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

Sabrina Tartu*[a], Paco Bustamante[b], Frédéric Angelier[a], Ádám Z. Lendvai[c], Børge Moe[d], Pierre Blévin[a], Claus Bech[e], Geir W. Gabrielsen[f], Jan Ove Bustnes[g], Olivier Chastel[a]

[a] Centre d'Etudes Biologiques de Chizé, UMR 7372 CNRS-Université La Rochelle, Villiers-en-Bois, France

[b] Littoral Environnement et Sociétés, UMR 7266 CNRS-Université La Rochelle, La Rochelle, France

[c] Department of Evolutionary Zoology, University of Debrecen, Hungary

[d] Norwegian Institute for Nature Research, Trondheim, Norway

[e] Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

[f] Norwegian Polar Research Institute, FRAM – High North Research Centre for Climate and the Environment, Tromsø, Norway

[g] Norwegian Institute for Nature Research, FRAM – High North Research Centre for Climate and the Environment, Tromsø, Norway

*Corresponding author: tartu.sabrina@gmail.com

Running head: Mercury, stress and parental hormone in a seabird

In preparation for Functional Ecology

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 baseline PRL and blood Hg concentrations in males. Moreover, Hg concentration was negatively related to breeding success in chick-rearing males.
5. Second, to study the effect of a chronic increase of stress 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 that an increase of 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, yet 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,

the underlying mechanisms associating CORT and Hg with PRL, might be more complex than a single interaction of two factors.

Key-words: Arctic; Black-legged kittiwake; breeding success; contaminants; corticosterone; endocrine disruptors; parenting hormone; parental investment.

Introduction

Parental investment is governed by a trade-off between the benefits and costs of resource allocation to current versus future reproduction (Clutton-Brock, 1991; Stearns, 1992). When facing stressful conditions, such as inclement weather, food deprivation or predation risk, breeding adults have to take the decision to either continuing to care for their offspring or to desert current reproduction, thereby favouring their own survival. In vertebrates, adjustments of behaviour to environmental changes are often mediated by physiology, and more 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.

With regard to endocrine mechanisms, glucocorticoid hormones (cortisol, corticosterone, CORT) have been recognised to play a major role for the modulation of parental investment in vertebrates and have been widely studied in bird species: during stressful events the release of stress hormones trigger physiological and behavioural adjustments that shift energy investment away from reproduction and redirects 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 (see 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 are growing evidences 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 to defer breeding in black-legged kittiwakes (thereafter kittiwake; *Rissa trydactyla*, Tartu et al. 2013), with a higher occurrence of temporary egg desertion in snow petrels *Pagodroma nivea* Tartu et al. 2015) and in highly Hg polluted great northern divers *Gavia immer*, chicks spent less time back-riding (Nocera & Taylor 1998). 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 *Diomedoea 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. 2015). This study suggested that, at least in this seabird species, Hg shall disrupt PRL secretion (Tartu et al. 2015). 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.

We investigated the relationship between total blood Hg (comprising both organic and inorganic Hg), plasma baseline 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 baseline PRL concentrations would decrease with increasing Hg concentration in blood (**Figure 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 effect 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. 2015), 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, these environmental stressors combined to 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 to other environmental stressors have a synergistic effect on PRL, then 1) the negative relationship between Hg contamination and baseline PRL would be steeper in the presence of CORT (**Fig. 1B**) and 2) 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 111 incubating kittiwakes (56 females and 55 males) and from July 10th to July 27th, 41 chick-rearing kittiwakes (19 females and 22 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 (*ca.* 0.2 mL) immediately after capture, from the alar vein with a 1 mL heparinised syringe and a 25-gauge needle to assess 'baseline 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 sec ± 12 sec (SD).

