Relationships between contaminants and hormones involved in breeding decisions in polar seabirds

Sabrina Tartu

▶ **To cite this version:**

Sabrina Tartu. Relationships between contaminants and hormones involved in breeding decisions in polar seabirds. Agricultural sciences. Université de La Rochelle, 2014. English. NNT : 2014LAROS022 . tel-01175495

HAL Id: tel-01175495
https://theses.hal.science/tel-01175495

Submitted on 10 Jul 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

University of La Rochelle

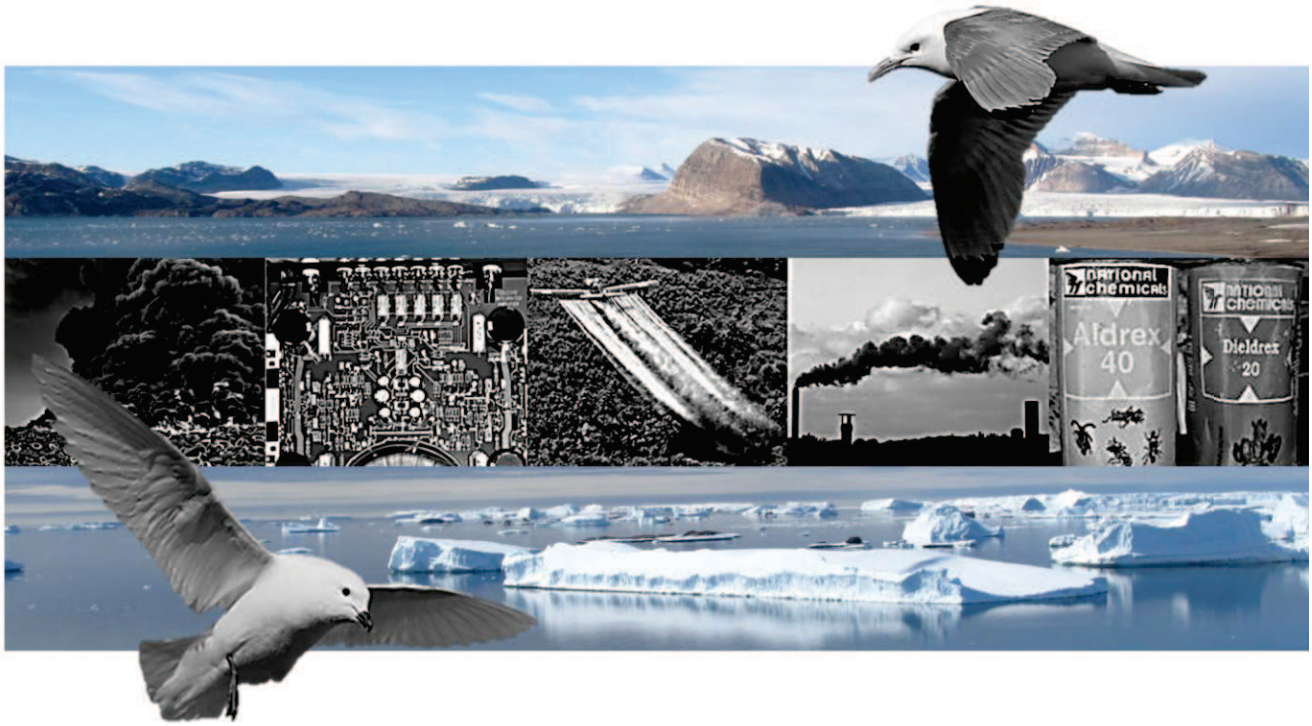
Doctoral school Gay-Lussac, Sciences pour l'environnement

Thesis submitted for the degree of Philosophiae doctor

Specialty: Biologie de l'environnement, des populations, écologie

Defended by Sabrina TARTU on October 17th 2014

Relationships between contaminants and hormones involved in breeding decisions in polar seabirds



Prepared at Centre d'études biologiques de Chizé, UMR 7372
Thesis advisor: Olivier CHASTEL

Jury composition:

Reviewers:	Mary-Ann OTTINGER, University of Houston
	Renaud SCHEIFLER, University of Franche-Comté
Examiners:	Paco BUSTAMANTE, University of La Rochelle
	Samuel CARO, Centre d'écologie fonctionnelle et évolutive - CNRS
Advisor:	Olivier CHASTEL, Centre d'études biologiques de Chizé - CNRS
President:	Pierre MIRAMAND, University of La Rochelle

Table of contents

Table of contents 2
Acknowledgments/ Remerciements 4
Abstract 5
Résumé 5
LIST OF PUBLICATION 6
CONTRIBUTION TO OTHER STUDIES 7
CONGRESS PARTICIPATION 8
ABBREVIATIONS 9
And no birds sing. 10
1. INTRODUCTION 11
1.1. Contaminants in polar regions 11
1.1.1. Types of contaminants, global distillation and bioaccumulation 11
1.2. Hazardous biological effects and endocrine disruption 17
1.3. Breeding decision and targeted hormones 20
1.3.1. From pre-laying to egg laying: role of LH and CORT in reproductive decisions 24
1.3.2. Parental phase, from incubation to chick rearing: the role of PRL and CORT 25
2. OBJECTIVES AND METHODS 27
2.1. Overall and specific objectives 27
2.1.1. Seabirds, biomonitors of marine environment contamination 27
2.1.2. Thesis objectives 28
2.2. Methods 30
2.2.1. Study sites and species 30
2.2.2. Blood sampling, morphometrics and fitness measurements 32
2.2.3. Hormone manipulations 34
2.2.4. Hormone and contaminant assays 38
3. DISCUSSION 39
3.1. The pre-laying phase: from mating to egg laying 40
3.1.1. Hg, endocrine correlates and fitness 43
3.1.2. POPs, endocrine correlates and fitness 45
3.2. The parental phase: from incubation to chick rearing 47
3.2.1. Contaminants and age 50
3.2.2. Hg, endocrine correlates and fitness 52

3.2.3. POPs, endocrine correlates and fitness 54
3.3. Consequences and specific action of contaminants 58
3.4. Conclusions and perspectives 62
REFERENCES 66
PAPER I 78
PAPER II 83
PAPER III 94
PAPER IV 103
PAPER V 113
PAPER VI 126
PAPER VII 135
PAPER VIII 150
APPENDIX 158

Acknowledgments/ Remerciements

First of all I would like to thank **Mary-Ann Ottinger**, **Renaud Scheifler**, **Samuel Caro**, **Paco Bustamante** and **Pierre Miramand** for accepting to judge this thesis.

Olivier, je ne te remercierais jamais assez de m'avoir offert l'opportunité de travailler sur ce projet avec toi, et de t'être investis à ce point dans ma petite carrière.

Fred, à nous trois on forme l'équipe de choc. Je te remercie pour tes interventions scientifiques et pour ton amitié. Merci de m'avoir fourni un toit et Phantom quand j'étais dans le besoin.

Je remercie toute l'équipe du projet Polartop : **Paco Bustamante**, **Christophe Barbraud**, **Yves Cherel**, **Hélène Budzinski** et **Pierre Labadie**. Merci à vous pour tous ces échanges très enrichissants.

Tussen takk to the Norwegian team for your help and advises in fieldwork and manuscript writing: **Jan Ove Bustnes**, **Geir Gabrielsen**, **Børge Moe**, **Dorte Herzke**, **Claus Bech**, **Sveinn Are Hanssen**, **Nanette Verboven**.

And also thank you to all the other seabird experts that collaborated with me in this work: **Frédéric Angelier**, **Aurélie Goutte**, **Pierre Blévin**, **Adam Lendvai** and **Jonathan Verreault**.

Merci aux directeurs qui se sont succédés au Centre d'études biologiques de Chizé **Vincent Bretagnolle, Xavier Bonnet** et **Christophe Guinet** pour m'avoir accueillie au CEBC.

Je tiens à remercier de nombreuses personnes du CEBC, qui ont toujours été présentes pour faciliter la vie d'un thésard. Les Dames responsables des analyses biologiques et de l'administration : **Colette Trouvé**, **Charline Parenteau**, **Stéphanie Dano**, **Evelyse Rouby**, **Delphine Bonnet**, **Martine Lacalle** et **Annie Télias**. Et les Hommes responsables de notre survie : **Christophe Coiffet** et **Arnaud Viollet**, merci pour les délicieux mets préparés chaque jour.

Un grand merci à toute **l'équipe Ecophy** que je suis fière d'avoir pu intégrer.

Et puis tout cela n'aurait pas été aussi agréable sans les belles personnes que j'ai croisé :

JB merci d'avoir guidé mes premiers pas dans le monde de la recherche, de m'avoir soutenue et épaulée. Merci aux amis des premiers jours : **Boen G**, **Aurélie A**, **Kévin LR**, **Jade VG**, **Matthieu L**, **Aurélie G**.

Pierre B, tout d'abord "fieldworker", tu es devenu mon stagiaire, puis un « vrai » héros polaire, maintenant tu vas me prendre mon bureau. Beau chemin parcouru. Je remercie les oiseaux polaires de t'avoir placé sur ma route, ce fut une très belle découverte.

Et évidemment merci à la dream team, je ne pensais pas trouver autant de gens bien dans un si petit endroit. Des plus matures aux juvéniles: **Fabrice LB**, **Aurélien P**, **Paul T**, **Sophie JDG**, **Alice C**, **Alizée M**, **Laurie T**, **Andréaz D**, **Baptiste P**, **Marina F**, **Aymeric F**, **Yves LB**, **Joffrey J**, **Christophe S**, **Rémy F**.

Un grand merci à **Anne Lindsey** qui a nettement amélioré l'anglais de ce manuscrit.

Je tiens à remercier ma famille, **mes parents, mon frère et sa douce**. Vous qui m'avez donné les moyens d'arriver jusqu'ici, c'est grâce à vous que j'écris aujourd'hui cette thèse. Thank you to all the larger family (**Celestino, Chazal, Espina and Tartu)**. Thank you to those who were able to attend my defense and to those who participated in the "banquet".

Et puis le meilleur pour la fin, **Baptiste**, puisque tu n'aimes pas les éloges je serais brève: merci pour tout.

And for those that I forgot, I may have other opportunities to thank you.

Abstract

In order to maximise fitness, individuals will have to take several decisions that shall match with environmental conditions (whether to breed or not, when to breed, what level of parental investment). These decisions are mediated by hormones: such as luteinizing hormone (LH), a pituitary hormone involved in the onset of breeding, stress hormones (corticosterone, CORT) and prolactin (PRL) a pituitary hormone involved in the expression of parental care. Environmental contaminants are present world-wide, and also in Polar Regions. Since many contaminants are endocrine disruptors, they may impair breeding decisions, but evidences are scarce for wildlife. The aim of this thesis was to assess the relationships between some legacy persistent organic pollutants (POPs: PCBs, organochlorine pesticides), mercury (Hg) and hormones (LH, CORT, PRL) in different Arctic and Antarctic seabird species. Results show that POPs and Hg clearly interact with LH, CORT and PRL secretion. These contaminants appear to target different hormones: increasing Hg was related to decreasing pituitary hormone secretion (LH and PRL); whereas increasing PCBs were linked to an exacerbated CORT response to an acute stress. Hg disrupted LH secretion by probably suppressing GnRH input to the pituitary; PCBs seem to act at the adrenal level by probably stimulating ACTH receptors. Legacy POPs and Hg are therefore able to disrupt reproductive decisions and to impact fitness: elevated Hg levels were linked to skipped reproduction and poor incubation behavior; elevated PCB levels may make individuals more susceptible to environmental perturbations. The long-term consequences of contaminant exposure for seabirds are discussed in the context of the environmental challenges affecting polar regions.

Key-words: legacy POPs; Mercury; endocrine disruption; fitness; seabirds, Arctic, Antarctica

Résumé

De façon à maximiser leur valeur sélective, les individus doivent prendre différentes décisions qui doivent concorder aux conditions environnementales (Se reproduire ou pas ? Quand ? Quel investissement parental ?). Ces décisions sont fortement influencées par la sécrétion de certaines hormones comme l'hormone lutéinisante (LH) déclencheur de la reproduction, l'hormone de stress (CORT) et la prolactine (PRL), pour l'expression des soins parentaux. Les contaminants chimiques et naturels sont omniprésents et ont même été retrouvés en zones polaires. De nombreux contaminants sont des perturbateurs endocriniens et pourraient altérer les décisions de reproduction, mais les preuves manquent pour la faune sauvage. Cette thèse vise donc à identifier les liens entre certains polluants organiques persistants dits 'd'héritage' (POPs: PCBs, pesticides organochlorés), le mercure (Hg) et les hormones LH, CORT et PRL chez plusieurs espèces d'oiseaux marins Arctiques et Antarctiques. Les résultats obtenus ont démontré que ces contaminants interagissent avec ces hormones, et de façon spécifique: le Hg réprimerait la sécrétion d'hormones hypophysaires (LH, PRL) tandis que les PCBs stimuleraient la production de CORT. Plus précisément, le Hg pourrait affecter la sécrétion de LH en réprimant l'apport de GnRH à l'hypophyse et les PCBs pourraient stimuler le nombre de récepteurs à l'ACTH au niveau des glandes surrénales. Le Hg et les PCBs peuvent donc altérer les décisions de reproduction et impacter la valeur sélective: des taux élevés de Hg sont associés à la suppression de la reproduction et à un moindre investissement parental, tandis que la présence de PCBs pourrait exacerber la susceptibilité aux stress environnemental. Les conséquences à long-terme de l'exposition des oiseaux marins à ces contaminants sont discutées dans le cadre des changements globaux qui affectent les Pôles.

Mots-clés: POPs d'héritage; Mercure; perturbation endocrine; valeur sélective ; oiseaux marins ; Arctique ; Antarctique

LIST OF PUBLICATION

This thesis is principally based on the following papers that are indicated in the text by Roman numerals:

I. Tartu, S., Goutte, A., Bustamante, P., Angelier, F., Moe, B., Clément-Chastel, C., Bech, C., Gabrielsen, G.W., Bustnes, J.O. & Chastel, O. (2013) **To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird.** *Biology Letters*, 9, 20130317.

II. Tartu, S., Bustamante, P., Goutte, A., Cherel, Y., Weimerskirch. H., Bustnes J.O., & Chastel, O. (2014) **Age-related mercury contamination and relationship with luteinizing hormone in a long-lived Antarctic bird.** *PLoS ONE,* 9(7): e103642.

III. Tartu, S., Angelier, F., Herzke, D., Moe, B., Bech, C., Gabrielsen, G. W., Bustnes J.O. & Chastel, O. (2014). **The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes.** *Science of The Total Environment*, 476, 553-560.

IV. Tartu, S., Wingfield, J.C., Bustamante, P., Angelier, F., Budzinski, H., Labadie P., Bustnes J.O., Weimerskirch. H., & Chastel, O. (2015) **Corticosterone, prolactin and egg neglect behaviour in relation to mercury and legacy POPs in a long-lived Antarctic bird.** *Science of The Total Environment,* 505, 180-188.

V. Tartu, S., Angelier F., Bustamante P., Lendvai, A. Z., Blévin P., Moe, B., Bech C., Gabrielsen, G. W., Bustnes J.O., & Chastel, O. **Co-stressors: does corticosterone reinforce the endocrine consequences of mercury exposure in an Arctic seabird?** *In preparation for Functional ecology*.

VI. Tartu, S., Angelier A., Bustnes J.O., Moe, B., Hanssen S.A., Herzke D., Gabrielsen, G. W., Bustnes J.O., Verboven N., Verreault J., Budzinski, H., Labadie P., Wingfield J.C. & Chastel, O. (2015) **Polychlorinated biphenyl exposure and corticosterone secretion in seven polar seabirds.** *Environmental pollution*, 197, 173-180.

VII. Tartu, S., Lendvai, A. Z.., Blévin P., Herzke, D., Bustamante P., Moe, B., Gabrielsen, G. W., Bustnes J.O., & Chastel, O. **Increased adrenal responsiveness of Arctic-breeding black-legged kittiwakes in relation to polychlorinated biphenyl exposure.** *General and Comparative Endocrinology,* in press.

VIII. Tartu, S., Blévin, P., Gabrielsen, G. W., Ellis H., Bustnes J.O., Herzke, D., & Chastel, O. (2014) **Endocrine and fitness correlates of long-chain perfluorinated carboxylates exposure in an Arctic seabird.** *Environmental science and technology,* 48(22), 13504-13510.

CONTRIBUTION TO OTHER STUDIES

Blévin, P., **Tartu, S.,** Angelier, F., Leclaire, S., Bustnes, J. O., Moe, B., Herzke D., Gabrielsen G.W. & Chastel, O. (2014). Integument colouration in relation to persistent organic pollutants and body condition in arctic breeding black-legged kittiwakes. *Science of the Total Environment*, *470*, 248-254.

Carravieri, A., Bustamante, P., **Tartu, S.,** Meillère, A., Labadie, P., Budzinski, H., Peluhet, L., Barbraud, C., Weimerskirch, H., Chastel, O., Cherel, Y. (2014). Wandering Albatrosses Document Latitudinal Variations in the Transfer of Persistent Organic Pollutants and Mercury to Southern Ocean Predators. *Environmental science and technology,* 48, 14746–14755.

Goutte, A., Angelier, F., Bech, C., Clément-Chastel, C., Dell'Omo, G., Gabrielsen, G. W., Lendvai A.Z., Moe B., Noreen E., Pinaud D., **Tartu S.,** & Chastel, O. (2014). Annual variation in the timing of breeding, pre-breeding foraging areas and corticosterone levels in an Arctic population of black-legged kittiwakes. *Mar Ecol Prog Ser*, *496*, 233-247.

Schultner, J., Moe, B., Chastel, O., **Tartu, S.,** Bech, C., & Kitaysky, A. S. (2014). Corticosterone mediates carry-over effects between breeding and migration in the kittiwake Rissa tridactyla. *Mar Ecol Prog Ser*, *496*, 125-133.

Reviewer activity: Environmental pollution

CONGRESS PARTICIPATION

Oral communication

2011 **Polar seabirds in a changing environment: a mechanistic approach.** 10th Ny-Ålesund seminar, Kjeller, Norway (25-26 October). Young scientist travel grant from the Norwegian Research Council.

2012 **Mercury suppresses breeding readiness in an Arctic seabird**. 8th Ecology and behavior meeting, Chizé, France (2-6 April)

2013 **Effects of pollutants on reproduction, hormones and phenotype in female black-legged kittiwakes**. Kittiwake workshop, NTNU, Trondheim, Norway (20-22 March)

2013 **Influences of persistent organic pollutants on the stress response of an Antarctic long-lived bird**. 9th Ecology and behavior meeting, Strasbourg, France (22-26 April)

2014 **Mercury and persistent organic pollutants as endocrine disruptors in polar seabirds.** SETAC Europe 24th Annual Meeting, Basel, Switzerland (11-15 May)

Poster presentation

2012 **Mercury suppresses breeding readiness in an Arctic seabird.** 10th International Symposium of Avian Endocrinology, Gifu, Japan (5-9 June)

ABBREVIATIONS

ACTH	Adrenocoticotrophic hormone
ACTH-R	Adrenocoticotrophic hormone receptors
BFR	Brominated flame retardants
CBG	Corticosteroid-binding globulin
CORT	Corticosterone
CRH	Corticotropin-releasing hormone
DA	Dopamine
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DEX	Dexamethasone
E2	Estradiol
EDC	Endocrine disrupting chemicals
FSH	Follicle-stimulating hormone
GC-ECD	Gas Chromatography - Electron capture detector
GC-MS	Gas Chromatography - Mass Spectrometry
GnRH	Gonadotrophic releasing hormone
GR	Glucocorticoid receptors
HCB	Hexachlorocyclobenzene
HCH	Hexachlorocyclohexane
Hg	Mercury
HPA (axis)	Hypothalamo-pituitary-adrenal (axis)
HPG (axis)	Hypothalamo-pituitary-gonad (axis)
LH	Luteinizing hormone
MeHg	Methyl mercury
OCP	Organochlorine pesticides
PBDE	Polybrominated diether
PCB	Polychlorinated biphenyls
PFAS	Poly- and perfluorinated alkyl substances
PFCA	Perfluorinated carboxylates
POPs	Persistent organic pollutants
PRL	Prolactin

CEBC	Centre d'études biologiques de Chizé
EPOC	Environnements et Paléoenvironnements Océaniques et Continentaux, University of Bordeaux
IPEV	Institut Paul Emile Victor, French polar institute
LIENSs	Littoral environnement et Sociétés, University of La Rochelle
NILU	Norsk Institutt for luftforskning, Norwegian Institute for air research

And no birds sing.

"After several years of DDT spray, the town is almost devoid of robins and starlings; chickadees have not been on my shelf for two years, and this year the cardinals are gone too […]. It is hard to explain to the children that the birds have been killed off, when they have learned in school that a Federal law protects the birds from killing or capture."

Rachel Carson, Silent spring, 1962.

1. INTRODUCTION

1.1. Contaminants in polar regions

1.1.1. Types of contaminants, global distillation and bioaccumulation

Human activities have released an immeasurable amount of contaminants into the environment. This worldwide contamination was or not intentional. Persistent organic pollutants (POPs) are a large group of man-made chemicals that are persistent in the environment; POPs have long half-lives in soils, sediments, air and biota (Harrad 2009). In addition to being persistent, the combination of their resistance to metabolism and their lipophilicity makes them able to bioaccumulate inside organisms (bioaccumulation) and be transferred from a lower to a higher position along the food web (biomagnification) (Walker et al. 2012). POPs are also highly volatile and toxic. Their hazardous effects on health will be discussed further. Given all these properties POPs have been in the focus of attention during the last decades, and under the Stockholm Convention 12 chlorinated chemical substances were banned or severely restricted (Stockholm convention 2001). The Stockholm Convention was originally signed by 92 nations and the European Community in Stockholm, Sweden in May 2001, and came into force in May 2004 with over 150 countries then signing the Convention. The Stockholm Convention established an initial list of 12 key POP chemicals (the so-called dirty dozen) for which signatories are required to reduce the risks to human health and the environment arising from their release. Ratifying parties are required to take (legal and/or administrative) measures to eliminate or heavily restrict the production and use of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), and to minimize the unintentional production and release of POPs. The 12 POPs that were targeted (**Figure 1**) include eight pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, and

toxaphene), two industrial chemicals (polychlorinated biphenyls and hexachlorobenzene) and two combustion by-products (dioxins and furans).

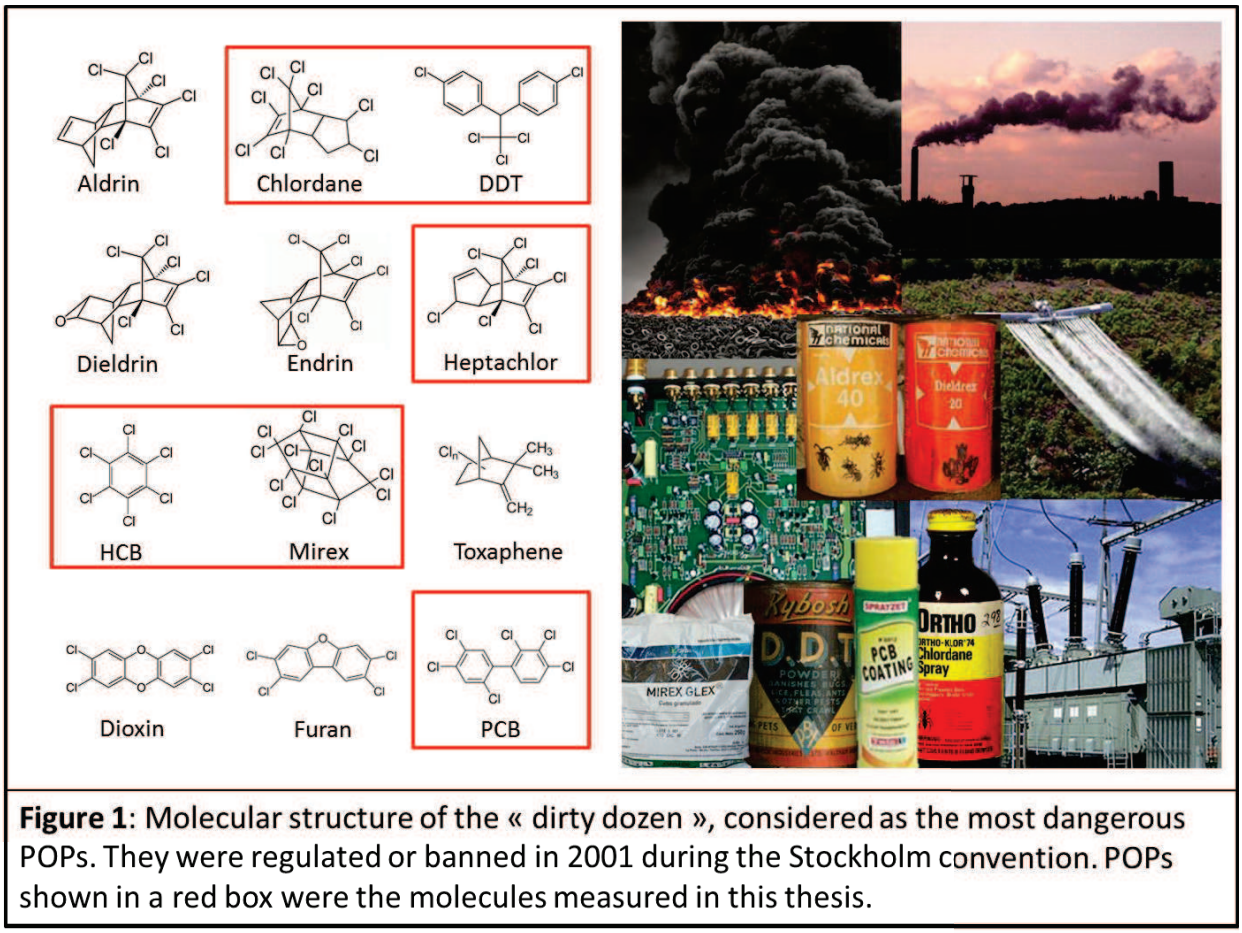


Figure 1: Molecular structure of the « dirty dozen », considered as the most dangerous POPs. They were regulated or banned in 2001 during the Stockholm convention. POPs shown in a red box were the molecules measured in this thesis.

DDT, for example, was widely used during World War II to protect soldiers and civilians from malaria, typhus, and other diseases spread by insects. After the war, DDT was further used to control disease, and it was sprayed on a variety of agricultural crops, especially cotton. DDT application against mosquitoes is nowadays maintained in several countries to control malaria. Its stability, its persistence (as much as 50% can remain in the soil 10-15 years after application), and its widespread use have resulted in DDT residues being possibly found anywhere; residual DDT has even been detected in Antarctica and in the Arctic (George & Frear 1966; AMAP 2004). Perhaps the best-known toxic effect of DDT is eggshell thinning among birds, especially birds of prey (Bitman et al. 1970; Wiemeyer et al. 1984). Its impact on bird populations led to bans in many countries during the 1970s (Stockholm

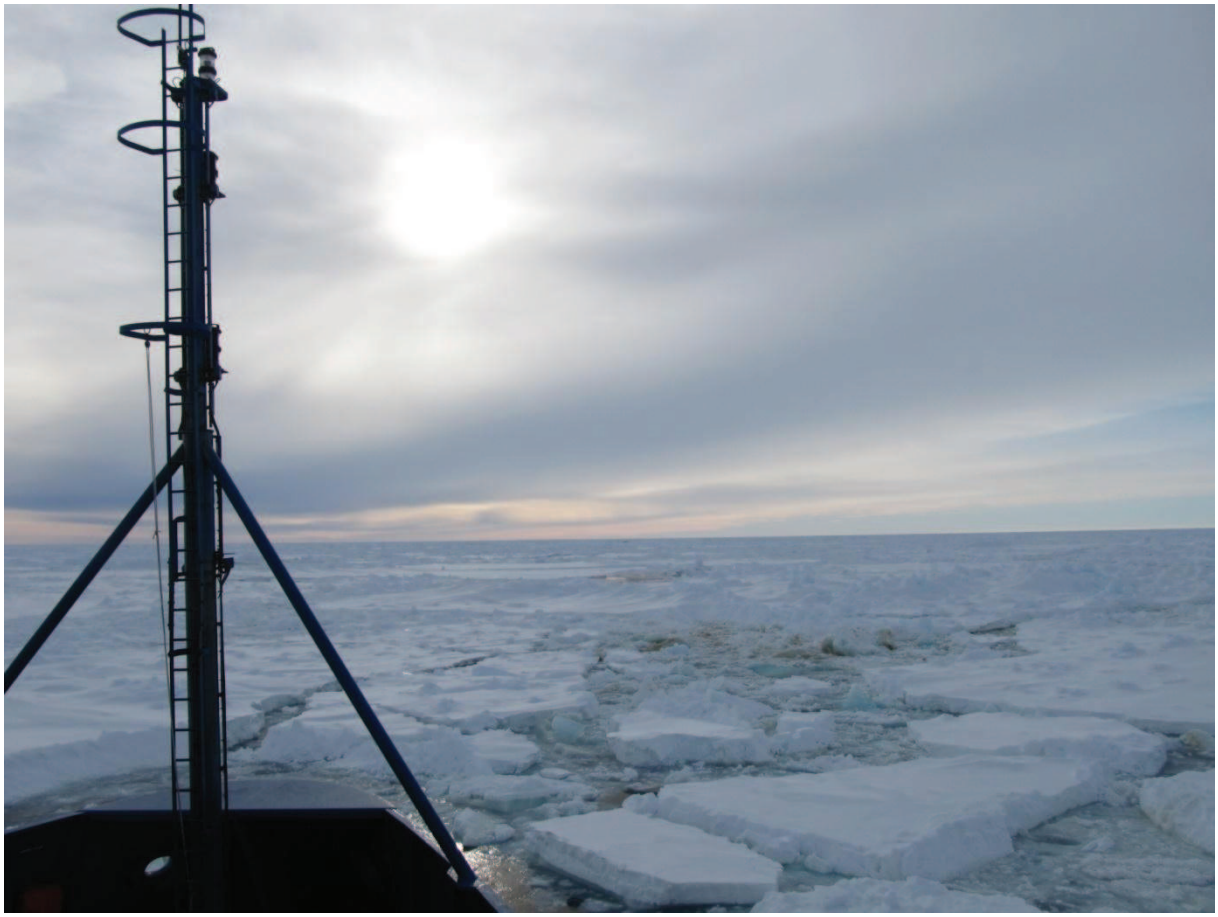
Convention 2001). Although its use was banned in many countries, it is still detected in humans and wildlife worldwide (Turusov et al. 2002). PCBs have also been widely studied; they are used in industry as heat exchange fluids, in electric transformers and capacitors, and as additives in paint, carbonless copy paper, and plastics. Of the 209 different PCB types, 13 exhibit a dioxin-like toxicity (Walker et al. 2012). Their persistence in the environment implies the degree of chlorination, and half-lives can vary from 10 days to 1.5 years. These 12 restricted or banned chemicals (the dirty dozen) are referred to as **"legacy POPs"** due to their long history of use and release into the environment (Stockholm Convention 2001). Numerous other POPs are however of concern, and regulations for these were made only recently, if at all. It is the case of brominated flame retardants (BFR) which are chemicals used as flame retardants and bearing a general resemblance to PCB mixtures in their properties (Walker et al. 2012), or of poly- and perfluorinated alkyl substances (PFAS) which have been used as surface-active agents in a vast number of manufactured and consumer products (e.g., fire-fighting foam and impregnating agents for carpets, papers, and textiles; Jenssen & Leffers 2008). PFAS are extremely persistent in the environment and accumulate in the blood, liver and kidneys (Jones et al. 2003; Jensen & Leffers 2008). Nowadays, human exposure to PFAS in blood reaches the highest values observed as regards an exogenous chemical (Jensen & Leffers 2008). These chemicals were recently discovered in the environment and are known or suspected to cause adverse effects to humans and wildlife (Muir & de Wit 2010). They are referred to as **"emerging POPs"**. Consequently, while a decreasing trend in legacy POPs can be measured in the environment some emerging POPs show dramatic increasing trends (e.g., in Arctic seabird eggs; Helgason et al. 2011). Metabolism of POPs occurs mainly in the liver via a two-phase process. These processes are catalyzed by liver enzymes such as cytochromes P450 (Nebert & Gonzalez 1987). Metabolism-resistant lipophilic substances are selectively accumulated. In addition to

detoxification, the enzymatic processes can also create reactive intermediates or metabolites that are biologically active (Walker et al. 2012). For example, in living organisms DDT is metabolized to DDE, which is lipophilic and toxic (World Health Organization 1989).

In addition to all human-produced chemicals, some compounds are naturally present on Earth as is the case with metals. Metals are ubiquitous but human activities have increased their emission through mining and smelting, making them pollutants. A contaminant will be referred to as a "pollutant" when it exceeds normal background levels and can cause harm (Walker et al. 2012). However, in the present thesis whether or not a contaminant is a pollutant, the designation "contaminant" is used as a general term. Although there is no clear definition for a heavy metal, heavy metals are commonly defined as those with a specific density exceeding 5g/cm^3 (Järup 2003). Nevertheless, the term was replaced by a classification scheme that considers chemistry rather than relative density (Nieboer & Richardson 1980). The main threats to human and wildlife health induced by heavy metals are associated with exposure to lead, cadmium, mercury (Hg) and arsenic. Contrary to POPs, heavy metals are non-biodegradable. Detoxification consists in hiding active metal ions within a protein or depositing them in insoluble forms in intracellular granules for long-term storage or excretion (Walker et al. 2012). In this thesis the focus was on Hg, a non-essential heavy metal. Nowadays, the emission of Hg is almost exclusively the result of anthropogenic activity (Walker et al. 2012). Hg can become organic through the action of bacteria; its methylated form Hg is very stable and can also bioaccumulate and biomagnify in the food web (Atwell et al. 1998). The organic form of Hg is highly toxic as it passes the blood-brain barrier owing to its lipid solubility (Zahir et al. 2005). After the Minamata Bay tragedy in 1950, extra focus was directed towards the toxic effects of Hg on health (Kudo et al. 1998; Zahir et al. 2005): a chemical factory had released Hg into Minamata Bay, Japan, resulting in

the death of more than 100 people and the paralysis of several thousands of people through fish consumption (Kudo et al. 1998).

Despite the restrictions placed on legacy POPs and the awareness of Hg toxicity, these contaminants are still recorded in large amounts in organisms distant from industrialized areas such as polar regions.



The French Polar Institute's ship "The Astrolabe" approaching Adélie Land.

"*If any area of the world were to be free of pesticidal chemicals, it would seem probable that the Antarctic would be such a place, because there are literally no pests and relatively few animals or plants on the continent, and there is no knowledge of any application of pesticides there*" (George & Frear 1966).

The primary aim of studies monitoring contaminants in polar regions was to establish background concentrations in pristine areas (George & Frear 1966; Clausen et al. 1975). However, very high levels of environmental contaminants sometimes exceeding those found in industrialized cities were reported in air, soil and animal tissues (Wania & MacKay 1993). Polar regions (the Arctic and the Antarctic), though very distant from industrialized areas and agricultural activity, are the fallout regions for long-range transport contaminants (POPs and Hg). Polar regions are contaminated by air masses from mid-latitudes due to several physical-chemical properties of contaminants such as volatility, temperature and "global distillation" (Wania & MacKay 1993; Ariya et al. 2004; Lu et al. 2008). Indeed, a contaminant's volatility and the ambient temperature will significantly influence its distribution (Wania & MacKay 1993). Polar regions are characterized by very cold temperatures, which trigger the condensation of contaminants present in the atmosphere (**Figure 2A**; Wania & MacKay, 1993; Ariya et al. 2004; Lu et al. 2008), and the life of contaminants is thereby greatly prolonged (George & Frear 1966). These contaminants are then deposited into soil, water, aerosols, snow and ice (Wania & MacKay, 1993; Ariya et al. 2004) with an ultimate sink in the ocean (Walker et al. 2012). The transfer of contaminants to organisms occurs via the food web (Atwell et al. 1998; Bargagli et al. 1998; Borgå et al. 2001). And, as previously mentioned, most contaminants can bioaccumulate inside organisms and biomagnify along the food web (**Figure 2B**; Atwell et al. 1998; Bargagli et al. 1998; Borgå et al. 2001).

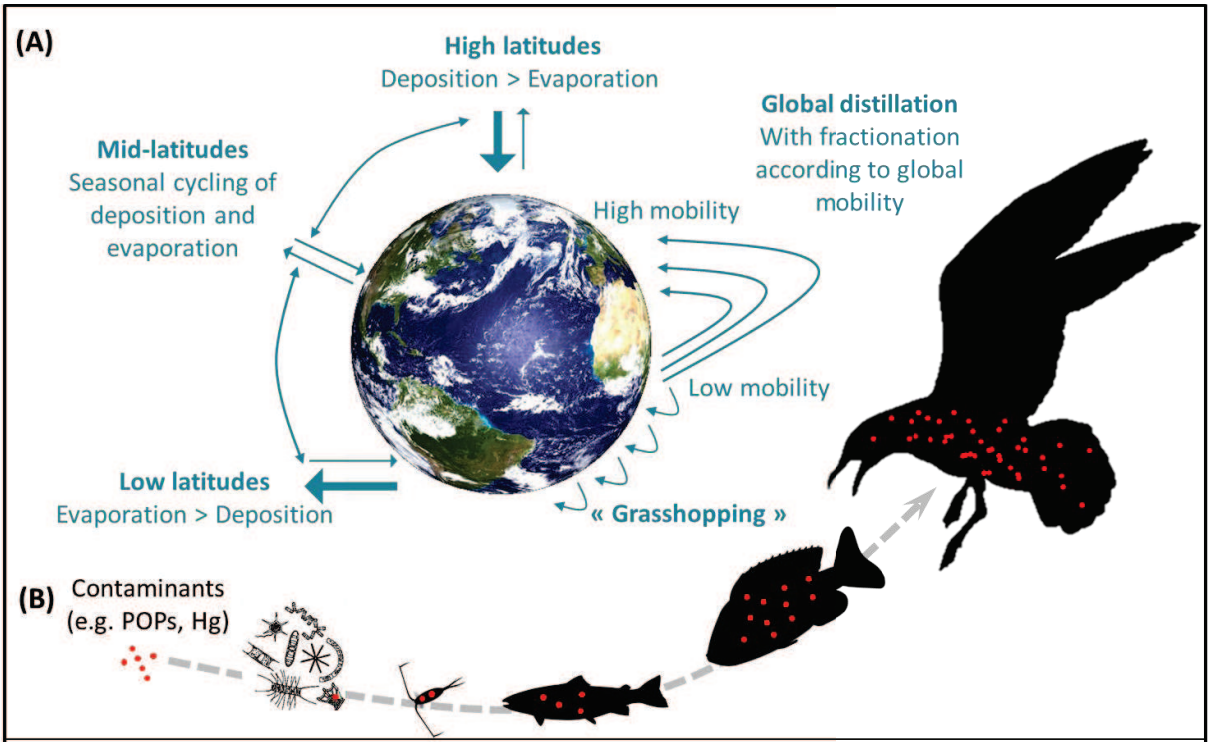


Figure 2: (A) Global distillation of contaminants, adapted from Wania & McKay, 1994. **(B)** Contaminants bioaccumulate inside organisms and biomagnify along the food web.

Consequently, marine apex predators such as polar bears, seals, whales and seabirds are particularly vulnerable to the hazardous biological effects of contaminants (Wolfe et al. 1998; Gabrielsen et al. 2007; Tan et al. 2009; Letcher et al. 2010; Verreaut et al. 2010).

1.2. Hazardous biological effects and endocrine disruption

Two methods are generally used to assess the harmful effects of contaminants (Gabrielsen 2007; Walker et al. 2012). The first one consists in extrapolating from levels in the wild and comparing them with those known to cause toxic effects in laboratory species (Ottinger et al. 2002). The second method is to investigate correlations between biomarker endpoints (e.g., immunity, hormones, pathologies, reproduction, development, etc.) and tissue levels of contaminants (Gabrielsen 2007; Muir & de Wit 2010). However, cause and effects are difficult to assess in such studies. In field studies, it is indeed complex to determine a causal relationship between an observed effect and a specific contaminant (Gabrielsen 2007; Muir &

de Wit 2010): the effect could simply result from other confounding factors. Experimental studies on captive animals have set threshold levels beyond which adverse effects are known to be observable. Nevertheless, most laboratory studies use either a unique compound at high doses or a mixture of several compounds, which is never representative of exposure in the wild. Correlational studies can better inform on the relationships between contaminants and biomarkers, individuals being usually exposed for a long period to many contaminants at sometimes low doses. Correlational studies highlighted the fact that a wide range of hazardous biological effects were ascribed to legacy POPs and Hg (Wolfe et al. 1998; Gabrielsen et al. 2007; Tan et al. 2009; Letcher et al. 2010; Verreaut et al. 2010).

For example, DDT can induce eggshell thinning in birds (e.g., Bitman et al. 1970; Cooke 1973); Hg and POPs may impact breeding capacities, for instance causing abnormal breeding behaviors, reduced fertility or poor breeding success (Harrison et al. 1997; Wolfe et al. 1998; Taylor & Harrison 1999; Bustnes et al. 2003a, 2007; Gabrielsen 2007; Fredericks & Jayasena 2009; Letcher et al. 2010; Verreault et al. 2010). Globally, hazardous effects of contaminants were reported on reproduction, neurotoxicity, immunotoxicity, genotoxicity; and hazardous effects on the hormonal system were also observed (Colborn et al. 1993; Wolfe et al. 1998). Since the 1940s, the effects of endocrine-disrupting chemicals (EDC) are evident in several raptor species (Ratcliffe 1970). Broken eggs attributable to parental destruction were found in nests of peregrines (*Falco peregrinus*), Eurasian sparrowhawks (*Accipiter nisus*) and Scottish golden eagles (*Aquila chrysaetos*), and occurred concurrently with a decrease in eggshell weight (Ratcliffe 1970). This reproductive failure due to eggshell thinning was the consequence of contamination by DDE, a DDT metabolite (Ratcliffe 1970). This is probably the first example of endocrine disruption in wildlife. A plausible mechanism of action was only discovered recently: DDT metabolites could enhance estrogenic metabolism, and eggshell thinning in avian wildlife could result from a functional malformation in the shell

gland, induced by embryonic exposure to estrogenic substances (**Figure 3**; Dawson et al. 2000; Berg et al. 2004; Holm et al. 2006).

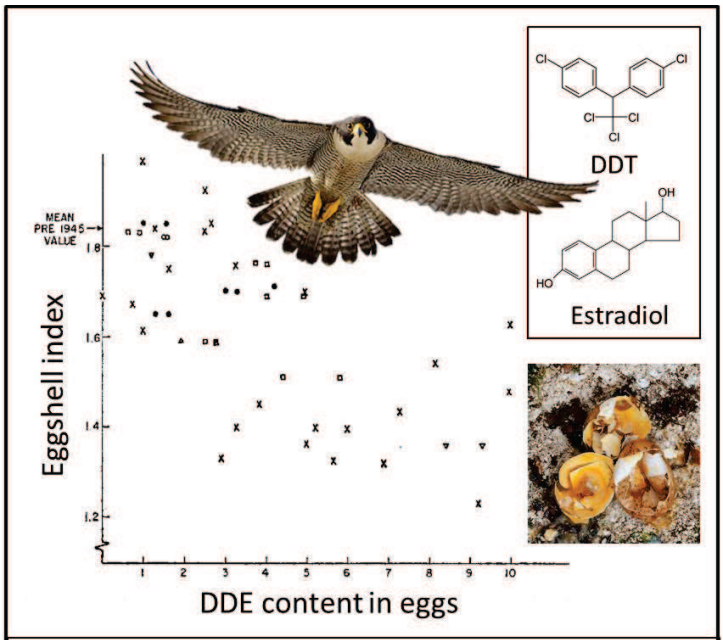


Figure 3: The DDE-induced eggshell thinning in Peregrine falcons can be considered as the first example of endocrine disruption in wildlife. Eggshell thinning may result from the capacity of DDE to mimic endogenous estradiol. Adapted from Peakall et al. 1976.

In 1962 Rachel Carson published "Silent Spring", which led to increasing public concern about chemicals that could have harmful effects on wildlife and human health (Carson 1962). Then, in 1979 a series of conferences was launched, bringing together a group of expert scientists dealing with "chemically induced alterations in sexual development" with a specific focus on estrogens in the environment (e.g., McLachlan et al. 1984). Yet, it was only in the mid-1990s that Colborn et al. (1996), by publishing their book "Our stolen future", brought worldwide attention to scientific discoveries about endocrine disruption and to the fact that common contaminants can interfere with hormonal pathways. This book played a key role in raising public awareness and concern about endocrine disruption and motivated many environmental groups to call for the banning or strict regulation of all man-made EDCs.

Endocrine disruptors can be chemicals which, 1) as a consequence of their molecular structure, bind to hormone receptors, and consequently mimic or antagonize the action of the natural ligand, 2) indirectly affect hormone concentrations by altering their synthesis or metabolism, or 3) interfere with signaling between different components of the hypothalamus-pituitary-endocrine axis (**Figure 4**; Dawson 2000). EDCs can act at very low concentrations, and their action has widely been investigated in laboratory studies (e.g., Amaral Mendes 2002; Ottinger et al. 2005, 2008). Yet, the effects of EDCs on wildlife were studied to a lesser extent and mainly focus on reproductive and thyroid hormones (Colborn et al 1993; Giesy et al. 2003; Tyler et al. 1998; Vos et al. 2008; Tan et al. 2009; Tyler et al. 1998; Ottinger et al. 2013). However, the relationships between contaminants, especially legacy POPs and Hg, and some hormones highly involved in breeding decisions have rarely been studied in free-ranging bird species (Franceschini et al. 2009; Wada et al. 2009; Bergman et al. 2013; Verreault et al. 2008; Herring et al. 2012; Nordstad et al. 2012; Verboven et al. 2010). **Specifically, in this thesis I focused on the capacity of legacy POPs and Hg to act as endocrine disruptors, on the hormonal mechanisms related to breeding decision.**

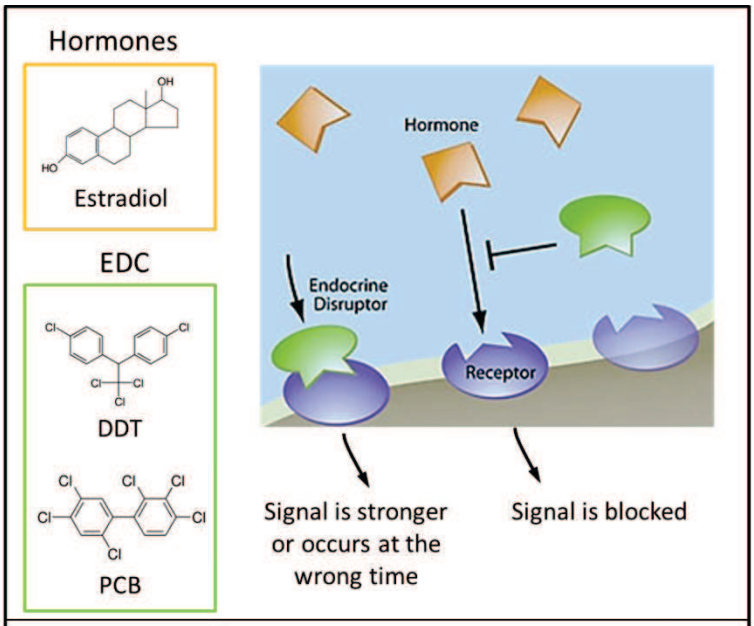


Figure 4: Example of endocrine disrupting chemical (EDC) action. Because of structural similarities (e.g. estradiol and DDT or PCBs), EDC can activate or block the signal of endogenous hormones.

1.3. Breeding decision and targeted hormones

In the present study, the term "breeding decision" refers to the different choices an individual has to make along the breeding cycle (**Figure 5**). Some decisions have to be made at the beginning of the cycle (hereinafter referred to as "the pre-laying phase"), such as "whether or not to breed", "when to lay eggs". And other decisions will be made during the parental phase (incubation and chick-rearing period), such as "what level of parental investment to devote" or "whether or not to abandon reproduction".

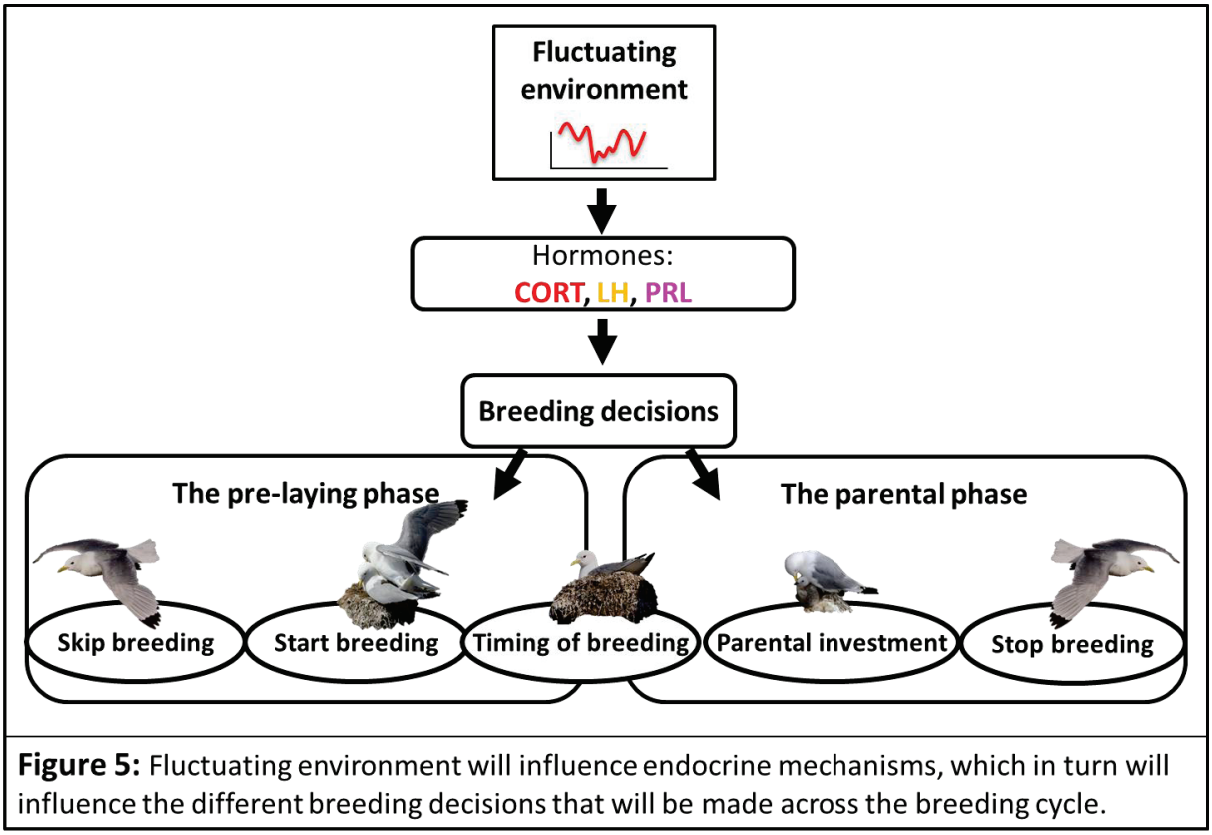


Figure 5: Fluctuating environment will influence endocrine mechanisms, which in turn will influence the different breeding decisions that will be made across the breeding cycle.

For example, in the case of predation risk or food shortage during the parental phase, important decisions have to be made: to abandon reproduction or continue to provide care to the brood. This would refer to the evolutionary trade-offs made by an individual, which will favor either its own survival or that of its brood (Drent & Daan 1980). Of course, these decisions are significantly influenced by environmental conditions. For example, if environmental conditions are good, an individual will be able to store enough energy to breed and perhaps successfully raise its offspring. If conditions are poor, all the available energy of

an individual will be directed towards its survival rather than reproduction, especially in long-lived organisms that will not jeopardize their survival and will act as "prudent parents" (Drent & Daan 1980; Bókony et al. 2009). Accordingly, some physiological mechanisms essential to the onset of breeding will not be activated. Indeed, as previously mentioned, all of these decisions are under hormonal influence. Hormones are chemical messengers that facilitate communication within the vertebrate endocrine system. They regulate a wide array of physiological processes such as growth, maintenance, reproduction, energy availability (reviewed in Lister & van der Kraak 2001). In the framework of breeding decisions, **I will specifically focus on three hormones: a hormone involved in the onset of breeding (luteinizing hormone, LH), a hormone involved in the expression of parental care (prolactin, PRL) and stress hormones (glucocorticoids), with particular attention to the latter.** As previously mentioned, the relationships between legacy POPs, Hg and these three hormones are poorly investigated in free-ranging organisms and will be the main topic of this thesis.

In vertebrates, the onset of breeding involves the activation of the hypothalamic-pituitary-gonadal axis (hereinafter referred to as HPG axis) from environmental cues such as increasing day light and increasing temperature (Schaper et al. 2012; Caro et al. 2013) but also social stimulation (Caro et al. 2007): these cues will activate the expression of a neuro-hormone, Gonadotropin Releasing Hormone (GnRH), which triggers LH secretion from the anterior pituitary. LH secretion is pulsatile and reaches higher levels during the pre-laying phase. LH, together with follicle-stimulating hormone (FSH), promotes gonadal maturation, sex steroid secretion and, in turn, sexual behaviors (**Figure 6**; Norris & Carr 2013).

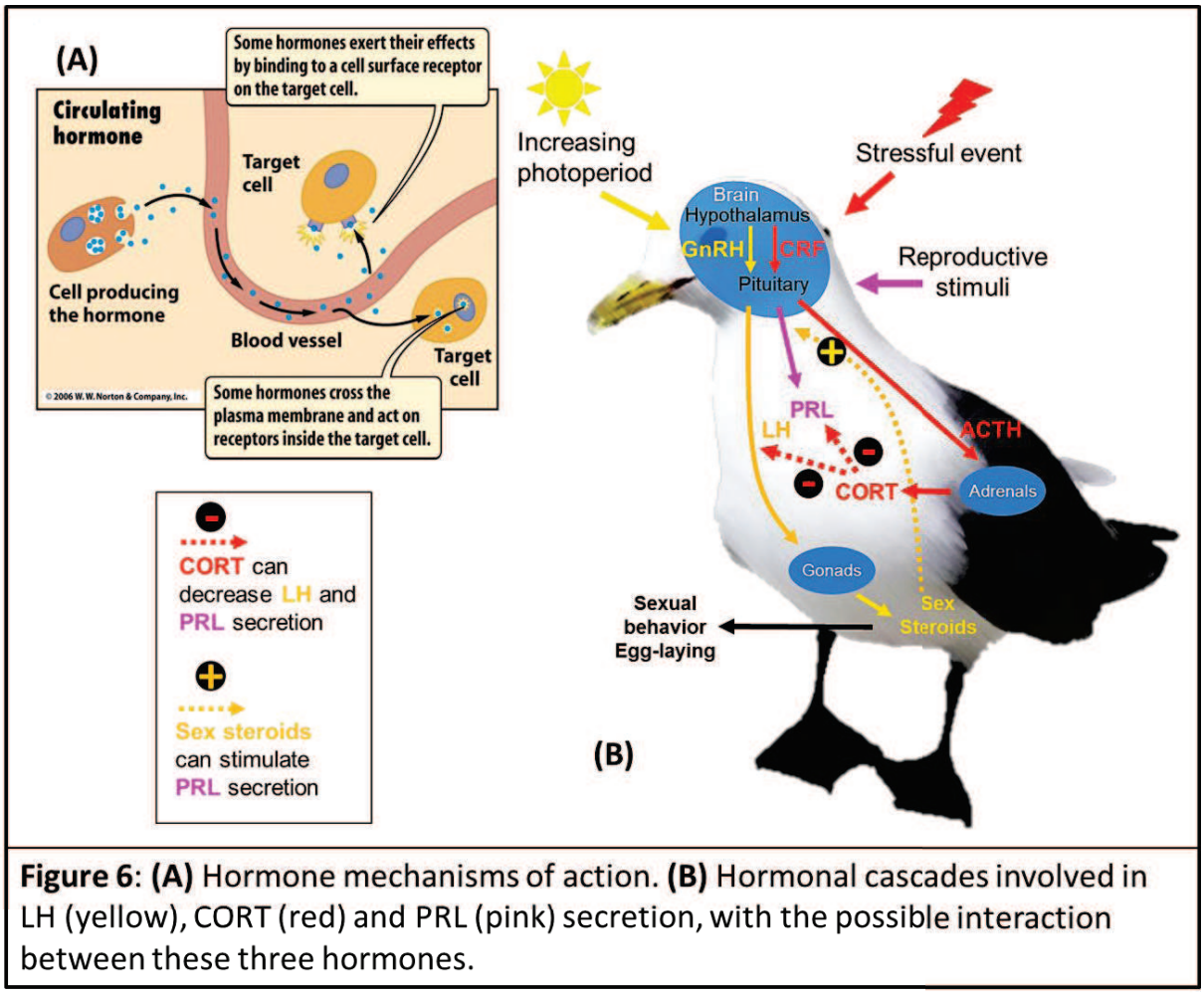


Figure 6: **(A)** Hormone mechanisms of action. **(B)** Hormonal cascades involved in LH (yellow), CORT (red) and PRL (pink) secretion, with the possible interaction between these three hormones.

PRL is an anterior pituitary hormone involved in more actions than that of all other pituitary hormones combined (Bole-Feysot et al. 1998); it is historically known as the lactation hormone in mammals (Stricker & Grueter 1928). In vertebrates, PRL is highly involved in parental care, such as mucus production or fanning behavior in fish (Blüm & Fiedler 1965; Ruiter et al. 1986), and in birds PRL induces nest building, nest attendance, incubation and brooding behaviors (**Figure 6**; Janik & Buntin 1985).

Finally, stress hormones, such as glucocorticoids, are produced in the case of a stressful event (e.g., inclement weather, food shortage, predation). At the endocrine level, an environmental disturbance will stimulate the hypothalamic–pituitary–adrenal (HPA) axis: specifically, the corticotropin-releasing hormone (CRH) will be released from the hypothalamus and will then stimulate adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary, which in

turn will activate the synthesis of glucocorticoid hormones (cortisol, corticosterone: CORT) from the adrenal cortex (**Figure 6**; Wingfield 2013). In birds, up to 90% of CORT released into the bloodstream will bind to corticosteroid-binding globulin (CBG) and will be transported to target cells. Concurrently, glucocorticoids will provide negative feedback signals for ACTH and CRH release (Wingfield 2013). This hormonal cascade triggers an array of physiological and behavioral adjustments that shift energy investment away from reproduction and redirect it towards survival (Wingfield & Sapolsky 2003).

LH, PRL and CORT significantly mediate fitness-related traits. Fitness is defined as the "reproductive power", the rate of energy conversion into offspring (e.g., Brown et al. 1993). For example, in birds, egg-laying date, incubation length, number of offspring that hatched or number of offspring that survived can be used as a proxy of breeding success and therefore fitness.

1.3.1. From pre-laying to egg laying: role of LH and CORT in reproductive decisions

The timing of breeding is significantly constrained by LH and CORT. Several studies explored the respective roles of LH and CORT in relation to reproduction (Schoech et al. 1996; Goutte et al. 2010a, 2010b, 2011a).

In female polar seabirds such as black-legged kittiwakes (*Rissa tridactyla*) and snow petrels (*Pagodroma nivea*), the decision to breed or not, a phenomenon frequently observed in seabirds (Chastel et al. 1993, 1995; Cam et al. 1998), is associated to higher concentrations of baseline CORT during the pre-laying phase (Goutte et al. 2010a, 2010b).

In female kittiwakes, low LH concentrations (induced by a GnRH injection) were also related to non-breeding events and, interestingly, LH decreased with increasing baseline CORT (Goutte et al. 2010b). In contrast, in snow petrels of both genders, it was rather the inability to

maintain high LH concentrations (30 min. following GnRH injection) that predicted the probability to forgo breeding (Goutte et al. 2011a). Additionally, snow petrels' age was also related to baseline LH concentrations and GnRH-induced LH concentrations after 30 minutes: baseline LH concentrations increased with increasing age and LH concentrations 30 minutes after GnRH injection were lower in very young and very old snow petrels (Goutte et al. 2011a).

With regard to egg-laying dates, in female kittiwakes the first egg-laying date is negatively correlated to body condition (i.e., the better the condition, the earlier a female will lay), whereas egg-laying dates are positively related to testosterone concentrations in male kittiwakes (Goutte et al. 2010b). In kittiwakes, the male provides food to the female prior to egg laying (e.g., Kempenaers et al. 2007). One may thus suppose that high testosterone concentrations would result in increased activity to provide food, which would directly increase the female's body condition, or in an increased mating effort (Kempenaers et al. 2007). Both cases result in an advanced egg-laying date. In snow petrels, egg-laying date was positively related to baseline CORT (Goutte et al. 2010a).

1.3.2. Parental phase, from incubation to chick rearing: the role of PRL and CORT

In incubating or chick-rearing birds, both PRL and CORT can mediate several behaviors and their secretion is believed to be modulated according to brood value (Chastel et al. 2005; Angelier & Chastel 2009; Angelier et al. 2013).

During stressful events CORT enables behavioral adjustments that shift energy investment away from reproduction and redirects it towards self-preservation and hence survival (Ricklefs & Wikelski 2002; Wingfield & Sapolsky 2003; Angelier & Wingfield 2013). CORT is therefore strongly connected with fitness traits such as breeding success, individual quality

and survival (Kitaysky et al. 1999; Breuner et al. 2008; Angelier et al. 2009a, 2010; Bonier et al. 2009; Bókony et al. 2009; Goutte et al. 2010, 2011b; Breuner 2011).

Additionally, the hormone prolactin (PRL) can also mediate this life-history trade-off between reproduction and survival in free-living birds (reviewed in Angelier & Chastel 2009). The release of this anterior pituitary hormone stimulates and facilitates parental behavior such as egg incubation and brood provisioning (Buntin 1996). In response to acute stress, circulating PRL levels have been shown to decrease in several bird species (Chastel et al. 2005; Angelier & Chastel 2009; Angelier et al. 2013), and this could ultimately trigger nest desertion if PRL levels remain low during a prolonged period (Angelier et al. 2007, 2009b; Angelier & Chastel 2009; Heidinger et al. 2010). Importantly, this decrease in PRL levels varies between individuals and life-history stages, suggesting that birds are able to attenuate their PRL response to acute stress in order to ensure that reproduction is not inhibited when the fitness value of the current reproductive event is high ("brood value hypothesis"; Bókony et al. 2009; Lendvai et al. 2007).

Hence, both CORT and PRL are very likely to mediate parental effort and parental investment in birds (Koch et al. 2004; Chastel et al. 2005; Criscuolo et al. 2005; Angelier et al. 2007, 2009b, 2013; Groscolas et al. 2008) and any disruption of these hormones may alter an individual's ability to adjust reproductive decisions to environmental conditions (Jenssen 2005).

As previously mentioned, the relationships between Hg, legacy POPs, LH, PRL and CORT have rarely been studied in free-ranging species. Only three studies on wild bird species have focused on the relationships between Hg and CORT (Franceschini et al. 2009; Wada et al. 2009; Herring et al. 2012), two on the relationships between POPs and CORT (Verboven et al. 2010; Nordstad et al. 2012), and only one has investigated the relationships between POPs

and PRL (Verreault et al. 2008). To date, no study was aimed at studying the relationships between Hg and PRL; there is thus a clear gap in studies investigating the relationships between contaminants and some hormones involved in reproductive decisions.

2. OBJECTIVES AND METHODS

2.1. Overall and specific objectives

2.1.1. Seabirds, biomonitors of marine environment contamination

Seabirds in particular are good biomonitors of marine environment contamination (Furness 1993). Firstly, top predators accumulate elevated amounts of contaminants (Atwell et al. 1998; Borgå et al. 2003). Secondly, seabirds are long-lived animals; they would thus accumulate contaminants in their tissues over a long lifespan, with more time for the hazardous effects of contaminants to become visible (Rowe et al. 2008). Seabirds are also "capital breeders": they need to accumulate energetic reserves through fat, to be able to breed (e.g., Chastel et al. 2005). Body condition (which reflects energetic storage) is therefore a crucial factor for reproductive success (Chastel et al. 1995) and, given the fact that many contaminants are lipophilic, seabirds may be more vulnerable to contaminant hazards.

In this thesis, I mainly studied two polar seabird species: the Arctic breeding black-legged kittiwake (*Rissa tridactyla,* hereinafter referred to as 'kittiwake') and the Antarctic snow petrel (*Pagodroma nivea*). Species' ecology and study sites are described in detail in the following section **"2.2.1 Study sites and species"**. Both species are colonial, tame and large enough (~400g) for large amounts of blood (1.5ml) to be sampled without inducing health hazards.

Kittiwakes are subjected to a long-term hormonal survey (IPEV prog 330 to O. Chastel; Goutte et al. 2014a) and snow petrels to a long-term ringing survey running from 1964 to the present day (IPEV prog 109 to H. Weimerskirch). As a consequence, many snow petrels are of known age. Very few studies have investigated the influence of age on contaminants in adult bird species, and the patterns are often contradictory (Furness & Hutton, 1979; Hutton 1981; Thompson et al. 1991; Bustnes et al. 2003b). Besides, hormonal secretion of PRL, CORT and LH are tightly related to age in long-lived seabirds such as snow petrels (Angelier et al. 2007; Goutte et al. 2010b, 2011a). Therefore, one objective of this thesis was to incorporate age into the study of endocrine disruption. I specifically tested if some age classes would be more susceptible to endocrine disruptors in snow petrels.

Additionally, kittiwakes and snow petrels are known to bear significant amounts of Hg and POPs (Nettleship & Peakall, 1987; Savinova et al. 1995; Bargagli et al. 1998; Campbell et al. 2005; Xie et al. 2008; Corsolini et al. 2011; Nordstad et al. 2012; Goutte et al. 2013). These significant amounts of contaminants can increase baseline CORT secretion and alter coloration of carotenoid-based integuments in kittiwakes (Nordstad et al. 2012; Blévin et al. 2014).

CORT and POP data were also available for 5 other species feeding at different trophic positions: two Arctic species sampled in Svalbard by Norwegian and Canadian collaborators, the common eider *Somateria mollissima* and the glaucous gull *Larus hyperboreus*, and three Antarctic species, the wandering albatross *Diomedea exulans* sampled in Crozet Islands, the cape petrel *Daption capense* and the south polar skua *Catharacta maccormicki* sampled in Adélie Land.

2.1.2. Thesis objectives

As previously mentioned, contaminants are well-known endocrine disruptors but most studies have mainly focused on disruption of sex steroids or thyroid hormones (e.g., Tyler et al. 1998; Tan et al. 2009). Breeding decisions are mediated by hormones, among which LH, PRL and CORT can predict decisions such as whether or not to breed, when to breed, what parental investment to make (e.g., Goutte et al. 2010a, 2010b; Goutte et al. 2011a; Angelier & Chastel 2009). However, the relationships between contaminants and LH, PRL or CORT have seldom been studied in free-ranging seabirds (Verreault et al. 2008; Verboven et al. 2010; Nordstad et al. 2012). The aim of the present thesis was to investigate the relationships between POPs (OCPs and PCBs), Hg and hormones involved in breeding decisions (LH, PRL and CORT), and also identify which contaminant would act on the HPA axis. These investigations were carried out during two periods of the breeding cycle, the pre-laying phase and the parental phase.

