differences indicated that all organochlorines except *trans*- and *cis*-nonachlor, all PFASs except PFTeA, and Hg concentrations significantly differed between sexes, with males having higher contamination levels than females. The LMMs indicated that males incubated the egg at a higher T_{mean} compared with their female partners (LMM, $F_{1,19} = 9.518$, $p = 0.006$; Figure 2). Mean plasma prolactin concentrations, brood patch size, and body mass of female and male incubating adult kittiwakes are given in Table 2. The LMMs with nest identity as a random factor to test sex-related differences indicated no significant differences between sexes for baseline prolactin concentrations, or brood patch size (Table 2). However, as expected, males were significantly heavier than their female partners (Table 2).

Incubation temperatures and contaminants

According to the model selection, the model including oxychlordane was the best fit model in males (ΔAICc = 5.77; Table 3). Specifically, we observed a negative and highly significant relationship between oxychlordane concentrations in blood and T_{min} in males (linear model, slope = -3×10^{-3}; $p = 0.001$; $R^2 = 0.45$; Figure 3), indicating a lower T_{min} with increasing oxychlordane concentrations. To a lesser extent, both models with HCB or mirex as explanatory variables were also better than the null model (ΔAICc from null model > 2; Table 3). Specifically, we observed a significant negative relationship between blood HCB and mirex concentrations and T_{min} in males (linear model, slope = -1×10^{-3}; $p = 0.023$; $R^2 = 0.26$ for HCB; linear model, slope = -5×10^{-3}; $p = 0.029$; $R^2 = 0.24$ for mirex). Concentrations of PFASs and Hg were not related to T_{min} in males (Table 3). Finally, model selection also indicated a significant effect of body mass on T_{min} (ΔAICc from null model > 2; Table 3), with heavier males having a higher T_{min} (linear model, slope = 0.109; $p = 0.021$; $R^2 = 0.26$). It is worth noting that oxychlordane concentrations and body mass were significantly and negatively correlated in males ($r_{pearson} = -0.62$; $p = 0.004$; $n = 20$). Running an additive model including oxychlordane and body mass simultaneously did not improve predictions of T_{min} compared with the model with oxychlordane only ($AICc_{(oxychlordane)}$: 107.08/$AICc_{(oxychlordane + body\ mass)}$: 109.67). We found no significant relationships between contaminants and body mass on T_{min} in females (Table 3 and Figure 3).

The AICc model selection that explained T_{mean} variations based on contaminant concentrations and body mass is presented in Table 4. We found no significant relationships between contaminant concentrations and T_{mean}, in either males, or females. However, the model including body mass was considered the best predictor in males among the set of candidate models (ΔAICc = 3.65; Table 4), whereas for females no relationship was found. Indeed, there was a significant positive relationship between body mass and T_{mean} in males (linear model, slope = 0.049; $p = 0.018$; $R^2 = 0.28$).

The AICc model selection that explained T_{max} variations based on contaminant concentrations and body mass is presented in Table 5. There was no significant relationship between contaminant concentrations and T_{max}, in either males or females. However, the model including body mass was considered the best predictor in males (ΔAICc = 5.97; Table 5), whereas for females, there was no relationship. There was a significant positive relationship between body mass and T_{max} in males (linear model, slope = 0.056; $p = 0.006$; $R^2 = 0.36$).

The AICc model selection that explained T_{modal} variations based on contaminant concentrations and body mass is presented in Table 6. There was no significant effect of contaminant concentrations and body mass on T_{modal}, in either males or females.

Baseline prolactin, brood patch, and contaminants

We observed a relationship between oxychlordane concentrations and T_{min} in males but not in females. Consequently, we examined relationships between oxychlordane concentrations and baseline prolactin levels, and the size of the brood patch to evaluate potential underlying mechanisms. Baseline prolactin

TABLE 1: Organochlorines, perfluoroalkyl substances (PFASs; ng/g wet wt), and Hg (μg/g dry wt) mean concentrations ± standard deviation (SD) and limits of detection (LODs) for male and female incubating kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway[a]