In 2013, we conducted a follow-up experimental study only on males, as 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 baseline PRL and Hg concentrations. Immediately after the first blood sample (2 min 21 sec ± 20 sec, SD), male kittiwakes were randomly allocated either to a treatment or a control group and were implanted subcutaneously either with a CORT (25mg/pellet 15 days release, G111, N=22) or a placebo (15 days release, C111, N=21) biodegradable pellet. 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 (~5mm) on the nape of the kittiwakes with a sterilized surgical scalpel and inserted the pellet with a sterilized bent clip. The incision was then sutured with surgical glue (3M Vetbond) and disinfected with aluminium spray (Vetoquinol Aluspray). The operation lasted for approximately 10 min. The implantation day was denoted as ‘day 0’. To validate the CORT treatment, we recaptured 4 CORT and 4 control birds (different individuals each time) at days 1, 2, 3, 7 that were subjected to a ‘baseline’ blood sample. At day 11, we succeeded to recapture 16 CORT and 20 control birds out of the 43 implanted birds. They were sampled for baseline concentrations and blood Hg.

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 metal rings and PVC plastic bands engraved with a three-digit code and fixed to the bird’s tarsus for identification from a distance. Using a mirror at the end of an 8 m fishing rod, we checked the whole plot (*ca*. 117 nests) every two days to monitor the number of hatchlings (thereafter ‘number of eggs that 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 telescope. 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 that hatched’ and ‘the number of chicks that survived’ 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 lab, 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 lab, 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 4 different reference 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 lab 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). Recoveries ranged from 99.16 ± 0.77 %. Hg concentrations are expressed in µg/g dry weight (dw).

STATISTICAL ANALYSES

All analyses were performed using R 2.13.1 (R Development 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, $F_{1,149}$=59.6, P<0.001) and in incubating birds compared to chick-rearing birds (GLM, $F_{1,149}$=54.9, P<0.001). Males bore higher blood Hg concentrations than females during the incubation and chick-rearing period (sex × breeding stage: GLM, $F_{1,149}$=5.8, P=0.017). In 2012, we found no significant relationships between Hg and baseline CORT concentrations neither in male nor female kittiwakes nor at any breeding stage (GLM, F<3.3, P>0.075).

In male kittiwakes, baseline PRL concentrations were negatively associated with blood Hg concentrations, regardless of the breeding stage (incubation: GLM, $F_{1,50}$=4.5, P=0.039, **Figure 2A**; chick-rearing: $F_{1,18}$=10.7, P=0.004, **Figure 3A**), whereas in female kittiwakes baseline PRL concentrations were unrelated to blood Hg concentrations neither during incubation nor chick-rearing period (GLM, F<16, P>0.230 for all tests, **Fig. 2B, Fig. 3B**). Blood Hg concentrations during the incubation period were unrelated to the number of eggs that hatched in both sexes (GLM, $F_{1,43}$<0.1, P>0.718). In chick-rearing kittiwakes, all the sampled birds had a two eggs' clutch, and the number of chicks that survived was either 1 or 2. Blood Hg concentrations during the chick-rearing period were higher in males that successfully raised one chick compared to those which were able to successfully raise two chicks (GLM, χ^2=6.3, P=0.012, **Figure 4A**), this relationship was not observed in chick-rearing females (GLM, χ^2=0.1, P=0.822, **Fig. 4B**).