Firstly, during the pre-laying phase, I investigated the relationships between Hg and LH through a GnRH challenge. LH is a crucial hormone for the onset of breeding, therefore I also explored how age could interact with this relationship. I also examined the relationships between POPs and CORT in female pre-laying kittiwakes.

Secondly, during the parental phase, I tested whether POPs and Hg were related to CORT and PRL secretion. I conducted a study on the relationships between CORT and PCBs in 7 polar seabirds. Then, I tested whether different aspects of the HPA axis of incubating male kittiwakes were related to Hg, PCB and OCP concentrations in blood. To do so, I first measured the CORT response to a restraint stress protocol. Birds were then injected with exogenous dexamethasone (DEX, a potent CORT agonist) to test the efficiency of the negative feedback. And finally, birds were injected with exogenous ACTH, a protocol used in several bird species to test adrenal responsiveness. The last experiment was to test whether the hazardous effects of contaminants could be exacerbated during a stressful situation. To do so,

I experimentally increased CORT levels by implanting CORT or placebo pellets and measured the effects of increased stress response on contaminant/hormones relationships.

2.2. Methods

2.2.1. Study sites and species

Most of the data used in this thesis were collected during 3 fieldwork sessions (1.5 months in 2011, 1.5 months in 2012, and 3 weeks in 2013) in Svalbard (Arctic) and one 2-month fieldwork session in Adélie Land (Antarctica, from December 2012 to January 2013).

Snow petrels in Adélie Land

The study was carried out on Ile des Pétrels, Pointe Géologie Archipelago, Adélie Land (140° 01′ E, 66° 40′ S), Antarctica. Snow petrels (250–500g; **Figure 7**) are very long-lived birds with low annual fecundity and high adult life expectancy (Chastel et al. 1993; Jenouvrier et al. 2005). Like all procellariiform species, breeding pairs lay only one egg per season, with no replacement clutch in case of failure (Chastel et al. 1993; Barbraud & Weimerskirch 2001). Age at first breeding is 10 years on average and birds breed annually, although a significant proportion of birds skip the breeding season (Chastel et al. 1993). Nest and mate fidelity is high (Bried et al. 2003). Males and females provide roughly similar amounts of parental care (incubation of the egg and care for the chick). Birds arrive on the breeding ground in early November. The courtship and mating period typically lasts about 2 weeks. Then, individuals of both genders forage at sea to accumulate resources and females build up reserves for the egg (pre-laying exodus). The laying period ranges from late November to mid-December. Within one breeding season, egg-laying dates differ between individuals over a 2-week period (Barbraud et al. 2000). The ongoing snow petrel population mark-recapture programme began in 1964 (see Chastel et al. 1993; Barbraud & Weimerskirch 2001 for detailed methodology).

Each chick of the monitored colonies was banded prior to fledging every year since 1964. Part of the current population is thus of known age.

Figure 7: **(A)** Snow petrels nest into crevices and are caught by hand. **(B)** Their age is determined by the number that appears on the metal ring placed on the tarsus. **(C)** brooding snow petrel and its chick.

Kittiwakes in Svalbard

The study was carried out in a colony at Kongsfjorden, Svalbard (12° 13′ E, 78° 54′ N), 7km southeast of Ny-Ålesund, Norway. Kittiwakes (340-500g, **Figure 8**) are colonial seabirds that breed on cliffs throughout the northern parts of the Pacific and Atlantic, including the Barents Sea region up to the Svalbard Archipelago (Anker-Nilssen et al., 2000). At Kongsfjorden, kittiwakes generally arrive at the colony in early April. April and May are devoted to nest building, nest defence and mating. Egg laying generally starts in mid-June; clutch size varies from 1 to 3 eggs but in most cases only 2 eggs are laid. Eggs are incubated by both parents and incubation lasts approximately 27 days (Coulson 2011). After hatching, the first 10 to 15

days are energetically costly for the parents; they have to alternate nest attendance in order to keep the chick warm, with foraging at sea. Once the chick is thermally independent the parents are more rarely observed on the nest and mainly come back to feed their chicks. Kittiwakes fledge at around 40 days of age (late August/early September), and start breeding at the age of 3 to 4 years (Coulson 2011).

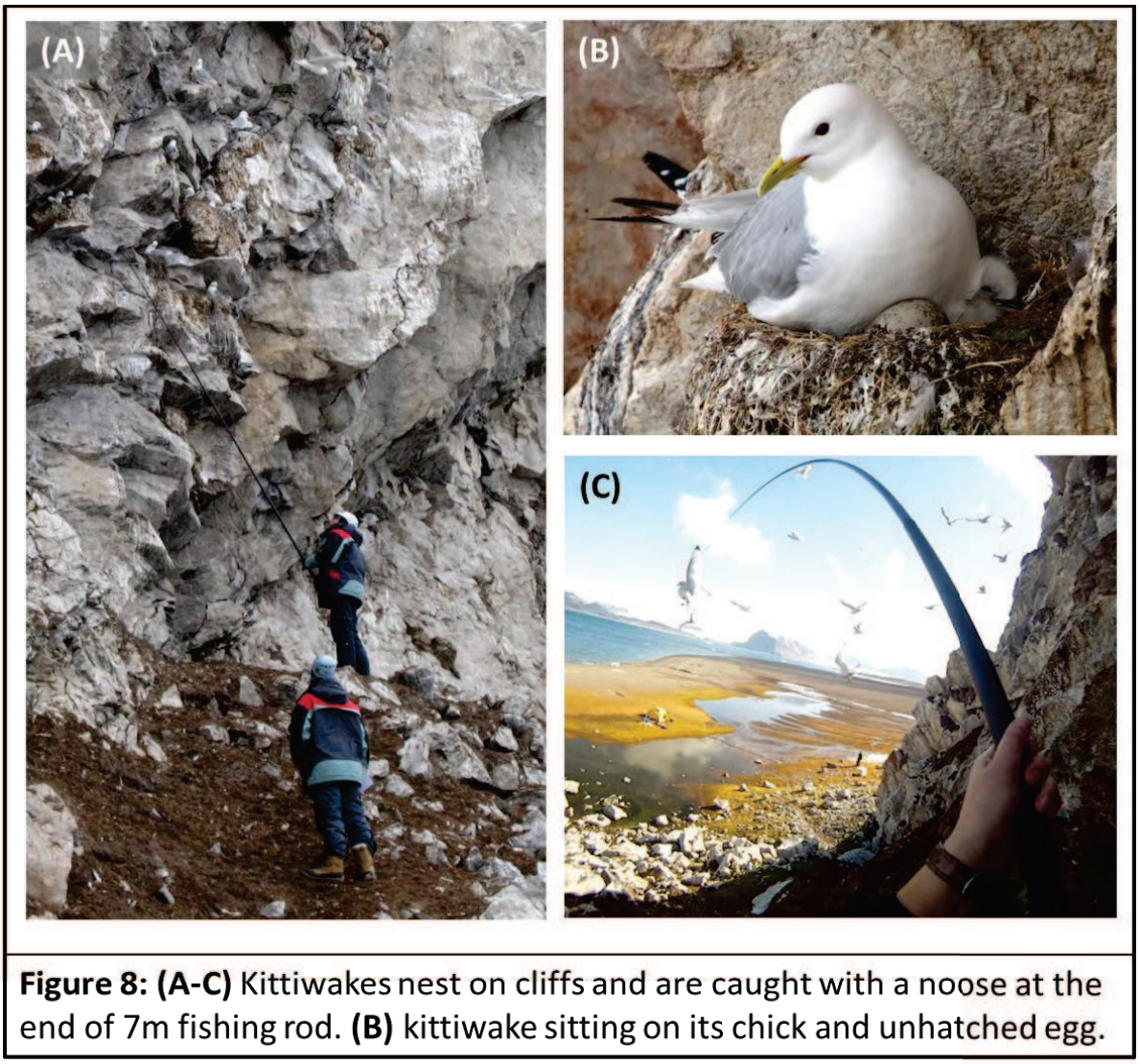


Figure 8: (A-C) Kittiwakes nest on cliffs and are caught with a noose at the end of 7m fishing rod. **(B)** kittiwake sitting on its chick and unhatched egg.

2.2.2. Blood sampling, morphometrics and fitness measurements

Snow petrels were caught by hand on their nest whereas kittiwakes were captured on their nest with a noose at the end of a 7-m fishing rod (**Figure 7-8**). Immediately after capture (i.e., within 3 min.), both species are subjected to a standardized capture/restraint stress protocol as

described by Wingfield (1994): an initial blood sample (300µl) is collected from the alar vein using a 1-ml heparinized syringe and a 25-gauge needle. This initial blood sample reflects baseline hormone concentration (e.g., Chastel et al. 2005). After the initial blood sample collection, birds are placed into a cloth bag and a subsequent sample (1.5ml) is collected 30 minutes after capture (**Figure 9**). This blood sample facilitates the measurement of hormone modulation when an individual is subjected to stress, and is also used to measure contaminants. After centrifugation hormones are measured in plasma, Hg concentration is determined in red blood cells (~200µl) and POP concentration in plasma/whole blood (~1ml) depending on the lab. Focal birds are then marked on the forehead with a small spot using a water erasable marker in order to distinguish them from their partner from a distance.

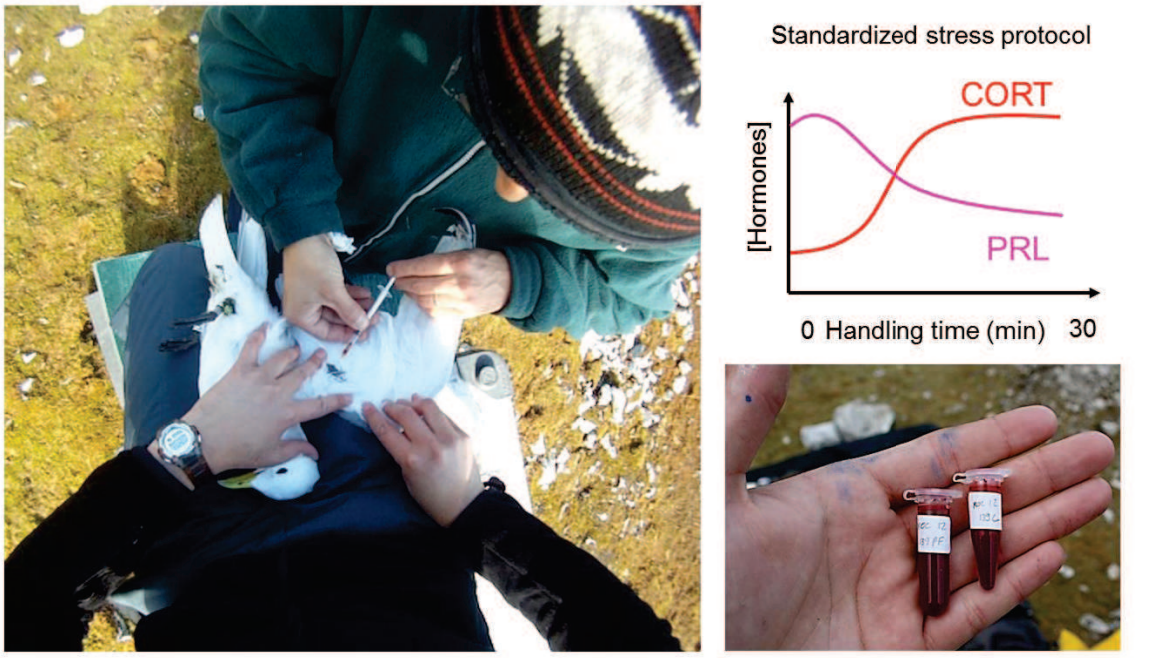


Figure 9: Birds are sampled for blood within 3 minutes (baseline levels) and after 30 minutes (stress-induced levels). CORT concentrations will increase and concurrently PRL concentrations will decrease.

Birds were individually marked with metal bands (snow petrels and kittiwakes) and PVC plastic bands engraved with a three-digit code (for kittiwakes), fixed to the bird's tarsus, for identification from a distance. Birds were weighed to the nearest 2g using a Pesola spring

balance, and their skull length (head + bill) was measured to the nearest 0.5mm with a sliding calliper. For each individual, I calculated a body condition index by using the scaled mass index equation (Peig & Green 2009). Every two days, all nests were checked to monitor breeding decision (at least one egg laid or no egg laid) and egg-laying dates. Then, with the same technique, I checked the nest content every 2 or 3 days to monitor the number of eggs that hatched (hatching success) and the number of chicks that reached 40 days of age for snow petrels or at least 12 days of age for kittiwakes (hereinafter referred to as "breeding success").

2.2.3. Hormone manipulations

GnRH challenge **[PAPER I; II]**

A first blood sample was collected from the alar vein with a syringe immediately after capture to determine baseline LH concentrations in plasma. Then, birds were immediately injected with 0.1ml of a GnRH solution in the second alar vein ([Gln8], Sigma Lot 121H04314). The GnRH was dissolved in a physiological solution to yield a dosage of 0.6mg for 0.1ml (about 1.5mg/ kg body mass in 1ml of 0.9% saline solution). A GnRH injection elicits a maximal LH increase after 10 minutes, and this response documents the ability of the pituitary to release LH. Measuring LH levels 30 minutes following the GnRH challenge can provide information on an individual's capacity to maintain high LH concentrations. Blood samples were therefore collected from alar veins 10 and 30 minutes following the GnRH injection to measure LH concentrations in plasma (**Figure 10**).

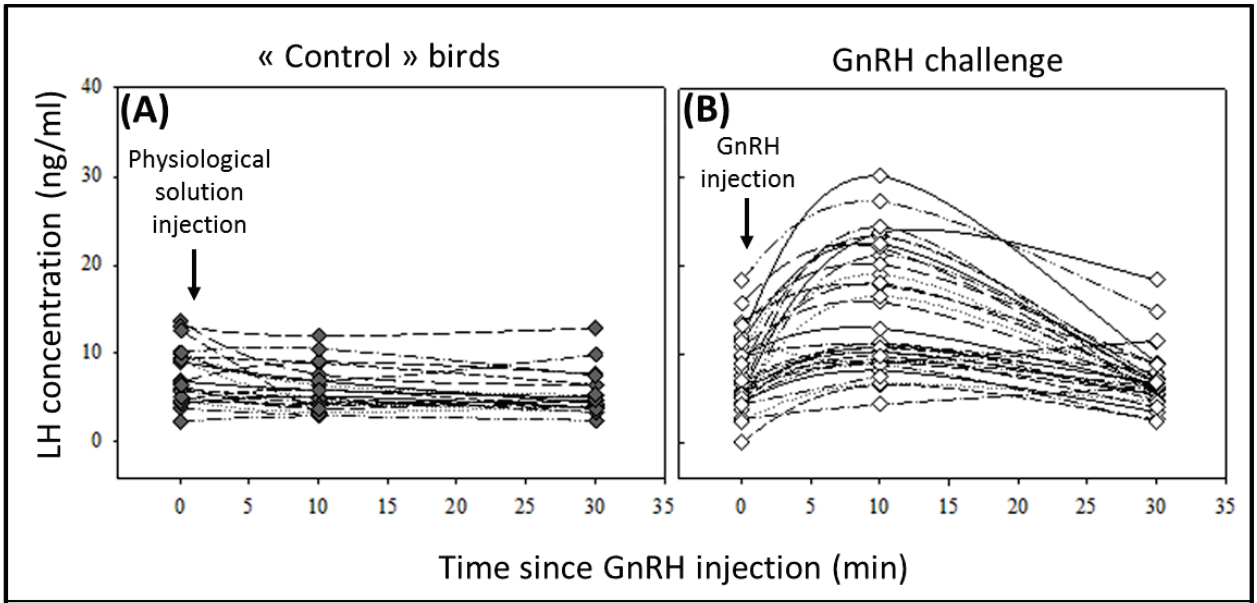


Figure 10: Effects of **(A)** physiological solution injection and **(B)** GnRH injection on circulating concentrations of LH in plasma. Kittiwakes and snow petrels were blood sampled before injection then 10 and 30 minutes after injection.

CORT pellet implantations **[PAPER V]**

Immediately after the first blood sample, male kittiwakes were implanted subcutaneously either with a CORT (25mg/pellet 15-day release, G111, N=22) or a placebo (25mg/pellet 15-day release, C111, N=21) pellet. Pellets were obtained from Innovative Research of America (Sarasota) and surgical equipment was sterilized with 90% alcohol. A small incision (~5mm) was made on the nape of the neck using a sterilized surgical scalpel and the pellet was inserted with a sterilized bent clip. The incision was then sutured with surgical glue (3M Vetbond) and disinfected with aluminum spray (Vetoquinol Aluspray, **Figure 11**).

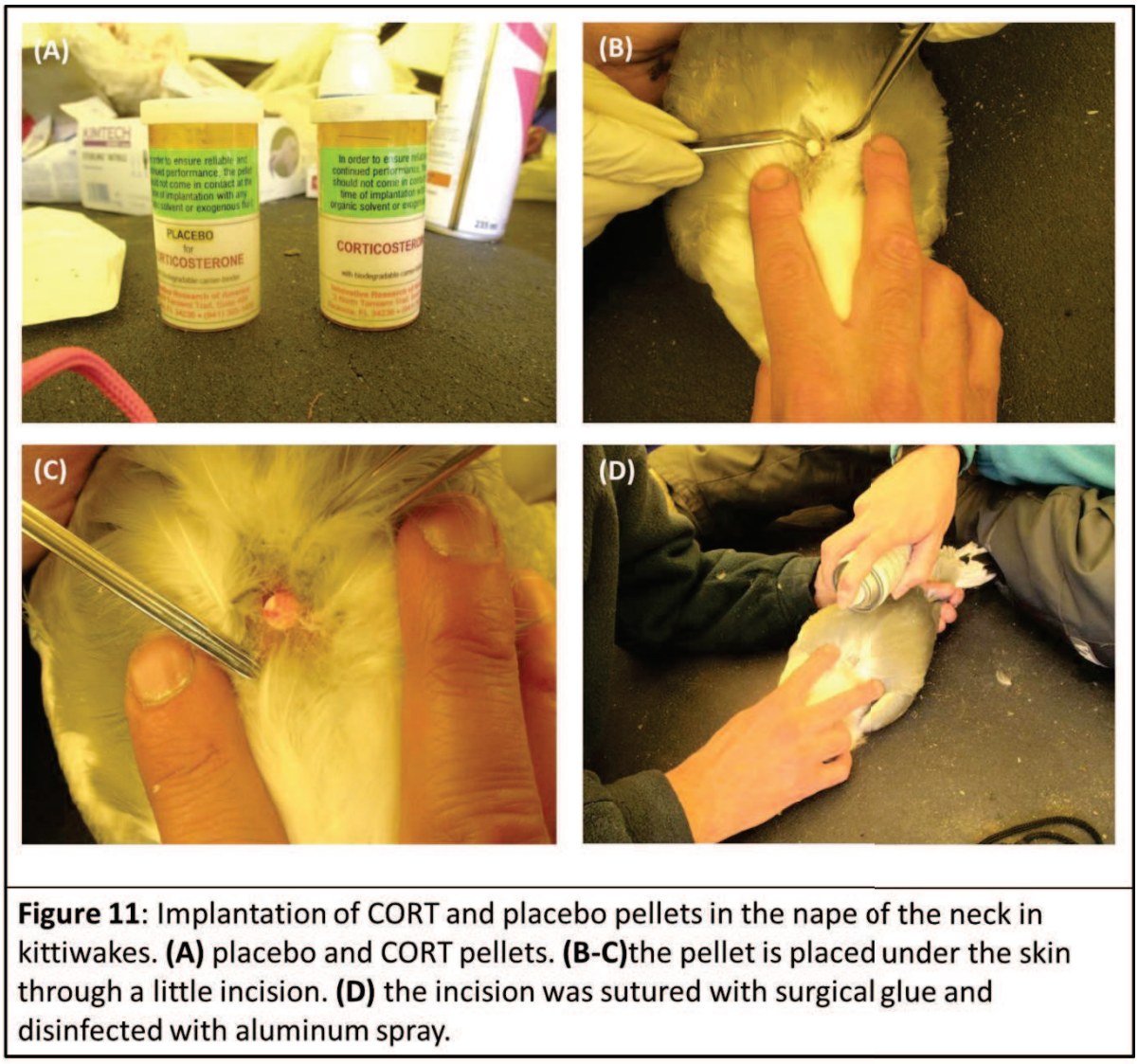


Figure 11: Implantation of CORT and placebo pellets in the nape of the neck in kittiwakes. **(A)** placebo and CORT pellets. **(B-C)**the pellet is placed under the skin through a little incision. **(D)** the incision was sutured with surgical glue and disinfected with aluminum spray.

The operation lasted about 10 minutes and the implantation day was denoted as "day 0". To validate the CORT treatment, 4 CORT and 4 placebo birds (different ones each time) were recaptured on day 1, 2, 3, 7 and were subjected to a "baseline" blood sampling. On day 11, most kittiwakes were recaptured and sampled for baseline and stress-induced hormone concentrations (**Figure 12**).

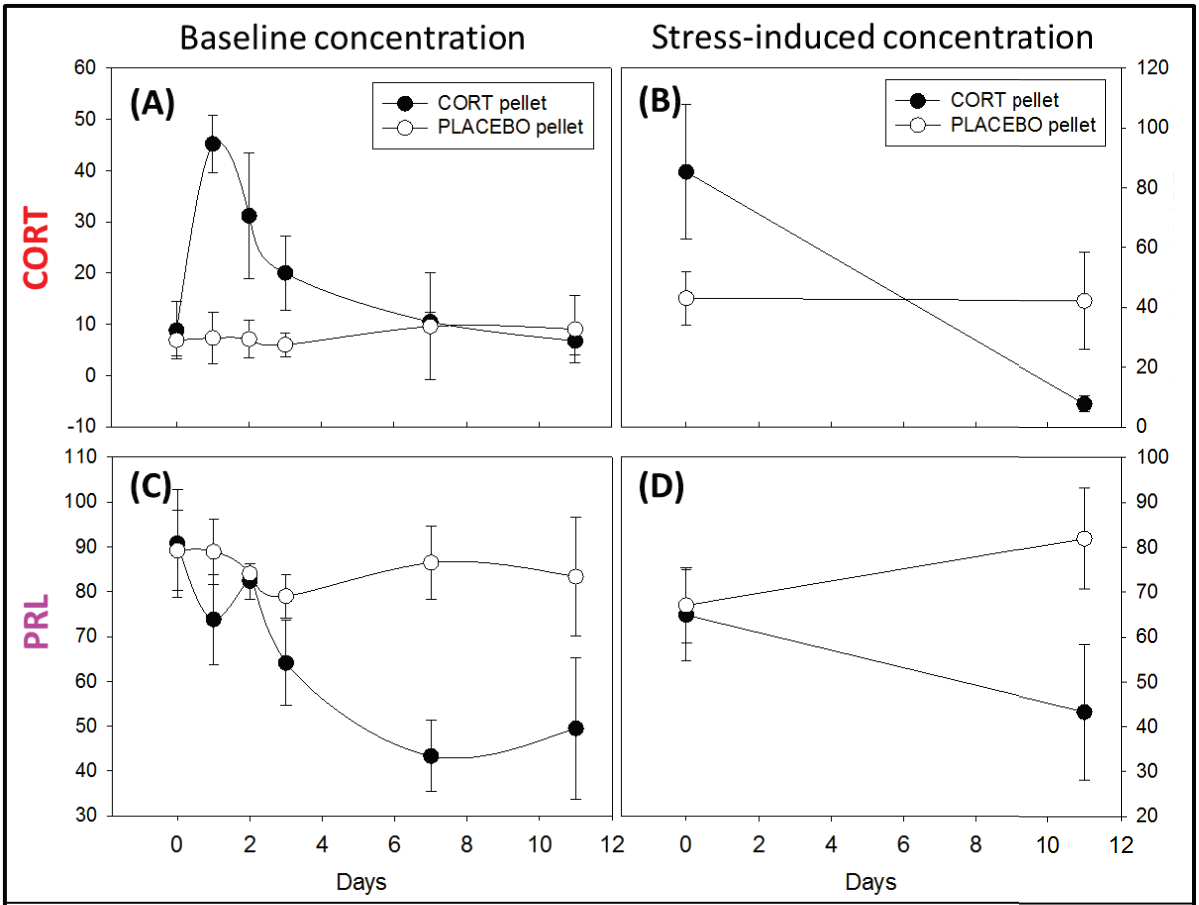


Figure 12: Effects of CORT and placebo pellet implantation during 11 days on **(A-B)** baseline and stress-induced CORT concentrations and **(C-D)** baseline and stress-induced PRL concentrations in incubating male kittiwakes.

Testing HPA axis activity **[PAPER VII]**

After 30 minutes, at the end of the capture/restraint stress protocol, birds were injected with DEX then put back into the cloth bag. Fifteen minutes later, I collected a third blood sample and then immediately injected ACTH, after which the birds were replaced into the bag again. Fifteen minutes following the ACTH injections, I collected a fourth and final blood sample. All injections were performed intramuscularly in the pectoral muscle. Concentrations were adapted to a 400-g body mass, which is the average body mass of incubating male kittiwakes. I therefore injected 0.2ml of DEX (Dexazone 2mg/ml Virbac, France) and 0.1ml of porcine ACTH (Sigma Aldrich, 100IU A6303 dissolved in 0.5ml Ringer's physiological solution), to obtain doses of 1mg/kg dexamethasone (DEX) and 50IU/kg ACTH, which are considered as

sufficient to elicit maximal CORT decrease and increase (**Figure 13**). The stress series were shortened compared to those used in laboratory studies: blood samples were collected 15 minutes after each injection versus 45 minutes normally used to elicit maximal CORT responses induced by DEX and ACTH (Schmidt et al. 2012). I shortened the stress series to avoid leaving the nests unattended for a long period (which may increase the risk of the nest being predated).

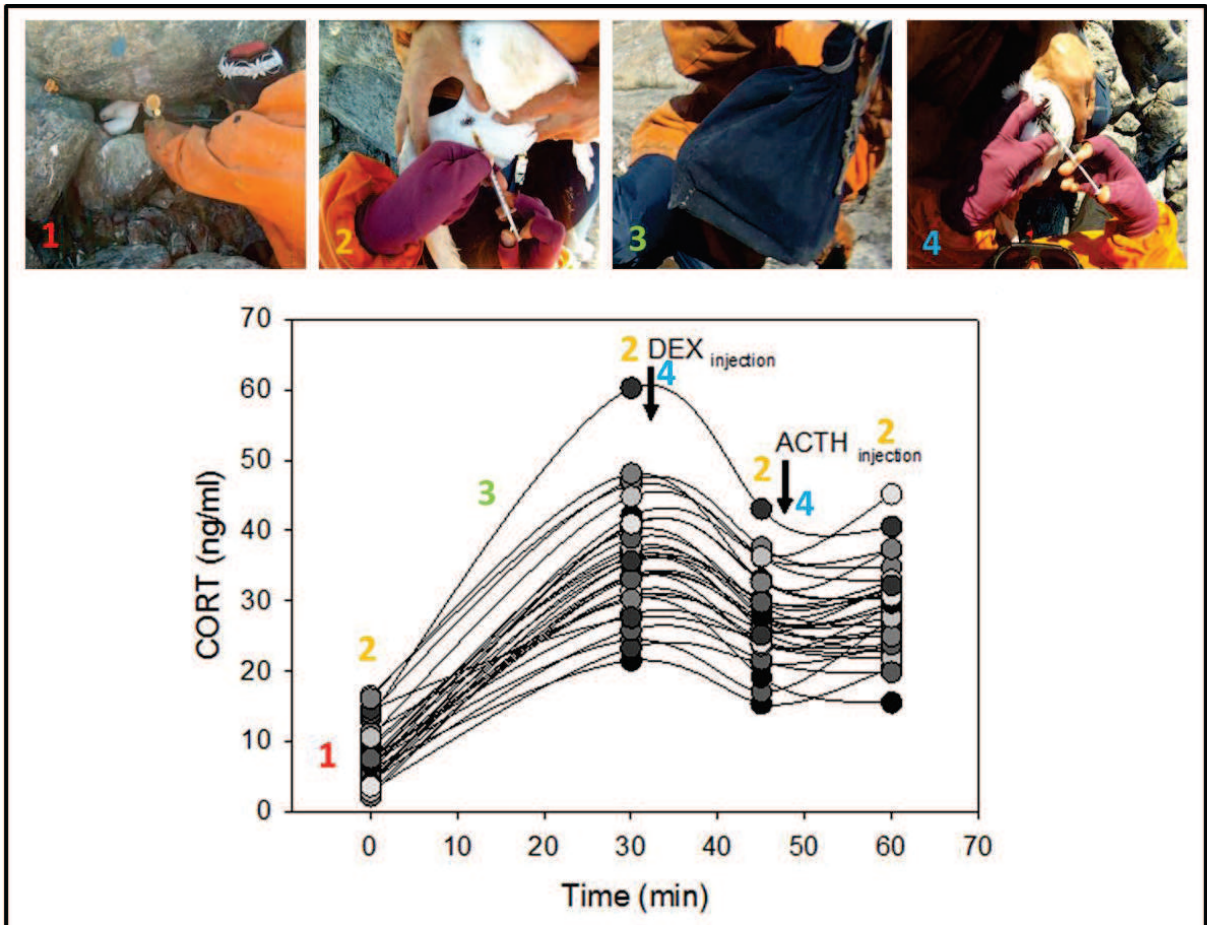


Figure 13: Testing the HPA axis activity. 1: bird catching. 2: blood sampling. 3: handling stress. 4: DEX or ACTH injections.

2.2.4. Hormone and contaminant assays

Hormones were measured in plasma by radioimmunoassay at the CEBC (Villiers-en-Bois, France) and Hg was measured in red blood cells using an Advanced Hg Analyzer

spectrophotometer at LIENSs (La Rochelle, France). For kittiwakes, POPs were measured in whole blood samples by GC-MS at NILU (Tromsø, Norway) and, for snow petrels, POPs were measured in plasma by GC-ECD at EPOC (Bordeaux, France). For kittiwakes, the targeted compounds included 13 PCBs (CB-28, -52, -99, -101, -105, -118, -128, -138, -153, -180, -183, -187 and -194) and 10 OCPs (*p,p'*-DDE, α-, β-, γ-HCH, HCB, oxychlordane, trans-, cis-chlordane, trans-, cis-nonachlor); and for snow petrels: 7 indicator PCBs (CB-28, -52, -101, -118, -138, -153 and -180) and 11 OCPs (HCB, Gamma HCH, Heptachlore, cis-chlordane, trans-nonachlor, 2,4' DDE, 4,4' DDE, 4,4' DDD, 2,4' DDT, 4,4' DDT and mirex). Detailed methods are described for the LH assay and GnRH challenge in [**PAPER I; II**], for the CORT assay in [**PAPER III; IV; VI; VII**], and for the PRL assay in [**PAPER IV; V**]. Total blood Hg is found in [**PAPER I; II; IV; V**], POPs measured at EPOC in [**PAPER IV**], and POPs measured at NILU in [**PAPER III; VI; VII**].

3. DISCUSSION

This section is organized in two parts: the pre-laying phase and the parental phase. Due to differences in energy cost across the breeding cycle, diet and body condition will vary, which in turn will modify contaminant concentrations in blood (**Figures 14, 15)**.

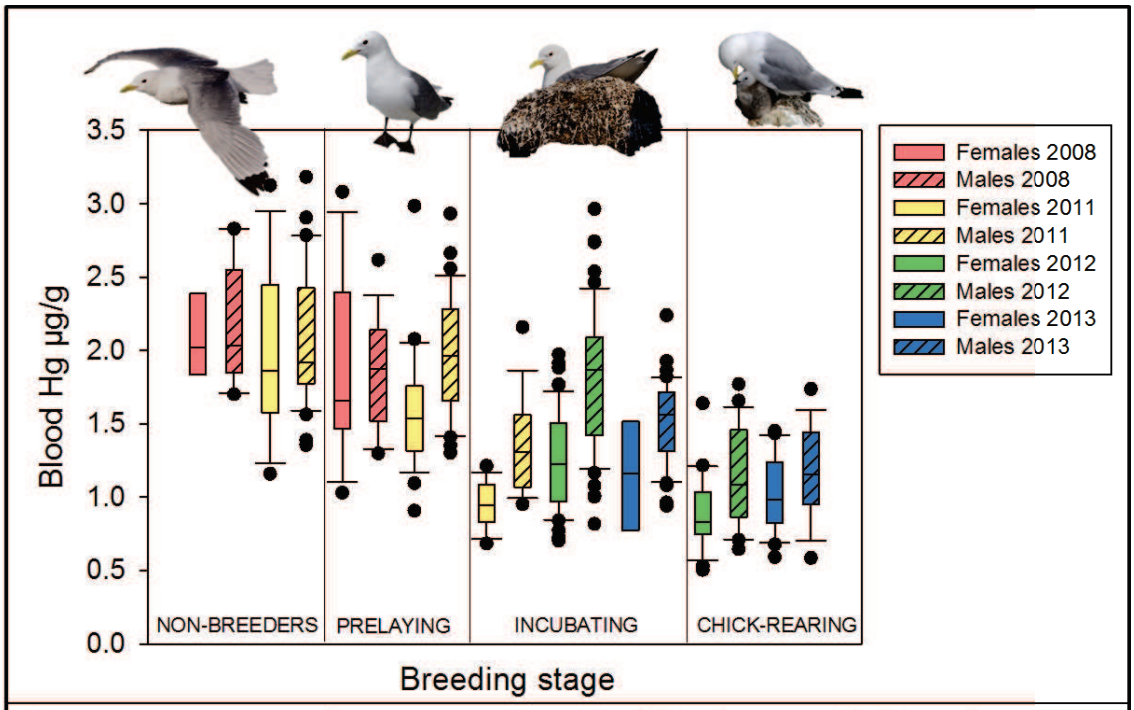


Figure 14: Variation of Hg concentrations in blood across the breeding cycle in 4 different years. Plain boxes denote females and striped boxes denote males. Different colored boxes represent different years.

Additionally, hormone concentrations - mostly those involved in breeding decisions - are released cyclically according to the physiological function they trigger (mating, egg laying, incubating, chick feeding, etc.; **Figures 16, 17**). The different breeding decisions would therefore be perfectly timed in order to adjust the individuals' needs to environmental resource availability. Given these variations, the relationships between contaminants and hormones may differ according to the breeding cycle. To illustrate the main results, I mostly used kittiwake-related data as these were obtained from 3 successive years including at least two phases of the breeding cycle each year.

3.1. The pre-laying phase: from mating to egg laying

The pre-laying phase is the part of the breeding cycle that precedes the parental phase (once the egg is laid); it thus includes several behaviors such as arrival at the breeding site, mate

choice, nest building and egg laying. During this pre-laying phase, several reproductive decisions have to be made: “whether or not to breed”, “which partner to choose”, “when to start egg laying”, “how many eggs to lay”, etc. For the pre-laying phase, the focus was on investigating the relationships between contaminants, LH and CORT. Several questions were addressed: **Can Hg and some legacy POPs alter the breeding decisions that should be made during the pre-laying phase? If so, are they related to the same hormones? And what can be the underlying disrupted mechanism?** The study on snow petrels provided the opportunity to investigate an additional question, concerning the relationships between age and contaminants. **Can contaminant concentrations in blood vary with the age of individuals?**

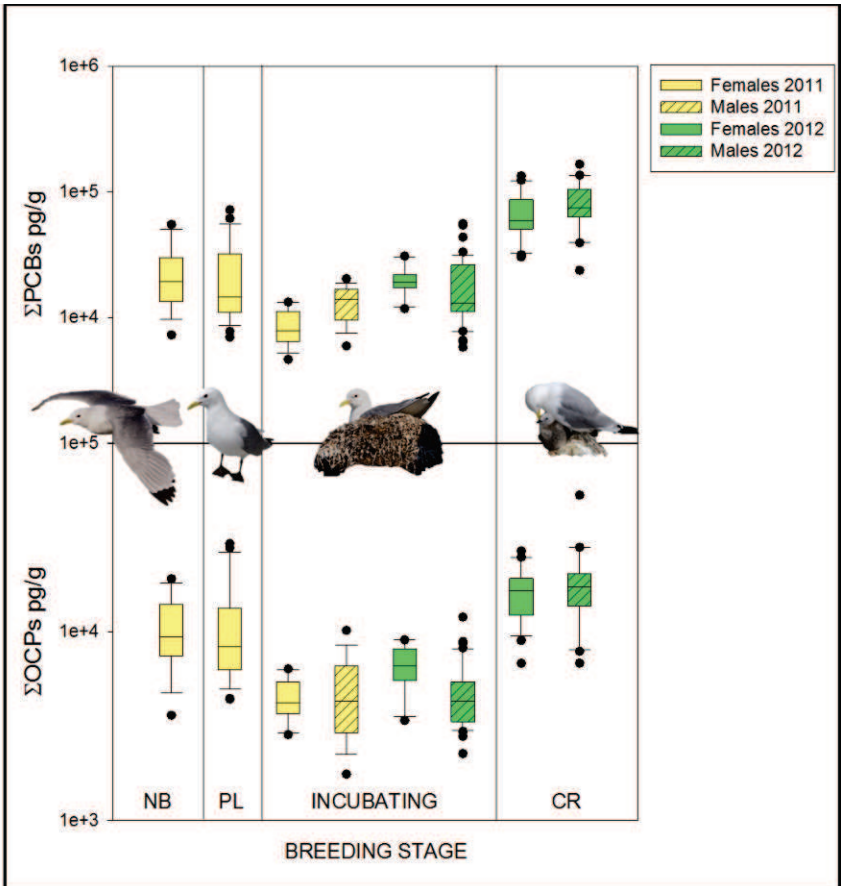


Figure 15: Variation of blood PCBs (upper panel) and OCPs (lower panel) concentrations across the breeding cycle in two different years. Plain boxes denote females and striped boxes denote males. Boxes of different colors are for different years. NB=non-breeders; PL=Pre-laying; CR=Chick-rearing

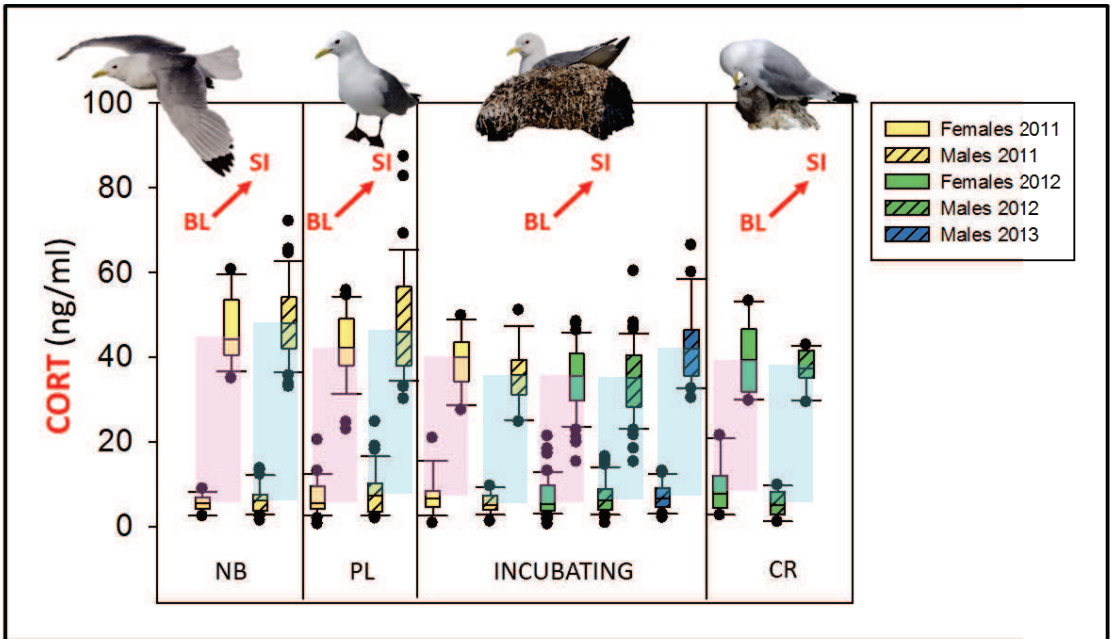


Figure 16: Variation of plasma CORT concentrations across the breeding cycle in three different years. The CORT stress response is illustrated by pink rectangles for females and blue rectangles for males, arrow indicates the direction of CORT variation. Plain boxes denote females and striped boxes denote males. Different colored boxes represent different years. BL= Baseline; SI= Stress-induced; NB=non-breeders; PL=Pre-laying; CR=Chick-rearing

In pre-laying kittiwakes, the decision to breed or not could be predicted according to Hg concentration in blood **[PAPER I].** Indeed, kittiwakes that would defer reproduction had a higher Hg concentration than birds that would breed, in both sexes **[PAPER I].** The association of Hg with non-breeding events was likely to be related to LH in kittiwakes and snow petrels **[PAPER I; II]**. In pre-laying male snow petrels, blood Hg decreased with increasing age and this relationship was more likely to be connected with age and trophic niche segregation between the sexes **[PAPER II]**. POPs were measured in pre-laying female kittiwakes only, and POP concentrations in blood were not related to mating decisions **[PAPER III]**. However, body condition was associated with non-breeding events; female kittiwakes that would defer breeding had a lower body condition **[PAPER III]**. And interestingly, a negative association between body condition and POPs was found only in

females that would not breed **[PAPER III]**. CORT secretion induced by handling stress increased with increasing PCBs in pre-laying female kittiwakes **[PAPER III]**. Clutch size and breeding success were not related to Hg or POPs measured during the pre-laying period **[PAPER I; II; III]**. However, in female kittiwakes, the egg-laying date was brought forward with increasing blood OCPs **[PAPER III]**.

3.1.1. Hg, endocrine correlates and fitness

To provide information on the underlying mechanism that could associate Hg with LH, exogenous GnRH was injected into kittiwakes and snow petrels (GnRH is the precursor of LH) to test whether the release of LH from the pituitary gland was disrupted by Hg **[PAPER I; II]**. In kittiwakes and snow petrels, Hg did not suppress the pituitary's ability to release LH **[PAPER I; II]**. And the subsequent LH release following GnRH injection in relation to Hg differed according to gender, breeding status or age **[PAPER I; II]**. In kittiwakes, relationships between LH and Hg were exacerbated in birds that would not breed and, in snow petrels, in individuals that were up to 23 years old; non-breeding kittiwakes and young snow petrels being more Hg-contaminated than birds from other statuses or age groups, respectively **[PAPER I; II]**. Indeed, in snow petrels, younger birds were more Hg-contaminated than older ones, and there was more evidence for this in males **[PAPER II].** Hg contamination principally occurs via food intake and, in snow petrels, the analysis of stable isotopes used as a proxy for the trophic niche revealed an age- and sex-related trophic niche segregation **[PAPER II]**. Indeed, individuals from different age groups seem to feed in different trophic niches where Hg bio-availability varies **[PAPER II].** In both studies, the results obtained suggest a problem originating from the hypothalamus, which may not release enough GnRH in response to high Hg exposure **[PAPER I; II]**.

Hg can deposit in the hypothalamus (Oliveira et al. 2006) and increase dopamine (DA) concentration, which is known to alter GnRH signaling (Crump & Trudeau 2008). Additionally, Oliveira et al. (2006) observed a decrease in GnRH content in the medial hypothalamus of rats fed methyl Hg (MeHg). In Hg-contaminated cutthroat trout (*Oncorhynchus clarki*), a down-regulation of GnRH genes with a concomitant up-regulation of GnRH receptor genes was described (Crump & Trudeau 2008). Therefore, Hg could decrease GnRH content in the hypothalamus and also alter its signaling, and this could be the reason why Hg prevents the most contaminated birds from releasing enough LH. In non-breeding kittiwakes, this lack of GnRH stimulation may have resulted in an increase in GnRH receptors on the pituitary, and consequently in an over-release of LH following a GnRH injection **[PAPER I]**. However, in female kittiwakes that would not breed, baseline LH levels increased with increasing blood Hg. Hg is able to mimic estrogen activity by binding and activating estrogen receptors (Zhang et al. 2008). And interestingly, in estrogen-treated mammals, an increase in the pituitary's number of GnRH receptors was observed (e.g., Turzillo et al. 1995). With regard to this estrogen-activity mimic by Hg, in female birds, two feedback mechanisms of estrogens on LH release can occur: low levels of estrogen resulting in a negative feedback action on LH secretion while high estrogen level results would entail a positive feedback action increasing baseline LH levels (Stetson 1972). Consequently, high Hg levels could slightly increase LH secretion in female kittiwakes that would not breed. However, measured LH levels were still significantly below that of females able to breed **[PAPER I]**. Within the snow petrel class most contaminated by Hg (the younger ones), I did not observe such an over-release of LH **[PAPER II]**. However, in snow petrels that were up to 23 years old, LH was negatively associated with Hg **[PAPER II]**. And this could explain why a large proportion of snow petrels do not breed each year (Goutte et al. 2011a). Endocrine responses and sensitivity to Hg contamination could be highly species-specific. For

example, average Hg concentrations in blood in brown skuas were much higher than in south polar skuas (8.2 ± 0.2 and 2.2 ± 0.2 $\mu g \bullet g^{-1}$ dw, respectively); however, south polar skuas suffered from higher Hg-induced breeding failure than their close relatives (Goutte et al. 2014b). It is thus possible that kittiwakes could be more sensitive to Hg than snow petrels and would react more strongly to slightly lower Hg concentrations.

Although my study was correlational and would require experimental manipulation of contaminants (Frederick & Jayasena 2011; Hammerschmidt et al. 2002), there is evidence of causal effects since Hg administration can alter pairing in white ibises (*Eudocimus albus*), suppress spawning in fathead minnows (*Pimephales promelas*) (Frederick & Jayasena 2011; Hammerschmidt et al. 2002) and decrease baseline LH and testosterone in laboratory rats (Ramalingam et al. 2003). Moreover, there is some evidence that Hg can deposit in the hypothalamus and alter GnRH content and signaling (Oliveira et al. 2006; Crump & Trudeau 2009). The onset of breeding is significantly controlled by an increase in circulating LH in plasma (Follet et al. 1975; Sharp 1975). If these concentrations are not high enough individuals will be less receptive to mating behaviors (Bentley et al. 2006). Hg measured in pre-laying kittiwakes' blood was not related to egg-laying date, hatching success, breeding success or return rate the following year **[PAPER I]**. However, Hg clearly correlates with the decision to breed or not, which is a major fitness penalty **[PAPER I]**.

3.1.2. POPs, endocrine correlates and fitness

In female kittiwakes, the decision to breed or not was not related to POPs **[PAPER III].** Yet, as found in other studies (Chastel et al. 1995; Goutte et al. 2010a, 2011c), female kittiwakes that did not breed had a lower body-condition index **[PAPER III]**. Interestingly, in females that would not breed, decreasing condition was associated with increasing blood POPs **[PAPER III]**. Organic pollutants are lipophilic; if body fat reserves are low, organic

pollutants can be redistributed through the bloodstream and be more likely to migrate to sensitive vital organs such as the brain, kidneys and liver (Fuglei et al. 2007; Henriksen et al. 1996). It is therefore plausible that the most polluted non-breeding female kittiwakes may be in lower body condition because they are expending more energy in detoxification than breeders.

Stress-induced CORT concentrations were positively related to PCBs (not to OCPs) in pre-laying female kittiwakes **[PAPER III]**. This relationship will be discussed in the next section (**3.2. The parental phase: from incubation to chick rearing**).

Surprisingly, female kittiwakes with the highest OCP concentrations laid their first egg earlier in the season **[PAPER III]**. Similar relationships were found in some populations of glaucous gulls (Bustnes et al. 2003a) and great black-backed gulls (Bustnes et al. 2008). This negative relationship between OCP contamination and egg-laying date may not be causal: e.g., females laying early could be of better quality, forage at a higher trophic level and hence be more exposed to pesticide contamination. Along the same lines, this correlation could be the result of age-related processes since, in birds, old females often lay earlier than young ones (e.g., Goutte et al. 2010b) and those older females may possibly bear higher POP levels. In my study, no kittiwakes were of known age; therefore I was unable to test for a possible influence of age on POP levels. Another possibility could be that some OCPs (e.g., *p,p'*-DDE) could disrupt the secretion of some hormones involved in oviposition (e.g., prostaglandin). Prostaglandin synthesis, a major hormone involved in oviposition, can be inhibited by *p,p'*-DDE in ducks (Lundholm 1997), which is inconsistent with my results yet consistent with those of Bustnes et al. (2007, 2008). However, many pollutants have a non-linear dose-response relationship (Calabrese 2010; Heinz et al. 2012) and several studies have pictured an inverted U-shaped effect (e.g., Love et al. 2003). A low contaminant dose may enhance a

hormone's synthesis while high doses may inhibit it. This hypothesis would need further experimental evidence, and is worth exploring.

POPs measured in pre-laying female kittiwakes were not related to the number of eggs laid (clutch size) or breeding success **[PAPER III]**. However, another study exploring the same dataset as in **[PAPER III]** reported a negative relationship between POPs and carotenoid-based integument coloration, which could possibly penalize mate choice. In seabirds that provide bi-parental care, sexual selection is mutual (Jones & Hunter 1993) and this is probably also the case in kittiwakes (Doutrelant et al. 2013). Coloration of carotenoid-based integuments may be involved in the choice of partner in kittiwakes. Carotenoid pigments are important for immunity and as antioxidants; carotenoid-based colors have been reported to provide honest signals of an individual's quality (Leclaire et al. 2011, 2013). The coloration of carotenoid-based integuments was negatively associated with the sum of POPs (PCBs and OCPs; Blévin et al. 2014). Specifically, in female kittiwakes that would breed, eye-ring saturation (used as a proxy for carotenoid density) decreased with increasing POPs (Blévin et al. 2014), which could be the consequence of reduced immunity. There could thus be a trade-off between the need to express colored ornamentation and detoxification processes, both requiring carotenoid mobilization.

3.2. The parental phase: from incubation to chick rearing

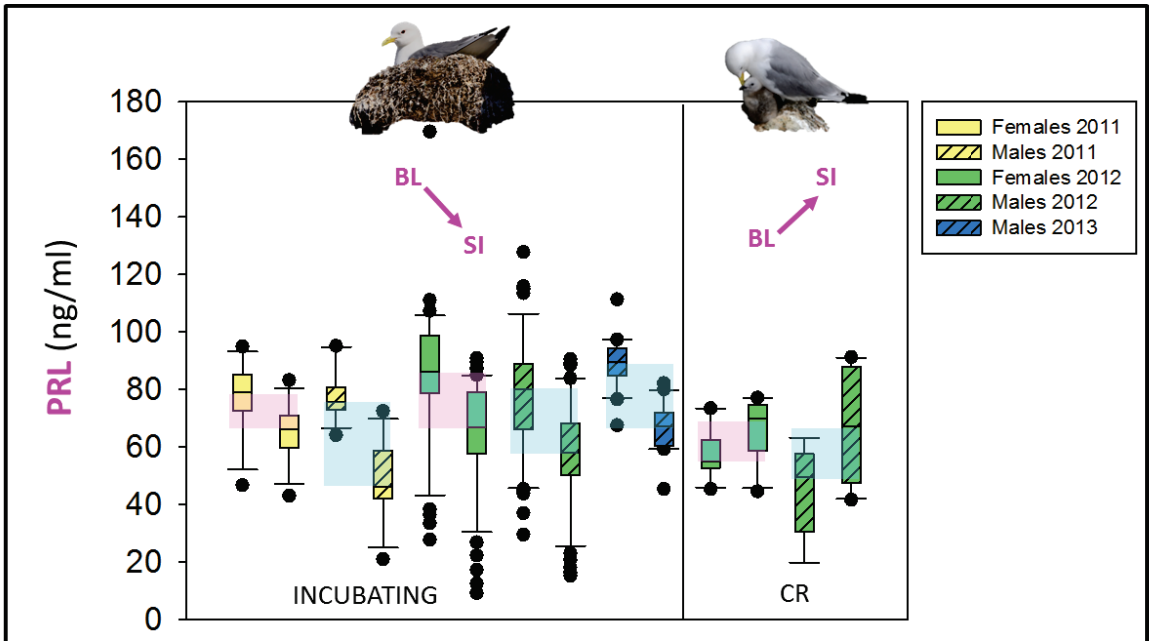


Figure 17: Variation of plasma PRL concentrations during the parental phase in three different years. The PRL stress response is illustrated by pink rectangles for females and blue rectangles for males, arrows indicate the direction of PRL variation. Plain boxes denote females and striped boxes denote males. Different colored boxes represent different years. BL= Baseline; SI= Stress-induced; CR=Chick-rearing

As previously mentioned, the parental phase begins once the first egg is laid and lasts until the chick has fledged. Several decisions have to be made during the parental phase, such as "what investment to make in egg incubation or in food provisioning to the chick" and, if environmental conditions become too harsh once parenting has begun, individuals can also choose to "abandon breeding". During the parental phase two hormones are highly involved in these decisions, PRL and CORT, both of which can be modulated in response to a stressful event (**Figures 16, 17**). The questions addressed were the same as during the pre-laying phase: **Do Hg and some legacy POPs affect PRL and CORT? Will these contaminants target the same endocrine systems? What could be the underlying mechanisms that could relate contaminants to hormones involved in breeding decisions? Can contaminant concentrations in blood vary with the age of individuals?**

During the parental phase, in known-age snow petrels, neither Hg nor POPs were related to the age of individuals **[PAPER IV]**. Hg was related to PRL but POPs were not **[PAPER IV].** Indeed, PRL concentrations induced by handling stress (after 30 min. of manipulation) were significantly lower with increasing Hg, in snow petrels and kittiwakes **[PAPER IV; V]**. Increasing Hg concentrations were related to an increased egg-neglect behavior in male snow petrels and to decreased breeding success in male kittiwakes **[PAPER IV; V]**. Moreover, when male kittiwakes were subjected to an increased stress (CORT pellet implantation), the decrease in PRL with increasing Hg was exacerbated **[PAPER V]**.

As in pre-laying female kittiwakes **[PAPER III]**, I observed positive associations between CORT and POPs (especially PCBs) in incubating male kittiwakes and snow petrels **[PAPER IV; VI]**. In male yet not in female incubating kittiwakes, baseline and stress-induced CORT increased with increasing PCBs **[PAPER VI]**, and stress-induced CORT was positively associated with POPs (PCBs and OCPs) in incubating snow petrels **[PAPER IV]**. When exploring the relationships between circulating CORT and PCB levels in seabird species that feed at different trophic positions, baseline CORT significantly increased as a function of Σ_7PCBs in male kittiwakes, and a positive trend was found in female wandering albatrosses **[PAPER VI]**. Stress-induced CORT levels were positively related to Σ_7PCBs in male kittiwakes and a positive trend was found in male snow petrels, whereas in male glaucous gulls I found a negative association between stress-induced CORT and Σ_7PCBs **[PAPER VI]**. The general pattern including the 7 species shows a positive trend between baseline CORT and Σ_7PCBs, whereas a significant inverted U-shaped pattern associates stress-induced CORT with Σ_7PCBs **[PAPER VI]**. HPA axis activity in male kittiwakes was investigated via injections of DEX and ACTH **[PAPER VII].** DEX provided information on the negative feedback functionality of CORT on the brain while ACTH provided information on adrenal responsiveness. CORT decrease following DEX injection was not related to contaminants

whereas the ACTH injection resulted in an over-release of CORT, in male kittiwakes most contaminated by PCBs **[PAPER VII]**. As regards fitness, I observed a delayed hatching date in male kittiwakes most contaminated by POPs **[PAPER VII]**.

3.2.1. Contaminants and age

Although snow petrels are very long-lived and thus exposed to contaminants over many years, no evidence was found that contamination was age-related, neither for POPs nor Hg. POPs and Hg measured in blood (plasma and red blood cells, respectively) can be correlated with levels found in storage organs and also adipose tissues, in birds but also chelonians and humans (Henriksen et al. 1998; Pauwels et al. 2000; Wayland et al. 2001; Henny et al. 2002; Keller et al. 2004; van de Merwe et al. 2010; Szumiło et al. 2013; Fromant et al. unpublished data). Contaminant concentration in blood may thus be a good proxy for the contaminant burden in other organs.

The relationship between Hg and age in seabirds is often contradictory, for example liver Hg was found to decrease, increase or be unrelated to age (Furness & Hutton 1979; Hutton 1981; Thompson et al. 1991). For blood, the relationship between Hg contamination and age is also unclear: no relationship was found between age and Hg contamination (Gonzáles-Solís et al. 2002; Tavares et al. 2013), but in pre-breeding snow petrels and incubating/chick-rearing cape petrels a negative relationship was found between blood Hg and age (**Figure 18A**).

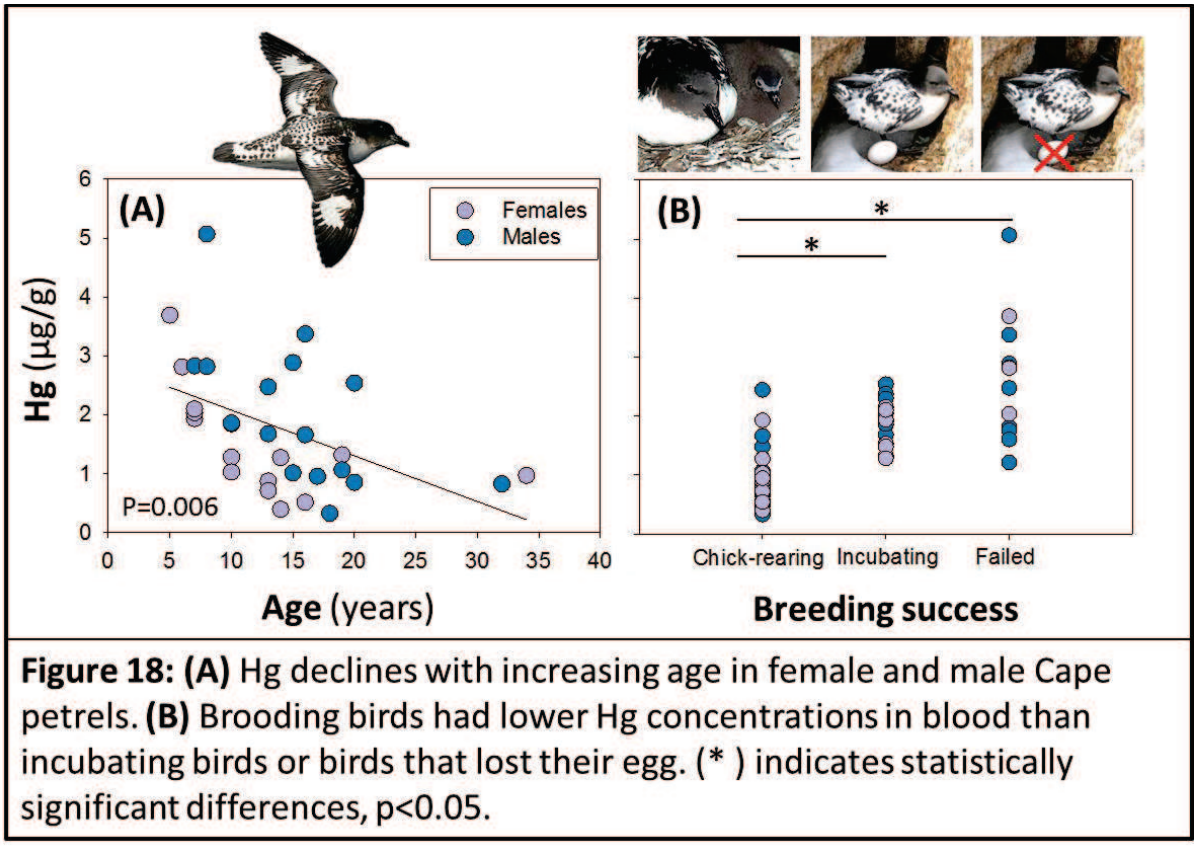


Figure 18: (A) Hg declines with increasing age in female and male Cape petrels. **(B)** Brooding birds had lower Hg concentrations in blood than incubating birds or birds that lost their egg. (*) indicates statistically significant differences, p<0.05.

However, these relationships were more likely the result of an age-related change in feeding ecology. With regard to POPs, it seems that in several bird species concentrations in different tissues and blood increase until a steady state is reached, often before the age of breeding (Donaldson et al. 1997; Newton et al. 1981; van den Brink et al. 1998). As for breeding birds, no age-related POP accumulation was observed in most studies (Bustnes et al. 2003b; Newton et al. 1981). In the present study, all snow petrels were breeders, and presumably they had already reached their steady-state levels.

Besides, seabirds can biotransform PCBs and eliminate POPs through their preen gland (Borgå et al. 2005; Henriksen et al. 1996; Solheim 2010). Similarly, Hg can be excreted through feather growth (Bearhop et al. 2000). These mechanisms could partially explain the lack of association between blood contaminants and increasing age. Also, I had to remain cautious as I hypothesized that in snow petrels, as in other bird species, blood contaminant

levels would be representative of levels in internal tissues (Henriksen et al. 1998; Wayland et al. 2001; Henny et al. 2002; Szumiło et al. 2013; Fromant et al. unpublished data). However, I have no evidence for this relationship in snow petrels. Additionally, following food intake or lipid mobilization, contaminant levels in blood may fluctuate more than those in adipose tissues or liver and this could mask a hypothetical contaminant/age relationship.

3.2.2. Hg, endocrine correlates and fitness

All the results obtained in the present thesis seem to indicate that Hg is more concerning than some of the legacy POPs I analyzed here. Indeed, most of the hazardous relationships between contaminants and fitness were found for Hg (egg neglect, breeding success, etc.).

To date, no studies describing relationships between PRL and Hg have been conducted on wildlife. The only few human studies describing such relationships were aimed at studying neurotoxicity of Hg, and baseline PRL was used to ascertain possible interference of Hg with neurotransmitters (Barregård et al 1994; Lucchini et al. 2002, 2003; Carta et al. 2003). As previously mentioned, Hg increases DA secretion, a neurotransmitter secreted in the *zona incerta* area of the hypothalamus (similarly to GnRH) and the main antagonist of PRL (Ben-Jonathan & Hnasko 2001; Crump & Trudeau 2008). The negative relationships between Hg and PRL observed in kittiwakes and snow petrels **[PAPER IV; V]** are thus likely to be influenced by DA secretion (Ben-Jonathan & Hnasko 2001; Grattan & Kokay 2008). Indeed, it seems that organic and inorganic Hg can stimulate spontaneous DA release in laboratory rodents (Faro et al. 1997, 2000, 2002, 2003, 2007; Minnema et al. 1989) but also in wild larvae of a fish species (*Fundulus heteroclitus*; Zhou et al. 1999) and in wild American minks *Mustela vison* (Basu et al. 2005). The negative relationship observed between PRL and Hg is thus more likely to be indirect and will rely on an effect of Hg on the dopaminergic system. These studies were correlational and would nevertheless greatly benefit from further

experimental support. The reason for the relationships between Hg and PRL being more visible in males **[PAPER IV; V]** could be related to gender effects of Hg on DA. This was described in mice where MeHg altered locomotor activity in males only (Giménez-Llort et al. 2001), and/or through protective effects of estradiol (which is higher in females) on Hg toxicity as reported in female mice (Olivera et al. 2006). Low PRL levels are associated with reduced nest attendance, greater latency prior to coming back to the nest after short-term stress, and reduced breeding success (Angelier et al. 2009a; Angelier & Chastel 2009).

With regard to fitness, Hg concentrations measured in incubating snow petrels were higher in males that were more likely to leave their egg unattended. High Hg concentrations in blood in chick-rearing male kittiwakes were associated with lower breeding success **[PAPER IV; V].** In cape petrels, Hg concentration was higher in males and females that failed reproduction compared to chick-rearing birds **(Figure 18B)**. Hazardous effects of Hg in free-ranging species, especially Hg hazards affecting breeding success, have been widely described in free-living populations and in laboratory experiments (see Tan et al. 2009 for a review). For example, 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). Moreover, MeHg experimental feeding to black ducks (*Anas rubripes*) induced lower clutch size, hatched eggs and duckling survival (Finley & Stendell 1978). However, the mechanisms underlying these relationships often remained poorly understood. The fact that Hg can modify PRL secretion could be a key element to understand the relationships between Hg and reproductive failure.

In extreme environments such as polar regions, individuals often experience harsh and unpredictable environmental conditions, and adopt different 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 too poor (e.g., Angelier et

al. 2007; Goutte et al. 2011a). Such behaviors (i.e., to refrain from breeding or abandon reproduction) are mediated by CORT release during stressful events that will shift energy investment away from reproduction and redirect it towards self-preservation and hence survival (Angelier & Wingfield 2013; Ricklefs & Wikelski 2002; Wingfield & Sapolsky 2003). By mimicking a short (~11-day) stressful event **[PAPER V],** I tested whether the PRL/Hg relationships would be affected. The action of the CORT pellet resulted in a decrease in baseline and stress-induced PRL and, with regard to stress-induced PRL, the decrease was stronger in male kittiwakes most contaminated by Hg. This exacerbated decrease in PRL in birds most contaminated by Hg is difficult to interpret, but could result from an additive effect of CORT and Hg, both being related to decreasing PRL concentrations. This result suggests that, for some contaminants (at least Hg), environmental stressors (such as food shortage, disturbances, habitat destruction and more generally ongoing climate change) may increase the hazardous biological effects of contaminants. Jenssen (2006) described the interaction between endocrine disruptors and climate change as a "worst case combination for Arctic marine species".

3.2.3. POPs, endocrine correlates and fitness

Other seabird studies investigating the relationships between CORT and POPs have reported associations between CORT and PCBs yet not OCPs (Lorenzen et al. 1999; Nordstad et al. 2012). The increase in baseline CORT with increasing PCBs observed in male kittiwakes could result from an increased metabolism with an increasing PCB burden. Indeed, as a result of contamination, the organism may increase the energy allocated to detoxification (Parkinson & Ogilvie 2008). Some cytochrome P450 enzymes used to metabolize contaminants are in part regulated by CORT (Helgason et al. 2010). These enzymes convert contaminants into

more hydrophilic compounds, which are more easily conjugated and excreted (Bandiera 2001; Pascussi et al. 2003).

In captive American kestrels, PCB administration resulted in decreased baseline CORT concentrations; however, PCB concentrations in liver were associated with baseline CORT in an inverted U-shaped pattern (Love et al. 2003). In American kestrels, baseline CORT started to decline when PCB concentrations in liver reached 20µg/g ww and, in free-ranging species highly contaminated by PCBs, such as glaucous gulls, PCBs in liver ranged from 0.1 to 2.9µg/g ww (Savinova et al. 1995). PCB levels measured in contaminated wild seabirds are thus still much below those reached in American kestrels; therefore it can be suggested that the PCB threshold level that would induce a decline in baseline CORT is not reached in wild seabirds.