		Males ($n = 20$)	Females ($n = 20$)		
	LOD	Mean ± SD	Mean ± SD	$F_{1,19}$	p value
Organochlorines					
Oxychlordane[b]	286 10^{-3}	1.431 ± 0.864	0.983 ± 0.318	5.552	0.029*
trans-Nonachlor[b]	18.4 10^{-3}	0.078 ± 0.048	0.079 ± 0.069	0.308	0.585
cis-Nonachlor[b]	17.6 10^{-3}	0.059 ± 0.033	0.03 ± 0.050	0.032	0.861
Mirex[b]	31.4 10^{-3}	0.790 ± 0.398	0.491 ± 0.219	12.836	0.002*
HCB[b]	525 10^{-3}	3.230 ± 1.486	2.083 ± 0.610	9.629	0.006*
p,p′-DDE[b]	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[b]	270.5 10^{-3}	7.330 ± 3.338	2.102 ± 1.028	100.094	<0.001*
PFNA[b]	20.5 10^{-3}	0.949 ± 0.450	0.511 ± 0.233	18.21	<0.001*
PFDcA	36.9 10^{-3}	1.207 ± 0.507	0.489 ± 0.228	42.608	<0.001*
PFUnA[*b]	88.5 10^{-3}	5.783 ± 1.933	2.911 ± 0.882	58.694	<0.001*
PFTrA[b]	133.1 10^{-3}	7.367 ± 2.197	2.779 ± 1.200	101.031	<0.001*
PFTeA	24.8 10^{-3}	0.497 ± 0.399	0.370 ± 0.305	2.021	0.171
Trace element					
Hg[b]	5 10^{-3}	2.004 ± 0.591	1.426 ± 0.377	20.325	<0.001*

[a]Sex-related differences were tested using linear mixed effects models with nest identity as a random factor. The organochlorines were measured in whole blood, PFASs in plasma, and Hg in red blood cells.
[b]Indicates a log10 transformation.
*Significant p value.
HCB = hexachlorobenzene; *p,p′*-DDE = dichlorodiphenyldichloroethylene (17 males); ∑PCBs = ∑polychlorinated biphenyls (-28, -99, -105, -118, -138, -153, -180, -183, -187); PFOSlin = perfluorooctane sulfonate; PFNA = perfluorononanoate; PFDcA = perfluorodecanoate; PFUnA = perfluoroundecanoate; PFTrA = perfluorotridecanoate; PFTeA = perfluorotetradecanoate; Hg = mercury.

levels in males were not significantly related to oxychlordane concentrations (log10-transformed; linear model, slope = –16.21; $p = 0.47$; Figure 4), to brood patch size (linear model, slope = 0.039; $p = 0.15$), or to body mass (linear model, slope = 0.475; $p = 0.07$). Baseline prolactin levels in females were not significantly related to oxychlordane concentrations (linear model, slope = -6.10^{-3}; $p = 0.50$), to brood patch size (linear model, slope = 0.042; $p = 0.23$), or to body mass (linear model, slope = 0.044; $p = 0.67$). However, we found a highly significant negative relationship between oxychlordane concentrations and size of the brood patch in males but not in females (log10-transformed; linear model, slope = -5×10^{-5}; $p = 0.16$). Thus, the most oxychlordane contaminated males had the smallest brood patch (linear model, slope = -2×10^{-3}; $p = 2 \times 10^{-4}$; $R^2 = 0.53$; Figure 4). Body mass and size of the brood patch were also positively related in males (linear model, slope = 0.067; $p = 0.029$; $R^2 = 0.24$) but not in females (log10-transformed; linear model, slope = 4×10^{-4}; $p = 0.404$). Importantly, the size of the brood patch was positively and significantly related to T_{min} in males (linear model, slope = 1.178; $p = 1 \times 10^{-4}$; $R^2 = 0.56$; Figure 5).

The size of the LBP and CBP were marginally correlated to the size of the RBP (LBP, $r_{spearman} = 0.45$; $p = 0.13$; $n = 13$ and CBP, $r_{spearman} = 0.51$; $p = 0.078$; $n = 13$). We assume that our results for the RBP could also be relevant for the LBP and CBP.

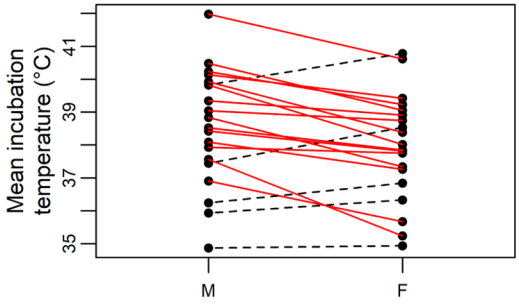

FIGURE 2: Mean incubation temperature (T_{mean}) of both partners of adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway in each investigated nest. Solid red lines indicate pairs with males incubating at a higher temperature than females. Dashed black lines indicate pairs with females incubating at a higher temperature than males.