VALIDATION OF THE EXPERIMENTAL CORT TREATMENT, EFFECT ON CORT, PRL AND Hg

On the day of implantation (day 0), Hg, baseline CORT and PRL concentrations were not significantly different between the two groups (GLM, $F_{1,41}$<2.16, P>0.154). Baseline CORT concentrations were significantly related to the sampling day (GLMM, $F_{5,61}$=4.5, P=0.002, **Figure 5A**), to the interaction of sampling day and treatment (GLMM, $F_{5,61}$=6.6, P<0.001, **Fig. 5A**) but not to the treatment alone (GLMM, $F_{1,41}$=2.0, P=0.168). Specifically, baseline CORT significantly rise within 1 day, plasma CORT concentrations reached at this time (45.22 ± 5.66 ng/ml) were similar to capture-restraint induced CORT concentrations measured in incubating male kittiwakes in 2013 (43.03 ± 8.94 ng/ml), 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, baseline CORT started to decrease, but remained significantly higher compared to controls until reaching concentrations similar to controls at days 7 and 11. Baseline PRL concentrations were significantly related to sampling day, treatment and interaction (GLMM, $F_{5,61}$=4.9, P<0.001, $F_{1,41}$=40.4, P<0.001 and $F_{5,61}$=3.1, P=0.015, respectively): baseline PRL concentrations remained unchanged in controls (day 0: 89.19 ± 8.94 ng/ml, day 11: 83.36 ± 13.25 ng/ml, **Fig. 5B**) while these concentrations significantly decreased over 11 days in the CORT birds (day 0: 90.80 ± 11.96 ng/ml, day 11: 50.55 ± 15.67 ng/ml, **Fig. 5B**). Contrary to what was expected, the CORT increase was not constant over 15 days. It rather triggered a 3 days long CORT surge with following 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 baseline PRL concentration at day 11 (GLMM, P>0.05 for all tests). Additionally, treatment did not influence body condition at day 11 (GLM, $F_{1,35}$=2.1, P=0.160).

RELATIONSHIPS BETWEEN Hg AND PRL AFTER AN EXPERIMENTAL INCREASE OF CORT DURING 11 DAYS

Baseline PRL changes between day 0 and day 11 (baseline PRL day 11 – baseline PRL day 0), were only related to the treatment (GLM, $F_{1,32}$=49.4, P<0.001). They were not related to Hg concentrations at day 0 nor to the interaction of Hg day 0 with treatment (GLM, $F_{1,32}$<0.1, P>0.830). Baseline PRL concentrations measured at day 11 were not related to blood Hg concentrations at day 11 (GLM, $F_{1,33}$<0.1, P=0.832), however they were significantly related to the treatment ($F_{1,33}$=35.6, P<0.001, **Figure 6**) and to the interaction of the treatment and Hg at day 11 ($F_{1,33}$=5.3, P=0.028, **Fig. 6**). Specifically, in control birds at day 11, baseline PRL significantly decreased with increasing Hg concentrations (GLM, $F_{1,18}$=4.5, P=0.048), whereas no relationship was found between Hg and PRL in the CORT group.

EFFECTS OF THE CORT TREATMENT AND Hg CONTAMINATION ON REPRODUCTIVE PERFORMANCE

Hatching success was significantly higher in the controls than in the CORT birds (GLM, $F_{1,36}$=5.4, P=0.026), but this relationship was independent of Hg concentrations at day 0 or interaction between Hg and treatment (GLM, F<0.5, P>0.474 for all tests). In all experimental birds (CORT and controls), breeding success was not associated with Hg concentrations at day 0 (GLM, F<2.1, P>0.155 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 CORT birds non-observed out of 22 implanted vs 3 control birds non-observed out of 21 implanted, GLM, χ^2=3.9, P=0.048). We found no effect of blood Hg concentrations in 2013 or interaction of Hg and treatment (GLM, Hg 2013: χ^2<0.1, P=0.820; Hg 2013 × treatment: χ^2=0.9, P=0.355) on return rate. Hatching and breeding success in 2014 were not affected by the treatment, Hg concentrations in the previous year and interactions (GLM, χ^2<0.2, P>0.664 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 baseline 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. With regard to the experimental manipulation of CORT concentrations, we observed, as in 2012, a negative relationship between plasma baseline 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. 2015). 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 neuro-transmitter 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 2015) could be related to sex-specific effects of Hg. Indeed, endocrine disruption could depend on the concentrations of circulating hormone. 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, they therefore adopt different life-history strategies in order to cope with environmental stressors. Long-lived organisms such as seabirds may refrain from breeding or desert reproduction when environmental conditions are too 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 redirects 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 baseline 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 down-regulation 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 because CORT levels already down-regulated PRL levels to such low levels that Hg contamination did not have a further detectable effect. Maybe if we tested the PRL/Hg relationship when baseline CORT was still elevated (i.e. days 1, 2 or 3) we would observe a steepened PRL/Hg relationship in CORT birds. To better illustrate a possible synergistic effect between CORT and Hg, further studies would be needed, using either lower concentrations in CORT implantation, to avoid a down-regulation of the HPA axis or to perform blood samples on the tested birds within 3 days, when baseline CORT is still elevated. With regard to 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 et al. 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 in 2013 ($P<0.03$ 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 parental effects of PRL, however 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 of PRL concentrations with increasing blood Hg concentrations may not only affect parenting but also a multitude of other biological and physiological aspects for birds. Increasing environmental stressors in Polar Regions, such as anthropogenic disturbance, ongoing climate change or the presence of a multitude of environmental contaminants (Clarke & Harris 2003; Smetacek & Nicol 2005; Gabrielsen 2007), could therefore modify food availability and thus increase stress levels. Although we were not able to show a synergistic effect between CORT and Hg, additional experiments would be needed.