With regard to stress-induced CORT levels, the positive relationship between stress-induced CORT and PCBs more likely results from an increase in ACTH-receptors (ACTH-R) on the adrenal glands than from a loss of negative feedback from CORT on the hypothalamus or the pituitary. Indeed, when investigating HPA axis activity in relation to POPs (PCBs and OCPs) in male kittiwakes **[PAPER VII]**, I observed that CORT secretion following a DEX injection was not related to contaminants. As previously mentioned, DEX will saturate the GR of the hypothalamus and the pituitary, which will result in a CORT decrease. CORT decrease following DEX injection was not related to PCBs or to OCPs **[PAPER VII]**. This may indicate that environmental contaminants do not alter the functioning of GR in the brain. However, adrenal responsiveness increased in kittiwakes most contaminated by PCBs. An ACTH injection resulted in an over-release of CORT in male kittiwakes most contaminated by PCBs; this relationship was not observed with OCPs **[PAPER VII]**. Administration of a standardized ACTH dose is an alternative approach to measure the stress response that is specifically due to variation in adrenal cortex sensitivity to ACTH. Response to exogenous

ACTH may also provide a more accurate measurement of maximum glucocorticoid production than response to restraint stress (Wada et al. 2007; Schmidt et al. 2012). Interestingly, in some birds, ACTH injection was not effective and CORT levels even decreased in birds least contaminated by PCBs **[PAPER VII]**. These results are surprising but could be the consequence of too short a time for ACTH action, in the present study. Indeed, the time necessary to elicit a maximal CORT release following ACTH injection was 45 minutes (e.g., Schmidt et al. 2012) and my experimental kittiwakes were only exposed for 15 minutes. If this hypothesis could be verified this would support the fact that adrenal activity of males most contaminated by PCBs is exacerbated.

One possible explanation for the over-release of CORT in relation to PCBs could be related to an increase in the number of ACTH receptors (ACTH-R) on the adrenals. ACTH is one of the few polypeptide hormones having a positive trophic effect on its own receptors (Beuschlein et al. 2001; Penhoat et al. 1989). An increase in ACTH-R in birds most contaminated by PCBs may thus be the consequence of an excess of ACTH input to adrenals. This suggests that PCBs may alter the functioning of the pituitary by stimulating ACTH release and/or that PCBs may mimic ACTH and bind to ACTH-R, which in that case would mobilize more ACTH-R from the adrenals of the most contaminated individuals. However, this study being correlational, I cannot confirm without experimental evidence that the observed relationship is not the consequence of other intrinsic or extrinsic factors. Subsequently, beyond a PCB-contamination threshold level, as suggested by the pattern observed in male glaucous gulls, stress-induced CORT levels decrease. In the present study, glaucous gulls' baseline CORT concentrations were almost as high as stress-induced CORT concentrations. For example, in male glaucous gulls, baseline CORT averages 10.8ng/ml and stress-induced CORT 16.5ng/ml. This decrease in stress-induced CORT may reflect a permanent saturation of

ACTH-R in individuals most contaminated by PCBs, as a result of a chronic rise in baseline CORT.

As regards fitness, an enhanced stress response in adults favors survival at the expense of parental investment (Wingfield & Sapolsky 2003). In seabird species bearing low to moderate PCB contamination such as kittiwakes and wandering albatrosses the sum of POPs (PCBs and OCPs) affected long-term breeding success (Goutte et al. 2014c; Goutte et al. unpublished data on Svalbard kittiwakes). In addition to this, I observed a delayed hatching date in the most contaminated (PCBs and OCPs) male kittiwakes **[PAPER VII]**. The same pattern was found in female south polar skuas *Catharacta maccormicki* (Bustnes et al. 2007). A longer incubation period could be the result of a non-optimal egg temperature (Hepp et al. 2006; Verboven et al. 2009), thus an impaired incubating behavior. An experimental study conducted on American kestrels, *Falco sparverius,* reported that PCB administration resulted in longer incubation periods and altered incubation behaviors in males especially (Fisher et al. 2006). In the study conducted by Fisher et al. (2006), an asynchrony was observed between incubation bouts completed by females and males; the eggs were left unattended for a longer period and eggs given inadequate heating may have taken longer to hatch (Fisher et al. 2006). In free-ranging species, such as glaucous gulls, the proportion of time away from the nest site and the number of these absences were positively related to blood PCBs (Bustnes et al. 2001). Also, still in glaucous gulls, the most contaminated individuals were less able to maintain an optimal nest temperature (Verboven et al. 2009). Consequently, the relationship observed in male kittiwakes could result from longer or more frequent recesses during the incubation period as a consequence of a high PCB and OCP burden.

When PCB contamination becomes too high, other fitness consequences may be observed. Indeed, a chronic rise in baseline CORT would result in an array of deleterious biological effects such as suppression of reproductive function and behavior, immune system

suppression, muscle wasting, growth suppression, and neuronal cell death (e.g., Sapolsky et al. 2000). Interestingly, negative effects of POPs (PCBs and OCPs) on survival have been described in glaucous gulls (Sagerup et al. 2009; Verreault et al. 2010; Erikstad et al. 2013) but not in south polar skuas (Goutte et al. unpublished data) or in moderately or lowly polluted seabirds such as wandering albatrosses and kittiwakes (Goutte et al. 2014c; Goutte et al. unpublished data).

Overall, the total data obtained seem to suggest that, firstly, PCBs interact with ACTH-R, increasing their numbers on the adrenals, which would result in an increased stress response. If PCB contamination becomes higher, this could be measured by an increase in baseline CORT, as a result of detoxification processes. At these points, negative impacts of PCBs on breeding success and breeding probability can be observed in seabird species. Yet, beyond a PCB-contamination threshold level, the detoxification process may result in a chronic rise in baseline CORT and, as is the case with glaucous gulls, seabirds become unable to raise CORT levels when subjected to an additional stress. At this point the effects of PCBs, added to other contaminants such as OCPs, on survival can be observed.

3.3. Consequences and specific action of contaminants

In the present study, I never observed POPs and Hg affecting the same hormones. This suggests that POPs and Hg may not target the same endocrine functions and thus biological pathways: Hg would rather interact indirectly with pituitary hormones via DA and estrogen disruption while PCBs would rather affect HPA axis hormones. OCP action is not as clear. PCBs' and OCPs' structure is very close, yet their actions are not always similar. In kittiwakes, PCBs were only related to CORT and OCPs were related to the egg-laying date. However, for some fitness-related traits, PCBs and OCPs had a similar action as they increased with decreasing body condition. They delayed egg hatching in male kittiwakes and,

in snow petrels, they led to increased stress-induced CORT. Moreover, in other bird studies, relationships were found between Hg and CORT (Franceschini et al. 2009; Herring et al. 2012; Wada et al. 2009): in female cape petrels, baseline CORT significantly decreased with increasing Hg, and a positive trend was found between stress-induced CORT and Hg (**Figure 19**). Furthermore, a study also reported significant relationships between PRL and POPs: in glaucous gulls, baseline PRL levels and the rate of decrease in PRL levels tended to vary negatively with several organohalogen contaminants in males only (Verreault et al. 2008). Parental investment strategies are considered to represent a trade-off between the benefits of investment in current offspring and costs related to future reproduction. Due to their high reproductive value, long-lived organisms are predicted to be more reluctant to increase their parental effort. The various Hg- and PCB-related actions that occur during the parental phase combine to shift the available energy away from parental investment. Indeed, Hg decreases PRL and PCBs increase CORT. Consequently, Hg contamination reduces nest attendance and breeding success. However, Hg/breeding-success relationships appear to be complex in birds since administration of a low Hg dose could even enhance breeding in mallards *Anas platyrhynchos* (Heinz et al. 2010). Many contaminants have a non-linear dose-response relationship (Calabrese 2010; Heinz et al. 2012) and several studies have pictured an inverted U-shaped effect associating contaminants with hormones (e.g., Love et al. 2003), where a low dose of contaminants may enhance a biological function while high doses may

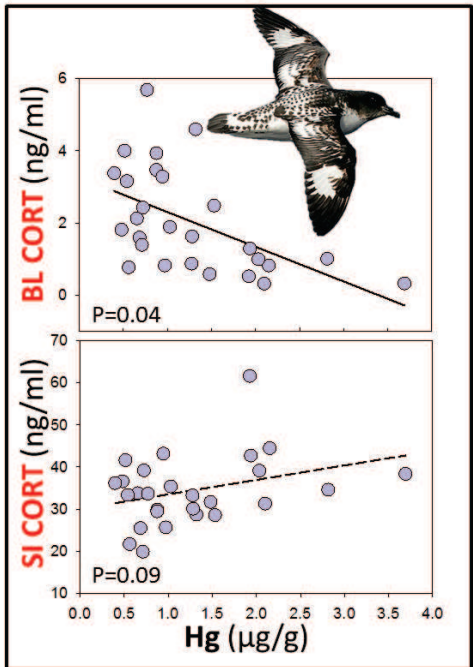


Figure 19: Baseline (BL) and stress-induced (SI) CORT concentrations in relation to blood Hg in female Cape petrels.

inhibit it. All of these mechanisms combined once again highlight the complexity of contaminant-hormones-fitness relationships.

In this PhD project, each contaminant was analyzed separately. Yet, in reality, each contaminant acts in synergy with a considerable amount of other contaminants, and most probably for a large proportion of them we are not yet aware of their presence in polar species. "Cocktail effects" are referred to when the additive action of several contaminants may result in health hazards, which was not observed by analyzing individual chemicals (e.g., Kortenkamp 2007; Celander 2011). In the wild, it is difficult to assess the cocktail effect of contaminants. Indeed, studying this effect involves the experimental administration of contaminants, which may not be feasible with protected free-living models, for obvious ethical reasons.

Contaminants show different temporal trends according to the hemisphere or the species. For example, Hg increases in some seabird species (Braune 2007; Mallory & Braune, 2012) but declines in others (Scheifler et al. 2009). With regard to POPs, in the Arctic the levels of several legacy POPs are now decreasing, however several emerging POPs show important increasing trends in wildlife (Braune 2007; Helgason et al. 2011) yet ecotoxicological data on these compounds are lacking (Petrovic et al. 2004). Some PFAS, for example, tend to increase over time in several Arctic mammal and seabird species (Butt et al. 2007, 2010; Helgason et al. 2011). With regard to the potential endocrine disrupting properties of PFAS, laboratory studies have shown that some PFAS have estrogenic, androgenic and thyroid-like activity (Liu et al. 2007; la Farré et al. 2008; Jensen & Leffers 2008; Benninghoff et al. 2011), however their impact on wildlife is virtually unknown. During this PhD project, I had the opportunity to analyze the relationships between PFAS and CORT in chick-rearing kittiwakes' plasma **[PAPER VIII]**. Interestingly, I found the reverse pattern of those found between baseline CORT and legacy POPs **[PAPER VIII]**: baseline CORT decreased with

increasing long-chain perfluorinated carboxylates (PFCAs), the most prevalent PFAS in Arctic species (Butt et al. 2007). This preliminary study was the first, to my knowledge, to report a relationship between long-chain PFCAs and CORT in a free-ranging species.

Another interesting result is that POPs and Hg were never related in kittiwakes or snow petrels (neither in gender nor breeding stage). This suggests a different source of contamination via food. The negative trend observed in Hg concentration in blood across the kittiwakes' breeding cycle (**Figure 14**) suggests that kittiwakes shift to prey feeding at a lower trophic position than that of the prey they feed on at the pre-laying period. Indeed, a shift in foraging area was observed between the pre-laying and the incubation period (Goutte et al. 2014a). During the pre-laying period, when the energetic needs are high, consumption of bigger preys, such as fish (higher in the food web), might be essential to ensure mating, nest defense and egg laying, which would thus result in higher Hg contamination. Also, prey composition changes throughout the summer in the Kongsfjord area (Walkusz et al 2009), and kittiwakes switch to a diet mainly containing gastropods (Mehlum & Gabrielsen 1993). This diet mainly composed of low trophic position prey could explain declining Hg concentrations across the breeding season. Hg concentration reaches the lowest levels during chick rearing, which could reflect the fact that kittiwakes feed their chicks with fish they catch while they feed themselves with gastropods. Concurrently, blood POPs seem to increase throughout the parental phase (**Figure 15**). This increase in POPs probably results from a decrease in fatty tissues and hence the release of POPs into blood, as already reported in common eiders and kittiwakes (Bustnes et al. 2012; Nordstad et al. 2012). Given that contaminant concentrations in blood can significantly vary during the breeding season, they may not provide the same data (e.g., relationships with hormones or fitness) depending on the breeding stage at which they were collected. Indeed, blood measurements performed at the beginning of the breeding

season may be less reliable to predict reproductive traits occurring at the end of the season such as breeding success (Lanctot et al. 2003).

3.4. Conclusions and perspectives

This thesis aimed to identify the specific relationships between legacy POPs, Hg and three hormones involved in reproductive decisions, in free-ranging polar seabirds. I was able to highlight significant relationships and specific actions of contaminants on these hormones (**Figure 20**).

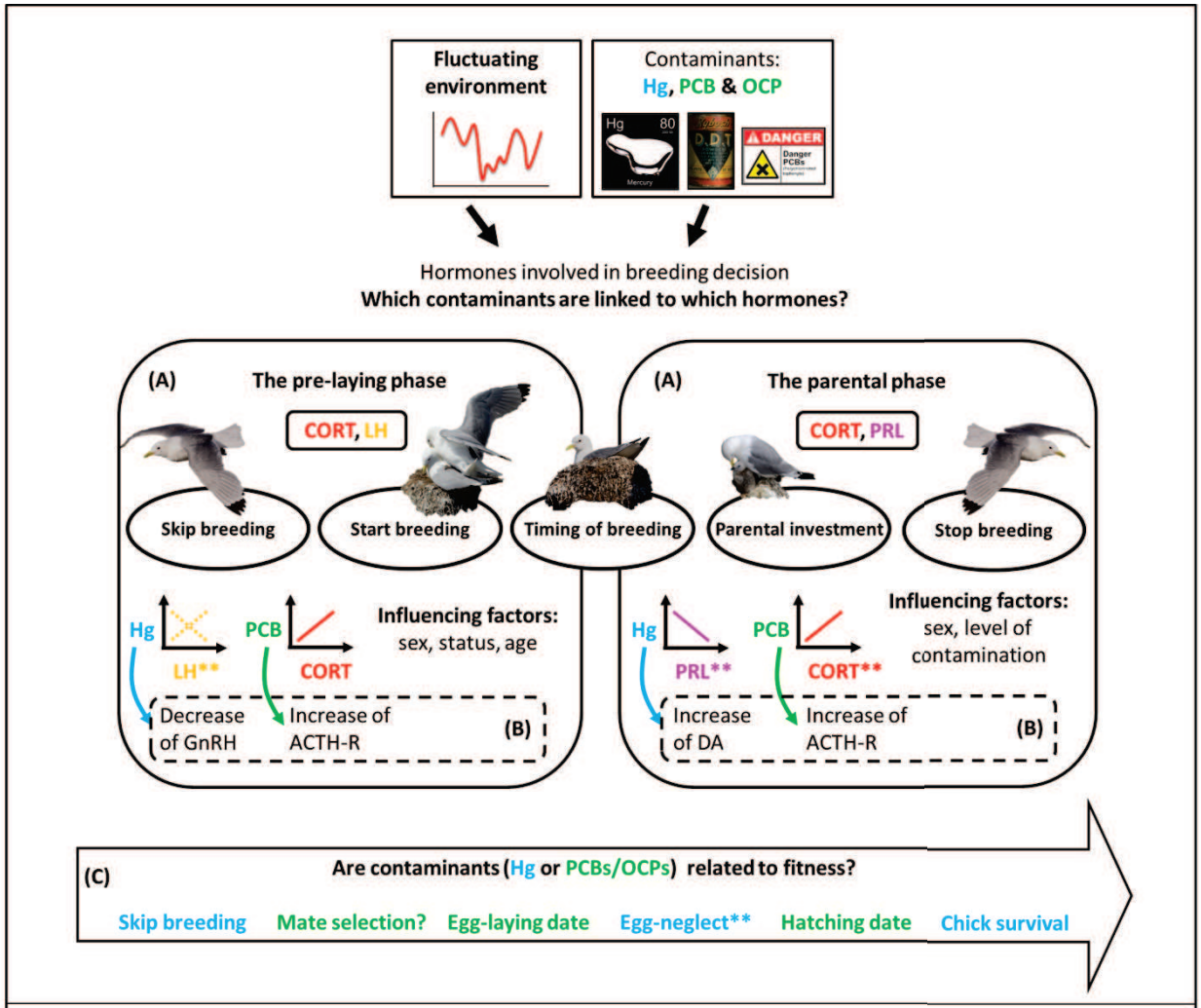


Figure 20: Summary of the principal results, **(A)** contaminant/hormones relationships within the breeding cycle, **(B)** Possible disrupted mechanisms and **(C)** relationships between contaminants and fitness. Each contaminant and each hormone is depicted by a different color. Pictures refer to kittiwakes but are also attributable to snow petrels when followed by (**).

This work also emphasizes the complexity of studying contaminant-hormone relationships within an uncontrolled environment but provides interesting findings regarding the possible impact of contaminants on fitness, as well as an understanding of the hazardous effects of contaminants on wildlife. I point out the fact that contaminants act at low concentrations; kittiwakes and snow petrels are indeed poorly to moderately contaminated (**Figures 21, 22**).

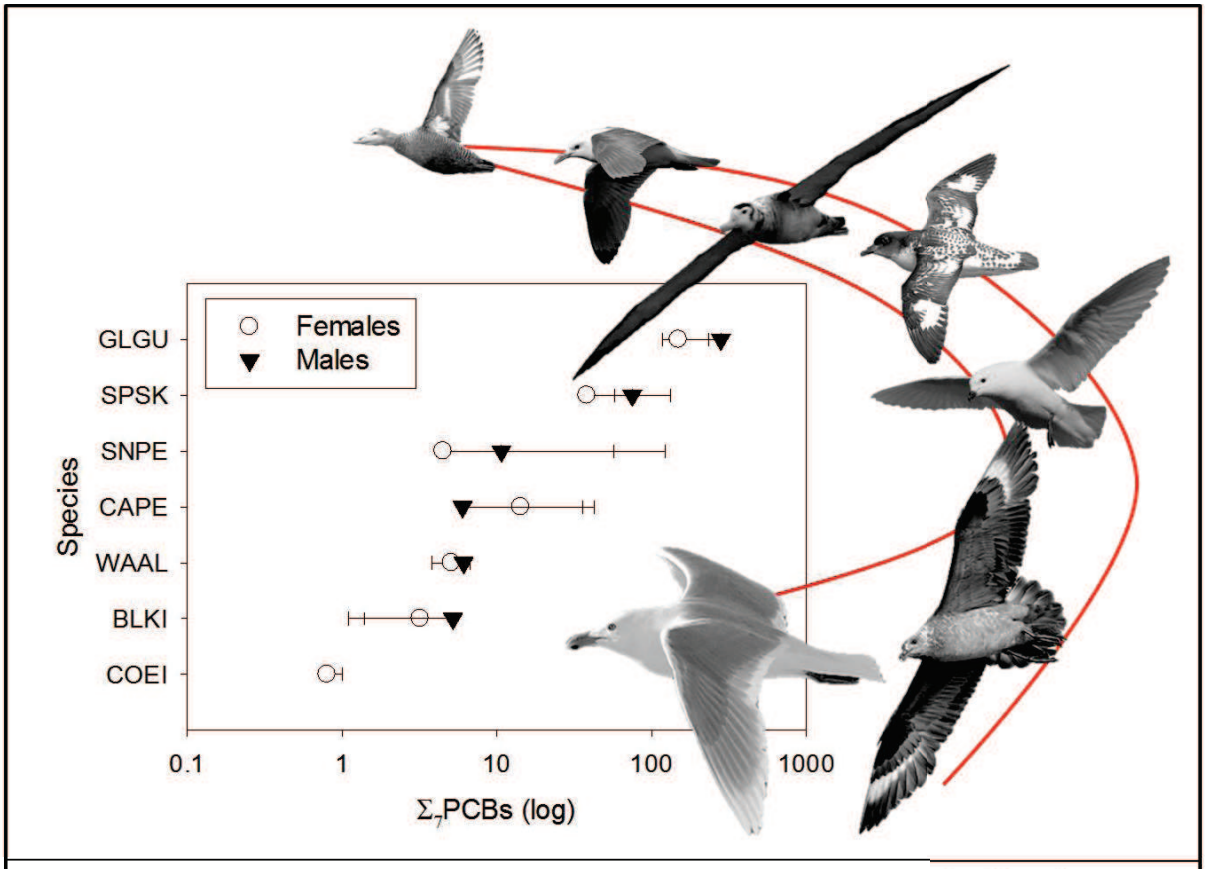


Figure 21: Levels of indicator PCBs in seven polar seabirds. Open circles denote females and filled triangles denote males. GLGU= Glaucous gulls; SPSK= South Polar skuas; SNPE= Snow petrels; CAPE= Cape petrels; WAAL= Wandering albatrosses; BLKI= Black-legged kittiwakes and COEI= Common eiders.

Yet, even at those concentration levels relationships with altered hormones and impacted fitness are visible; moreover, the relationships between Hg and PRL have been reported to be exacerbated when individuals were subjected to additional stress **[PAPER V]**. **Therefore, when considering the ongoing disturbances that affect polar regions such as climate change and anthropogenic disturbances** (Clarke & Harris 2003; Smetacek & Nicol 2005;

Moline et al. 2008) **added to the presence of EDCs that interact with hormones involved in breeding decisions, the maintenance of polar seabird populations may be jeopardized.**

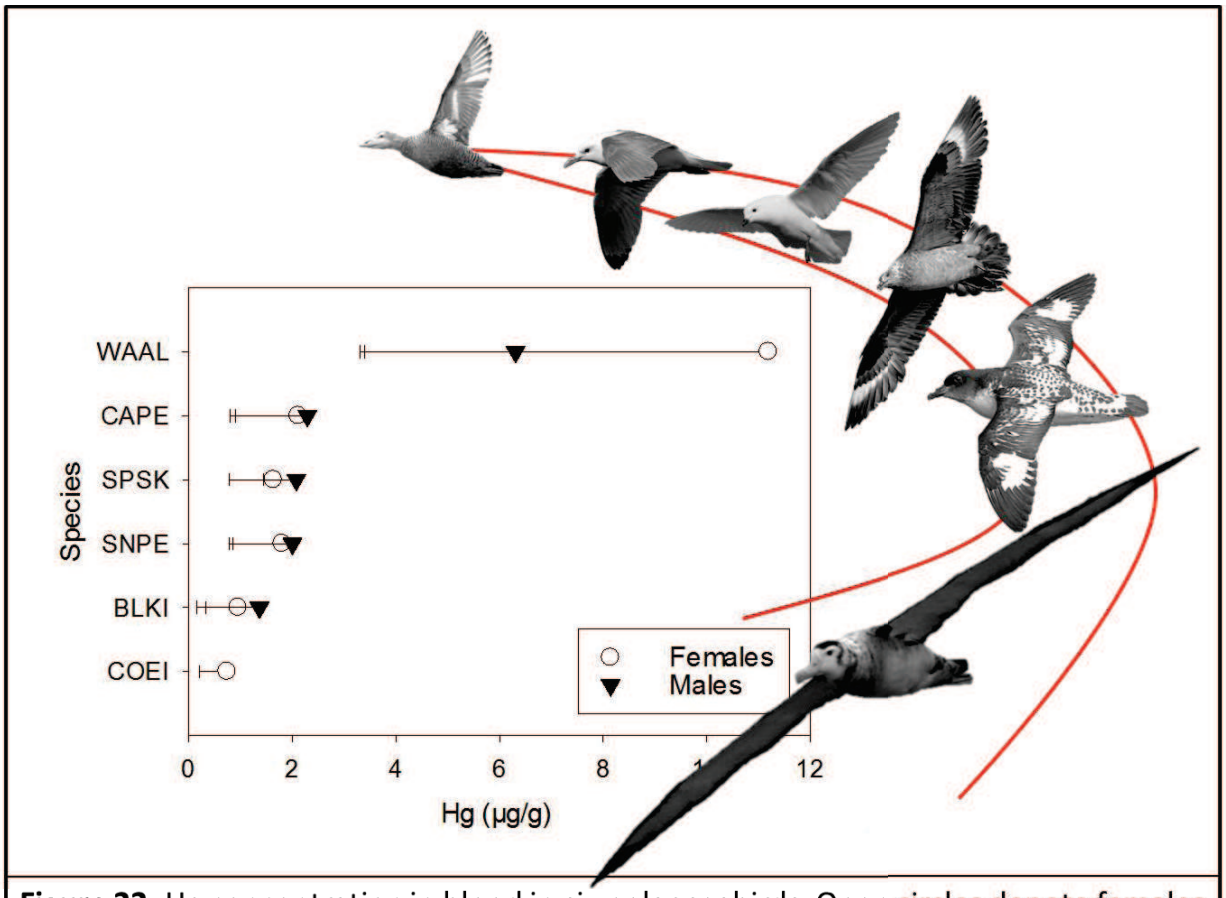


Figure 22: Hg concentration in blood in six polar seabirds. Open circles denote females and filled triangles denote males. GLGU= Glaucous gulls; SPSK= South Polar skuas; SNPE= Snow petrels; CAPE= Cape petrels; WAAL= Wandering albatrosses; BLKI= Black-legged kittiwakes and COEI= Common eiders.

Several questions arose from the present project. For example, I have not tested the relationships between legacy POPs and LH or GnRH whereas it has been reported that PCB126 could decrease hypothalamic GnRH (Ottinger et al. 2009) nor between contaminants and FSH, which is another hormone highly involved in the onset of breeding. Moreover, I only focused on one heavy metal - Hg - whereas several other heavy metals are of concern. For example, there is a lack of ecotoxicological data concerning cadmium exposure in wildlife (Burger 2008). Cadmium is a non-essential heavy metal that comes from natural and anthropogenic sources; it is known to be extremely toxic and ubiquitous in the environment

(Larison, et al. 2000). Also, cadmium can mimic estradiol (E2) by activating E2 receptors, and stimulate PRL secretion from anterior pituitary cells in female rats (Ronchetti et al. 2013). Among the Barents Sea seabird community, kittiwakes from Ny-Ålesund (i.e., the kittiwake colony used as a study model in the present thesis) bear the near highest concentrations of liver cadmium (Savinov et al 2003). It would thus be worth investigating the relationships between cadmium, hormones and fitness in kittiwakes.

Another interesting issue lacking information concerns the effects of Hg on sex ratio in birds especially. Indeed, female birds can excrete Hg into their eggs (Evers et al. 2003) and, in birds, sex differentiation is associated to E2 secretion (Adkins 1975; Balthazart & Ball 1995). Hg is able to bind and activate E2 receptors (Zhang et al. 2007), therefore this could result in a female-biased population in birds highly contaminated by Hg. To date, only one study was aimed at studying the relationships between Hg and sex ratio in complete broods of three species: the aquatic-feeding belted kingfisher *Megaceryle alcyon*, the terrestrial-feeding eastern bluebird *Sialia sialis*, and the tree swallow *Tachycineta bicolor*, which feeds from both aquatic and terrestrial sources (Bouland et al. 2012). Nestling sex ratios were shifted toward the production of females in all three species on Hg-contaminated sites when compared to uncontaminated reference sites (Bouland et al. 2012). Further research is thus required in bird populations highly contaminated by Hg. Indeed, the female-biased sex ratio induced by Hg might jeopardize the maintenance of Hg-exposed populations.

REFERENCES

Adkins EK. Hormonal basis of sexual differentiation in the Japanese quail. Journal of Comparative and Physiological Psychology. 1975; 89(1):61‑71.

Amaral Mendes JJ. The endocrine disrupters: a major medical challenge. Food and Chemical Toxicology. 2002;40(6):781-788.

AMAP, 2004. AMAP Assessment 2002: Persistent Organic Pollutants in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xvi +310 pp.

Angelier F, Clément-Chastel C, Gabrielsen GW, Chastel O. Corticosterone and time–activity budget: An experiment with Black-legged kittiwakes. Hormones and Behavior. 2007a; 52(4):482-491.

Angelier F, Moe B, Weimerskirch H, Chastel O. Age-specific reproductive success in a long-lived bird: do older parents resist stress better? Journal of Animal Ecology. 2007b ; 76(6):1181-91.

Angelier F, Chastel O. Stress, prolactin and parental investment in birds: a review. Gen Comp Endocrinol. 2009; 163(1-2):142-148.

Angelier F, Clément-Chastel C, Welcker J, Gabrielsen GW, Chastel O. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. Functional Ecology. 2009a; 23(4):784-93.

Angelier F, Moe B, Blanc S, Chastel O. What Factors Drive Prolactin and Corticosterone Responses to Stress in a Long-Lived Bird Species (Snow Petrel Pagodroma nivea)? Physiological and Biochemical Zoology. 2009b; 82(5):590-602.

Angelier F, Wingfield JC, Weimerskirch H, Chastel O. Hormonal correlates of individual quality in a long-lived bird: a test of the 'corticosterone–fitness hypothesis'. Biol Lett. 2010; 6(6):846-849.

Angelier F, Wingfield JC, Trouvé C, de Grissac S, Chastel O. Modulation of the prolactin and the corticosterone stress responses: Do they tell the same story in a long-lived bird, the Cape petrel? General and Comparative Endocrinology. 2013; 182:7-15.

Angelier F, Wingfield JC. Importance of the glucocorticoid stress response in a changing world: Theory, hypotheses and perspectives. General and Comparative Endocrinology. 2013; 190:118-128.

Anker-Nilssen T. The status of marine birds breeding in the Barents Sea region 2000 Norsk Polarinstitutt report; 214 pp.

Ariya PA, Dastoor AP, Amyot M, Schroeder WH, Barrie L, Anlauf K, et al. The Arctic: a sink for mercury. Tellus B. 2004; 56(5):397‑403.

Atwell L, Hobson KA, Welch HE. Biomagnification and bioaccumulation of mercury in an arctic marine food web : insights from stable nitrogen isotope analysis. Canadian journal of fisheries and aquatic sciences. 1998; 55(5):1114-1121.

Balthazart J, Ball GF. Sexual differentiation of brain and behavior in birds. Trends in Endocrinology & Metabolism. 1995; 6(1):21‑29.

Bandiera SM. Cytochrome P450 enzymes as biomarkers of PCB exposure and modulators of toxicity. PCB: Recent Advances in Environmental Toxicology and Health Effects (Robertson LW, Hansen LG, eds) Lexington, KY: The University Press of Kentucky. 2001;185-192.

Barbraud C, Lormée H, LeNevé A. Body size and determinants of laying date variation in the Snow Petrel Pagodroma nivea. Journal of Avian Biology. 2000; 31(3):295‑302.

Barbraud C, Weimerskirch H. Contrasting effects of the extent of sea-ice on the breeding performance of an Antarctic top predator, the Snow Petrel Pagodroma nivea. Journal of Avian Biology. 2001; 32(4):297‑302.

Barregård L, Lindstedt G, Schütz A, Sällsten G. Endocrine function in mercury exposed chloralkali workers. Occup Environ Med. 1994; 51(8):536‑540.

Bargagli R, Monaci F, SanchezHernandez JC, Cateni D. Biomagnification of mercury in an Antarctic marine coastal food web. Mar Ecol Prog Ser. 1998; 169:65-76.

Basu N, Klenavic K, Gamberg M, O'Brien M, Evans D, Scheuhammer AM, et al. Effects of mercury on neurochemical receptor-binding characteristics in wild mink. Environmental Toxicology and Chemistry. 2005; 24(6):1444-50.

Bearhop S, Ruxton GD, Furness RW. Dynamics of mercury in blood and feathers of great skuas. Environmental Toxicology and Chemistry. 2000; 19(6):1638-1643.

Benninghoff AD, Bisson WH, Koch DC, Ehresman DJ, Kolluri SK, Williams DE. Estrogen-Like Activity of Perfluoroalkyl Acids In Vivo and Interaction with Human and Rainbow Trout Estrogen Receptors In Vitro. Toxicol Sci. 2011; 120(1):42-58.

Ben-Jonathan N, Hnasko R. Dopamine as a Prolactin (PRL) Inhibitor. Endocrine Reviews. 2001; 22(6):724 763.

Bentley GE, Jensen JP, Kaur GJ, Wacker DW, Tsutsui K, Wingfield JC. Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH). Hormones and Behavior. 2006; 49(4):550‐555.

Bergman Å, Heindel JJ, Jobling S, Kidd KA, Zoeller RT. State of the science of endocrine disrupting chemicals 2012: an assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme and World Health Organization. 2013; xxv + 260 pp.

Berg C, Blomqvist A, Holm L, Brandt I, Brunström B, Ridderstråle Y. Embryonic exposure to oestrogen causes eggshell thinning and altered shell gland carbonic anhydrase expression in the domestic hen. Reproduction. 2004; 128(4):455‐461.

Beuschlein F, Fassnacht M, Klink A, Allolio B, Reincke M. ACTH-receptor expression, regulation and role in adrenocortial tumor formation. Eur J Endocrinol. 2001; 144(3):199-206.

Bitman J, Cecil HC, Fries GF. DDT-Induced Inhibition of Avian Shell Gland Carbonic Anhydrase: A Mechanism for Thin Eggshells. Science. 1970;168(3931):594-596.

Blévin P, Tartu S, Angelier F, Leclaire S, Bustnes JO, Moe B, et al. Integument colouration in relation to persistent organic pollutants and body condition in arctic breeding black-legged kittiwakes (Rissa tridactyla). Science of The Total Environment. 2014; 470–471:248-254.

Blüm V, Fiedler K. Hormonal control of reproductive behavior in some Cichlid fish. General and Comparative Endocrinology. 1965; 5(2):186‐196.

Bókony V, Lendvai ÁZ, Liker A, Angelier F, Wingfield JC, Chastel O. Stress Response and the Value of Reproduction: Are Birds Prudent Parents? The American Naturalist. 2009; 173(5):589-598.

Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and Its Receptor: Actions, Signal Transduction Pathways and Phenotypes Observed in PRL Receptor Knockout Mice. Endocrine Reviews. 1998; 19(3):225‐268.

Bonier F, Martin PR, Moore IT, Wingfield JC. Do baseline glucocorticoids predict fitness? Trends in Ecology & Evolution. 2009; 24(11):634-642.

Borgå K, Gabrielsen G., Skaare J. Biomagnification of organochlorines along a Barents Sea food chain. Environmental Pollution. 2001; 113(2):187-198.

Borgå K, Wolkers H, Skaare JU, Hop H, Muir DCG, Gabrielsen GW. Bioaccumulation of PCBs in Arctic seabirds: influence of dietary exposure and congener biotransformation. Environmental Pollution. 2005; 134(3):397‐409.

Bouland AJ, White AE, Lonabaugh KP, Varian-Ramos CW, Cristol DA. Female-biased offspring sex ratios in birds at a mercury-contaminated river. Journal of Avian Biology. 2012; 43(3):244-251.

Brasso RL, Cristol DA. Effects of mercury exposure on the reproductive success of tree swallows (Tachycineta bicolor). Ecotoxicology. 2008; 17(2):133-141.

Braune BM. Temporal trends of organochlorines and mercury in seabird

eggs from the Canadian Arctic, 1975–2003. Environmental Pollution. 2007; 148(2):599-613.

Breuner CW, Patterson SH, Hahn TP. In search of relationships between the acute adrenocortical response and fitness. General and Comparative Endocrinology. 2008; 157(3):288‐295.

Breuner CW. Stress and reproduction in birds. Hormones and reproduction of vertebrates. 2011; 4:129‐51.

Bried J, Pontier D, Jouventin P. Mate fidelity in monogamous birds: a re-examination of the Procellariiformes. Animal Behaviour. 2003; 65(1):235‐246.

Brown JH, Marquet PA, Taper ML. Evolution of body size: consequences of an energetic definition of fitness. American Naturalist. 1993; 573‐584.

Buntin JD. Parental Care: Evolution, Mechanisms, And Adaptive Significance: Parental Care: Evolution, Mechanisms, And Adaptive Significance. 1996; Eds Academic Press; 715 pp.

Burger J. Assessment and management of risk to wildlife from cadmium. Science of The Total Environment. 2008; 389(1):37‐45.

Bustnes JO., Bakken V, Erikstad KE., Mehlum F, Skaare JU. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. Journal of Applied Ecology. 2001; 38(4):791-801.

Bustnes JO, Erikstad KE, Skaare JU, Bakken V, Mehlum F. Ecological effects of organochlorine pollutants in the arctic: a study of the glaucous gull. Ecological Applications. 2003a; 13(2):504-515.

Bustnes JO, Bakken V, Skaare JU, Erikstad KE. Age and accumulation of persistent organochlorines: A study of arctic-breeding glaucous gulls (Larus hyperboreus). Environmental Toxicology and Chemistry. 2003b; 22(9):2173-9.

Bustnes JO, Tveraa T, Varpe Ø, Henden JA, Skaare JU. Reproductive performance and organochlorine pollutants in an Antarctic marine top predator: The south polar skua. Environment International. 2007; 33(7):911‐918.

Bustnes JO, Fauchald P, Tveraa T, Helberg M, Skaare JU. The potential impact of environmental variation on the concentrations and ecological effects of pollutants in a marine avian top predator. Environment International. 2008; 34(2):193‐201.

Bustnes JO, Moe B, Hanssen SA, Herzke D, Fenstad AA, Nordstad T, et al. Temporal Dynamics of Circulating Persistent Organic Pollutants in a Fasting Seabird under Different Environmental Conditions. Environ Sci Technol. 2012; 46(18):10287-10294.

Butt CM, Mabury SA, Muir, Braune BM. Prevalence of Long-Chained Perfluorinated Carboxylates in Seabirds from the Canadian Arctic between 1975 and 2004. Environ Sci Technol. 2007; 41(10):3521-3528.

Butt CM, Berger U, Bossi R, Tomy GT. Levels and trends of poly- and perfluorinated compounds in the arctic environment. Science of The Total Environment. 2010; 408(15):2936-2965.

Calabrese EJ. Hormesis is central to toxicology, pharmacology and risk assessment. Hum Exp Toxicol. 2010; 29(4):249-261.

Cam E, Hines JE, Monnat J-Y, Nichols JD, Danchin E. Are adult nonbreeders prudent parents? The kittiwake model. Ecology. 1998; 79(8):2917‐2930.

Campbell LM, Norstrom RJ, Hobson KA, Muir DCG, Backus S, Fisk AT. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). Science of The Total Environment. 2005; 351–352:247‐263.

Caro SP, Lambrechts MM, Balthazart J, Perret P. Non-photoperiodic factors and timing of breeding in blue tits: Impact of environmental and social influences in semi-natural conditions. Behav Processes. 2007; 75(1):1-7.

Caro SP, Schaper SV, Hut RA, Ball GF, Visser ME. The Case of the Missing Mechanism: How Does Temperature Influence Seasonal Timing in Endotherms? PLoS Biol. 2013; 11(4):e1001517.

Carson R. Silent Spring. 1962; Eds Houghton Mifflin Harcourt, 400 pp.

Carta P, Flore C, Alinovi R, Ibba A, Tocco MG, Aru G, et al. Sub-clinical neurobehavioral abnormalities associated with low level of mercury exposure through fish consumption. Neurotoxicology. 2003; 24(4):617‑623.

Celander MC. Cocktail effects on biomarker responses in fish. Aquatic Toxicology. 2011; 105(3–4, Supplement):72-77.

Chastel O, Weimerskirch H, Jouventin P. High annual variability in reproductive success and survival of an Antarctic seabird, the snow petrel Pagodroma nivea. Oecologia. 1993; 94(2):278-285.

Chastel O, Weimerskirch H, Jouventin P. Body Condition and Seabird Reproductive Performance: A Study of Three Petrel Species. Ecology. 1995; 76(7):2240.

Chastel O, Lacroix A, Weimerskirch H, Gabrielsen GW. Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird. Hormones and Behavior. 2005; 47(4):459-466.

Clarke A, Harris CM. Polar marine ecosystems: major threats and future change. Environmental Conservation. 2003; 30(01):1‑25.

Clausen J, Berg O. The content of polychlorinated hydrocarbons in Arctic ecosystems. Pure and Applied Chemistry. 1975; 42(1-2):223‑232.

Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect. 1993; 101(5):378-384.

Colborn T, Dumanoski D, Myers JP. Our stolen future: are we threatening our fertility, intelligence, and survival?: a scientific detective story. 1996; Eds Plume, 316 pp.

Cooke A. Shell thinning in avian eggs by environmental pollutants. Environmental Pollution. 1973; 4(2):85‑152.

Corsolini S, Borghesi N, Ademollo N, Focardi S. Chlorinated biphenyls and pesticides in migrating and resident seabirds from East and West Antarctica. Environment International. 2011; 37(8):1329‑1335.

Coulson JC. The Kittiwake. 2011.Eds T & AD Poyser, 320 pp.

Criscuolo F, Chastel O, Bertile F, Gabrielsen GW, Maho YL, Raclot T. Corticosterone alone does not trigger a short term behavioural shift in incubating female common eiders Somateria mollissima, but does modify long term reproductive success. Journal of Avian Biology. 2005; 36(4):306‑12.

Crump KL, Trudeau VL. Mercury-induced reproductive impairment in fish. Environmental Toxicology and Chemistry. 2009; 28(5):895-907.

Dawson A. Mechanisms of Endocrine Disruption with Particular Reference to Occurrence in Avian Wildlife: A Review. Ecotoxicology. 2000; 9(1-2):59-69.

De Ruiter AJH, Wendelaar Bonga SE, Slijkhuis H, Baggerman B. The effect of prolactin on fanning behavior in the male three-spined stickleback, Gasterosteus aculeatus L. General and Comparative Endocrinology. 1986; 64(2):273‑283.

Donaldson GM, Braune BM, Gaston AJ, Noble DG. Organochlorine and Heavy Metal Residues in Breast Muscle of Known-Age Thick-Milled Murres (Uria lomvia) from the Canadian Arctic. Arch Environ Contam Toxicol. 1997; 33(4):430‑435.

Doutrelant C, Grégoire A, Gomez D, Staszewski V, Arnoux E, Tveraa T, et al. Colouration in Atlantic puffins and blacklegged kittiwakes: monochromatism and links to body condition in both sexes. Journal of Avian Biology. 2013; 44(5):451-460.

Drent RH, Daan S. The prudent parent: energetic adjustments in avian breeding. Ardea. 1980; 68(1-4):225-252.

Erikstad KE, Sandvik H, Reiertsen TK, Bustnes JO, Strøm H. Persistent organic pollution in a high-Arctic top predator: sex-dependent thresholds in adult survival. Proc R Soc B. 2013; 280(1769):20131483.

Evers DC, Taylor KM, Major A, Taylor RJ, Poppenga RH, Scheuhammer AM. Common Loon Eggs as Indicators of Methylmercury Availability in North

America. Ecotoxicology. 2003; 12(1-4):69‑81.

Faro LRF, Durán R, do Nascimento JLM, Alfonso M, Picanço-Diniz CW. Effects of Methyl Mercury on the in Vivo Release of Dopamine and Its Acidic Metabolites DOPAC and HVA from Striatum of Rats. Ecotoxicology and Environmental Safety. 1997; 38(2):95-98.

Faro LRF, Nascimento JLM do, José JMS, Alfonso M, Durán R. Intrastriatal Administration of Methylmercury Increases In Vivo Dopamine Release. Neurochem Res. 2000; 25(2):225-229.

Faro LRF, do Nascimento JLM, Alfonso M, Durán R. Mechanism of action of methylmercury on in vivo striatal dopamine release: Possible involvement of dopamine transporter. Neurochemistry International. 2002; 40(5):455-465.

Faro LRF, Durán R, Do Nascimento JLM, Perez-Vences D, Alfonso M. Effects of successive intrastriatal methylmercury administrations on dopaminergic system. Ecotoxicology and Environmental Safety. 2003; 55(2):173-177.

Faro LRF, Rodrigues KJA, Santana MB, Vidal L, Alfonso M, Durán R. Comparative effects of organic and inorganic mercury on in vivo dopamine release in freely moving rats. Brazilian Journal of Medical and Biological Research. 2007; 40(10):1361-1365.

Finley MT, Stendell RC. Survival and reproductive success of black ducks fed methyl mercury. Environmental Pollution. 1978;16(1):51-64.

Fisher SA, Bortolotti GR, Fernie KJ, Bird DM, Smits JE. Behavioral variation and its consequences during incubation for American kestrels exposed to polychlorinated biphenyls. Ecotoxicology and Environmental Safety. 2006; 63(2):226-235.

Follett BK, Farner DS, Mattocks Jr. PW. Luteinizing hormone in the plasma of white-crowned sparrows (Zonotrichia leucophrys gambelii) during artificial photostimulation. General and Comparative Endocrinology. 1975; 26(1):126‑134.

Franceschini MD, Custer CM, Custer TW, Reed JM, Romero LM. Corticosterone stress response in tree swallows nesting near polychlorinated biphenyl- and dioxin-contaminated rivers. Environmental Toxicology and Chemistry. 2008; 27(11):2326-2331.

Franceschini MD, Lane OP, Evers DC, Reed JM, Hoskins B, Romero LM. The corticosterone stress response and mercury contamination in free-living tree swallows, Tachycineta bicolor. Ecotoxicology. 2009; 18(5):514-521.

Frederick P, Jayasena N. Altered Pairing Behaviour and Reproductive Success in White Ibises Exposed to Environmentally Relevant Concentrations of Methylmercury. Proc R Soc B. 2011;278 (1713):1851-1857.

Fuglei E, Bustnes JO, Hop H, Mørk T, Björnfoth H, van Bavel B. Environmental contaminants in arctic foxes (Alopex lagopus) in Svalbard: Relationships with feeding ecology and body condition. Environmental Pollution. 2007; 146(1):128-138.

Furness RW. Birds as monitors of pollutants. In: Furness RW, Greenwood JJD, Birds as Monitors of Environmental Change. Eds Springer Netherlands; 1993. p. 86-143.

Furness R, Hutton M. Pollutant levels in the great skua Catharacta skua. Environmental Pollution (1970). 1979; 19(4):261‑268.

Gabrielsen GW. Levels and effects of persistent organic pollutants in arctic animals. In: Ørbæk DJB, Kallenborn DR, Tombre DI, Hegseth DEN, Falk-Petersen DS, Hoel DAH, éditeurs. Arctic Alpine Ecosystems and People in a Changing Environment. Springer Berlin Heidelberg; 2007. p. 377-412.

George JL, Frear DEH. Pesticides in the Antarctic. The Journal of Applied Ecology. 1966; 3:155-167.

Giesy JP, Feyk LA, Jones PD, Kannan K, Sanderson T. Review of the effects of endocrine-disrupting chemicals in birds. Pure and Applied Chemistry. 2003; 75(11-12):2287-2303.

Giménez-Llort L, Ahlbom E, Daré E, Vahter M, Ögren S-O, Ceccatelli S. Prenatal exposure to methylmercury changes dopamine-modulated motor activity

during early ontogeny: age and gender-dependent effects. Environmental Toxicology and Pharmacology. 2001; 9(3):61-70.

González-Solis J, Sanpera C, Ruiz X. Metals and selenium as bioindicators of geographic and trophic segregation in giant petrels Macronectes spp. Mar Ecol Prog Ser. 2002; 244:257‑264.

Goutte A, Angelier F, Chastel CC, Trouvé C, Moe B, Bech C, et al. Stress and the timing of breeding: Glucocorticoid-luteinizing hormones relationships in an arctic seabird. General and Comparative Endocrinology. 2010a; 169(1):108-116.

Goutte A, Antoine É, Weimerskirch H, Chastel O. Age and the timing of breeding in a long-lived bird: a role for stress hormones? Functional Ecology. 2010b; 24(5):1007-1016.

Goutte A, Angelier F, Welcker J, Moe B, Clément-Chastel C, Gabrielsen GW, et al. Long-term survival effect of corticosterone manipulation in Black-legged kittiwakes. General and Comparative Endocrinology. 2010c; 167(2):246-251.

Goutte A, Kriloff M, Weimerskirch H, Chastel O. Why Do Some Adult Birds Skip Breeding? A Hormonal Investigation in a Long-Lived Bird. Biol Lett. 2011a; 7(5):790-792.

Goutte A, Antoine É, Chastel O. Experimentally delayed hatching triggers a magnified stress response in a long-lived bird. Hormones and Behavior. 2011b; 59(1):167-173.

Goutte A, Clément-Chastel C, Moe B, Bech C, Gabrielsen GW, Chastel O. Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes. J Exp Biol. 2011c; 214(12):2005-2013.

Goutte A, Chevreuil M, Alliot F, Chastel O, Cherel Y, Eléaume M, et al. Persistent organic pollutants in benthic and pelagic organisms off Adélie Land, Antarctica. Marine Pollution Bulletin. 2013; 77(1–2):82‑89.

Goutte A, Angelier F, Bech C, Clément-Chastel C, Dell'Omo G, Gabrielsen GW, et al. Annual variation in the timing of breeding, pre-breeding foraging areas and corticosterone levels in an Arctic population of black-legged kittiwakes. Mar Ecol Prog Ser. 2014a; 496:233‑247.

Goutte A, Bustamante P, Barbraud C, Delord K, Weimerskirch H, Chastel O. Demographic responses to mercury exposure in two closely related Antarctic top predators. Ecology. 2014b; 95(4):1075‑1086.

Goutte A, Barbraud C, Meillère A, Carravieri A, Bustamante P, Labadie P, et al. Demographic consequences of heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the wandering albatross. Proc R Soc B. 2014c; 281(1787):20133313.

Grattan D, Kokay I. Prolactin: a pleiotropic neuroendocrine hormone. Journal of neuroendocrinology. 2008; 20(6):752‑763.

Groscolas R, Lacroix A, Robin J-P. Spontaneous egg or chick abandonment in energy-depleted king penguins: A role for corticosterone and prolactin? Hormones and Behavior. 2008; 53(1):51‑60.

Hammerschmidt CR, Sandheinrich MB, Wiener JG, Rada RG. Effects of Dietary Methylmercury on Reproduction of Fathead Minnows. Environ Sci Technol. 2002; 36(5):877‑883.

Harrad S. Persistent Organic Pollutants. John Wiley & Sons; 2009.

Harrison PTC, Holmes P, Humfrey CDN. Reproductive health in humans and wildlife: are adverse trends associated with environmental chemical exposure? Science of The Total Environment. 1997; 205(2–3):97‑106.

Heidinger BJ, Chastel O, Nisbet ICT, Ketterson ED. Mellowing with age: older parents are less responsive to a stressor in a long-lived seabird. Functional Ecology. 2010; 24(5):1037‑44.

Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR, Kondrad SL, Erwin CA. Hormesis Associated with a Low Dose of Methylmercury Injected into Mallard Eggs. Arch Environ Contam Toxicol. 2012; 62(1):141‑144.

Helgason LB, Arukwe A, Gabrielsen GW, Harju M, Hegseth MN, Heimstad ES, et al. Biotransformation of PCBs in Arctic seabirds: Characterization of phase I and

II pathways at transcriptional, translational and activity levels. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2010; 152(1):34-41.

Helgason LB, Gabrielsen GW, Sagerup K.Temporal trends and contaminant profiles of persistent organic pollutants (POPs) in seabird eggs from Northern Norway and Svalbard. 2011. In: Global Contamination Trends of Persistent Organic Chemicals. Loganathan and Lam (Eds.). Taylor and Francis Books / CRC Press, Boca Raton, Florida, USA.

Henny CJ, Hill EF, Hoffman DJ, Spalding MG, Grove RA. Nineteenth Century Mercury: Hazard to Wading Birds and Cormorants of the Carson River, Nevada. Ecotoxicology. 2002; 11(4):213-231.

Henriksen EO, Gabrielsen GW, Skaare JU. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (Rissa tridactyla), in relation to mobilization of body-lipids associated with reproduction. Environmental Pollution. 1996; 92(1):27-37.

Henriksen EO, Gabrielsen GW, Utne Skaare J. Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gulls, Larus hyperboreus. Chemosphere. 1998; 37(13):2627-2643.

Hepp GR, Kennamer RA, Johnson MH. Maternal effects in Wood Ducks: incubation temperature influences incubation period and neonate phenotype. Functional Ecology. 2006; 20(2):308‐314.

Herring G, Ackerman JT, Herzog MP. Mercury Exposure May Suppress Baseline Corticosterone Levels in Juvenile Birds. Environ Sci Technol. 2012; 46(11):6339-6346.

Holm L, Blomqvist A, Brandt I, Brunström B, Ridderstråle Y, Berg C. Embryonic exposure to o,p′-DDT causes eggshell thinning and altered shell gland carbonic anhydrase expression in the domestic hen. Environmental Toxicology and Chemistry. 2006; 25(10):2787‐2793.

Hutton M. Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. Environmental Pollution Series A, Ecological and Biological. 1981; 26(2):129‐145.

Jackson AK, Evers DC, Etterson MA, Condon AM, Folsom SB, Detweiler J, et al. Mercury Exposure Affects the Reproductive Success of a Free-Living Terrestrial Songbird, the Carolina Wren (Thryothorus ludovicianus). The Auk. 2011; 128(4):759‐769.

Janik DS, Buntin JD. Behavioural and physiological effects of prolactin in incubating ring doves. J Endocrinol. 1985; 105(2):201‐209.

Järup L. Hazards of heavy metal contamination. Br Med Bull. 12 janv 2003; 68(1):167-182.

Jenouvrier S, Barbraud C, Weimerskirch H. Long-term contrasted responses to climate of two Antarctic seabird species. Ecology. 2005; 86(11):2889‐2903.

Jensen AA, Leffers H. Emerging endocrine disrupters: perfluoroalkylated substances. International Journal of Andrology. 2008; 31(2):161-9.

Jenssen BM. Endocrine-Disrupting Chemicals and Climate Change: A Worst-Case Combination for Arctic Marine Mammals and Seabirds? Environ Health Perspect. 2006; 114(S-1):76-80.

Jones PD, Hu W, De Coen W, Newsted JL, Giesy JP. Binding of perfluorinated fatty acids to serum proteins. Environmental Toxicology and Chemistry. 2003; 22(11):2639‐2649.

Jones IL, Hunter FM. Mutual sexual selection in a monogamous seabird. Nature. 1993; 362(6417):238‐239.

Keller JM, Kucklick JR, Harms CA, McClellan-Green PD. Organochlorine contaminants in sea turtles: correlations between whole blood and fat. Environmental Toxicology and Chemistry. 2004; 23(3):726‐738.

Kempenaers B, Lanctot RB, Gill VA, Hatch SA, Valcu M. Do females trade copulations for food? An experimental study on kittiwakes (Rissa tridactyla). Behavioral Ecology. 2007; 18(2):345‐353.

Kitaysky AS, Wingfield JC, Piatt JF. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged

Kittiwakes. Functional Ecology. 1999; 13(5):577-84.
Koch KA, Wingfield JC, Buntin JD. Prolactin-induced parental hyperphagia in ring doves: are glucocorticoids involved? Hormones and Behavior. 2004; 46(4):498‐505.
Kortenkamp A. Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals. Environmental Health Perspectives. 2007; 115(Suppl 1):98-105.
Kudo A, Fujikawa Y, Miyahara S, Zheng J, Takigami H, Sugahara M, et al. Lessons from Minamata mercury pollution, Japan — After a continuous 22 years of observation. Water Science and Technology. 1998; 38(7):187-193.
la Farré M, Pérez S, Kantiani L, Barceló D. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. TrAC Trends in Analytical Chemistry. 2008; 27(11):991‐1007.
Larison JR, Likens GE, Fitzpatrick JW, Crock JG. Cadmium toxicity among wildlife in the Colorado Rocky Mountains. Nature. 2000; 406(6792):181‐183.
Lanctot RB, Hatch SA, Gill VA, Eens M. Are corticosterone levels a good indicator of food availability and reproductive performance in a kittiwake colony? Hormones and Behavior. 2003; 43(4):489-502.
Leclaire S, White J, Arnoux E, Faivre B, Vetter N, Hatch SA, et al. Integument coloration signals reproductive success, heterozygosity, and antioxidant levels in chick-rearing black-legged kittiwakes. Naturwissenschaften. 2011; 98(9):773‐782.
Leclaire S, Blanchard P, White J, Hatch SA, Danchin É. Symmetry of Black Wingtips is Related to Clutch Size and Integument Coloration in Black-Legged Kittiwakes (Rissa tridactyla). The Auk. 2013; 130(3):541‐547.
Lendvai ÁZ, Giraudeau M, Chastel O. Reproduction and modulation of the stress response: an experimental test in the house sparrow. Proc R Soc B. 2007; 274(1608):391-397.
Letcher RJ, Bustnes JO, Dietz R, Jenssen BM, Jørgensen EH, Sonne C, et al. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Science of The Total Environment. 2010; 408(15):2995-3043.
Liu C, Du Y, Zhou B. Evaluation of estrogenic activities and mechanism of action of perfluorinated chemicals determined by vitellogenin induction in primary cultured tilapia hepatocytes. Aquatic Toxicology. 2007; 85(4):267‐277.
Lister AL, Van Der Kraak GJ. Endocrine disruption: why is it so complicated? Water Quality Research Journal of Canada. 2001; 36(2):175‐190.
Lorenzen A, Moon TW, Kennedy SW, Glen GA. Relationships between environmental organochlorine contaminant residues, plasma corticosterone concentrations, and intermediary metabolic enzyme activities in Great Lakes herring gull embryos. Environ Health Perspect. 1999; 107(3):179‐186.
Love OP, Shutt LJ, Silfies JS, Bortolotti GR, Smits JEG, Bird DM. Effects of Dietary PCB Exposure on Adrenocortical Function in Captive American Kestrels (Falco sparverius). Ecotoxicology. 2003; 12(1-4):199-208.
Lu JY, Schroeder WH, Barrie LA, Steffen A, Welch HE, Martin K, et al. Magnification of atmospheric mercury deposition to polar regions in springtime: The link to tropospheric ozone depletion chemistry. Geophys Res Lett. 2001; 28(17):3219‐3222.
Lucchini R, Cortesi I, Facco P, Benedetti L, Camerino D, Carta P, et al. Neurotoxic effect of exposure to low doses of mercury. Med Lav. 2002; 93(3):202-214.
Lucchini R, Calza S, Camerino D, Carta P, Decarli A, Parrinello G, et al. Application of a Latent Variable Model for a Multicenter Study on Early Effects Due to Mercury Exposure. NeuroToxicology. 2003; 24(4–5):605-616.
Lundholm C. DDE-induced eggshell thinning in birds: Effects of p,p′-DDE on

the calcium and prostaglandin metabolism of the eggshell gland. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 1997; 118(2):113-128.

Mallory ML, Braune BM. Tracking contaminants in seabirds of Arctic Canada: Temporal and spatial insights. Marine Pollution Bulletin. 2012; 64(7):1475‐1484.

McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. Hormones and behavior. 2003; 43(1):2‐15.

Mclachlan JA, Korach KS, Newbold RR, Degen GH. Diethylstilbestrol and Other Estrogens in the Environment. Toxicol Sci. 1984; 4(5):686‐691.

Mehlum F, Gabrielsen GW. The diet of high-arctic seabirds in coastal and ice-covered, pelagic areas near the Svalbard archipelago. Polar Research. 1993; 12(1):1‐20.

Minnema DJ, Cooper GP, Greenland RD. Effects of methylmercury on neurotransmitter release from rat brain synaptosomes. Toxicology and Applied Pharmacology. 1989; 99(3):510-521.

Moline MA, Karnovsky NJ, Brown Z, Divoky GJ, Frazer TK, Jacoby CA, et al. High Latitude Changes in Ice Dynamics and Their Impact on Polar Marine Ecosystems. Annals of the New York Academy of Sciences. 2008; 1134(1):267-319.

Muir DCG, de Wit CA. Trends of legacy and new persistent organic pollutants in the circumpolar arctic: Overview, conclusions, and recommendations. Science of The Total Environment. 2010; 408(15):3044-3051.

Nebert DW, Gonzalez FJ. P450 Genes: Structure, Evolution, and Regulation. Annual Review of Biochemistry. 1987; 56(1):945-993.

Nettleship DN, Peakall DB. Organochlorine residue levels in three high arctic species of colonially-breeding seabirds from Prince Leopold Island. Marine Pollution Bulletin. 1987; 18(8):434‐438.

Newton I, Bogan J, Marquiss M. Organochlorine contamination and age in sparrowhawks. Environmental Pollution Series A, Ecological and Biological. 1981; 25(2):155‐160.

Nieboer E, Richardson DH. The replacement of the nondescript term ‘heavy metals’ by a biologically and chemically significant classification of metal ions. Environmental Pollution Series B, Chemical and Physical. 1980; 1(1):3-26.

Nordstad T, Moe B, Bustnes JO, Bech C, Chastel O, Goutte A, et al. Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (Rissa tridactyla) across their breeding cycle. Environmental Pollution. 2012; 164(0):219-226.

Norris DO, Carr JA. Vertebrate Endocrinology. Academic Press; 2013. 580p.

Oliveira FRT, Ferreira JR, dos Santos CMC, Macêdo LEM, de Oliveira RB, Rodrigues JA, et al. Estradiol reduces cumulative mercury and associated disturbances in the hypothalamus–pituitary axis of ovariectomized rats. Ecotoxicology and Environmental Safety. 2006; 63(3):488-493.

Ottinger MA, Abdelnabi M, Quinn M, Golden N, Wu J, Thompson N. Reproductive consequences of EDCs in birds: What do laboratory effects mean in field species? Neurotoxicology and Teratology. 2002; 24(1):17-28.

Ottinger MA, Quinn Jr. MJ, Lavoie E, Abdelnabi MA, Thompson N, Hazelton JL, et al. Consequences of endocrine disrupting chemicals on reproductive endocrine function in birds: Establishing reliable end points of exposure. Domestic Animal Endocrinology. 2005; 29(2):411-419.

Ottinger MA, Lavoie E, Thompson N, Barton A, Whitehouse K, Barton M, et al. Neuroendocrine and behavioral effects of embryonic exposure to endocrine disrupting chemicals in birds. Brain Research Reviews. 2008; 57(2):376-385.

Ottinger MA, Lavoie ET, Thompson N, Bohannon M, Dean K, Quinn Jr. MJ. Is the gonadotropin releasing hormone system vulnerable to endocrine disruption in birds? General and Comparative Endocrinology. 2009; 163(1–2):104-108.

Ottinger MA, Carro T, Bohannon M, Baltos L, Marcell AM, McKernan M, et al. Assessing effects of environmental chemicals on neuroendocrine systems: Potential mechanisms and functional outcomes. General and Comparative Endocrinology. 2013; 190:194‑202.

Parkinson A, Ogilvie BW. Biotransformation of xenobiotics. Casarett and Dull's Toxicology: The Basic Science of Poisons. McGraw Hill, USA: C.D. Klaassen; 2008.

Pascussi JM, Gerbal-Chaloin S, Drocourt L, Maurel P, Vilarem MJ. The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. Biochimica et Biophysica Acta (BBA) - General Subjects. 2003; 1619(3):243-253.

Pauwels A, Covaci A, Weyler J, Delbeke L, Dhont M, De Sutter P, et al. Comparison of persistent organic pollutant residues in serum and adipose tissue in a female population in Belgium, 1996–1998. Archives of environmental contamination and toxicology. 2000; 39(2):265‑270.

Peig J, Green AJ. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos. 2009; 118(12):1883-1891.

Penhoat A, Jaillard C, Saez JM. Corticotropin positively regulates its own receptors and cAMP response in cultured bovine adrenal cells. PNAS. 1989; 86(13):4978-4981.

Petrovic M, Eljarrat E, Alda MJL de, Barceló D. Endocrine disrupting compounds and other emerging contaminants in the environment: A survey on new monitoring strategies and occurrence data. Anal Bioanal Chem. 2004; 378(3):549-562.

Ramalingam V, Vimaladevi V, Rajeswary S, Suryavathi V. Effect of mercuric chloride on circulating hormones in adult albino rats. J Environ Biol.2003; 24(4):401-404.

Ratcliffe D. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. Journal of Applied Ecology. 1970; 67‑115.

Ricklefs RE, Wikelski M. The physiology/life-history nexus. Trends in Ecology & Evolution. 2002; 17(10):462‑468.

Ronchetti SA, Miler EA, Duvilanski BH, Cabilla JP. Cadmium Mimics Estrogen-Driven Cell Proliferation and Prolactin Secretion from Anterior Pituitary Cells. PLoS ONE. 2013; 8(11):e81101.

Rowe CL. « The Calamity of So Long Life »: Life Histories, Contaminants, and Potential Emerging Threats to Long-lived Vertebrates. BioScience. 2008; 58(7):623.

Sagerup K, Helgason LB, Polder A, Strøm H, Josefsen TD, Skåre JU, et al. Persistent organic pollutants and mercury in dead and dying glaucous gulls (Larus hyperboreus) at Bjørnøya (Svalbard). Science of The Total Environment. 2009; 407(23):6009-6016.

Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions 1. Endocrine reviews. 2000; 21(1):55‑89.

Savinov VM, Gabrielsen GW, Savinova TN. Cadmium, zinc, copper, arsenic, selenium and mercury in seabirds from the Barents Sea: levels, inter-specific and geographical differences. Science of The Total Environment. 2003; 306(1–3):133-158.

Savinova TN, Polder A, Gabrielsen GW, Skaare JU. Chlorinated hydrocarbons in seabirds from the Barents Sea area. Science of The Total Environment. 1995; 160–161:497‑504.

Schaper SV, Dawson A, Sharp PJ, Gienapp P, Caro Samuel P., Visser ME. Increasing Temperature, Not Mean Temperature, Is a Cue for Avian Timing of Reproduction. The American Naturalist. 2012; 179(2):E55-E69.

Scheifler R, Gauthier-Clerc M, Bohec CL, Crini N, Cœurdassier M, Badot P-M, et al. Mercury concentrations in king penguin (Aptenodytes patagonicus) feathers at Crozet Islands (sub-Antarctic): Temporal trend between 1966–1974 and 2000–2001. Environmental Toxicology and Chemistry. 2005; 24(1):125-128.

Schmidt KL, Furlonger AA, Lapierre JM, MacDougall-Shackleton EA, MacDougall-Shackleton SA. Regulation of the HPA axis is related to song complexity and measures of phenotypic quality in song sparrows. Hormones and Behavior. 2012; 61(4):652‐659.

Schoech SJ, Mumme RL, Wingfield JC. Delayed breeding in the cooperatively breeding Florida scrub-jay (Aphelocoma coerulescens): inhibition or the absence of stimulation? Behav Ecol Sociobiol. 1996; 39(2):77-90.

Sharp PJ. A Comparison of Variations in Plasma Luteinizing Hormone Concentrations in Male and Female Domestic Chickens (gallus Domesticus) from Hatch to Sexual Maturity. J Endocrinol. 1975; 67(2):211‐223.

Solheim SA. The Preen Gland - an Organ for Excretion of Persistent Organic Pollutants in Black-legged Kittiwake (Rissa tridactyla). 2010. Master of Science, University of Bergen.

Stetson MH. Feedback Regulation by Oestradiol of Ovarian Function in Japanese Quail. J Reprod Fertil. 1972; 31(2):205-213.

Stricker P, Grueter R. Action du lobe anterieur de l'hypophyse sur la montee laiteuse. CR Soc Biol. 1928; 99:1978‐1980.

