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Mercury exposure, stress and prolactin secretion in an Arctic seabird: an experimental study

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Summary

1. Life-history theory predicts that long-lived organisms should reduce parental effort under inclement environmental conditions in order to favour long-term survival.
2. Seabirds are long-lived top predators often exposed to environmental endocrine disrupting chemicals such as mercury (Hg). Hg-contaminated birds show disrupted parental behaviour.
3. Avian parental behaviour is governed by two key hormones in birds: corticosterone (CORT, a glucocorticoid hormone) and prolactin (PRL, a pituitary hormone involved in parental care). Any disruption of these hormones may alter the ability of an individual to adjust parental behaviour to environmental conditions.
4. The first aim of this study was to describe the relationships between blood Hg concentrations, plasma PRL and reproductive performance in Arctic black-legged kittiwakes (*Rissa tridactyla)*. We a found negative relationship between plasma initial PRL and blood Hg concentrations in males. Moreover, Hg concentration was negatively related to breeding success in chick-rearing males.
5. Secondly, to study the effect of a chronic increase in CORT levels on the Hg–PRL relationship, we experimentally increased stress with CORT pellet implantation. We predicted that Hg and CORT would act synergistically on PRL and an increase in CORT concentration would steepen the Hg–PRL relationship. However, adding CORT did not steepen the Hg–PRL relationship. Hatching success was significantly lower in CORT-implanted males than in controls, and breeding success was not reduced in CORT-implanted male kittiwakes with high levels of blood Hg.
6. Our results suggest that Hg may impair reproductive performance through a disruption of PRL secretion. Contrary to our prediction, Hg and CORT did not act synergistically and the underlying mechanisms associating CORT and Hg with PRL might be more complex than a single interaction between two factors.

Key-words: arctic, black-legged kittiwake, breeding success, contaminants, corticosterone, endocrine disruptors, parental investment, parenting hormone

Introduction

Parental investment is governed by a trade-off between the benefits and costs of resource allocation to current vs. future reproduction (Clutton-Brock 1991; Stearns 1992). When facing stressful conditions, such as inclement weather, food deprivation or predation risk, breeding adults have to decide whether to keep caring for their offspring or to desert their brood, thereby favouring their own survival. Adjustments of behaviour to environmental changes are often mediated by physiology, and more

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specifically by hormonal mechanisms which orchestrate life-history decisions in vertebrates (Flinn *et al*. 1996; Nunes *et al*. 2001; Ricklefs & Wikelski 2002; Storey *et al*. 2006; O'Connor *et al*. 2011). Thus, investigating the hormonal regulation of parental behaviour is relevant to evaluate how parents modulate their parental investment according to specific environmental conditions.

Glucocorticoid hormones (cortisol, corticosterone, CORT) play a major role in the modulation of parental investment in vertebrates (Bonier *et al*. 2009) and have been widely studied in birds (e.g. Wingfield & Kitaysky 2002). During stressful events, the release of glucocorticoids triggers physiological and behavioural adjustments that shift energy investment away from reproduction and redirect it towards self-preservation and hence survival (Kitaysky, Wingfield & Piatt 2001; Angelier *et al*. 2009; Bókony *et al*. 2009). Far less studied, the hormone prolactin (PRL) can also mediate the life-history trade-off between reproduction and survival in free-living birds (Storey *et al*. 2006; Angelier & Chastel 2009). The release of this pituitary hormone facilitates parental behaviours such as egg incubation and brood provisioning (Buntin 1996). During a stressful situation, in concert with the increase in CORT, circulating PRL has been shown to decrease in several bird species (Angelier & Chastel 2009) and this could ultimately trigger nest desertion if PRL levels remain low during a prolonged period (e.g. Angelier & Chastel 2009; Spée *et al*. 2010, 2011).

Therefore, PRL secretion plays a key role in mediating parental investment in birds (Angelier & Chastel 2009) and any disruption of PRL may alter the ability of an individual to adjust reproductive decisions to environmental conditions. There is growing evidence that some environmental contaminants may be able to impair reproductive decisions. For example, elevated mercury (Hg) concentrations in blood, a non-essential trace metal, have been associated with a higher probability of skipping breeding in black-legged kittiwakes *Rissa tridactyla* (kittiwake henceforth) (Tartu *et al*. 2013) and a higher occurrence of temporary egg desertion in snow petrels *Pagodroma nivea* (Tartu *et al*. 2015b). Furthermore, highly Hg-polluted great northern divers *Gavia immer* chicks spent less time back-riding (Nocera & Taylor 1998), which probably indicates lower parental commitment. Such impaired reproductive decisions/behaviours can have negative fitness consequences: free-ranging Carolina wrens *Thryothorus ludovicianus* and tree swallows *Tachycineta bicolor* that reproduced in Hg-contaminated areas produced fewer fledglings (Brasso & Cristol 2008; Jackson *et al*. 2011). Additionally, long-term breeding success was negatively impacted by Hg in wandering albatrosses *Diomedea exulans,* south polar skuas *Catharacta maccormicki* and brown skuas *Catharacta lonnbergi* (Goutte *et al*. 2013, 2014) and breeding probability was negatively impacted by Hg in kittiwakes (Goutte *et al*. 2015).

