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Contaminants, prolactin and parental care in an Arctic seabird: contrasted associations of perfluoroalkyl substances and organochlorine compounds with egg-turning behavior

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Abstract : Incubating eggs represents a trade-off for parent birds between spending enough time fasting to take care of the clutch and to get enough nutrients for self-maintenance. It is believed that the pituitary hormone prolactin plays an important role in such allocation processes. Incubation does not solely imply the active warming of the eggs but also the active egg-turning to facilitate absorption of albumen by the embryo, reduce malposition and prevent the embryo from adhering to the inner shell membrane. However, how prolactin secretion is related to egg-turning behaviors is presently poorly addressed. In addition, several environmental contaminants can affect parental care behaviors through their endocrine disrupting properties but the effects of such contaminants on egg-turning behaviors remain so far unexplored. Using artificial eggs equipped with miniaturized data loggers, we investigated the relationships between egg-turning behaviors, prolactin secretion and contaminants burden in Arctic black-legged kittiwakes (*Rissa tridactyla*). Specifically, we examined the relationships between blood concentrations of poly- and perfluoroalkyl substances (PFASs), organochlorines (OCs), mercury (Hg), plasma prolactin levels and both egg-turning frequency and angular change. We also incorporated baseline corticosterone levels since this glucocorticoid is known to affect parental care. Plasma prolactin levels were positively related to angular change in female kittiwakes while corticosterone was not related to egg-turning behaviors in either sex. Hg was not related to egg-turning behaviors in either sex. We found contrasting associations between OCs and PFASs, since polychlorinated biphenyls (PCBs) were negatively associated with angular change in females, contrary to linear perfluorooctanesulfonate (PFOSlin) and perfluoroalkyl carboxylic acids (PFCAs) which were positively related to egg-turning frequency and angular change in both sexes. Additionally, PFASs concentrations were positively related to prolactin levels in female kittiwake. The possible stimulation of prolactin secretion by PFASs could therefore make adult kittiwakes to allocate more time taking care of their eggs, and thus possibly modify the trade-off between spending enough time caring for the clutch and obtaining enough nutrients at sea.

Keywords: Perfluoroalkyl substances; Organochlorine compounds; Mercury; Prolactin; Corticosterone; Egg-loggers; Incubation behaviors; Black-legged kittiwake

1. Introduction

Incubation, as the chick-rearing period, is a costly stage during the avian reproductive cycle (Deeming, 2002; Monaghan and Nager, 1997). This is especially the case for pelagic seabirds which alternate long periods of fasting when incubating the eggs, with distant foraging trips at sea to replenish their body reserves. Thus, incubating parents face a trade-off between spending enough time caring for the clutch and obtaining enough nutrients for self-maintenance during foraging trips. Too much time off the eggs might cause them not to hatch or may impair offspring quality on the long-term (Berntsen and Bech, 2016; Lindström, 1999), whereas too little time feeding could cause malnutrition. Furthermore, incubation does not solely require a bird to sit on eggs and fast, but also involves different parental behaviors like active egg-turning and egg-warming which are both considered as key determinants for egg hatchability (Decuyper and Michels, 1992; Elibol and Brake, 2004, 2006a; Funk and Forward, 1953; Poulsen, 1953; Tona et al., 2005a; Tullett and Deeming, 1987; Van Schalkwyk et al., 2000). Indeed, egg-turning behavior requires an optimal turning rate with appropriate angular changes to i) facilitate absorption of albumen by the embryo, ii) reduce malposition (e.g. head in the small end of the egg), and iii) prevent the embryo from adhering to the inner shell membrane (Deeming and Ferguson, 1991, Deeming, 2000; Elibol and Brake, 2004, 2006b; Eycleshymer, 1907; New, 1957; Tullett and Deeming, 1987). Accordingly, a lack or decrease of egg-turning can retard or prevent albumen absorption and gas exchanges resulting in an abnormal chick development with lower growth rate, a decrease in oxygen consumption, and overall, leading to delayed incubation or reduced hatching success (Deeming and Ferguson, 1991, Deeming, 2000, Deeming, 2002; Elibol and Brake, 2006a; Funk and Forward, 1953; New, 1957; Pearson et al., 1996; Robertson, 1961; Tazawa, 1980; Tona et al., 2005b; Van Schalkwyk et al., 2000; Wilson et al., 2003; Yoshizaki and Saito, 2002). In birds, initiation and maintenance of parental

care behaviors are orchestrated by a cocktail of different hormones acting synergistically and it is believed that the pituitary hormone prolactin plays an important role, together with glucocorticoids, in the allocation process between incubation effort and the need to collect enough nutrients for self-maintenance (Angelier et al., 2016; Angelier and Chastel, 2009; Buntin, 1996; Sockman et al., 2006; Thierry et al., 2013; Vleck, 2002; Vleck and Vleck, 2011). However, the influence of prolactin secretion on the expression of egg-turning behavior is presently poorly understood.

The required conditions for an optimal incubation and hatchability have been extensively studied in the poultry industry for commercial consumption (King' Ori, 2011; Lundy, 1969; Tona et al., 2005a). By comparison, our understanding of environmental factors that influence incubation behaviors in free-ranging birds is lacking. Previous investigations have shown that some environmental contaminants can negatively modulate parental care behaviors through their disrupting endocrine functions. With regard to the incubation period, field and experimental studies showed that exposure to organochlorines (OCs) and mercury (Hg) can be related to reduced nest attendance (Bustnes et al., 2001, 2005; Fisher et al., 2006; Fox et al., 1978; Tartu et al., 2015a), lowered incubation temperatures (Blévin et al., 2018; Fox et al., 1978; Peakall and Peakall, 1973; Verboven et al., 2009), increased egg predation and/or decreased nest defense (Fox et al., 1978; Fox and Donald, 1980; Helberg et al., 2005), and delayed hatching (Fisher et al., 2006; Kubiak et al., 1989; McArthur et al., 1983). However, there is a gap of knowledge for egg-turning behaviors and to date, only one study conducted on Forster terns (*Sterna forsteri*) has investigated the potential effects of exposure to Hg on egg-turning frequency (Taylor et al., 2018), but found no evidence of Hg-related impairment. Furthermore, to the best of our knowledge, effects of organic contaminants (pesticides, industrial compounds) on egg-turning behaviors remain so far unexplored.

Using the same egg-loggers as in Blévin et al. (2018) and described in detail in Shaffer et al. (2014), we investigated the relationships between egg-turning behaviors, plasma prolactin secretion and blood contaminants concentration in a seabird, the black-legged kittiwake, *Rissa tridactyla* (hereafter “kittiwakes”). In addition to recording incubation temperature, these egg-loggers consist of a triaxial accelerometer and a magnetometer, encapsulated in an artificial egg, recording continuous egg-turning behaviors in a three-dimensional orientation. Our study was conducted in Svalbard, where kittiwakes are exposed to various environmental contaminants already known as being detrimental for incubation behaviors (Blévin et al., 2018 [same dataset]; Tartu et al., 2015b). Among them, are the poly- and perfluoroalkyl substances (PFASs), the organochlorines (OCs) and mercury (Hg). Because corticosterone is known to disrupt prolactin secretion and to interact with incubation behavior expression (Angelier and Chastel, 2009; Angelier et al., 2009, Thierry et al., 2013), we also included baseline levels of this glucocorticoid hormone in the analysis. Using the same dataset, the present work comes to complete our recent publication where we investigated the consequences of contaminant exposure on egg incubation temperature (Blévin et al., 2018).

2. Material and methods

2.1. Study site and sampling

Fieldwork was conducted from 19th June to 12th July 2015 in a colony of black-legged kittiwakes at Kongsfjord, Svalbard (Fig. 1; 78°54'N; 12°13'E). Our study was performed on 20 nests where we caught both males and females (i.e. n = 40; 20 males and 20 females), using a noose attached to the end of a long telescopic pole. Upon capture, a blood sample (~0.5 mL) was collected within 3 min to assess baseline prolactin and corticosterone levels for each bird.

Additional blood (~2 mL) was sampled and stored separately to measure contaminant concentrations. Blood sampling was performed during the incubation period and within the time frame of egg-loggers deployment (detailed in Section 2.3 and Table S1 in supplementary materials). All the birds were then weighed to the nearest 5 g using a Pesola spring balance to know their body mass. Body morphometrics were also determined by measuring the skull length (head + bill) using a sliding caliper with an accuracy of 0.1 mm. These two morphometric measurements were used later to calculate the Scaled Mass Index (SMI) for each kittiwake, as a proxy of body condition, following methods developed and detailed in Peig and Green (2009, 2010). Each bird was also assessed for brood patch size as presented in Blévin et al. (2018). Blood samples were stored in ice while in the field. Upon returning to the lab, aliquots of whole blood and both plasma and red blood cells obtained after centrifugation were kept frozen at -20°C until lab analyses.

2.2. Hormone assay, molecular sexing and contaminant analysis

Prolactin and corticosterone assays as well as molecular sexing were conducted at the Centre d'Etudes Biologiques de Chizé (CEBC), France. Hormones were analyzed using radio-immuno-assay as detailed in Blévin et al. (2018) for prolactin, and Tartu et al. (2015b) for corticosterone. Prolactin levels were already presented in Blévin et al. (2018). Bleeding time was short (i.e. time elapsed from capture to the end of the first blood sampling: 2.48 ± 0.52 min (SD), on average) and was not related to baseline prolactin levels (linear mixed effect model, LMM, $F_{1,19} = 0.61$, $p = 0.45$) and baseline corticosterone levels (LMM, $F_{1,19} = 0.01$, $p = 0.94$). Blood sampling time (Table S1 in supplementary materials) was not related to baseline prolactin levels (linear mixed effect model, LMM, $F_{1,19} = 0.23$, $p = 0.64$) and baseline corticosterone levels (LMM, $F_{1,19} = 0.28$, $p = 0.60$). Corticosterone measurements were done

within one single assay (intra assay coefficient of variation: 6.4%, n = 5 duplicates). Kittiwakes were sexed from red blood cells as described in Fridolfsson and Ellegren (1999).

The detailed methodology of contaminant analyses and quality insurance have been extensively described in Blévin et al. (2018). Contaminant concentrations are already presented in Blévin et al. (2018) and Haar et al. (2018) (only for females). PFASs were analysed from plasma at the Norwegian Institute for Air Research (NILU) in Tromsø, Norway, following Hanssen et al. (2013). Concentrations below the limit of detection (LoD) were assigned by $\frac{1}{2}$ LoD value but only compounds detected in at least 70% of the data set were kept for further statistical analyses. Consequently, PFASs remaining for further investigations were the perfluoroalkyl carboxylic acids (PFCAs): perfluorononanoate (PFNA), perfluorodecanoate (PFDCa), perfluoroundecanoate (PFUnA), perfluorotridecanoate (PFTrA), perfluorotetradecanoate (PFTeA), and only one perfluoroalkyl sulfonic acid: linear perfluorooctanesulfonate (PFOSlin). OCs were analyzed from whole blood at NILU following Herzke et al. (2009) and concentrations below LoD were processed following the same procedure than PFASs. Consequently, OCs remaining for further investigations were the organochlorine pesticides: mirex, hexachlorobenzene (HCB), p,p'-dichlorodiphenyldichloroethylene (DDE), oxychlordane, trans-, cisonachlor, and the polychlorinated biphenyls: PCBs -28, -99, -105, -118, -138, -153, -180, -183, -187 (expressed here as the Σ PCBs). Concentrations of p,p'-DDE were missing for 3 males because of injection issues into the gas chromatography-mass spectrometry (GC/MS). Total mercury (Hg) was analyzed at the Littoral Environment et Sociétés laboratory (LIENSs) in La Rochelle, France, from freeze-dried and powdered red blood cells as described in Bustamante et al. (2006).