Stockholm Convention on Persitent Organic Pollutants: http://chm.pops.int/

Szumiło E, Szubska M, Meissner W, Bełdowska M, Falkowska L. Mercury in immature and adults Herring Gulls (Larus argentatus) wintering on the Gulf of Gdańsk area. Ocean and Hydro. 2013; 42(3):260-267.

Tan SW, Meiller JC, Mahaffey KR. The endocrine effects of mercury in humans and wildlife. Critical Reviews in Toxicology. 2009; 39(3):228-269.

Tavares S, Xavier JC, Phillips RA, Pereira ME, Pardal MA. Influence of age, sex and breeding status on mercury accumulation patterns in the wandering albatross Diomedea exulans. Environmental Pollution. 2013; 181:315-320.

Taylor MR, Harrison PTC. Ecological effects of endocrine disruption: Current evidence and research priorities. Chemosphere. 1999; 39(8):1237‐1248.

Thompson DR, Hamer KC, Furness RW. Mercury Accumulation in Great Skuas Catharacta skua of Known Age and Sex, and Its Effects Upon Breeding and Survival. The Journal of Applied Ecology. 1991; 28(2):672.

Turusov V, Rakitsky V, Tomatis L. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environ Health Perspect. 2002; 110(2):125-128.

Turzillo AM, DiGregorio GB, Nett TM. Messenger Ribonucleic Acid for Gonadotropin-Releasing Hormone Receptor and Numbers of Gonadotropin-Releasing Hormone Receptors in Ovariectomized Ewes After Hypothalamic-Pituitary Disconnection and Treatment with Estradiol. J Anim Sci. 1995; 73(6):1784‐1788.

Tyler CR, Jobling S, Sumpter JP. Endocrine Disruption in Wildlife: A Critical Review of the Evidence. Critical Reviews in Toxicology. 1998; 28(4):319-361.

Van de Merwe JP, Hodge M, Olszowy HA, Whittier JM, Lee SY. Using blood samples to estimate persistent organic pollutants and metals in green sea turtles (< i> Chelonia mydas). Marine pollution bulletin. 2010; 60(4):579‐588.

Van den Brink NW, van Franeker JA, de Ruiter-Dijkman EM. Fluctuating concentrations of organochlorine pollutants during a breeding season in two Antarctic seabirds: Adelie penguin and southern fulmar. Environmental Toxicology and Chemistry. 1998; 17(4):702-9.

Verboven N, Verreault J, Letcher RJ, Gabrielsen GW, Evans NP. Nest temperature and parental behaviour of Arctic-breeding glaucous gulls exposed to persistent organic pollutants. Animal Behaviour. 2009; 77(2):411-418.

Verboven N, Verreault J, Letcher RJ, Gabrielsen GW, Evans NP. Adrenocortical function of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. General and Comparative Endocrinology. 2010; 166(1):25-32.

Verreault J, Gabrielsen GW, Bustnes JO. The Svalbard Glaucous Gull as Bioindicator Species in the European Arctic: Insight from 35 Years of Contaminants Research. In: Whitacre DM, editor. Reviews of Environmental Contamination and Toxicology Volume 205. Springer New York; 2010. p. 77-116.

Verreault J, Verboven N, Gabrielsen GW, Letcher RJ, Chastel O. Changes in prolactin in a highly organohalogen contaminated Arctic top predator seabird, the glaucous gull. General and Comparative Endocrinology. 2008; 156(3):569-576.

Vos JG, Dybing E, Greim HA, Ladefoged O, Lambré C, Tarazona JV, et al. Health Effects of Endocrine-Disrupting Chemicals on Wildlife, with Special Reference to the European Situation. Critical Reviews in Toxicology. 2000; 30(1):71‑133.

Wada H, Hahn TP, Breuner CW. Development of stress reactivity in white-crowned sparrow nestlings: Total corticosterone response increases with age, while free corticosterone response remains low. General and Comparative Endocrinology. 2007; 150(3):405‑413.

Wada H, Cristol DA, McNabb FMA, Hopkins WA. Suppressed Adrenocortical Responses and Thyroid Hormone Levels in Birds near a Mercury-Contaminated River. Environ Sci Technol. 2009; 43(15):6031-6038.

Walker CH, Sibly RM, Hopkin SP, Peakall DB. Principles of Ecotoxicology, Fourth Edition. CRC Press.

Walkusz W, Kwasniewski S, Falk-Petersen S, Hop H, Tverberg V, Wieczorek P, et al. Seasonal and spatial changes in the zooplankton community of Kongsfjorden, Svalbard. Polar Research. 2009; 28(2):254‑281.

Wania F, Mackay D. Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. Ambio. 1993; 10‑18.

Wayland M, Garcia-Fernandez AJ, Neugebauer E, Gilchrist HG. Concentrations of Cadmium, Mercury and Selenium in Blood, Liver and Kidney of Common Eider Ducks from the Canadian Arctic. Environ Monit Assess. 2001; 71(3):255‑267.

Wiemeyer SN, Lamont TG, Bunck CM, Sindelar CR, Gramlich FJ, Fraser JD, et al. Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs—1969–79—and their relationships to shell thinning and reproduction. Arch Environ Contam Toxicol. 1984; 13(5):529-549.

Wingfield J. Modulation of the adrenocortical response to stress in birds. Perspectives in comparative endocrinology. 1994; 520-528.

Wingfield JC, Sapolsky RM. Reproduction and Resistance to Stress: When and How. Journal of Neuroendocrinology. 2003; 15(8):711-24.

Wingfield JC. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. Functional Ecology. 2013; 27(1):37‑44.

Wolfe MF, Schwarzbach S, Sulaiman RA. Effects of mercury on wildlife: a comprehensive review. Environmental toxicology and chemistry. 1998; 17(2):146‑160.

World Health Organization. DDT and its derivatives : environmental aspects. 1989, http://apps.who.int//iris/handle/10665/40018

Xie Z, Zhang P, Sun L, Xu S, Huang Y, He W. Microanalysis of metals in barbs of a snow petrel (Pagodroma Nivea) from the Antarctica using synchrotron radiation X-ray fluorescence. Marine Pollution Bulletin. 2008; 56(3):516‑524.

Zahir F, Rizwi SJ, Haq SK, Khan RH. Low dose mercury toxicity and human health. Environmental Toxicology and Pharmacology. 2005; 20(2):351‑360.

Zhang X, Wang Y, Zhao Y, Chen X. Experimental study on the estrogen-like effect of mercuric chloride. BioMetals. 2008; 21(2):143‑150.

Zhou T, Rademacher DJ, Steinpreis RE, Weis JS. Neurotransmitter levels in two populations of larval Fundulus heteroclitus after methylmercury exposure. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 1999; 124(3):287-294.

PAPER I

To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird.

Downloaded from rsbl.royalsocietypublishing.org on May 31, 2013

biology letters

rsbl.royalsocietypublishing.org

Research

Cite this article: Tartu S, Goutte A, Bustamante P, Angelier F, Moe B, Clément-Chastel C, Bech C, Gabrielsen GW, Bustnes JO, Chastel O. 2013 To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biol Lett 9: 20130317. http://dx.doi.org/10.1098/rsbl.2013.0317

Received: 9 April 2013
Accepted: 8 May 2013

Subject Areas:
behaviour, ecology, environmental science

Keywords:
intermittent breeding, mercury, GnRH challenge, luteinizing hormone, black-legged kittiwake

Author for correspondence:
Sabrina Tartu
e-mail: tartu@cebc.cnrs.fr

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2013.0317 or via http://rsbl.royalsocietypublishing.org.

Royal Society Publishing
Informing the science of the future

Physiology

To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird

Sabrina Tartu[1], Aurélie Goutte[1], Paco Bustamante[2], Frédéric Angelier[1], Børge Moe[3], Céline Clément-Chastel[1], Claus Bech[4], Geir Wing Gabrielsen[5], Jan Ove Bustnes[3] and Olivier Chastel[1]

[1]Centre d'Etudes Biologiques de Chizé (CEBC), UPR 1934-CNRS, 79360 Beauvoir-sur-niort, France
[2]Littoral Environnement Société (LIENSs), UMR 7266-CNRS, Université de La Rochelle, 17000 La Rochelle, France
[3]Norwegian Institute for Nature Research, Fram Center, 9296 Tromsø, Norway
[4]Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway
[5]Norwegian Polar Research Institute, Fram Center, 9296 Tromsø, Norway

Mercury, a ubiquitous toxic element, is known to alter expression of sex steroids and to impair reproduction across vertebrates but the mechanisms underlying these effects are not clearly identified. We examined whether contamination by mercury predicts the probability to skip reproduction in black-legged kittiwakes (*Rissa tridactyla*) from Svalbard. We also manipulated the endocrine system to investigate the mechanism underlying this relationship. During the pre-laying period, we injected exogenous GnRH (gonadotropin-releasing hormone) to test the ability of the pituitary to release luteinizing hormone (LH, a key hormone for the release of sex steroids and hence breeding) in relation to mercury burden. Birds that skipped reproduction had significantly higher mercury concentration in blood than breeders. Endocrine profiles of these birds also varied based on breeding status (breeders versus non-breeders), mercury contamination and sex. Specifically, in skippers (birds that did not breed), baseline LH decreased with increasing mercury concentration in males, whereas it increased in females. GnRH-induced LH levels increased with increasing mercury concentration in both sexes. These results suggest that mercury contamination may disrupt GnRH input to the pituitary. Thus, high mercury concentration could affect the ability of long-lived birds to modulate their reproductive effort (skipping or breeding) according to ongoing environmental changes in the Arctic, thereby impacting population dynamics.

1. Introduction

Mercury is a ubiquitous toxic element of both natural and anthropogenic sources. In its methylated form, mercury can impair reproduction and disrupt the expression of oestradiol and testosterone across vertebrates [1,2]. However, the mechanisms underlying these effects are not clearly identified [3]. In response to increased day length, gonadotropin-releasing hormone (GnRH) is secreted and triggers luteinizing hormone (LH) release from the pituitary gland. LH, in concert with follicle-stimulating hormone (FSH), promotes gonadal maturation, sex steroid secretion and in turn, the onset of reproduction. It is conceivable that mercury acts primarily on the ability of the pituitary to secrete gonadotropin hormones (LH and FSH), altering sex steroids release and ultimately impairing reproductive behaviour. Mercury may also suppress GnRH in the hypothalamus, thereby reducing LH production. Clearly, there is a need for studies investigating the mechanisms of LH suppression by mercury [3] and subsequent repercussions for breeding animals.

© 2013 The Author(s) Published by the Royal Society. All rights reserved.

Downloaded from rsbl.royalsocietypublishing.org on May 31, 2013

Recent investigations have highlighted a major role for LH on skipped breeding behaviour (non-breeding by individuals that previously bred), a common feature in long-lived birds [4,5]. In some seabirds, skipping individuals show either low levels of baseline LH (black-legged kittiwake, *Rissa tridactyla* [4]), or fail to maintain elevated LH levels after a GnRH injection (snow petrel, *Pagodroma nivea* [5]). LH secretion also appears to be sensitive to environmental stressors in kittiwakes [4]. This opens the yet unexplored possibility that some endocrine disruptors like mercury may play a role in skipping behaviour, by altering the release and secretion of LH.

Here, we test whether mercury concentrations (i) are linked to skipping behaviour, and (ii) affect patterns of LH release during the pre-breeding period in an Arctic population of black-legged kittiwakes. To evaluate LH release, we challenged the pituitary with an exogenous injection of GnRH. Kittiwakes provide an excellent model to address these questions, as they bear elevated mercury levels in Svalbard [6] and a significant proportion of adults skip breeding each year [4]. We predicted that high mercury concentration in blood would (i) be linked to a high probability to skip breeding, and (ii) impair baseline and/or GnRH-induced LH levels.

2. Material and methods

The study was conducted at Kongsfjorden, Svalbard (78°54′ N, 12°13′ E) from 20 May to 6 June 2008 (52 birds) and from 21 May to 7 June in 2011 (104 birds) during the pre-breeding period. Birds were caught on the nests and a blood sample was collected immediately after capture. In 2008, we performed a GnRH challenge immediately after the first blood sampling, kittiwakes were injected with 0.1 ml of a solution of GnRH (see the electronic supplementary material, figure S1). Blood samples were collected from the alar veins 10 and 30 min after the GnRH injection to measure baseline and GnRH-induced levels of LH (both sexes) and testosterone (males only) as detailed in the electronic supplementary material. Total mercury from all 2008 and 2011 samples was measured at LIENSs from lyophilized red blood cells, by atomic absorption spectrophotometry on 5–10 mg aliquots [7]. Mercury concentrations are expressed in $\mu g\, g^{-1}$ dry weight. In both years, focal birds were measured as described in Goutte *et al*. [4] to calculate a scaled mass index and observed daily. The nest content was checked every two days, to monitor whether birds had engaged in breeding (at least one egg laid) or whether they had skipped (no egg laid). We also monitored fitness, including date of first egg-laying, clutch size and the number of chicks that reached 12 days post-hatch (hereafter breeding success). To test the effects of mercury and scaled mass index on these reproductive parameters, we pooled data from 2008 and 2011 (no birds were sampled twice). Then, for 2008, we tested the effects of mercury concentration and interaction with sex, on baseline and GnRH-induced hormone levels in skipping and breeding birds. Data are available from the Dryad Digital Repository: doi.org/10.5061/dryad.4ff07. All statistical analyses were performed using R v. 2.13.1 and we used generalized linear models (GLM) with a normal/binomial error distribution and an identity/logit link function to test our biological assumptions. Model selection was performed by a step-down approach starting from the global model including all the independent variables.

3. Results

Sex and year did not explain reproductive decisions (skippers versus breeders; GLM, all p-values > 0.10). Mercury predicted the likelihood of breeding, as skippers had significantly elevated mercury levels compared with breeders ($\chi^2 = 14.06$, $p = 0.001$, figure 1*a*,*b*). This was true in both sexes (females: $\chi^2 = 4.41$, $p = 0.036$; males: $\chi^2 = 4.61$, $p = 0.032$). There was no interaction between mercury and either sex, year or scaled mass index (GLM, all p-values > 0.87). Among birds that bred, pre-breeding mercury concentration did not predict first egg-laying date, clutch size or breeding success (all p-values > 0.18).

In birds that bred, baseline LH levels were higher in females than in males ($F_{1,34} = 5.9$, $p = 0.02$), but were unrelated to mercury levels ($F_{1,33} = 0.08$, $p = 0.783$; mercury × sex: $F_{1,32} = 1.5$, $p = 0.23$, figure 1*c*,*d*). GnRH-induced LH levels were not linked to either sex ($F_{1,17} = 3.23$, $p = 0.09$) or mercury levels ($F_{1,17} = 0.88$, $p = 0.361$; mercury × sex: $F_{1,17} = 2.72$, $p = 0.118$) in breeders.

In skipping birds, baseline LH levels were not affected by sex ($F_{1,12} = 0.43$, $p = 0.523$) but significantly and negatively correlated to mercury levels in males, and positively in females (mercury × sex: $F_{1,12} = 19$, $p < 0.001$; figure 1*e*,*f*). GnRH-induced LH levels significantly increased with increasing mercury concentration in skipping males and females (mercury: $F_{1,10} = 21.6$, $p < 0.001$; mercury × sex: $F_{1,8} = 0.07$, $p = 0.805$, figure 1*e*,*f*). Baseline testosterone levels tended to decrease in skipping males ($F_{1,7} = 5.92$, $p = 0.051$) but not in breeding males ($F_{1,19} < 0.01$, $p = 0.97$). GnRH-induced testosterone levels were not related to mercury levels neither in skipping ($F_{1,5} = 0.1$, $p = 0.761$) nor in breeding males ($F_{1,9} = 0.6$, $p = 0.458$).

4. Discussion

Long-lived seabirds often skip reproduction in certain years, and our previous investigations in kittiwakes demonstrate that LH levels are lower in the birds which choose to skip breeding (skippers, [4]). Could environmental toxicants influence LH and/or GnRH levels, and therefore reproductive decisions? We evaluated a possible role for mercury contamination in non-breeding black-legged kittiwakes, and investigated the endocrine mechanisms that underlie this decision.

As expected, total blood mercury measured after arrival significantly predicted the likelihood of breeding. Although our study was correlational and would require experimental manipulation of contaminants [1,8], there is support for causal effects because experimental mercury administration can alter pairing in white ibises (*Eudocimus albus*) and suppress spawning in fathead minnows (*Pimephales promelas*) [1,8]. In our study, the most contaminated males and females were less likely to breed although there was a large overlap in mercury levels between skipping and breeding birds. Some of the skipping birds may have been young or low-quality individuals [5], and therefore be intrinsically more sensitive to mercury. In birds that did breed, mercury had no effect on egg-laying dates, clutch size and breeding success. In breeders, total blood mercury averaged 1.8 $\mu g\, g^{-1}$ (range: 0.91–3.08 $\mu g\, g^{-1}$), which could be below a threshold level beyond which breeding success is significantly impaired as shown in other bird species [9]. However, mercury–breeding success relationships appear complex in birds since administration of a low mercury dose could even enhance breeding in mallards *Anas platyrhynchos* [10].

Mercury concentration predicted LH levels in skippers, but patterns were different among males and females, and varied between baseline and GnRH-induced LH. Baseline LH and testosterone decreased with increasing blood

Downloaded from rsbl.royalsocietypublishing.org on May 31, 2013

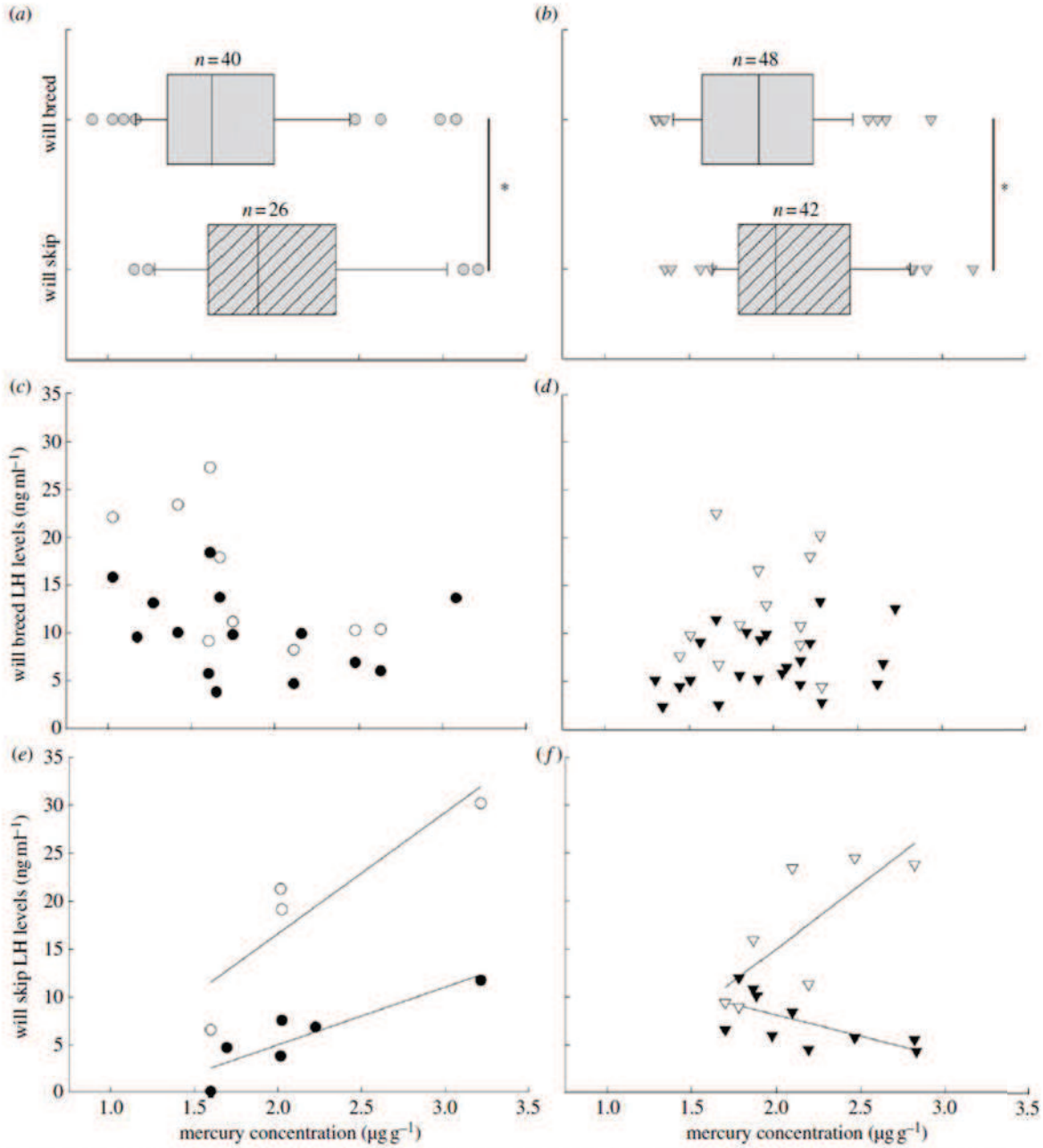


Figure 1. Pre-breeding mercury (μg g $^{-1}$ dwt) and LH levels (ng ml $^{-1}$) in breeding and skipping black-legged kittiwakes. Breeding birds (empty boxes) had lower mercury levels in blood than birds that would skip breeding (striped boxes), both in (*a*) females and (*b*) males. Boxes represent median, 25th and 75th percentiles and outliers. (*c–d*) In breeders, baseline and GnRH-induced LH levels were not affected by mercury. (*e*) In skipping females (circles), baseline LH increased with increasing mercury, whereas in (*f*) skipping males (triangles), baseline LH decreased with increasing mercury. GnRH-induced LH levels, in both skipping males and females, increased with increasing mercury levels (*e–f*). Filled circles and triangles denote baseline, and open circles and triangles denote GnRH-induced.

mercury concentration in males, as found in mercury-fed laboratory rats [11]. Conversely, in skipping females, LH and mercury were positively related (although levels are still significantly lower than in breeding females [4]). These data suggest that mercury–LH relationships are complex and LH disruption could also be the result of differential negative feedback from mercury given its oestrogen-like effect [2].

To test the functionality of the pituitary, we performed GnRH challenges in breeders and skippers. Based on previous work [4], we assume that the dose of GnRH we used was sufficient to elicit a significant increase of LH 10 min after injection, and interpretation of our results is based on this assumption. If mercury is suppressing LH secretion directly at the pituitary, we would expect LH response to GnRH to be suppressed in skippers. Contrary to our predictions, skippers were clearly able to release LH, and increased LH three- to fivefold above baseline (breeders did showed a slight LH increase). Although based on correlations, it suggests that mercury may act at the hypothalamic level, disrupting GnRH synthesis or secretion. Evidence from other vertebrates demonstrates that mercury can accumulate in the hypothalamus and alter GnRH content and signalling [3,12]. In response to GnRH decline, the pituitary may have upregulated GnRH receptors, explaining the large increase

Downloaded from rsbl.royalsocietypublishing.org on May 31, 2013

in LH with GnRH injection. Thus in kittiwakes, mercury contamination could lead to a decline in GnRH release, the subsequent increase of pituitary GnRH receptors explaining why the disrupted pituitary over-releases LH when experimentally challenged by GnRH. Although we originally hypothesized that mercury was targeting the pituitary, the induced LH response also points to a problem at the hypothalamus. It is additionally possible that mercury could bind to LH [13] making LH less able to activate gonadal receptors. Further experiments evaluating LH and GnRH expression in the brain would be necessary to meter out these possibilities.

Long-lived birds often skip breeding when foraging conditions are poor, modulating their reproductive effort according to environmental conditions [14]. The present situation in the Arctic is of concern: mercury levels in seabirds are increasing [15] and if combined with rapid climate change, we are facing a worst-case scenario [16]. Ultimately, the likelihood to skip breeding may become too high to maintain current population levels.

This study was approved by the French Veterinary Services (permit number 79-2).

This project was supported by Institut Polaire Français (IPEV project 330 to O.C.) and by Agence National de la Recherche (ANR project PolarTop to O.C.). The authors thank C. Breuner and three anonymous referees for useful comments, K. Sagerup, A. Fenstad, C. Trouvé and C. Churlaud for their great assistance in field and laboratory work.

References

1. Frederick P, Jayasena N. 2011 Altered pairing behaviour and reproductive success in white ibises exposed to environmentally relevant concentrations of methylmercury. *Proc. R. Soc. B* **278**, 1851–1857. (doi:10.1098/rspb.2010.2189)
2. Tan SW, Meiller JC, Mahaffey KR. 2009 The endocrine effects of mercury in humans and wildlife. *Crit. Rev. Toxicol.* **39**, 228–269. (doi:10.1080/10408440802233259)
3. Crump KL, Trudeau VL. 2009 Mercury-induced reproductive impairment in fish. *Environ. Toxicol. Chem.* **28**, 895–907. (doi:10.1897/08-151.1)
4. Goutte A, Angelier F, Clément-Chastel C, Trouvé C, Moe B, Bech C, Gabrielsen GW, Chastel O. 2010 Stress and the timing of breeding: glucocorticoid–luteinizing hormones relationships in an Arctic seabird. *Gen. Comp. Endocrinol.* **169**, 108–116. (doi:10.1016/j.ygcen.2010.07.016)
5. Goutte A, Kriloff M, Weimerskirch H, Chastel O. 2011 Why do some adult birds skip breeding? A hormonal investigation in a long-lived bird. *Biol. Lett.* **7**, 790–792. (doi:10.1098/rsbl.2011.0196)
6. Jæger I, Hop H, Gabrielsen GW. 2009 Biomagnification of mercury in selected species from an Arctic marine food web in Svalbard. *Sci. Total Environ.* **407**, 4744–4751. (doi:10.1016/j.scitotenv.2009.04.004)
7. Bustamante P, Lahaye V, Durnez C, Churlaud C, Caurant F. 2006 Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: influence of geographical origin and feeding ecology. *Sci. Total Environ.* **368**, 585–596. (doi:10.1016/j.scitotenv.2006.01.038)
8. Hammerschmidt CR, Sandheinrich MB, Wiener JG, Rada RG. 2002 Effects of dietary methylmercury on reproduction of fathead minnows. *Environ. Sci. Technol.* **36**, 877–883. (doi:10.1021/es011120p)
9. Evers D *et al.* 2008 Adverse effects from environmental mercury loads on breeding common loons. *Ecotoxicology* **17**, 69–81. (doi:10.1007/s10646-007-0168-7)
10. Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR. 2010 Enhanced reproduction in mallards fed a low level of methylmercury: an apparent case of hormesis. *Environ. Toxicol. Chem.* **29**, 650–653. (doi:10.1002/etc.64)
11. Ramalingam V, Vimaladevi V, Rajeswary S, Suryavathi V. 2003 Effect of mercuric chloride on circulating hormones in adult albino rats. *J. Environ. Biol.* **24**, 401–404.
12. Oliveira FRT, Ferreira JR, dos Santos CMC, Macêdo LEM, de Oliveira RB, Rodrigues JA, do Nascimento JLM, Faro LRF, Diniz DLWP. 2006 Estradiol reduces cumulative mercury and associated disturbances in the hypothalamus–pituitary axis of ovariectomized rats. *Ecotoxicol. Environ. Saf.* **63**, 488–493. (doi:10.1016/j.ecoenv.2004.12.024)
13. Laks DR. 2010 Luteinizing hormone provides a causal mechanism for mercury 194 associated disease. *Med. Hypotheses* **74**, 698–701. (doi:10.1016/j.mehy.2009.10.036)
14. Cubaynes S, Doherty PF, Schreiber EA, Gimenez O. 2011 To breed or not to breed: a seabird's response to extreme climatic events. *Biol. Lett.* **7**, 303–306. (doi:10.1098/rsbl.2010.0778)
15. Mallory ML, Braune BM. 2012 Tracking contaminants in seabirds of Arctic Canada: temporal and spatial insights. *Mar. Pollut. Bull.* **64**, 1475–1484. (doi:10.1016/j.marpolbul.2012.05.012)
16. Jenssen BM. 2006 Endocrine-disrupting chemicals and climate change: a worst-case combination for Arctic marine mammals and seabirds? *Environ. Health Perspect.* **114**, 76–80. (doi:10.1289/ehp.8057)

PAPER II

Age-related mercury contamination and relationship with luteinizing hormone in a long-lived Antarctic bird.

OPEN ACCESS Freely available online

PLOS | ONE

Age-Related Mercury Contamination and Relationship with Luteinizing Hormone in a Long-Lived Antarctic Bird

Sabrina Tartu[1]*, Paco Bustamante[2], Aurélie Goutte[1], Yves Cherel[1], Henri Weimerskirch[1], Jan Ove Bustnes[3], Olivier Chastel[1]

1 Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 CNRS-Université de la Rochelle, Villiers-en-Bois, France, **2** Littoral Environnement Société (LIENSs), UMR 7266 CNRS-Université La Rochelle, La Rochelle, France, **3** Norwegian Institute for Nature Research, FRAM – High North Research Centre on Climate and the Environment, Tromsø, Norway

Abstract

Seabirds, as long-lived top predators, accumulate contaminants such as mercury (Hg), an established endocrine disruptor. In long lived species hormonal secretion varies with age; therefore, Hg-induced endocrine disruption may be exacerbated in some age classes. Here we investigated relationships between blood total Hg and luteinizing hormone (LH, a key pituitary hormone for the onset of breeding), in pre-laying known-age (11–45 years old) snow petrels (*Pagodroma nivea*) from Adélie Land, Antarctica. We predicted that 1) blood Hg would increase with advancing age as a consequence of bio-accumulation; and that 2) increasing blood Hg would be related to decreased concentrations of LH in the most Hg-contaminated individuals. Hg concentrations were higher in females than in males ($p<0.001$), and contrary to our prediction, decreased with advancing age in males ($p=0.009$) and tended to do so in females ($p=0.06$). The analysis of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) suggested that this unexpected pattern could originate from age and sex-related variations in trophic niche, and hence Hg exposure. Regarding LH, our prediction was only supported in young birds (≤23 years) where baseline LH was inversely correlated with Hg concentrations ($p=0.04$). Hg burden did not predict baseline LH or GnRH-induced LH in birds that were more than 23 years old. These results show that age and contaminants may interfere with major endocrine mechanisms and, together with other recent studies, support the view that Hg could be connected to LH secretion and could then impair the fitness of long-lived birds.

Citation: Tartu S, Bustamante P, Goutte A, Cherel Y, Weimerskirch H, et al. (2014) Age-Related Mercury Contamination and Relationship with Luteinizing Hormone in a Long-Lived Antarctic Bird. PLoS ONE 9(7): e103642. doi:10.1371/journal.pone.0103642

Editor: Andrew Iwaniuk, University of Lethbridge, Canada

Received December 10, 2013; **Accepted** July 5, 2014; **Published** July 29, 2014

Copyright: © 2014 Tartu et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Financial support was provided by the Agence Nationale de la Recherche (program POLARTOP, O. Chastel), the Institut Polaire Français Paul Emile Victor (IPEV, program no. 109, H. Weimerskirch), and the Terres Australes et Antarctiques Françaises (TAAF). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: tartu.sabrina@gmail.com

Introduction

Mercury (Hg) is a ubiquitous contaminant, present in aquatic biota with elevated concentrations in fish, some mammals and seabirds [1], [2]. Diet is the principal route of contamination: Hg bio-accumulates into individuals [3], [4], [5], [6] and bio-magnifies along the food web [7], [8]. Seabirds are top predators which often bear elevated levels of Hg, and are thus considered good bio-monitors to describe Hg bio-accumulation and bio-magnification in the marine environment [9]. Seabirds are also long-lived organisms, and therefore would accumulate Hg in their tissues over a long life-span [10]. Although it has been well established that seabird nestlings show lower Hg concentrations than adults [11], very few studies have examined the influence of age on Hg contamination in adults and the patterns are not clear [12], [13], [14], [15], [16]. In this study, we report the results of a cross-sectional study on known-age adult snow petrels *Pagodroma nivea*, a small (≈400 g) but very long-lived Antarctic seabird (≈50 years) [17]. We made use of an exceptional long-term banding study (1964- to present, [17]) to identify the age of each bird and measured blood total Hg concentrations. Blood Hg concentrations reflect dietary exposure over the last several weeks and also a complex equilibrium between ingestion, deposition and mobilization [10], [18], [19]. In seabirds, blood Hg is strongly correlated to Hg concentrations in storage organs [20], [21], [22]; blood Hg is thus considered as a good proxy of overall Hg burden. Given the ability of Hg to bio-accumulate we would expect blood Hg concentrations to be positively related to age in adult snow petrels. As mentioned previously, diet is the principal route of Hg contamination and to further interpret the possible Hg/age relationship, we used the isotopic niche as a proxy of the trophic niche, with $\delta^{13}C$ and $\delta^{15}N$ values indicating the birds' foraging habitats and trophic position, respectively [23]. Indeed this possible Hg/age relationship could originate from an age-related change in trophic niche. Snow petrels forage in close association with ice-pack and during summer they mainly feed their chicks with fish (up to 95% of the preys [24]). To our knowledge no studies have described the trophic niche of snow petrels during the inter-breeding period, and there is no evidence of age and/or sex related trophic niche segregation.

Taking age into consideration is crucial when considering the consequences of Hg contamination. Indeed, several studies on vertebrates have reported breeding impairment from Hg including suppression of reproduction, reduced clutch size, poor breeding performances or unusual courtship behavior [25], [26], [27], [28],

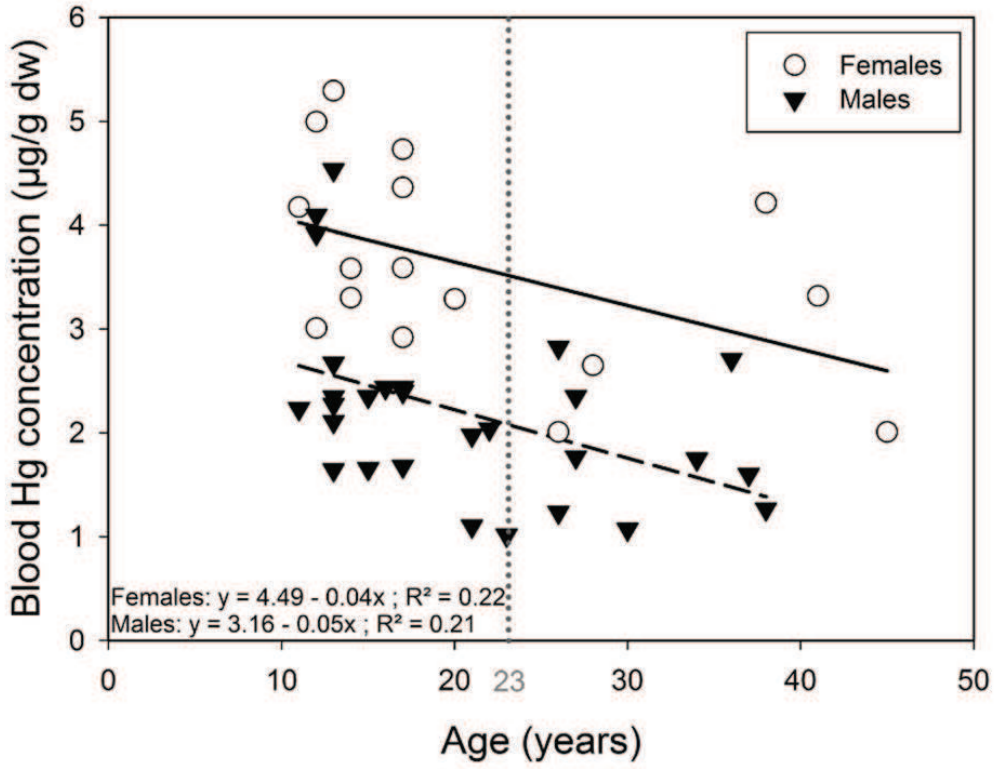


Figure 1. Blood Hg concentration in relation to age in pre-laying snow petrels. Hg concentration significantly decreased with increasing age in males (black triangles). For females (white circles) this relationship was close to statistical significance (P = 0.06). Solid line refers to linear regression for females and dashed line to linear regression for males. The vertical dashed line shows the change point inferred by the 'broken sticks' determination process for blood Hg concentration in relation with age (23 years old).
doi:10.1371/journal.pone.0103642.g001

[29]. Indeed, in its methylated form, Hg is highly toxic and is known to disrupt the reproductive endocrine system [25]. For instance, in an Arctic seabird, the black-legged kittiwake *Rissa tridactyla*, a recent study has reported that Hg could interfere with the secretion of luteinizing hormone (LH) [29] a major hormone for the onset of breeding [30]. In vertebrates, the onset of breeding involves the activation of the hypothalamic-pituitary-gonadal axis (hereafter HPG axis): increasing day length activates the expression of a neuro-hormone, the Gonadotropin Releasing Hormone (GnRH) which triggers the secretion of LH from the pituitary gland. LH, in concert with follicle-stimulating hormone (FSH), promotes gonadal maturation, sex steroid secretion and in turn, sexual behaviors [30]. In black-legged kittiwakes plasma baseline LH concentrations of non-breeding males were negatively correlated to blood Hg concentrations [29]. By using the injection of exogenous GnRH, a common and powerful method to test the functionality of the pituitary through its ability to release LH [31], [32], [33], the authors suggested that Hg contamination may disrupt GnRH secretion from the hypothalamus in black-legged kittiwakes [29]. The effect of Hg on LH secretion is supported by experimental investigations: Hg-fed male rats had decreased concentrations of LH and testosterone [34]. LH secretion could thus be a potential target for Hg. In addition LH secretion is also closely related to age across vertebrates [35] including long-lived seabirds like the snow petrel [33]. Regarding our study model, the snow petrel, we therefore predict a negative relationship between blood Hg and baseline LH in the most contaminated age class. To investigate the underlying mechanism of such possible relationship, we used a GnRH challenge to test the ability of the pituitary to release LH in relation to Hg. If GnRH-induced LH concentrations are negatively correlated to blood Hg, this would indicate that Hg affects the ability of the pituitary to properly release LH.

Materials and Methods

Ethics statement

Animals were cared for in accordance with the guidelines of the ethics committee of the Institut Polaire Français Paul Emile Victor (IPEV) that specifically approved this study (Program no. 109, H. Weimerskirch).

Capture, blood sampling and GnRH-challenge

The study was conducted on Adélie land (66°40′ S, 140°01′ E), Antarctica. Many snow petrels were of known age, because chicks have been banded each year since 1964 [17]. Twenty-nine males and 16 females, from 11 to 45 years old, were handled during the pre-laying period (i.e. the courtship and mating period, a few days after arrival to the colony), from 11 to 23 November 2008. Only one bird was captured at each nest and the sex of the birds was previously determined by molecular sexing [36]. Snow petrels were caught by hand and a first blood sample was collected from the alar vein with a syringe immediately after capture (mean ± S.D.: 3 min ± 5 s) to determine baseline plasma LH concentra-

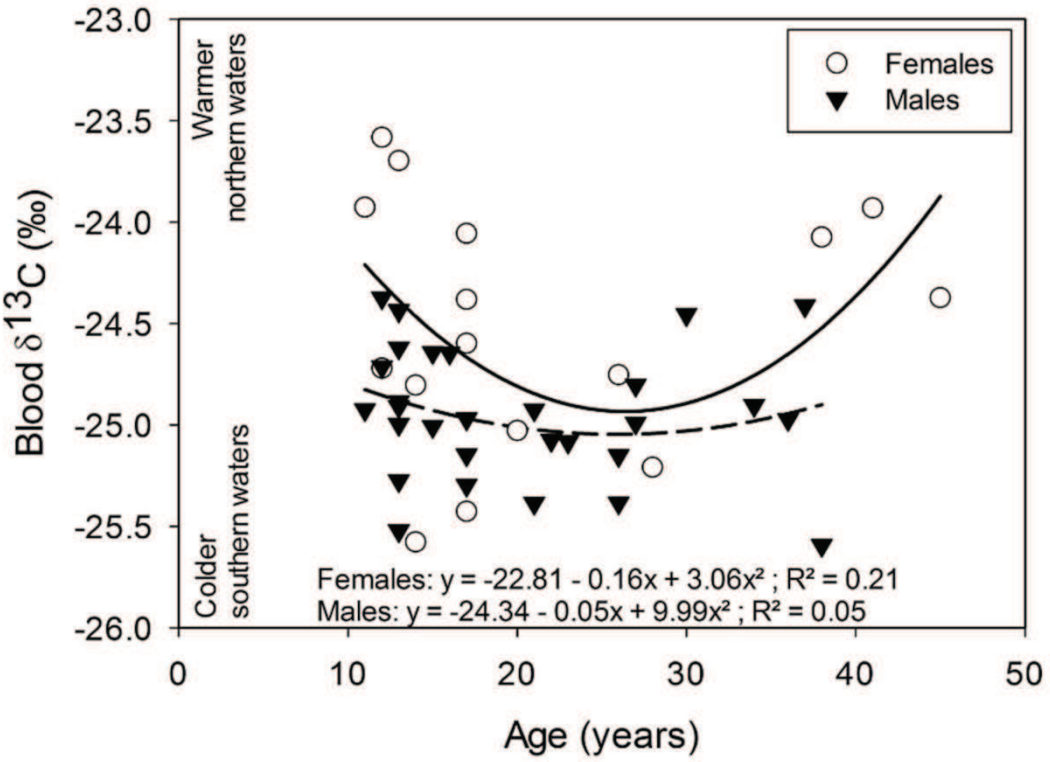


Figure 2. Blood $\delta^{13}C$ values in relation to age in pre-laying snow petrels. $\delta^{13}C$ values quadratically varied with age and this was more pronounced in females (white circles) than in males (black triangles). Solid line refers to quadratic regression for females and dashed line to quadratic regression for males.
doi:10.1371/journal.pone.0103642.g002

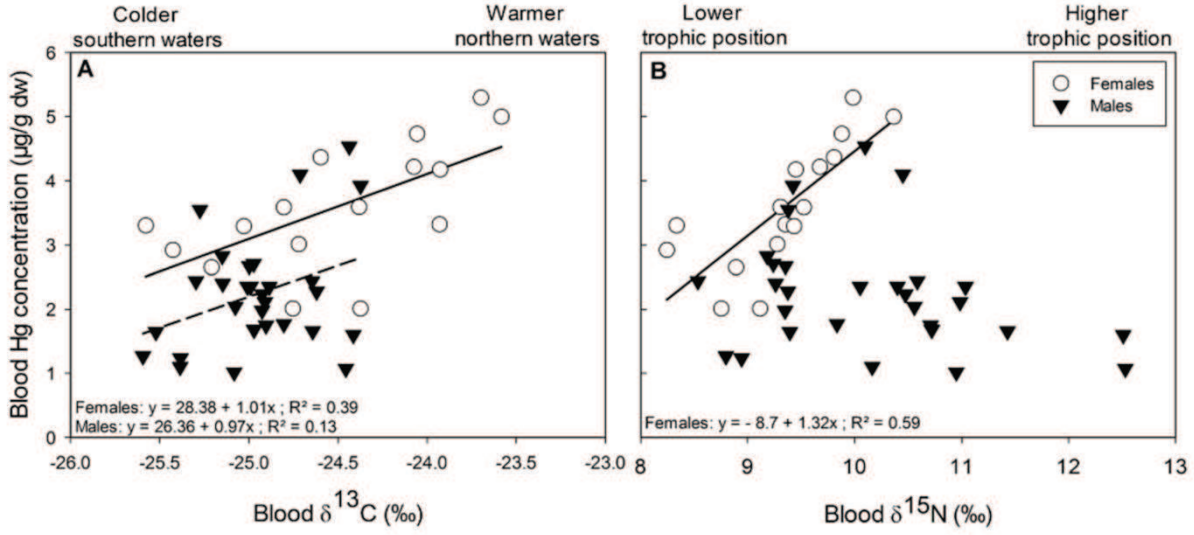


Figure 3. Blood Hg concentration in relation with blood $\delta^{13}C$ and $\delta^{15}N$ values in pre-laying snow petrels. (A) Hg concentration increased with increasing $\delta^{13}C$ values for females and this relationship was close to significance for males ($P = 0.06$), while (B) Hg concentration increased with increasing $\delta^{15}N$ values in females only (white circle). Solid line refers to linear regression for females and dashed line to linear regression for males.
doi:10.1371/journal.pone.0103642.g003

Table 1. Blood Hg concentrations, $\delta^{13}C$ and $\delta^{15}N$ values in pre-laying snow petrels in relation to sex.

Blood values	Males	Females	Sex differences			
			SS*	Df	F	p-value
Hg ($\mu g \cdot g^{-1}$ ww)	2.2±0.9 (1.0; 4.5)	3.6±1.0 (2.0; 5.3)	18.7	1,43	21.9	>0.001
$\delta^{13}C$ (‰)	−25±0.3 (−25.6; −24.4)	−24.5±0.6 (−25.6; −23.6)	1.9	1,43	10.1	>0.001
$\delta^{15}N$ (‰)	9.3±0.6 (8.5; 12.5)	10.1±1.0 (8.2; 10.4)	6.5	1,43	8.3	0.006

*SS = Sum of squares.
Values are means ± SD with ranges in parentheses.
doi:10.1371/journal.pone.0103642.t001

tions. Then, 40 birds were immediately injected with 0.1 ml of a GnRH solution in the second alar vein ([Gln8], Sigma Lot 121H04314). The GnRH was dissolved in a physiological solution to yield a dosage of 0.6 mg for 0.1 ml (about 1.5 mg kg^{-1} body mass in 1 ml of 0.9% saline solution, as validated in this species [33]). In snow petrels, a GnRH injection elicits a maximal increase of LH after 10 minutes, and this response documents the ability of the pituitary to release LH. In this species, measuring LH levels 30 minutes post-GnRH challenge can inform on an individual's capacity to maintain elevated LH concentrations [33]. Blood samples were therefore collected from alar veins at 10 and 30 min after the GnRH injection to measure plasma LH concentrations. LH changes from 0 to 10 min post-GnRH injection $[(LH_{t-10} - LH_{t-0})/LH_{t-0}]$ and LH changes from 10 to 30 min post-GnRH injection $[(LH_{t-30} - LH_{t-10})/LH_{t-10}]$ were used as indicators of different individuals' abilities to release LH. Blood samples were centrifuged and plasma (for LH assay) and red blood cells (for total Hg and stable isotopes assays) were stored at −20°C. Adults were weighted to the nearest 2 g using a spring balance and their skull length (head+bill) was measured to the nearest 0.5 mm using a caliper. In snow petrels, skull length appears to be a reliable measurement of the overall size of a bird [36] and adult scaled mass index was then calculated [37].

Hg analysis

Total Hg was measured as described in [38] from freeze-dried and powdered red blood cells (hereafter called blood) in an Advanced Hg Analyzer spectrophotometer (Altec AMA 254). Prior and after freeze-drying, blood samples were weighed to determine the percentage of water in blood to facilitate comparisons with other studies. At least two aliquots ranging from 5 to 10 mg were analyzed 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 $\mu g \cdot g^{-1}$). Hg concentrations are expressed in $\mu g \cdot g^{-1}$ dry weight (dw).

Stable isotopes analysis

The isotopic niche of snow petrels was used as a proxy of their trophic niche during the pre-breeding period, as blood cells is a metabolically active tissue that integrates periods of weeks before sampling [39], [40] and [41]. The isotopic method was validated in the southern Indian Ocean, with $\delta^{13}C$ values of seabirds indicating their foraging habitats [42], [43] and their $\delta^{15}N$ values increasing with trophic level [44]. Before isotopic analysis, blood samples were freeze-dried and powdered. Sub-samples of homogenized blood powder were then weighed (~0.4 mg) with a microbalance and packed in tin containers. Relative abundances

Table 2. Modelling the effects of age (years), age^2, sex and interactions as a function of blood $\delta^{13}C$ and $\delta^{15}N$ values (‰) in pre-laying snow petrels.

Dependent variable	Independent variable	SS*	Df	F	p-value
a) $\delta^{13}C$	**Age^2**	1.0	1,32	5.0	**0.033**
	Age	0.9	1,32	4.5	**0.041**
	Sex	1.5	1,32	7.5	**>0.001**
	Age^2×Age	0.8	1,32	4.1	0.052
	Age^2×Sex	0.7	1,32	3.7	0.063
	Age×Sex	0.7	1,32	3.4	0.074
	Age^2×Age×Sex	>0.1	1,32	>0.1	0.862
b) $\delta^{15}N$	Age^2	>0.1	1,32	>0.1	0.809
	Age	>0.1	1,32	>0.1	0.876
	Sex	5.3	1,32	6.1	**0.019**
	Age^2×Age	0.2	1,32	0.2	0.644
	Age^2×Sex	0.6	1,32	0.7	0.421
	Age×Sex	0.7	1,32	0.8	0.369
	Age^2×Age×Sex	>0.1	1,32	>0.1	0.927

*SS = Sum of squares.
doi:10.1371/journal.pone.0103642.t002

Table 3. Modelling the relationships between blood $\delta^{13}C$, $\delta^{15}N$ values (‰) and blood Hg concentration (μg·g ^{1} dw) in male and female pre-laying snow petrels.

Dependent variable	Sex	Independent variable	SS	Df	F	p-value
Hg	Males	$\delta^{13}C$	2.8	1,27	3.9	0.060
		$\delta^{15}N$	1.7	1,27	2.2	0.150
	Females	**$\delta^{13}C$**	5.7	1,14	9.1	**0.009**
		$\delta^{15}N$	8.6	1,14	20.6	**>0.001**

*SS = Sum of squares.

doi:10.1371/journal.pone.0103642.t003

of C and N isotopes were determined with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyzer (Thermo Scientific Flash EA 1112). Isotopic results are presented in the δ notation relative to Vienna PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicate measurement errors <0.10 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. The C:N mass ratios of the samples were calculated as the ratio between the mass percentages in carbon and nitrogen. The consistently low C:N values of blood cells verified that low lipid content did not necessitate lipid extraction [45].

Hormone assays

Plasma LH concentrations were assayed by radioimmunoassay as validated for snow petrels [33]. The lowest detectable concentration of LH was 0.05 ng·ml ^{1} and the intra-assay coefficient of variation was 6.2% (three duplicates). In 4 birds plasma levels were too low to measure LH.

Statistics

All analyses were performed using R v. 2.13.1. We used GLM with normal error and identity link function to explain: (i) blood Hg concentration in relation to age, blood $\delta^{13}C$ and $\delta^{15}N$, scaled mass index, and interactions with sex, (ii) blood $\delta^{13}C$ and $\delta^{15}N$ values in relation to age, age^2, scaled mass index and interactions with sex (linear and/or quadratic). To address the interaction between LH, Hg and age, the dataset was split into two groups using a recently developed Bayesian approach ('broken stick' modelling technique [46], [47], based on beta regression analysis with 'betareg' package in R, [48]. This method will infer the 'age limit' where blood Hg concentration starts translates statistically as a change-point in the distribution of blood Hg concentration versus age. To estimate the age limit of the change-point T_i, in blood Hg concentrations we used a profile likelihood approach [47]: the likelihood was computed for each age. The value of T_i that maximized the likelihood was thus evaluated, and an approximate confidence interval for T_i was computed with a Likelihood Ratio Test with 1 df. This method bypasses the need to manually inspect the data for identification of a change-point, which could only be uncertain in our case owing to the high variance observed in individual blood Hg concentrations. Thanks to this method we were able to distinguish a 'break' in Hg distribution at 23 years old in our data set. Thus we considered two groups: birds that were until 23 years old (N = 31) and birds that were more than 23 (N = 14) hereafter '≤23 y.o.' and '> 23 y.o.', respectively), blood Hg concentrations were significantly higher in '≤23 y.o' birds than in the '>23 y.o.' ones (GLM, $F_{1,43} = 4.8$, $p = 0.034$). Finally (iii) absolute plasma baseline LH concentrations, LH changes from 0 to 10 minutes, and changes from 10 to 30 minutes were tested as functions of blood Hg concentration (linear) in relation to age classes ('≤23 y.o.' and '> 23 y.o.'). We performed all our model selection starting from the most parameterized model that included all the variables/factors and their interactions and we removed step by step the non-significant interactions, variables or factors. Selected models were checked for assumptions and values were log-transformed when necessary, we used GLM with normal error and identity link function to explain LH concentrations in relation with Hg and interaction with sex. Values are means + SD.

Results

Hg concentrations in relation to age, sex and trophic niche

In snow petrels dw blood was equivalent to 35.5+0.3% of ww blood and Hg concentration averaged 2.7+1.1 (range: 1.0; 5.3) μg·g ^{1} dw During the pre-laying period, blood Hg concentrations were not related to sampling date (GLM, $F_{1,43} = 0.5$, $p = 0.488$) and were higher in females than in males (**Table 1**). Blood Hg concentration decreased with increasing age in males (GLM, $F_{1,27} = 7.9$, $p = 0.009$, **Fig. 1**) and this negative relationship was only close to statistical significance in females (GLM, $F_{1,14} = 4.0$, $p = 0.06$, **Fig. 1**). Females had significantly higher $\delta^{13}C$ and lower $\delta^{15}N$ values than males (**Table 1**). A significant U-shaped relationship between blood $\delta^{13}C$ values and age was found, i.e. young and old birds had higher $\delta^{13}C$ values than middle-aged ones (**Fig. 2, Table 2**). Blood $\delta^{15}N$ values were not related to age (**Table 2**). Blood Hg concentrations increased with increasing blood $\delta^{13}C$ in females while in males the similar pattern was found but only close to statistical significance (**Fig. 3A, Table 3**). Regarding the relationships between blood Hg and $\delta^{15}N$ values, a positive relationship was found in females only (**Fig. 3B, Table 3**). Scaled mass index was not related to blood Hg concentration, $\delta^{13}C$ or $\delta^{15}N$ values and interactions with sex ($p > 0.099$ for all tests).

Hg, age and plasma LH

Following GnRH injections, absolute LH concentrations (baseline: 8.4+0.5 ng·ml ^{1}) significantly increased (10 min: 11.5+0.8 ng·ml ^{1}), then decreased (30 min: 9.1+0.8 ng·ml ^{1}; generalized linear mixed model (GLMM), time as factor: $F_{2,61} = 12.57$, $p < 0.001$) these LH variations were not different between sexes or age classes (≤23 y.o. versus >23 y.o.) ($p > 0.08$ for all tests). Absolute plasma LH concentrations (baseline, after 10 and after 30 minutes) were significantly higher in males compared to females ($p < 0.009$ for all tests) and were not related to sampling date ($p > 0.123$ for all tests) or to scaled mass index ($p > 0.07$ for all tests). In birds that were ≤23 y.o., plasma baseline LH significantly decreased with increasing Hg concentration in blood (**Fig. 4A**, **Table 4**) and LH changes from 0 to 10 minutes and

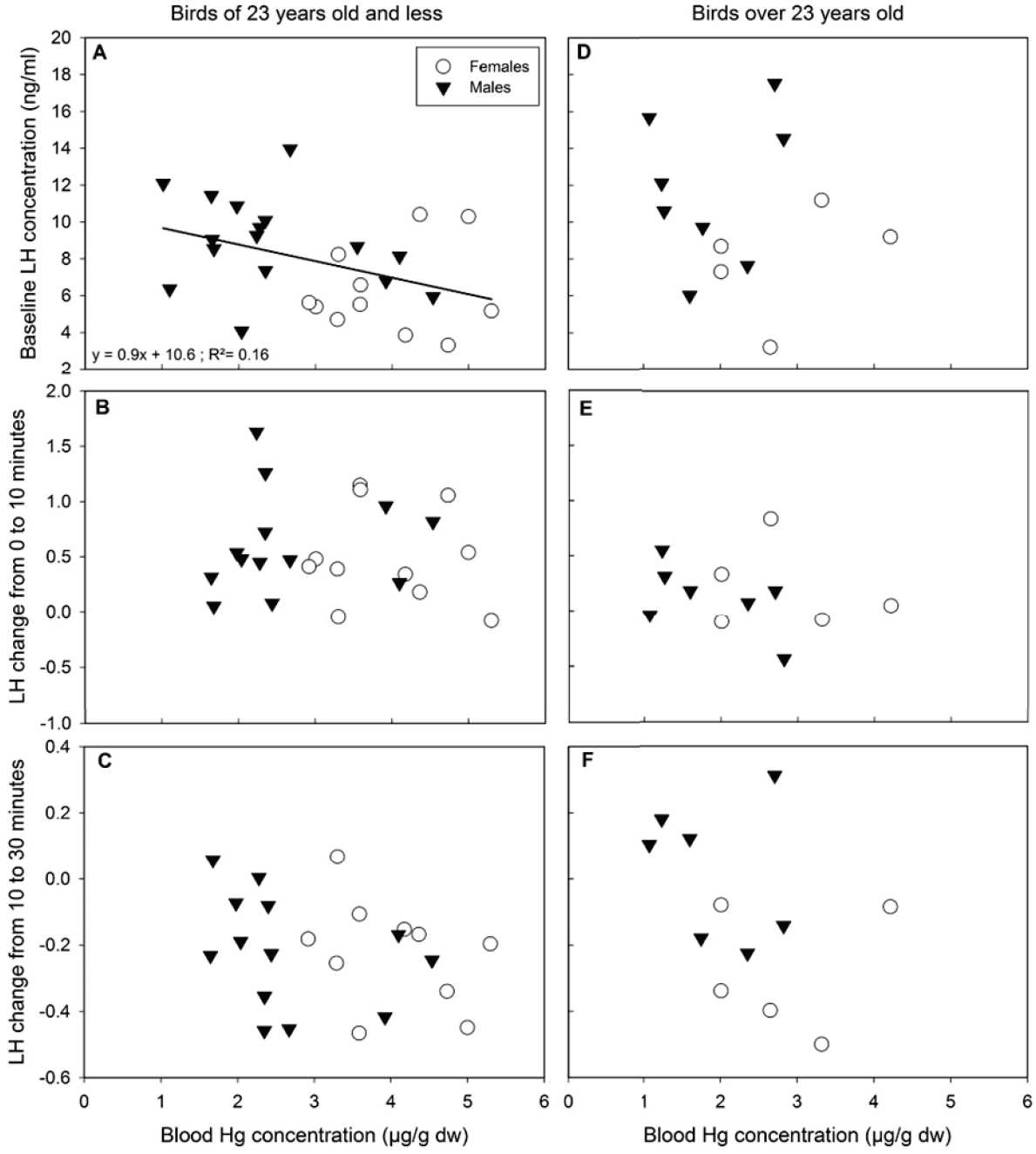


Figure 4. Plasma LH concentrations in relation to blood Hg concentration. In pre-laying snow petrels, baseline LH (A) decreased with increasing Hg concentration in individuals ≤23 years old (left panel) but not in individuals >23 years old (right panel, D). LH changes from 0 to 10 and from 10 to 30 minutes following GnRH challenge were not related to blood Hg concentrations in any age class (B-C-E-F). Solid line refers to linear regression for both sexes.
doi:10.1371/journal.pone.0103642.g004

from 10 to 30 minutes following the GnRH challenge were unrelated to blood Hg concentration (**Fig. 4B–C**, **Table 4**). In birds that were >23 y.o., plasma baseline LH concentration, LH changes from 0 to 10 minutes and from 10 to 30 minutes, were unrelated to blood Hg concentration (**Fig. 4D–E–F**, **Table 4**).

Table 4. Modelling the relationships between blood Hg concentration a) baseline plasma LH concentrations, b) LH change from 0 to 10 minutes and c) LH change from 10 to 30 minutes in pre-laying snow petrels ≤23 years old and >23 years old.

Independent variable: Hg	≤23 years old				>23 years old			
Dependent variable	**SS**	**Df**	**F**	**p-value**	**SS**	**Df**	**F**	**p-value**
a) Baseline LH	31	1,25	5	**0.041**	0.8	1,11	0	0.830
b) LH change from 0 to 10 minutes	0	1,21	0	0.713	0.1	1,10	1	0.429
c) LH change from 10 to 30 minutes	0	1,19	1	0.269	0.1	1,9	2	0.205

SS = Sum of squares.

doi:10.1371/journal.pone.0103642.t004

Discussion

Hg exposure, trophic niche and age

In pre-laying snow petrels, blood Hg concentrations were in the range of concentrations found in other species from the Southern ocean [16], [49], [50] and equivalent to those reported at the same location (Adélie Land) in south polar skuas *Catharacta maccormicki* ($2.2+0.2$ $\mu g \cdot g^{-1}$ dw [51]). Blood Hg concentrations were higher in females than in males. In birds, Hg sex differences are often observed and are more likely to be related to trophic guild than other extrinsic or intrinsic factors [52]. In the present study we have an evidence of trophic guild difference between sexes; indeed stable isotopes analyses showed that foraging habitat ($\delta^{13}C$ values) was positively related to blood $\delta^{13}C$ values especially in females. According to the latitudinal $\delta^{13}C$ gradient in oceanic waters of the Southern Ocean [42], [43], blood $\delta^{13}C$ values suggest the use of a high Antarctic habitat [53]. More precisely, the $\delta^{13}C$ values close to -23 ‰ depict warmer waters at more northern latitudes and the $\delta^{13}C$ values close to -26 ‰ colder waters at more southern latitudes, but still within the Antarctic zone [53]. Hg distribution, and hence its bioavailability is known to be heterogeneous in the Southern Ocean [54]. The positive relationship between $\delta^{13}C$ values and Hg concentrations means that Hg might be more bioavailable for snow petrels at more northern latitudes ($\delta^{13}C$ values close to -23 ‰).

Trophic position, as depicted by blood $\delta^{15}N$ values, was positively related to blood Hg concentration in females only. The enrichment of Hg with increasing trophic level has been described in food-webs and species communities [8], [9] but more rarely within a given species. For example, Hg enrichment with increasing $\delta^{15}N$ values was found in chicks but not in adults of great skuas *Catharacta skua* [19]. In Adélie land, snow petrels feed their chicks mainly with fish [24], but the adults' diet is still unknown. When compared to other Antarctic seabirds and their prey [53], the large range of blood $\delta^{15}N$ values suggests that self-feeding breeding adults fed from a krill- to a fish-based diet, depending on individuals. The higher blood $\delta^{15}N$ values of males indicate they fed more on fish than females. However and unexpectedly despite their higher trophic level, males are less Hg contaminated than females, suggesting once more that females may feed in areas where Hg is more bioavailable. This is in agreement with the $\delta^{13}C$ values-Hg concentration relationship. Alternatively, detoxification processes could be more efficient in males. Noticeably, their stable isotope values indicate that the most contaminated males fed on fish at more northern latitudes than the others, suggesting again a latitudinal effect on Hg availability [54]. To summarize, females probably fed at more northern latitudes and at a lower trophic position compared to males. However, females were more Hg contaminated and Hg contamination was positively related to foraging habitat and trophic position, while in males no relationships were found. These results could be the consequence of sex-specific foraging preferences [55], [56] or maybe sex-specific physiological constraints. Indeed, females are 20% lighter than males consequently they could be more sensitive to the harsh Antarctic climate during winter and constraint to forage in more northern latitudes where Hg is more bioavailable.

Contrary to our prediction blood Hg concentration decreased with increasing age in pre-laying snow petrels, especially in males. The relationship between Hg and age in seabirds is poorly documented and often contradictory. For example, in organs involved in storage and detoxification as the liver, Hg concentration may decrease, increase, with advancing age or may not be related to age [12], [13] and [15]. In excretory tissues as feathers, Hg reaches a plateau until individuals start breeding, and then

feather Hg concentration decreases [16]. Regarding blood Hg, two studies on Procellariiforms found no relationship between Hg and age [16], [50]. However in south polar skuas, breeding experience (which is tightly related to age) was negatively related to blood Hg [51], as found in our study. This pattern could originate from an age-related change in trophic niche i.e. older individuals would forage in areas where Hg is less bioavailable and/or at a lower trophic level than younger ones. Indeed, trophic niche segregations by age, has been observed in some Procellariform species [55], [56]. In our study, a quadratic relationship was found between foraging habitat ($\delta^{13}C$ values) and age. This suggests that, compared to middle-aged birds, young and old individuals foraged at higher latitudes which may be more Hg contaminated. Isotopic signature of blood represents the isotopic niche during the last month preceding sampling [53]. As birds have been sampled in November (a few days after their arrival on the breeding area) those values may mirror the trophic niche used during late winter and suggest that young and old snow petrels do not use the same foraging areas than middle-aged birds at that time. And why older individuals bear lower Hg concentrations than younger birds could be the consequence of an increase in Hg excretion rate with increasing age or the selective disappearance of the most contaminated individuals over time. One may assume that Hg excretion opportunities increase with age; as older individuals had more opportunities to excrete Hg, through molting, and egg-laying for females, than younger birds. But there is no evidence for supporting such an assumption. Also, our study was cross-sectional, and since in its methylated form Hg is highly toxic, the possibility that most of the highly contaminated birds died selectively over time cannot be excluded. In Adult common loons (*Gavia immer*) blood Hg concentrations of 2–3 $\mu g \cdot g^{-1}$ (ww) are considered at moderate risk to effects of Hg contamination [57]. In snow petrels ww blood Hg concentrations would reach 1.6 $\mu g \cdot g^{-1}$ and 1.9 $\mu g \cdot g^{-1}$, for the most contaminated male and female respectively; these concentrations are close to the threshold level for moderate risk in common loons and snow petrels could maybe be more sensitive to lower Hg concentrations. However, in free-living birds, Hg has so far never been reported as affecting survival probabilities, even in highly contaminated areas [51], [58], [59], [60], [61].

LH secretion in relation to age and Hg

In the present study, plasma baseline LH decreased with increasing blood Hg in individuals until 23 y.o., which were those that bore the higher concentrations of blood Hg. This negative relationship between plasma baseline LH and blood Hg could either originate from a Hg-related dysfunction of the pituitary (i.e. an inability to produce enough LH) and/or of the hypothalamus (i.e. a lack of endogenous GnRH production). Since LH changes (0–10 min and 10–30 min) following an exogenous input of GnRH were not related to Hg, this means that Hg does not disrupt the capability of the pituitary to release LH in birds until 23 y.o.: their pituitary is thus functional. This rather suggests, as found in black-legged kittiwakes, of a problem originating from the hypothalamus, which may not release enough GnRH in response to high Hg exposure [29]. However, contrary to black-legged kittiwakes [29], Hg contamination did not result in an over-release of LH post-GnRH injection in snow petrels. In black-legged kittiwakes, the over-release of LH was interpreted as an increase of GnRH receptors on the pituitary in response to the low level of GnRH secreted by the hypothalamus [29]. In snow petrels, the concentrations of blood Hg are slightly higher than in black-legged kittiwakes (2.7 ± 1.1 $\mu g \cdot g^{-1}$ against 2.0 ± 0.5 $\mu g \cdot g^{-1}$, respectively) and LH secretion post-GnRH injection was less pronounced in snow petrels compared to kittiwakes (averagely 1.4-fold and 2.0-fold, respectively). It is always difficult to compare different species as hazardous effects of Hg could be highly species-specific [51]: e.g. average blood Hg concentrations in brown skuas were much higher than in south polar skuas (8.2 ± 0.2 and 2.2 ± 0.2 $\mu g \cdot g^{-1}$ dw, respectively) however south polar skuas suffered from higher Hg-induced breeding failure than their closely relative [51]. Thus, it is possible that black-legged kittiwakes could be more sensitive to Hg than snow petrels and would react more strongly to slightly lower Hg concentrations. In birds that were more than 23 y.o., blood Hg concentrations were lower than in the ≤23 age class, and no relationship appeared between plasma LH and blood Hg concentrations. However sample size was low for individuals aged above 23, and such interpretation must be taken cautiously. The onset of breeding is importantly controlled by an increase of circulating LH in the plasma [62], [63], if these concentrations are not high enough individuals will be less receptive to mating behaviors [64]. In snow petrels a large proportion of young individuals do not breed each year [65], this non-breeding behavior could thus be related to Hg contamination.