Acknowledgements

This study was supported by the Institut Polaire Français (IPEV project 330 to O. Chastel), the Agence Nationale de la Recherche (ANR project PolarTop to O. Chastel) and the CPER 13 (Contrat de Projet Etat Région) through the funding of the AMA. The authors thank C. Parenteau, C. Trouvé, S. Dano, C. Churlaud and M. Brault-Favrou for their excellent technical assistance in hormones assays, molecular sexing and Hg assays, two anonymous referees and an associate editor for their constructive and helpful comments.

Data Accessibility

Data are deposited in Dryad repository:

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1 Statistical analyses

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Figure caption

Figure 1: Predicted relationship between plasma baseline prolactin (PRL) and blood mercury (Hg) levels in black-legged kittiwakes: A) we predict that baseline PRL would be negatively associated with Hg. B) if Hg contamination has 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.

Figure 2: Relationships between baseline PRL concentrations and blood Hg concentrations in 2012's male (A) and female (B) incubating kittiwakes. Small R^2 suggest 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 regression.

Figure 3: Relationships between baseline PRL concentrations and blood Hg concentrations in 2012's male (A) and female (B) chick-rearing kittiwakes. Closed triangles denote females and open circles denote males; solid line refers to statistically significant linear regression for males.

Figure 4: Relationships between baseline Hg concentrations in 2012's male (A, open circles) and female (B, closed triangles) chick-rearing kittiwakes in relation to the number of chicks that survived. All sampled birds had a two eggs' clutch with at least one chick that survived. * denotes significant difference.

Figure 5: PRL concentrations (ng/ml) at day 11 in relation to Hg concentrations in blood at day 11 (µg/g 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.