2.3. Experimental design and data processing

The 20 nests investigated initially contained a natural clutch size of two eggs. We removed and replaced one of these two eggs by an artificial egg containing a triaxial accelerometer, a magnetometer and a temperature sensor to record orientation and angular changes in three dimensions (i.e. roll, pitch and yaw; as described in Clatterbuck et al., 2017; Kelsey et al., 2016; Shaffer et al., 2014; Taylor et al., 2018), as well as core egg temperature (as presented in Blévin et al., 2018). These loggers provided accurate (sensing 1-2° angular changes orientation) and continuous records (~1 sec) of egg-turning behaviors during the whole duration of deployment in a nest. The accuracy of egg-loggers was tested and verified by comparing a video of an egg being turned manually with a virtual animation (as shown in Shaffer et al., 2014 [supplementary information]) created from the data recorded by its logger. Artificial eggs were designed to approximate size and shape of kittiwake eggs using 3D printing technology and painted with non-toxic water-based paint to mimic natural kittiwake eggs (see Blévin et al., 2018 for more details). All natural eggs removed from the nest were candled and determined as being fertile. These eggs were further dissected to estimate the age of the embryo (n = 12). Egg-loggers were deployed in the nest between 7 and 10 days, during the first half of the incubation period (Table S1 in supplementary materials). All incubating birds readily accepted the artificial egg and showed no abnormal responses (e.g. egg rejection). Because each partner was marked with different color dye spots on the forehead, we could easily delineate the incubation bouts of each parent with a telescope from a distance. Once the egg-loggers deployed, each nest was regularly checked every day (around 5 times per day and checks were not conducted at night) to establish which partner was incubating the eggs. Once the experiment completed, artificial eggs were removed from the nest, leaving only one natural egg in the nest.

Egg-logger data were processed using purpose-built routines in MATLAB (The Mathworks, Natick, MA, USA) following methods developed and detailed in Shaffer et al. (2014). Raw accelerometer and magnetometer data were converted to 3-2-1 Euler angles to estimate instantaneous egg movement in 3 dimensions (i.e. roll, pitch and yaw). Only rotations exceeding an angular change of 10° were considered as significant egg-turning events (Beaulieu et al., 2010; Shaffer et al., 2014). For each kittiwake, we estimated i) the egg-turning frequency by dividing the sum of all events detected per bird by the duration of the record for this bird (expressed on an hourly basis) and ii) the overall angular change, by averaging the value of all egg-tuning events per bird (expressed in degrees). It is worth noting that incubation behavior was recorded per nest, making the duration of egg-turning behaviors for both partners dependent of each other (both partners do not incubate in the same time). The day of egg deployment and recording bouts during our presence in the colony were excluded from the dataset in order to avoid any biased data.

2.4. Statistics

All statistical analyses were performed using R 3.3.1 (R core Team, 2018). Linear mixed effect models (LMMs) with the nest identity as a random factor were used to test whether egg-turning behaviors (i.e. egg-turning frequency and angular change), hormone levels (i.e. baseline prolactin and corticosterone), and body condition differed between sexes. We used the restricted maximum likelihood estimation (REML) method to avoid any potential biased statistical estimations (Zuur et al., 2009). We first tested the relationships between (a) hormones and (b) contaminants with egg-turning behaviors using linear models (LMs). Each contaminant was considered separately to avoid any collinearity problems. These analyses have been conducted for each sex separately as males were more contaminated than females (Table 1 in Blévin et al.,

2018) and because both sexes can react in very different ways to environmental stressors like PFASs, OCs and Hg contamination (e.g. Blévin et al., 2016, 2017a, 2018; Goutte et al., 2015; Tartu et al., 2013, 2014, 2016). The relationships between egg-turning behaviors and SMI were also tested since body condition may impact the release of fat soluble contaminants and may play a substantial role. Multiple testing can potentially lead to misleading results, indicating statistical significance in situations where there is none. Consequently, we performed bootstrapping (i.e. resampling method, as detailed in Westfall and Young, 1993; Chernick, 2011) from the dataset of significant relationships and then assessed diagnostic plot to corroborate the results (Figs. S1-S9 in supplementary materials). Then, we examined the relationships between the contaminants and hormones of interest (i.e. only those significantly related to egg-turning behaviors) with LMs. Diagnostic plots were performed on residuals (Zuur et al., 2009) and the significance level was $\alpha \leq 0.05$.

We also explored how the contrasting abundance of contaminants (i.e. Σ PFASs and Σ PCBs as detailed in discussion and Fig. 5) in blood are related to the different responses on egg-turning behaviors. We thus divided the dataset in 4 groups (“high PFASs/high PCBs”, “high PFASs/low PCBs”, “low PFASs/high PCBs” and “low PFASs/low PCBs”). Each bird has been attributed to one group by comparing its contaminant concentration relative to the average value (e.g. “low PFASs” if the Σ PFAS concentrations of the bird is below the average Σ PFAS

3. Results

Sex specific egg-turning frequency and angular change are shown in Table 1. LMMs did not indicate any statistical differences of egg-turning behaviors between males and females. LMMs did not indicate any statistical differences for prolactin concentrations in plasma between males and females ($F_{1,19} = 0.08$, $p = 0.78$). Corticosterone concentrations in plasma (females: $8.40 \pm$

4.58 ng/mL (SD); males: 9.15 ± 5.10 (SD) ng/mL) were not statistically different between sex ($F_{1,19} = 0.30$, $p = 0.59$). Prolactin levels were positively and significantly related to the angular change in females but not in males (Fig. 2; LMs, females: $F_{1,18} = 5.64$, $p = 0.03$; males: $F_{1,18} = 0.20$, $p = 0.66$). Furthermore, prolactin levels were not significantly related to egg-turning frequency in either sex (LMs, females: $F_{1,18} = 0.49$, $p = 0.50$; males: $F_{1,18} = 2.60$, $p = 0.12$). Corticosterone levels were not significantly related to egg-turning frequency (LMs, females: $F_{1,18} = 0.55$, $p = 0.47$; males: $F_{1,18} = 0.07$, $p = 0.79$) and angular change (LMs, females: $F_{1,18} = 0.91$, $p = 0.35$; males: $F_{1,18} = 2.73$, $p = 0.12$) in either sex. Contaminant and prolactin concentrations have been detailed extensively in Blévin et al. (2018) and thus will not be further described and discussed here.

Individual recording duration of egg-turning behaviors (19.83 ± 9.38 (SD) hours per bird, ranging from 4.64 to 43.07 h) did not influence angular change (LMM, $F_{1,19} = 1.44$, $p = 0.25$) but was positively related to the egg-turning frequency (LMM, $F_{1,19} = 4.35$, $p = 0.05$). Consequently, we controlled for the effect of recording duration on egg-turning frequency in further statistical analyses. Egg-turning frequency was positively and significantly related to PFOSlin and PFNA concentrations in female kittiwakes (Table 2). In other words, the most PFOSlin and PFNA-contaminated females turned their eggs more frequently compared to the less contaminated females. OCs, Hg and SMI were not significantly related to egg-turning frequency in either sex (Table 2). Angular change was positively and significantly related to PFOSlin, PFNA, PFUnA and PFTrA concentrations in females, but negatively and significantly related to the Σ PCBs concentrations in female kittiwakes (Table 2; Fig. 3). In other words, the most PFASs contaminated females turned their eggs, on average, with higher amplitude while the most PCBs-contaminated females turned their eggs with lower amplitude (Fig. 3). Concentrations of PFASs and PCBs were not significantly correlated in female kittiwakes (PFOSlin/ Σ PCBs: $r_{\text{pearson}} = -0.29$; $p\text{-value} = 0.21$; Σ PFCAAs/ Σ PCBs: $r_{\text{pearson}} = -0.37$; $p\text{-value} =$

0.11). In males, we observed a positive and significant relationship between angular change and PFNA (Table 2; Fig. 3). Hg and SMI were never associated with angular change in either sex (Table 2). In addition, to control for potential changes in egg-turning behaviors that may have occurred across the incubation period, we used the age of embryo as a proxy of incubation stage. Age of embryo was only available for 12 eggs, thus eliminating the possibility of using this variable in statistical analyses conducted on the whole dataset. Embryo age did not influence angular change (LMM, $F_{1,10} = 0.77$, $p = 0.40$) but was positively related to the egg-turning frequency (LMM, $F_{1,10} = 5.99$, $p = 0.03$). We thus performed a second run of statistical analysis with a subset (12 males and 12 females), controlling for age of embryo on egg-turning frequency in the significant models from the first statistical run and found a similar result.

We further examined the relationships between the contaminants and the hormones that were significantly related to egg-turning behaviors. In females, except for PFTrA, PFOSlin, PFNA, PFUnA were positively and significantly related to baseline levels of prolactin whereas PCBs were not (Fig. 4; LMs, PFOSlin: $F_{1,18} = 7.25$, $p = 0.01$; PFNA: $F_{1,18} = 8.40$, $p = <0.01$; PFUnA: $F_{1,18} = 8.10$, $p = 0.01$; PFTrA: $F_{1,18} = 1.08$, $p = 0.31$; Σ PCBs: $F_{1,18} = 1.56$, $p = 0.23$). In males, PFNA and PFTeA were not related to baseline levels of prolactin (LMs, PFNA: $F_{1,18} = 1.85$, $p = 0.19$; PFTeA: $F_{1,18} = 0.50$, $p = 0.49$).

4. Discussion

Using the same dataset with similar experimental design, the present work comes to complete our recent publication where we investigated the consequences of contaminant exposure on egg incubation temperature (Blévin et al., 2018). In this study, egg-turning behaviors were not associated to egg-incubation temperature (Figs. S10 & S11 in supplementary materials). Indeed, as pointed out and discussed in Shaffer et al. (2014), Larids shuffle their feet and body

while rotating around the nest cup. Moreover, we did not observe any kittiwakes using their bill to turn their eggs as is the case for other species (Deeming, 2002). Such behavior enables egg-turning without the parent lifting off the egg, thus minimizing exposure of eggs to ambient temperature. As a result, egg-turning does not incur egg temperature loss in kittiwakes and therefore, contaminant exposure can potentially impact incubation temperature and egg-turning behaviors independently.