Hg is a well-established endocrine disruptor in vertebrates, interfering with thyroid, adrenal and reproductive systems (Tan, Meiller & Mahaffey 2009). Given the relationships between Hg and parental investment, it is conceivable that Hg exposure could alter PRL secretion. The Hg–PRL relationships have principally been explored in human studies with inconsistent patterns: increased, decreased or unchanged serum PRL concentrations in relation to increasing Hg concentrations (Barregård *et al*. 1994; Lucchini *et al*. 2002; Carta *et al*. 2003). In birds, only a handful of studies have reported negative association between some environmental contaminants and PRL (i.e. petroleum and organohalogen pollutants, Cavanaugh *et al*. 1983; Verreault *et al*. 2008). To date, only one study has investigated the relationship between Hg and PRL: in male snow petrels, PRL concentrations decreased with increasing blood Hg concentrations (Tartu *et al*. 2015b). This study suggested that, at least in this seabird species, Hg can disrupt PRL secretion (Tartu *et al*. 2015b). Given the scarcity of studies on Hg–PRL relationships in free-living birds, more studies are needed to confirm the potential role of Hg in avian PRL disruption.

Here, we investigated the relationship between total blood Hg (comprising both organic and inorganic Hg), plasma initial PRL concentrations and reproductive performance in Arctic breeding kittiwakes (Svalbard archipelago). The Arctic is considered a sink for Hg deposition (Ariya *et al*. 2004), and marine apex predators, such as seabirds, are particularly exposed to Hg through their diet (reviewed in Dietz *et al*. 2013). The first aim of this study was to describe the natural covariation between blood Hg and PRL concentrations, and reproductive performance. If Hg functions as an endocrine disruptor in this species, we predicted that plasma initial PRL concentrations would decrease with increasing Hg concentration in blood (Fig. 1a) and that kittiwakes bearing high levels of blood Hg would have lower reproductive performance. The second aim of this study was to test the effects of an additional stressor on the PRL–Hg relationship. Experimentally elevated CORT levels are known to decrease PRL concentrations and breeding success in kittiwakes (Angelier *et al*. 2009). Because a recent seabird study has reported decreased PRL secretion in relation to blood Hg concentrations (Tartu *et al*. 2015b), we asked whether the negative effect of elevated CORT levels on PRL levels can be influenced by blood Hg concentrations. As the Arctic is facing multiple environmental challenges including increasing anthropogenic disturbance and rapid climate and habitat changes (Clarke & Harris 2003; Smetacek & Nicol 2005; Gabrielsen 2007), these environmental stressors combined with contaminants, such as Hg, may have additive or synergistic negative effects on wildlife (Jenssen 2006; Hooper *et al*. 2013). To test this hypothesis, we experimentally increased plasma CORT concentrations through the implantation of exogenous CORT pellets, to mimic stressful conditions. We predicted that if Hg contamination combined with other environmental stressors has a synergistic effect on PRL, then (i) the negative relationship between Hg contamination and PRL would be steeper in

the presence of CORT (Fig. 1b) and (ii) the negative effect of higher Hg blood concentrations on breeding success would be magnified by the CORT treatment.

Materials and methods

ETHIC STATEMENT AND STUDY AREA

The sampling of birds was approved by the Governor of Svalbard, and national guidelines for ethical treatment of experimental animals were followed (NARA, FOTS id 4214, 5264, 6363). The study was conducted at Kongsfjorden, Svalbard (78°54′N, 12°13′E), during three consecutive breeding seasons from 2012 to 2014.

BLOOD SAMPLING AND CORT IMPLANT

In 2012 from June 19th to July 4th, we caught 92 incubating kittiwakes (48 females and 44 males) and from July 10th to July 27th, 38 chick-rearing kittiwakes (17 females and 21 males). Birds were caught on their nest with a noose at the end of a 5-m fishing rod. We collected a first blood sample (*c.* 0·2 mL) immediately after capture, from the alar vein with a 1-mL heparinized syringe and a 25-gauge needle to assess 'initial PRL' (Chastel *et al.* 2005) and Hg concentrations. Bleeding time (i.e. time elapsed from capture to the end of the first blood sample) was on average 2 min 28 ± 12 s (SD).