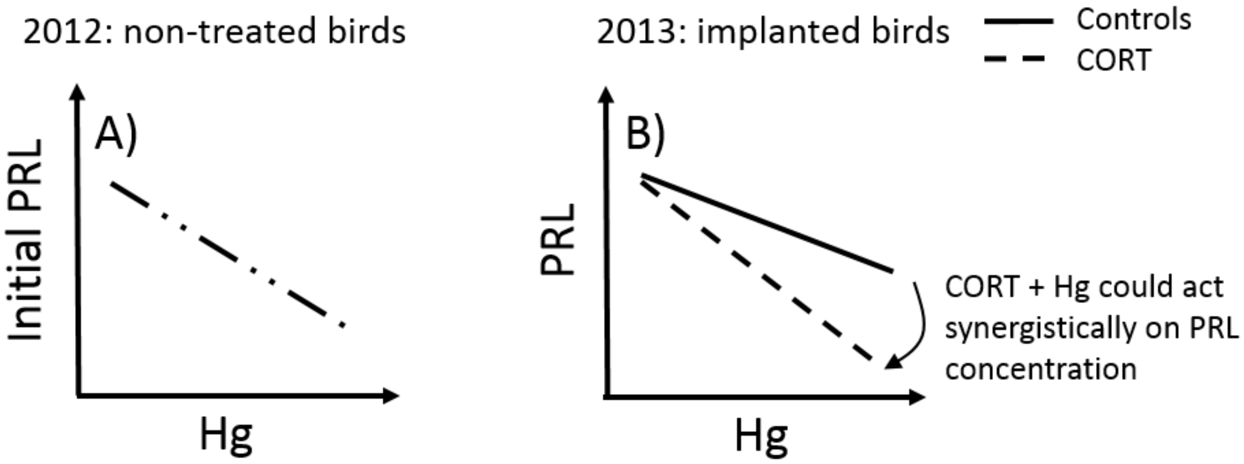