The goal of the present study was to investigate the relationships between three groups of contaminants (i.e. PFASs, OCs and Hg), hormone levels (i.e. prolactin and corticosterone) and egg-turning behaviors (i.e. frequency and angular change) in incubating Arctic kittiwakes. Our results revealed that prolactin levels were positively and significantly related to the angular change in females but not in males. Corticosterone levels were not associated with egg-turning behaviors in either sex. Furthermore, we found contrasting associations between PFASs and OCs since PFOSlin and PFCAs were positively related to egg-turning frequency and angular change in females and PFNA to angular change in males, contrary to Σ PCBs which was negatively associated with angular change in females. Furthermore, Hg and SMI were not related to either incubation behaviors which is consistent with the results of a previous study on Forster terns (Taylor et al., 2018). Because PFASs and PCBs are known endocrine disruptors (DeWitt, 2015; Giesy et al., 2003; Jensen and Leffers, 2008; Khetan, 2014; Tyler et al., 1998), we investigated underlying hormonal intermediary by focusing on prolactin, a hormone highly involved in incubation behavior (Angelier and Chastel, 2009; Angelier et al., 2016; Vleck, 1998, 2002). Interestingly, PFASs were positively related to the plasma prolactin levels in female kittiwakes, thus corroborating the positive relationships between PFASs and egg-turning behaviors (at least for females). Consequently, PFASs and PCBs, through their endocrine disrupting properties, could alter egg-turning behaviors of kittiwakes.

4.1. Prolactin and egg-turning

Prolactin is the major controller of parental behavior (Riddle, 1963). Accordingly, several correlational and experimental studies highlighted the predominant role of prolactin in the set-up and maintenance of incubation behaviors (Angelier et al., 2016; Buntin, 1996; Lynn, 2016; Sockman et al., 2006; Vleck, 2002). For example, Sockman et al. (2000) demonstrated that a moderate experimental increase in prolactin concentration induces a better incubation commitment (i.e. percent day incubating) in American kestrels (*Falco sparverius*). Furthermore, previous research on kittiwakes from the same colony reported that experimentally induced low prolactin levels were associated with reduced nest attendance during the chick-rearing period (Angelier et al., 2009). Our study suggested a positive effect of prolactin secretion on egg angular changes in female kittiwakes, providing new understanding about the role of prolactin on parental behaviors in wild birds. Currently, only one additional study conducted on Adélie penguins (*Pygoscelis adeliae*) has examined prolactin levels and egg-turning behavior (Thierry et al., 2013). Surprisingly, they determined that birds with lowered prolactin levels following implants of self-degradable bromocriptine pellets turned their eggs more frequently compared to a control group. However, the authors state that their result was likely attributed to a shift from a prone to an upright body posture of treated birds, rather than a direct effect of a prolactin decrease, which favored egg-turning events. Although we observed a relationship between prolactin and angular change, egg-turning frequency was not significantly influenced by prolactin concentration so further study is needed in other species.

4.2. Parental care and contaminants

Surprisingly, PFOSlin and PFCAs concentrations were positively related to egg-turning frequency and angular change, suggesting a beneficial effect of PFASs on incubation behaviors in kittiwakes (especially in females). This result is unexpected since it is widely accepted that environmental contaminants are rather associated with adverse effects in living organisms. To the best of our knowledge, the effects of PFASs on reproductive behaviors of birds have never been investigated which makes comparison with other studies impossible. However, although correlative, several recent studies revealed that PFASs could have some “beneficial” effects on the physiology of Arctic seabirds, including lower stress levels (i.e. baseline corticosterone, Tartu et al., 2014), reduced ageing rate (i.e. telomere length dynamic, Blévin et al., 2017b) and higher energy expenditure allocated for self-maintenance (i.e. basal metabolic rate, Blévin et al., 2017a; thyroid hormones, Melnes et al., 2017). Thus, a suggested “positive” effect of PFASs on egg-turning behaviors is in line with previous findings but still remains purely speculative, warranting further experimental investigations.

In contrast to PFASs, the effects of OCs contamination like PCBs on reproductive behaviors are better understood and several studies conducted *in natura* or experimentally revealed an array of detrimental effects in birds. For example, an experimental study where captive American kestrels received a mixture of PCBs showed longer incubation periods and altered incubation behaviors like a reduced nest attendance in treated groups (Fisher et al., 2006). In glaucous gulls (*Larus hyperboreus*), another Arctic seabird, exposure to PCBs was found to be associated with reduced nest attendance (i.e. longer and/or more frequent absences from the nest site during incubation period; Bustnes et al., 2001) and lowered nest temperature (Verboven et al., 2009). In addition, delayed hatching date in relation to PCBs exposure in male kittiwakes (females were not investigated) from the same study colony was also reported (Tartu

et al., 2015b). As a result, potential detrimental effects of PCBs on reproductive behaviors in birds appear consistent across species. Similarly, in the same birds and using the same exact loggers, we found that high blood levels of other OCs (i.e. oxychlorane and to a lesser extent, mirex and HCB), were associated with a lower egg incubation temperature (Blévin et al., 2018). By focusing on the fine-scale egg-turning behavior and because we reported a negative association between angular change and PCBs in females, this study improves our knowledge of the way through which OCs could impact reproduction of birds.

4.3. Some contrasted patterns between PFASs and OCs

This study revealed contrasted associations between PFASs and OCs with angular change in female kittiwakes. Interestingly, such contrasted patterns between PFASs and OCs have already been highlighted in Arctic seabirds for several physiological endpoints, including hormone levels (e.g. corticosterone, thyroid hormones; Melnes et al., 2017; Nordstad et al., 2012; Tartu et al., 2014, 2015b), ageing (i.e. telomere length; Blévin et al., 2016, 2017b) and energy expenditure (i.e. basal metabolic rate; Blévin et al., 2017a). The contrasted physico-chemical properties of the proteinophilic PFASs and lipophilic OCs are consistent with this observation. Indeed, PFASs are known to preferentially accumulate in protein rich tissues (e.g. liver, blood) whereas OCs are stored in adipose tissues before being released into the bloodstream during periods of accelerated lipid mobilization (Aas et al., 2014; Bustnes et al., 2010; Findlay and DeFreitas, 1971; Henriksen et al., 1995; Jones et al., 2003; Kelly et al., 2009; Luebker et al., 2002; Routti et al., 2013; Vanden Heuvel et al., 1992; Verreault et al., 2005). Consequently, we posit that PFASs and OCs could target physiological functions through very different modes of action, without mechanistic interlinkage, potentially resulting in mixed or contrasted responses. Another potential explanation could rely on a possible confounding effect between

contaminants. However, PFASs (i.e. PFOSlin and PFCAs) were not significantly related to Σ PCBs in female kittiwakes in the present study, thus partly excluding this hypothesis. Finally, since PFASs and PCBs are both circulating in kittiwake's blood at the same time, we explored how the contrasting abundance of PFASs and PCBs in blood are related to the different responses on egg angular change, following different Σ PFAS/ Σ PCB combinations (Fig. 5). The resulting sample size in each group is very low, precluding performing any robust statistical analyses and requiring cautiousness in graphical interpretation. Nevertheless, highly PFASs-contaminated females appear to turn their eggs with the greatest amplitude only in birds with low PCB concentrations. Conversely, highly PCBs-contaminated females appear to turn their egg with the lowest amplitude only in birds with low PFASs concentrations. In addition, both "high PFASs/high PCBs" and "low PFASs/low PCBs" contaminated groups display intermediate responses, suggesting that both PFASs and PCBs may impact angular change and that their effect might offset each other. However, a larger sample size is needed to test if these patterns are statistically significant. Furthermore, kittiwakes are obviously exposed to a complex cocktail of contaminants which were not measured in this study and future experimental research focusing on structurally opposed chemicals is essential to better understand and clarify the underlying mechanisms through which contaminants, and especially PFASs, influence health of living organisms.

4.4. Contaminants and prolactin

Given its key role in mediating parental behaviors, we investigated the possible disruption of prolactin secretion originating from PFASs and PCBs exposure, both known as endocrine disruptors (DeWitt, 2015; Giesy et al., 2003; Jensen and Leffers, 2008; Khetan, 2014; Tyler et al., 1998). Interestingly, we found a positive and significant relationship between PFASs and

prolactin levels in female kittiwakes which is consistent with the previously suggested positive effect of PFASs on egg-turning behaviors. Thus, through a possible increase of prolactin secretion, the most PFAS-contaminated kittiwakes (at least females) could be more attentive to their eggs by exhibiting greater angular changes when turning. However, the endocrine disrupting property of PFASs on prolactin secretion remains unknown and has never been explored in birds. Similarly, in humans, exposure to PFOA and PFOS has been found to be positively associated with the level of prolactin (Zhang et al., 2018), but the possible mechanisms linking PFASs burden to prolactin secretion were not known. Our previous studies have shown that in kittiwakes, exposure to long-chain PFCAs is associated with decreased baseline corticosterone levels (Tartu et al., 2014). Glucocorticoids are well known to depress prolactin secretion (Angelier et al., 2009). Thus, in most PFASs-contaminated individuals, depressed corticosterone levels may enhance prolactin secretion and in turn, stimulate egg-turning behaviors. Alternatively, it could also be that, independent of an endocrine disrupting effect, exposure to PFASs directly stimulates egg-turning behaviors, and in turn, that such change in behavior is feeding back onto prolactin levels. Clearly, additional experimental and correlational studies are needed to confirm this relationship and to understand the endocrine mechanisms linking PFASs to prolactin in avian models. In contrast, PCB concentrations were not associated with plasma prolactin level in kittiwakes although Fig. 4 suggests a negative trend in females. In glaucous gulls, PCBs (and other OCs) have been associated with a decrease plasma prolactin concentration in males only, although these associations (including PCBs) did not adhere with the criterion of significance (Verreault et al., 2008). Furthermore, one previous study performed on snow petrels (*Pagodroma nivea*) did not report any effects of OCs (including PCBs) on prolactin levels (Tartu et al., 2015a). Given these inconsistencies, it is thus impossible to make affirmative conclusions so we can only speculate that PCBs exposure may decrease prolactin levels in kittiwakes. Moreover, because initiation and maintenance of

incubation behaviors is orchestrated by a complex ‘cocktail’ of different reproductive hormones acting synergistically (Angelier et al., 2016; Buntin, 1996; Lynn, 2016; Sockman et al., 2006; Vleck, 2002; Vleck and Vleck, 2011), further experimental studies i) focusing on sex steroids (e.g. testosterone, estradiol, progesterone) and ii) on different doses of administered PCBs may provide greater clarity about endocrine mechanisms targeted by PCBs and toxicity thresholds.

5. Conclusion

The present study, in combination with recent findings reported in Blévin et al. (2018), suggests some subtle effects of environmental contaminants on incubation behaviors (i.e. egg-turning behaviors and incubation temperature) in Arctic kittiwakes. In turn, such variations of egg-tuning behaviors and/ or incubation temperature may impact the reproductive success of kittiwakes (Blévin et al., 2018). Because our study is purely correlative, causality is not possible to establish and cautious interpretation is warranted. Further studies with a similar fine scale approach should be conducted in the future and would enable to better understand the way through which contaminants are challenging bird reproduction. Experimental designs to compare incubation behaviors of untreated birds and contaminant-exposed birds would enable to confirm such effects and quantify the extent to which incubation behaviors are altered. Egg-loggers are thus promising tools to study the fine scale consequences of exposure to environmental contaminants on reproductive behaviors in free-living birds.