In 2013, we conducted a follow-up experimental study only on males, because male kittiwakes bear higher levels of Hg and they seem to be more sensitive to Hg contamination (Tartu *et al.* 2013). From June 27th to July 11th, we caught 43 incubating males to determine initial PRL (which was measured in 42 samples, plasma volume was too small in one sample) and Hg concentrations. Immediately after the first blood sample (2 min 21 ± 20 s, SD), male kittiwakes were randomly allocated to either a treatment or a control group and were implanted subcutaneously with either a CORT (25 mg pellet per 15-day release, G111, $n = 22$) or a placebo (15-day release, C111, $n = 21$) biodegradable pellet without anaesthesia. These groups are referred to as CORT and control, respectively. We obtained pellets from Innovative Research of America (Sarasota), and surgical equipment was sterilized with 90% alcohol. We performed a small incision (*c.* 5 mm) on the nape of the kittiwakes with a sterilized surgical scalpel and inserted the pellet with a sterilized bent clip. The incision was then closed with surgical glue (3M Vetbond3M, St. Paul, MN, USA) and disinfected with aluminium spray (Vetoquinol Aluspray, Vetoquinol UK Limited, Buckingham, UK). The operation lasted for *c.* 10 min. The implantation day was denoted as 'day 0'. To validate the CORT treatment, we recaptured 4–5 CORT and four control birds (different individuals each time) at days 1, 2, 3 and 7 that were subjected to a blood sampling. At day 11, we recaptured 17 CORT and 20 control birds out of the 43 implanted birds. They were sampled for PRL concentrations and blood Hg.

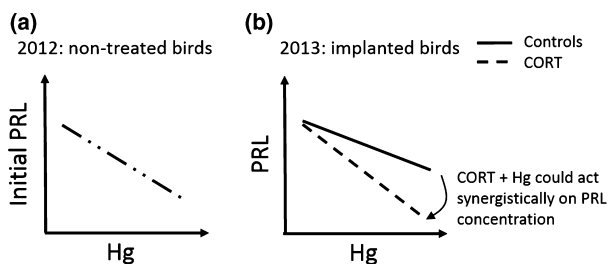

Fig. 1. Predicted relationship between plasma initial prolactin (PRL) and blood mercury (Hg) levels in black-legged kittiwakes: (a) we predict that initial PRL would be negatively associated with Hg. (b) If Hg contamination and stress hormone (corticosterone, CORT) act synergistically on PRL, then the negative relationship between Hg contamination and PRL would be steeper in CORT-implanted birds compared to controls. Long dash-dotted line refers to non-treated birds, solid line to control birds and dashed line to CORT birds.

BODY CONDITION, HATCHING SUCCESS, BREEDING SUCCESS AND RETURN RATE

We weighed kittiwakes to the nearest 2 g using a Pesola spring balance, and we measured their skull length (head+bill) to the nearest 0·5 mm with a sliding calliper. For each bird, we calculated a scaled mass index as a measure of body condition (Peig & Green 2009). Kittiwakes were individually marked with a numbered metal ring and a plastic ring engraved with a three-digit code to allow for identification from a distance. Using a mirror at the end of an 8-m fishing rod, we checked the whole plot (117 nests) every two days to monitor the number of hatchlings (thereafter 'number of eggs hatched' ranging between 0 and 3) and the number of chicks that reached at least 12 days old (thereafter 'number of chicks successfully raised' ranging between 0 and 3). In 2014, we monitored the 'return rate' of the implanted kittiwakes from 2013 by reading plastic rings using a spotting scope. The entire nesting colony was checked twice a day from June 25th to July 1st. Apparent adult survival rate in the present colony is around 85% [82–88%] (Goutte *et al.* 2015), and resighting probabilities of seabirds at breeding colonies are high because of high site fidelity (e.g. Gauthier, Milot & Weimerskirch 2012). We also monitored 'the number of eggs hatched' and 'the number of chicks successfully raised' of the kittiwakes implanted in 2013, using the same protocol as in the previous years.

MOLECULAR SEXING AND HORMONE ASSAY

We centrifuged blood samples; plasma was separated and stored at −20 °C until assayed. After centrifugation, red blood cells were kept frozen for Hg analysis as well for molecular sexing. The sex was determined by polymerase chain reaction amplification of part of two highly conserved genes (CHD) present on the sex chromosomes. Analyses were carried out at the Chizé laboratory, UMR 7372 (CNRS, Université de La Rochelle), as detailed in Weimerskirch, Lallemand & Martin (2005). Plasma concentrations of CORT and PRL were determined from the 2012 and 2013 samples by radioimmunoassay at Chizé laboratory, as previously validated for kittiwakes from this population (Chastel *et al.* 2005). All samples were run in one assay for both hormones. To measure intra-assay variation, we included four different reference samples 10 times in the CORT and PRL assays. From this, the intra-assay variation was 6·7% for total CORT and 7·8% for PRL.

HG DETERMINATION IN BLOOD CELLS

We measured total Hg from the 2012 and 2013 samples at Littoral Environnement et Sociétés laboratory as described by Bustamante *et al.* (2006) from freeze-dried and powdered red blood cells (hereafter called 'blood') in an Advanced Hg Analyzer Spectrophotometer (Altec AMA 254). At least two aliquots ranging from 5 to 10 mg were analysed for each individual, and quality assessment was measured by repeated analyses of certified reference material TORT-2 (lobster hepatopancreas, NRCC; certified value 0·27 ± 0·06 μg g^{-1}). Recoveries ranged from 99·16 ± 0·77%. Hg concentrations are expressed in μg g^{-1} dry weight (dw).

STATISTICAL ANALYSES

All analyses were performed using R 2.13.1 (R Core Team 2011) and are detailed in supporting information (see Appendix S1).