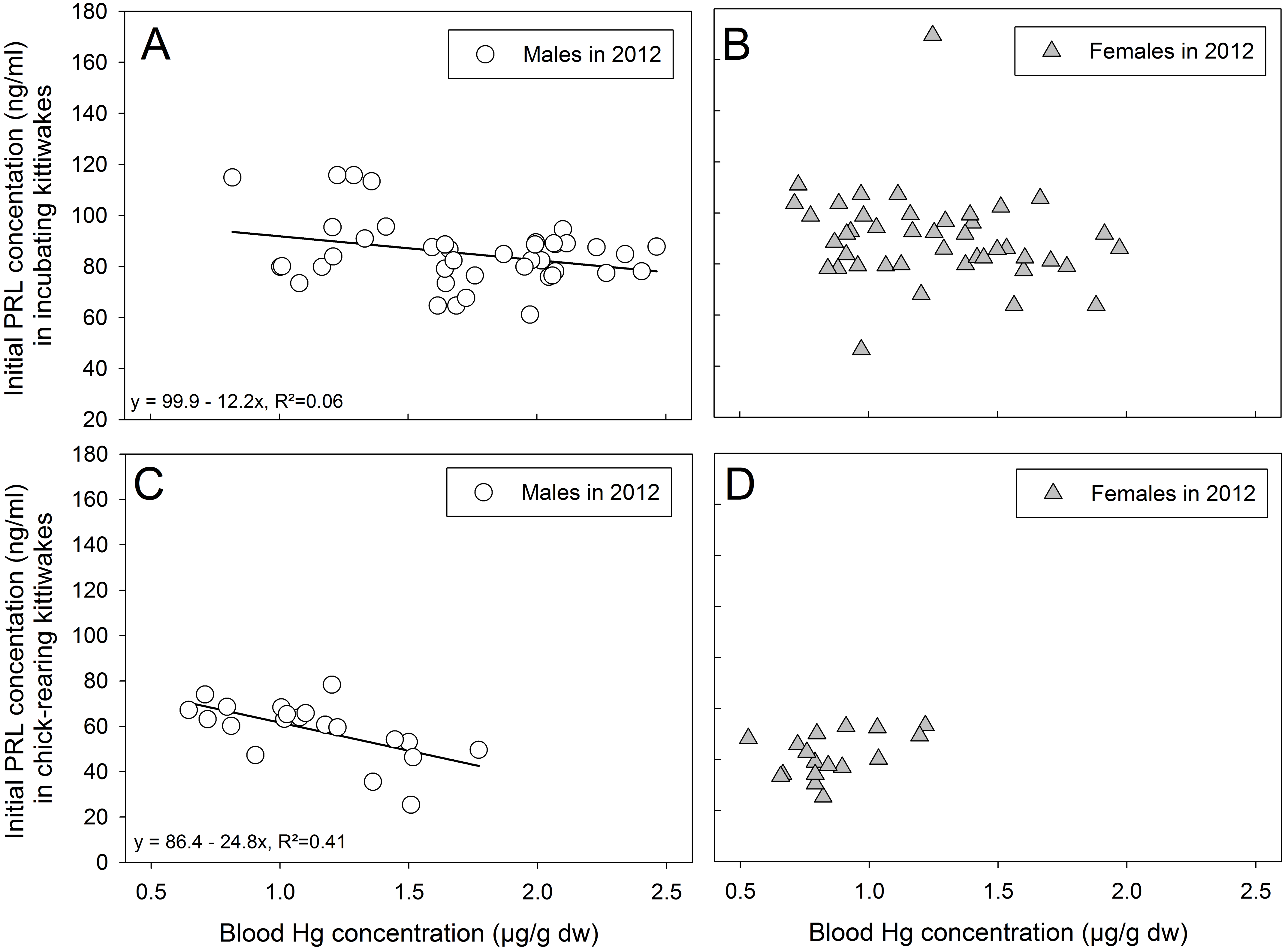
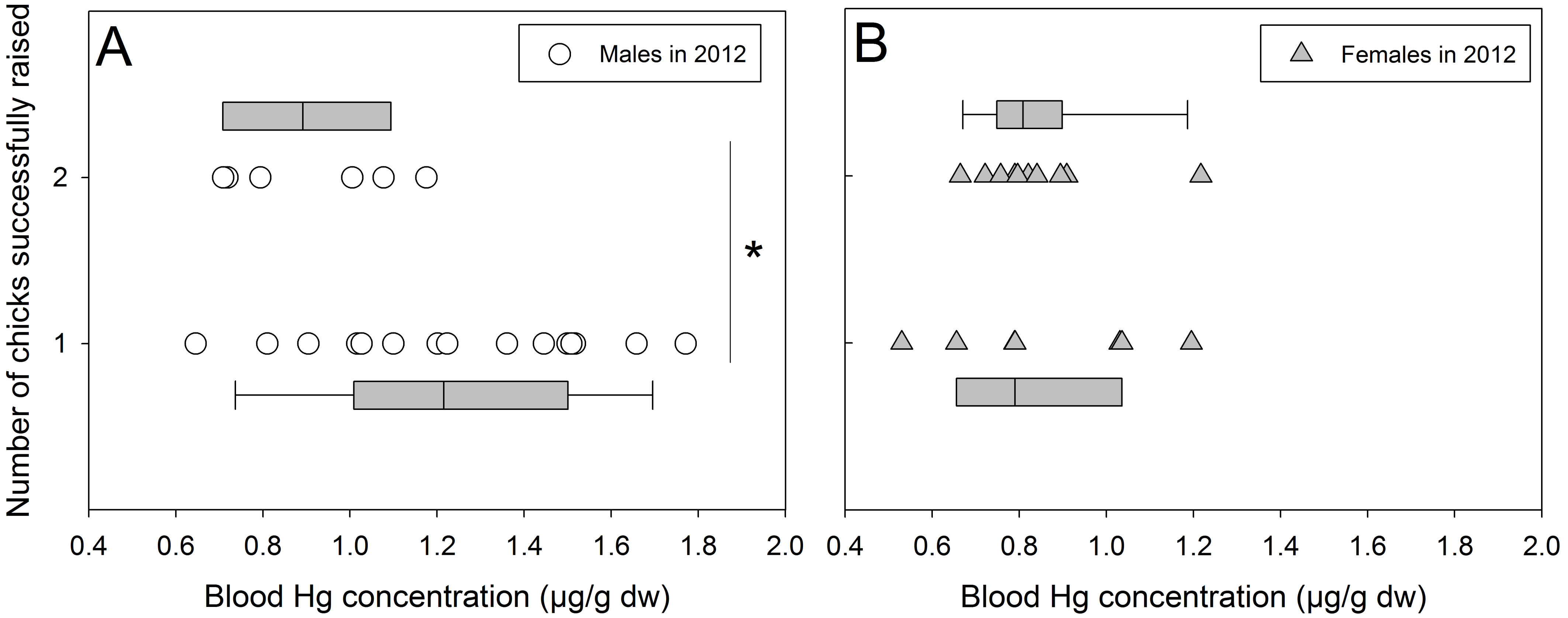