Conclusion

This study shows the importance of considering age in eco-toxicological studies. We reported an unexpected negative relationship between age and blood Hg concentrations in adult snow petrels and the use of stable isotopes suggests that this pattern could originate from an age and sex-related variation in late wintering areas, and hence Hg bioavailability. It is now possible to map migration and winter distribution of relatively small birds such as snow petrels via miniature loggers (geolocation sensors, GLS, e.g. [66]). These devices could thus open new research possibilities to confirm the influence of age on the wintering areas used by this Antarctic species and to document how migratory strategies and winter distribution can affect the uptake of contaminants. We also showed that the age of the birds and their contaminant burden could influence the secretion of a key pituitary hormone for the onset of breeding (LH). This result, together with recent findings on black-legged kittiwakes, supports the view that Hg is tightly connected to LH secretion and then can impair the decision to breed [29] and long-term reproductive success [51] in polar seabirds. Anthropogenic Hg emissions are expected to rise in the coming decades [67], probably resulting in increasing Hg exposure for Antarctic top predators. This, coupled with a wide range of environmental perturbations in Antarctic regions could exacerbate the demographic responses of top predators to Hg [51].

Acknowledgments

The authors thank M. Kriloff and E. Antoine for their help in the field, C. Trouvé, C. Parenteau, S. Dano and C. Churlaud for their assistance in hormone and Hg assays, C. Barbraud for his help in statistical analyses, J.B. Thiébot and M. Authier for providing the R code for the 'broken sticks' method and C. Breuner for improving the English and the manuscript. We also thank two anonymous referees for their constructive comments.

Author Contributions

Conceived and designed the experiments: AG OC. Performed the experiments: AG. Analyzed the data: ST. Contributed reagents/materials/analysis tools: PB HW JOB. Wrote the paper: ST AG OC.

References

1. Scheuhammer AM, Meyer MW, Sandheinrich MB, Murray MW (2007) Effects of Environmental Methylmercury on the Health of Wild Birds, Mammals, and Fish. AMBIO: A Journal of the Human Environment 36: 12–19. doi:10.1579/0044-7447(2007)36[12:EOEMOT]2.0.CO;2.
2. Gabrielsen GW, Sydnes L (2009) Pollution in the Barents Sea. Ecosystem Barents Sea. Tapir Academic Press.
3. Koeman JH, van de Ven WSM, de Goeij JJM, Tjioe PS, van Haaften JL (1975) Mercury and selenium in marine mammals and birds. Science of The Total Environment 3: 279–287. doi:10.1016/0048-9697(75)90052-2.
4. Honda K, Fujise Y, Tatsukawa R, Itano K, Miyazaki N (1986) Age-related accumulation of heavy metals in bone of the striped dolphin, Stenella coeruleoalba. Marine Environmental Research 20: 143–160. doi:10.1016/0141-1136(86)90045-0.
5. Wagemann R (1989) Comparison of Heavy Metals in Two Groups of Ringed Seals (Phoca hispida) from the Canadian Arctic. Can J Fish Aquat Sci 46: 1558–1563. doi:10.1139/f89-198.
6. Dietz R, Riget F, Born E. (2000) Geographical differences of zinc, cadmium, mercury and selenium in polar bears (Ursus maritimus) from Greenland. Science of The Total Environment 245: 25–47. doi:10.1016/S0048-9697(99)00431-3.
7. Bargagli R, Monaci F, SanchezHernandez JC, Cateni D (1998) Biomagnification of mercury in an Antarctic marine coastal food web. Mar Ecol Prog Ser 169: 65–76. doi:10.3354/meps169065.
8. Blévin P, Carravieri A, Jaeger A, Chastel O, Bustamante P, et al. (2013) Wide Range of Mercury Contamination in Chicks of Southern Ocean Seabirds. PLoS ONE 8: e54508. doi:10.1371/journal.pone.0054508.
9. Furness RW (1993) Birds as monitors of pollutants. In: Furness RW, Greenwood JJD, editors. Birds as Monitors of Environmental Change. Springer Netherlands. 86–143.
10. Rowe CL (2008) "The Calamity of So Long Life": Life Histories, Contaminants, and Potential Emerging Threats to Long-lived Vertebrates. BioScience 58: 623. doi:10.1641/B580709.
11. Carravieri A, Cherel Y, Blévin P, Brault-Favrou M, Chastel O, et al. (2014) Mercury exposure in a large subantarctic avian community. Environmental Pollution 190: 51–57. doi:10.1016/j.envpol.2014.03.017.
12. Furness R, Hutton M (1979) Pollutant levels in the great skua Catharacta skua. Environmental Pollution (1970) 19: 261–268. doi:10.1016/0013-9327(79)90118-6.
13. Hutton M (1981) Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. Environmental Pollution Series A, Ecological and Biological 26: 129–145. doi:10.1016/0143-1471(81)90043-X.
14. Furness RW, Lewis SA, Mills JA (1990) Mercury levels in the plumage of red-billed gulls Larus novaehollandiae scopulinus of known sex and age. Environmental Pollution 63: 33–39. doi:10.1016/0269-7491(90)90101-H.
15. Thompson DR, Hamer KC, Furness RW (1991) Mercury Accumulation in Great Skuas Catharacta skua of Known Age and Sex, and Its Effects Upon Breeding and Survival. The Journal of Applied Ecology 28: 672. doi:10.2307/2404575.
16. Tavares S, Xavier JC, Phillips RA, Pereira ME, Pardal MA (2013) Influence of age, sex and breeding status on mercury accumulation patterns in the wandering albatross Diomedea exulans. Environmental Pollution 181: 315–320. doi:10.1016/j.envpol.2013.06.032.
17. Chastel O, Weimerskirch H, Jouventin P (1993) High annual variability in reproductive success and survival of an Antarctic seabird, the snow petrel Pagodroma nivea. Oecologia 94: 278–285. doi:10.1007/BF00341328.
18. Bearhop S, Ruxton GD, Furness RW (2000) Dynamics of mercury in blood and feathers of great skuas. Environmental Toxicology and Chemistry 19: 1638–1643. doi:10.1002/etc.5620190622.
19. Bearhop S, Waldron S, Thompson D, Furness R (2000) Bioamplification of Mercury in Great Skua Catharacta skua Chicks: the Influence of Trophic Status as Determined by Stable Isotope Signatures of Blood and Feathers. Marine Pollution Bulletin 40: 181–185. doi:10.1016/S0025-326X(99)00205-2.
20. Gochfeld M (1980) Tissue distribution of mercury in normal and abnormal young Common Terns. Marine Pollution Bulletin 11: 362–366. doi:10.1016/0025-326X(80)90284-2.
21. Wayland M, Garcia-Fernandez AJ, Neugebauer E, Gilchrist HG (2001) Concentrations of Cadmium, Mercury and Selenium in Blood, Liver and Kidney of Common Eider Ducks from the Canadian Arctic. Environ Monit Assess 71: 255–267. doi:10.1023/A:1011850000360.
22. Henny CJ, Hill EF, Hoffman DJ, Spalding MG, Grove RA (2002) Nineteenth Century Mercury: Hazard to Wading Birds and Cormorants of the Carson River, Nevada. Ecotoxicology 11: 213–231. doi:10.1023/A:1016327602656.
23. Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. Frontiers in Ecology and the Environment 5: 429–436. doi:10.1890/060150.1.
24. Ridoux V, Offredo C (1989) The diets of five summer breeding seabirds in Adélie Land, Antarctica. Polar Biol 9: 137–145. doi:10.1007/BF00297168.
25. Tan SW, Meiller JC, Mahaffey KR (2009) The endocrine effects of mercury in humans and wildlife. Critical Reviews in Toxicology 39: 228–269. doi:10.1080/10408440802233259.
26. Drevnick PE, Sandheinrich MB (2003) Effects of Dietary Methylmercury on Reproductive Endocrinology of Fathead Minnows. Environ Sci Technol 37: 4390–4396. doi:10.1021/es034252m.
27. Frederick P, Jayasena N (2011) Altered pairing behaviour and reproductive success in white ibises exposed to environmentally relevant concentrations of methylmercury. Proc R Soc B 278: 1851–1857. doi:10.1098/rspb.2010.2189.
28. Evers DC, Savoy LJ, DeSorbo CR, Yates DE, Hanson W, et al. (2008) Adverse effects from environmental mercury loads on breeding common loons. Ecotoxicology 17: 69–81. doi:10.1007/s10646-007-0168-7.
29. Tartu S, Goutte A, Bustamante P, Angelier F, Moe B, et al. (2013) To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biol Lett.
30. Norris DO, Carr JA (2013) Vertebrate Endocrinology. Academic Press. 599 p.
31. Schoech SJ, Mumme RL, Wingfield JC (1996) Delayed breeding in the cooperatively breeding Florida scrub-jay (Aphelocoma coerulescens): inhibition or the absence of stimulation? Behav Ecol Sociobiol 39: 77–90. doi:10.1007/s002650050269.
32. Goutte A, Angelier F, Chastel CC, Trouvé C, Moe B, et al. (2010) Stress and the timing of breeding: Glucocorticoid-luteinizing hormones relationships in an arctic seabird. General and Comparative Endocrinology 169: 108–116. doi:10.1016/j.ygcen.2010.07.016.
33. Goutte A, Kriloff M, Weimerskirch H, Chastel O (2011) Why do some adult birds skip breeding? A hormonal investigation in a long-lived bird. Biol Lett 7: 790–792. doi:10.1098/rsbl.2011.0196.
34. Ramalingam V, Vimaladevi V, Rajeswary S, Suryavathi V (2003) Effect of mercuric chloride on circulating hormones in adult albino rats. J Environ Biol 24: 401–404.
35. Vom Saal FS, Finch CE (1988) Reproductive senescence: Phenomena and mechanisms in mammals and selected vertebrate. The physiology of reproduction. New York.
36. Angelier F, Moe B, Weimerskirch H, Chastel O (2007) Age-specific reproductive success in a long-lived bird: do older parents resist stress better? Journal of Animal Ecology 76: 1181–1191. doi:10.1111/j.1365-2656.2007.01295.x.
37. Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118: 1883–1891. doi:10.1111/j.1600-0706.2009.17643.x.
38. Bustamante P, Lahaye V, Durnez C, Churlaud C, Caurant F (2006) Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: Influence of geographical origin and feeding ecology. Science of The Total Environment 368: 585–596. doi:10.1016/j.scitotenv.2006.01.038.
39. Hobson KA, Clark RG (1993) Turnover of ^{13}C in Cellular and Plasma Fractions of Blood: Implications for Nondestructive Sampling in Avian Dietary Studies. The Auk 110: 638–641. doi:10.2307/4088430.
40. Hobson KA, Clark RG (1992) Assessing Avian Diets Using Stable Isotopes II: Factors Influencing Diet-Tissue Fractionation. The Condor 94: 189–197. doi:10.2307/1368808.
41. Bearhop S, Waldron S, Votier SC, Furness RW (2002) Factors That Influence Assimilation Rates and Fractionation of Nitrogen and Carbon Stable Isotopes in Avian Blood and Feathers. Physiological and Biochemical Zoology 75: 451–458. doi:10.1086/342800.
42. Cherel Y, Hobson KA (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Marine Ecology Progress Series 329: 281–287.
43. Jaeger A, Lecomte VJ, Weimerskirch H, Richard P, Cherel Y (2010) Seabird satellite tracking validates the use of latitudinal isoscapes to depict predators' foraging areas in the Southern Ocean. Rapid Communications in Mass Spectrometry 24: 3456–3460. doi:10.1002/rcm.4792.
44. Cherel Y, Fontaine C, Richard P, Labat J-P (2010) Isotopic niches and trophic levels of myctophid fishes and their predators in the Southern Ocean. Limnology and oceanography 55: 324–332.
45. Cherel Y, Hobson KA, Hassani S (2005) Isotopic Discrimination between Food and Blood and Feathers of Captive Penguins: Implications for Dietary Studies in the Wild. Physiological and Biochemical Zoology 78: 106–115. doi:10.1086/425202.
46. Thiebot JB, Authier M, Trathan PN, Bost C-A (2014) Gentlemen first? "Broken stick" modelling reveals sex-related homing decision date in migrating seabirds. Journal of Zoology 292: 25–30. doi:10.1111/jzo.12080.
47. Authier M, Martin C, Ponchon A, Steelandt S, Bentaleb I, et al. (2012) Breaking the sticks: a hierarchical change-point model for estimating ontogenetic shifts with stable isotope data. Methods in Ecology and Evolution 3: 281–290. doi:10.1111/j.2041-210X.2011.00162.x.
48. Cribari-Neto L, Zeileis A (2010) Beta Regression in R. J Stat Softw 34: 1–24.
49. Anderson O, Phillips R, McDonald R, Shore R, McGill R, et al. (2009) Influence of trophic position and foraging range on mercury levels within a seabird community. Marine Ecology Progress Series 375: 277–288. doi:10.3354/meps07784.
50. González-Solís J, Sanpera C, Ruiz X (2002) Metals and selenium as bioindicators of geographic and trophic segregation in giant petrels Macronectes spp. Mar Ecol Prog Ser 244: 257–264. doi:10.3354/meps244257.

51. Goutte A, Bustamante P, Barbraud C, Delord K, Weimerskirch H, et al. (2013) Demographic responses to mercury exposure in two closely-related Antarctic top predators. Ecology.
52. Robinson SA, Lajeunesse MJ, Forbes MR (2012) Sex Differences in Mercury Contamination of Birds: Testing Multiple Hypotheses with Meta-Analysis. Environ Sci Technol 46: 7094–7101. doi:10.1021/es204032m.
53. Cherel Y (2008) Isotopic niches of emperor and Adélie penguins in Adélie Land, Antarctica. Mar Biol 154: 813–821. doi:10.1007/s00227-008-0974-3.
54. Cossa D, Heimbürger L-E, Lannuzel D, Rintoul SR, Butler ECV, et al. (2011) Mercury in the Southern Ocean. Geochimica et Cosmochimica Acta 75: 4037–4052. doi:10.1016/j.gca.2011.05.001.
55. Lecomte VJ, Sorci G, Cornet S, Jaeger A, Faivre B, et al. (2010) Patterns of aging in the long-lived wandering albatross. PNAS 107: 6370–6375. doi:10.1073/pnas.0911181107.
56. Thiers L, Delord K, Barbraud C, Phillips RA, Pinaud D, Weimerskirch (2014) Foraging zones of the two sibling species of giant petrels in the Indian Ocean throughout the annual cycle: implication for their conservation. Marine Ecology Progress Series 499: 233–248.
57. Champoux L, Masse DC, Evers D, Lane OP, Plante M, et al. (2006) Assessment of mercury exposure and potential effects on common loons (Gavia immer) in Québec. In: Hanson AR, Kerekes JJ, editors. Limnology and Aquatic Birds. Developments in Hydrobiology. Springer Netherlands. 263–274.
58. Mitro MG, Evers DC, Meyer MW, Piper WH (2008) Common Loon Survival Rates and Mercury in New England and Wisconsin. The Journal of Wildlife Management 72: 665–673. doi:10.2193/2006-551.
59. Wayland M, Drake KL, Alisauskas RT, Kellett DK, Traylor J, et al. (2008) Survival rates and blood metal concentrations in two species of free-ranging North American sea ducks. Environmental Toxicology and Chemistry 27: 698–704. doi:10.1897/07-321.1.
60. Hallinger KK, Cornell KL, Brasso RL, Cristol DA (2011) Mercury exposure and survival in free-living tree swallows (Tachycineta bicolor). Ecotoxicology 20: 39–46. doi:10.1007/s10646-010-0554-4.
61. Goutte A, Barbraud C, Meillère A, Carravieri A, Bustamante P, et al. (2014) Demographic consequences of heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the wandering albatross. Proc. R. Soc. B 20133313.http://dx.doi.org/10.1098/rspb.2013.3313.
62. Follett BK, Farner DS, Mattocks Jr PW (1975) Luteinizing hormone in the plasma of white-crowned sparrows (Zonotrichia leucophrys gambelii) during artificial photostimulation. General and Comparative Endocrinology 26: 126–134. doi:10.1016/0016-6480(75)90223-3.
63. Sharp PJ (1975) A Comparison of Variations in Plasma Luteinizing Hormone Concentrations in Male and Female Domestic Chickens (gallus Domesticus) from Hatch to Sexual Maturity. J Endocrinol 67: 211–223. doi:10.1677/joe.0.0670211.
64. Bentley GE, Jensen JP, Kaur GJ, Wacker DW, Tsutsui K, et al. (2006) Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH). Hormones and Behavior 49: 550–555. doi:10.1016/j.yhbeh.2005.12.005.
65. Goutte A, Antoine É, Weimerskirch H, Chastel O (2010) Age and the timing of breeding in a long-lived bird: a role for stress hormones? Functional Ecology 24: 1007–1016. doi:10.1111/j.1365-2435.2010.01712.x.
66. Frederiksen M, Moe B, Daunt F, Phillips RA, Barrett RT, et al. (2012) Multicolony tracking reveals the winter distribution of a pelagic seabird on an ocean basin scale. Diversity and Distributions 18: 530–542. doi:10.1111/j.1472-4642.2011.00864.x.
67. Streets DG, Zhang Q, Wu Y (2009) Projections of Global Mercury Emissions in 2050. Environmental Science & Technology 43: 2983–2988. doi:10.1021/es802474j.

PAPER III

The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes.

Author's personal copy

Science of the Total Environment 476–477 (2014) 553–560

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes

Sabrina Tartu [a,*], Frédéric Angelier [a], Dorte Herzke [b], Børge Moe [c], Claus Bech [d], Geir W. Gabrielsen [e], Jan Ove Bustnes [c], Olivier Chastel [a]

[a] Centre d'Etudes Biologiques de Chizé (CEBC), UPR 1934-CNRS, F-79360, France
[b] Norwegian Institute for Air Research, FRAM — High North Research Centre on Climate and the Environment, N-9296 Tromsø, Norway
[c] Norwegian Institute for Nature Research, FRAM — High North Research Centre on Climate and the Environment, N-9296 Tromsø, Norway
[d] Department of Biology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway
[e] Norwegian Polar Research Institute, FRAM Centre High North Research on Climate and the Environment, N-9296 Tromsø, Norway

HIGHLIGHTS

- We examined relationships between POPs, reproduction and CORT secretion in a seabird.
- POP levels were negatively related to body condition in non-breeding females only.
- Females with high levels of pesticides laid their eggs earlier.
- Females with high levels of PCBs released more CORT when subjected to a stress.
- High POP levels were not associated with poor breeding success.

ARTICLE INFO

Article history:
Received 9 December 2013
Received in revised form 16 January 2014
Accepted 17 January 2014
Available online 1 February 2014

Keywords:
Persistent organic pollutants
Corticosterone
Stress response
Reproduction
Arctic seabirds

ABSTRACT

High levels of environmental pollutants such as persistent organic pollutants (POPs) including PCB and DDT have been found in the Arctic and many of those pollutants may impair reproduction through endocrine disruption. Nevertheless, their effects on stress hormones remain poorly understood, especially in free-ranging birds. Corticosterone, the principal glucocorticoid in birds, can indirectly impair reproduction. The aim of the present study was to examine the relationships between POPs and reproduction through their potential consequences on different reproductive traits (breeding decision, egg-laying date, breeding success) and corticosterone secretion (baseline and stress-induced levels). We addressed those questions in an Arctic population of female black-legged kittiwakes during the pre-breeding stage and measured several legacy POPs (PCBs and pesticides: HCB, *p,p'*-DDE, CHL) in whole blood. POP levels were not related to breeding decision neither to breeding success, whereas females with high levels of pesticides laid their eggs earlier in the season. We found a negative relationship between POP levels and body condition index in non-breeding females. Black-legged kittiwakes with higher levels of PCB showed stronger adrenocortical response when subjected to a capture-handling stress protocol. We suggest that PCBs may disrupt corticosterone secretion whereas the positive relationship between pesticides and egg-laying date could either originate from a direct effect of pesticides or may be related to other confounding factors such as age or individual's quality. Although no direct negative reproduction output of POPs was found in this study, it is possible that the most contaminated individuals would be more sensitive to environmental stress and would be less able to maintain parental investment than less polluted individuals.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Environmental pollutants, such as persistent organic pollutants (POPs: pesticides, PCBs), have received an increasing attention during the last 30 years. The Arctic is considered as a sink for environmental pollution, and for some compounds, levels may exceed that of industrialized cities (Gabrielsen and Henriksen, 2001). Because of bioaccumulation into organisms and bio-magnification along the food chain, marine apex predators such as seals, whales and seabirds are particularly vulnerable (Letcher et al., 2010; Vallack et al., 1998). Among free-living vertebrates, highly polluted individuals show decreased breeding capacities, such as abnormal breeding behaviour, reduced fertility or poor breeding success (Bustnes et al., 2003a, 2007; Colborn et al.,

* Corresponding author.
E-mail address: tartu@cebc.cnrs.fr (S. Tartu).

0048-9697/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2014.01.060

Author's personal copy

1993; Gabrielsen, 2007; Harrison et al., 1997; Taylor and Harrison, 1999; Verreault et al., 2010). Such breeding impairment could originate from the ability of POPs to act as endocrine disruptors and thus, to alter the functioning of major endocrine axes (Ottinger et al., 2013; Tyler et al., 1998). Indeed those substances are able to mimic, antagonize, alter or modify endogenous' hormone functions (e.g. Amaral Mendes, 2002). In free-living vertebrates, several studies have found significant relationships between POPs and reproductive hormones such as steroids (Colborn et al., 1993; Giesy et al., 2003; Vos et al., 2000) and more recently hypothalamic and pituitary hormones (Verreault et al., 2008). Other hormones, such as those from the hypothalamic–pituitary–adrenal (HPA) axis, and especially glucocorticoids, are however known to affect reproductive behaviours and to mediate major reproductive decisions in vertebrates (reviewed in Wingfield and Sapolsky, 2003). Studies on laboratory mammals have documented a number of effects of chemicals on glucocorticoids (Odermatt and Gumy, 2008) but effects of POPs on stress hormones have been poorly studied in wildlife (Bergman et al., 2012). Hence, the concern for endocrine disruptors should also be directed towards the glucocorticoid system (Dawson, 2000; Johansson et al., 1998). Glucocorticoids (cortisol, corticosterone) are released in response to stressful events (food shortage, predation, and pathogens) to adjust life-history strategies in relation to environmental conditions and to individual physiological state (Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). Indeed, the release of glucocorticoids during stressful events triggers physiological and behavioural adjustments that shift energy investment away from reproduction and redirects it towards survival (Wingfield and Sapolsky, 2003). Stress hormones have therefore a strong connection with fitness traits such as breeding success, individual quality and survival (Angelier et al., 2009, 2010; Bókony et al., 2009; Bonier et al., 2009; Breuner et al., 2008; Goutte et al., 2010b, 2011b; Kitaysky et al., 1999). Importantly, this means that a disruption of glucocorticoid secretion may alter the ability of an individual to adjust breeding decisions (to breed or not, when to breed) to environmental conditions. However, only a few studies have explored the impact of pollutants on both baseline and stress induced glucocorticoid levels, which depict different physiological functions: baseline corticosterone levels (CORT, the major glucocorticoid in birds) mirror the activity and metabolic rate and reflect the ratio between energy available and energy needed (Landys et al., 2006), while stress-induced CORT can be used as an index of the sensitivity to stress of an individual, this value can be modulated in order to maximize either survival, or reproduction (Bókony et al., 2009; Lendvai et al., 2007). Regarding contaminant/HPA axis, the pattern seems clear in fish: i.e. individuals from polluted sites (heavy metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls) are unable to elevate their cortisol levels (reviewed in Hontela, 2005). Studies on wild birds remain sparse and the pattern is less clear (reviewed in Verreault et al., 2010), mainly because they are difficult to compare as pollutants have been measured in different tissues (i.e. muscles, liver or feathers). And even when comparing two studies on the effects of POPs on CORT secretion, both measured in blood of Arctic seabirds, no clear pattern appeared. In black-legged kittiwakes *Rissa tridactyla* (hereafter 'kittiwakes') sampled early in the breeding season (April), increasing blood levels of PCBs were related to an increase of baseline CORT levels, and this relationship did not appear during the incubation period (Nordstad et al., 2012). In incubating glaucous gulls *Larus hyperboreus*, increasing blood POP levels (among which PCBs but also several pesticides) resulted in an increase of baseline CORT (Verboven et al., 2010). Moreover, male glaucous gulls subjected to a standardized stress-protocol, had decreased levels of stress-induced CORT with increasing POPs (Verboven et al., 2010). Thus the nature of POPs-CORT relationships could therefore depend on the type of pollutants, gender, types of tissue sampled and the reproductive status of the individuals. Although CORT is considered a keystone hormone for allocation processes and reproductive effort (Wingfield and Sapolsky, 2003), there is a lack of studies investigating POPs-CORT-fitness in free-living organisms.

The aim of the present study was to consider the relationships between POPs and reproduction through their potential effects on reproductive traits and corticosterone secretion (baseline and stress-induced levels). We addressed those questions on female kittiwakes during the pre-breeding stage and measured POP levels (PCBs and pesticides: HCB, *p,p′*-DDE, CHL) from whole blood samples. In female kittiwakes CORT predicts breeding decision (Goutte et al., 2010a), egg-laying date (Goutte et al., 2011b) and breeding success (Goutte et al., 2011b). Thus, we investigated if blood POP levels would be related to 1) reproductive traits (the decision to breed or not, egg-laying date and breeding success) and 2) CORT secretion (baseline and stress-induced levels).

2. Materials and methods

2.1. Study area and birds

Our study was conducted in a colony of kittiwakes at Kongsfjorden, Svalbard (78°54′N, 12°13′E), 7 km southeast of Ny-Ålesund, Norway. Kittiwakes are colonial seabirds that breed on cliffs throughout the northern parts of the Pacific and Atlantic, including the Barents Sea region up to the Svalbard Archipelago (Anker-Nilssen et al., 2000). We studied kittiwakes in one plot of around 117 pairs breeding on cliff ledges at heights of 5–10 m. Female kittiwakes were sampled from 19 May to 7 June 2011, during the pre-laying period (i.e. copulations and nest building period), a key period for reproductive decisions during which female kittiwakes appear highly sensitive to stressors (Goutte et al., 2010a, 2011b; Tartu et al., 2013).

2.2. Capture and blood sampling

Forty-seven females were caught on the nests with a noose at the end of a 5 m fishing rod. A first blood sample (*ca.* 0.3 ml) was collected immediately after capture, from the alar vein with a 1 ml heparinised syringe and a 25-gauge needle to assess baseline CORT levels. Bleeding time (i.e. time elapsed from capture to the end of the first blood sample: 2 min 55 ± 34 (SD) seconds, on average) did not affect CORT levels (GLM, $F_{1,45} = 1.79$, $p = 0.190$). Kittiwakes were then placed into cloth bags and subsequent blood samples (*ca.* 0.3 ml) were collected from the alar vein at 30 min to assess stress-induced CORT levels.

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. Birds were weighed to the nearest 2 g using a Pesola spring balance, and their skull length (head + bill) was measured to the nearest 0.5 mm with a sliding calliper. For each individual, we calculated an index of body condition by using the residuals from a linear regression of body mass against skull length (GLM, $F_{1,46} = 7.05$, $p = 0.01$). Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observation and then released. 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 breeding decision (at least one egg is laid or no egg laid) and egg-laying dates. Then, with same technique, we checked the nest content every 2 or 3 days to monitor the number of chicks that reached at least 12 days of age per active nest (hereafter called 'breeding success').

2.3. Molecular sexing and hormone assay

Blood samples were centrifuged, and plasma and red blood cells were separated and stored at −20 °C until used respectively in hormone assays or molecular sexing, at the Centre d'Etudes Biologiques de Chizé (CEBC). Molecular sexing was performed as detailed in Weimerskirch et al. (2005). Plasma concentrations of CORT were determined by radioimmunoassay at the CEBC, as described by Lormée et al. (2003). Baseline CORT levels were not related to sampling date (GLM, $F_{1,45} = 0.27$, $p = 0.610$) or time of the day (GLM, $F_{1,45} = 0.33$, $p = 0.571$) and neither were

Author's personal copy

stress-induced levels (sampling date: GLM, $F_{1,45} = 0.55$, $p = 0.460$; time of the day: GLM, $F_{1,45} = 0.72$, $p = 0.399$). The lowest detectable concentration for CORT was 0.53 ng/ml. Only one assay was performed and the intra-assay coefficient of variation was 6.7% (N = 5 duplicates).

2.4. POPs analyses

POPs were analysed from 45 whole blood samples at the Norwegian Institute for Air Research (NILU) in Tromsø, for two individuals blood volumes were too low for POP measurements. The following compounds were analysed: the PCBs (CB-28, -52, -99, -101, -105, -118, -128, -138, -153, -180, -183, -187 and -194), and the pesticides (*p,p′*-DDE, α-, β-, γ-HCH, HCB, oxychlordane, trans-, cis-chlordane, trans-, cis-nonachlor). The compounds chosen for further investigation were the ΣPCBs (CB-99, -105, -118, -128, -138, -153, -180, -183, -187 and -194), and the ΣPesticides (*p,p′*-DDE, HCB, oxychlordane, trans-chlordane, trans-, cis-nonachlor). To a blood sample of 0.5 to 1.5 ml, a 100 μl internal standard solution was added (13C-labelled compounds from Cambridge Isotope Laboratories: Woburn, MA, USA). The sample was extracted twice with 6 ml of n-hexane, after denaturation with ethanol and a saturated solution of ammonium sulphate in water. Matrix removal on florisil columns, separation on an Agilent Technology 7890 GC and detection on an Agilent Technology 5975C MSD were performed as described by Herzke et al. (2009). The limit for detection was threefold the signal-to-noise ratio, and for the compounds investigated the limit ranged from 0.4 to 122 pg/g wet weights (ww). For validation of the results, blanks (clean and empty glass tubes treated like a sample, 3 in total) were run for every 10 samples, while standard reference material (3 in total, 1589 a human serum from NIST) was run for every 10 samples. The accuracy of the method was within the 70 and 108% range.

2.5. Statistical analyses

All statistical analyses were performed using R 2.8.0 (R Development Core Team, 2008). We used generalised linear models (GLM) with a normal, binomial or Poisson error distribution and an identity, logit or log link function, respectively, to test our biological assumptions. To test the relationships between different groups of POPs (pesticides and PCBs), we categorised POP compounds based on chemical structure similarities. We summed their concentrations in four classes as follows: PCBs (n = 10 congeners), CHL (n = 4 compounds), *p,p′*-DDE and HCH. Because CHL, *p,p′*-DDE and HCH were all correlated (Pearson correlation coefficients, R = 0.70, 0.83 and 0.82) we grouped them into a sum of pesticides (Pesticides). To describe the total blood contaminant concentration of individual kittiwakes we used the sum of POPs (POPs) including PCBs and Pesticides as they were also highly correlated (R = 0.79). First, we tested the relationships between body condition, organic pollutants (using POPs, PCBs and Pesticides) and breeding decision, egg-laying date and breeding success. We also tested the relationships between organic pollutants and body condition index in non-breeders and breeders separately. Second, we checked for relationships between organic pollutants (using POPs, PCBs and Pesticides), breeding decision and interaction, and baseline and stress-induced CORT. We performed statistical tests by using absolute CORT levels to facilitate the comparison with other published results. Prior to this, we checked for possible statistical effects of bleeding time, sampling date, time elapsed between blood sampling and egg-laying, hour of the day, body condition and breeding decision on baseline and stress-induced CORT levels ($p > 0.05$ for all tests). Diagnostic plots were used to assess whether the data sufficiently met the assumptions of the linear model, and dependent continuous variables were log-10 transformed when necessary. Values are mean ± SD.

3. Results

3.1. Relationships between organic pollutants, body condition and reproductive traits

The probability to breed was significantly related to the body condition as non-breeding females had a lower body-condition than breeding females (Fig. 1A; Table 1). However, the probability of breeding was not related to POPs, PCBs or Pesticides (Fig. 1B; Table 1). Additionally, in non-breeding females, we found a negative and significant relationship between body condition and POPs (GLM, $F_{1,17} = 12.90$, $p = 0.002$), the same relationship was found between PCBs (GLM, $F_{1,17} = 12.46$, $p = 0.003$) and Pesticides (GLM, $F_{1,17} = 8.85$, $p = 0.008$) on body condition index (Fig. 2), while no relationship was observed in breeding females ($p > 0.51$ for all tests, Fig. 2).

In females that bred, egg-laying date was negatively related to Pesticides: females that laid early had higher levels of pesticides than females that laid later in June (Fig. 3; Table 1). This relationship did not appear when considering body condition or PCBs. No relationships were found between body condition, POPs, PCBs or Pesticides and breeding success (Table 1).

3.2. Relationships between organic pollutants and CORT secretion

Baseline and stress induced CORT levels (log transformed) were not related to body condition ($p > 0.05$ for all tests) or breeding decision (Table 2). We did not find any relationship between POPs, PCBs or Pesticides on baseline CORT levels (Fig. 4A; Table 2). However, we found a positive relationship between PCBs and stress-induced CORT levels. The most polluted individuals released more CORT when subjected to a capture-handling stress protocol (Fig. 4B; Table 2), and this relation was not found when using POPs only or Pesticides.

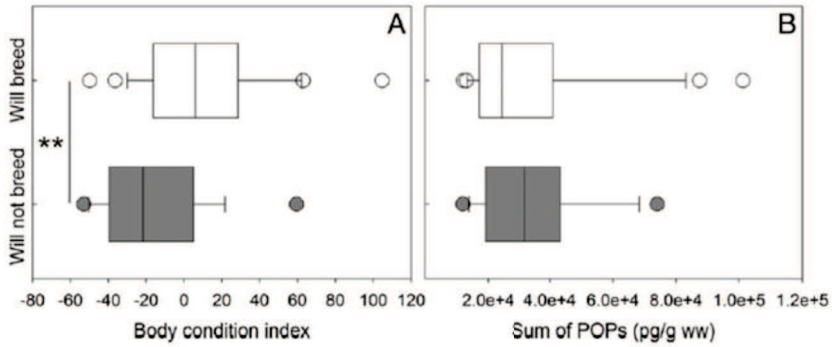


Fig. 1. Body-condition index (A) was higher in breeding (empty boxes) than in non-breeding (filled boxes, **: $p < 0.02$) female black-legged kittiwakes, although blood POP levels (pg/g ww, B) did not differ between breeding and non-breeding females.

Author's personal copy

Table 1
Relationships between body condition index (N = 47), organic pollutants (pg/g ww, N = 45) as Σ POPs, Σ PCBs and Σ Pesticides and reproductive traits: a) breeding decision (will breed or will not breed), b) egg-laying date (days of June) and c) breeding success in pre-laying female black-legged kittiwakes. Egg-laying dates were available for all breeding females (N = 28), while breeding success was not available for two of them. Numbers in bold indicate significant p-values ($p < 0.05$).

	Independent variable	Sum of squares	Df	F or χ^2	p-Value
a) Breeding decision	Body condition	–	1.45	5.60	**0.018**
	Σ POPs	–	1.43	0.00	0.953
	Σ PCBs	–	1.43	0.01	0.942
	Σ Pesticides	–	1.43	0.14	0.707
b) Egg-laying date (log)	Body condition	0.20	1.26	2.36	0.136
	Σ POPs	0.30	1.24	4.08	0.055
	Σ PCBs	0.21	1.24	2.69	0.114
	Σ Pesticides	0.53	1.24	8.12	**0.009**
c) Breeding success	Body condition	–	1.24	0.19	0.663
	Σ POPs	–	1.22	0.10	0.752
	Σ PCBs	–	1.22	0.07	0.785
	Σ Pesticides	–	1.22	0.16	0.694

F tests were used for models with normal distribution (Egg-laying date).
χ^2 tests were used for models with binomial (Breeding decision) and Poisson (Breeding success) distribution.

4. Discussion

Although kittiwakes from colonies in Svalbard are exposed to POPs (Nordstad et al., 2012; Savinova et al., 1995), the present study did not reveal any negative impact on the decision to breed or not, and breeding success. Nevertheless, females with the higher levels of pesticides laid eggs earlier. We found a negative relationship between POP levels and body condition index in non-breeding females. We also found that individuals bearing the higher levels of PCB would have a stronger adrenocortical response when subjected to a capture-handling stress protocol, and this did not appear when testing the relationship between Pesticides only and CORT secretion.

4.1. Relationships between organic pollutants and reproductive traits

4.1.1. Breeding decision and body-condition index

In kittiwakes, and in many long-lived seabird species, a significant proportion of adult birds will not breed in a given year (Goutte et al., 2010a, 2011c). In a recent study we have shown that moderate levels of mercury could be linked to non-breeding events in kittiwakes from Svalbard (Tartu et al., 2013). In our study, however the decision to breed or not was unrelated to blood POP levels (neither to pesticides nor PCBs). Although Svalbard kittiwakes bear significant amounts of pesticides and PCBs in their blood (Nordstad et al., 2012, this study), their POP levels were about 10-fold lower compared to levels in glaucous gulls, the most polluted arctic seabird species (Borgà et al., 2001; Bustnes et al., 2003b; Gabrielsen et al., 1995). Thus it is possible that POP levels found in pre-laying kittiwakes were not high enough to alter reproduction. Another possibility is that pesticides and PCBs, contrary to mercury, may not interfere with hormonal pathways involved in non-breeding behaviour, such as GnRH (gonadotropin-releasing hormone) and luteinizing hormone (LH, Tartu et al., 2013).

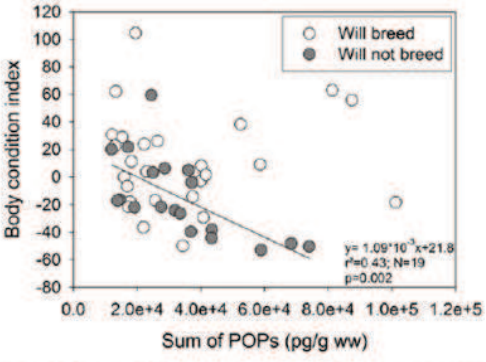


Fig. 2. Relationship between blood POP concentration (POPs pg/g ww) and body condition index in breeding female black-legged kittiwakes (empty circles) and non-breeding (filled circles). Body condition index decreased with increasing POP levels in non-breeding females (solid line).

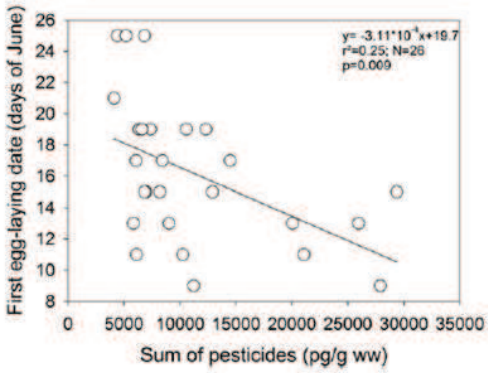


Fig. 3. Relationship between pesticide concentration in blood (Pesticides pg/g ww) and first-egg laying date (days of June) in female black-legged kittiwakes. The females with the higher levels of pesticides (CHL, *p,p'*-DDE and HCB) laid earlier than those with lower levels.

As found in other seabird species females that did not breed had a lower body condition index (Chastel et al., 1995; Goutte et al., 2010a, 2010c). Decreasing condition in non-breeding females was associated with increasing blood POP levels. Organic pollutants are lipophilic; if body fat reserves are low, organic pollutants can be redistributed through the bloodstream and more likely to migrate to sensitive vital organs as brain, kidneys and liver (Fuglei et al., 2007; Henriksen et al., 1996). This pollutant redistribution has been confirmed in several bird species: emaciated individuals had higher levels of POPs in liver, blood and brain than individuals in better body condition (Bogan and Newton, 1977; Bustnes et al., 2010, 2012; Kenntner et al., 2003). In female kittiwakes breeding in northern Norway, average body mass decreases 20% from the beginning to the end of the breeding period, and this body mass loss was accompanied with a 4-fold increase of PCB levels in the brain (Henriksen et al., 1996). Thus birds in poor body condition are more sensitive to environmental pollution since pollutants are more available in sensitive organs. If vital organs are harmed, detoxification process would be more important, increasing the individual's metabolism. Thus, we can suppose that the most polluted non-breeders may therefore be in lower body condition because they are expending more energy in detoxification than breeders. Our study highlights that in pre-laying female kittiwakes, POP-condition relationships differ according to breeding decision. However, a question is why such relationship was found in non-breeding birds only although all females despite their breeding decision had, on average, similar blood concentrations of POPs? Maybe non-breeding individuals reached a threshold condition below which POPs/body condition relationships became apparent or non-breeding birds may also be poor quality individuals (Cam et al., 1998), which may be less able to physiologically deal with a given dose of POPs. Also, POPs may act as endocrine disruptors, and according to the physiological state of an individual, hormonal levels vary, especially if individuals chose to breed or not. Because in kittiwakes, endocrine levels differ between breeders and non-breeders (Goutte et al., 2010a; Tartu et al., 2013) this could have an effect on the potential toxicity and threat of endocrine disrupting chemicals. It has been suggested, in studies on rats, that oestrogens have a protective effect on methyl-mercury threat on the brain (Oliveira

Author's personal copy

Table 2
Relationships between organic pollutants (pg/g ww) as Σ POPs, Σ PCBs and Σ Pesticides and a) baseline and b) stress-induced CORT levels (ng/ml) in pre-laying female black-legged kittiwakes. Numbers in bold indicate significant p-values ($p < 0.05$).

Dependant variable	Independent variable	Sum of squares	Df	F	p-Value
a) Baseline CORT (log)	Breeding decision	0.00	1.41	0.01	0.931
	Σ POPs	0.63	1.41	1.95	0.170
	Σ POPs × breeding decision	0.02	1.41	0.07	0.788
	Σ PCBs	0.48	1.41	1.45	0.235
	Σ PCBs × breeding decision	0.02	1.41	0.05	0.820
	Σ Pesticides	0.95	1.41	3.03	0.089
	Σ Pesticides × breeding decision	0.03	1.41	0.11	0.741
b) Stress-induced CORT (log)	Breeding decision	0.14	1.42	3.42	0.072
	Σ POPs	0.14	1.42	3.43	0.071
	Σ POPs × breeding decision	0.03	1.41	0.74	0.395
	Σ PCBs	0.17	1.42	4.11	**0.049**
	Σ PCBs × breeding decision	0.02	1.41	0.49	0.488
	Σ Pesticides	0.07	1.41	1.60	0.213
	Σ Pesticides × breeding decision	0.04	1.41	0.97	0.331

et al., 2006). Therefore, if some pollutants mimic the effects of sex steroids, and if these latter are endogenously more present in the organism, the pollutants' harm could be less effective. To our knowledge, a protective effect of reproductive hormones on POPs has never been observed. Further studies comparing POP levels in the Hypothalamo-Pituitary-Gonad axis of breeders and non-breeders, which include measurements of sex steroids, would be important to test this supposition.

4.1.2. Egg-laying date and breeding success

In birds, breeding at the right time is one of the most important factors for successful reproduction in a fluctuating environment (e.g. Lack, 1968) and in kittiwakes, late breeding is usually associated with low breeding success (Goutte et al., 2011b; Moe et al., 2009). Studies on free-living great black-backed gulls (*Larus marinus*) and Antarctic skuas (*Catharacta maccormicki*) have reported that the most polluted individuals had a delayed egg-laying date (Bustnes et al., 2007; Helberg et al., 2005). In our study we found the reverse pattern: females with the highest levels of pesticides laid their eggs earlier in the season, as found in some populations of glaucous gulls (Bustnes et al., 2003b) and great black-backed gulls (Bustnes et al., 2008).

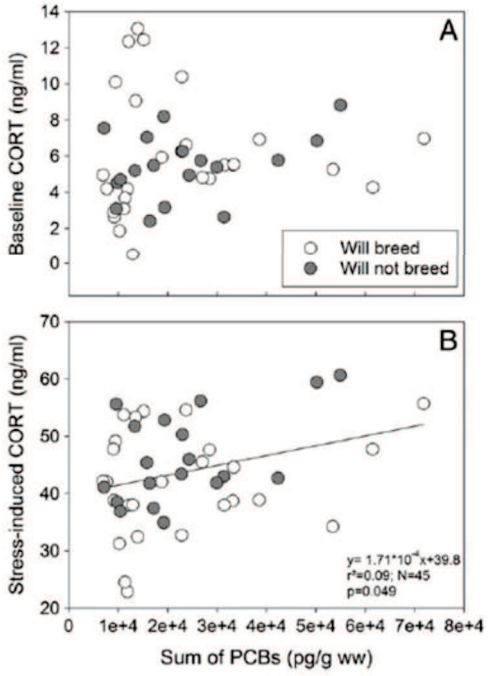


Fig. 4. Relationship between PCBs concentration in blood (PCBs pg/g ww) and baseline (A) and stress-induced (B) CORT levels (ng/ml) in female black-legged kittiwakes. Baseline CORT levels were not related to PCB levels whereas stress-induced CORT levels increased with increasing PCB levels. Empty circles refer to breeding females and filled circles to non-breeding females.

This negative relationship between pesticide contamination and egg-laying date may not be causal: e.g. females laying early could be of better quality, forage at a higher trophic level and hence be more exposed to pesticide contamination. In the same line of idea, this correlation could be the result of age related processes since in birds old females often lay earlier than young ones (e.g. Goutte et al., 2010c) and those older females may possibly bear higher POP levels. In our study, no birds were of known age, so we were not able to test for a possible influence of age on POP levels. However the few studies that have explored these relationships have failed to find a relationship between age and POP concentration (Bustnes et al., 2003a; Tartu et al. unpublished data).

In mammals, some studies have described relationships between organochlorine pesticides and preterm birth (Longnecker et al., 2001; Saxena et al., 1981). DDT metabolites are able to impede the binding of some sex steroids (androgen or progesterone) to their receptor through indirect or direct paths, shortening the duration of gestation (Klotz et al., 1997; Lyon and Glenister, 1980). Some of the pesticides present in kittiwakes could also mimic or stimulate the secretion of some hormones involved in oviposition. Prostaglandin synthesis, a major hormone involved in oviposition, can be inhibited by *p,p′*-DDE in ducks (Lundholm, 1997), which is not coherent with our results, but consistent with Bustnes et al. (2007, 2008). However, many pollutants have a non-linear dose–response relationship (Calabrese, 2010; Heinz et al., 2012) and several studies have pictured an inverted U-shaped effect (e.g. Love et al., 2003), low dose of pollutant may enhance the synthesis of a hormone while important doses may inhibit it. The amount of DDE measured into the eggs to inhibit prostaglandin in Lundholm (1997) was averagely 3000-fold that of *p,p′*-DDE levels found in female kittiwakes' blood (this study) and 200-fold that of *p,p′*-DDE levels found in Svalbard kittiwakes' eggs (Barrett et al., 1996). We may speculate that in kittiwakes low dose of *p,p′*-DDE may stimulate prostaglandin synthesis, which would in this case initiate an early oviposition. Because *p,p′*-DDE and prostaglandin are tightly linked (Lundholm, 1997) and that the relationship between egg-laying date and *p,p′*-DDE was statistically the more significant among all other pesticides (i.e. HCB, chlordanes), we suppose there could exist a positive relationship between low levels of *p,p′*-DDE and prostaglandin secretion. This hypothetical disruption of prostaglandin from pesticides could however be advantageous in Polar Regions. Indeed, in regions where breeding season is short, breeding early is beneficial (Perrins, 1970).

Our study did not reveal any negative impact of persistent organic pollutants on breeding success. Average breeding success during our study was quite good, 1.26 ± 0.65 chicks reached more than 12 days old per active nest, against less than 1 chick, 7 years over 11, during the period from 1997 to 2008, excluding 2001 (Moe et al., 2009),

Author's personal copy

suggesting favourable foraging condition at sea. It is therefore possible that birds were able to cope with POP contamination without visible reproductive penalties.

4.2. Relationships between organic pollutants and CORT secretion

In the present study, stress-induced levels were higher in individuals that had the higher levels of PCBs, and this was not true for pesticides. The adrenal gland is one of the most common targets for chemically induced lesions (Rosol et al., 2001). Because of several characteristics as: its large blood supply, its lipophilicity (allowing the accumulation of lipophilic compounds), its high concentration of cytochrome P450 that can also bioactivate toxicants, and its capacity to synthesize all major classes of steroids (Falco et al., 2007; Harvey and Everett, 2003; Hinson and Raven, 2006; Rosol et al., 2001). Adrenal cells concentrate a number of toxic agents, as DDT (Lund et al., 1988) and PCB metabolites (Brandt and Bergman, 1987), those toxicants may remain inactive caught into the adrenal tissue until a period of particularly high adrenal steroid demand, as the breeding period. During the breeding period, the body mass loss may make available contaminants stored in different organs and body reserves, which at their turn would cause damage. Indeed, reduced food intake enhances biotransformation of halogenated organic contaminants and formation of metabolites which have greater toxicological impacts compared to parent POPs (Routti et al., 2013). In our study, stress-induced CORT levels increased with increasing levels of circulating PCBs. This could reflect a dysfunction coming from the adrenals (e.g. up-regulation of ACTH receptors, pollutants mimicking ACTH etc.) and/or this could mirror a dysfunction coming from the brain (e.g. pituitary loss of negative feedback from CORT on the pituitary). Relationships between PCBs and adrenocortical functions have been experimentally highlighted: PCBs can alter adrenocortical steroidogenesis, down-regulate the number of brain glucocorticoid receptors and some PCB metabolites (i.e. hydroxylor methyl PCBs) can bind competitively to glucocorticoid receptors (Aluru et al., 2004; Johansson et al., 1998; Xu et al., 2006). In addition, it seems that high concentration of PCB126 could sensitize the regulation of ACTH on adrenocortical cells by increasing ACTH receptor levels (Li and Wang, 2005), which could result in an increase of CORT secretion. Thus it is possible that some PCB congeners present in kittiwakes could act similarly by increasing the number of ACTH receptors, thus increasing CORT secretion in the most polluted individuals.

Contrary to our findings on kittiwakes, stress-induced CORT levels in the highly polluted glaucous gull decreased with increasing POP levels (Verboven et al., 2010). As mentioned previously, environmental pollutants can have a non-linear dose–response relationship (Heinz et al., 2012; Love et al., 2003), the lower stress response observed in highly polluted glaucous gulls may also be the consequence of a negative feed-back from higher baseline CORT levels (Verboven et al., 2010) due to an increased allostatic load when resources are allocated to biotransformation, detoxification and excretion of contaminants (Parkinson and Ogilvie, 2008). Finally, the lack of relationship between baseline CORT and PCBs in our study compared to that of Nordstad et al. (2012) could be explained by the difference between the two sampling periods: in the study of Nordstad et al. (2012), pre-breeding female kittiwakes were sampled in April, their blood PCB levels were average 14,900 pg/g and baseline CORT levels averaged 7.2 ± 1 ng/ml. In the present study, female kittiwakes were sampled in May–June, their blood PCB levels were on average 23,030 pg/g and baseline CORT levels averaged 6.13 ± 3 ng/ml. Although the compounds entering in the PCBs were not completely identical they were very close (11 PCB congeners in Nordstad et al. (2012), 10 PCB congeners in the present study, among which 8 were similar). Those findings suggest that the relationships between PCBs and CORT secretion may highly depend of the levels of PCBs present in blood, and probably also to the sampling period.

Our study adds new evidence that PCBs are linked to CORT secretion disruption. To elucidate which aspects of the HPA axis are involved in mediating contaminant-related changes in the stress response, experimental ACTH injection would be useful to investigate if contaminant-related enhancement occurs at the level of the adrenal gland, or rather at the level of the pituitary, hypothalamus or perception of the stressor. An enhanced stress response is often the consequence of poor early-life experience across vertebrates as low body mass at birth, food restriction, and maternal deprivation (Banerjee et al., 2012; Heath and Dufty, 1998; Kitaysky et al., 2001; Müllner et al., 2004; Phillips and Jones, 2006), while in adults it often mirrors poor fitness related traits as poor parental investment (Angelier et al., 2009; Bókony et al., 2009; Goutte et al., 2011a; Lendvai et al., 2007) or an impacted survival (Blas et al., 2007; Goutte et al., 2010b; Romero, 2012). Still, as mentioned previously, in our study we failed to relate POP levels to breeding success. Environmental conditions were apparently favourable but in case of poor foraging conditions when CORT secretion is stimulated (Kitaysky et al., 1999; Lanctot et al., 2003), it is likely that the most contaminated individuals would be more sensitive to environmental stress and would be less able to maintain parental investment than less polluted ones.

5. Conclusion

Although in this study we did not find any direct negative reproductive outputs of POPs, no information was available about the chicks or parents' survival. Also, females have been blood sampled during the pre-laying phase, and blood measures performed at the beginning of the breeding season may be less reliable to predict reproductive traits occurring at the end of the season as breeding success (Lanctot et al., 2003). This may be particularly true for POPs as levels vary significantly between the different breeding phases (Nordstad et al., 2012). Moreover a previous study on pre-laying kittiwakes has shown that the decision to defer breeding is highly related to a disruption of GnRH secretion from the hypothalamus by mercury (Tartu et al., 2013). Thus, brought together, all the effects of POPs added to the threat of other environmental pollutants could have important impacts on the long-term, depending on a birds' quality. A point to underline is that our study is correlative; we cannot confirm if there is a causal effect of POPs on reproductive traits or CORT. Feeding different concentrations of POPs to captive individuals (Love et al., 2003; Lundholm, 1997) or using silastic tubes filled with PCBs (Van den Steen et al., 2007) is a method that could enlighten on the causal effects of pollutants, yet this would be difficult to perform in free-ranging protected species. Moreover, we only measured legacy POPs, already known to be importantly related to several physiological parameters (Gabrielsen, 2007; Gabrielsen and Henriksen, 2001). In the Arctic, several emerging POPs (e.g. Perfluorinated compounds (PFC), brominated flame retardants) show increasing trends (Braune and Letcher, 2013; Braune et al., 2007; Butt et al., 2007; de Wit et al., 2006; Verreault et al., 2007). Given this perspective, in top predators those emerging POPs could be in higher levels than legacy POPs (e.g. PFC, Nøst et al., 2012) and their relationships with the parameters we measured in this study may be exacerbated, taking them into account could provide further awareness on the way environmental pollution may affect free-ranging populations.

Conflict of interest

The authors declare having no conflict of interest.

Acknowledgements

This project was supported by Institut Polaire Français (IPEV project 330 to O. Chastel), Agence National de la Recherche (ANR project PolarTop to O. Chastel) and COPOL (GW Gabrielsen & JO Bustnes). This study was approved by the French and Norwegian Ethic committees and by the Governor of Svalbard. The authors thank K. Sagerup, A. J. Haugerud, A. Fenstad, V. Garcia Matarranz, and L. Guéry for their wonderful help in the field, and C. Parenteau, C. Trouvé, and S. Dano, for

Author's personal copy

their excellent technical assistance in hormones assays and molecular sexing. This article benefited greatly from the comments of two anonymous referees.

References

Aluru N, Jorgensen EH, Maule AG, Vijayan MM. PCB disruption of the hypothalamus-pituitary-interrenal axis involves brain glucocorticoid receptor downregulation in anadromous Arctic charr. Am J Physiol Regul Integr Comp Physiol 2004;287(4):787–93.

Amaral Mendes JJ. The endocrine disrupters: a major medical challenge. Food Chem Toxicol 2002;40(6):781–8.

Angelier F, Clément-Chastel C, Welcker J, Gabrielsen GW, Chastel O. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. Funct Ecol 2009;23(4):784–93.

Angelier F, Wingfield JC, Weimerskirch H, Chastel O. Hormonal correlates of individual quality in a long-lived bird: a test of the 'corticosterone-fitness hypothesis'. Biol Lett 2010;6(6):846–9.

Anker-Nilssen T, Bakken V, Strøm H, Golovkin AN, Bianki VV, Tatarinkova IP. The status of marine birds breeding in the Barents sea region. Tromsø: Norwegian Polar Institute; 2000. 1–213.

Banerjee SB, Arterbery AS, Fergus DJ, Adkins-Regan E. Deprivation of maternal care has long-lasting consequences for the hypothalamic–pituitary–adrenal axis of zebra finches. Proc R Soc B 2012;279(1729):759–66.

Barrett RT, Skaare JU, Gabrielsen GW. Recent changes in levels of persistent organochlorines and mercury in eggs of seabirds from the Barents Sea. Environ Pollut 1996;92(1):13–8.

Bergman A, Heindel JJ, Jobling S, Kidd KA, Zoeller RT. State of the science of endocrine disrupting chemicals. United Nations Environment Programme; 2012.

Blas J, Bortolotti GR, Tella JL, Baos R, Marchant TA. Stress response during development predicts fitness in a wild, long lived vertebrate. PNAS 2007;104(21):8880–4.

Bogan JA, Newton I. Redistribution of DDE in sparrowhawks during starvation. Bull Environ Contam Toxicol 1977;18(3):317–21.

Bókony V, Lendvai ÁZ, Liker A, Angelier F, Wingfield JC, Chastel O. Stress response and the value of reproduction: are birds prudent parents? Am Nat 2009;173(5):589–98.

Bonier F, Martin PR, Moore IT, Wingfield JC. Do baseline glucocorticoids predict fitness? Trends Ecol Evol 2009;24(11):634–42.

Borgå K, Gabrielsen G, Skaare J. Biomagnification of organochlorines along a Barents Sea food chain. Environ Pollut 2001;113(2):187–98.

Brandt I, Bergman Å. PCB methyl sulphones and related compounds: identification of target cells and tissues in different species. Chemosphere 1987;16(8–9):1671–6.

Braune BM, Letcher RJ. Perfluorinated sulfonate and carboxylate compounds in eggs of seabirds breeding in the Canadian Arctic: temporal trends (1975–2011) and interspecies comparison. Environ Sci Technol 2013;47(1):616–24.

Braune BM, Mallory ML, Grant Gilchrist H, Letcher RJ, Drouillard KG. Levels and trends of organochlorines and brominated flame retardants in Ivory Gull eggs from the Canadian Arctic, 1976 to 2004. Sci Total Environ 2007;378(3):403–17.

Breuner CW, Patterson SH, Hahn TP. In search of relationships between the acute adrenocortical response and fitness. Gen Comp Endocrinol 2008;157(3):288–95.

Bustnes JO, Bakken V, Skaare JU, Erikstad KE. Age and accumulation of persistent organochlorines: a study of arctic-breeding glaucous gulls (*Larus hyperboreus*). Environ Toxicol Chem 2003a;22(9):2173–9.

Bustnes JO, Erikstad KE, Skaare JU, Bakken V, Mehlum F. Ecological effects of organochlorine pollutants in the arctic: a study of the glaucous gull. Ecol Appl 2003b;13(2):504–15.

Bustnes JO, Tveraa T, Varpe Ø, Henden JA, Skaare JU. Reproductive performance and organochlorine pollutants in an Antarctic marine top predator: the south polar skua. Environ Int 2007;33(7):911–8.

Bustnes JO, Fauchald P, Tveraa T, Helberg M, Skaare JU. The potential impact of environmental variation on the concentrations and ecological effects of pollutants in a marine avian top predator. Environ Int 2008;34(2):193–201.

Bustnes JO, Moe B, Herzke D, Hanssen SA, Nordstad T, Sagerup K, et al. Strongly increasing blood concentrations of lipid-soluble organochlorines in high arctic common eiders during incubation fast. Chemosphere 2010;79(3):320–5.

Bustnes JO, Moe B, Hanssen SA, Herzke D, Fenstad AA, Nordstad T, et al. Temporal dynamics of circulating persistent organic pollutants in a fasting seabird under different environmental conditions. Environ Sci Technol 2012;46(18):10287–94.

Butt CM, Mabury SA, Muir, Braune BM. Prevalence of long-chained perfluorinated carboxylates in seabirds from the Canadian Arctic between 1975 and 2004. Environ Sci Technol 2007;41(10):3521–8.

Calabrese EJ. Hormesis is central to toxicology, pharmacology and risk assessment. Hum Exp Toxicol 2010;29(4):249–61.

Cam E, Hines JE, Monnat J-Y, Nichols JD, Danchin E. Are adult non-breeders prudent parents? The kittiwake model. Ecology 1998;79(8):2917–30.

Chastel O, Weimerskirch H, Jouventin P. Body condition and seabird reproductive performance: a study of three petrel species. Ecology 1995;76(7):2240.

Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 1993;101(5):378–84.

Dawson A. Mechanisms of endocrine disruption with particular reference to occurrence in avian wildlife: a review. Ecotoxicology 2000;9(1–2):59–69.

De Wit CA, Alaee M, Muir DCG. Levels and trends of brominated flame retardants in the Arctic. Chemosphere 2006;64(2):209–33.

Falco MD, Sciarrillo R, Capaldo A, Russo T, Gay F, Valiante S, et al. The effects of the fungicide methyl thiophanate on adrenal gland morphophysiology of the lizard, *Podarcis sicula*. Arch Environ Contam Toxicol 2007;53(2):241–8.

Fuglei E, Bustnes JO, Hop H, Mørk T, Björnfoth H, van Bavel B. Environmental contaminants in arctic foxes (*Alopex lagopus*) in Svalbard: relationships with feeding ecology and body condition. Environ Pollut 2007;146(1):128–38.

Gabrielsen GW. Levels and effects of persistent organic pollutants in arctic animals. In: Ørbæk DJB, Kallenborn DR, Tombre DI, Hegseth DEN, Falk-Petersen DS, Hoel DAH, editors. Arctic alpine ecosystems and People in a Changing Environment. Berlin Heidelberg: Springer; 2007.

Gabrielsen GW, Henriksen E. Persistent organic pollutants in Arctic animals in the Barents Sea area and at Svalbard: levels and effects. Mem Natl Inst Polar Res Spec Issue 2001;54:349–64.

Gabrielsen GW, Skaare JU, Polder A, Bakken V. Chlorinated hydrocarbons in glaucous gulls (*Larus hyperboreus*) in the southern part of Svalbard. Sci Total Environ 1995;160–161:337–46.

Giesy JP, Feyk LA, Jones PD, Kannan K, Sanderson T. Review of the effects of endocrine-disrupting chemicals in birds. Pure Appl Chem 2003;75(11–12):2287–303.

Goutte A, Angelier F, Chastel CC, Trouvé C, Moe B, Bech C, et al. Stress and the timing of breeding: glucocorticoid-luteinizing hormones relationships in an arctic seabird. Gen Comp Endocrinol 2010a;169(1):108–16.

Goutte A, Angelier F, Welcker J, Moe B, Clément-Chastel C, Gabrielsen GW, et al. Long-term survival effect of corticosterone manipulation in black-legged kittiwakes. Gen Comp Endocrinol 2010b;167(2):246–51.

Goutte A, Antoine É, Weimerskirch H, Chastel O. Age and the timing of breeding in a long-lived bird: a role for stress hormones? Funct Ecol 2010c;24(5):1007–16.

Goutte A, Antoine É, Chastel O. Experimentally delayed hatching triggers a magnified stress response in a long-lived bird. Horm Behav 2011a;59(1):167–73.

Goutte A, Clément-Chastel C, Moe B, Bech C, Gabrielsen GW, Chastel O. Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes. J Exp Biol 2011b;214(12):2005–13.

Goutte A, Kriloff M, Weimerskirch H, Chastel O. Why do some adult birds skip breeding? A hormonal investigation in a long-lived bird. Biol Lett 2011c;7(5):790–2.

Harrison PTC, Holmes P, Humfrey CDN. Reproductive health in humans and wildlife: are adverse trends associated with environmental chemical exposure? Sci Total Environ 1997;205(2–3):97–106.

Harvey PW, Everett DJ. The adrenal cortex and steroidogenesis as cellular and molecular targets for toxicity: critical omissions from regulatory endocrine disrupter screening strategies for human health? J Appl Toxicol 2003;23(2):81–7.

Heath JA, Dufty Alfred M. Body condition and the adrenal stress response in captive American Kestrel juveniles. Physiol Zool 1998;71(1):67–73.

Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR, Kondrad SL, Erwin CA. Hormesis associated with a low dose of methyl-mercury injected into mallard eggs. Arch Environ Contam Toxicol 2012;62(1):141–4.

Helberg M, Bustnes JO, Erikstad KE, Kristiansen KO, Skaare JU. Relationships between reproductive performance and organochlorine contaminants in great black-backed gulls (*Larus marinus*). Environ Pollut 2005;134(3):475–83.

Henriksen EO, Gabrielsen GW, Skaare JU. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (*Rissa tridactyla*), in relation to mobilization of body-lipids associated with reproduction. Environ Pollut 1996;92(1):27–37.

Herzke D, Nygård T, Berger U, Huber S, Røv N. Perfluorinated and other persistent halogenated organic compounds in European shag (*Phalacrocorax aristotelis*) and common eider (*Somateria mollissima*) from Norway: a suburban to remote pollutant gradient. Sci Total Environ 2009;408(2):340–8.

Hinson JP, Raven PW. Effects of endocrine-disrupting chemicals on adrenal function. Best Pract Res Clin Endocrinol Metab 2006;20(1):111–20.

Hontela A. Chapter 12 adrenal toxicology: environmental pollutants and the HPI axis. In: Mommsen TP, Moon TW, editors. Biochemistry and molecular biology of fishes. Elsevier; 2005.

Johansson M, Nilsson S, Lund BO. Interactions between methylsulfonyl PCBs and the glucocorticoid receptor. Environ Health Perspect 1998;106(12):769–72.

Kenntner N, Krone O, Oehme G, Heidecke D, Tataruch F. Organochlorine contaminants in body tissue of free-ranging white-tailed eagles from northern regions of Germany. Environ Toxicol Chem 2003;22(7):1457–64.

Kitaysky AS, Wingfield JC, Piatt JF. Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. Funct Ecol 1999;13(5):577–84.

Kitaysky AS, Kitaiskaia EV, Wingfield JC, Piatt JF. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. J Comp Physiol B 2001;171(8):701–9.

Klotz DM, Ladlie BL, Vonier PM, McLachlan JA, Arnold SF. o, p′-DDT and its metabolites inhibit progesterone-dependent responses in yeast and human cells. Mol Cell Endocrinol 1997;129(1):63–71.

Lack D. Ecological adaptations for breeding in birds. London: Methuen; 1968.

Lanctot RB, Hatch SA, Gill VA, Eens M. Are corticosterone levels a good indicator of food availability and reproductive performance in a kittiwake colony? Horm Behav 2003;43(4):489–502.

Landys MM, Ramenofsky M, Wingfield JC. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen Comp Endocrinol 2006;148(2):132–49.