Results

RELATIONSHIPS BETWEEN HG, CORT, PRL AND REPRODUCTIVE PERFORMANCE IN 2012

In 2012, blood Hg concentrations were significantly higher in male than in female kittiwakes (GLM, $F_{1,127} = 47{\cdot}7$, $P < 0{\cdot}001$) and in incubating birds compared to chick-rearing birds (GLM, $F_{1,127} = 47{\cdot}3$, $P < 0{\cdot}001$). Males bore higher blood Hg concentrations than females during the incubation and chick-rearing period (sex × breeding stage: GLM, $F_{1,127} = 5{\cdot}1$, $P = 0{\cdot}026$). In 2012, we found no significant relationships between Hg and initial CORT concentrations in neither male nor female kittiwakes nor at any breeding stage (GLM, $F < 3{\cdot}3$, $P > 0{\cdot}075$).

In male kittiwakes, initial PRL concentrations were negatively associated with blood Hg concentrations, regardless of the breeding stage (incubation: GLM, $F_{1,39} = 4{\cdot}2$, $P = 0{\cdot}047$, Fig. 2a; chick-rearing: $F_{1,18} = 10{\cdot}7$, $P = 0{\cdot}004$, Fig. 2c), whereas in female kittiwakes, initial PRL concentrations were unrelated to blood Hg concentrations during neither incubation nor chick-rearing period (GLM, $F < 16$, $P > 0{\cdot}230$ for all tests, Fig. 2b, d). Blood Hg concentrations during the incubation period were unrelated to the number of eggs that hatched in both sexes (GLM, $F_{1,43} < 0{\cdot}1$, $P > 0{\cdot}718$). In chick-rearing kittiwakes, all sampled birds had a clutch with two eggs, and the number of successfully raised chicks was either 1 or 2. Blood Hg concentrations during the chick-rearing period were higher in males that successfully raised one chick compared with those that raised two chicks (GLM, $\chi^2 = 6{\cdot}3$, $P = 0{\cdot}012$, Fig. 3a), while the latter relationship was not observed in chick-rearing females (GLM, $\chi^2 = 0{\cdot}1$, $P = 0{\cdot}822$, Fig. 3b).

VALIDATION OF THE EXPERIMENTAL CORT TREATMENT: EFFECT ON CORT, PRL AND HG

On the day of implantation (day 0), Hg, initial CORT and PRL concentrations were not significantly different between