Our study provides new evidences that different contaminants (here PCBs and PFASs) can have opposite effects on physiology and behavior. This study also points to a possible stimulation of prolactin secretion by PFASs. Given its key role in mediating parental investment in birds, any disruption of prolactin by PFASs, could therefore modify incubation effort and more generally act on the trade-off for parent birds between spending enough time caring for the clutch and

obtaining enough nutrients for self-maintenance. However, the potential enhancing effect of PFASs on egg-turning, via stimulated prolactin levels, has to be confirmed in other studies and require investigations of the energetic cost of egg-turning behavior, together with the long-term consequences (survival, breeding frequency) on this possible PFASs-related increased incubation effort.

Conflict of interest

The authors declare no conflicts of interest.

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Table 1. Egg-turning frequency (number of hourly turns) and angular change (degrees) for female and male incubating kittiwakes, *Rissa tridactyla*, from Kongsfjord, Svalbard.

	Females (n = 20)	Males (n = 20)		
	Mean ± SD	Mean ± SD	<i>F</i> _{1,19}	p-value
Turning Frequency	2.37 ± 0.71	2.09 ± 0.82	1.45	0.24
Angular change	41.45 ± 7.53	43.08 ± 10.16	0.37	0.55

Table 2. Output of LMs examining relationships between contaminants, body condition (SMI), and egg-turning behaviors in female and male incubating kittiwakes, *Rissa tridactyla*, from Kongsfjord, Svalbard.

Predictor	Frequency*			Angular change		
	Estimate \pm SE	p-value	R ²	Estimate \pm SE	p-value	R ²
Females (n = 20)						
PFOSlin	$3.10^{-4} \pm 1.10^{-4}$	0.05	0.25	$3.10^{-3} \pm 2.10^{-3}$	0.05	0.19
PFNA	$2.10^{-3} \pm 6.10^{-4}$	0.03	0.29	$2.10^{-2} \pm 7.10^{-3}$	0.03	0.23
PFDCa	$6.10^{-4} \pm 7.10^{-4}$	0.44	0.08	$1.10^{-2} \pm 7.10^{-3}$	0.06	0.19
PFUnA	$3.10^{-4} \pm 2.10^{-4}$	0.12	0.17	$4.10^{-3} \pm 2.10^{-3}$	0.05	0.20
PFTra	$1.10^{-4} \pm 1.10^{-4}$	0.41	0.08	$3.10^{-3} \pm 1.10^{-3}$	0.01	0.30
PFTeA	$6.10^{-4} \pm 5.10^{-4}$	0.25	0.12	$1.10^{-2} \pm 5.10^{-3}$	0.07	0.18
Σ PCBs	$-3.10^{-6} \pm 3.10^{-5}$	0.90	0.05	$-6.10^{-4} \pm 2.10^{-4}$	0.03	0.23
Oxychlorane	$3.10^{-4} \pm 5.10^{-4}$	0.64	0.06	$-7.10^{-3} \pm 5.10^{-3}$	0.18	0.10
<i>trans</i> -Nonachlor	$1.10^{-3} \pm 3.10^{-3}$	0.64	0.06	$-3.10^{-2} \pm 2.10^{-2}$	0.22	0.08
<i>cis</i> -Nonachlor	$3.10^{-3} \pm 3.10^{-3}$	0.37	0.09	$-4.10^{-2} \pm 3.10^{-2}$	0.32	0.05
<i>p,p'</i> -DDE	$1.10^{-4} \pm 1.10^{-4}$	0.48	0.07	$-1.10^{-3} \pm 1.10^{-3}$	0.20	0.09
Mirex	$-4.10^{-5} \pm 8.10^{-4}$	0.96	0.05	$-8.10^{-3} \pm 8.10^{-3}$	0.31	0.06
HCB	$3.10^{-4} \pm 3.10^{-4}$	0.33	0.10	$-3.10^{-3} \pm 3.10^{-3}$	0.30	0.06
Hg	$-1.10^{-1} \pm 4.10^{-1}$	0.83	0.05	-3.98 ± 4.61	0.40	0.04
SMI	$2.10^{-3} \pm 7.10^{-3}$	0.80	0.05	$3.10^{-2} \pm 8.10^{-2}$	0.69	0.01
Males (n = 20)						
PFOSlin	$-8.10^{-5} \pm 7.10^{-5}$	0.26	0.10	$1.10^{-3} \pm 7.10^{-4}$	0.16	0.11
PFNA	$-5.10^{-4} \pm 5.10^{-4}$	0.35	0.08	$1.10^{-2} \pm 4.10^{-3}$	0.01	0.31
PFDCa	$-2.10^{-4} \pm 5.10^{-4}$	0.68	0.04	$8.10^{-3} \pm 4.10^{-3}$	0.08	0.16
PFUnA	$-1.10^{-4} \pm 1.10^{-4}$	0.35	0.08	$2.10^{-3} \pm 1.10^{-3}$	0.08	0.16
PFTra	$3.10^{-5} \pm 9.10^{-5}$	0.78	0.04	$2.10^{-3} \pm 1.10^{-3}$	0.10	0.14
PFTeA	$-1.10^{-3} \pm 5.10^{-4}$	0.05	0.24	$7.10^{-3} \pm 6.10^{-3}$	0.25	0.07
Σ PCBs	$2.10^{-5} \pm 1.10^{-5}$	0.10	0.18	$3.10^{-5} \pm 2.10^{-4}$	0.86	< 0.01
Oxychlorane	$1.10^{-4} \pm 2.10^{-4}$	0.53	0.05	$3.10^{-3} \pm 3.10^{-3}$	0.32	0.05
<i>trans</i> -Nonachlor	$6.10^{-3} \pm 4.10^{-3}$	0.14	0.15	$4.10^{-2} \pm 5.10^{-2}$	0.47	0.03
<i>cis</i> -Nonachlor	$3.10^{-3} \pm 6.10^{-3}$	0.60	0.05	$5.10^{-2} \pm 7.10^{-2}$	0.48	0.03
<i>p,p'</i> -DDE**	$2.10^{-4} \pm 1.10^{-4}$	0.06	0.28	$1.10^{-3} \pm 1.10^{-3}$	0.47	0.04
Mirex	$6.10^{-4} \pm 5.10^{-4}$	0.25	0.10	$9.10^{-3} \pm 6.10^{-3}$	0.14	0.12
HCB	$1.10^{-4} \pm 1.10^{-4}$	0.27	0.10	$8.10^{-4} \pm 2.10^{-3}$	0.64	0.01
Hg	$-5.10^{-1} \pm 3.10^{-1}$	0.13	0.15	2.38 ± 4.02	0.56	0.02
SMI	$-1.10^{-2} \pm 1.10^{-2}$	0.19	0.13	$-8.10^{-2} \pm 1.10^{-1}$	0.53	0.02

Significant variables are in bold.

* Controlled for recording duration in each model.

** Sample size = 17 (3 males are missing because of injection issues into the GC/MS).

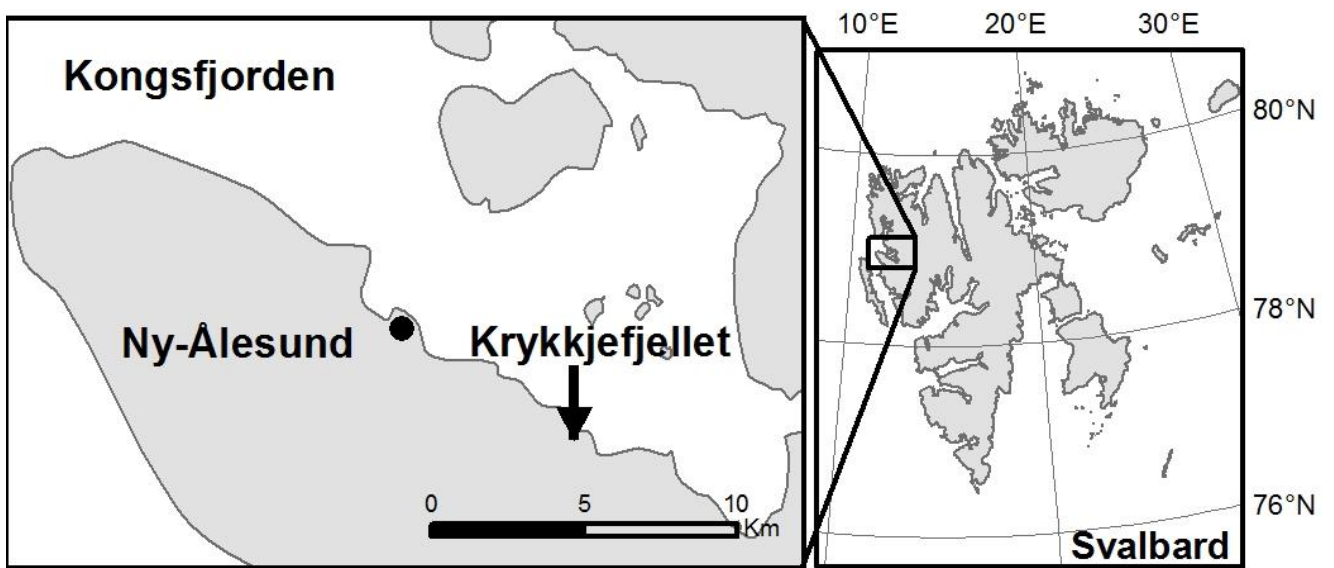


Figure 1. Location of the study area (Kongsfjord, Svalbard) and the sampling site (Krykkjefjellet).

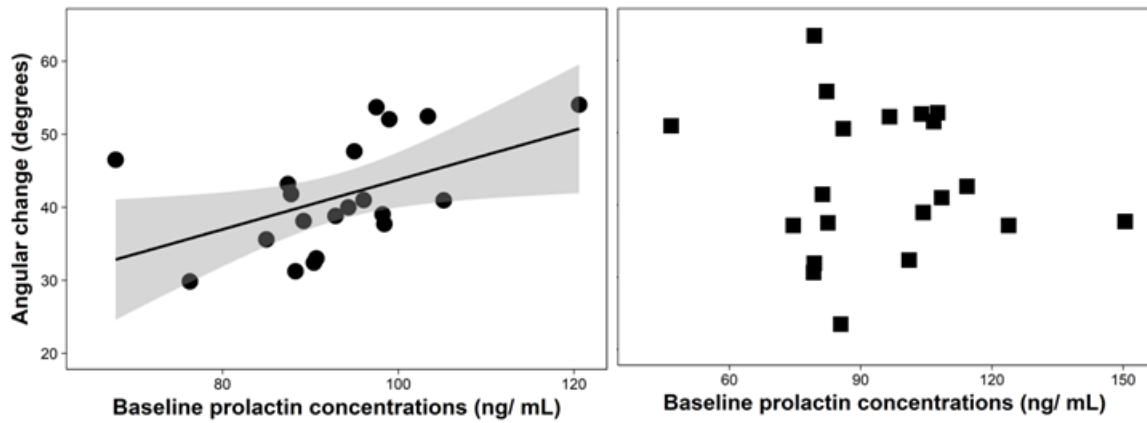


Figure 2. Plasma prolactin levels and egg angular change were positively associated in (a) females ($n = 20$) incubating kittiwakes, *Rissa tridactyla*, from Kongsfjord, Svalbard. In contrast, plasma prolactin levels and egg angular change were not significantly associated in (b) males ($n = 20$). The significant relationship is indicated by a solid regression line with standard error (SE, shaded area).