Consequences for hatching success

Because there was a relationship between oxychlordane concentrations and T_{min} in males, we evaluated the

TABLE 2: Plasma baseline prolactin concentrations (ng/mL), brood patch size (cm^2), and body mass (g) for male and female incubating kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway[a]

	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

[a]Sex-related differences were tested using linear mixed effects models with nest identity as a random factor.
SD = standard deviation.

TABLE 3: The bias-adjusted Akaike's information criteria values (AICc) model selection to explain minimum incubation temperature (T_{min}) variations based on organochlorines, perfluoroalkyl substances (PFASs), Hg concentrations, and body mass in male and female kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway[a]

LMs (T_{min}~)	AICc	ΔAICc[b]	*Wi*[c]
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

[a]Effects of contaminants and body mass on T_{min} were tested using linear models. Organochlorines were measured in whole blood, PFASs in plasma, and Hg in red blood cells. Only the 5 best ranked and the null models are presented.
[b]Scaled ΔAICc; ΔAICc = 0 is interpreted as the best fit to the data among the models.
[c]Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00. LM = linear model; HCB = hexachlorobenzene; *p,p*′-DDE = dichlorodiphenyldichloroethylene; PFTrA = perfluorotridecanoate; *Wi* = AICc weights.

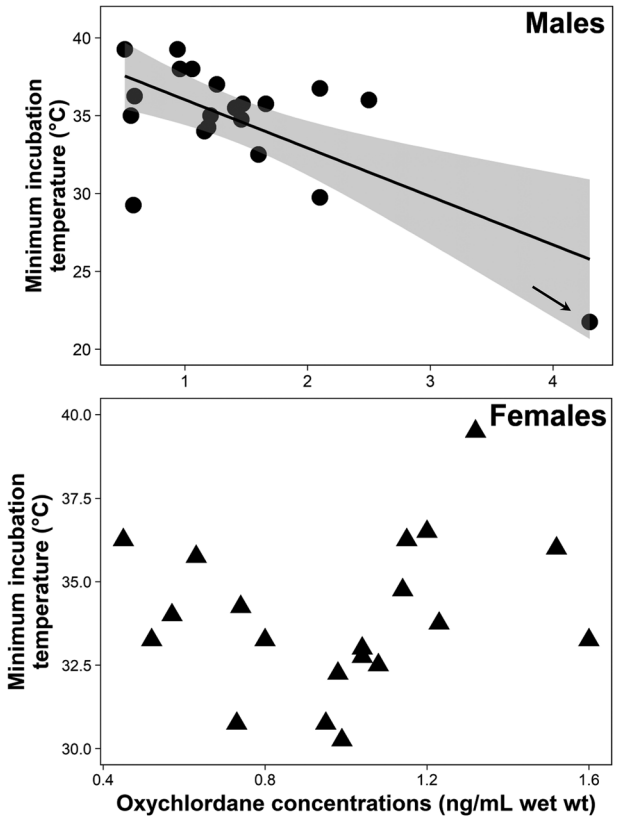

FIGURE 3: Relationships between oxychlordane concentrations and the minimum incubation temperature in male and female adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway. Oxychlordane concentrations were measured in whole blood. The arrow indicates one individual with a fairly strong relative statistical power (see *Limitations of the study and other potential confounding factors* section for more details).

TABLE 4: The bias-adjusted Akaike's information criteria values (AICc) model selection to explain mean incubation temperature (T_{mean}) variations based on organochlorines, perfluoroalkyl substances (PFASs), Hg concentrations and body mass in male and female kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway[a]

LMs (T_{mean}~)	AICc	ΔAICc[b]	*Wi*[c]
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

[a]Effects of contaminants and body mass on T_{mean} were tested using linear models. Organochlorines were measured in whole blood, PFASs in plasma, and Hg in red blood cells. Only the 5 best ranked and the null models are presented.
[b]Scaled ΔAICc; ΔAICc = 0 is interpreted as the best fit to the data among the models.
[c]Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00. LM = linear model; HCB = hexachlorobenzene; PFOSlin = perfluorooctane sulfonate; PFNA = perfluorononanoate; PFTrA = perfluorotridecanoate; *p,p*′-DDE = dichlorodiphenyldichloroethylene; *Wi* = AICc weights.

consequences of T_{min} variations on hatching success. There was a positive and marginally significant relationship between T_{min} and the probability that the remaining egg in the experimental nests successfully hatched (GLM, $Z = 1.932$; $p = 0.053$; Figure 6). As a result, the lower the T_{min} was, the lower the hatching success.