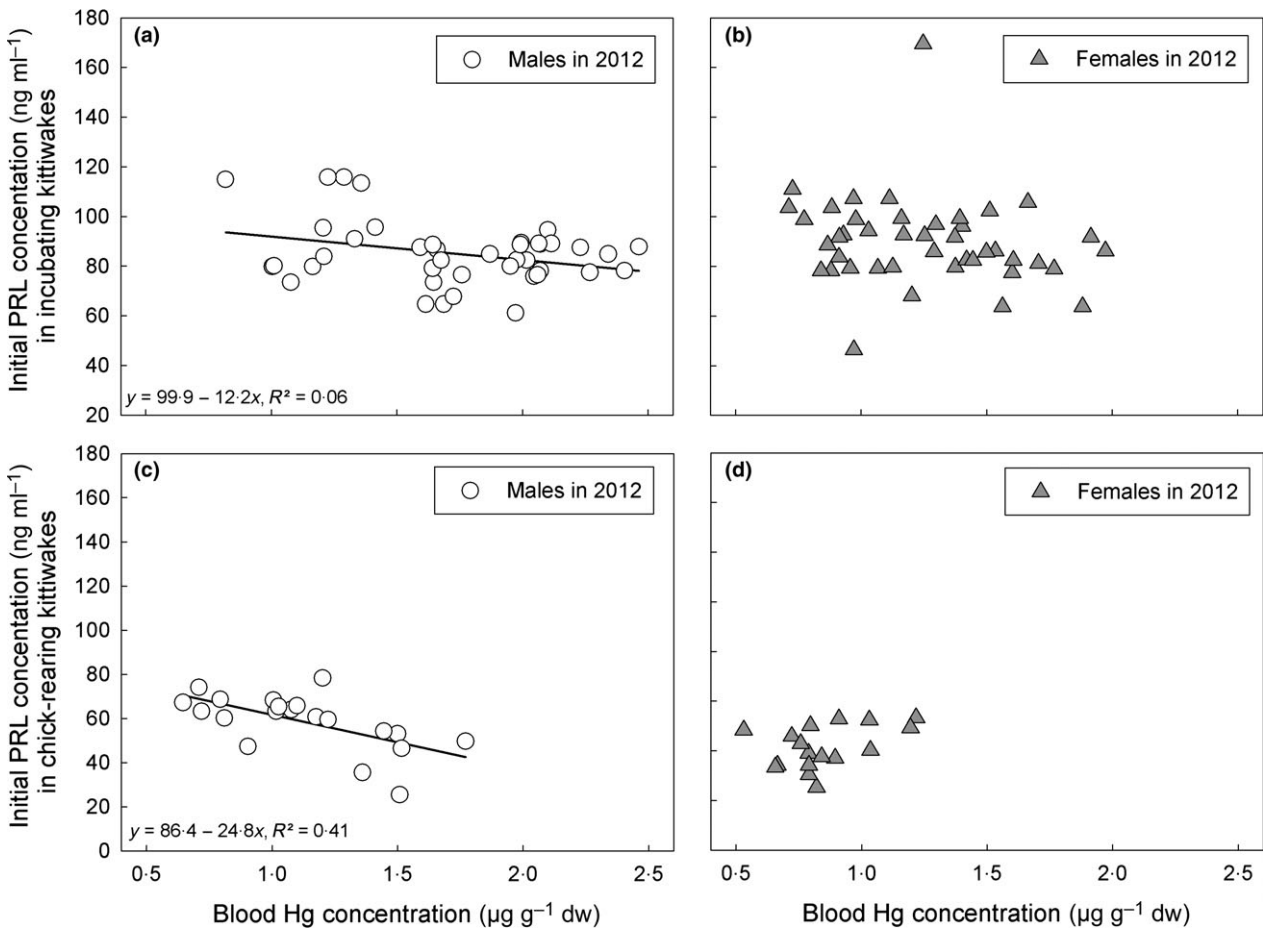

Fig. 2. Relationships between initial PRL concentrations and blood Hg concentrations in 2012's male (a) and female (b) incubating kittiwakes, and male (c) and female (d) chick-rearing kittiwakes. Small R^2 suggests that several other factors not taken into account may also influence PRL secretion. Closed triangles denote females and open circles denote males; solid lines refer to statistically significant linear regressions for males.

the treatment and control groups (GLM, $F_{1,41} < 2{\cdot}16$, $P > 0{\cdot}154$). CORT concentrations were significantly related to the sampling day (GLMM, $F_{5,61} = 4{\cdot}5$, $P = 0{\cdot}002$, Fig. 4a), to the interaction between sampling day and treatment (GLMM, $F_{5,61} = 6{\cdot}6$, $P < 0{\cdot}001$, Fig. 4a), but not to the treatment alone (GLMM, $F_{1,41} = 2{\cdot}0$, $P = 0{\cdot}168$). Specifically, CORT significantly increased within 1 day: plasma CORT concentrations reached at this time ($45{\cdot}22 \pm 5{\cdot}66$ ng mL^{-1}) were similar to capture/restraint-induced CORT concentrations measured in incubating male kittiwakes in 2013 ($43{\cdot}03 \pm 8{\cdot}94$ ng mL^{-1}), and to unmanipulated CORT concentrations observed in breeding kittiwakes when food shortages and stressful events occur (Kitaysky, Wingfield & Piatt 1999). At days 2 and 3, CORT started to decrease, but remained significantly higher compared to controls until reaching concentrations similar to controls at days 7 and 11. PRL concentrations were significantly related to sampling day, treatment and interaction (GLMM, $F_{5,61} = 4{\cdot}9$, $P < 0{\cdot}001$, $F_{1,41} = 40{\cdot}4$, $P < 0{\cdot}001$ and $F_{5,61} = 3{\cdot}1$, $P = 0{\cdot}015$, respectively): PRL concentrations remained unchanged in controls (day 0: $89{\cdot}19 \pm 8{\cdot}94$ ng mL^{-1}, day 11: $83{\cdot}36 \pm 13{\cdot}25$ ng mL^{-1}, Fig. 4b), while these concentrations significantly decreased over 11 days in the CORT birds (day 0: $90{\cdot}80 \pm 11{\cdot}96$ ng mL^{-1}, day 11: $50{\cdot}55 \pm 15{\cdot}67$ ng mL^{-1}, Fig. 4b). Contrary to what we expected, the CORT increase was not constant over 15 days. It rather triggered a 3-day-long CORT surge with kinetics of CORT and PRL very similar to the ones reported previously in the same species implanted with Silastic tubes filled with crystallized CORT (Angelier *et al.* 2007, 2009).

Body condition, calculated from biometric measurements taken on day 0, treatment and interactions did not influence PRL concentration at day 11 (GLMM, $P > 0{\cdot}05$ for all tests). Additionally, treatment did not influence body condition at day 11 (GLM, $F_{1,35} = 2{\cdot}1$, $P = 0{\cdot}160$).

RELATIONSHIPS BETWEEN HG AND PRL AFTER AN EXPERIMENTAL INCREASE IN CORT DURING 11 DAYS

Prolactin changes between day 0 and day 11 (PRL day 11 – PRL day 0) were only related to the treatment (GLM, $F_{1,32} = 49{\cdot}4$, $P < 0{\cdot}001$). They were not related to Hg concentrations at day 0 nor to the interaction of Hg day 0 with treatment (GLM, $F_{1,32} < 0{\cdot}1$, $P > 0{\cdot}830$). PRL concentrations measured at day 11 were not related to blood Hg concentrations at day 11 (GLM, $F_{1,33} < 0{\cdot}1$, $P = 0{\cdot}832$); however, they were significantly related to the treatment ($F_{1,33} = 35{\cdot}6$, $P < 0{\cdot}001$, Fig. 5) and to the interaction between the treatment and Hg at day 11 ($F_{1,33} = 5{\cdot}3$, $P = 0{\cdot}028$, Fig. 5). Specifically, in control birds at day 11, PRL significantly decreased with increasing Hg concentrations (GLM, $F_{1,18} = 4{\cdot}5$, $P = 0{\cdot}048$), whereas no relationship was found between Hg and PRL in the CORT group.