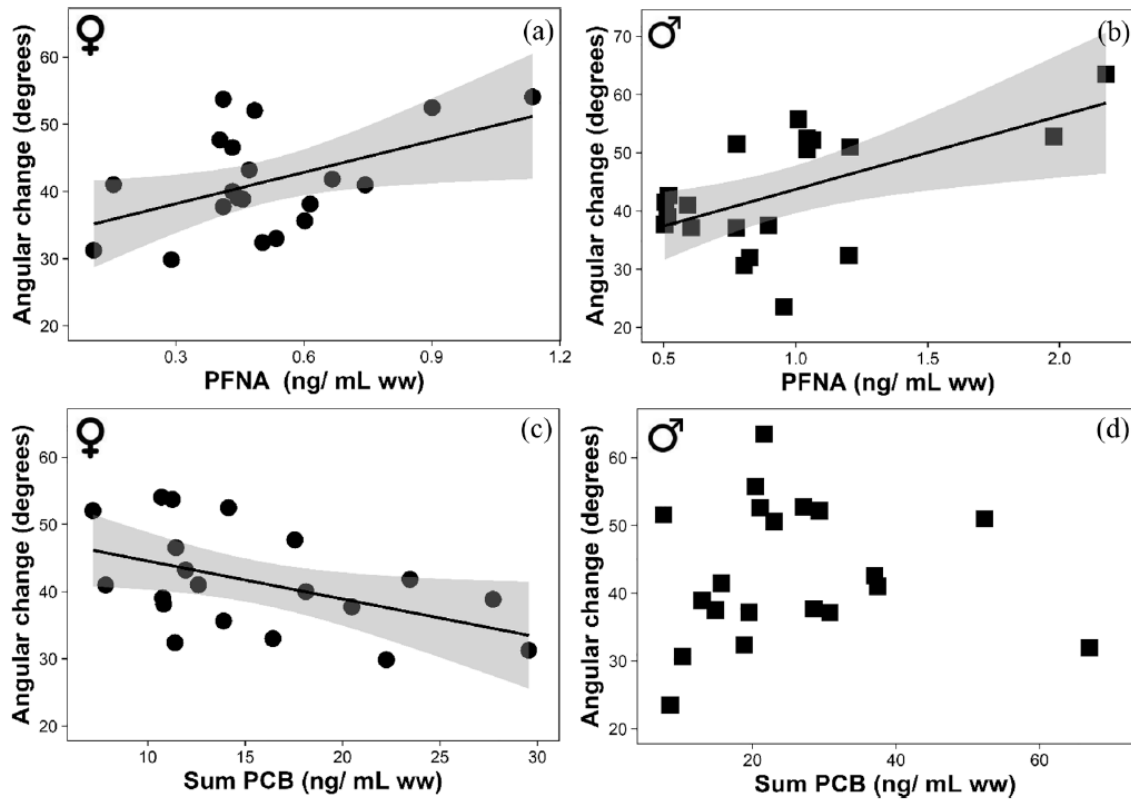


Figure 3. Plasma PFNA concentrations and egg angular change were positively associated in both (a) females (n = 20) and (b) males (n = 20) incubating kittiwakes, *Rissa tridactyla*, from Kongsfjord, Svalbard. In contrast, blood Σ PCBs and egg angular change were negatively associated in (c) females, and (d) no significant relationship was found in males. Significant relationships are indicated by solid regression lines with standard errors (SE, shaded area).

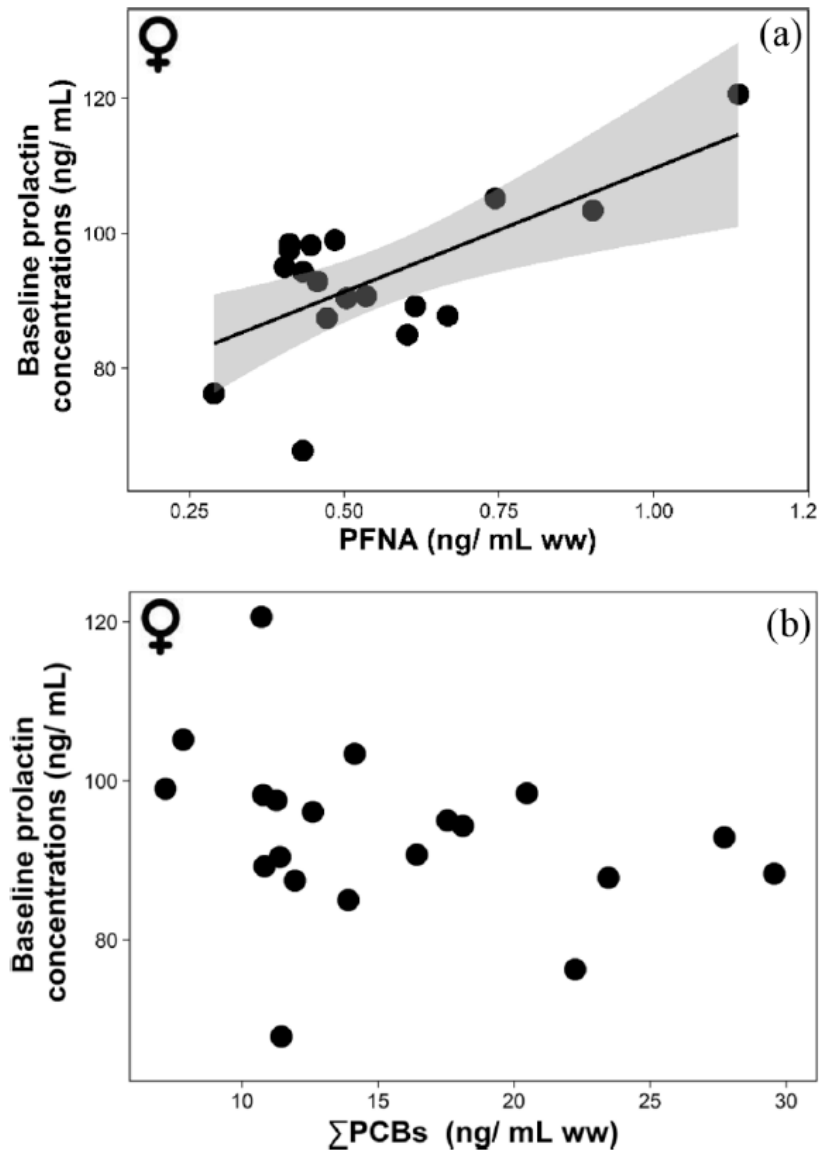


Figure 4. Plasma prolactin levels and plasma PFNA concentrations were positively associated in (a) females ($n = 20$) incubating kittiwakes, *Rissa tridactyla*, from Kongsfjord, Svalbard. By contrast, plasma prolactin levels and blood Σ PCBs concentrations were not significantly associated in (b) females ($n = 20$). The significant relationship is indicated by a solid regression line with standard error (SE, shaded area).

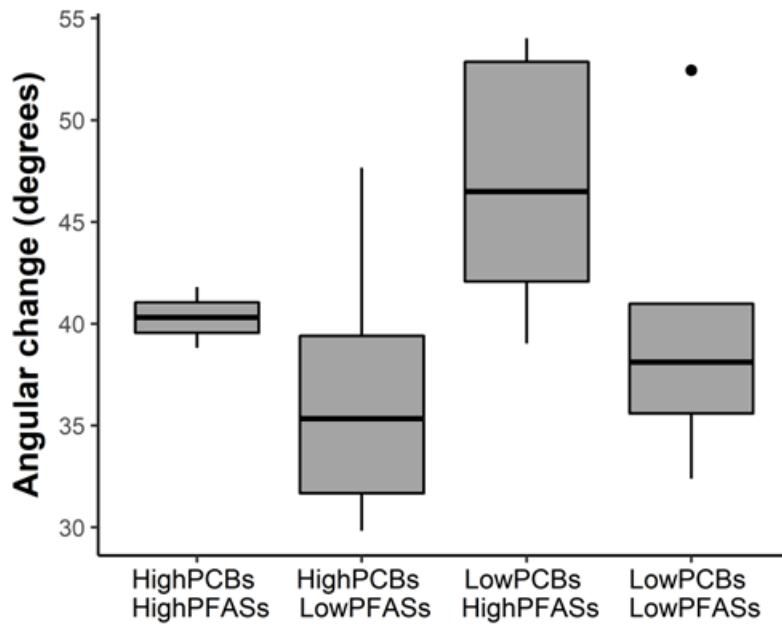


Figure 5. Inter-group comparison of angular change in response to different combination of contaminant concentrations in incubating female kittiwakes, *Rissa tridactyla*, from Kongsfjord, Svalbard. The female dataset has been divided in 4 groups: high PFASs/high PCBs (n = 2), high PFASs/low PCBs (n = 7), low PFASs/high PCBs (n = 6) and low PFASs/low PCBs (n = 5). Each female has been attributed to one group. The band near the middle of the box corresponds to the median (the 50th percentile). The bottom and top of the box corresponds to the lower and upper quartiles (25th and 75th percentile, respectively).

Supplementary materials for

Contaminants, prolactin and parental care in an Arctic seabird: Contrasted associations of perfluoroalkyl substances and organochlorine compounds with egg-turning behavior

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Table S1. Date (day.month.year) of egg-logger deployment, retrieval, blood sampling and blood sampling time (UTC +1; 24h format) for each kittiwake considered in this study (ranked per nest Id and sex)

Nest Id	Sex	Egg-logger deployment	Egg-logger retrieval	Blood sampling date	Blood sampling time
1	F	27.06.2015	06.07.2015	27.06.2015	12:05
1	M	27.06.2015	06.07.2015	27.06.2015	12:37
2	F	27.06.2015	06.07.2015	30.06.2015	11:25
2	M	27.06.2015	06.07.2015	27.06.2015	15:00
3	F	27.06.2015	06.07.2015	30.06.2015	20:53
3	M	27.06.2015	06.07.2015	27.06.2015	~14:00 (deducted value based on last and next sampling)
4	F	27.06.2015	06.07.2015	30.06.2015	11:51
4	M	27.06.2015	06.07.2015	27.06.2015	12:20
5	F	20.06.2015	26.06.2015	20.06.2015	15:30
5	M	20.06.2015	26.06.2015	24.06.2015	10:20
6	F	28.06.2018	06.07.2015	28.06.2015	12:10
6	M	28.06.2018	06.07.2015	03.07.2015	18:48
7	F	28.06.2015	06.07.2015	30.06.2015	10:11
7	M	28.06.2015	06.07.2015	28.06.2015	11:51
8	F	20.06.2015	26.06.2015	22.06.2015	17:12
8	M	20.06.2015	26.06.2015	20.06.2015	14:50
9	F	20.06.2015	26.06.2015	22.06.2015	10:50
9	M	20.06.2015	26.06.2015	20.06.2015	14:17
10	F	20.06.2015	26.06.2015	22.06.2015	16:47
10	M	20.06.2015	26.06.2015	20.06.2015	13:33
11	F	27.06.2015	06.07.2015	27.06.2015	11:02
11	M	27.06.2015	06.07.2015	30.06.2015	12:12
12	F	19.06.2015	25.06.2015	19.06.2015	16:56
12	M	19.06.2015	25.06.2015	19.06.2015	16:35
13	F	19.06.2015	25.06.2015	19.06.2015	15:23
13	M	19.06.2015	25.06.2015	22.06.2015	09:33
14	F	19.06.2015	25.06.2015	19.06.2015	16:05
14	M	19.06.2015	25.06.2015	24.06.2015	12:10
15	F	19.06.2015	25.06.2015	21.06.2015	10:41