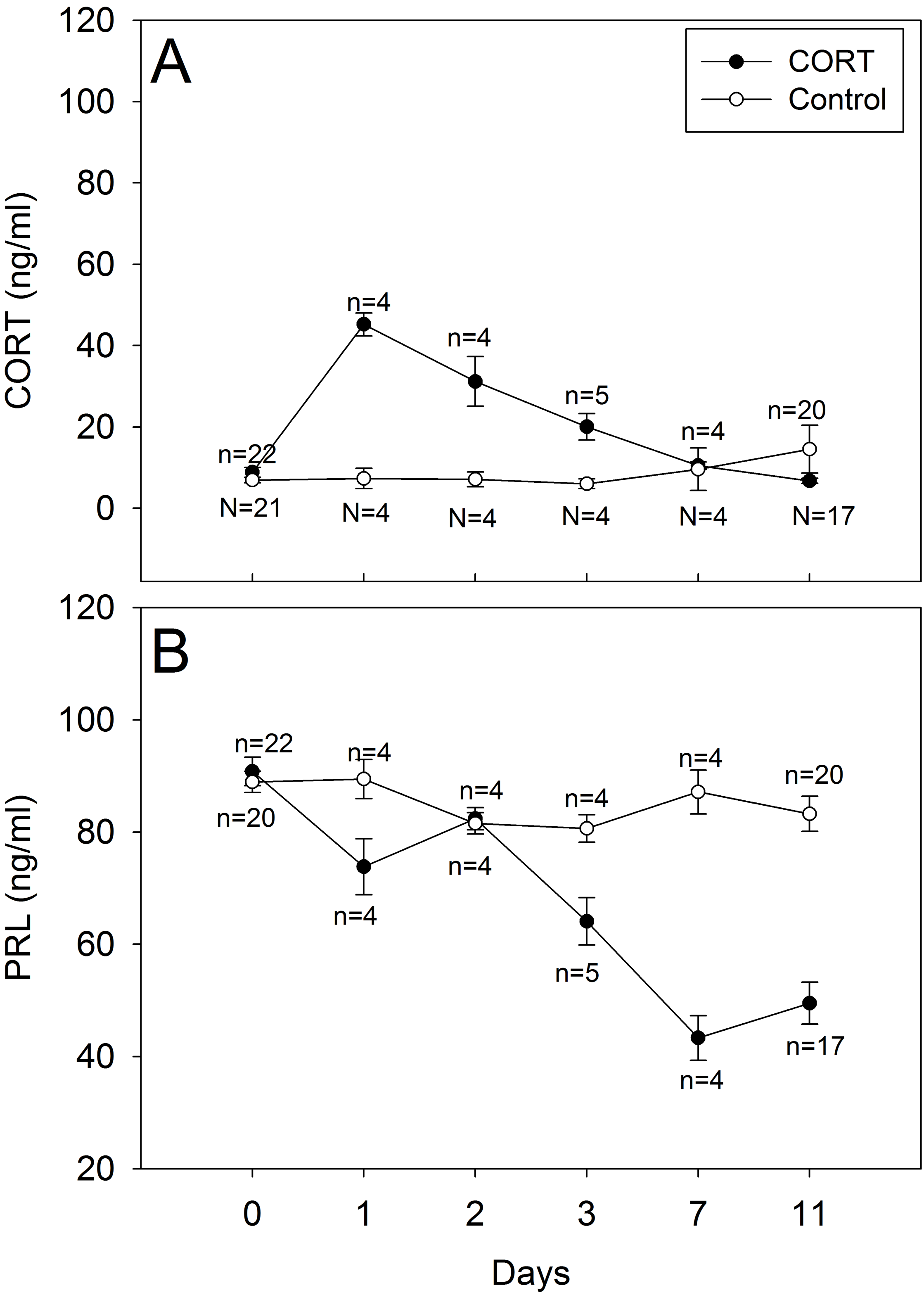