EFFECTS OF THE CORT TREATMENT AND HG CONTAMINATION ON REPRODUCTIVE PERFORMANCE

The number of hatched eggs was significantly higher in the controls than in the CORT birds (GLM, $F_{1,36} = 5{\cdot}4$, $P = 0{\cdot}026$), but this relationship was independent of Hg concentrations at day 0 or interaction between Hg and treatment (GLM, $F < 0{\cdot}5$, $P > 0{\cdot}474$ for all tests). In all experimental birds (CORT and controls), the number of chicks successfully raised was not associated with Hg concentrations at day 0 (GLM, $F < 2{\cdot}1$, $P > 0{\cdot}155$ for all tests).

EFFECTS OF CORT IMPLANT AND HG ON RETURN RATE, HATCHING AND BREEDING SUCCESS IN 2014

In 2014, significantly less CORT-implanted male kittiwakes were resighted compared to control males (10

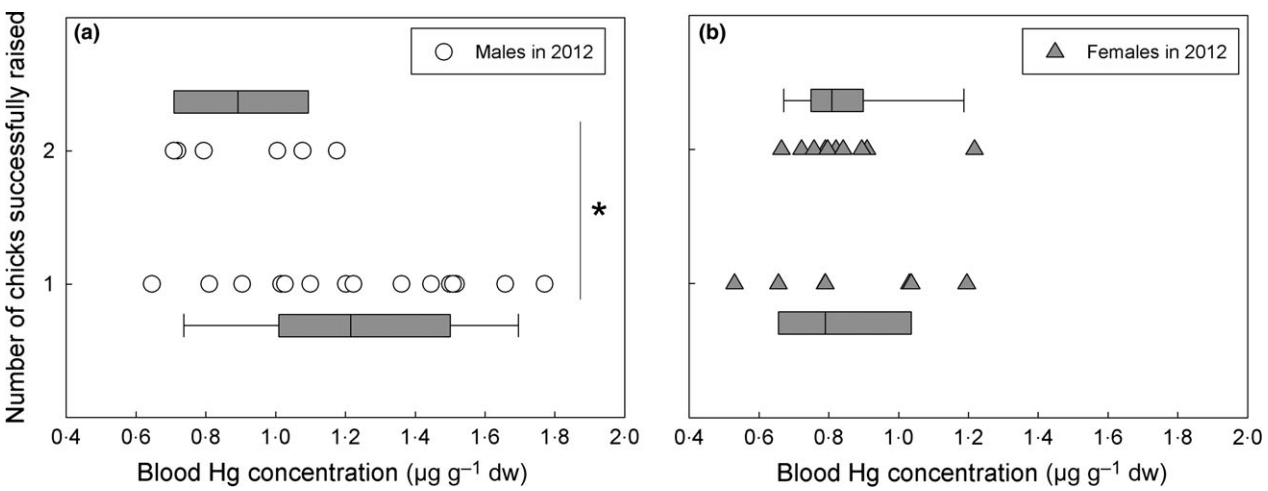

Fig. 3. Relationships between Hg concentrations in 2012's male (a, open circles) and female (b, closed triangles) chick-rearing kittiwakes in relation to the number of chicks successfully raised. All sampled birds had a two-egg clutch with at least one chick that survived. Two females that successfully raised one chick had very close Hg values (0·790 and 0·789 µg g^{-1}); consequently, their triangles overlap. Boxes represent median, first and second quartiles, whiskers indicate the 10th and 90th percentiles. *denotes significant difference.

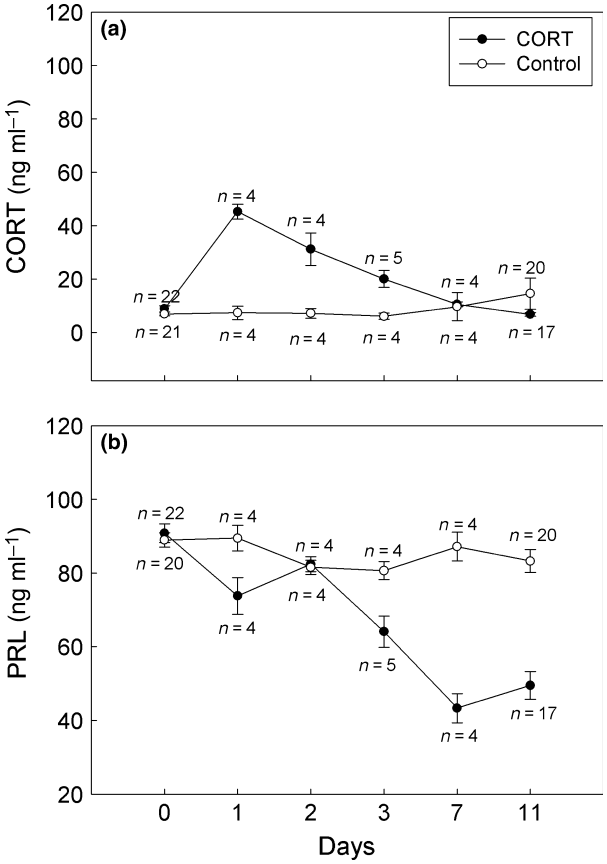

Fig. 4. Effects of CORT and control pellets on CORT (a) and PRL (b) concentrations, in incubating male kittiwakes, in 2013. Open circles denote control-implanted males, and closed circles denote CORT-implanted males. Values are mean ± standard error and '*n*' denotes sample sizes.