TABLE 5: The bias-adjusted Akaike's information criteria values (AICc) model selection to explain maximum incubation temperature (T_{max}) variations based on organochlorines, perfluoroalkyl substances (PFASs), Hg concentrations, and body mass in male and female incubating kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway[a]

LMs (T_{max}~)	AICc	ΔAICc[b]	*Wi*[c]
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

[a]Effects of contaminants and body mass on T_{max} were tested using linear models. Organochlorines were measured in whole blood, PFASs in plasma, and Hg in red blood cells. Only the 5 best ranked and the null models are presented.
[b]Scaled ΔAICc; ΔAICc = 0 is interpreted as the best fit to the data among the models.
[c]Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00. LM = linear model; ∑PCBs = ∑polychlorinated biphenyls; HCB = hexachlorobenzene; PFOSlin = perfluorooctane sulfonate; PFTrA = perfluorotridecanoate; PFDcA = perfluorodecanoate; *Wi* = AICc weights.

TABLE 6: The bias-adjusted Akaike's information criteria values (AICc) model selection to explain modal incubation temperature (T_{modal}) variations based on organochlorines, perfluoroalkyl substances (PFASs), Hg concentrations, and body mass in male and female incubating kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway[a]

LMs (T_{modal}~)	AICc	ΔAICc[b]	*Wi*[c]
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

[a]Effects of contaminants and body mass on T_{modal} were tested using linear models. Organochlorines were measured in whole blood, PFASs in plasma, and Hg in red blood cells. Only the 5 best ranked and the null models are presented.
[b]Scaled ΔAICc; ΔAICc = 0 is interpreted as the best fit to the data among the models.
[c]Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.
LM = linear model; PFTeA = perfluorotetradecanoate; PFNA = perfluorononanoate; PFOSlin = perfluorooctane sulfonate; *p,p*′-DDE = dichlorodiphenyldichloroethylene; HCB = hexachlorobenzene.

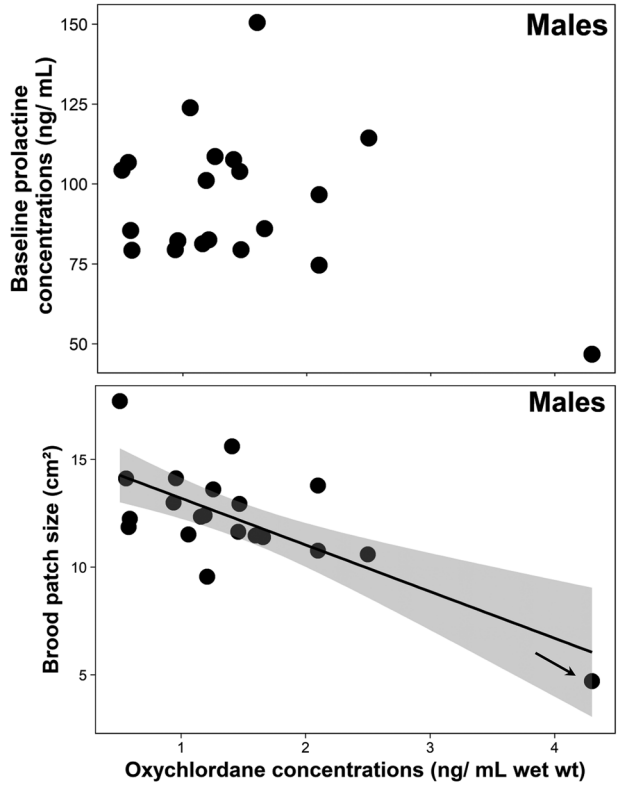

FIGURE 4: Relationships between oxychlordane concentrations, baseline prolactin levels, and brood patch size in male incubating adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway. Oxychlordane concentrations were measured in whole blood and baseline prolactin in plasma. Brood patch size here reflects the size of the right brood patch. The arrow indicates one individual with a fairly strong relative statistical power (see *Limitations of the study and other potential confounding factors* section for more details).