15	M	19.06.2015	25.06.2015	19.06.2015	15:09
16	F	19.06.2015	25.06.2015	22.06.2015	09:50
16	M	19.06.2015	25.06.2015	19.06.2015	14:45
17	F	19.06.2015	25.06.2015	19.06.2015	18:03
17	M	19.06.2015	25.06.2015	19.06.2015	14:26
18	F	19.06.2015	25.06.2015	24.06.2015	09:50
18	M	19.06.2015	25.06.2015	19.06.2015	18:15
19	F	19.06.2015	25.06.2015	19.06.2015	13:47
19	M	19.06.2015	25.06.2015	19.06.2015	15:47
20	F	27.06.2015	06.07.2015	27.06.2015	16:12
20	M	27.06.2015	06.07.2015	30.06.2015	21:14

Figure S1. Bootstrapping from the dataset of the significant relationship between PFOSlin and egg-turning frequency for female kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient ($r = 0.46$) between PFOSlin and egg-turning frequency in female kittiwakes.

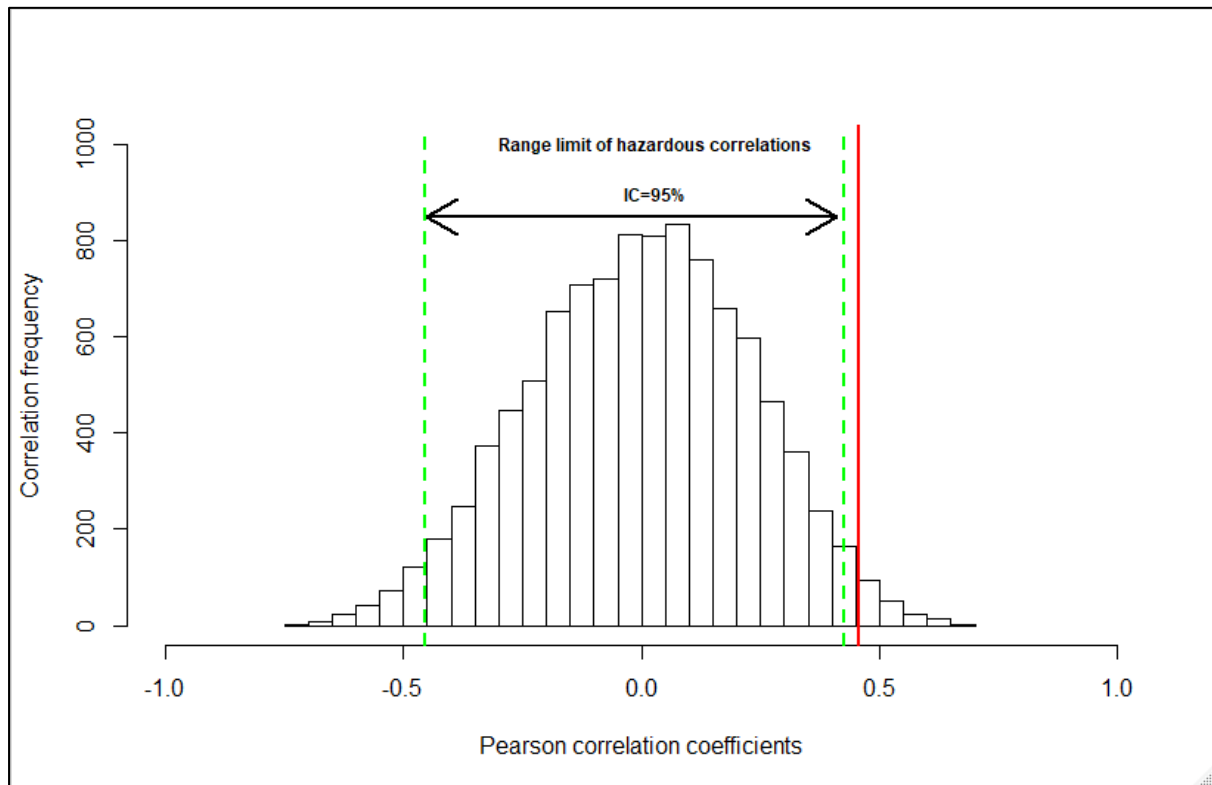


Figure S2. Bootstrapping from the dataset of the significant relationship between PFNA and egg-turning frequency for female kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient ($r = 0.43$) between PFNA and egg-turning frequency in female kittiwakes.

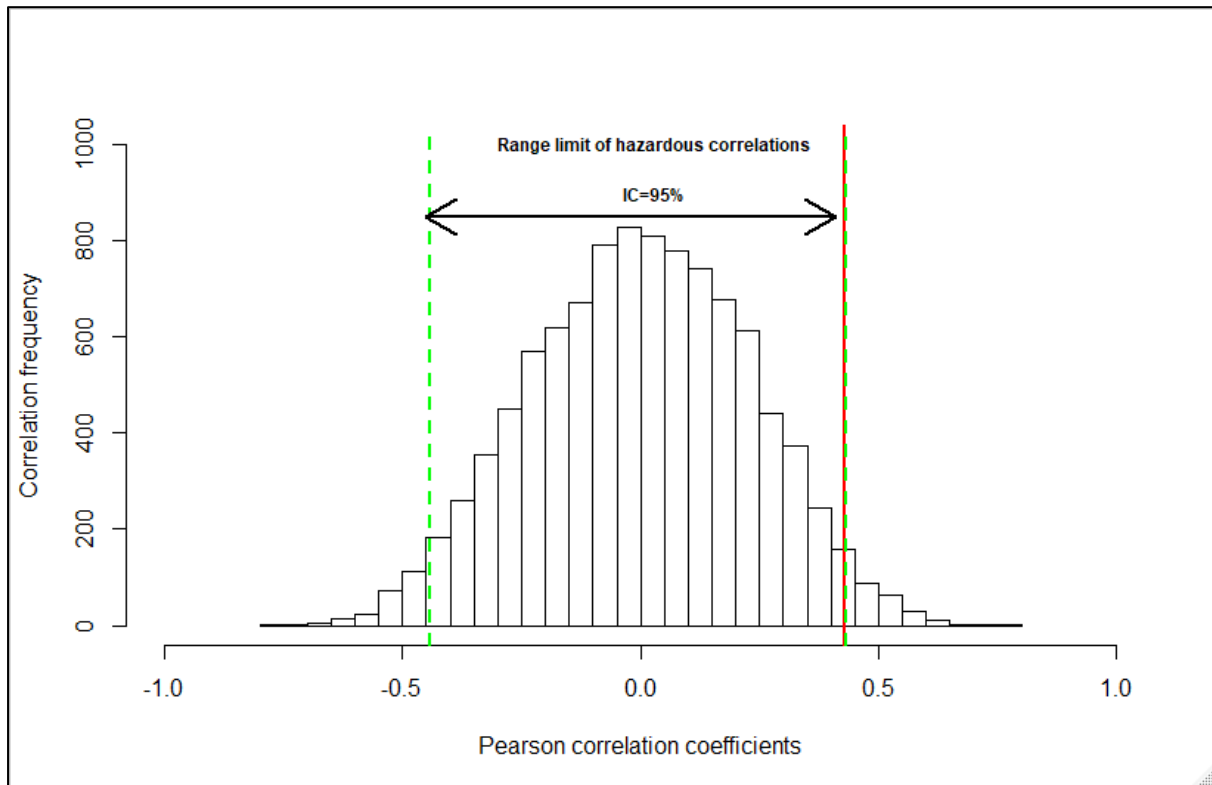


Figure S3. Bootstrapping from the dataset of the significant relationship between PFOSlin and egg-angular change for female kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient ($r = 0.44$) between PFOSlin and egg-angular change in female kittiwakes.

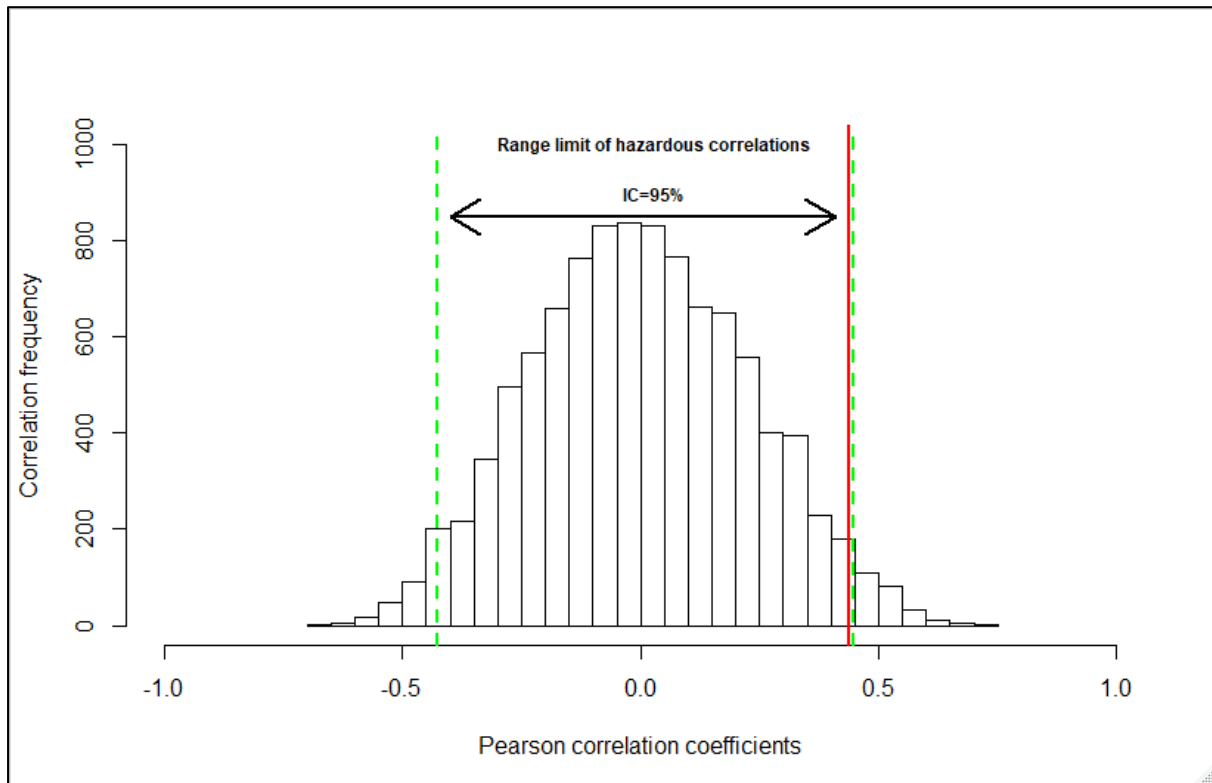


Figure S4. Bootstrapping from the dataset of the significant relationship between PFNA and egg-angular change for female kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient ($r = 0.48$) between PFNA and egg-angular change in female kittiwakes.