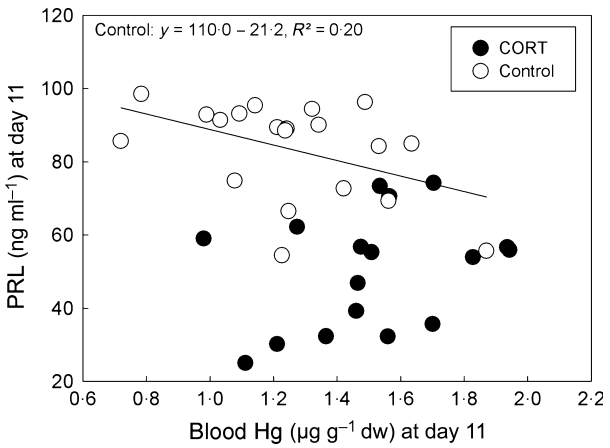

Fig. 5. PRL concentrations (ng mL^{-1}) at day 11 in relation to Hg concentrations in blood at day 11 (μg g^{-1} dw). Data shown are 2013's incubating male kittiwakes, open circles denote controls, and closed circles denote CORT-implanted birds. Solid line refers to statistically significant linear regressions.

CORT birds non-observed out of 22 implanted vs. three control birds non-observed out of 21 implanted, GLM, $\chi^2 = 3{\cdot}9$, $P = 0{\cdot}048$). We found no effect of blood Hg concentrations in 2013 or interaction between Hg and treatment (GLM, Hg 2013: $\chi^2 < 0{\cdot}1$, $P = 0{\cdot}820$; Hg 2013 × treatment: $\chi^2 = 0{\cdot}9$, $P = 0{\cdot}355$) on return rate. The number of hatched eggs or chicks successfully raised in 2014 was not affected by the treatment, Hg concentrations in the previous year and interactions (GLM, $\chi^2 < 0{\cdot}2$, $P > 0{\cdot}664$ for all tests).

Discussion

The aim of this study was to investigate the relationships between blood Hg and PRL concentrations in breeding kittiwakes. In line with our first prediction, we report a negative relationship between plasma initial PRL and blood Hg concentrations during incubating and chick-rearing periods in 2012 in male kittiwakes. Furthermore, in 2012, blood Hg concentrations measured in chick-rearing males were negatively related to breeding success. Regarding the experimental manipulation of CORT concentrations, we observed, as in 2012, a negative relationship between plasma PRL and blood Hg in control males. However, contrary to our prediction, the experimental CORT increase did not steepen the PRL–Hg relationship at day 11.

RELATIONSHIP BETWEEN PRL AND HG

Similarly to our findings, stress-induced PRL concentrations were negatively related to increasing blood Hg concentrations in males of an Antarctic seabird, the snow petrel (Tartu *et al.* 2015b). Such negative relationships between plasma PRL and blood Hg observed in those two polar seabirds (i.e. kittiwakes and snow petrels) add new evidence that Hg seems to disrupt the secretion of pituitary hormones. This finding is also corroborated by other studies showing that increased Hg concentrations inhibit efficient production of another pituitary hormone, the luteinizing hormone (Tartu *et al.* 2013, 2014). Nonetheless, the possible mechanisms underlying these relationships still need to be clarified. Dopamine, a neurotransmitter and potent inhibitor of PRL, may play a significant role in the negative relationship between Hg and PRL (Ben-Jonathan & Hnasko 2001). It seems that organic and inorganic Hg can stimulate the spontaneous release of dopamine in laboratory rodents (Faro *et al.* 2007), but also in wild larvae of a fish (the mummichog *Fundulus heteroclitus*, Zhou *et al.* 1999) and in wild American minks *Mustela vison* (Basu *et al.* 2005). Consequently, the negative relationship observed between PRL and Hg is more likely to be indirect and could rely on an effect of Hg on the dopaminergic system. However, a causal relationship between dopamine and Hg has never been reported in birds, and the studies reporting decreased PRL secretion in relation to blood Hg in seabirds are correlational and would greatly benefit from further experimental investigations. The reason for the relationships between Hg and PRL being more visible in males as observed in snow petrels (Tartu *et al.* 2015b)

could be related to sex-specific effects of Hg. Indeed, endocrine disruption could depend on the concentrations of circulating hormones. For example, estradiol (which is higher in females) exhibits protective properties on Hg toxicity as reported in mice (Oliveira *et al.* 2006). In Svalbard kittiwakes, high blood Hg concentrations were associated with low PRL concentrations, and in chick-rearing male kittiwakes, elevated Hg concentrations were associated with lower breeding success. Consequently, the lower reproductive performance observed in highly Hg-contaminated birds may result from a disruption of PRL secretion.