2012: non-treated birds
A)
Initial PRL
Hg
2013: implanted birds
Controls
CORT
B)
PRL
CORT + Hg could act synergistically on PRL concentration
Hg

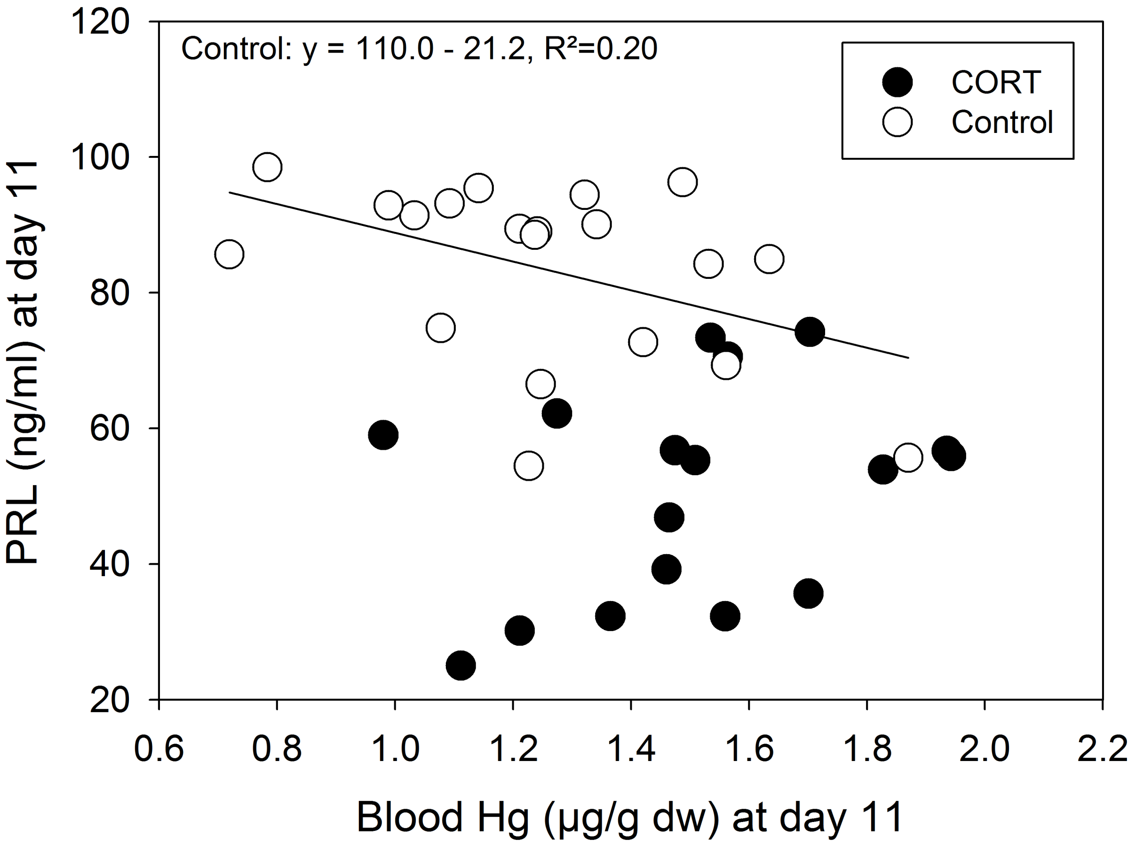
Figure 1



A
Males in 2012
y = 99.9 - 12.2x, R²=0.06
B
Females in 2012
C
Males in 2012
y = 86.4 - 24.8x, R²=0.41
D
Females in 2012
Initial PRL concentation (ng/ml) in incubating kittiwakes
Initial PRL concentation (ng/ml) in chick-rearing kittiwakes
Blood Hg concentration (µg/g dw)
Blood Hg concentration (µg/g dw)
180
160
140
120
100
80
60
40
20
0
0.5
1.0
1.5
2.0
2.5


A
Males in 2012
B
Females in 2012
Number of chicks successfully raised
2
1
*
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Blood Hg concentration (µg/g dw)
Blood Hg concentration (µg/g dw)


A
B
CORT
Control
CORT (ng/ml)
PRL (ng/ml)
Days
0
20
40
60
80
100
120
1
2
3
7
11
n=22
n=4
n=4
n=5
n=4
n=20
N=21
N=4
N=4
N=4
N=4
N=17
n=22
n=20
n=4
n=4
n=4
n=4
n=4
n=5
n=4
n=4
n=20
n=17



Control: y = 110.0 - 21.2, R²=0.20
CORT
Control
PRL (ng/ml) at day 11
120
100
80
60
40
20
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Blood Hg (µg/g dw) at day 11