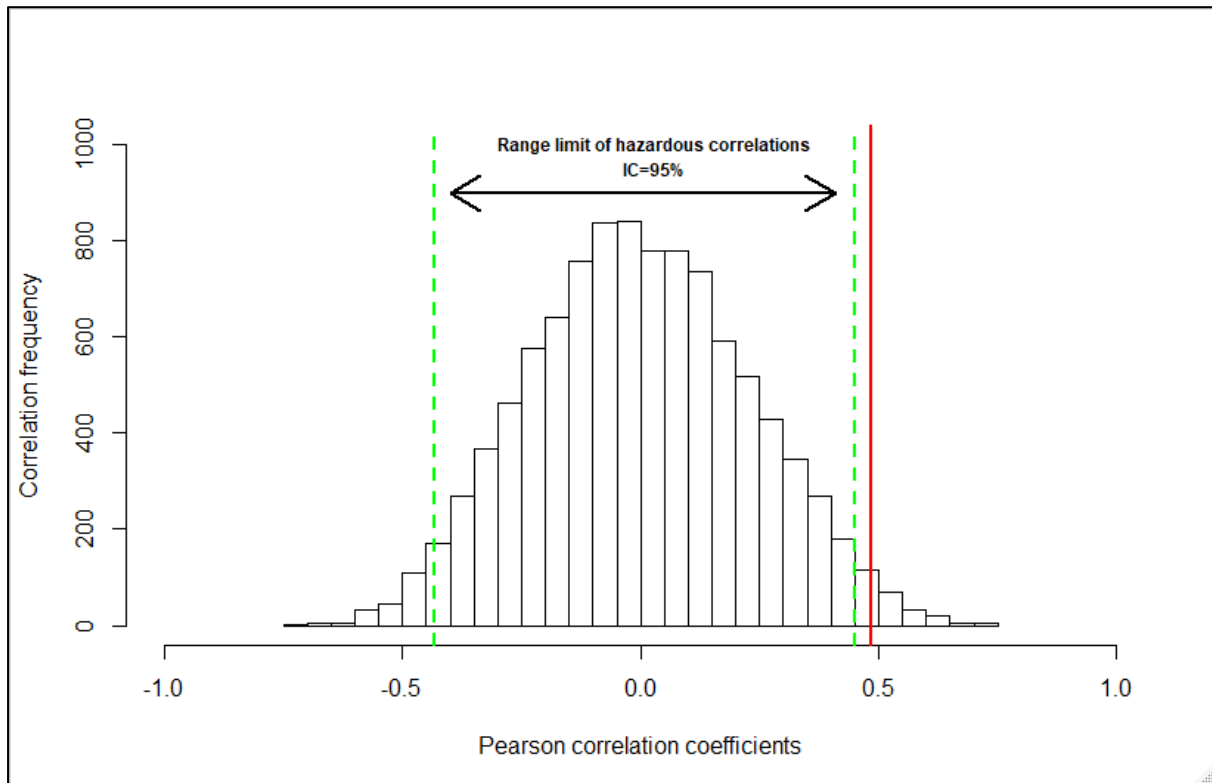


Figure S5. Bootstrapping from the dataset of the significant relationship between PFUnA and egg-angular change for female kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient ($r = 0.44$) between PFUnA and egg-angular change in female kittiwakes.

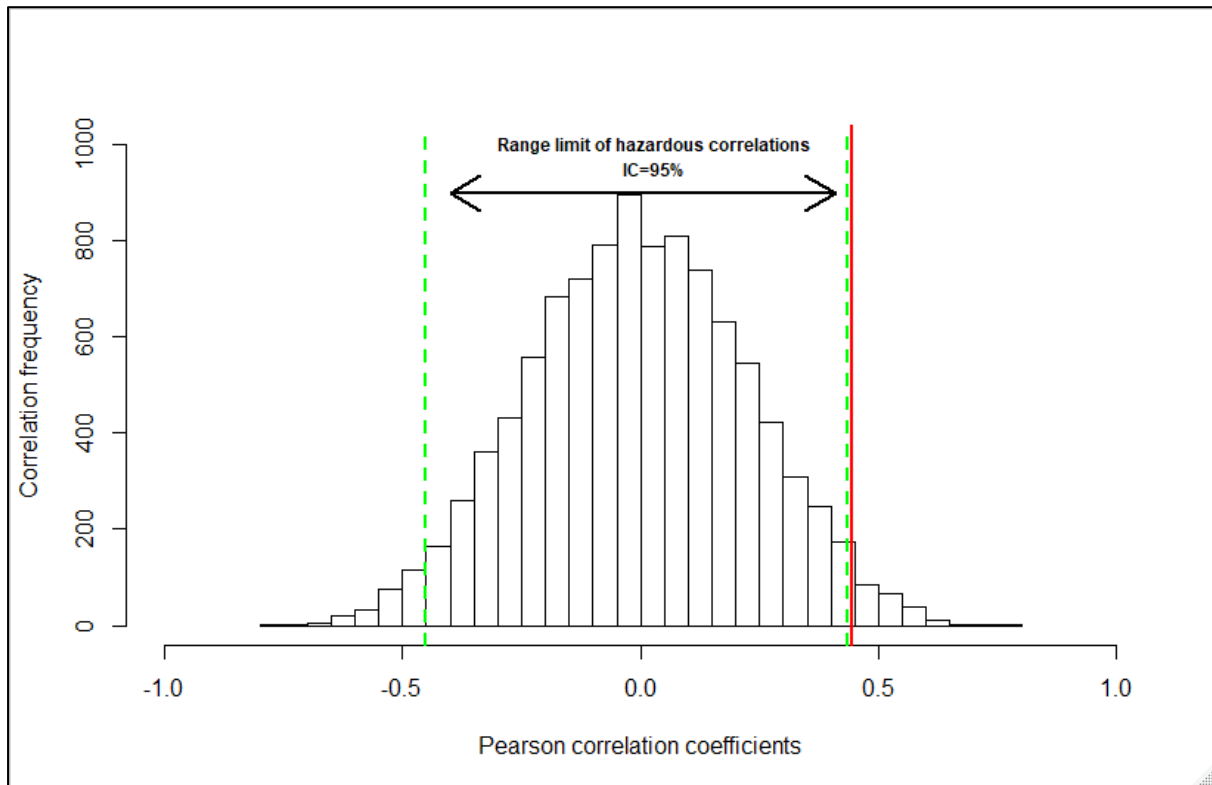


Figure S6. Bootstrapping from the dataset of the significant relationship between PFTrA and egg-angular change for female kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient ($r = 0.54$) between PFTrA and egg-angular change in female kittiwakes.

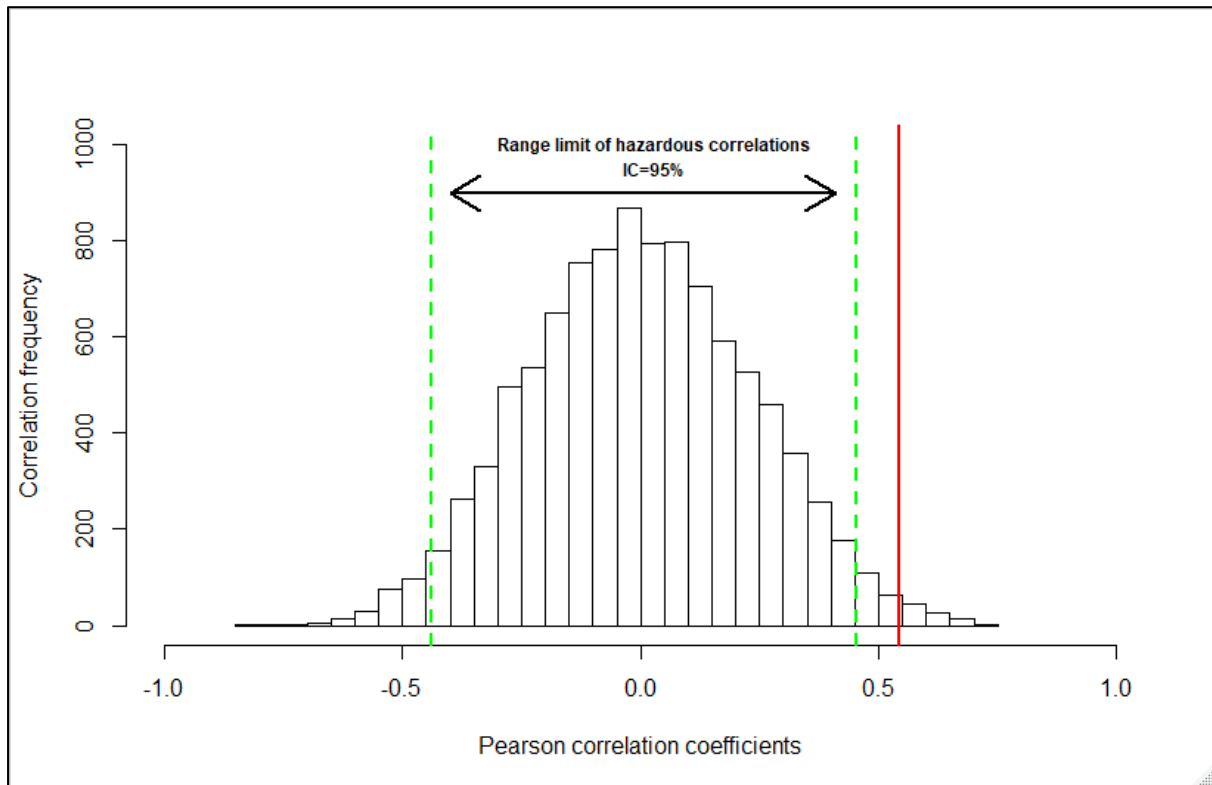


Figure S7. Bootstrapping from the dataset of the significant relationship between Σ PCBs and egg-angular change for female kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient ($r = -0.48$) between Σ PCBs and egg-angular change in female kittiwakes.