DISCUSSION

Using temperature thermistors placed into artificial eggs, our results reveal that among the organochlorines considered, only blood levels of oxychlordane, the main metabolite of the chlorinated pesticides termed chlordanes, were negatively related to T_{min} in male kittiwakes. Levels of PFASs and Hg were unrelated to T_{inc} in kittiwakes. Moreover, our study suggests a possible underlying mechanism between T_{inc} and contaminants because we report a highly significant and negative association between blood oxychlordane concentrations and the size of the brood patch in males. Such effects on T_{inc} could induce deleterious consequences for egg hatchability.

Incubation temperature and contaminants

Contaminants such as organochlorines, PFASs, and Hg are ubiquitous and toxic to wildlife. There is now clear evidence of 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 the 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 ∑PCBs, ∑DDTs, and a number of quantitatively minor persistent organic pollutant (POP) classes (total-(α)-hexabromocyclododecane, ∑polybrominated diphenyl ether [PBDE], ∑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). In addition,

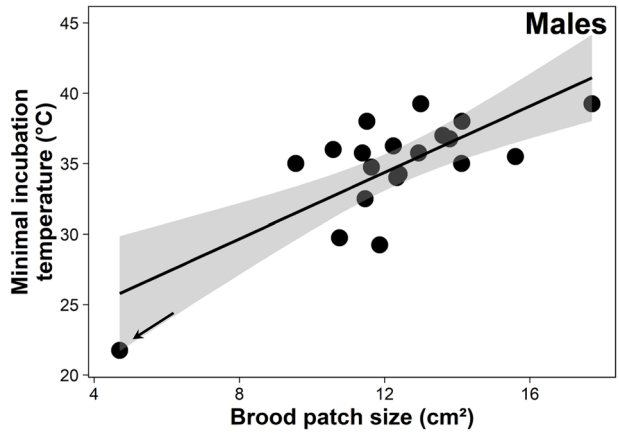

FIGURE 5: Relationships between brood patch size and minimum incubation temperature in male incubating adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard, Norway. Brood patch size here reflects the size of the right brood patch. The arrow indicates one individual with a fairly strong relative statistical power (see *Limitations of the study and other potential confounding factors* section for more details).

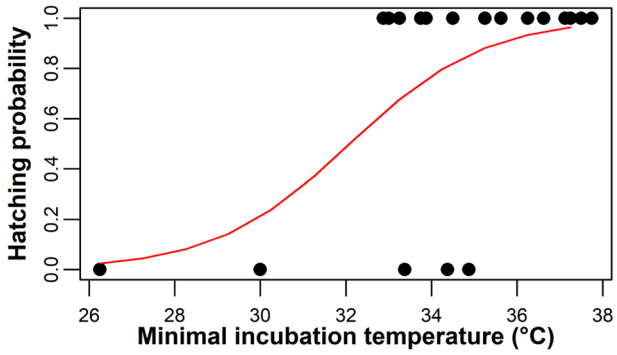

FIGURE 6: Hatching probability (0 = not hatched; 1 = hatched) of the remaining eggs in the experimental nests in relation to the minimal incubation temperature (T_{min}). The T_{min} was calculated by meaning the minimal incubation temperature of both partners in each nest.

exposure to ∑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 with Svalbard kittiwakes, along with the findings of previous studies, seem to highlight some potential associations between some organochlorines 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), who found no relationship 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.

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 the 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 nonmutually 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), it is relevant to investigate relationships between contaminants and the size of the brood patch. In this context, a reduction in size of the brood patch in the most oxychlordane-contaminated male kittiwakes logically decreases the amount of heat transferred to their eggs. This reasoning is consistent with an experimental study on American kestrels (*Falco sparverius)* in which smaller brood patches were observed in males exposed to PCBs compared with controls (Fisher et al. 2006b).