Lendvai ÁZ, Giraudeau M, Chastel O. Reproduction and modulation of the stress response: an experimental test in the house sparrow. Proc R Soc B 2007;274(1608):391–7.

Letcher RJ, Bustnes JO, Dietz R, Jenssen BM, Jørgensen EH, Sonne C, et al. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci Total Environ 2010;408(15):2995–3043.

Li L-A, Wang P-W. PCB126 induces differential changes in androgen, cortisol, and aldosterone biosynthesis in human adrenocortical H295R cells. Toxicol Sci 2005;85(1):530–40. [5 jan].

Author's personal copy

Longnecker MP, Klebanoff MA, Zhou H, Brock JW. Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. Lancet 2001;358(9276):110–4.
Lormée H, Jouventin P, Trouve C, Chastel O. Sex-specific patterns in baseline corticosterone and body condition changes in breeding red-footed boobies *Sula sula*. Ibis 2003;145(2):212–9.
Love OP, Shutt LJ, Silfies JS, Bortolotti GR, Smits JEG, Bird DM. Effects of dietary PCB exposure on adrenocortical function in captive American kestrels (*Falco sparverius*). Ecotoxicology 2003;12(1–4):199–208.
Lund BO, Bergman Å, Brandt I. Metabolic activation and toxicity of a DDT-metabolite, 3-methylsulphonyl-DDE, in the adrenal zona fasciculata in mice. Chem Biol Interact 1988;65(1):25–40.
Lundholm C. DDE-induced eggshell thinning in birds: effects of p, p′-DDE on the calcium and prostaglandin metabolism of the eggshell gland. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1997;118(2):113–28.
Lyon MF, Glenister PH. Reduced reproductive performance in androgen-resistant Tfm/Tfm female mice. Proc R Soc Lond B 1980;208(1170):1–12.
Moe B, Stempniewicz L, Jakubas D, Angelier F, Chastel O, Dinessen F, et al. Climate change and phenological responses of two seabird species breeding in the high-Arctic. Mar Ecol Prog Ser 2009;393:235–46.
Müllner A, Eduard Linsenmair K, Wikelski M. Exposure to ecotourism reduces survival and affects stress response in hoatzin chicks (*Opisthocomus hoazin*). Biol Conserv 2004;118(4):549–58.
Nordstad T, Moe B, Bustnes JO, Bech C, Chastel O, Goutte A, et al. *Relationships between* POPs and baseline corticosterone levels in black-legged kittiwakes (*Rissa tridactyla*) across their breeding cycle. Environ Pollut 2012;164:219–26.
Nøst TH, Helgason LB, Harju M, Heimstad ES, Gabrielsen GW, Jenssen BM. Halogenated organic contaminants and their correlations with circulating thyroid hormones in developing Arctic seabirds. Sci Total Environ 2012;414:248–56.
Odermatt A, Gumy C. Glucocorticoid and mineralocorticoid action: why should we consider influences by environmental chemicals? Biochem Pharmacol 2008;76(10):1184–93.
Oliveira FRT, Ferreira JR, dos Santos CMC, Macêdo LEM, de Oliveira RB, Rodrigues JA, et al. Estradiol reduces cumulative mercury and associated disturbances in the hypothalamus-pituitary axis of ovariectomized rats. Ecotoxicol Environ Saf 2006;63(3):488–93.
Ottinger MA, Carro T, Bohannon M, Baltos L, Marcell AM, McKernan M, et al. Assessing effects of environmental chemicals on neuroendocrine systems: potential mechanisms and functional outcomes. Gen Comp Endocrinol 2013;190:194–202.
Parkinson A, Ogilvie BW. Biotransformation of xenobiotics. Casarett and Dull's toxicology: the basic science of poisons. McGraw Hill, USA: C.D. Klaassen; 2008.
Perrins CM. The timing of birds' breeding seasons. Ibis 1970;112(2):242–55.
Phillips DIW, Jones A. Fetal programming of autonomic and HPA function: do people who were small babies have enhanced stress responses? J Physiol 2006;572(1):45–50.
R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2008. ISBN 3-900051-07-0. http://www.R-project.org.
Ricklefs RE, Wikelski M. The physiology/life-history nexus. Trends Ecol Evol 2002;17(10):462–8.
Romero LM. Using the reactive scope model to understand why stress physiology predicts survival during starvation in Galápagos marine iguanas. Gen Comp Endocrinol 2012;176(3):296–9.
Rosol TJ, Yarrington JT, Latendresse J, Capen CC. Adrenal gland: structure, function, and mechanisms of toxicity. Toxicol Pathol 2001;29(1):41–8.
Routti H, Helgason LB, Arukwe A, Wolkers H, Heimstad ES, Harju M, et al. Effect of reduced food intake on toxicokinetics of halogenated organic contaminants in herring gull (*Larus argentatus*) chicks. Environ Toxicol Chem 2013;32(1):156–64.
Savinova TN, Polder A, Gabrielsen GW, Skaare JU. Chlorinated hydrocarbons in seabirds from the Barents Sea area. Sci Total Environ 1995;160–161:497–504.
Saxena MC, Siddiqui MKJ, Seth TD, Murti CRK, Bhargava AK, Kutty D. Organochlorine pesticides in specimens from women undergoing spontaneous abortion, premature or full-term delivery. J Anal Toxicol 1981;5(1):6–9.
Tartu S, Goutte A, Bustamante P, Angelier F, Moe B, Clément-Chastel C, et al. To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biol lett 2013;9(4).
Taylor MR, Harrison PTC. Ecological effects of endocrine disruption: current evidence and research priorities. Chemosphere 1999;39(8):1237–48.
Tyler CR, Jobling S, Sumpter JP. Endocrine disruption in wildlife: a critical review of the evidence. Crit Rev Toxicol 1998;28(4):319–61.
Vallack HW, Bakker DJ, Brandt I, Broström-Lundén E, Brouwer A, Bull KR, et al. Controlling persistent organic pollutants—what next? Environ Toxicol Pharmacol 1998;6(3):143–75.
Van den Steen E, Covaci A, Jaspers VLB, Dauwe T, Voorspoels S, Eens M, et al. Experimental evaluation of the usefulness of feathers as a non-destructive biomonitor for polychlorinated biphenyls (PCBs) using silastic implants as a novel method of exposure. Environ Int 2007;33(2):257–64.
Verboven N, Verreault J, Letcher RJ, Gabrielsen GW, Evans NP. Adrenocortical function of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. Gen Comp Endocrinol 2010;166(1):25–32.
Verreault J, Berger U, Gabrielsen GW. Trends of perfluorinated alkyl substances in herring gull eggs from two coastal colonies in northern Norway: 1983–2003. Environ Sci Technol 2007;41(19):6671–7.
Verreault J, Verboven N, Gabrielsen GW, Letcher RJ, Chastel O. Changes in prolactin in a highly organohalogen contaminated Arctic top predator seabird, the glaucous gull. Gen Comp Endocrinol 2008;156(3):569–76.
Verreault J, Gabrielsen GW, Bustnes JO. The Svalbard glaucous gull as bioindicator species in the European Arctic: insight from 35 years of contaminants research. In: Whitacre DM, editor. Rev Environ Contam Toxicol, vol. 205. New York: Springer; 2010.
Vos JG, Dybing E, Greim HA, Ladefoged O, Lambré C, Tarazona JV, et al. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. CRC Crit Rev Toxicol 2000;30(1):71–133.
Weimerskirch H, Lallemand J, Martin J. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. J Anim Ecol 2005;74(2):285–91.
Wingfield JC, Sapolsky RM. Reproduction and resistance to stress: when and how. J Neuroendocrinol 2003;15(8):711–24.
Xu Y, Yu RMK, Zhang X, Murphy MB, Giesy JP, Lam MHW, et al. Effects of PCBs and MeSO2–PCBs on adrenocortical steroidogenesis in H295R human adrenocortical carcinoma cells. Chemosphere 2006;63(5):772–84.

PAPER IV

Corticosterone, prolactin and egg neglect behaviour in relation to mercury and legacy POPs in a long-lived Antarctic bird.

Science of the Total Environment 505 (2015) 180–188

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird

S. Tartu [a,*], F. Angelier [a], J.C. Wingfield [b], P. Bustamante [c], P. Labadie [d,e], H. Budzinski [d,e], H. Weimerskirch [a], J.O. Bustnes [f], O. Chastel [a]

[a] UMR 7372, CNRS-Université de La Rochelle, Villiers-en-bois, France
[b] Department of Neurobiology, Physiology and Behavior, University of CA, Davis, USA
[c] Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-Université de la Rochelle, La Rochelle, France
[d] Université de Bordeaux, EPOC/LPTC, UMR 5805, F-33400 Talence, France
[e] CNRS, EPOC/LPTC, UMR 5805, F-33400 Talence, France
[f] Norwegian Institute for Nature Research, FRAM — High North Research Centre on Climate and the Environment, NO-9296 Tromsø, Norway

HIGHLIGHTS

- We measured legacy POPs and Hg in the blood of adult snow petrels from Antarctica.
- POPs or Hg concentrations were not related to age.
- Stress-induced CORT concentrations were positively related to POP concentrations.
- Stress-induced PRL concentrations were negatively related to Hg concentrations.
- Hg concentrations were higher in males that neglected their egg.

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 1 October 2014
Accepted 2 October 2014
Available online xxxx

Editor: F. Riget

Keywords:
Snow petrel
Mercury
Persistent organic pollutants
Age
Reproduction

ABSTRACT

Seabirds often have high loads of contaminants. These contaminants have endocrine disrupting properties but their relationships with some endocrine mechanisms are still poorly investigated in free-living organisms. This is the case for the stress response which shifts energy investment away from reproduction and redirects it towards survival. In birds, this stress response is achieved through a release of corticosterone and is also accompanied by a decrease in circulating prolactin, an anterior pituitary hormone widely involved in regulating parental cares. We measured blood concentrations of some legacy persistent organic pollutants (POPs) and mercury (Hg) and examined their relationships with the corticosterone and prolactin responses of known-age (9–46 years old) incubating snow petrels (*Pagodroma nivea*) to a standardized capture/handling stress protocol. In this Antarctic seabird, we also investigated whether high contaminant burden correlates with a higher occurrence of egg neglect, a frequently observed behavior in snow petrels. POPs and Hg were unrelated to age. Stress-induced corticosterone concentrations were positively related to POPs in both sexes, and stress-induced prolactin concentrations were negatively related to Hg in males. Egg-neglect behavior was not related to POPs burden, but males with higher Hg concentrations were more likely to neglect their egg. This suggests that in birds, relationships between age and contaminants are complex and that even low to moderate concentrations of POPs and Hg are significantly related to hormonal secretion. In this Antarctic species, exposure to legacy POPs and Hg could make individuals more susceptible to environmental stressors such as ongoing disturbances in Polar Regions.
© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The parental phase is energy-demanding (Drent and Daan 1980) and individuals adopt different life-history strategies in order to cope with environmental stressors such as food shortage, predation or poor weather. In extreme environments, such as Polar Regions, animals often experience harsh and unpredictable environmental conditions, and as a result long-lived organisms such as seabirds may refrain from breeding or desert their brood (e.g. Angelier et al., 2007; Goutte et al., 2011a). At the physiological level, the release of glucocorticoid hormones (cortisol, corticosterone: CORT) during stressful events triggers physiological and behavioral adjustments that shift energy investment away from reproduction and redirects it towards self-preservation and

* Corresponding author.
E-mail address: tartu.sabrina@gmail.com (S. Tartu).

http://dx.doi.org/10.1016/j.scitotenv.2014.10.008
0048-9697/© 2014 Elsevier B.V. All rights reserved.

hence survival (Angelier and Wingfield, 2013; Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). Stress hormones have therefore a strong connection to fitness traits such as breeding success, individual quality and survival (Angelier et al., 2009a, 2010; Bonier et al., 2009; Bókony et al., 2009; Breuner et al., 2008; Goutte et al., 2010, 2011b; Kitaysky et al., 1999). Additionally, the hormone prolactin (PRL) can also mediate this life-history trade-off between reproduction and survival in free-living birds (reviewed in Angelier and Chastel, 2009). The release of this anterior pituitary hormone stimulates and facilitates parental behavior such as egg incubation and brood provisioning (Buntin, 1996). In response to acute stress, circulating PRL levels has been shown to decrease in several bird species (Angelier et al., 2013; Chastel et al., 2005), and this could ultimately trigger nest desertion if PRL levels remain low during a prolonged period (Angelier et al., 2007, 2009b; Angelier and Chastel, 2009; Heidinger et al., 2010). Importantly, this decrease in PRL levels varies between individuals and life-history stages, suggesting that birds can attenuate their PRL response to acute stress to ensure that reproduction is not inhibited when the fitness value of the current reproductive event is high (the 'brood value hypothesis'; Bókony et al., 2009; Lendvai et al., 2007). Thus, both CORT and PRL are very likely to mediate parental effort and parental investment in birds (Angelier et al., 2007, 2009b, 2013; Chastel et al., 2005; Criscuolo et al., 2005; Groscolas et al., 2008; Koch et al., 2004) and any disruption of these major endocrine cascades may alter the ability of an individual to adjust reproductive decisions to environmental conditions (Jenssen, 2005; Tartu et al., 2013).

In addition to extreme environmental conditions, climate change and anthropogenic disturbances (Clarke and Harris, 2003; Moline et al., 2008; Smetacek and Nicol, 2005), polar species are subjected to environmental pollution. Indeed, despite their remote location, polar areas are the fall-out region of contaminants which undergo long range transport such as persistent organic pollutants (POPs) and heavy metals (e.g. mercury: Hg). Indeed, because of climate characteristics, contaminants accumulate in the polar environment, where they may be bio-accumulated and for some compounds bio-magnified (Bargagli, 2008; Gordeev, 2002; Risebrough et al., 1976; Wania and Mackay, 1996; Wania, 2003). Moreover, long-lived organisms are thought to be highly sensitive to contaminants (Rowe, 2008), but there are surprisingly few data on the effect of age on contaminant levels, and it is not clear if seabirds accumulate POPs with increasing age (Bustnes et al., 2003).

Marine apex predators, such as seabirds, are particularly exposed (Gabrielsen, 2007; Rowe, 2008; van den Brink, 1997) and several studies have reported breeding impairments in highly polluted seabirds (Bustnes et al., 2001, 2003, 2007; Tartu et al., 2013; Verboven et al., 2009). Such breeding impairments could originate from the ability of contaminants to act as endocrine disruptors and thus, to alter the functioning of endocrine axes (Guillette and Gunderson, 2001; Ottinger et al., 2002, 2013; Tan et al., 2009; Tartu et al., 2013, 2014; Tyler et al., 1998). Experimental studies have documented some effects of chemicals on glucocorticoids (Love et al., 2003; Odermatt and Gumy, 2008), but the effects of contaminants on stress hormones in free-living organisms such as seabirds have rarely been studied (Bergman et al., 2013; Nordstad et al., 2012; Tartu et al., 2014; Verboven et al., 2010). It is therefore difficult to draw a general pattern of the relationships between contaminants and stress hormones.

Regarding the effects of contaminants on PRL, the knowledge is even poorer and only one study (Verreault et al., 2008) has addressed relationships between PRL secretion and POPs. In glaucous gulls (*Larus hyperboreus*) baseline PRL levels and the rate of decrease in PRL levels tended to vary negatively with organohalogen contaminants in males only (Verreault et al., 2008). Furthermore, numerous compounds are potential environmental contaminants (e.g. heavy metals, POPs), which may have different effects on hormones of the hypothalamo–pituitary–adrenal (HPA) axis, such as CORT but also PRL secretion. There is thus a need to determine whether different environmental contaminants can disrupt these hormones in free-living organisms. The aim of this study was to investigate the potential roles of environmental contaminants such as Hg and some legacy POPs (i.e. polychlorinated biphenyls: PCBs; organochlorine pesticides: OCPs; and polybrominated diphenyl ethers: PBDEs) on two major endocrine mechanisms: stress hormones from the HPA axis: CORT, and a key pituitary 'parental hormone': PRL. The snow petrel (*Pagodroma nivea*) a contaminated Antarctic seabird (Xie et al., 2008; Corsolini et al., 2011; Goutte et al., 2013) provides an ideal species to address these questions. In this long-lived species (until ~50 years old, Chastel et al., 1993), CORT and PRL responses to acute stress are modulated in relation to parental investment and incubation commitment (Angelier et al., 2007; Goutte et al., 2011c). For example, low stress-induced PRL levels are associated with a high probability of egg neglect, a frequently observed behavior in snow petrels (Angelier et al., 2007). Further, thanks to an exceptional long-term banding survey (1964–present; Barbraud and Weimerskirch, 2001; Chastel et al., 1993), many snow petrels are of known age, making it possible to address the effect of age on contaminant burden.

In that context, we investigated if POPs and/or Hg concentrations were related to 1) age, 2) CORT and/or PRL secretion and 3) parenting through egg-neglect behavior. We predicted that POPs and/or Hg: 1) would increase with increasing age as a result of bio-accumulation; 2) would increase CORT and decrease PRL secretion; 3) would be higher in individuals that neglected their egg.

2. Materials and methods

2.1. Ethics statement

Animals were cared for in accordance with the guidelines of the ethics committee of the Institut Polaire Français Paul Emile Victor (IPEV) that specifically approved this study (Program no. 109, H. Weimerskirch).

2.2. Study site, blood sampling and body-condition

Snow petrels are Antarctic seabirds with a delayed sexual maturity (~10 years of age), a low fecundity (one egg per clutch and a maximum of one clutch per year) and a long lifespan (~50 years old) (Chastel et al., 1993). Adult males and females were handled during the 2010 late incubation period (8–21 January). A total of 49 birds (27 males and 22 females) were caught in 49 different nests and age was known for 47 of them (9–46 years old). Birds were captured by hand and were then bled according to the standardized capture/restraint stress protocol described by Wingfield (1994). Immediately after capture (i.e. within 3 min), an initial blood sample (300 μl) was collected from the alar vein with a 1-ml heparinized syringe and a 25-gauge needle. These initial blood samples were considered to reflect baseline levels of CORT and PRL (Chastel et al., 2005; Romero and Reed, 2005; thereafter called 'baseline' sample). After collection of the initial blood samples birds were placed into cloth bags, and a subsequent sample (300 μl) was collected 30 min after capture (thereafter called 'stress-induced' sample). During handling of the adult birds, their eggs were covered with cotton and kept warm. After these blood samples, each bird was put back in its nest. Snow petrels are tame and usually resume parental duties as soon as returned to their nest (e.g. Angelier et al., 2007). After this acute stress protocol, petrels were left undisturbed at their nest for 20 min and were then captured again and blood sampled within 3 min of recapture (thereafter called 'post-stress' sample) to monitor how quickly hormone levels may return to baseline after a stressor. This blood sample was taken before CORT and PRL concentrations returned to normal, allowing us to effectively monitor the stress recovery. All birds were weighed to the nearest 2 g using a spring balance and their skull length (head + bill) was measured to the nearest 0.5 mm. Body condition index (thereafter 'body condition') was calculated as the residuals between body mass and skull length (regression: $F_{1,47} = 20.28$,

$p < 0.001$, $R^2 = 0.35$). After capture and blood sample, each nest was monitored twice a day until the manipulated petrel was relieved by its mate. We were therefore able to know whether a bird neglected its egg during the incubation bout following capture/restraint stress protocol (thereafter called 'egg neglect behavior'). Leaving eggs unattended temporarily is common in Procellariiform birds (Boersma and Wheelwright, 1979; Chaurand and Weimerskirch, 1994). Distant foraging and unpredictable weather increase the probability to delay an individual's returning to relieve its incubating partner (Boersma and Wheelwright, 1979). Eggs left unattended for a long period are less likely to hatch successfully (Boersma and Wheelwright, 1979; Angelier et al., 2007). In snow petrels were egg-neglect is often observed (Angelier et al, 2007), both parents incubate the single egg four bouts lasting ca. 4 to 8 days while the partner is feeding at sea (Ryan and Watkins, 1989). In two sampled birds, the egg was predated during the incubation bout following the capture/restraint stress protocol. Egg-neglect data were available for 47 birds.

2.3. Molecular sexing and hormone assay

Blood samples were centrifuged, and plasma was decanted and stored at −20 °C until assayed. After centrifugation, red cells were kept frozen for molecular sexing as well as for Hg determination. The sex was determined by polymerase chain reaction amplification of part of two highly conserved genes (CHD) present on the sex chromosomes at UMR 7372, CNRS-Université de La Rochelle, as detailed in Weimerskirch et al. (2005). Plasma concentrations of CORT were determined first by radioimmunoassay at UMR 7372, CNRS-Université de La Rochelle, as previously described (Lormée et al., 2003). Plasma concentrations of PRL were determined with the remaining plasma by a heterologous radioimmunoassay at UMR 7372, CNRS-Université de La Rochelle, as detailed in Cherel et al. (1994). The PRL assay has previously been validated in snow petrels (Angelier et al., 2007). All samples were run in one assay for both hormones. To measure intra-assay variation, we included 4 different referents 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. CORT and PRL concentrations were measured in baseline, stress-induced and post-stress samples.

2.4. Organic pollutants determination in plasma

POPs were measured in plasma samples collected from 15 females and 21 males only, since in 13 birds the remaining plasma volumes were too low. The targeted compounds included 7 indicator PCBs (CB-28, -52, -101, -118, -138, -153 and -180), 11 OCPs (HCB, Gamma HCH, Heptachlore, cis-chlordane, trans-nonachlor, 2,4′ DDE, 4,4′ DDE, 4,4′ DDD, 2,4′ DDT, 4,4′ DDT and mirex) and two PBDE (BDE-47 and BDE-99). Certified solutions containing all analytes in isooctane at 2 $ng \cdot \mu l^{-1}$ each were obtained from LGC Standards (Molsheim, France). To a plasma sample of 100 μl, internal standards (1 ng each) were added gravimetrically: CB-30, -103, -155 and -198 were used to quantify PCBs, p,p′-DDT-d8 was used to quantify OCPs and F-BDE-47 was used to quantify BDE-47 and BDE-99; standards were provided by either Dr. Ehrenstorfer GmbH, Cambridge Isotope Laboratory (via Cluzeau Info Labo, Sainte-Foy-La-Grande, France) or Chiron (via BCP Instruments, Irigny, France). POPs were extracted with 1 ml of pentane:dichloromethane (90:10; v/v); after centrifugation (2000 rpm, 2 min at 4 °C), the organic layer was collected and the operation was repeated. Both extracts were combined and purified on an acid silica gel column (40% H_2SO_4). After extract loading, analytes were eluted with 3 × 5 ml of pentane/dichloromethane (90/10; v/v). Extracts were then concentrated using a RapidVap vacuum evaporation system from Labconco (Kansas City, MO, USA) to a volume of 1 ml and further concentrated under a gentle stream of nitrogen (40 °C) after addition of 100 μl of isooctane as solvent keeper. Octachloronaphtalene (1 ng) was finally added to determine the recovery rate for each internal standard, for each sample (68–108%). Final extracts were analyzed by gas chromatography coupled with electron capture detection (GC-ECD) as described elsewhere (Tapie et al., 2011).

Quality control consisted in the analysis of standard solutions (NIST SRM 2261 and SRM 2262) and of procedural blanks (clean and empty glass tubes treated like a sample, one blank for 8 samples). Recoveries for standard solutions ranged from 89 to 104% with standard deviations lower than 13% ($n = 4$). Chicken plasma samples (Sigma-Aldrich, St. Quentin Fallavier, France) spiked with all analytes (3 $ng \cdot g^{-1}$ each) were analyzed; the recovery rates were in the range 77–103% with coefficients of variation lower than 17% ($n = 5$), except for CB-52 (22%) and mirex (29%). POP concentrations were blank corrected and the detection limit (LoD) was set at two times the mean blank value; for analytes that were not detected in blanks, LoD was determined as the concentration with a signal to noise ratio of 3 in spiked chicken plasma samples. Overall, LoDs ranged from 0.03 to 0.34 $ng \cdot g^{-1}$ wet weight (ww). Additionally, plasma total lipids were measured on an aliquot of 10 μl by the *sulfo-phospho-vanillin* (*SPV*) method for colorimetric determination (Frings et al., 1972).

2.5. Hg determination in blood cells

Total Hg was measured as described in details in Bustamante et al. (2006). Briefly, 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 analyzed 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 $\mu g \cdot g^{-1}$; with recoveries of 98 to 102%) and blanks, empty sample container, run every 20 samples. Hg concentrations are expressed in $\mu g \cdot g^{-1}$ dry weight (dw).

2.6. Statistical analyses

All analyses were performed using R 2.13.1 (http://r-project.org/). We first tested inter-correlations between the different families of POPs detected by using linear models (LM). Second, we used generalized linear model (GLM) with normal errors and an identity link function to test whether POPs or Hg were influenced by sex, body-condition and age (dependent variable: POPs and Hg, independent factor and variables: sex, body-condition and age). Third, we tested whether CORT and PRL kinetics differed between male and females by using repeated measures GLM with the time of sampling (baseline, stress-induced and post-stress levels) as the repeated measures; (dependent variable: CORT and PRL concentrations, independent factors: sex, time and their interaction). Fourth, we tested whether CORT concentration (baseline, stress-induced and post-stress) was related to POPs and Hg (dependent variable: CORT, independent factor and variables: POPs, sex, age, Hg, body-condition and their interaction with sex). For PRL we analyzed males and females separately (dependent variable: PRL, independent variables: POPs, age, Hg, body-condition) because in incubating snow petrels, females bear higher PRL concentrations than males (Angelier et al., 2007). Finally, we tested if the probability of neglecting the egg was related to POPs and Hg in males and females separately (dependent variable: egg neglect; independent variables: POPs, age, Hg, body-condition). To test the relationships between contaminants and egg-neglect behavior (yes or no) we used GLM with binomial error and logit link. Dependent continuous variables were previously tested for normality with a Shapiro–Wilk test and were log-transformed when necessary. Selected models were then checked for assumptions, that is, constancy of variance and residual normality. We performed all our model selection starting from the most general model that included all the variables/factors of interest and their interactions and we removed step by step the non-significant interactions, variables or factors. For POPs statistical analyses, concentrations below LoD were assigned LoD

value, and only compounds detected in at least 70% of the individuals were included into the sum of POPs (Noël et al., 2009).

3. Results

3.1. Contaminants: concentrations and compounds

Out of the 20 POP targeted compounds, 15 could be detected but the concentrations of four OCPs (cis-chlordane, trans-nonachlor, heptachlor and 2,4′-DDE) and one PBDE (BDE-99) were systematically below LoD. The most abundant compounds were the PCBs, with the PCBs 101 and 118 reaching the highest concentrations, closely followed by CB-138 and CB-153 (Table 1). Of the OCPs, HCB had the highest concentrations followed by 4,4′-DDE (Table 1). Only four PCBs (-CB101, -118, -138 and -153) and four OCPs (HCB, gamma HCH, 4,4′ DDE and 2,4′ DDT) were detected in at least 70% of the individuals and were thus included into the analyses. PCBs and OCPs were positively correlated (PCBs vs. OCPs: LM, $F_{1,34} = 18.1$, $p < 0.001$, $R^2 = 0.35$). Thus the global pollutant burden was described as a sum of POPs (hereafter 'POPs'). In incubating snow petrels, blood Hg averaged 1.91 ± 0.75 $\mu g \cdot g^{-1}$ dw, specifically in males 1.94 ± 0.77 $\mu g \cdot g^{-1}$ dw (range: 0.89–4.01) and in females 1.87 ± 0.73 $\mu g \cdot g^{-1}$ dw (range: 0.74–3.70).

3.2. Relationship between contaminants, sex, body-condition and age

During the incubation period, POPs was not statistically different between male and female snow petrels (GLM, $F_{1,34} = 3.21$, $p = 0.082$). Hg concentrations were not related to sex neither (GLM, $F_{1,47} = 0.12$, $p = 0.734$). POPs and Hg concentration, respectively, were unrelated to the body-condition index (POPs: $F_{1,34} = 1.05$, $p = 0.313$, interaction with sex: $F_{1,33} = 2.18$, $p = 0.149$; Hg: $F_{1,46} = 3.30$, $p = 0.076$, interaction with sex: $F_{1,45} = 0.14$, $p = 0.714$). POPs were not related to age ($F_{1,34} = 0.88$, $p = 0.355$, interaction with sex: $F_{1,33} = 0.61$, $p = 0.441$), and neither were Hg concentrations ($F_{1,45} = 2.05$, $p = 0.159$ interaction with sex: $F_{1,44} = 0.16$, $p = 0.693$). Finally, POPs and Hg were not related ($F_{1,34} = 2.09$, $p = 0.157$).

3.3. CORT and PRL kinetics: response and recovery to acute stress protocol

CORT concentrations significantly increased over 30 min from 4.7 ± 3.4 to 37.8 ± 8.6 $ng \cdot ml^{-1}$ and then declined 20 min post-stress to 23.6 ± 12.6 $ng \cdot ml^{-1}$ (GLMM, time as factor, $F_{2,94} = 269.98$, $p < 0.001$), without sex difference (sex: $F_{1,47} = 0.28$, $p = 0.600$; time × sex interaction: $F_{2,94} = 0.08$, $p = 0.926$). PRL concentrations significantly decreased over time (GLMM, time as factor, $F_{2,94} =$

Table 1
Concentrations of plasma persistent organic pollutants and blood Hg ($\mu g \cdot g^{-1}$) of incubating female and male snow petrels. For POPs, concentrations are given in wet-weight ($ng \cdot g^{-1}$ ww) in the first rows and lipid-weight ($ng \cdot g^{-1}$ lw) in the second rows.

	Females			Males		
	Mean	Standard deviation	Range (min–max)	Mean	Standard deviation	Range (min–max)
Lipids (%)	0.68	0.1	(0.48–0.88)	0.7	0.1	(0.50–0.94)
CB-50/28	0.4	0.6	(<0.10–2.1)	1.2	2.5	(<0.10–10.9)
	65.2	92.1	(<14.4–322.7)	152.2	286.7	(<14.4–1160.8)
CB-52	3.8	9.5	(<0.04–36.6)	10.0	16.3	(<0.04–63.1)
	617.1	1494.2	(<5.2–5719.4)	1300.2	1953.0	(<5.2–6709.3)
CB-101	6.9	14.4	(<0.02–57.4)	24.0	40.5	(<0.02–167.9)
	1082.1	2251.5	(<3.1–8976.5)	3151.6	5019	(<3.1–20,730.2)
CB-118	6.6	19.0	(<0.04–73.9)	22.7	38.4	(<0.04–150.6)
	1045	2960.4	(<5.4–11,543.5)	2963.4	4802.2	(<5.4–18,587.5)
CB-138	2.8	6.8	(<0.02–26.5)	12.1	17.5	(<0.02–52.4)
	441.5	1067	(<2.5–4136.9)	1601.4	2261.5	(<2.5–6469.2)
CB-153	2.6	6.3	(<0.04–24.6)	9.8	13.9	(<0.04–44.5)
	414.9	988.0	(<6.0–3845.6)	1297.4	1780.2	(<6.0–5488.8)
CB-180	0.2	0.6	(<0.02–2.2)	1.1	1.6	(<0.02–5.4)
	37	88.4	(<3.2–338.8)	145.8	204.3	(<3.2–666.0)
PCBs*	12.6	28.0	(1.6–110.7)	47.0	72.0	(1.8–270.2)
	1975.5	4379.2	(211.4–17,297.8)	5394.6	8427	(222.2–33,354.2)
HCB	1.3	1.0	(<0.01–3.2)	2.2	1.6	(<0.01–4.8)
	192.6	153.2	(<1.2–395.0)	296.6	223.9	(<1.2–779.1)
Gamma HCH	0.1	0.1	(<0.02–0.3)	0.2	0.1	(<0.02–0.5)
	16.9	19.8	(<2.1–47.8)	21.7	16.8	(<2.1–56.5)
4,4′-DDE	0.6	1.0	(<0.01–4.0)	0.4	0.4	(<0.01–1.2)
	92.7	168	(<1.7–669.3)	53.9	51.9	(<1.7–192.3)
4,4′-DDD	0.1	0.1	(<0.05–0.4)	0.1	0.2	(<0.05–0.9)
	8.9	17.5	(<7.3–65.7)	18.2	27.9	(<7.3–114.7)
2,4′-DDT	0.3	0.7	(<0.03–2.8)	1.1	1.9	(<0.03–6.6)
	47.4	114.9	(<4.6–433.5)	155.9	266.8	(<4.6–1018.7)
4,4′-DDT	0.3	0.6	(<0.02–2.2)	1.3	2.5	(<0.02–9.0)
	40.2	92.0	(<2.7–337.2)	176.3	350.8	(<2.7–1388.9)
Mirex	0.2	0.3	(<0.02–1.2)	0.6	1.0	(<0.02–3.4)
	26.8	49.3	(<2.6–190.4)	78.1	124.2	(<2.6–420.5)
OCPs**	2.3	1.8	(0.3–6.2)	3.9	2.6	(0.3–10.4)
	349.5	286.7	(33.1–1027.7)	531.9	375.3	(43.6–1593.1)
BDE-47	0.1	0.2	(<0.03–0.7)	0.2	0.3	(<0.03–0.9)
	10.2	29.8	(<4.3–115.4)	27.4	35.5	(<4.3–95.7)
POPs***	14.9	29.0	(2.3–116.1)	50.8	73.5	(3.9–275.3)
	2325.1	4533.2	(244.5–18,134.5)	5926.5	8645.2	(456.1–33,985.0)
Hg	1.8	0.7	(0.7–3.7)	1.9	0.8	(0.9–4.0)

* PCBs = CB101 + CB138 + CB153 + CB180.
** OCPs = HCB + -HCH + 4,4′-DDE + 2,4′-DDT.
*** POPs = PCBs + OCPs.

144.26, $p < 0.001$) and females had higher PRL concentrations than males (sex: $F_{1,47} = 152.72$, $p < 0.001$; time × sex interaction: $F_{2,94} = 3.79$, $p = 0.026$). In females, PRL decreased from 239.5 ± 36.3 to 169.4 ± 32.1 ng·ml^{-1} after 30 min and until 165.4 ± 30.8 ng·ml^{-1} 20 min post-stress. In males PRL concentrations decreased from 139.5 ± 30.7 to 90.9 ± 24.7 over 30 min and they reached 83.4 ± 24.0 ng·ml^{-1} 20 min post-stress.

3.4. Relationships between contaminants and CORT concentrations

CORT absolute concentrations (baseline, stress-induced and post-stress) were not related to sex, neither to age, Hg, body-condition and their interaction with sex (Table 2). POPs were not related to baseline CORT (Fig. 1A, Table 2), but increasing concentration of POPs was positively related to increasing concentration of stress-induced and post-stress CORT (Fig. 1B–C, Table 2).

3.5. Relationships between contaminants, PRL concentrations and egg-neglect

In females we did not find any relationship between PRL (baseline, stress-induced and post-stress) and POPs, age, Hg, or body-condition ($p > 0.08$ for all tests). In males baseline and post-stress PRL concentrations were not related to POPs, age, Hg, or body-condition ($p > 0.07$ for all tests), but increasing blood Hg concentration was related to decreasing stress-induced PRL concentration: i.e. after 30 min restraint the most contaminated males were less likely to maintain high concentrations of PRL (GLM, $F_{1,25} = 5.6$, $p = 0.0263$; Fig. 2). Eleven females and eight males were observed neglecting their egg, out of 21 and 26, respectively. Blood Hg concentration was higher in males that were more likely to neglect their egg (GLM, $\chi^2 = 5.4$, $p = 0.019$, Fig. 3) a relationship not found in females (GLM, $\chi^2 = 0.1$, $p = 0.796$, Fig. 3). Finally, egg neglect behavior was not related to POPs, age or body-condition in any sex ($p > 0.4$ for all tests).

Table 2
Relationships between contaminants (POPs and Hg), sex, age, body-condition and interaction with sex as a function of CORT concentrations (ng·ml^{-1}) in incubating snow petrels: a) baseline, b) stress-induced and c) post-stress. N is the number of birds of each variable. Degrees of freedom vary between measures of CORT because model selection was performed by starting from the most general model that included all the variables/factors of interest and their interactions and we removed step by step the non-significant interactions, variables or factors.

Dependent variables	N	Independent variables	*Df*	F	p-Value
a) Baseline CORT	36	Log (POPs)	1,27	0.14	0.715
	49	Sex	1,47	1.2	0.279
	47	Age	1,44	0.2	0.66
	49	Hg	1,42	0.09	0.767
	48	Body condition	1,39	1.73	0.195
	36	Log (POPs) × Sex	1,26	0.46	0.505
	47	Age × Sex	1,43	3.49	0.068
	49	Hg × Sex	1,41	3.29	0.077
	49	Body condition × Sex	1,38	0.05	0.819
b) Stress-induced CORT	36	**Log (POPs)**	**1,34**	**6.1**	**0.019**
	49	Sex	1,33	0.48	0.495
	47	Age	1,30	0.02	0.9
	49	Hg	1,28	0.01	0.915
	48	Body condition	1,31	2.82	0.103
	36	Log (POPs) × Sex	1,26	0	0.989
	47	Age × Sex	1,29	0.64	0.429
	49	Hg × Sex	1,27	0.21	0.653
	49	Body condition × Sex	1,32	3.97	0.055
d) Post-stress CORT	36	**Log (POPs)**	**1,34**	**5.17**	**0.029**
	49	Sex	1,33	1.2	0.282
	47	Age	1,30	0.33	0.57
	49	Hg	1,27	0.03	0.863
	48	Body condition	1,31	1.37	0.25
	36	log (POPs) × Sex	1,32	1.51	0.228
	47	Age × Sex	1,29	1.34	0.257
	49	Hg × Sex	1,26	0	0.955
	49	Body condition × Sex	1,28	0.18	0.676

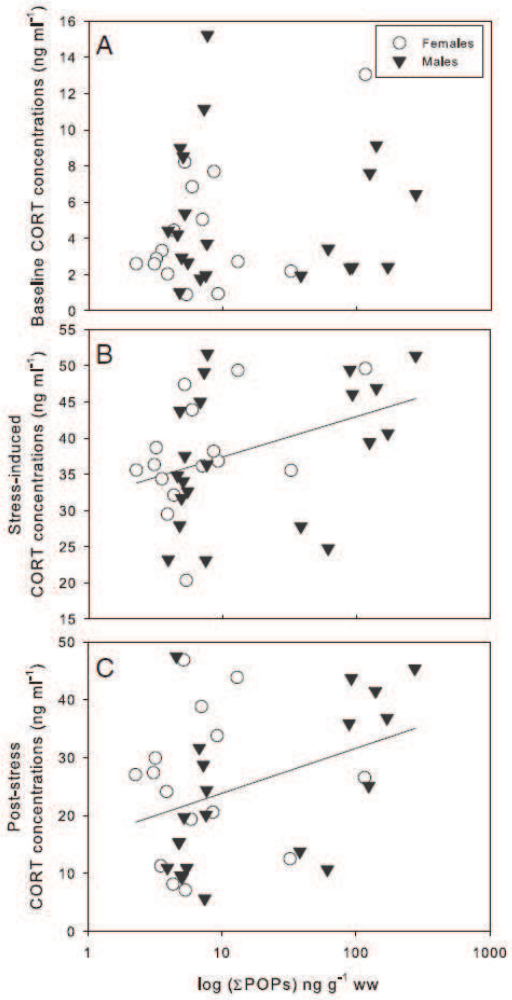


Fig. 1. Relationships between plasma POP concentrations (ng·g^{-1} wet weight) and plasma CORT concentrations (ng·ml^{-1}) in incubating snow-petrels (A) baseline, (B) stress-induced and (C) post-stress. White circles denote females and black triangles denote males. The solid line refers to statistically significant linear regression.

4. Discussion

The present study is the first to report plasma POP concentrations in the long-lived, Antarctic snow petrel. Firstly, there were no relationships between plasma POPs or blood Hg and age, suggesting that long-lived seabird are able to eliminate much of their contaminant burden. Secondly, POPs and Hg seem related to different hormonal pathways involved in reproductive decisions; POPs may disrupt the HPA axis whereas Hg was related to PRL secretion in males and consequently to egg-neglect behavior.

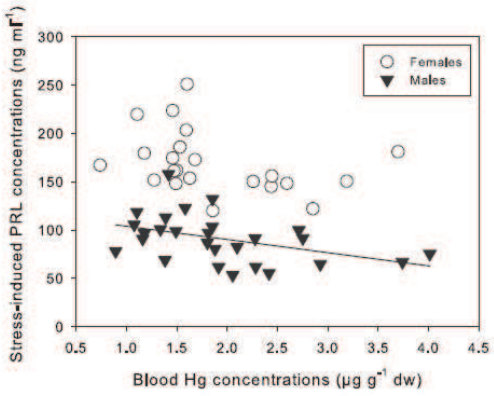


Fig. 2. Relationship between total blood Hg (µg·g^{-1} dry weight) and stress-induced plasma PRL concentrations (ng·ml^{-1}) in incubating snow-petrels. White circles denote females and black triangles denote males. The solid line refers to statistically significant linear regression for males.

4.1. Contaminants and age

Although snow petrels are very long-lived and thus exposed to contaminants over many years, no evidence was found that contamination was age-related, neither for POPs nor Hg. POPs and Hg measured in blood (plasma and red blood cells, respectively) can be correlated to levels found in storage organs and also adipose tissues, in birds but also chelonians and humans (Henriksen et al., 1998; Henny et al., 2002; Pauwels et al., 2000; Wayland et al., 2001; Keller et al., 2004; van de Merwe et al., 2010; Szumiło et al., 2013). Thus, blood contaminant concentration may be a good proxy of contaminant burden in other organs.

The relationship between Hg and age in seabirds is often contradictory, for example liver Hg was found to decrease, increase or be unrelated to age (Furness and Hutton, 1979; Hutton, 1981; Thompson et al., 1991). For blood, the relationship between Hg contamination and age is also not clear: no relationship was found between age and Hg contamination (Gonzáles-Solís et al., 2002; Tavares et al., 2013) but in pre-breeding snow petrels and incubating cape petrels (*Daption capense*), a negative relationship was found between blood Hg and age. This relationship was, however more likely the result of an age-related change in feeding ecology. With regard to POPs, it seems that in seabirds, concentrations in different tissues and blood increase until a steady-state is reached, often before the age of breeding (Donaldson et al., 1997; Newton et al., 1981; van den Brink et al., 1998), and for breeding birds, most studies have not observed any age-related POP accumulation (Bustnes et al., 2003; Newton et al., 1981). In this study, all snow petrels were breeders, and presumably they had already reached their steady-state levels.

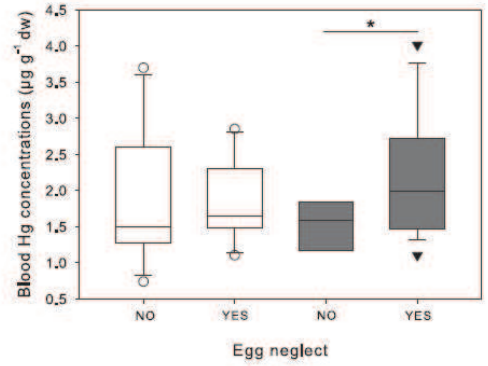


Fig. 3. Relationship between blood Hg (µg·g^{-1} dry weight) and egg-neglect behavior in incubating snow-petrels. The empty boxes denote females and the filled boxes denote males (*: $p < 0.02$).

Besides, seabirds can biotransform PCBs and eliminate POPs through their preen gland (Borgå et al., 2005; Henriksen et al., 1996; Solheim, 2010), in the same line Hg can be excreted through feather growth (Bearhop et al., 2000). These mechanisms could partially explain the lack of association between blood contaminants and increasing age. Also we have to remain cautious as we hypothesized that in snow petrels, as in other bird species, blood contaminants would represent levels in internal tissues (Henny et al., 2002; Szumiło et al., 2013; Wayland et al., 2001; Henriksen et al., 1998). However, we have no evidence for this relationship in snow petrels. Additionally, following food intake or lipid mobilization, contaminant levels in blood may fluctuate more than those in adipose tissues or liver and this could mask a hypothetical contaminant/age relationship.

4.2. POPs: concentrations and relationship with the CORT stress response

Very few studies have examined blood concentrations of POPs in adult Antarctic seabirds, and among the few studies, comparisons are made difficult due to different analytical methods. Indeed, POPs are often described as a "sum of compounds" and the compounds taken into the sum vary among studies. However, some OCPs are often reported individually since their detrimental effects have been well identified. This is the case of the HCB a relatively volatile compound (Calamari et al., 1991) principally used in fungicide formulations (Barber et al., 2005). Higher concentrations of this compound are commonly found in species restricted to the Antarctic region than those in temperate regions (e.g. van den Brink, 1997). HCB was the OCP with the highest concentrations in incubating snow petrels, but much lower than the concentrations found in other high-Antarctic species such as south polar skuas (*Catharacta maccormicki*) from Svarthamaren (71° 53′ S, 05° 10′ E) in Dronning Maud Land (Antarctica): i.e. HCB concentrations were 10-fold lower, and mirex concentrations 100-fold lower (Bustnes et al., 2006, 2007). These results could be the consequence of a different trophic level, toxicokinetic factors (e.g. metabolism, clearance rate), compound-specific physicochemical properties (K_{ow}, half-life) or depend on the bio-availability of contaminants in the breeding area (Walker et al., 2012).

The Arctic is more contaminated by POPs than Antarctica (Bustnes et al., 2006; Choi et al., 2008), HCB concentrations in snow petrels were slightly lower than those measured in plasma of incubating black-legged kittiwakes (*Rissa tridactyla*) from Svalbard: 1.85 ± 1.41 ng·g^{-1} ww versus 2.5 ± 0.44 ng·g^{-1} ww respectively. In comparison, incubating glaucous gulls (*Larus hyperboreus*) had HCB concentrations in plasma much higher than snow petrels (Verreault et al., 2005): on average ~400 ng·g^{-1} lipid weight whereas in snow petrels concentrations given in lipid weight average ~200 ng·g^{-1}. In both black-legged kittiwakes and glaucous gulls there is evidence of CORT disruption by POPs (Nordstad et al., 2012; Verboven et al., 2010). Indeed, in both species increasing POP concentrations were related to higher baseline CORT concentrations and for male glaucous gulls, higher POP concentrations were related to decreasing stress-induced CORT concentrations. In the present study, increasing POPs were not related to baseline CORT, but to stress-induced and post-stress CORT concentrations. Hence, the most polluted birds released more CORT when subjected to a handling stress protocol, and those concentrations remained high 20 min post-stress. These results are in accordance with the recent finding that POPs, and especially PCBs are associated with a higher adrenocortical response to an acute stress in pre-laying female black-legged kittiwakes (Tartu et al., 2014). However, although post-stress CORT concentrations were admittedly higher in the most contaminated snow petrels they did not decrease more slowly than in less polluted birds,

indicating that negative feedback from CORT on the hypothalamus and the pituitary was functional.

One possible explanation for the over-release of CORT could be related to an increase of the number of adreno-corticotrophic-hormone (ACTH) receptors (ACTH-R) on the adrenals. ACTH is one of the few polypeptide hormones having a positive trophic effect on its own receptors (Beuschlein et al., 2001; Penhoat et al., 1989). Thus, an increase of ACTH-R in the most POP contaminated snow petrels may be the consequence of an excess of ACTH input to adrenals. This suggests that POPs may alter the functioning of the pituitary by stimulating ACTH release and/or that POPs may mimic ACTH and bind to ACTH-R, which in that case would mobilize more ACTH-R from the adrenals of the most contaminated individuals. However this study is correlational, we cannot confirm without experimental support that the observed relationship is not the consequence of other intrinsic or extrinsic factors. Yet, an exacerbated secretion of CORT in response to a stressful event often mirrors poor fitness related traits as lowered parental investment (Angelier et al., 2009a; Bókony et al., 2009; Goutte et al., 2011b; Lendvai et al., 2007) or an impacted survival (Blas et al., 2007; Goutte et al., 2010; Romero, 2012). Nevertheless we did not find any relationship between POPs and parenting in terms of PRL concentration contrary to the study of Verreault et al. (2008) or egg-neglect behavior.

4.3. Hg: concentrations and relationships with stress-induced PRL and egg neglecting

Hg concentrations in incubating snow-petrels were within the range of those measured in the blood of south polar skuas breeding in Adélie land (2.15 ± 0.17 $\mu g \cdot g^{-1}$ dw, Goutte et al., 2014). In comparison with an Arctic breeding seabird, we also found comparable concentrations in incubating black-legged kittiwakes (average 1.6 ± 0.5 $\mu g \cdot g^{-1}$ dw). Contrary to other studies on free-ranging birds (Franceschini et al., 2009; Herring et al., 2012; Wada et al., 2009), we did not find any relationship between Hg and CORT secretion. Hg is well-known for its negative effects on breeding (reviewed in Tan et al., 2009). However, to the best of our knowledge, no studies have described relationships between Hg and PRL in free-living organisms. In humans, urinary Hg concentration was negatively correlated to plasma PRL (De Burbure and Bernard, 2006; Lucchini et al., 2002, 2003). In the present study, we found a similar relationship in incubating male snow petrels: increasing Hg concentrations were related to decreasing stress-induced PRL concentrations.

PRL is an anterior pituitary hormone, and a previous study on polar seabirds has described relationships between Hg and another anterior pituitary hormone: luteinizing hormone (LH, Tartu et al., 2013). Hg seemed to disrupt LH secretion via a lack of Gonadotropin-Releasing-Hormone (GnRH) input from the hypothalamus (Tartu et al., 2013). GnRH release is controlled by an area of the hypothalamus called *zona incerta* (Ben-Jonathan and Hnasko, 2001). Interestingly, this area also participates in the secretion of dopamine, a neuro-transmitter which is the principal antagonist of PRL (reviewed in Ben-Jonathan and Hnasko, 2001). Moreover, it has been well established that organic and inorganic Hg can stimulate the spontaneous release of dopamine in laboratory rodents (Faro et al., 1997, 2000, 2007; Minnema et al., 1989) but also in wild larvae of a fish (*Fundulus heteroclitus*, Zhou et al., 1999) and in wild American minks *Mustela vison*, where Hg induced a decrease of dopaminergic receptors and ligand affinity interpreted as an adaptive mechanism to prevent the hyperstimulation of the dopaminergic system (Basu et al., 2005). Additionally, when subjected to a stress, dopamine concentrations in blood increase (e.g. Finlay and Zigmond, 1997). Stress-induced dopamine synthesis in male snow petrels may thus be enhanced by Hg contamination, and result in a decrease of stress-induced PRL concentrations but not baseline or post-stress PRL concentrations. The fact that the most polluted birds quickly decrease their PRL concentrations when exposed to stress may highly affect their parental investment: they would be more likely to neglect their egg than less polluted birds. This goes together with the fact that in males, where PRL concentrations were lower than in females, the most polluted individuals were more likely to neglect their egg. In females, PRL concentrations and egg-neglect behavior were not related to Hg, maybe their PRL concentrations remained sufficiently high to prevent egg-neglect, a behavior associated with poor hatching success and chick mortality (Boersma and Wheelwright, 1979; Angelier et al., 2007).

5. Conclusion

In conclusion, there were no relationships between age and POPs or Hg, which is in line with most other studies. However we report significant relationships between contaminants and hormones involved in reproductive decisions. Over time, the action of POPs and Hg may jeopardize the maintenance of long-lived species populations. Indeed in long-lived species, that are expected to maximize their own survival rather than that of their brood, an exacerbated stress response as a consequence of POPs contamination and a decrease of PRL for the most Hg polluted males, are additional threats that may encourage individuals to refrain from breeding or desert their brood. To confirm the reported relationships, this study would greatly benefit from further experimental support.

Role of the funding source

This project was funded by Agence Nationale de la Recherche (ANR PolarTop, O. Chastel), the French Polar Institute (IPEV, Program 109, H. Weimerskirch) and the 7th Research Program of the European Community FP7/2007–2013 (Marie-Curie Fellowship, no. 237034, F. Angelier). The funders played no part in the design, analysis, or reporting of the study.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

The authors thank M. Kriloff, S. Lucchini, F. Amélineau, E. Cottin, and A.M. Thierry for their help in the field, C. Trouvé, C. Parenteau, S. Ruault, C. Churlaud, A. Carravieri, L. Peluhet and P. van Delft for their assistance in hormone, Hg and POPs assays and three anonymous referees for their very useful comments on a previous version of the manuscript.

References

Angelier F, Chastel O. Stress, prolactin and parental investment in birds: a review. Gen Comp Endocrinol 2009;163:142–8. http://dx.doi.org/10.1016/j.ygcen.2009.03.028.
Angelier F, Wingfield JC. Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. General and Comparative Endocrinology, 10th International Symposium on Avian. Endocrinology 2013;190:118–28.
Angelier F, Moe B, Weimerskirch H, Chastel O. Age-specific reproductive success in a long-lived bird: do older parents resist stress better? J Anim Ecol 2007;76:1181–91. http://dx.doi.org/10.1111/j.1365-2656.2007.01295.x.
Angelier F, Clément-Chastel C, Welcker J, Gabrielsen GW, Chastel O. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. Funct Ecol 2009a;23:784–93. http://dx.doi.org/10.1111/j.1365-2435.2009.01545.x.
Angelier F, Moe B, Blanc S, Chastel O. What factors drive prolactin and corticosterone responses to stress in a long-lived bird species (snow petrel *Pagodroma nivea*)? Physiol Biochem Zool 2009b;82:590–602. http://dx.doi.org/10.1086/592846.
Angelier F, Wingfield JC, Weimerskirch H, Chastel O. Hormonal correlates of individual quality in a long-lived bird: a test of the "corticosterone–fitness hypothesis". Biol Lett 2010;6:846–9. http://dx.doi.org/10.1098/rsbl.2010.0376.
Angelier F, Wingfield JC, Trouvé C, de Grissac S, Chastel O. Modulation of the prolactin and the corticosterone stress responses: do they tell the same story in a long-lived bird, the Cape petrel? Gen Comp Endocrinol 2013;182:7–15. http://dx.doi.org/10.1016/j.ygcen.2012.10.008.
Barber JL, Sweetman AJ, van Wijk D, Jones KC. Hexachlorobenzene in the global environment: emissions, levels, distribution, trends and processes. Sci Total Environ 2005;349:1–44. http://dx.doi.org/10.1016/j.scitotenv.2005.03.014.

Barbraud C, Weimerskirch H. Contrasting effects of the extent of sea-ice on the breeding performance of an Antarctic top predator, the Snow Petrel *Pagodroma nivea*. J Avian Biol 2001;32:297–302. http://dx.doi.org/10.1111/j.0908-8857.2001.320402.x.
Bargagli R. Environmental contamination in Antarctic ecosystems. Sci Total Environ 2008; 400:212–26. http://dx.doi.org/10.1016/j.scitotenv.2008.06.062.
Basu N, Klenavic K, Gamberg M, O'Brien M, Evans D, Scheuhammer AM, et al. Effects of mercury on neurochemical receptor-binding characteristics in wild mink. Environ Toxicol Chem 2005;24:1444–50. http://dx.doi.org/10.1897/04-048R.1.
Bearhop S, Ruxton GD, Furness RW. Dynamics of mercury in blood and feathers of great skuas. Environ Toxicol Chem 2000;19:1638–43. http://dx.doi.org/10.1002/etc.5620190622.
Ben-Jonathan N, Hnasko R. Dopamine as a prolactin (PRL) inhibitor. Endocr Rev 2001;22: 724–63. http://dx.doi.org/10.1210/er.22.6.724.
Bergman Å, Heindel JJ, Jobling S, Kidd KA, Zoeller RT. State of the science of endocrine disrupting chemicals 2012: an assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme and World Health Organization; 2013 [xxv + 260 pp.].
Beuschlein F, Fassnacht M, Klink A, Allolio B, Reincke M. ACTH-receptor expression, regulation and role in adrenocortical tumor formation. Eur J Endocrinol 2001;144: 199–206. http://dx.doi.org/10.1530/eje.0.1440199.
Blas J, Bortolotti GR, Tella JL, Baos R, Marchant TA. Stress response during development predicts fitness in a wild, long lived vertebrate. Proc Natl Acad Sci U S A 2007;104: 8880–4. http://dx.doi.org/10.1073/pnas.0700232104.
Boersma PD, Wheelwright NT. Egg neglect in the Procellariiformes: reproductive adaptations in the Fork-tailed Storm-Petrel. Condor 1979;157–165.
Bókony V, Lendvai ÁZ, Liker A, Angelier F, Wingfield JC, Chastel O. Stress response and the value of reproduction: are birds prudent parents? Am Nat 2009;173:589–98. http://dx.doi.org/10.1086/593131.
Bonier F, Martin PR, Moore IT, Wingfield JC. Do baseline glucocorticoids predict fitness? Trends Ecol Evol 2009;24:634–42. http://dx.doi.org/10.1016/j.tree.2009.04.013.
Borgå K, Wolkers H, Skaare JU, Hop H, Muir DCG, Gabrielsen GW. Bioaccumulation of PCBs in Arctic seabirds: influence of dietary exposure and congener biotransformation. Environ Pollut 2005;134:397–409. http://dx.doi.org/10.1016/j.envpol.2004.09.016.
Breuner CW, Patterson SH, Hahn TP. In search of relationships between the acute adrenocortical response and fitness. Gen Comp Endocrinol 2008;157:288–95. http://dx.doi.org/10.1016/j.ygcen.2008.05.017.
Buntin JD. Parental care: evolution, mechanisms, and adaptive significance. Academic Press; 1996.
Bustamante P, Lahaye V, Durnez C, Churlaud C, Caurant F. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: influence of geographical origin and feeding ecology. Sci Total Environ 2006;368:585–96. http://dx.doi.org/10.1016/j.scitotenv.2006.01.038.
Bustnes JO, Bakken V, Erikstad KE, Mehlum F, Skaare JU. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. J Appl Ecol 2001;38:791–801. http://dx.doi.org/10.1046/j.1365-2664.2001.00633.x.
Bustnes JO, Bakken V, Skaare JU, Erikstad KE. Age and accumulation of persistent organochlorines: a study of Arctic-breeding glaucous gulls (*Larus hyperboreus*). Environ Toxicol Chem 2003;22:2173–9. http://dx.doi.org/10.1897/02-456.
Bustnes JO, Tveraa T, Henden JA, Varpe Ø, Janssen K, Skaare JU. Organochlorines in Antarctic and Arctic avian top predators: a comparison between the south polar skua and two species of Northern Hemisphere gulls. Environ Sci Technol 2006;40:2826–31. http://dx.doi.org/10.1021/es051920q.
Bustnes JO, Tveraa T, Varpe Ø, Henden JA, Skaare JU. Reproductive performance and organochlorine pollutants in an Antarctic marine top predator: the south polar skua. Environ Int 2007;33:911–8. http://dx.doi.org/10.1016/j.envint.2007.04.010.
Calamari D, Bacci E, Focardi S, Gaggi C, Morosini M, Vighi M. Role of plant biomass in the global environmental partitioning of chlorinated hydrocarbons. Environ Sci Technol 1991;25:1489–95. http://dx.doi.org/10.1021/es00020a020.
Chastel O, Weimerskirch H, Jouventin P. High annual variability in reproductive success and survival of an Antarctic seabird, the snow petrel *Pagodroma nivea*. Oecologia 1993;94:278–85. http://dx.doi.org/10.1007/BF00341328.
Chastel O, Lacroix A, Weimerskirch H, Gabrielsen GW. Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird. Horm Behav 2005;47:459–66. http://dx.doi.org/10.1016/j.yhbeh.2004.10.009.
Chaurand T, Weimerskirch H. Incubation routine, body mass regulation and egg neglect in the blue petrel *Halobaena caerulea*. Ibis 1994;136:285–90.
Cherel Y, Mauget R, Lacroix A, Gilles J. Seasonal and fasting-related changes in circulating gonadal steroids and prolactin in king penguins, *Aptenodytes patagonicus*. Physiol Zool 1994;67:1154–73.
Choi S-D, Baek S-Y, Chang Y-S, Wania F, Ikonomou MG, Yoon Y-J, et al. Passive air sampling of polychlorinated biphenyls and organochlorine pesticides at the Korean Arctic and Antarctic research stations: implications for long-range transport and local pollution. Environ Sci Technol 2008;42:7125–31. http://dx.doi.org/10.1021/es801004p.
Clarke A, Harris CM. Polar marine ecosystems: major threats and future change. Environ Conserv 2003;30:1–25. http://dx.doi.org/10.1017/S0376892903000018.
Corsolini S, Borghesi N, Ademollo N, Focardi S. Chlorinated biphenyls and pesticides in migrating and resident seabirds from East and West Antarctica. Environ Int 2011;37: 1329–35.
Criscuolo F, Chastel O, Bertile F, Gabrielsen GW, Maho YL, Raclot T. Corticosterone alone does not trigger a short term behavioural shift in incubating female common eiders *Somateria mollissima*, but does modify long term reproductive success. J Avian Biol 2005;36:306–12. http://dx.doi.org/10.1111/j.0908-8857.2005.03371.x.
De Burbure C, Bernard A. Prolactin changes as a consequence of chemical exposure: de Burbure and Bernard respond. Environ Health Perspect 2006;114:A574.
Donaldson GM, Braune BM, Gaston AJ, Noble DG. Organochlorine and heavy metal residues in breast muscle of known-age thick-milled murres (*Uria lomvia*) from the Canadian Arctic. Arch Environ Contam Toxicol 1997;33:430–5. http://dx.doi.org/10.1007/s002449900273.
Drent RH, Daan S. The prudent parent: energetic adjustments in avian breeding. Ardea 1980;68(1–4):225–52.
Faro LRF, Durán R, do Nascimento JLM, Alfonso M, Picanço-Diniz CW. Effects of methyl mercury on the in vivo release of dopamine and its acidic metabolites DOPAC and HVA from striatum of rats. Ecotoxicol Environ Saf 1997;38:95–8. http://dx.doi.org/10.1006/eesa.1997.1567.
Faro LRF, do Nascimento JLM, José JMS, Alfonso M, Durán R. Intrastriatal administration of methylmercury increases in vivo dopamine release. Neurochem Res 2000;25:225–9. http://dx.doi.org/10.1023/A:1007571403413.
Faro LRF, Rodrigues KJA, Santana MB, Vidal L, Alfonso M, Durán R. Comparative effects of organic and inorganic mercury on in vivo dopamine release in freely moving rats. Braz J Med Biol Res 2007;40:1361–5. http://dx.doi.org/10.1590/S0100-879X2006005000157.
Finlay JM, Zigmond MJ. The effects of stress on central dopaminergic neurons: possible clinical implications. Neurochem Res 1997;22:1387–94. http://dx.doi.org/10.1023/A:1022075324164.
Franceschini MD, Lane OP, Evers DC, Reed JM, Hoskins B, Romero LM. The corticosterone stress response and mercury contamination in free-living tree swallows, *Tachycineta bicolor*. Ecotoxicology 2009;18:514–21. http://dx.doi.org/10.1007/s10646-009-0309-2.
Frings CS, Fendley TW, Dunn RT, Queen CA. Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. Clin Chem 1972;18:673–4.
Furness R, Hutton M. Pollutant levels in the great skua *Catharacta skua*. Environ Pollut 1979;19:261–8. http://dx.doi.org/10.1016/0013-9327(79)90118-6.
Gabrielsen GW. Levels and effects of persistent organic pollutants in arctic animals. In: Ørbæk DJB, Kallenborn DR, Tombre DI, Hegseth DEN, Falk-Petersen DS, Hoel DAH, editors. Arctic Alpine ecosystems and people in a changing environment. Springer Berlin Heidelberg; 2007. p. 377–412.
Gonzâles-Solís J, Sanpera C, Ruiz X. Metals and selenium as bioindicators of geographic and trophic segregation in giant petrels *Macronectes* spp. Mar Ecol Prog Ser 2002; 244:257–64. http://dx.doi.org/10.3354/meps244257.
Gordeev V. Pollution of the Arctic. Reg Environ Chang 2002;3:88–98. http://dx.doi.org/10.1007/s10113-002-0041-4.
Goutte A, Angelier F, Welcker J, Moe B, Clément-Chastel C, Gabrielsen GW, et al. Long-term survival effect of corticosterone manipulation in Black-legged kittiwakes. Gen Comp Endocrinol 2010;167:246–51. http://dx.doi.org/10.1016/j.ygcen.2010.03.018.
Goutte A, Kriloff M, Weimerskirch H, Chastel O. Why do some adult birds skip breeding? A hormonal investigation in a long-lived bird. Biol Lett 2011a;7:790–2. http://dx.doi.org/10.1098/rsbl.2011.0196.
Goutte A, Antoine E, Chastel O. Experimentally delayed hatching triggers a magnified stress response in a long-lived bird. Horm Behav 2011b;59:167–73. http://dx.doi.org/10.1016/j.yhbeh.2010.11.004.
Goutte A, Clément-Chastel C, Moe B, Bech C, Gabrielsen GW, Chastel O. Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes. J Exp Biol 2011c;214:2005–13. http://dx.doi.org/10.1242/jeb.051979.
Goutte A, Chevreuil M, Alliot F, Chastel O, Cherel Y, Eléaume M, et al. Persistent organic pollutants in benthic and pelagic organisms off Adélie Land, Antarctica. Mar Pollut Bull 2013;77:82–9.
Goutte A, Bustamante P, Barbraud C, Delord K, Weimerskirch H, Chastel O. Demographic responses to mercury exposure in two closely-related Antarctic top predators. Ecology 2014. http://dx.doi.org/10.1890/13-1229.1.
Groscolas R, Lacroix A, Robin J-P. Spontaneous egg or chick abandonment in energy-depleted king penguins: a role for corticosterone and prolactin? Horm Behav 2008; 53:51–60. http://dx.doi.org/10.1016/j.yhbeh.2007.08.010.
Guillette LJ, Gunderson MP. Alterations in development of reproductive and endocrine systems of wildlife populations exposed to endocrine-disrupting contaminants. Reproduction 2001;122:857–64. http://dx.doi.org/10.1530/rep.0.1220857.
Heidinger BJ, Chastel O, Nisbet ICT, Ketterson ED. Mellowing with age: older parents are less responsive to a stressor in a long-lived seabird. Funct Ecol 2010;24:1037–44. http://dx.doi.org/10.1111/j.1365-2435.2010.01733.x.
Henny CJ, Hill EF, Hoffman DJ, Spalding MG, Grove RA. Nineteenth century mercury: hazard to wading birds and cormorants of the Carson River, Nevada. Ecotoxicology 2002; 11:213–31. http://dx.doi.org/10.1023/A:1016327602656.
Henriksen EO, Gabrielsen GW, Skaare JU. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (*Rissa tridactyla*), in relation to mobilization of body-lipids associated with reproduction. Environ Pollut 1996;92:27–37. http://dx.doi.org/10.1016/0269-7491(95)00087-9.
Henriksen EO, Gabrielsen GW, Utne Skaare J. Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gulls, *Larus hyperboreus*. Chemosphere 1998;37:2627–43. http://dx.doi.org/10.1016/S0045-6535(98)00162-3.
Herring G, Ackerman JT, Herzog MP. Mercury exposure may suppress baseline corticosterone levels in juvenile birds. Environ Sci Technol 2012;46:6339–46. http://dx.doi.org/10.1021/es300668c.
Hutton M. Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. Environ Pollut Ser A Ecol Biol 1981;26:129–45. http://dx.doi.org/10.1016/0143-1471(81)90043-X.
Jenssen BM. Endocrine-disrupting chemicals and climate change: a worst-case combination for Arctic marine mammals and seabirds? Environ Health Perspect 2005;114: 76–80. http://dx.doi.org/10.1289/ehp.8057.
Keller JM, Kucklick JR, Harms CA, McClellan-Green PD. Organochlorine contaminants in sea turtles: correlations between whole blood and fat. Environ Toxicol Chem 2004; 23:726–38. http://dx.doi.org/10.1897/03-254.