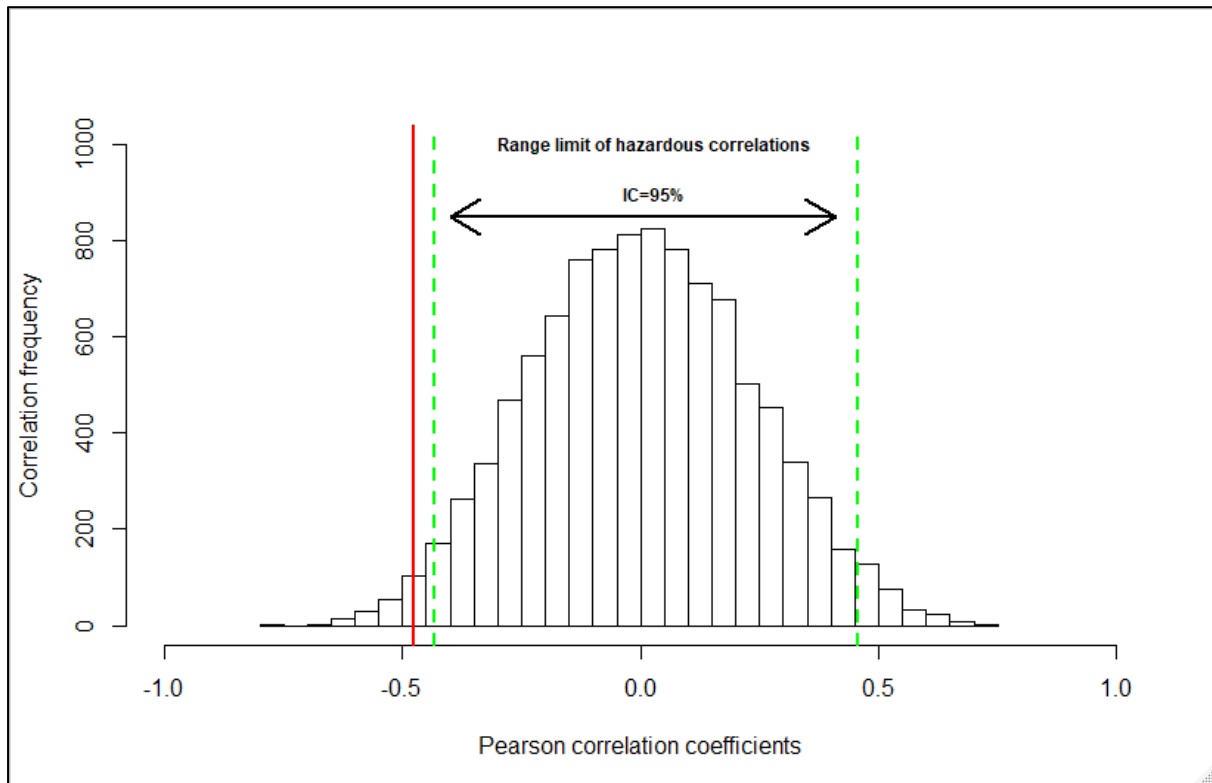


Figure S8. Bootstrapping from the dataset of the significant relationship between PFNA and egg-angular change for male kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient ($r = 0.56$) between PFNA and egg-angular change for male kittiwakes.

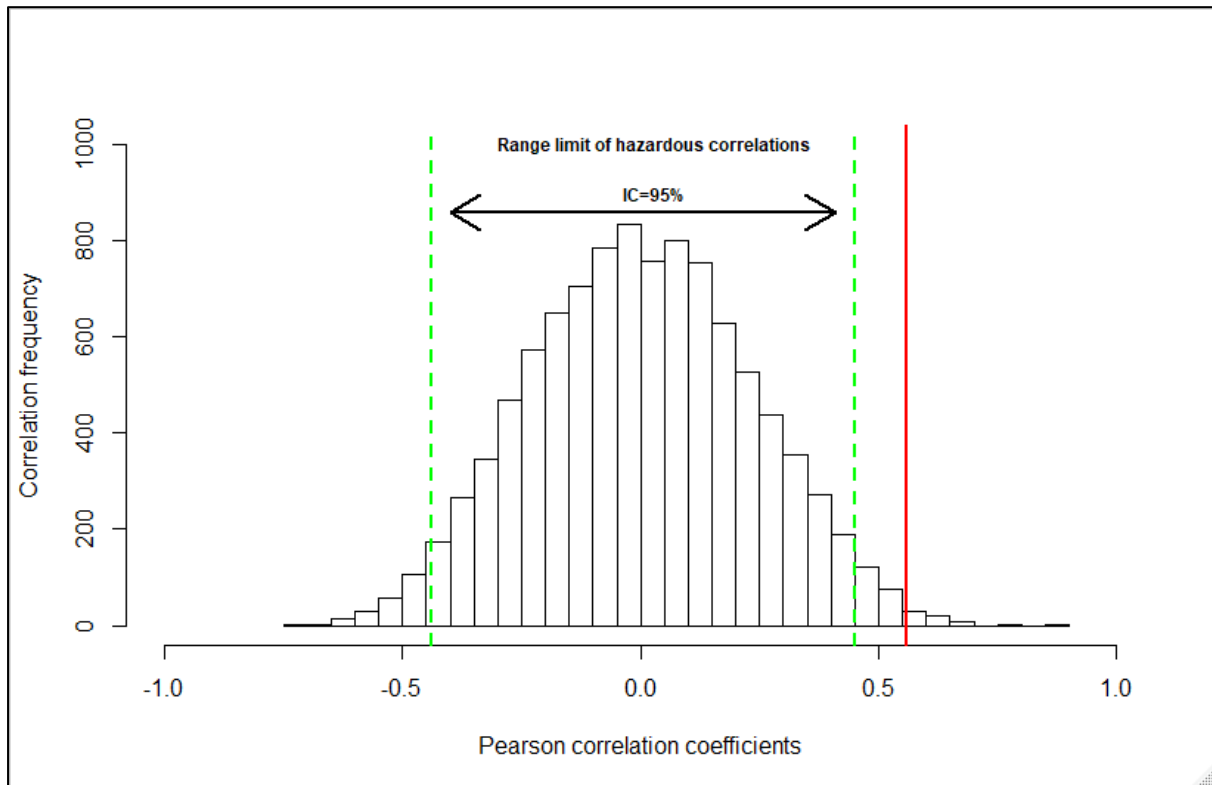


Figure S9. Bootstrapping from the dataset of the significant relationship between PFTeA and egg-turning frequency for male kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient ($r = 0.49$) between PFTeA and egg-turning frequency for male kittiwakes.

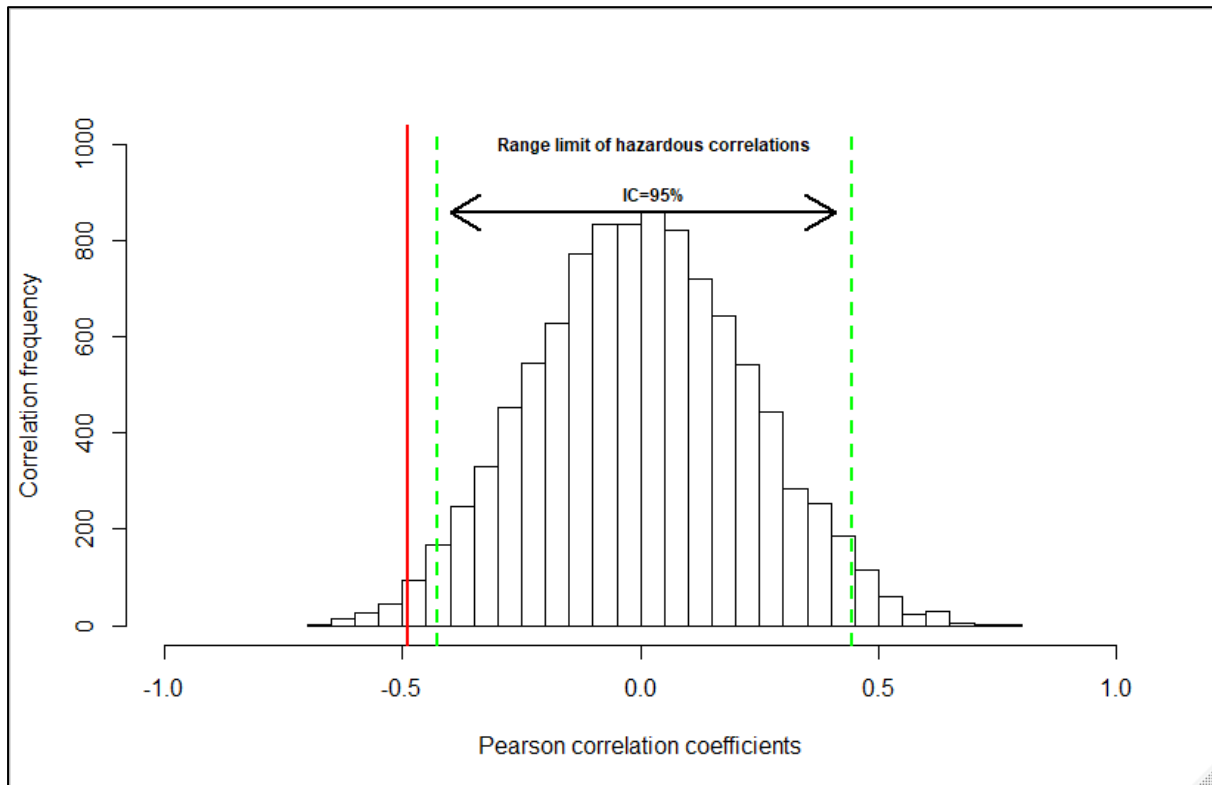


Figure S10. Relationships between egg angular change and a) mean incubation temperature and b) minimum incubation temperature in female and male incubating kittiwakes, *Rissa tridactyla*, from Kongsfjord, Svalbard. Incubation temperature data are published in Blévin et al., 2018. White symbols correspond to males and black symbols to females.

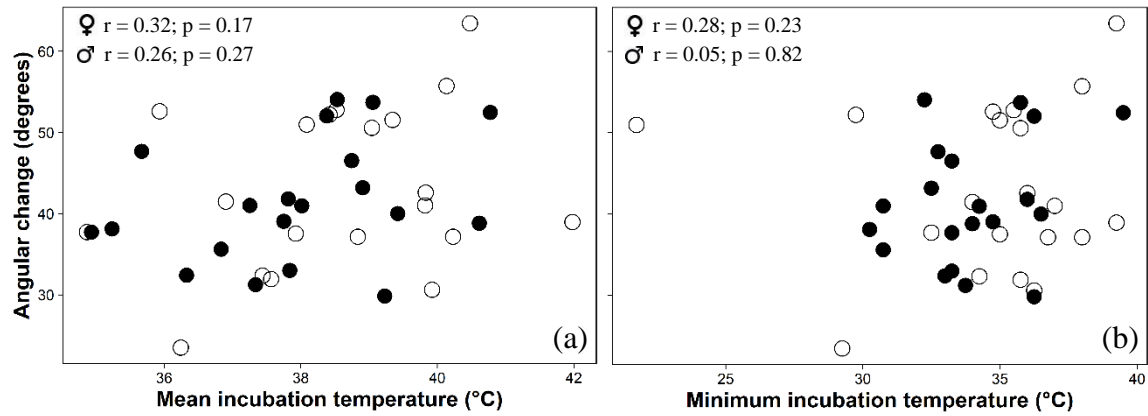


Figure S11. Relationships between egg-turning frequency and a) mean incubation temperature and b) minimum incubation temperature in female and male incubating kittiwakes, *Rissa tridactyla*, from Kongsfjord, Svalbard. Incubation temperature data are published in Blévin et al., 2018. White symbols correspond to males and black symbols to females.