Because incubation behaviors (including brood patch formation) are triggered by an array of different hormones (Buntin 1996; Lea and Klandorf 2002; Vleck 2002; Sockman et al. 2006; Angelier and Chastel 2009; Vleck and Vleck 2011; Lynn 2016) and because of the potential endocrine-disrupting properties of some organochlorines, reproductive hormones like prolactin could be key in explaining why the most oxychlordane-contaminated male kittiwakes exhibited a reduced brood patch and a lowered T_{inc}. However, we did not observe a relationship between prolactin levels and brood patch size, or to oxychlordane concentrations in male kittiwakes. Several explanations could account for this discrepancy. First, relationships between prolactin and contaminants could be dose dependent. A previous study on glaucous gulls revealed some negative relationships, although only marginally significant, between blood concentrations of several organochlorines and plasma prolactin secretions (Verreault et al. 2008). However, levels of chlordanes in glaucous gulls (44.0 ± 7.0 ng/g wet wt; reported as the sum of heptachlor epoxide, oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, and *cis*-nonachlor) were approximately 28 times higher than those of our kittiwakes (1.569 ± 0.908 ng/g wet wt; reported as the sum of oxychlordane, *trans*-nonachlor, and *cis*-nonachlor). Second, the establishment and maintenance of incubation behaviors (including brood patch formation) is orchestrated by a complex cocktail of different reproductive hormones acting synergistically (Buntin 1996; Lea and Klandorf 2002; Vleck 2002; Sockman et al. 2006; Vleck and Vleck 2011; Angelier et al. 2016; Lynn 2016), and further studies focusing on sex steroids (e.g., testosterone, estradiol, progesterone) may provide greater clarity about which endocrine mechanisms are involved in a reduced brood patch size and lowered T_{inc} in response to oxychlordane contamination. Finally, the timing of blood sampling for prolactin assays could have been conducted too late in the season for comparison with the timing of brood patch formation or the maximum amount of prolactin secretion. Although brood patch formation is initiated only a few days before egg-laying (Lea and Klandorf 2002), our sampling for prolactin assessment was performed several days after egg-laying. Moreover, it has been suggested that prolactin levels in altricial pelagic seabird species remain high in a relatively steady state throughout incubation and sometimes even during the chick-rearing period, as a strategy to achieve parental care even though parents need to undertake prolonged foraging trips at sea (Vleck 1998, 2002; Lormée et al. 2000; Angelier et al. 2016), thus partly excluding this scenario.

Sex-related differences

When each nest is considered separately, our study indicates that male parents generally incubate their eggs at a higher temperature (T_{mean}) compared with their female partners, possibly because of a potential difference between sexes regarding energetic expenditure and thus heat production: males are heavier than females (by ~8% in the present study). Furthermore, both basal and field metabolic rates have been shown to scale with body mass in kittiwakes from the same colony (Elliott et al. 2013; Welcker et al. 2013; Blévin et al. 2017) and in Arctic glaucous gulls (Verreault et al. 2007). Finally, results from the model selection given in the present study indicate a significant contribution of body mass to several T_{inc} parameters (T_{min}, T_{mean}, T_{max}) in male kittiwakes. Hence the fact that males incubate at a higher temperature than their female partners is likely related to difference in body mass.

The relationship between oxychlordane and T_{inc} was sex dependent; 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: incubation behaviors of males experimentally exposed to PCBs were more disrupted than those of females under 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; females are able to excrete contaminants into their eggs. Indeed, several correlational and experimental studies have shown that females can significantly lower their contaminant body burden by excretion 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, thus posing a greater challenge for males in coping with costly reproductive tasks.

What are the consequences for hatching success?

The T_{inc} is critically important for egg hatchability (Funk and Forward 1953; Decuypere and Michels 1992), and several studies have reported reduced hatching success of eggs incubated at suboptimal temperatures (Webb 1987; Feast et al. 1998; Deeming and Ferguson 1991; French 2000; Moraes et al. 2004; Mortola 2006; Nord and Nilsson 2011, 2012; DuRant et al. 2013). The reduced T_{inc} reported in the present study in the most contaminated kittiwakes could impair hatchability by decreasing hatching probability. However, we cannot completely rule out another possible nonmutually exclusive hypothesis, which relies on a delay in hatching in response to low T_{inc} events. Although kittiwakes displayed a high synchrony in the date of hatching (Mehlum 2006), our fieldwork was completed within a few days after the peak of hatching (~5 d), so it is conceivable that some eggs we considered to be nonviable in fact hatched soon after we stopped monitoring nest contents. This is entirely consistent with previous investigations showing an extended incubation period in eggs incubated below the optimal temperature range (Webb 1987; Deeming and Ferguson 1991; Feast et al. 1998; Martin 2002; Mortola 2006; Martin et al. 2007; Ardia et al. 2010; Nord and Nilsson 2011, 2012; DuRant et al. 2013). An experimental study on wood ducks (*Aix sponsa*) revealed that low T_{inc} resulted in prolonged incubation periods and lower hatching success (Hepp et al. 2006). To sum up, even though further investigations are needed, we assume that a reduced T_{min} in the most oxychlordane-contaminated kittiwakes could impair egg hatchability, either by lengthening the incubation period or reducing hatching success, or both mechanisms.