Kitaysky AS, Wingfield JC, Piatt JF. Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. Funct Ecol 1999;13:577–84. http://dx.doi.org/10.1046/j.1365-2435.1999.00352.x.
Koch KA, Wingfield JC, Buntin JD. Prolactin-induced parental hyperphagia in ring doves: are glucocorticoids involved? Horm Behav 2004;46:498–505. http://dx.doi.org/10.1016/j.yhbeh.2004.02.012.
Lendvai AZ, Giraudeau M, Chastel O. Reproduction and modulation of the stress response: an experimental test in the house sparrow. Proc R Soc B 2007;274:391–7. http://dx.doi.org/10.1098/rspb.2006.3735.
Lormée H, Jouventin P, Trouve C, Chastel O. Sex-specific patterns in baseline corticosterone and body condition changes in breeding red-footed boobies *Sula sula*. Ibis 2003;145:212–9. http://dx.doi.org/10.1046/j.1474-919X.2003.00106.x.
Love OP, Shutt LJ, Silfies JS, Bortolotti GR, Smits JEG, Bird DM. Effects of dietary PCB exposure on adrenocortical function in captive American kestrels (*Falco sparverius*). Ecotoxicology 2003;12:199–208. http://dx.doi.org/10.1023/A:1022502826800.
Lucchini R, Cortesi I, Facco P, Benedetti L, Camerino D, Carta P, et al. Neurotoxic effect of exposure to low doses of mercury. Med Lav 2002;93:202–14.
Lucchini R, Calza S, Camerino D, Carta P, Decarli A, Parrinello G, et al. Application of a latent variable model for a multicenter study on early effects due to mercury exposure. Neurotoxicology 2003;24:605–16. http://dx.doi.org/10.1016/S0161-813X(03)00048-2.
Minnema DJ, Cooper GP, Greenland RD. Effects of methylmercury on neurotransmitter release from rat brain synaptosomes. Toxicol Appl Pharmacol 1989;99:510–21. http://dx.doi.org/10.1016/0041-008X(89)90158-0.
Moline MA, Karnovsky NJ, Brown Z, Divoky GJ, Frazer TK, Jacoby CA, et al. High latitude changes in ice dynamics and their impact on polar marine ecosystems. Ann N Y Acad Sci 2008;1134:267–319. http://dx.doi.org/10.1196/annals.1439.010.
Newton I, Bogan J, Marquiss M. Organochlorine contamination and age in sparrowhawks. Environ Pollut Ser A Ecol Biol 1981;25:155–60. http://dx.doi.org/10.1016/0143-1471(81)90016-7.
Noël M, Barrett-Lennard L, Guinet C, Dangerfield N, Ross PS. Persistent organic pollutants (POPs) in killer whales (*Orcinus orca*) from the Crozet Archipelago, southern Indian Ocean. Mar Environ Res 2009;68:196–202. http://dx.doi.org/10.1016/j.marenvres.2009.06.009.
Nordstad T, Moe B, Bustnes JO, Bech C, Chastel O, Goutte A, et al. Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (*Rissa tridactyla*) across their breeding cycle. Environ Pollut 2012;164:219–26. http://dx.doi.org/10.1016/j.envpol.2012.01.044.
Odermatt A, Gumy C. Glucocorticoid and mineralocorticoid action: why should we consider influences by environmental chemicals? Biochem Pharmacol 2008;76:1184–93. http://dx.doi.org/10.1016/j.bcp.2008.07.019.
Ottinger MA, Abdelnabi M, Quinn M, Golden N, Wu J, Thompson N. Reproductive consequences of EDCs in birds: what do laboratory effects mean in field species? Neurotoxicol Teratol 2002;24:17–28. http://dx.doi.org/10.1016/S0892-0362(01)00195-7.
Ottinger MA, Carro T, Bohannon M, Baltos L, Marcell AM, McKernan M, et al. Assessing effects of environmental chemicals on neuroendocrine systems: potential mechanisms and functional outcomes. Gen Comp Endocrinol 2013;190:194–202. http://dx.doi.org/10.1016/j.ygcen.2013.06.004.
Pauwels A, Covaci A, Weyler J, Delbeke L, Dhont M, Sutter PD, et al. Comparison of persistent organic pollutant residues in serum and adipose tissue in a female population in Belgium, 1996–1998. Arch Environ Contam Toxicol 2000;39:265–70. http://dx.doi.org/10.1007/s002440010104.
Penhoat A, Jaillard C, Saez JM. Corticotropin positively regulates its own receptors and cAMP response in cultured bovine adrenal cells. Proc Natl Acad Sci U S A 1989;86:4978–81.
Ricklefs RE, Wikelski M. The physiology/life-history nexus. Trends Ecol Evol 2002;17:462–8. http://dx.doi.org/10.1016/S0169-5347(02)02578-8.
Risebrough RW, De Lappe BW, Schmidt TT. Bioaccumulation factors of chlorinated hydrocarbons between mussels and seawater. Mar Pollut Bull 1976;7:225–8. http://dx.doi.org/10.1016/0025-326X(76)90266-6.
Romero LM. Using the reactive scope model to understand why stress physiology predicts survival during starvation in Galápagos marine iguanas. Gen Comp Endocrinol 2012;176:296–9. http://dx.doi.org/10.1016/j.ygcen.2011.11.004.
Romero LM, Reed JM. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp Biochem Physiol A Mol Integr Physiol 2005;140:73–9. http://dx.doi.org/10.1016/j.cbpb.2004.11.004.
Rowe CL. "The calamity of so long life": life histories, contaminants, and potential emerging threats to long-lived vertebrates. Bioscience 2008;58:623–31. http://dx.doi.org/10.1641/B580709.
Ryan PG, Watkins BP. Snow petrel breeding biology at an inland site in continental Antarctica. Colon Waterbirds 1989;12:176. http://dx.doi.org/10.2307/1521338.
Smetacek V, Nicol S. Polar ocean ecosystems in a changing world. Nature 2005;437:362–8. http://dx.doi.org/10.1038/nature04161.
Solheim SA. The preen gland — an organ for excretion of persistent organic pollutants in black-legged kittiwake (*Rissa tridactyla*). Master thesis University of Bergen; 2010.
Szumiło E, Szubska M, Meissner W, Bełdowska M, Falkowska L. Mercury in immature and adults herring gulls (*Larus argentatus*) wintering on the Gulf of Gdańsk area. Ocean Hydro 2013;42:260–7. http://dx.doi.org/10.2478/s13545-013-0082-y.
Tan SW, Meiller JC, Mahaffey KR. The endocrine effects of mercury in humans and wildlife. Crit Rev Toxicol 2009;39:228–69. http://dx.doi.org/10.1080/10408440802233259.
Tapie N, Menach KL, Pasquaud S, Elie P, Devier MH, Budzinski H. PBDE and PCB contamination of eels from the Gironde estuary: from glass eels to silver eels. Chemosphere 2011;83:175–85. http://dx.doi.org/10.1016/j.chemosphere.2010.12.044.
Tartu S, Goutte A, Bustamante P, Angelier F, Moe B, Clément-Chastel C, et al. To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biol Lett 2013;9:20130317. http://dx.doi.org/10.1098/rsbl.2013.0317.
Tartu S, Angelier F, Herzke D, Moe B, Bech C, Gabrielsen GW, et al. The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. Sci Total Environ 2014;476–477:553–60. http://dx.doi.org/10.1016/j.scitotenv.2014.01.060.
Tavares S, Xavier JC, Phillips RA, Pereira ME, Pardal MA. Influence of age, sex and breeding status on mercury accumulation patterns in the wandering albatross *Diomedea exulans*. Environ Pollut 2013;181:315–20. http://dx.doi.org/10.1016/j.envpol.2013.06.032.
Thompson DR, Hamer KC, Furness RW. Mercury accumulation in great skuas *Catharacta skua* of known age and sex, and its effects upon breeding and survival. J Appl Ecol 1991;28:672–84.
Tyler CR, Jobling S, Sumpter JP. Endocrine disruption in wildlife: a critical review of the evidence. Crit Rev Toxicol 1998;28:319–61. http://dx.doi.org/10.1080/10408449891344236.
Van de Merwe JP, Hodge M, Olszowy HA, Whittier JM, Lee SY. Using blood samples to estimate persistent organic pollutants and metals in green sea turtles (*Chelonia mydas*). Mar Pollut Bull 2010;60:579–88.
Van den Brink NW. Directed transport of volatile organochlorine pollutants to Polar Regions: the effect on the contamination pattern of Antarctic seabirds. Sci Total Environ 1997;198:43–50. http://dx.doi.org/10.1016/S0048-9697(97)05440-5.
Van den Brink NW, van Franeker JA, de Ruiter-Dijkman EM. Fluctuating concentrations of organochlorine pollutants during a breeding season in two Antarctic seabirds: Adelie penguin and southern fulmar. Environ Toxicol Chem 1998;17:702–9. http://dx.doi.org/10.1002/etc.5620170426.
Verboven N, Verreault J, Letcher RJ, Gabrielsen GW, Evans NP. Nest temperature and parental behaviour of Arctic-breeding glaucous gulls exposed to persistent organic pollutants. Anim Behav 2009;77:411–8. http://dx.doi.org/10.1016/j.anbehav.2008.10.022.
Verboven N, Verreault J, Letcher RJ, Gabrielsen GW, Evans NP. Adrenocortical function of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. Gen Comp Endocrinol 2010;166:25–32. http://dx.doi.org/10.1016/j.ygcen.2009.11.013.
Verreault J, Letcher RJ, Muir DCG, Chu S, Gebbink WA, Gabrielsen GW. New organochlorine contaminants and metabolites in plasma and eggs of glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. Environ Toxicol Chem 2005;24:2486. http://dx.doi.org/10.1897/05-067R.1.
Verreault J, Verboven N, Gabrielsen GW, Letcher RJ, Chastel O. Changes in prolactin in a highly organohalogen contaminated Arctic top predator seabird, the glaucous gull. Gen Comp Endocrinol 2008;156:569–76. http://dx.doi.org/10.1016/j.ygcen.2008.02.013.
Wada H, Cristol DA, McNabb FMA, Hopkins WA. Suppressed adrenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. Environ Sci Technol 2009;43:6031–8. http://dx.doi.org/10.1021/es803707f.
Walker CH, Sibly RM, Hopkin SP, Peakall DB. Principles of ecotoxicology. 4th ed. CRC Press; 2012.
Wania F. Assessing the potential of persistent organic chemicals for long-range transport and accumulation in Polar Regions. Environ Sci Technol 2003;37:1344–51. http://dx.doi.org/10.1021/es026019e.
Wania F, MacKay D. Peer reviewed: tracking the distribution of persistent organic pollutants. Environ Sci Technol 1996;30:390A–6A. http://dx.doi.org/10.1021/es962399q.
Wayland M, Garcia-Fernandez AJ, Neugebauer E, Gilchrist HG. Concentrations of cadmium, mercury and selenium in blood, liver and kidney of common eider ducks from the Canadian Arctic. Environ Monit Assess 2001;71:255–67. http://dx.doi.org/10.1023/A:1011850000360.
Weimerskirch H, Lallemand J, Martin J. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. J Anim Ecol 2005;74:285–91. http://dx.doi.org/10.1111/j.1365-2656.2005.00922.x.
Wingfield J. Modulation of the adrenocortical response to stress in birds. Perspectives in comparative endocrinology; 1994. p. 520–8.
Wingfield JC, Sapolsky RM. Reproduction and resistance to stress: when and how. J Neuroendocrinol 2003;15:711–24. http://dx.doi.org/10.1046/j.1365-2826.2003.01033.x.
Xie Z, Zhang P, Sun L, Xu S, Huang Y, He W. Microanalysis of metals in barbs of a snow petrel (*Pagodroma nivea*) from the Antarctica using synchrotron radiation X-ray fluorescence. Mar Pollut Bull 2008;56:516–24. http://dx.doi.org/10.1016/j.marpolbul.2007.11.015.
Zhou T, Rademacher DJ, Steinpreis RE, Weis JS. Neurotransmitter levels in two populations of larval *Fundulus heteroclitus* after methylmercury exposure. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1999(124):287–94. http://dx.doi.org/10.1016/S0742-8413(99)00077-8.

male snow petrels may thus be enhanced by Hg contamination, and result in a decrease of stress-induced PRL concentrations but not baseline or post-stress PRL concentrations. The fact that the most polluted birds quickly decrease their PRL concentrations when exposed to stress may highly affect their parental investment: they would be more likely to

Angelier F, Wingfield JC, Trouvé C, de Grissac S, Chastel O. Modulation of the prolactin and the corticosterone stress responses: do they tell the same story in a long-lived bird, the Cape petrel? Gen Comp Endocrinol 2013;182:7–15. http://dx.doi.org/10.1016/j.ygcen.2012.10.008.
Barber JL, Sweetman AJ, van Wijk D, Jones KC. Hexachlorobenzene in the global environment: emissions, levels, distribution, trends and processes. Sci Total Environ 2005;349:1–44. http://dx.doi.org/10.1016/j.scitotenv.2005.03.014.

PAPER V

Co-stressors: does corticosterone reinforce the endocrine consequences of mercury exposure in an Arctic seabird?

Co-stressors: does corticosterone reinforce the endocrine consequences of mercury exposure in an Arctic seabird?

SABRINA TARTU[1], FRÉDÉRIC ANGELIER[1], PACO BUSTAMANTE[2], ADAM Z. LENDVAI[3], BØRGE MOE[4], PIERRE BLEVIN[1], CLAUS BECH[5], GEIR W. GABRIELSEN[6], JAN OVE BUSTNES[4], OLIVIER CHASTEL[1]

[1]*Centre d'Etudes Biologiques de Chizé UMR7372 CNRS-Université La Rochelle, F-79360, France*
[2] *Littoral Environnement Société (LIENSs), CNRS-Université La Rochelle, F-17000, France*
[3]*Department of Biological Sciences (0406), Derring Hall RM 4102, Virginia Tech 1405 Perry Street*
[4]*Norwegian Institute for Nature Research, FRAM – High North Research Centre on Climate and the Environment, N-9296 Tromsø, Norway*
[5]*Department of Biology, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway*
[6]*Norwegian Polar Research Institute, FRAM Centre High North Research on Climate and the Environment N-9296 Tromsø, Norway*
Corresponding author: tartu.sabrina@gmail.com

Abstract

In extreme environments, such as Polar Regions, nest desertion is a common behavioral response of seabirds. The decision to abandon current reproduction during unfavorable conditions is thought to be selected in order to favor the long-term survival of these long-lived organisms, and is governed by two key hormones: prolactin (PRL, a hormone involved in parental care) and corticosterone (CORT, a stress hormone). Any disruption of these endocrine mechanisms may therefore alter the ability of an individual to adjust reproductive decisions to environmental conditions. Additionally to ongoing environmental changes, polar seabirds are exposed to increasing concentration of some contaminants such as mercury (Hg), a well-known endocrine disruptor. However, there is to date no available study investigating Hg- PRL relationships in free-living animals. The first aim of this study was therefore to investigate the relationships between plasma PRL and blood Hg concentrations and their consequences for reproductive performances in Svalbard black-legged kittiwakes Rissa tridactyla. We reported negative relationships between plasma baseline (males only), stress-induced PRL (both sexes) and blood Hg levels during incubation and chick-rearing period; Hg concentrations were negatively related to breeding success in chick-rearing males. Then we tested the hypothesis that stressors and hence associated CORT release could reinforce the negative relationship between PRL and Hg. To do so, we implanted incubating males bearing various levels of Hg with subcutaneous CORT pellets, and predicted that the negative relationships between Hg and PRL should be more pronounced in the CORT-implanted group, compared to the placebo one. Additionally, the most Hg contaminated individuals of the CORT-implanted group should suffer the highest breeding failure. According to our prediction, the experimental CORT increase appeared to reinforce the Hg-related decrease in stress-induced PRL concentration over an 11 days period. However, the experimental CORT increase did not reinforce Hg-related breeding failure, yet hatching success was significantly lower in CORT implanted males. Our results strongly suggest that Hg shall impair incubating behavior and hence reproductive performances trough a disruption of PRL secretion. The possible long-term consequences of the interactions between Hg and environmental stressors are discussed in the light of the unprecedented challenges currently affecting Arctic seabirds.

Key-words: Prolactin, parental care, stress, Mercury, breeding success, Arctic, Black-legged kittiwake

Introduction

Polar breeding seabird species are exposed to extreme variation in abiotic parameters such as temperature, wind, precipitation, and snow cover (Martin and Wiebe, 2004). In such environments, phenotypes with the greatest capacity to buffer harsh conditions and maintain a constant reproductive output will have superior mean fitness (Boyce, 1979). The modulation of parental care is a common behavioral response of polar seabirds in order to maximize their fitness (Chastel et al. 1993; Kitaysky et al. 2001, Groscolas et al., 2008). At the ultimate level, the decision to abandon current reproduction during unfavorable conditions should be selected in order to favor the long-term survival of these long-lived organisms (Williams 1966; Houston et al. 2005). At the physiological level, the modulation of avian parental care is governed by prolactin (PRL), a pituitary hormone which facilitates egg incubation, brood-guarding and provisioning (Buntin 1996). Studies on polar seabirds have shown that environmental stressors or prolonged energy constraints can affect the secretion of this hormone (Chastel et al., 2005; Cockrem et al. 2006; Angelier et al., 2007; Groscolas et al., 2008; Angelier and Chastel 2009). More precisely, the release of glucocorticoid hormones (corticosterone: CORT in birds) during stressful events (Wingfield and Sapolsky, 2003) has been experimentally shown to be associated with a reduction in plasma PRL concentrations (Criscuolo et al. 2005; Angelier et al. 2009; Spée et al. 2011) . Thus, PRL and CORT play a key role in mediating parental investment in birds (Angelier and Chastel 2009) and any disruption of these endocrine mechanisms may alter the ability of an individual to adjust reproductive decisions to environmental conditions (Jenssen 2006; Tartu et al. 2013).

An additional challenge to polar breeding seabirds is the growing exposure to environmental contaminants such as mercury (Hg, Bargagli et al. 1998; Ariya et al. 2004). In its methylated form (MeHg) Hg is very stable and can also bioaccumulate and biomagnify in the food web (Atwell et al. 1998; Bargagli et al. 1998; Mallory & Braune, 2012). MeHg is highly toxic as it passes the blood-brain barrier owing to its lipid solubility (Zahir et al. 2005), and the Arctic more particularly is considered as a sink for Hg deposition (Ariya et al. 2004). Marine apex predators, such as seabirds, are particularly exposed (reviewed in Dietz et al. 2013) and several studies have reported breeding impairments in highly Hg contaminated species (Wolfe et al. 1998; Evers et al. 2008; Tartu et al. 2013; Goutte et al. 2013, 2014; Dietz et al. 2013). Such breeding impairments could originate from the ability of Hg to alter the functioning of endocrine axes (Tan et al. 2009; Tartu et al. 2013, 2014).

However, there is to our knowledge no available study investigating Hg- PRL relationships in free-living animals. Indeed, studies focusing on the relationships between contaminants and PRL are scare and only one study (Verreault et al. 2008) has reported a negative relationship between PRL secretion and blood persistent organic pollutant levels in an Arctic seabird, the Glaucous Gull (*Larus hyperboreus*). Some human studies have focused on Hg neurotoxicity, and have explored the relationships between Hg and PRL as a proxy of neuroendocrine functions (Barregård et al. 1994 Lucchini et al. 2002; Carta et al. 2003). These latter studies have reported increased, decreased or unchanged serum PRL with increased organic Hg (Barregård et al. 1994 Lucchini et al. 2002; Carta et al. 2003). Regarding birds, Hg-PRL relationships deserve investigation because this heavy metal seems to be associated with the quality of parental cares (Nocera and Taylor, 1998) and to be tightly linked to the secretion of another pituitary hormone (luteinizing hormone, Tartu et al. 2013, 2014). PRL

may therefore be a target for Hg which may impair its secretion.

In this study we investigated the relationships between Hg and PRL as a proxy of parental care, in breeding black-legged kittiwakes *Rissa tridactyla* (hereafter 'kittiwakes' a seabird which in Svalbard bears significant levels of Hg in blood (Tartu et al. 2013). The first aim of the present study was, using correlative data to investigate the relationships between baseline and stress-induced PRL concentrations, Hg concentrations in blood and reproductive performances. We predicted that plasma PRL concentrations would decrease with increasing Hg concentration in blood and the kittiwakes most contaminated with Hg would experience poor reproductive performances. The second aim of this study was to test, the hypothesis that CORT could reinforce the possible negative relationship between PRL and Hg levels. As mentioned above, the Arctic is currently facing multiple environmental challenges such as increasing disturbance and rapid climate and habitat changes, it is thus possible that these environmental stressors could make organisms more sensitive to contaminants, such as Hg (Jenssen 2006; Hooper et al. 2013).To do so, we experimentally increased plasma CORT levels through the implantation of exogenous CORT which is known to decrease PRL levels in kittiwakes (Angelier et al. 2009). We therefore predicted that the negative relationships between Hg and PRL should be more pronounced in the CORT-implanted group, compared to the placebo group. Additionally, the most Hg-contaminated male kittiwakes from the CORT-implanted group should suffer the highest breeding failure.

Material and method

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 5264). The study was conducted at Kongsfjorden, Svalbard (78°54′N, 12°13′E) during two breeding seasons: in 2012 from June 19th to 4th July during the incubation period and from July 10th to 27th during the chick-rearing period; in 2013 birds were caught during the late incubation to early chick-rearing from June 27th to July 11th. In 2012 male and female kittiwakes were sampled whereas in 2013 only males were caught.

Blood sampling and CORT implant

In 2012, 111 incubating (56 females and 55 males) and 41 chick-rearing (19 females and 22 males) were caught on their nest with a noose at the end of a 5 m fishing rod. A first blood sample (*ca.* 0.2 mL) was collected immediately after capture, from the alar vein with a 1 mL heparinised syringe and a 25-gauge needle to assess 'baseline PRL' and Hg concentrations. Bleeding time (i.e. time elapsed from capture to the end of the first blood sample: 2min 28 ± 12 (SD) seconds, on average). Incubating birds were then placed into a cloth bag and a second blood samples (*ca*. 0.2 mL) was collected from the alar vein at 30 minutes (30min 08 ± 1min 15) to assess 'stress-induced PRL' concentrations (Chastel et al. 2005). Chick-rearing birds were not subjected to the stress protocol, in order not to leave theirs chicks unattended for a long period; therefore only baseline PRL levels were available.

In 2013, we focused the experimental study on males only since they bear higher Hg levels than females (Tartu et al. 2013). Forty-three incubating males were caught and subjected to a similar capture-restraint stress protocol to determine baseline, stress-induced PRL and Hg concentrations. Immediately after the first blood sample male kittiwakes were implanted subcutaneously either with a CORT (25mg/pellet 15 days release, G111, N=22) or a placebo (25mg/pellet 15 days release, C111, N=21) pellet. Pellets were obtained

from Innovative research of America (Sarasota) and surgical equipment was sterilized with 90% alcohol. A small incision (~5mm) was done on the nape with a sterilized surgical scalpel and the pellet was inserted with a sterilized bent clip. The incision was then sutured with surgical glue (3M Vetbond) and disinfected with aluminum spray (Vetoquinol Aluspray). The operation lasted for roughly 10 min and the implantation day was denoted as "day 0". To validate the CORT treatment, 4 CORT and 4 placebo birds (different kittiwakes each time) were recaptured at day 1, 2, 3, 7 and were subjected to a 'baseline' blood sampling. At day 11 we were able to recapture 16 CORT birds and 20 placebo birds, all recaptured kittiwakes were sampled for baseline and stress-induced PRL concentrations. Birds that received a CORT pellet will be referred to as the 'CORT' group, while the birds that received a placebo pellet will be referred as the 'Placebo' group.

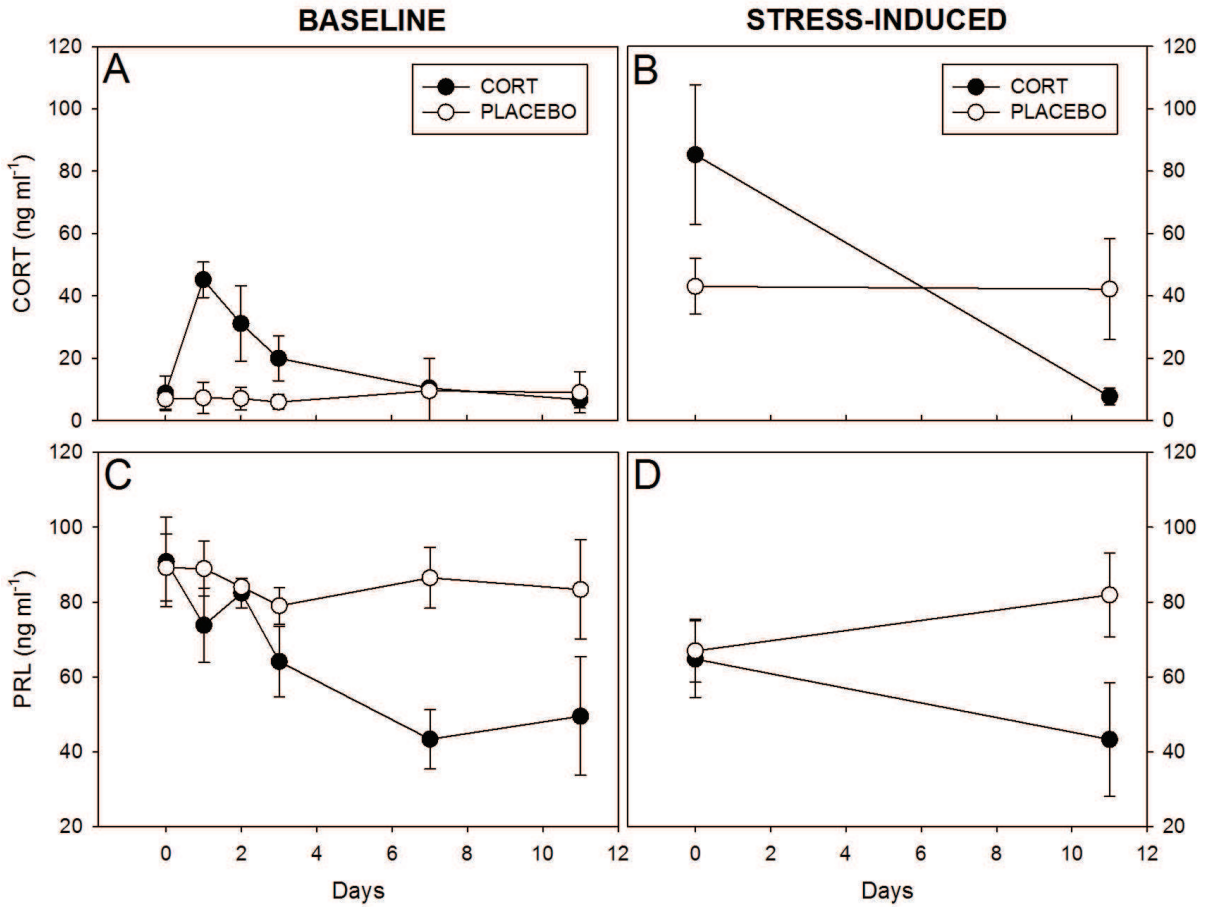


Figure 1: Effect of CORT and placebo pellets on baseline (A-C) and stress-induced (B-D) CORT and PRL concentrations, respectively, in 2013's incubating males. Open circles denote Placebo implanted male kittiwakes and closed circles denote CORT implanted male kittiwakes. Values are mean ± error standard.

Validation of the implants

Generalized linear mixed models (GLMM), with individual identity as a random factor, were used to validate the effects of the treatment (CORT and Placebo pellets) on CORT and PRL secretion. In the 'CORT' group, baseline CORT concentration were significantly related to the treatment day (GLMM, $_{F5,30}$=45.2, P<0.001, **Fig. 1A**). Specifically, baseline CORT significantly peaked within 1 day (Tukey HSD, difference=1.9, CI 95% [0.9, 2.9], P<0.001). Plasma CORT levels reached at this time were similar

levels measured in previous experimental CORT manipulation of kittiwakes (Angelier et al. 2007, 2009) and to un-manipulated CORT levels observed in breeding kittiwakes when food shortages and stressful events occur (Kitaysky et al., 1999, 2001; Buck et al., 2007). At day 2 and day 3 baseline CORT started decreasing but remained significantly higher compared to original concentrations (Tukey HSD, $P<0.021$ for all tests) until reaching original concentrations at day 7 (Tukey HSD, difference=0.4, CI 95% [-0.6, 1.4], $P=0.879$). At day 11, baseline CORT levels were not different from those measured at day 0 (GLMM, $F_{1,16}=2.1$, $P=0.165$). Stress-induced CORT significantly decreased over 11 days (GLMM, $F_{1,15}=214.4$, $P<0.001$, **Fig. 1B**). In the 'Placebo' group baseline and stress-induced CORT remained unchanged over 11 days (GLMM, $F<0.9$, $P>0.410$, **Fig. 1A-B**).

With regards to PRL levels, in the 'CORT' group, both baseline and stress-induced PRL concentrations decreased from day 0 to day 11 (GLMM, $F>25.6$, $P<0.001$, **Fig. 1C-D**). In the 'Placebo' group over 11 days, baseline PRL remained unchanged (GLMM, $F_{1,19}=0.9$, $P=0.419$, **Fig. 1C**) and stress-induced PRL concentrations significantly increased (GLMM, $F_{1,19}=43.4$, $P<0.001$, **Fig. 1D**). The kinetics of CORT and PRL following experimental increase of CORT levels were very similar to the ones observed previously in the same species (Angelier et al. 2007, 2009).

Hatching success and breeding success

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. Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observation and then released. 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 eggs that hatched (thereafter 'hatching success') and the number of chicks that reached at least 12 days old (thereafter 'breeding success').

Molecular sexing and hormone assay

Blood samples were centrifuged, and plasma was decanted and stored at −20°C until assayed. After centrifugation, red cells were kept frozen 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 at UMR 7372, as detailed in Weimerskirch et al. (2005). Plasma concentrations of baseline and stress-induced CORT and PRL were measured by radioimmunoassay at UMR 7372, as previously validated in kittiwakes (Chastel et al. 2005). Plasma concentrations of PRL were determined with the remaining plasma by a heterologous radioimmunoassay at UMR 7372, as previously validated for kittiwakes from this area (Chastel et al. 2005). All samples were run in one assay for both hormones. To measure intra-assay variation, we included 4 different referents 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. CORT and PRL concentrations were measured in day 0, 1, 2, 3, 7 and 11 samples.

Hg determination in blood cells

Total Hg was measured 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 analyzed 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). Hg concentrations are expressed in µg/g dry weight (dw).

Statistical analyses

All analyses were performed using R 2.13.1 (http://r-project.org/). First, correlations between PRL and Hg were tested by using generalized linear model (GLM) with normal errors and an identity link function. Second, we analyzed if hatching and breeding success were related to Hg concentration by using GLMs with normal/binomial errors and an identity/logit link function. Third, with regards to the experimental increase of CORT, we tested, in a global model the effects of Hg at day 0, treatment (CORT or placebo pellet) and their interaction on baseline and stress-induced PRL decrease from day 0 to day 11 ($[PRL]_{day0}$-$[PRL]_{day11}$). Then, we focused on the CORT implanted birds and tested whether baseline and stress-induced PRL decrease were related to Hg at day 0, and if hatching success, breeding success and nest abandon would be increased in the most contaminated birds. To do so, we used GLMs with normal/binomial errors and an identity/logit link function. Dependent continuous variables were previously tested for normality with a Shapiro–Wilk test and were log-transformed when necessary. Selected models were then checked for assumptions, that is, constancy of variance and residual normality.

Results

1. *Relationships between Hg, PRL and parenting in 2012*

Baseline PRL was negatively associated to Hg in male kittiwakes regardless the breeding stage (incubation: GLM, $F_{1,50}$=4.5, P=0.039, **Fig. 2A**; chick-rearing: $F_{1,18}$=10.7, P=0.004, **Fig. 3**) whereas baseline PRL was unrelated to Hg in females (GLM, F<16, P>0.230 for all tests, **Fig. 2A, Fig. 3**). Stress-induced PRL was negatively associated to Hg in incubating male and female kittiwakes (GLM, males: $F_{1,52}$=5.3, P=0.026; females: $F_{1,50}$=7.0, P=0.011, **Fig. 1B**). Blood Hg concentrations during the incubation period were unrelated to hatching success in both genders (GLM, F1,43<0.1, P>0.718). However, blood Hg concentration during the chick-rearing

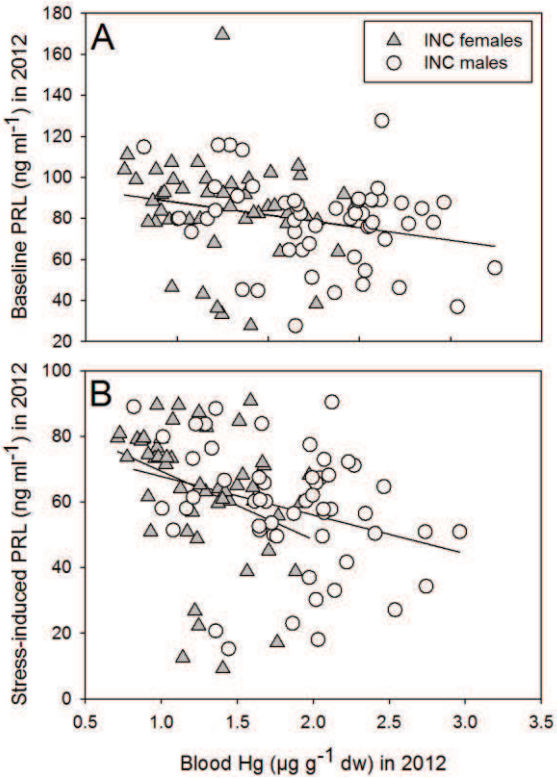


Figure 2: Relationships between baseline (A), stress-induced (B) PRL and blood Hg in 2012's incubating kittiwakes. Closed triangles denote females and open circles denote males; solid lines refer to statistically significant linear regression.

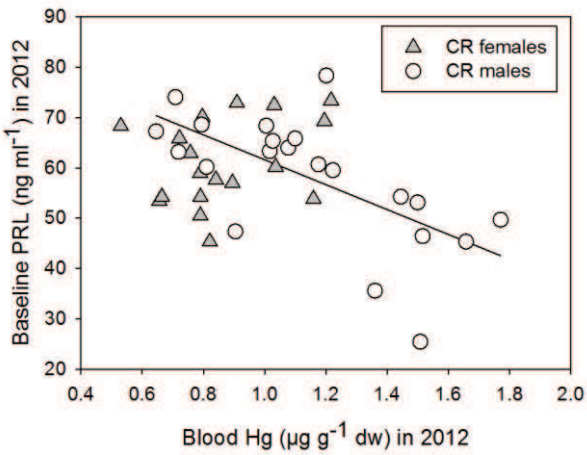


Figure 3: Relationships between baseline PRL and blood Hg in 2012's chick-rearing kittiwakes. Closed triangles denote females and open circles denote males; solid line refers to statistically significant linear regression for males.

period was higher in males that would successfully raise 1 chick instead of two over a 2 eggs clutch (GLM, χ^2=6.3, P=0.012, **Fig. 4**), this relationship was not observed in chick-rearing females (GLM, χ^2=0.1, P=0.822, **Fig. 4**).

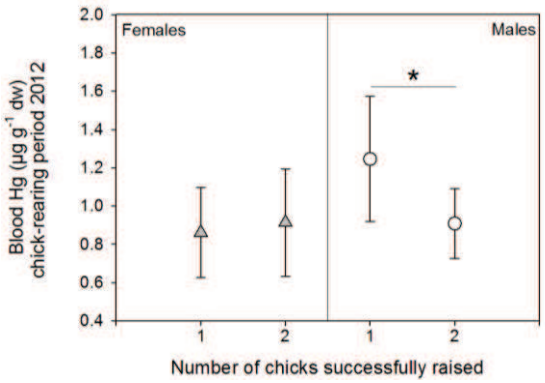


Figure 4: In 2012, blood Hg in female chick-rearing kittiwakes was not related to breeding success (i.e. the number of chicks successfully raised, left panel). However, male chick rearing kittiwakes with higher blood Hg concentrations were more likely to raise 1 chick instead of two (right panel). Closed triangles denote females and open circles males. Values are mean ± error standard, * denotes significant differences.

2. *Effects of an experimental increase in CORT levels on Hg/PRL relationships*

At day 0 PRL and Hg concentration were unrelated to Hg (GLM, F<0.2, P>0.676). The baseline PRL decrease from day 0 to day 11($[blPRL]_{day0}$-$[blPRL]_{day11}$), was significantly related to the treatment (CORT vs placebo; GLM, $F_{1,32}$=49.4, P<0.001), but not to Hg at day 0 ($F_{1,32}$<0.1, P=0.830) nor to the interaction of Hg at day 0 × treatment ($F_{1,32}$<0.1, P=0.988). Whereas stress-induced PRL decrease from day 0 to day 11($[siPRL]_{day0}$-$[siPRL]_{day11}$) was related to the treatment (GLM, $F_{1,32}$=52.7, P<0.001) and also to Hg at day 0 (F1,32=5.3, P=0.027): in males from the CORT group, the decrease of stress-induced PRL was more pronounced in the most Hg contaminated individuals (GLM, $F_{1,14}$=7.5, P=0.016, **Fig. 5**). However no significant relationship was found with regards to the interaction of Hg at day 0 × treatment ($F_{1,32}$=2.8, P=0.102). In the Placebo group, baseline and stress-induced PRL changes were not related to Hg at day 0 (GLM, F<0.9, P>0.360 for all tests; **Fig. 5**). At day 11 in 'Placebo' birds baseline PRL significantly decreased with increasing Hg (GLM, $F_{1,18}$=4.5, P=0.047, **Fig. 6A**), regarding stress-induced PRL a negative trend was observed (GLM, $F_{1,18}$=3.5, P=0.077, **Fig. 6B**). However in the 'CORT' birds, PRL and Hg were unrelated (GLM, F<1.6, P>0.232, **Fig. 6A-B**).

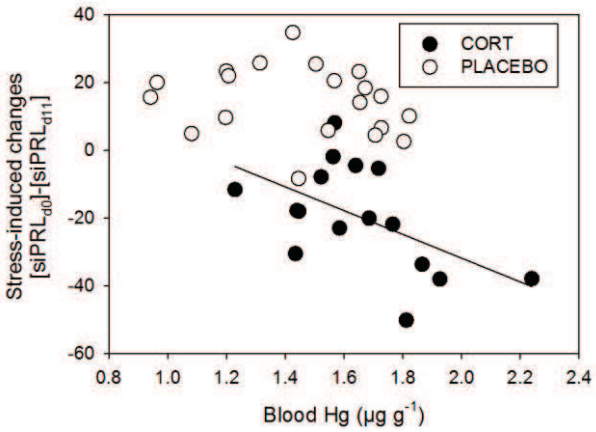


Figure 5: Stress-induced PRL decrease over 11 days in 'CORT' or Placebo implanted male kittiwakes in relation to initial blood Hg concentrations (day 0) in 2013's incubating males. Open circles denote Placebo implanted male kittiwakes and closed circles denote CORT implanted birds. Solid line refers to statistically significant linear regression for 'CORT' implanted male kittiwakes.

3. *Effects of the treatment and Hg contamination on reproductive performances*

Hatching success was significantly higher in the 'Placebo' birds than in 'CORT' birds (GLM, $F_{1,36}$=5.4, P=0.026) but this relationship was independent of Hg at day 0, or interaction between Hg and treatment (F<0.5, P>474 for all tests). Moreover, stress-induced, but not baseline, PRL concentrations at day 0 were positively related to hatching success (GLM, stress-induced PRL: $F_{1,36}$=7.7, P=0.009; baseline PRL: $F_{1,35}$=0.6, P=0.453). In all

experimental birds or in the 'CORT' implanted birds, breeding success was not associated to Hg at day 0 (GLM, $F<2.1$, $P>0.155$).

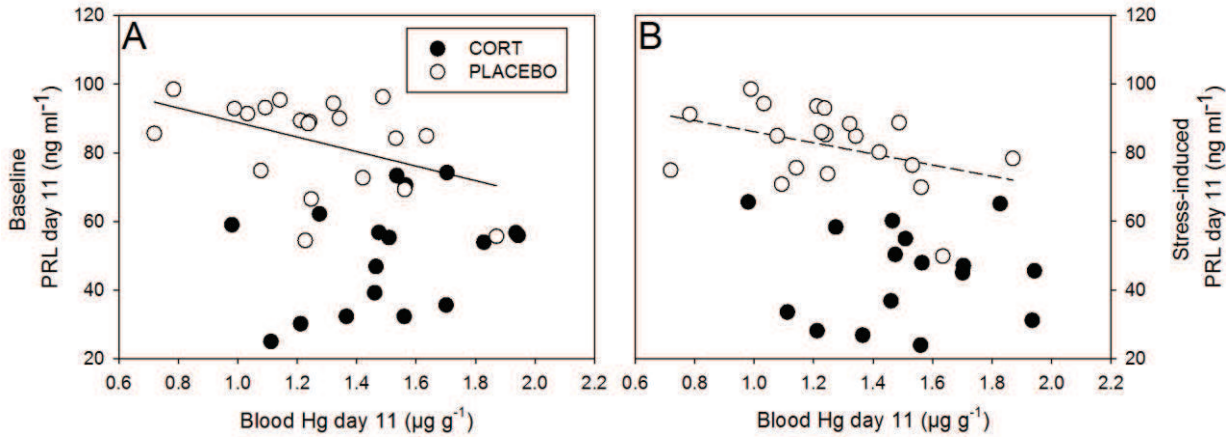


Figure 6: Relationships between baseline (A), stress-induced (B) PRL concentrations and blood Hg concentration in 2013's incubating male kittiwakes. Open circles denote 'Placebo' males and filled circles 'CORT' males; solid line refers to statistically significant linear regression and dashed line to linear regression close to statistical significance.

Discussion

The aim of the present study was to investigate the relationships between blood Hg and PRL in breeding kittiwakes. In line with our predictions, we reported a negative relationship between plasma baseline (males only) or stress-induced PRL (both sexes) and blood Hg levels during incubation and chick-rearing period; and Hg concentrations were negatively related to breeding success in chick-rearing males. Regarding the experimental manipulation of CORT levels via subcutaneous CORT pellets, according to our prediction, the experimental CORT increase appeared to reinforce the Hg-related decrease in stress-induced PRL concentration over an 11 days period. However, the experimental CORT increase did not reinforce Hg-related breeding failure, yet hatching success was significantly lower in the CORT implanted male kittiwakes, which was also importantly related to initial concentrations of stress-induced PRL (stress-induced PRL at day 0).

Relationship between PRL and Hg

To date no studies describing relationships between PRL and Hg have been conducted on free-living vertebrates. As previously mentioned, the only few human studies describing such relationships were aimed at studying neurotoxicity of Hg and baseline PRL was used to ascertain possible interference of Hg with neurotransmitters (Barregård et al 1994; Lucchini et al. 2002, 2003; Carta et al. 2003). In an Antarctic seabird, the snow petrel *Pagodroma nivea,* as found in the present study, stress-induced PRL levels were negatively related to increasing blood Hg in males especially (Tartu et al. unpublished). These negative relationships 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 (Tartu et al. 2013, 2014). But what could be the possible mechanisms underlying these relationships? Dopamine (DA), a neuro-transmitter and potent inhibitor of PRL, may play a significant role in this negative Hg-PRL relationship (Ben-Jonathan and Hnasko, 2001; Grattan & Kokay 2008). Indeed, it seems that organic and inorganic Hg can stimulate the spontaneous release

of dopamine in laboratory rodents (Faro et al. 1997, 2000, 2007; Minnema et al. 1989) but also in wild larvae of a fish (*Fundulus heteroclitus*, Zhou et al. 1999) and in wild American minks *Mustela vison* (Basu et al. 2005). The negative relationship observed between PRL and Hg is thus more likely to be indirect and will rely on an effect of Hg on the dopaminergic system. These studies were correlational and would nevertheless greatly benefit from further experimental support. The reason for the relationships between Hg and PRL being more visible in males (baseline PRL concentrations were never related to Hg in females) could be related to gender effects of Hg on DA. This was described in mice where MeHg altered locomotor activity in males only (Giménez-Llort et al. 2001), and/or through protective effects of estradiol (which is higher in females) on Hg toxicity as reported in female mice (Oliveira et al. 2006).

Fitness consequences of decreased PRL concentrations

In Svalbard kittiwakes, low PRL levels are associated to a reduced nest attendance, and a reduced breeding success (Angelier et al. 2009). Hg significantly decreases PRL concentrations, and in chick-rearing male kittiwakes elevated Hg concentrations were associated to lower breeding performances. Hg concentrations measured in incubating male snow petrels were higher in individuals that were more likely to leave their egg unattended (Tartu et al. unpublished). Similarly, poor parental behaviors have been reported in highly Hg-contaminated great northern divers (*Gavia immer*), (Nocera and Taylor 1998). Hazardous effects of Hg in free-ranging species, especially Hg hazards affecting breeding success, have been widely described in free-living populations and in laboratory experiments (see Tan et al. 2009 for a review). For example, 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). Moreover, MeHg experimental feeding to black ducks (*Anas rubripes*) induced lower clutch size, hatched eggs and duckling survival (Finley & Stendell 1978). Our results and these studies, all highlight the fact Hg shall impair incubating behavior and hence reproductive performances trough a disruption of PRL secretion. However, the mechanisms underlying these relationships often remained poorly understood. The possibility that Hg can disrupt PRL secretion could be a key element to understand the relationships between Hg and reproductive failure. And this study provides important support about the relationships between Hg, PRL and breeding success.

What happens when stress comes into play?

In extreme environments, such as Polar Regions, individuals often experience harsh and unpredictable environmental conditions, and individuals adopt different 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 too poor (e.g Angelier et al. 2007; Goutte et al. 2011). These behaviors (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 (Angelier and Wingfield, 2013; Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). By mimicking a stressful event we tested whether the potential disruptive effect of Hg on PRL could be reinforced. As reported earlier in the same species (Angelier et al. 2009), administration of exogenous CORT resulted in a decrease in baseline and stress-induced PRL. According to our predictions, the decrease in stress-induced

PRL was stronger in male kittiwakes most contaminated by Hg. This may originate from an additive effect of CORT and Hg, both being related to decreasing PRL concentrations (Angelier and Chastel, 2009). This result suggests that, for some contaminants (at least Hg), environmental stressors (such as food shortage, disturbances, habitat destruction and more generally ongoing climate change) may increase the hazardous biological effects of contaminants. Jenssen (2006) described the interaction between endocrine disruptors and climate change as a "worst case combination for Arctic marine species". Although in the 2012 correlative data, high Hg concentrations in blood of chick-rearing male kittiwakes were associated with poor reproductive performances, we could not observe an increased breeding failure in the most Hg contaminated males from the CORT group. 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 environmental conditions or even the breeding stage when the PRL and Hg measurements were performed. Indeed, blood Hg concentrations were higher in incubating males in 2012 compared to those measured in incubating males in 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 more observable in 2012 when conditions were supposedly poorer.

Conclusion

In the present study we focused on the biological effects of PRL as inducing parental behavior, however a spectrum of biological functions is associated to PRL such as water and electrolyte balance, growth and development, endocrinology and metabolism, brain and behavior, reproduction, immunoregulation and protection (Bole-Feysot et al. 1998). Thus a decrease of PRL with increasing Hg may not only affect parenting but also a multitude of other biological aspects. Our experimental CORT manipulation suggests that, in case of stressful event, the likely disruption of PRL by Hg decline is exacerbated. Thus with increasing environmental stressors in polar regions, such as anthropogenic disturbance, ongoing climate change, the presence of a multitude of environmental contaminants (Clarke and Harris 2003; Moline et al. 2008; Smetacek and Nicol 2005; Gabrielsen 2007) it may become difficult for polar species to maintain current population levels.

Acknowledgements: This project was supported by Institut Polaire Français (IPEV project 330 to O. Chastel), Agence National de la Recherche (ANR project PolarTop to O. Chastel). The authors thank C. Parenteau, C. Trouvé, S. Dano, M. Brault-Favrou for their excellent technical assistance in hormones assays, molecular sexing and Hg assays.

References

Angelier, F., Moe, B., Weimerskirch, H., Chastel, O., 2007. Age-specific reproductive success in a long-lived bird: do older parents resist stress better? Journal of Animal Ecology 76, 1181–1191. doi:10.1111/j.1365-2656.2007.01295.x

Angelier, F., Clément-Chastel, C., Welcker, J., Gabrielsen, G.W., Chastel, O., 2009. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. Functional Ecology 23, 784–793. doi:10.1111/j.1365-2435.2009.01545.x

Angelier, F., Chastel, O., 2009. Stress, prolactin and parental investment in birds: a review. Gen. Comp. Endocrinol. 163, 142–148. doi:10.1016/j.ygcen.2009.03.028

Angelier, F., Wingfield, J.C., 2013. Importance of the glucocorticoid stress response in a changing world: Theory, hypotheses and perspectives. General and Comparative Endocrinology, 10th International Symposium on Avian Endocrinology 190, 118–128. doi:10.1016/j.ygcen.2013.05.022

Ariya, P.A., Dastoor, A.P., Amyot, M., Schroeder, W.H., Barrie, L., Anlauf, K., Raofie, F., Ryzhkov, A., Davignon, D., Lalonde, J., 2004. The Arctic: a sink for mercury. Tellus B 56, 397–403.

Atwell, L., Hobson, K.A., Welch, H.E., 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web : insights from stable nitrogen isotope analysis. Canadian journal of fisheries and aquatic sciences 55, 1114–1121.

Bargagli, R., Monaci, F., SanchezHernandez, J.C., Cateni, D., 1998. Biomagnification of mercury in an Antarctic marine coastal food web. Mar Ecol Prog Ser 169, 65–76. doi:10.3354/meps169065

Barregård, L., Lindstedt, G., Schütz, A., Sällsten, G., 1994. Endocrine function in mercury exposed chloralkali workers. Occup Environ Med 51, 536–540. doi:10.1136/oem.51.8.536

Basu, N., Klenavic, K., Gamberg, M., O'Brien, M., Evans, D., Scheuhammer, A.M., Chan, H.M., 2005. Effects of mercury on neurochemical receptor-binding characteristics in wild mink. Environmental Toxicology and Chemistry 24, 1444–1450. doi:10.1897/04-048R.1

Ben-Jonathan, N., Hnasko, R., 2001. Dopamine as a Prolactin (PRL) Inhibitor. Endocrine Reviews 22, 724–763. doi:10.1210/er.22.6.724

Bole-Feysot, C., Goffin, V., Edery, M., Binart, N., Kelly, P.A., 1998. Prolactin (PRL) and Its Receptor: Actions, Signal Transduction Pathways and Phenotypes Observed in PRL Receptor Knockout Mice. Endocrine Reviews 19, 225–268. doi:10.1210/edrv.19.3.0334

Boyce, M.S., 1979. Seasonality and patterns of natural selection for life histories. American Naturalist 569–583.

Brasso, R.L., Cristol, D.A., 2008. Effects of mercury exposure on the reproductive success of tree swallows (Tachycineta bicolor). Ecotoxicology 17, 133–141. doi:10.1007/s10646-007-0163-z

Buck, C.L., O'Reilly, K.M., Kildaw, S.D., 2007. Interannual variability of Black-legged Kittiwake productivity is reflected in baseline plasma corticosterone. General and comparative endocrinology 150, 430–436.

Buntin, J.D., 1996. Parental Care: Evolution, Mechanisms, And Adaptive Significance: Parental Care: Evolution, Mechanisms, And Adaptive Significance. Academic Press.

Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C., Caurant, F., 2006. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: Influence of geographical origin and feeding ecology. Science of The Total Environment 368, 585–596. doi:10.1016/j.scitotenv.2006.01.038

Carta, P., Flore, C., Alinovi, R., Ibba, A., Tocco, M.G., Aru, G., Carta, R., Girei, E., Mutti, A., Lucchini, R., 2003. Sub-clinical neurobehavioral abnormalities associated with low level of mercury exposure through fish consumption. Neurotoxicology 24, 617–623.

Chastel, O., Weimerskirch, H., Jouventin, P., 1993. High annual variability in reproductive success and survival of an Antarctic seabird, the snow petrel Pagodroma nivea. Oecologia 94, 278–285. doi:10.1007/BF00341328

Chastel, O., Lacroix, A., Weimerskirch, H., Gabrielsen, G.W., 2005. Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird. Hormones and Behavior 47, 459–466. doi:10.1016/j.yhbeh.2004.10.009

Clarke, A., Harris, C.M., 2003. Polar marine ecosystems: major threats and future change. Environmental Conservation 30, 1–25. doi:10.1017/S0376892903000018

Cockrem, J., Potter, M., Candy, E., 2006. Corticosterone in relation to body mass in Adelie penguins (< i> Pygoscelis adeliae) affected by unusual sea ice conditions at Ross Island, Antarctica. General and comparative endocrinology 149, 244–252.

Criscuolo, F., Chastel, O., Bertile, F., Gabrielsen, G.W., Maho, Y.L., Raclot, T., 2005. Corticosterone alone does not trigger a short term behavioural shift in incubating female common eiders Somateria mollissima, but does modify long term reproductive success. Journal of Avian Biology 36, 306–312. doi:10.1111/j.0908-8857.2005.03371.x

Dietz, R., Sonne, C., Basu, N., Braune, B., O'Hara, T., Letcher, R.J., Scheuhammer, T., Andersen, M., Andreasen, C., Andriashek, D., 2013. What are the toxicological effects of mercury in Arctic biota? Science of the Total Environment 443, 775–790.

Evers, D., Savoy, L., DeSorbo, C., Yates, D., Hanson, W., Taylor, K., Siegel, L., Cooley, J., Bank, M., Major, A., Munney, K., Mower, B., Vogel, H., Schoch, N., Pokras, M., Goodale, M., Fair, J., 2008. Adverse effects from environmental mercury loads on breeding common loons. Ecotoxicology 17, 69–81. doi:10.1007/s10646-007-0168-7

Faro, L.R.F., Durán, R., do Nascimento, J.L.M., Alfonso, M., Picanço-Diniz, C.W., 1997. Effects of Methyl Mercury on the in Vivo Release of Dopamine and Its Acidic Metabolites DOPAC and HVA from Striatum of Rats. Ecotoxicology and Environmental Safety 38, 95–98. doi:10.1006/eesa.1997.1567

Faro, L.R.F., Nascimento, J.L.M. do, José, J.M.S., Alfonso, M., Durán, R., 2000. Intrastriatal Administration of Methylmercury Increases In Vivo Dopamine Release. Neurochem Res 25, 225–229. doi:10.1023/A:1007571403413

Faro, L.R.F., Rodrigues, K.J.A., Santana, M.B., Vidal, L., Alfonso, M., Durán, R., 2007. Comparative effects of organic and inorganic mercury on in vivo dopamine release in freely moving rats. Brazilian Journal of Medical and Biological Research 40, 1361–1365. doi:10.1590/S0100-879X2006005000157

Finley, M.T., Stendell, R.C., 1978. Survival and reproductive success of black ducks fed methyl mercury. Environmental Pollution (1970) 16, 51–64. doi:10.1016/0013-9327(78)90137-4

Gabrielsen, G.W., 2007. Levels and effects of persistent organic pollutants in arctic animals, in: Ørbæk, D.J.B., Kallenborn, D.R., Tombre, D.I., Hegseth, D.E.N., Falk-Petersen, D.S., Hoel, D.A.H. (Eds.), Arctic Alpine Ecosystems and People in a Changing Environment. Springer Berlin Heidelberg, pp. 377–412.

Giménez-Llort, L., Ahlbom, E., Daré, E., Vahter, M., Ögren, S.-O., Ceccatelli, S., 2001. Prenatal exposure to methylmercury changes dopamine-modulated motor activity during early ontogeny: age and gender-dependent effects. Environmental Toxicology and Pharmacology 9, 61–70. doi:10.1016/S1382-6689(00)00060-0

Goutte, A., Antoine, É., Chastel, O., 2011. Experimentally delayed hatching triggers a magnified stress response in a long-lived bird. Hormones and Behavior 59, 167–173. doi:10.1016/j.yhbeh.2010.11.004

Goutte, A., Bustamante, P., Barbraud, C., Delord, K., Weimerskirch, H., Chastel, O., 2013. Demographic responses to mercury exposure in two closely related Antarctic top predators. Ecology 95, 1075–1086. doi:10.1890/13-1229.1

Goutte, A., Barbraud, C., Meillère, A., Carravieri, A., Bustamante, P., Labadie, P., Budzinski, H., Delord, K., Cherel, Y., Weimerskirch, H., Chastel, O., 2014. Demographic consequences of heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the wandering albatross. Proc. R. Soc. B 281, 20133313. doi:10.1098/rspb.2013.3313

Grattan, D., Kokay, I., 2008. Prolactin: a pleiotropic neuroendocrine hormone. Journal of neuroendocrinology 20, 752–763.

Groscolas, R., Lacroix, A., Robin, J.-P., 2008. Spontaneous egg or chick abandonment in energy-depleted king penguins: A role for corticosterone and prolactin? Hormones and Behavior 53, 51–60. doi:10.1016/j.yhbeh.2007.08.010

Hooper, M.J., Ankley, G.T., Cristol, D.A., Maryoung, L.A., Noyes, P.D., Pinkerton, K.E., 2013. Interactions between chemical and climate stressors: A role for mechanistic toxicology in assessing climate change risks. Environmental Toxicology and Chemistry 32, 32–48.

Houston, A.I., Székely, T., McNamara, J.M., 2005. Conflict between parents over care. Trends in Ecology & Evolution 20, 33–38.

Jackson, A.K., Evers, D.C., Etterson, M.A., Condon, A.M., Folsom, S.B., Detweiler, J., Schmerfeld, J., Cristol, D.A., 2011. Mercury Exposure Affects the Reproductive Success of a Free-Living Terrestrial Songbird, the Carolina Wren (Thryothorus ludovicianus). The Auk 128, 759–769. doi:10.1525/auk.2011.11106

Jenssen, B.M., 2006. Endocrine-Disrupting Chemicals and Climate Change: A Worst-Case Combination for Arctic Marine Mammals and Seabirds? Environ Health Perspect 114, 76–80. doi:10.1289/ehp.8057

Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 1999. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. Functional Ecology 13, 577–584. doi:10.1046/j.1365-2435.1999.00352.x

Kitaysky, A.S., Kitaiskaia, E.V., Wingfield, J.C., Piatt, J.F., 2001. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. J Comp Physiol B 171, 701–709. doi:10.1007/s003600100230

Lucchini, R., Cortesi, I., Facco, P., Benedetti, L., Camerino, D., Carta, P., Urbano, M.L., Zaccheo, A., Alessio, L., 2002. Neurotoxic effect of exposure to low doses of mercury. Med Lav 93, 202–214.

Lucchini, R., Calza, S., Camerino, D., Carta, P., Decarli, A., Parrinello, G., Soleo, L., Zefferino, R., Alessio, L., 2003. Application of a Latent Variable Model for a Multicenter Study on Early Effects Due to Mercury Exposure. NeuroToxicology 24, 605–616. doi:10.1016/S0161-813X(03)00048-2

Mallory, M.L., Braune, B.M., 2012. Tracking contaminants in seabirds of Arctic Canada: Temporal and spatial insights. Marine Pollution Bulletin 64, 1475–1484. doi:10.1016/j.marpolbul.2012.05.012

Martin, K., Wiebe, K.L., 2004. Coping Mechanisms of Alpine and Arctic Breeding Birds: Extreme Weather and Limitations to Reproductive Resilience. Integr. Comp. Biol. 44, 177–185. doi:10.1093/icb/44.2.177

Minnema, D.J., Cooper, G.P., Greenland, R.D., 1989. Effects of methylmercury on neurotransmitter release from rat brain synaptosomes. Toxicology and Applied Pharmacology 99, 510–521. doi:10.1016/0041-008X(89)90158-0

Moline, M.A., Karnovsky, N.J., Brown, Z., Divoky, G.J., Frazer, T.K., Jacoby, C.A., Torres, J.J., Fraser, W.R., 2008. High Latitude Changes in Ice Dynamics and Their Impact on Polar Marine Ecosystems. Annals of the New York Academy of Sciences 1134, 267–319. doi:10.1196/annals.1439.010

Nocera, J.J., Taylor, P.D., 1998. In situ behavioral response of common loons associated with elevated mercury (Hg) exposure. Conservation Ecology 2.

Oliveira, F.R.T., Ferreira, J.R., dos Santos, C.M.C., Macêdo, L.E.M., de Oliveira, R.B., Rodrigues, J.A., do Nascimento, J.L.M., Faro, L.R.F., Diniz, D.L.W.P., 2006. Estradiol reduces cumulative mercury and associated disturbances in the hypothalamus–pituitary axis of ovariectomized rats. Ecotoxicology and Environmental Safety 63, 488–493. doi:10.1016/j.ecoenv.2004.12.024

Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. Trends in Ecology & Evolution 17, 462–468. doi:10.1016/S0169-5347(02)02578-8

Smetacek, V., Nicol, S., 2005. Polar ocean ecosystems in a changing world. Nature 437, 362–368. doi:10.1038/nature04161

Spée, M., Marchal, L., Lazin, D., Le Maho, Y., Chastel, O., Beaulieu, M., Raclot, T., 2011. Exogenous corticosterone and nest abandonment: a study in a long-lived bird, the Adélie penguin. Hormones and behavior 60, 362–370.

Tan, S.W., Meiller, J.C., Mahaffey, K.R., 2009. The endocrine effects of mercury in humans and wildlife. Critical Reviews in Toxicology 39, 228–269. doi:10.1080/10408440802233259

Tartu, S., Goutte, A., Bustamante, P., Angelier, F., Moe, B., Clément-Chastel, C., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2013. To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biology Letters 9. doi:10.1098/rsbl.2013.0317

Tartu, S., Bustamante, P., Goutte, A., Cherel, Y., Weimerskirch, H., Bustnes, J.O., Chastel, O., 2014. Age-Related Mercury Contamination and Relationship with Luteinizing Hormone in a Long-Lived Antarctic Bird. PloS one 9, e103642.

Verreault, J., Verboven, N., Gabrielsen, G.W., Letcher, R.J., Chastel, O., 2008. Changes in prolactin in a highly organohalogen contaminated Arctic top predator seabird, the glaucous gull. General and Comparative Endocrinology 156, 569–576. doi:10.1016/j.ygcen.2008.02.013

Weimerskirch, H., Lallemand, J., Martin, J., 2005. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. Journal of Animal Ecology 74, 285–291. doi:10.1111/j.1365-2656.2005.00922.x

Williams, G.C., 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. American naturalist 687–690.

Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and Resistance to Stress: When and How. Journal of Neuroendocrinology 15, 711–724. doi:10.1046/j.1365-2826.2003.01033.x

Wolfe, M.F., Schwarzbach, S., Sulaiman, R.A., 1998. Effects of mercury on wildlife: a comprehensive review. Environmental toxicology and chemistry 17, 146–160.

Zahir, F., Rizwi, S.J., Haq, S.K., Khan, R.H., 2005. Low dose mercury toxicity and human health. Environmental Toxicology and Pharmacology 20, 351–360. doi:10.1016/j.etap.2005.03.007

Zhou, T., Rademacher, D.J., Steinpreis, R.E., Weis, J.S., 1999. Neurotransmitter levels in two populations of larval Fundulus heteroclitus after methylmercury exposure. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 124, 287–294. doi:10.1016/S0742-8413(99)00077-8

PAPER VI

Polychlorinated biphenyl exposure and corticosterone secretion in seven polar seabirds.

Environmental Pollution 197 (2015) 173–180

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Polychlorinated biphenyl exposure and corticosterone levels in seven polar seabird species

S. Tartu [a, *], F. Angelier [a], J.O. Bustnes [b], B. Moe [c], S.A. Hanssen [b], D. Herzke [d], G.W. Gabrielsen [e], N. Verboven [e], J. Verreault [f], P. Labadie [g, h], H. Budzinski [g, h], J.C. Wingfield [i], O. Chastel [a]

[a] Centre d'études biologiques de Chizé (CEBC) – UMR 7372 ULR CNRS, Villiers-en-bois, France
[b] Norwegian Institute for Nature Research, FRAM – High North Research Centre for Climate and the Environment, Tromsø, Norway
[c] Norwegian Institute for Nature Research, Postboks 5685 Sluppen, N-7485 Trondheim, Norway
[d] Norwegian Institute for Air Research (NILU), FRAM – High North Research Centre for Climate and the Environment, N-9296 Tromsø, Norway
[e] Norwegian Polar Institute, FRAM – High North Research Centre for Climate and the Environment, N-9296 Tromsø, Norway
[f] Centre de recherche en toxicologie de l'environnement (TOXEN), Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succursale Centre-ville, Montreal, QC H3C 3P8, Canada
[g] Université de Bordeaux, EPOC/LPTC, UMR 5805, F-33400 Talence, France
[h] CNRS, EPOC/LPTC, UMR 5805, F-33400 Talence, France
[i] Department of Neurobiology, Physiology and Behaviour, University of California, Davis, USA

ARTICLE INFO

Article history:
Received 19 September 2014
Received in revised form
29 October 2014
Accepted 1 December 2014
Available online

Keywords:
Arctic
Antarctic
Birds
PCBs
Glucocorticoids
Stress

ABSTRACT

The role of polychlorinated biphenyls (PCBs) on exposure-related endocrine effects has been poorly investigated in wild birds. This is the case for stress hormones including corticosterone (CORT). Some studies have suggested that environmental exposure to PCBs and altered CORT secretion might be associated. Here we investigated the relationships between blood PCB concentrations and circulating CORT levels in seven free-ranging polar seabird species occupying different trophic positions, and hence covering a wide range of PCB exposure. Blood $\sum_7$PCB concentrations (range: 61–115,632 ng/g lw) were positively associated to baseline or stress-induced CORT levels in three species and negatively associated to stress-induced CORT levels in one species. Global analysis suggests that in males, baseline CORT levels generally increase with increasing blood $\sum_7$PCB concentrations, whereas stress-induced CORT levels decrease when reaching high blood $\sum_7$PCB concentrations. This study suggests that the nature of the PCB-CORT relationships may depend on the level of PCB exposure.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

In Polar Regions, increasing attention has been directed towards environmental contaminants and their potentially hazardous effects on susceptible wildlife species (Bargagli, 2008; Bustnes et al., 2003, 2007; Gabrielsen, 2007; Verreault et al., 2010; Wania, 2003; Letcher et al., 2010). Among environmental contaminants, several persistent organic pollutants (POPs) may exhibit endocrine disruptive properties, and may alter functions of several hormones (e.g. Amaral Mendes, 2002). For example, a number of studies have reported significant relationships between concentrations of POPs and plasma levels of reproductive hormones such as steroids and some pituitary hormones in free-living birds and mammals (Giesy et al., 2003; Vos et al., 2000; Jenssen, 2006; Gabrielsen, 2007; Verreault et al., 2008, 2010).