WHAT HAPPENS WHEN STRESS COMES INTO PLAY?

In extreme environments, such as Polar Regions, individuals often experience harsh and unpredictable environmental conditions; therefore, they adopt specific life-history strategies in order to cope with environmental stressors. Long-lived organisms such as seabirds may refrain from breeding or desert their brood when environmental conditions are poor (e.g. Clutton-Brock 1991; Stearns 1992). These behaviours (i.e. refrain from breeding or desert reproduction) are mediated by the release of CORT during stressful events that will shift energy investment away from reproduction and redirect it towards self-preservation and hence survival (Ricklefs & Wikelski 2002; Angelier & Wingfield 2013). By mimicking a stressful event, we tested whether the CORT-induced PRL decrease could be reinforced by elevated concentrations of Hg. As reported earlier in the same species (Angelier *et al.* 2009), administration of exogenous CORT resulted in a decrease in PRL concentrations. Nevertheless, contrary to our prediction, after 11 days of treatment, the PRL–Hg relationship was not steepened in CORT-implanted birds. In 2013, by artificially increasing CORT, we modified the natural physiological parameters of the birds: CORT elevation lowered PRL concentration and attenuated the PRL and CORT stress responses (i.e. the hormonal responses to capture–restraint protocol) (Angelier *et al.* 2009; Goutte *et al.* 2011). Attenuation of the CORT stress response after exogenous CORT administration shall result from a controlled downregulation of the HPA axis, in order to prevent the deleterious effects of chronic CORT secretion (Müller *et al.* 2009). The reason why PRL concentrations decrease may also be related to dopamine secretion. Indeed, in mice, the PRL decrease in relation to increasing stress is likely to be linked to a positive relationship between CORT and dopamine (Gala 1990; Piazza *et al.* 1996). Consequently, both CORT and Hg may interact with dopamine secretion, leading to a disruption of PRL secretion, yet we have no evidence for such a relationship in birds. One reason why CORT and Hg have not acted synergistically could be that CORT levels already downregulated PRL levels to such low levels that Hg contamination did not have a further detectable effect. Had we tested the PRL/Hg relationship when CORT was still elevated (i.e. days 1, 2 or 3), we might have observed a steepened PRL/Hg relationship in CORT birds. To better illustrate a possible synergistic effect between CORT and Hg, further studies need either to use lower concentrations in CORT implantation (to avoid a downregulation of the HPA axis) or to perform blood samples on the tested birds within 3 days. Regarding parenting behaviour, the inability to modulate CORT and PRL secretion may have lowered the bird's motivation to incubate which may have reduced hatching success. Additionally, CORT is known to increase self-foraging in breeding kittiwakes (Kitaysky, Wingfield & Piatt 2001; Angelier *et al.* 2007). It is thus possible that CORT-implanted males were more likely to self-forage and presumably go for longer foraging trips, leading to an asynchrony in incubating shifts. A behavioural modification in CORT-treated male kittiwakes may have constrained their partner to leave the nest unattended in order to feed themselves which may have resulted into a lower hatching success. Although in the 2012 correlative data, high Hg concentrations in blood of chick-rearing male kittiwakes were associated with poor reproductive performance, we did not observe an increased breeding failure in CORT-treated male kittiwakes most contaminated with Hg the year after. Since Hg, but also PRL, varies across the breeding cycle (Tartu *et al.* unpublished data), these Hg–fitness relationships could importantly rely on other factors such as environmental conditions or even the breeding stage when the blood sampling used to measure PRL and Hg was performed. Indeed, blood Hg concentrations were higher in incubating males in 2012 compared to 2013. Also in 2012, clutch size and hatching success were lower than those in 2013 ($P < 0{\cdot}03$ for all tests). Thus, the hazardous effects of Hg were probably more observable in 2012 when conditions were supposedly poorer.

Conclusion

In the present study, we focused on the relationship between Hg and PRL considered as a measure of parental commitment. We show Hg can disrupt PRL secretion in male kittiwakes which could lead to reduced chick survival. We were not able to highlight the fact Hg and CORT could act synergistically; however, blood sampling birds while CORT levels are still high and PRL decreases (e.g. day 1 or 3) could better inform on this hypothetical synergistic relationship. Yet the negative relationship between Hg and PRL is of concern, a spectrum of biological functions is associated with PRL such as water and electrolyte balance, growth and development, endocrinology and metabolism, brain and behaviour, reproduction, immunoregulation and protection (Bole-Feysot *et al.* 1998). Thus, a decrease in PRL concentrations with increasing blood Hg concentrations may affect not only parental commitment but also a multitude of other biological and physiological aspects for birds.

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Data accessibility

Data deposited in the Dryad Digital Repository: http://dx.doi:10.5061/dryad.tv50m (Tartu *et al.* 2015a).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Statistical analyses.