Limitations of the study and other potential confounding factors

Our study was conducted on a limited sample size, and the reported relationships, although statistically significant, appear to be partly influenced by one individual with a fairly strong relative statistical power (Cook's distance > 1; indicated with an arrow in Figures 3–5 and further discussed in the Supplemental Data). However, after removing this bird from the data set, we found similar results (see Supplemental Data). In addition, there was no valid reason to discard this bird from the data set. Hence, this male kittiwake was the most oxychlordane-contaminated bird of our study. It had the smallest brood patch, exhibited the lowest T_{inc}, failed at hatching, and was observed several times standing on the nest instead of incubating its eggs. Finally, when applying Bonferroni's outlier test (Hay-Jahans 2011; Fox 2016), this individual was not considered an outlier in our data set. Nevertheless, we want to be cautious with our findings; further investigations using a larger sample size will yield a wider range of contamination levels and will thus help to confirm or refute the reported relationships.

Among the different T_{inc} parameters we considered, only T_{min} was related to contaminant levels. One possible explanation involves the duration of recording periods (19.83 ± 9.38 [SD] h; range = 4.64–43.07 h). A longer duration for each record would ultimately result in more extreme temperature variations including the low T_{inc} events that have a stronger impact on T_{mean}. In this case, it would be possible to find relationships between contaminants and T_{mean}. Nevertheless, our study highlights the importance of focusing on several T_{inc} parameters (such as extreme values) for detecting any subtle effects.

One potential confounding effect is body mass, which may positively affect several T_{inc} parameters in males. Body mass and oxychlordane concentrations are negatively related in male kittiwakes. Previous research shows that birds (including kittiwakes), with high organochlorine burdens generally have poor body condition and are lighter in mass than birds with low organochlorine levels (Henriksen 1995; Henriksen et al. 1998,

2000; Helberg et al. 2005; Bustnes et al. 2017). When body mass decreases, the lipophilic organochlorines such as oxychlordane, previously stored in adipose tissues, are released into the blood circulation and become very toxic to the whole organism (Henriksen 1995; Borgå et al. 2007; Nøst et al. 2012; Routti et al. 2013). It is thus difficult to disentangle a potential confounding effect of body mass or a real impact of contaminants on T_{inc}.

Finally, being a metabolite itself, oxychlordane might not be the direct link in the mechanistic processes; such a link might come from the parent compounds (the so-called chlordanes), which cannot be measured with our sampling design, because they would be metabolized at time of sampling. Also, the metabolization process itself might play a role that would explain our observations. However, we cannot establish a causal order of the mechanistic relationships.

CONCLUSIONS

Chlordane has been listed as a legacy POP by the Stockholm Convention in 2004. It was used extensively as a pesticide for >35 yr, but this use decreased in the 1980s (US Department of Health and Human Services 1994). Oxychlordane (primary metabolite of the chlordanes) is considered extremely toxic for wildlife (Wiemeyer 1996; Bondy et al. 2003; Bustnes 2006; Erikstad et al. 2013). Indeed, recent studies have reported potential adverse effects of this chemical on thyroid hormones, energy expenditure, nest attendance, reproductive outputs, immune function, morphological traits, telomere length, and even survival rate in different seabird species (Bustnes et al. 2002, 2003, 2004, 2005; Verreault et al. 2004, 2007, 2010; Bustnes 2006; Blévin et al. 2016, 2017; Erikstad et al. 2013; Goutte et al. 2015). Our study, in combination with previous findings, highlights the high toxicity of this compound to wildlife even though it was found in a relatively small proportion compared with other organochlorines (<5% of the total organochlorines considered in the present study).

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4250.

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Data Accessibility—Research data pertaining to this article are located at figshare.com.

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