Relationships reported to date in a limited number of studies on wild bird species between POP levels and stress hormones (glucocorticoids) have been largely inconclusive: in black-legged kittiwakes *Rissa tridactyla* baseline CORT levels were positively associated to $\sum_{11}$PCB concentrations (Nordstad et al., 2012). Also, in the most PCB-exposed Arctic seabird species, the glaucous gull *Larus hyperboreus*, a higher POP burden (including 58 PCB congeners, organochlorine pesticides, brominated flame retardants and their metabolically-derived products) was associated with higher baseline CORT levels in both sexes (Verboven et al., 2010). Moreover, in studies of pre-laying female kittiwakes and incubating

* Corresponding author.
E-mail address: tartu.sabrina@gmail.com (S. Tartu).

http://dx.doi.org/10.1016/j.envpol.2014.12.007
0269-7491/© 2014 Elsevier Ltd. All rights reserved.

snow petrels *Pagodroma nivea*, which bear low to moderate PCB contamination, stress-induced CORT levels increased with increasing $\sum_{10}$PCB concentrations and $\sum$POPs (including 7 PCBs congeners and organochlorine pesticides), respectively (Tartu et al., 2014, 2015). On the other hand, stress-induced CORT levels decreased with increasing POPs (58 PCB congeners, organochlorine pesticides, brominated flame retardants and their metabolically-derived products) in male glaucous gulls that accumulate the highest levels of these contaminants among Arctic species (Verboven et al., 2010). This suggests that the nature of the relationship between POPs, and CORT secretion may be related to the levels of contamination. The major POP detected in wildlife are still the PCBs despite their global ban more than 30 years ago. PCBs bioaccumulate in top predators such as polar seabirds (Letcher et al., 2010; Corsolini et al., 2011) and occasionally high levels of these compounds accumulate in lipid-rich tissues. Since PCB may be a good proxy for POPs in general, the link between PCB levels and stress hormones therefore deserves more attention especially because of the major role of stress hormones in allostasis (McEwen and Wingfield, 2003; Angelier and Wingfield, 2013). For example, in an experimental study conducted on captive American kestrels *Falco spaverinus* dosed with PCBs, decreased levels of baseline and stress-induced CORT were reported compared to levels measured in the control group (Love et al., 2003). CORT secretion is regulated through a number of physiological mechanisms. At the endocrine level, a stressful event will trigger the release of corticotropin-releasing hormone (CRH) from the hypothalamus; CRH will then stimulate the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn will activate the synthesis of glucocorticoids from the adrenal cortex (Sapolsky et al., 2000; Wingfield, 2013). In birds, up to 90% of glucocorticoids released into the bloodstream will bind to corticosteroid-binding globulin (CBG) and will be transported to target cells. Concurrently, glucocorticoids will provide negative feedback signals for ACTH and CRH release (Wingfield, 2013). This hormonal cascade may trigger an array of physiological and behavioural adjustments that shift energy investment away from reproduction, and redirect it towards survival (Wingfield and Sapolsky, 2003). Glucocorticoids are therefore considered as major mediators of reproductive decisions in birds (reviewed in Wingfield and Sapolsky, 2003) and have a strong connection with fitness in some seabird species (Angelier et al., 2010; Goutte et al., 2011; Schultner et al., 2014). It is thus crucial to determine how both baseline and stress-induced glucocorticoid secretion can be influenced by ubiquitous and abundant environmental contaminants including PCBs. Baseline and stress-induced CORT levels (i.e. CORT levels measured in response to a capture/handling stress), may depict different physiological status: baseline CORT mirrors energetic state (Landys et al., 2006), while stress-induced CORT can be used to infer on an individual's sensitivity to stress. The CORT release following a stress can be modulated (elevated or low release) in order to maximize either survival or reproduction (Lendvai et al., 2007; Bókony et al., 2009).

The aim of the present study was to investigate the relationships between $\sum_{7}$PCB concentrations, plasma baseline CORT levels and stress-induced CORT levels in seven polar seabird species. We selected seabird species occupying different trophic positions that encompassed a wide range of plasma PCB levels (Letcher et al., 2010). These include the glaucous gull, the black-legged kittiwake, the common eider *Somateria mollissima*, these three species were sampled in the Norwegian Arctic (Bear Island and Kongsfjorden, 74° 22′N, 19° 05′E and 78°54′N, 12°13′E, respectively) the snow petrel, the cape petrel *Daption capense*, the south polar skua *Catharacta maccormicki*, the three species were sampled in Antarctica (Adélie land, 66°40′S, 140°01′E) and the wandering albatross *Diomedea exulans* which was sampled at Crozet Island (46° 24′ S, 51° 45′E) a subantarctic French territory. All species were sampled within a short period of time during the breeding period, that is, from late incubation to early chick-rearing (corresponding to the month of June for Arctic species, and early to late December for Antarctic and subantarctic species). Based on the previous reports on PCB/CORT relationships (Verboven et al., 2010; Nordstad et al., 2012; Tartu et al., 2014), we predicted that the relationships between PCB and CORT levels would differ between species according to their blood PCB levels: 1) baseline CORT concentrations would increase with increasing PCB levels, whereas 2) stress-induced CORT levels would increase in moderately contaminated species and decline in highly contaminated bird species.

0269-7491/© 2014 Elsevier Ltd. All rights reserved.

2. Material and methods

2.1. Ethics statement

Animals were handled in accordance with the national guidelines for ethical treatment of experimental animals from the Governor of Svalbard, the Norwegian Animal Research Authority (NARA), and the ethic committee of the Institut Polaire Français Paul Emile Victor (IPEV): Governor of Svalbard (2004/00481-12 to G.W. Gabrielsen and J. Verreault, (2007/00165) to S.A. Hanssen and B Moe; NARA 2006/16056 to G.W. Gabrielsen and J. Verreault, (2007/6072) to S.A. Hanssen and B. Moe, FOTS id 2086, 3319 to O. Chastel and IPEV programs no. 109 to H. Weimerskirch and no. 330 to O. Chastel.

2.2. Sampling year, study site and species

Two hundred eighty-six blood samples were available from three high Arctic seabird species: the black-legged kittiwake (hereafter 'kittiwakes', $N = 25$, 2011), the common eider ($N = 55$ females, 2007) and the glaucous gulls ($N = 38$, 2006) and four Antarctic species, the wandering albatross ($N = 75$, 2008), the snow petrel ($N = 35$, 2010), the cape petrel ($N = 27$, 2011), and the south polar skua ($N = 31$, 2003). Main diet and average body mass during late incubation to early chick-rearing are reported for all species in Table 1. Wandering albatrosses were not weighed but the average body mass of wandering albatrosses during incubation is around 8403 ± 642 g for females and 10,720 ± 966 g for males (Weimerskirch, 1995). Study sites, bird capture, and sampling protocols have been described in previous studies (Verboven et al., 2010; Bustnes et al., 2012; Angelier et al., 2013; Goutte et al., 2013; Tartu et al., 2014, 2015; Goutte et al., 2014). Because in seabirds blood CORT and PCB levels may vary between breeding phases (Nordstad et al., 2012), we selected blood samples of birds collected during late incubation and early chick-rearing periods. Briefly, a first blood sample (*ca.* 0.3 mL) for baseline CORT analysis was collected immediately after capture from the alar vein using a heparinized syringe and a gauge needle (Romero and Reed, 2005). Birds were then kept in opaque cloth bags during 30 min after which blood samples were collected immediately following previously described methods for stress-induced CORT analysis (e.g. Tartu et al., 2014). Stress-induced CORT levels were calculated by subtracting the baseline CORT concentrations from the CORT concentration following 30 min handling protocol: stress-induced CORT levels = ($CORT_{t=30\ min} - CORT_{t=0\ min}$). Wandering albatrosses and south polar skuas were not subjected to a capture/handling stress protocol and only baseline CORT levels are available.

2.3. Molecular sexing and hormone assay

Whole blood samples were centrifuged and plasma was stored

Table 1
Diet (see footnote references 1 to 7), parental care behaviour, as well as mean blood/plasma lipid content, body mass, and plasma concentrations of Σ_7PCBs, baseline and stress-induced CORT levels in females and males of seven seabirds species. First row values are mean (geometric for Σ_7PCBs) ± standard deviation (sd) and 2nd row range (min – max). Non-available data are referred to as 'na'.

Diet	Sex	GLGU	SPSK	SNPE	CAPE	WAAL	BLKI	COEI
		Fish, other seabird species (adult, chicks, eggs) (1,5)	Fish, other seabird species (adult, chicks, eggs) (1,5)	Marine invertebrates, crustaceans, fish, carrion (1,3)	Marine invertebrates, crustaceans, fish, carrion (1,3)	Cephalopods, fish (6)	Marine invertebrates, fish (4,5)	Benthic mollusks, crabs, urchins (1,2,7)
Parental care		Bi-parental	Bi-parental	Bi-parental	Bi-parental	Bi-parental	Bi-parental	Female only
Blood/plasma lipids (%)	Females	0.84 ± 0.22 0.40–1.26	0.6 ± 0.16 0.35–0.90	0.68 ± 1.11 0.48–0.88	0.2 ± 0.08 0.13–0.37	0.63 ± 0.12 0.50–0.99	0.26 ± 1.11 0.06–3.32	0.28 ± 0.09 0.15–0.49
	Males	0.79 ± 0.15 0.54–1.0	0.49 ± 0.18 0.24–0.78	0.70 ± 0.12 0.50–0.94	0.21 ± 0.09 0.10–0.51	0.60 ± 0.11 0.38–0.94	0.12 ± 0.04 0.07–0.23	na na
Body mass (g)	Females	1397 ± 118.6 1180–1620	1495 ± 92.4 1325–1700	393 ± 55.3 307–538	433 ± 39.5 365–525	na na	397 ± 15.9 375–430	1140 ± 112.9 1274–1829
	Males	1755 ± 103.5 1530–1920	1342 ± 97.3 1140–1540	444 ± 47.4 374–545	510 ± 60.5 420–640	na na	425 ± 20.7 390–471	na na
Σ_7PCBs (ng/g lw)	Females	17,850 ± 11,738 7089–51,068	6358 ± 9113 1604–29,383	660.2 ± 8904 85.2–33,666	7177 ± 17,531 1529–48,695	803.8 ± 622.1 144.0–2831	2757 ± 3447 140.4–14,125	558.2 ± 668.4 60.6–3346
	Males	35,357 ± 32,392 7062–115,632	15,193 ± 42812 1162–128,089	1531 ± 15,406 65.7–55,119	2803 ± 13,740 432.1–51,219	1017 ± 1055 125.8–4769	7956 ± 4821 4429–22,165	na na
Baseline CORT (ng/ml)	Females	13.6 ± 16.7 1.2–25.7	5.6 ± 4.9 2.7–23.2	4.1 ± 4.1 1.1–15.7	1.2 ± 1.0 0.5–3.6	4.3 ± 1.9 1.2–8.8	7.7 ± 6.2 1.0–27.0	6.0 ± 4.7 0.6–27.0
	Males	10.8 ± 11.2 1.0–35.1	7.7 ± 3.3 2.3–13.8	4.9 ± 4.5 1.2–18.3	1.7 ± 1.2 0.4–4.8	4.4 ± 2.2 2.0–10.6	6.9 ± 2.5 4.5–11.9	na na
Stress-induced CORT (ng/ml)	Females	27.0 ± 11.7 10.9–50.3	na na	39.1 ± 7.6 23.3–56.0	47.5 ± 7.6 37.5–59.5	na na	40 ± 10.4 19.5–55.5	34.4 ± 9.2 11.7–54.7
	Males	16.5 ± 15.4 1.3–59.5	na na	38.2 ± 9.7 22.6–56.4	42.0 ± 11.5 20.7–61.2	na na	37.6 ± 6.9 23.7–48.5	na na

(1)del Hoyo et al., 1992; (2)Guillemette et al., 1992; (3)Ainley et al., 1993; (4)Mehlum and Gabrielsen, 1993; (5)del Hoyo et al., 1996; (6)Cherel and Klages, 1998; (7)Varpe, 2010. COEI = common eider, SNPE = snow petrel; WAAL = wandering albatross; BLKI = kittiwake; CAPE = cape petrel; SPSK = south polar skua and GLGU = glaucous gull.

at −20 °C until assayed. Red blood cells were kept at −20 °C for molecular sexing (polymerase chain reaction amplification, Weimerskirch et al., 2005) at the CEBC, with the exception of common eiders (only females incubate) and glaucous gulls which were sexed based on morphometric measurements. Plasma concentrations of CORT were determined by radioimmunoassay for all species as described elsewhere (Lormée et al., 2003; Verboven et al., 2010). Radioimmunoassays were conducted at CEBC for all species except for glaucous gulls for which radioimmunoassays were conducted at the university of Glasgow veterinary school. For glaucous gull data, an inter-laboratory validation was conducted.

2.4. PCB analysis

For POPs, cross-validation between the different labs (EPOC/LPTC, NILU and the National Wildlife Research Centre) was not possible due to limited sample volumes, however, quality assurance and quality control procedures are performed routinely in NILU, the National Wildlife Research Centre and EPOC/LPTC using standard reference materials, method blanks, duplicate extractions and injections of authentic standards, and these labs met the established criteria for QA/QC (for details see Verboven et al. 2010; Goutte et al., 2014; Tartu et al., 2014). POPs analyses for kittiwakes and common eiders were conducted on whole blood samples at NILU with the method described in Tartu et al. (2014) by gas chromatography coupled with a mass spectrometer (GC–MS). The same method (GC–MS) was used for glaucous gulls, POPs were measured in plasma at the National Wildlife Research Centre, a detailed method was described in Verboven et al. (2010). Finally, for wandering albatrosses, snow petrels, cape petrels and south polar skuas, POPs were measured in plasma at EPOC/LPTC as described in Goutte et al. (2014) by gas chromatography coupled with electron capture detection. In the present study, we focused on 7 major PCB congeners (CB-28, -52, -101, -118, -138, -153 and -180) since they are the most abundant in the marine ecosystem and often the most bioaccumulative in a wide range of seabird species inhabiting the polar regions (Gabrielsen et al., 1995; Savinova et al., 1995). We used the $\sum_7$PCBs (i.e. $\sum$CB-28, -52, -101, -118, -138, -153 and -180) for further analyses.

2.5. Lipid determination

Lipids were determined in plasma on an aliquot of 10 μL by the *sulfo-phospho-vanillin* (*SPV*) reaction for colorimetric determination for cape petrels, snow petrels, south polar skuas and wandering albatrosses at EPOC/LPTC (Frings et al., 1972). For common eiders, kittiwakes and glaucous gulls, lipids were determined using a gravimetric method using the whole sample amount at NILU and National Wildlife Research Centre. In order to compare whole blood to plasma samples, PCB concentrations were converted to ng/g lipid weight (lw).

2.6. Statistics

We used generalized linear models (GLMs) with a gaussian error distribution to test whether CORT (baseline and stress-induced levels) and Σ_7PCB concentrations were different between males and females for each species. As consequences, using males and females separately, we used GLMs with a gaussian error distribution to test whether Σ_7PCB concentrations were related to 1) baseline CORT levels and 2) stress-induced CORT levels. Because our purpose was to describe a general pattern between Σ_7PCB concentrations and CORT levels, we calculated geometric means for Σ_7PCB concentrations, baseline and stress-induced CORT levels. Toxicity data are essentially lognormally distributed and the geometric mean is more appropriate (Posthuma et al., 2001). Following visual inspection of the data we tested whether Σ_7PCBs were 1) linearly related to baseline CORT or 2) quadratically or linearly related to stress-induced CORT levels, again by using a GLM with a gaussian error distribution. Dependent continuous variables were

log-10 transformed when necessary to achieve normality. All statistical analyses were performed using R 2.13.1 (R Development Core Team, 2008).

3. Results

3.1. Sex differences in baseline and stress-induced CORT levels

Baseline CORT levels were not different between sexes in any species (GLM, $F < 2.7$, $P > 0.115$). In glaucous gulls, females had higher stress-induced CORT levels than males (GLM, $F_{1,36} = 4.3$, $P = 0.045$), in snow petrels, kittiwakes and cape petrels stress-induced CORT levels were not different between females and males (GLM, $F < 1.2$, $P > 0.289$).

3.2. Sex difference in Σ_7PCBs concentrations

In kittiwakes, south polar skuas and glaucous gulls Σ_7PCB concentrations were significantly higher in males than in females (GLM, kittiwakes: $F_{1,23} = 8.7$, $P = 0.007$; south polar skuas: $F_{1,29} = 4.2$, $P = 0.048$; glaucous gulls: $F_{1,36} = 9.4$, $P = 0.004$). In snow petrels, wandering albatrosses and cape petrels Σ_7PCBs concentrations were not different between females and males (GLM, $F < 2.8$, $P > 0.108$).

3.3. Relationships between Σ_7PCBs concentrations and CORT levels

Statistics on the relationships between CORT levels (baseline and stress-induced) and Σ_7PCB concentration are given in Table 2. In male kittiwakes, both baseline and stress-induced CORT levels significantly increased with increasing Σ_7PCB concentrations (Table 2, Figs. 1J and 2G). Positive trend were observed between baseline CORT levels and Σ_7PCB concentrations in female wandering albatrosses (Table 2, Fig. 1C) as well as CORT stress-induced levels and Σ_7PCB concentrations in male snow petrels (Table 2, Fig. 2F). Moreover, a significant negative relationship was found between stress-induced CORT levels and Σ_7PCB concentrations in male glaucous gulls (Table 2, Fig. 2I). In common eiders, cape petrels and south polar skuas CORT (baseline and stress-induced) levels were not related to Σ_7PCB concentrations.

With regard to the global analysis using the geometric means for Σ_7PCB concentrations and CORT levels (one point per species and sex); in females, a positive trend associated Σ_7PCB concentrations and baseline CORT levels (GLM, Σ_7PCB, $F_{1,5} = 4.2$, $P = 0.095$, Fig. 3A). Stress-induced CORT levels were not associated to Σ_7PCB concentrations, to $(\Sigma_7\text{PCB})^2$ nor to $(\Sigma_7\text{PCB}) \times (\Sigma_7\text{PCB})^2$, (GLM, $F_{1,3} = 1.1$, $P = 0.378$; $F_{1,3} = 2.7$, $P = 0.201$; $F_{1,3} = 3.8$, $P = 0.147$, respectively, Fig. 3B). Significant relationships were observed in males only. Specifically, Σ_7PCB concentrations were positively associated to baseline CORT levels (GLM, $F_{1,4} = 14.3$, $P = 0.019$, Fig. 3C) and negatively associated to stress-induced CORT levels (GLM, $(\Sigma_7\text{PCB})$: $F_{1,2} = 59.1$, $P = 0.016$; $(\Sigma_7\text{PCB})^2$: $F_{1,2} = 87.4$, $P = 0.011$; $(\Sigma_7\text{PCB}) \times (\Sigma_7\text{PCB})^2$: $F_{1,2} = 73.4$, $P = 0.013$; Fig. 3D).

4. Discussion

This is, to our knowledge, the first study that comprehensively investigates the relationships between circulating CORT levels and PCB levels in multiple seabird species feeding at various trophic positions and thus exposed to various PCB concentrations. Baseline CORT levels significantly increased as a function of Σ_7PCB concentrations in male kittiwakes and a positive trend was observed in female wandering albatrosses. Stress-induced CORT levels were positively related to Σ_7PCB concentrations in male kittiwakes and a positive trend was observed in male snow petrels whereas stress-induced CORT levels were negatively related to Σ_7PCB concentrations in male glaucous gulls. Interestingly, Σ_7PCB concentrations were found to be unrelated to baseline or stress-induced CORT levels in common eiders, cape petrels and south polar skuas. The general pattern including all seven seabird species showed, in females, a positive trend between baseline CORT levels and Σ_7PCB concentrations whereas stress-induced CORT levels were unrelated to Σ_7PCB concentrations. In males, baseline CORT levels generally increase with increasing blood $\sum_7$PCB concentrations, whereas stress-induced CORT levels decrease when reaching high blood $\sum_7$PCB concentrations. However, caution should be made when interpreting this general pattern since only seven seabird species were included in this analysis. And several factors could not be taken into account such as species-specific differences in hormone regulation, diet composition, biotransformation of contaminants, but also individual nutritional status, phylogeny and life-history traits (which could also influence PCBs and CORT) and differences in methodology.

In mammals and fish, the modes of action of contaminants on glucocorticoids, including PCBs have been studied extensively (Odermatt et al., 2006). For example, certain methyl sulfone-containing PCB metabolites act as antagonists on human glucocorticoid receptors (GR, Johansson et al., 1998). Moreover, oral administration of a commercial PCB mixture resulted in a depression of the number of GR in the brain of Arctic charrs *Salvelinus*

Table 2
Relationships between log transformed $\sum_7$PCB concentrations and A) baseline and B) stress-induced CORT levels in seven female and six male seabird species.

Independent variable: $\sum_7$PCBs ng/g lw		Females				Males			
Dependent variable	Species	Df	*F*	*P*	Correlation	Df	*F*	*P*	Correlation
A) Baseline CORT	COEI	1.52	0.19	0.661		na	na	na	
	WAAL	1.27	4.10	0.053	(+)	1.44	0.02	0.886	
	BLKI	1.11	0.00	0.955		**1.10**	**10.83**	**0.008**	(+)
	CAPE	1.6	0.08	0.781		1.17	0.92	0.352	
	SNPE	1.12	2.11	0.172		1.19	1.33	0.263	
	SPSK	1.15	0.50	0.488		1.12	2.48	0.141	
	GLGU	1.22	2.89	0.103		1.12	1.23	0.290	
B) Stress-induced CORT	COEI	1.52	0.11	0.743		na	na	na	
	BLKI	1.11	1.00	0.339		**1.10**	**6.95**	**0.025**	(+)
	CAPE	1.6	0.43	0.535		1.17	0.00	0.970	
	SNPE	1.12	1.12	0.312		1.19	3.47	0.078	(+)
	GLGU	1.22	0.08	0.775		**1.12**	**5.95**	**0.031**	(−)

Numbers in bold are significant relationship ($P < 0.05$). Directions are given for significant relationships and trends ($P < 0.10$). COEI = common eider, SNPE = snow petrel; WAAL = wandering albatross; BLKI = kittiwake; CAPE = cape petrel; SPSK = south polar skua and GLGU = glaucous gull.

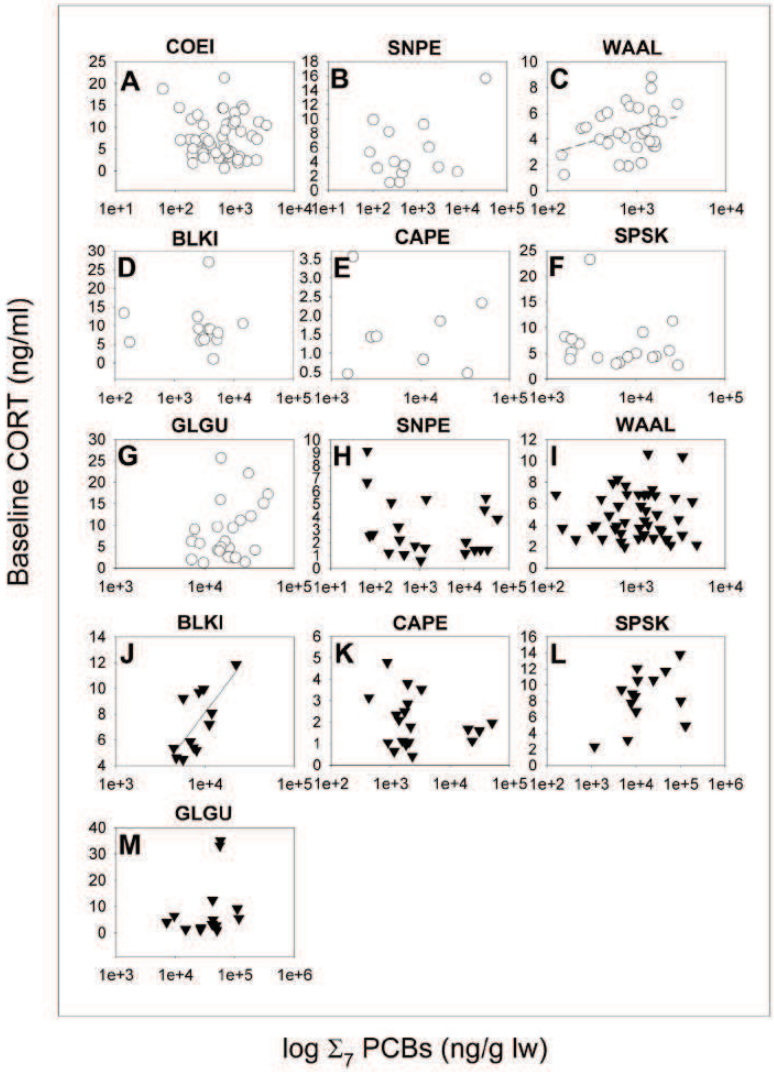


Fig. 1. Relationships between baseline CORT (ng/ml) and log-transformed $\sum_7$PCBs (ng/g lw) in female common eiders (A, COEI), female and male snow petrels (B, H; SNPE), wandering albatrosses (C, I; WAAL), kittiwakes (D, J; BLKI), cape petrels (E, K; CAPE), south polar skuas (F, L; SPSK) and glaucous gulls (G, M; GLGU). Solid line refers to a significant linear regressions ($P = 0.008$) and dashed line to a regression close to statistical significance ($P = 0.053$). Closed triangles denote males and open circles females.

alpinus (Aluru et al., 2004).

The increase in baseline CORT levels with increasing Σ_7PCB concentrations in the present study may be explained based on cytochrome P450 (CYP)-mediated enzymes activity. Some contaminants including PCBs have been shown to inhibit/stimulate CYP enzymes in the steroidogenesis pathway. Hence, PCBs may inhibit the metabolism of CORT (to aldosterone), thus elevating CORT, or stimulate metabolism of desoxy-CORT (to CORT), thus also elevating CORT (Xu et al., 2006). However, in captive American kestrels, the reverse pattern was found: oral PCB administration resulted in lower levels of baseline CORT concentrations compared to a control group, and liver PCB concentrations were associated with baseline CORT levels with an inverted U-shaped pattern (Love et al., 2003). Furthermore, baseline CORT declined when liver PCB concentrations reached 20 μg/g ww. Love et al. (2003) discussed this inverted U-shaped pattern as an apparent hormetic response of CORT to PCBs (Calabrese and Baldwin, 2003): adrenal monoxygenase (P-450 family) have the capacity to metabolize contaminants and may have produced toxic metabolites. The inverted U-shaped pattern may result in long-term damage of these toxic metabolites to the adrenal cortex: "remaining intact cells still produce a hermetic baseline CORT response in relation to liver PCB concentrations; however because the cortex has been damaged, there are fewer cells overall resulting in baseline levels depressed below those of controls" (Love et al., 2003).

For stress-induced CORT levels, we found in our study positive associations in two species (snow petrels and black-legged kittiwakes). Two possible explanations could support these positive

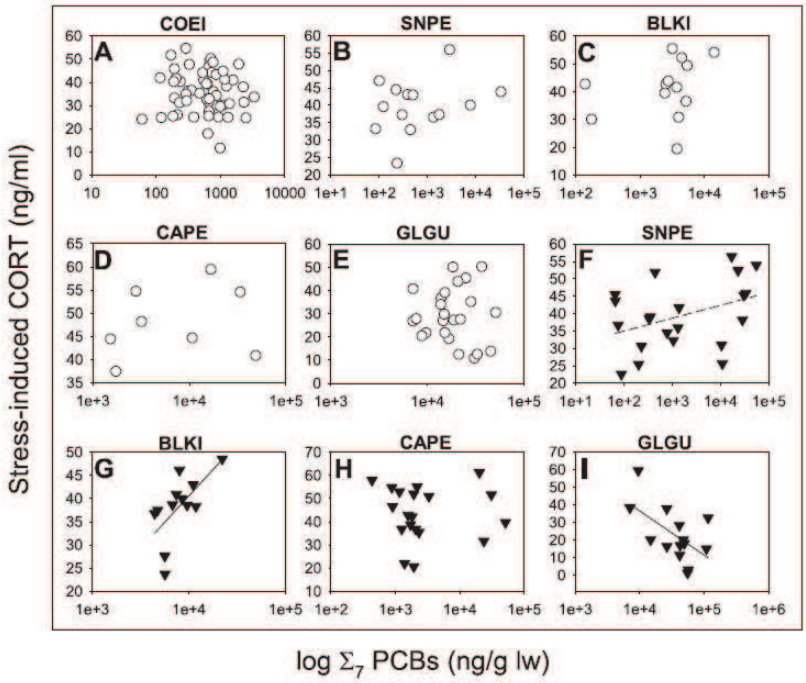


Fig. 2. Relationships between stress-induced CORT levels (ng/ml) and log-transformed $\sum_7$PCBs (ng/g lw) in female common eiders (A, COEI), female and male snow petrels (B, F; SNPE), kittiwakes (C, G; BLKI), cape petrels (D, H; CAPE) and glaucous gulls (E, I; GLGU). Solid lines refer to significant linear regressions ($P < 0.031$) and dashed line to a regression close to statistical significance ($P = 0.078$). Closed triangles denote males and open circles females.

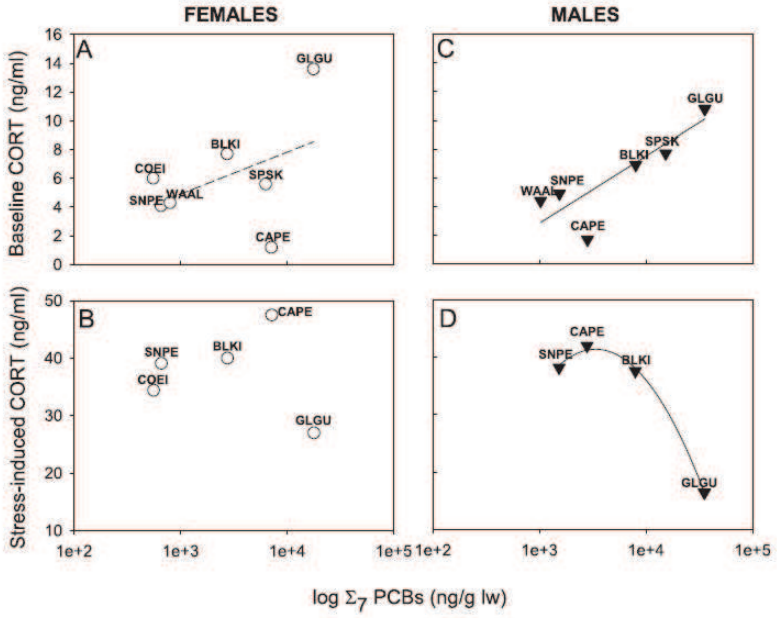


Fig. 3. Relationships between log-transformed $\sum_7$PCBs (ng/g lw), baseline CORT levels (ng/ml) in A) seven female and C) six male seabird species; and stress-induced CORT levels (ng/ml) in B) five female and C) and D) four male seabird species. Data represent geometric means for $\sum_7$PCBs, baseline CORT and stress-induced CORT levels. Solid line refers to significant relationship ($P < 0.05$) and dashed line to linear regression close to statistical significance ($P < 0.10$). Closed triangles denote males and open circles females. COEI = common eider, SNPE = snow petrel; WAAL = wandering albatross; BLKI = kittiwake; CAPE = cape petrel; SPSK = south polar skua and GLGU = glaucous gull.

relationships between PCB (and organochlorine pesticides) and stress-induced CORT levels: either they increase the ability of the adrenal glands to release CORT or they decrease the negative feedback capacity of CORT on the hypothalamus or the pituitary. In kittiwakes the capacity of CORT to decrease post-stress episode has been measured by dexamethasone injection (a potent CORT agonist), and the CORT concentrations measured following dexamethasone injection were not related to ∑PCB concentrations nor to ∑organochlorine pesticides (Tartu et al. unpublished data). However, the CORT levels measured in kittiwakes following an ACTH injection were positively associated to ∑PCBs but not to ∑organochlorine pesticides (Tartu et al. unpublished data). This suggests that in kittiwakes, increasing ∑PCB concentrations may increase the adrenal sensitivity. ACTH is one of the few polypeptide hormones having a positive trophic effect on its own receptors (Beuschlein et al., 2001; Penhoat et al., 1989). Thus, an increase of ACTH-R in the most PCB-exposed birds may be the consequence of an excess of ACTH stimulation to the adrenals. Alternatively, it may be possible that PCBs mimic ACTH and activate ACTH-R or increase ACTH secretion; both cases would result in an increase of ACTH-R, however we have no experimental support for such interpretation. An enhanced CORT stress response in adult birds may favour survival at the expense of parental investment (Wingfield and Sapolsky, 2003). Indeed, in kittiwakes and wandering albatrosses, even relatively low POP exposure was associated with a reduction in long-term breeding success (Goutte et al., 2014; Goutte at al. unpublished data). In male glaucous gulls, we found a negative association between stress-induced CORT levels and $\sum_7$PCB concentrations. In the present study, glaucous gulls' mean baseline CORT concentrations (10.8 ng/mL) were almost as high as that of CORT levels attained following a stressful episode (16.5 ng/mL). The relatively low stress-induced CORT levels in the most PCB exposed male glaucous gulls may suggest a permanent saturation of ACTH-R as a result of chronic elevation of baseline CORT. Chronic elevation of baseline CORT may result in an array of deleterious biological effects (Sapolsky et al., 2000) which can explain the negative effects of POPs on adult survival which have been reported in glaucous gulls (Erikstad et al., 2013). Interestingly, the strongest associations between CORT (baseline and stress-induced)levels and Σ_7PCB concentrations were only observed in males, which often bear higher levels of PCB compared to females. As suggested earlier, more species would be required to corroborate these patterns, although these may be confounded by several factors (e.g. differences in hormone regulation, diet composition, biotransformation of contaminants, individual nutritional status, phylogeny, life-history traits, differences in methodology, etc.) that would be necessary to investigate in future studies. Regardless, present meta-analysis investigation provides valuable insights onto the associations between CORT levels and ∑PCB concentrations in polar seabirds.

Additional controlled studies using a mechanistic approach are warranted to verify whether there is a causal linkage between PCB exposure and perturbation in CORT homeostasis in present seabirds such as wandering albatrosses, kittiwakes, snow petrels and glaucous gulls. Although present study focused solely on PCBs and polar seabirds, other contaminants such as brominated flame retardants have been shown to impair CORT levels (Verboven et al., 2010) and some bird species including gulls feeding in urban environment or raptors may be exposed to substantially higher contaminant levels (Chen and Hale, 2010; Gentes et al., 2012; Guerra et al., 2012). It is therefore crucial to better understand the effects contaminant exposure may have on CORT regulation, which may significantly impact the adaptability of free-ranging bird species in such a changing environment.

Acknowledgements

This study was funded by Agence Nationale de la Recherche (ANR PolarTop, O. Chastel), the French Polar Institute (IPEV, Program 109, H. Weimerskirch and 330, O. Chastel), the 7th research program of the European Community FP7/2007–2013 (Marie-Curie Fellowship, no. 237034, F. Angelier), the Norwegian Research Council through two International Polar Year projects (COPOL to J.O. Bustnes and G.W. Gabrielsen and 176046 BirdHealth to S.A. Hanssen) and by the Arctic field Grant. J.C. Wingfield was funded by USA National Science Foundation grant number IOS-0750540 and the University of California, Davis, Endowed Chair in Physiology. The authors thank many laboratory personnel and field workers for their valuable help in this study. This study was also carried out in the framework of the Cluster of Excellence COTE (ANR-10-LABX-45). The Aquitaine Region and the European Union (CPER A2E project) are acknowledged for their financial support. Europe is moving in Aquitaine with the European Regional Development Fund (FEDER).

References

Ainley, D.G., Ribic, C.A., Fraser, W.R., 1993. Does prey preference affect habitat choice in Antarctic seabirds? Mar. Ecol. Prog. Ser. 90, 207.
Aluru, N., Jorgensen, E.H., Maule, A.G., Vijayan, M.M., 2004. PCB disruption of the hypothalamus-pituitary-interrenal axis involves brain glucocorticoid receptor downregulation in anadromous Arctic charr. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287, R787–R793. http://dx.doi.org/10.1152/ajpregu.00091.2004.
Amaral Mendes, J.J., 2002. The endocrine disrupters: a major medical challenge. Food Chem. Toxicol. 40, 781–788. http://dx.doi.org/10.1016/S0278-6915(02)00018-2.
Angelier, F., Wingfield, J.C., Weimerskirch, H., Chastel, O., 2010. Hormonal correlates of individual quality in a long-lived bird: a test of the "corticosterone–fitness hypothesis". Biol. Lett. 6, 846–849. http://dx.doi.org/10.1098/rsbl.2010.0376.
Angelier, F., Wingfield, J.C., Trouvé, C., de Grissac, S., Chastel, O., 2013. Modulation of the prolactin and the corticosterone stress responses: do they tell the same story in a long-lived bird, the Cape petrels? Gen. Comp. Endocrinol. 182, 7–15. http://dx.doi.org/10.1016/j.ygcen.2012.10.008.
Angelier, F., Wingfield, J.C., 2013. Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives10th International Symposium on Avian Endocrinology. Gen. Comp. Endocrinol. 190, 118–128. http://dx.doi.org/10.1016/j.ygcen.2013.05.022.
Bargagli, R., 2008. Environmental contamination in Antarctic ecosystems. Sci. Total Environ. 400, 212–226. http://dx.doi.org/10.1016/j.scitotenv.2008.06.062.
Beuschlein, F., Fassnacht, M., Klink, A., Allolio, B., Reincke, M., 2001. ACTH-receptor expression, regulation and role in adrenocortial tumor formation. Eur. J. Endocrinol. 144, 199–206. http://dx.doi.org/10.1530/eje.0.1440199.
Bókony, V., Lendvai, A.Z., Liker, A., Angelier, F., Wingfield, J.C., Chastel, O., 2009. Stress response and the value of reproduction: are birds prudent parents? Am. Nat. 173, 589–598. http://dx.doi.org/10.1086/597610.
Bustnes, J.O., Erikstad, K.E., Skaare, J.U., Bakken, V., Mehlum, F., 2003. Ecological effects of organochlorine pollutants in the arctic: a study of the glaucous gull. Ecol. Appl. 13, 504–515. http://dx.doi.org/10.1890/1051-0761(2003)013[0504:EEOOPI]2.0.CO;2.
Bustnes, J.O., Tveraa, T., Varpe, Ø., Henden, J.A., Skaare, J.U., 2007. Reproductive performance and organochlorine pollutants in an Antarctic marine top predator: the south polar skua. Environ. Int. 33, 911–918. http://dx.doi.org/10.1016/j.envint.2007.04.010.
Bustnes, J.O., Moe, B., Hanssen, S.A., Herzke, D., Fenstad, A.A., Nordstad, T., Borgå, K., Gabrielsen, G.W., 2012. Temporal dynamics of circulating persistent organic pollutants in a fasting seabird under different environmental conditions. Environ. Sci. Technol. 46, 10287–10294. http://dx.doi.org/10.1021/es301746j.
Calabrese, E.J., Baldwin, L.A., 2003. HORMESIS: the dose-response revolution. Annu. Rev. Pharmacol. Toxicol. 43, 175–197. http://dx.doi.org/10.1146/annurev.pharmtox.43.100901.140223.
Chen, D., Hale, R.C., 2010. A global review of polybrominated diphenyl ether flame retardant contamination in birds. Environ. Int. 36, 800–811. http://dx.doi.org/10.1016/j.envint.2010.05.013.
Cherel, Y., Klages, N., 1998. A Review of the Food of Albatrosses. Albatross Biology and Conservation. Surrey Beatty, Chipping Norton, pp. 113–136.
Corsolini, S., Borghesi, N., Ademollo, N., Focardi, S., 2011. Chlorinated biphenyls and pesticides in migrating and resident seabirds from East and West Antarctica. Environ. Int. 37, 1329–1335.
del Hoyo, J., Elliott, A., Sargatal, J., Cabot, J., Collar, N.J., 1992. Handbook of the Birds of the World: Ostrich to Ducks. In: Handbook of the Birds of the World. Lynx Edicions.
del Hoyo, J., Elliott, A., Sargatal, J., Collar, N.J., 1996. Handbook of the Birds of the World: Hoatzin to Auks. In: Handbook of the Birds of the World. Lynx Edicions.

Erikstad, K.E., Sandvik, H., Reiertsen, T.K., Bustnes, J.O., Strøm, H., 2013. Persistent organic pollution in a high-Arctic top predator: sex-dependent thresholds in adult survival. Proc. R. Soc. B 280. http://dx.doi.org/10.1098/rspb.2013.1483, 20131483.
Frings, C.S., Fendley, T.W., Dunn, R.T., Queen, C.A., 1972. Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. Clin. Chem. 18, 673–674.
Gabrielsen, G.W., Skaare, J.U., Polder, A., Bakken, V., 1995. Chlorinated hydrocarbons in glaucous gulls (*Larus hyperboreus*) in the southern part of Svalbard. Sci. Total Environ. 160–161, 337–346. http://dx.doi.org/10.1016/0048-9697(95)04367-A.
Gabrielsen, G.W., 2007. Levels and effects of persistent organic pollutants in arctic animals. In: Ørbæk, D.J.B., Kallenborn, D.R., Tombre, D.I., Hegseth, D.E.N., Falk-Petersen, D.S., Hoel, D.A.H. (Eds.), Arctic Alpine Ecosystems and People in a Changing Environment. Springer Berlin Heidelberg, pp. 377–412.
Gentes, M.-L., Letcher, R.J., Caron-Beaudoin, É., Verreault, J., 2012. Novel flame retardants in urban-feeding ring-billed gulls from the St. Lawrence River, Canada. Environ. Sci. Technol. 46, 9735–9744. http://dx.doi.org/10.1021/es302099f.
Giesy, J.P., Feyk, L.A., Jones, P.D., Kannan, K., Sanderson, T., 2003. Review of the effects of endocrine-disrupting chemicals in birds. Pure Appl. Chem. 75, 2287–2303. http://dx.doi.org/10.1351/pac200375112287.
Goutte, A., Clément-Chastel, C., Moe, B., Bech, C., Gabrielsen, G.W., Chastel, O., 2011. Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes. J. Exp. Biol. 214, 2005–2013. http://dx.doi.org/10.1242/jeb.051979.
Goutte, A., Bustamante, P., Barbraud, C., Delord, K., Weimerskirch, H., Chastel, O., 2013. Demographic responses to mercury exposure in two closely-related Antarctic top predators. Ecology. http://dx.doi.org/10.1890/13-1229.1.
Goutte, A., Barbraud, C., Meillère, A., Carravieri, A., Bustamante, P., Labadie, P., Budzinski, H., Delord, K., Cherel, Y., Weimerskirch, H., Chastel, O., 2014. Demographic consequences of heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the wandering albatross. Proc. R. Soc. B 281, 20133313. http://dx.doi.org/10.1098/rspb.2013.3313.
Guerra, P., Alaee, M., Jiménez, B., Pacepavicius, G., Marvin, C., MacInnis, G., Eljarrat, E., Barceló, D., Champoux, L., Fernie, K., 2012. Emerging and historical brominated flame retardants in peregrine falcon (Falco peregrinus) eggs from Canada and Spain. Environ. Int. 40, 179–186. http://dx.doi.org/10.1016/j.envint.2011.07.014.
Guillemette, M., Ydenberg, R.C., Himmelman, J.H., 1992. The role of energy intake rate in prey and habitat selection of common eiders *Somateria mollissima* in winter: a risk-sensitive interpretation. J. Anim. Ecol. 61, 599–610.
Jenssen, B.M., 2006. Endocrine-disrupting chemicals and climate change: a worst-case combination for arctic marine mammals and seabirds? Environ. Health Perspect. 114, 76–80. http://dx.doi.org/10.1289/ehp.8057.
Johansson, M., Nilsson, S., Lund, B.O., 1998. Interactions between methylsulfonyl PCBs and the glucocorticoid receptor. Environ. Health Perspect. 106, 769–772.
Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen. Comp. Endocrinol. 148, 132–149. http://dx.doi.org/10.1016/j.ygcen.2006.02.013.
Lendvai, Á.Z., Giraudeau, M., Chastel, O., 2007. Reproduction and modulation of the stress response: an experimental test in the house sparrow. Proc. R. Soc. B 274, 391–397. http://dx.doi.org/10.1098/rspb.2006.3735.
Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995–3043. http://dx.doi.org/10.1016/j.scitotenv.2009.10.038.
Lormée, H., Jouventin, P., Trouve, C., Chastel, O., 2003. Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies Sula sula. Ibis 145, 212–219. http://dx.doi.org/10.1046/j.1474-919X.2003.00106.x.
Love, O.P., Shutt, L.J., Silfies, J.S., Bortolotti, G.R., Smits, J.E.G., Bird, D.M., 2003. Effects of dietary PCB exposure on adrenocortical function in captive American kestrels (Falco sparverius). Ecotoxicology 12, 199–208.
McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15.
Mehlum, F., Gabrielsen, G.W., 1993. The diet of high arctic seabirds in coastal and ice-covered, pelagic areas near the Svalbard archipelago. Polar Res. 12, 1–20.
Nordstad, T., Moe, B., Bustnes, J.O., Bech, C., Chastel, O., Goutte, A., Sagerup, K., Trouvé, C., Herzke, D., Gabrielsen, G.W., 2012. Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (*Rissa tridactyla*) across their breeding cycle. Environ. Pollut. 164, 219–226. http://dx.doi.org/10.1016/j.envpol.2012.01.044.

polar seabirds, other contaminants such as brominated flame retardants have been shown to impair CORT levels (Verboven et al., 2010) and some bird species including gulls feeding in urban environment or raptors may be exposed to substantially higher contaminant levels (Chen and Hale, 2010; Gentes et al., 2012; Guerra et al., 2012). It is therefore crucial to better understand the effects contaminant exposure may have on CORT regulation, which may significantly impact the adaptability of free-ranging bird species in such a changing environment.

Odermatt, A., Gumy, C., Atanasov, A.G., Dzyakanchuk, A.A., 2006. Disruption of glucocorticoid action by environmental chemicals: potential mechanisms and relevance. J. Steroid Biochem. Mol. Biol. 102, 222–231.
Penhoat, A., Jaillard, C., Saez, J.M., 1989. Corticotropin positively regulates its own receptors and cAMP response in cultured bovine adrenal cells. PNAS 86, 4978–4981.
Posthuma, L., S II, G.W., Traas, T.P., 2001. Species Sensitivity Distributions in Ecotoxicology. CRC Press.
R Development Core Team, 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.
Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 140, 73–79. http://dx.doi.org/10.1016/j.cbpb.2004.11.004.
Sapolsky, R., Romero, L., Munck, A., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55.
Savinova, T.N., Polder, A., Gabrielsen, G.W., Skaare, J.U., 1995. Chlorinated hydrocarbons in seabirds from the Barents Sea area. Sci. Total Environ. 160–161, 497–504. http://dx.doi.org/10.1016/0048-9697(95)04383-C.
Schultner, J., Moe, B., Chastel, O., Bech, C., Kitaysky, A.S., 2014. Migration and stress during reproduction govern telomere dynamics in a seabird. Biol. Lett. 10, 20130889.
Tartu, S., Angelier, F., Herzke, D., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2014. The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. Sci. Total Environ. 476–477, 553–560. http://dx.doi.org/10.1016/j.scitotenv.2014.01.060.
Tartu, S., Angelier, F., Wingfield, J.C., Bustamante, P., Labadie, P., Budzinski, H., Weimerskirch, H., Bustnes, J.O., Chastel, O., 2015. Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. Sci. Total Environ. 505, 180–188. http://dx.doi.org/10.1016/j.scitotenv.2014.10.008.
Varpe, O., 2010. Stealing bivalves from common eiders: kleptoparasitism by glaucous gulls in spring. Polar Biol. 33, 359–365.
Verboven, N., Verreault, J., Letcher, R.J., Gabrielsen, G.W., Evans, N.P., 2010. Adrenocortical function of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. Gen. Comp. Endocrinol. 166, 25–32. http://dx.doi.org/10.1016/j.ygcen.2009.11.013.
Verreault, J., Verboven, N., Gabrielsen, G.W., Letcher, R.J., Chastel, O., 2008. Changes in prolactin in a highly organohalogen contaminated Arctic top predator seabird, the glaucous gull. Gen. Comp. Endocrinol. 156, 569–576. http://dx.doi.org/10.1016/j.ygcen.2008.02.013.
Verreault, J., Gabrielsen, G.W., Bustnes, J.O., 2010. The Svalbard glaucous gull as bioindicator species in the European Arctic: insight from 35 years of contaminants research. In: Whitacre, D.M. (Ed.), Reviews of Environmental Contamination and Toxicology, vol. 205. Springer, New York, pp. 77–116.
Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambré, C., Tarazona, J.V., Brandt, I., Vethaak, A.D., 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. CRC Crit. Rev. Toxicol. 30, 71–133.
Wania, F., 2003. Assessing the potential of persistent organic chemicals for long-range transport and accumulation in polar regions. Environ. Sci. Technol. 37, 1344–1351. http://dx.doi.org/10.1021/es026019e.
Weimerskirch, H., 1995. Regulation of foraging trips and incubation routine in male and female wandering albatrosses. Oecologia 102, 37–43. http://dx.doi.org/10.1007/BF00333308.
Weimerskirch, H., Lallemand, J., Martin, J., 2005. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. J. Anim. Ecol. 74, 285–291. http://dx.doi.org/10.1111/j.1365-2656.2005.00922.x.
Wingfield, J.C., 2013. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. Funct. Ecol. 27, 37–44. http://dx.doi.org/10.1111/1365-2435.12039.
Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. J. Neuroendocrinol. 15, 711–724. http://dx.doi.org/10.1046/j.1365-2826.2003.01033.x.
Xu, Y., Yu, R.M.K., Zhang, X., Murphy, M.B., Giesy, J.P., Lam, M.H.W., Lam, P.K.S., Wu, R.S.S., Yu, H., 2006. Effects of PCBs and $MeSO_2$–PCBs on adrenocortical steroidogenesis in H295R human adrenocortical carcinoma cells. Chemosphere 63, 772–784. http://dx.doi.org/10.1016/j.chemosphere.2005.08.013.

Chen, D., Hale, R.C., 2010. A global review of polybrominated diphenyl ether flame retardant contamination in birds. Environ. Int. 36, 800–811. http://dx.doi.org/10.1016/j.envint.2010.05.013.
Cherel, Y., Klages, N., 1998. A Review of the Food of Albatrosses. Albatross Biology and Conservation. Surrey Beatty, Chipping Norton, pp. 113–136.
Corsolini, S., Borghesi, N., Ademollo, N., Focardi, S., 2011. Chlorinated biphenyls and pesticides in migrating and resident seabirds from East and West Antarctica. Environ. Int. 37, 1329–1335.
del Hoyo, J., Elliott, A., Sargatal, J., Cabot, J., Collar, N.J., 1992. Handbook of the Birds of the World: Ostrich to Ducks. In: Handbook of the Birds of the World. Lynx Edicions.
del Hoyo, J., Elliott, A., Sargatal, J., Collar, N.J., 1996. Handbook of the Birds of the World: Hoatzin to Auks. In: Handbook of the Birds of the World. Lynx Edicions.

PAPER VII

Increased adrenal responsiveness and delayed hatching date in relation to polychlorinated biphenyl exposure in Arctic-breeding black-legged kittiwakes (*Rissa tridactyla*).

Increased adrenal responsiveness and delayed hatching date in relation to polychlorinated biphenyl exposure in Arctic-breeding black-legged kittiwakes (*Rissa tridactyla*)

Sabrina Tartu[1], Adam Z. Lendvai[2], Pierre Blévin[1], Dorte Herzke[3], Paco Bustamante[4], Børge Moe[5], Geir Wing Gabrielsen[6], Jan Ove Bustnes[7] and Olivier Chastel[1]

[1]*Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372-ULR CNRS, F-79360, France*
[2]*Department of Biological Sciences (0406), Derring Hall RM 4102, Virginia Tech 1405 Perry Street*
[3]*Norwegian Institute for Air Research, FRAM High North Research Centre for Climate and the Environment N-9296 Tromsø, Norway*
[4]*Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-Université de la Rochelle, , 2 rue Olympe de Gouges 17000 La Rochelle, France*
[5]*Norwegian Institute for Nature Research, Postboks 5685 Sluppen, N-7485 Trondheim, Norway*
[6]*Norwegian Polar Institute, FRAM – High North Research Centre on Climate and the Environment, N-9296 Tromsø, Norway*
[7]*Norwegian Institute for Nature Research, FRAM – High North Research Centre for Climate and the Environment, N-9296 Tromsø, Norway*

[*]tartu.sabrina@gmail.com

Abstract

High levels of environmental contaminants such as polychlorinated biphenyl (PCBs), organochlorine pesticides (OCPs) and mercury (Hg) have been reported in some Arctic top predators such as seabirds. Chronic exposure to these contaminants might alter the response to environmental changes through interference with the regulation of corticosterone (CORT), a glucocorticoid stress hormone released by the hypothalamo-pituitary-adrenal (HPA) axis. Some relationships between CORT and environmental contaminants have been reported in some polar top predators. However no clear pattern appears and it is difficult to attribute these relationships to a dysfunction of the HPA axis or to other confounding effects. In order to explore the relationships between HPA axis activity and blood levels of PCBs, OCPs and Hg, we conducted an experimental study in an Arctic seabird, the black-legged kittiwakes *Rissa tridactyla* in Svalbard. Male kittiwakes were caught during the incubation period and were subjected to different stress series: 1) a capture-restraint stress protocol, 2) an injection of dexamethasone (DEX) that enabled to test the efficacy of the HPA negative feedback and 3) an injection of adrenocorticotropic hormone (ACTH) that informed on the adrenal responsiveness. The HPA axis activity was unrelated to OCPs and Hg. However, birds with high concentrations of ΣPCBs released more CORT after the ACTH injection. It is suggested that ΣPCBs may increase the number of ACTH-receptors on the adrenals. Also hatching date was delayed in males with higher concentrations of ΣPCBs and ΣOCPs. This study gives new evidence that PCBs and adrenal activity are tightly related. Thus high PCB burden may make individuals more prone to other stressors such as ongoing climate change.

Key-words: corticosterone; ACTH; dexamethasone; adrenal gland; PCBs; reproduction

1. Introduction

Species breeding in extreme environments, such as Polar Regions, are often subjected to a wide range of stressors (e.g. harsh weather, unpredictable food shortage, presence of predators) and an efficient response to these various stressors is vital to ensure self or brood survival (Wingfield et al. 2011; Wingfield 2013). At the endocrine level, an environmental perturbation will stimulate the hypothalamic–pituitary–adrenal (HPA) axis: specifically corticotropin-releasing hormone (CRH) will be released from the hypothalamus and will then stimulate the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn will activate the synthesis of glucocorticoid hormones (corticosterone, CORT for birds) from the adrenal cortex (Wingfield 2013). In birds, up to 90% of CORT released into the bloodstream will bind to corticosteroid-binding globulin (CBG) and will be transported to target cells. Concurrently, glucocorticoids will provide negative feedback signals for ACTH and CRH release (Wingfield 2013). This hormonal cascade will trigger an array of physiological and behavioral adjustments that shift energy investment away from reproduction and redirect it towards survival (Wingfield and Sapolsky, 2003). In birds, CORT has therefore a strong connection with fitness traits such as breeding success, individual quality and survival (Angelier et al. 2009a, 2010; Bonier et al. 2009; Bókony et al. 2009; Breuner et al. 2008; Goutte et al. 2010b, 2011b; Lendvai et al. 2007; Kitaysky et al. 1999; Schultner et al. 2014).

Free-ranging polar seabirds often accumulate various environmental contaminants (e.g. Gabrielsen 2007; Verreault et al. 2010), such as persistent organic pollutant (POPs) which includes polychlorinated biphenyl (PCBs) and organochlorine pesticides (OCPs) but also heavy metals such as mercury (Hg). Several environmental contaminants are endocrine disruptors (Ottinger et al. 2013; Tyler et al. 1998). Indeed those substances are able to mimic, antagonize, alter or modify endogenous' hormone functions (e.g. Amaral Mendes 2002). In free-living vertebrates, several studies have found significant relationships between contaminants and reproductive hormones such as steroids (Colborn et al 1993; Giesy et al. 2003; Vos et al. 2008) and more recently with hormones from the HPA axis (Verboven et al. 2010; Nordstad et al. 2012; Tartu et al. 2014). However the mechanisms through which contaminants may influence CORT regulation are poorly known, it is likely that such compounds disrupt one of the many steps of CORT regulation causing dysfunction of the HPA axis. For instance, energy costs related to e.g. detoxification of POPs (Parkinson and Ogilvie, 2008; Preston and Hoffman, 2008) might stimulate the CORT secretion by increasing the allostatic load (McEwen and Wingfield, 2003). Further, the adrenal gland is suspected to be vulnerable to hazardous effects of POPs due to its high lipid content and multiple sites for interference (Hinson and Raven, 2006; Odermatt and Gumy, 2008).

When it comes to other environmental contaminants such as Hg, studies of seabirds' eggs from the Canadian Arctic show an increasing trend of Hg levels in several species (Braune 2007; Mallory and Braune, 2012). Hg is a well-known endocrine disruptor (reviewed in Tan et al. 2009). However some studies on water birds have reported unclear patterns (Wayland et al 2002, Herring et al. 2012). Therefore, it is difficult to draw a general picture of contaminants-CORT relationships. Also, it is not clear which type of contaminants (Hg, PCBs or OCPs) is actually acting on the HPA axis.

The aim of this study was to investigate the specific role of Hg, PCBs and OCPs on the functioning of the HPA axis of an Arctic seabird, the black-legged kittiwake (*Rissa tridactyla*, hereafter

'kittiwake'). Svalbard kittiwakes bear significant amounts of blood Hg, PCBs and OCPs (Savinova et al. 1995; Nordstad et al., 2012, Tartu et al. 2013, 2014), and two recent studies have reported positive relationships between CORT concentrations and PCB burden (Nordstad et al., 2012, Tartu et al. 2014). For example, in the same breeding population where the present study was conducted, Tartu et al. (2014) found that kittiwakes with higher levels of PCBs had higher stress-induced CORT levels when subjected to a capture-handling protocol. However, it is still unknown whether this relationship is due to an increased adrenocortical responsiveness, a decreased negative feedback during acute stress, or both.

We specifically tested whether different aspects of the HPA axis of incubating male kittiwakes are related to blood Hg, PCBs and OCPs concentrations. Males only were chosen since they are more contaminated than females (Nordstad et al. 2012; Tartu et al. 2013) thus hypothetically more susceptible to the hazardous effects of contaminants. To do so, we measured the CORT response to a restraint stress protocol (Wingfield 1994), birds were then injected with exogenous dexamethasone (DEX, a potent CORT agonist, Astheimer et al.1994) to test the efficiency of the negative feedback. The purpose of the DEX injection was also to induce inhibitory feedback of the HPA axis, thereby reducing confounding effects of endogenous ACTH release. Birds were then injected with exogenous ACTH, a protocol used in several bird species to test the adrenal responsiveness (Rich and Romero 2005; Angelier et al. 2009b; Dickens et al. 2009; Schmidt et al. 2012). Specifically, if the exacerbated CORT release is connected to a higher adrenal activity in the most contaminated individuals, we should observe a positive relationship between blood contaminant concentrations and plasma ACTH-induced CORT concentrations. Furthermore, contaminants may represent stressors (Bustnes et al., 2006), because they may increase the allostatic load and impact fitness. We therefore investigated the consequences of Hg, PCBs and OCPs exposure on reproductive outputs such as hatching date and hatching success. If Hg, PCBs or OCPs stimulate CORT secretion, and since reproductive phenology and success are influenced by CORT levels in kittiwakes (Goutte et al. 2011a), we predict that the most contaminated individuals would have a delayed hatching and a lower hatching success compared to less contaminated kittiwakes.

2. Material and methods

2.1. Ethic statement and study area

The sampling of birds was approved by the Governor of Svalbard, and by the Norwegian Animal Research Authority (NARA, permit number 4214). The study was conducted at Kongsfjorden, Svalbard (78°54′N, 12°13′E) from June 19th to July 4th 2012 during the incubating period for kittiwakes.

2.2. CORT stress series: DEX and ACTH injections

Thirty-four male kittiwakes were caught on the nests with a noose at the end of a 5 m fishing rod. A first blood sample (*ca.* 0.2 ml) was collected immediately after capture, from the alar vein with a 1 mL heparinised syringe and a 25-gauge needle to assess baseline CORT concentrations ([$CORT_{BL}$] henceforth). Bleeding time (i.e. time elapsed from capture to the end of the first blood sample: 2min 24sec ± 28sec (SD) on average) was not related to $CORT_{BL}$ concentrations (GLM, $F_{1,32} = 0.7$, $P = 0.395$). Birds were then placed into a cloth bag and second blood sample (*ca.* 0.2 ml) was collected from the alar vein 30 minutes later (30min 13sec ± 1min 22sec) to assess stress-induced CORT concentrations [$CORT_{30min}$]. Immediately following this blood sample, (30min 58sec ± 1min 26sec), 30 birds were injected with

DEX then put back into the cloth bag. Fifteen minutes later, we collected a third blood sample ([$CORT_{45min}$], at 46min 43sec ± 2min 03sec) and then immediately injected ACTH (47min 29sec ± 2min 05sec), after which the birds were placed back into the bag again. 15 minutes following the ACTH injections, (at 63min 08sec ± 2min 33sec) we collected a fourth and final blood sample [$CORT_{60min}$]. The volume of the final blood sample was 1.5 ml, and this sample was used to measure the concentration of contaminants (see below). All injections were given intramuscularly in the pectoral muscle. Concentrations were adapted to a body mass of 400 g, which is the average body mass of incubating male kittiwakes. We therefore injected 0.2 ml of DEX (Dexazone 2mg/ml Virbac, France) and 0.1 ml porcine ACTH (Sigma Aldrich, 100IU A6303 dissolved in 0.5 ml Ringer's physiological solution), to obtain doses of 1mg/kg dexamethasone (DEX) and 50 IU/kg ACTH which are considered as sufficient to elicit maximal CORT decrease and increase, respectively (Rich and Romero 2005; Dickens et al. 2009; Schmidt et al. 2012).

The stress series were shortened compared to those used in laboratory studies: blood samples were collected 15min after each injection versus 45min normally used to elicit maximal CORT responses induced by DEX and ACTH (Schmidt et al. 2012). We shortened the stress series to avoid leaving the nests unattended for a long period (which may increase the risk of the nest being predated) sampled males were kept for 60min (in total, from baseline sampling to post ACTH injection sample) versus 120min in total in other studies (e.g. Schmidt et al. 2012). Four birds were used as control and injected Ringer's physiological solution to validate the effects of DEX and ACTH injections. We then calculated CORT induced changes following capture/handling protocol, DEX and ACTH injections. Stress-induced CORT: $CORT_{SI}$=[$CORT_{30min}$]-[$CORT_{BL}$]; $CORT_{DEX}$=[$CORT_{45min}$]-[$CORT_{30min}$] and $CORT_{ACTH}$=[$CORT_{60min}$]-[$CORT_{45min}$].

2.3. Hatching dates and hatching success

Kittiwakes were individually marked with metal rings and PVC plastic bands engraved with a three-letter code and fixed to the bird's tarsus for identification from a distance. Birds were weighed to the nearest 2 g using a Pesola spring balance, and their skull length (head+bill) was measured 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 and Green 2009). Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observation and then released. 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 exact hatching date for the first egg (thereafter called "hatching date") and the number of eggs that hatched. The exact hatching date of the first egg laid was obtained for 25 individuals (21 birds injected with DEX and ACTH and 4 birds injected with Ringer's physiological solution). We then considered the "hatching success" binomially: 0 = no eggs at all have hatched and 1 = at least one egg has hatched.

2.4. Molecular sexing and hormone assay

For the 34 focal birds, blood samples were centrifuged, and plasma was decanted and stored at −20°C until assayed. After centrifugation, red cells were kept frozen for molecular sexing as well as for Hg analysis. The sex was determined by polymerase chain reaction amplification of part of two highly conserved genes (CHD) present on the sex chromosomes at UMR 7372 - CNRS-Université de La Rochelle, as detailed in Weimerskirch et al. (2005). Plasma concentrations of CORT were determined by radioimmunoassay at UMR 7372 - CNRS-Université de La Rochelle, as previously described (Lormée et al. 2003). All samples were run in one assay,

to measure intra-assay variation, we included 4 different referents 10 times in the assay. From this, the intra-assay variation was 6.7%. Plasma CORT levels were measured in baseline, stress-, DEX- (or control) and ACTH-induced (or control) CORT samples (**Figure 1**).

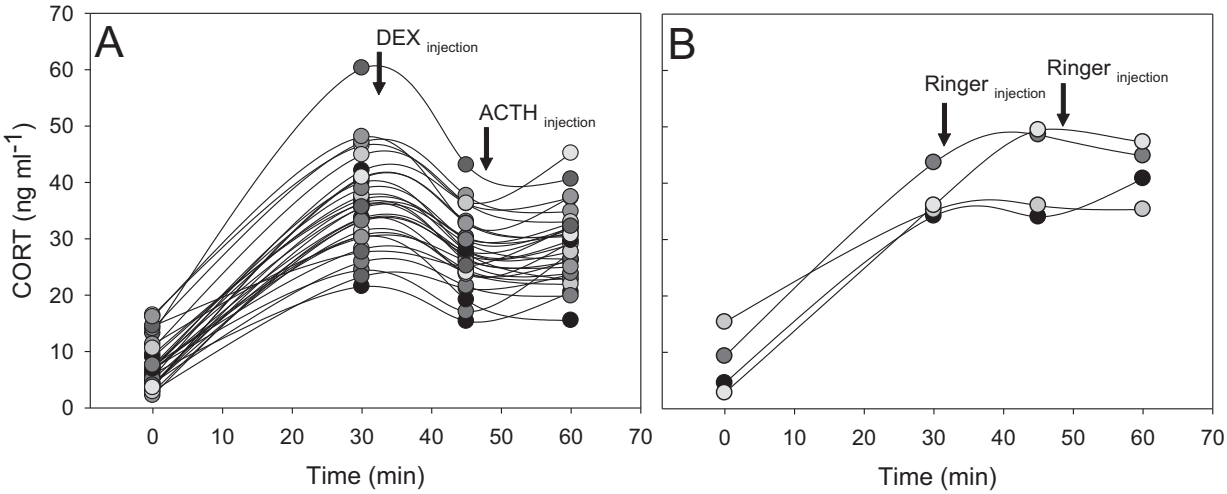


Figure 1: Stress series to characterize the hypothalamo-pituitary-adrenal (HPA) activity in incubating male black-legged kittiwakes. CORT increase from 0 to 30 min indicates the time of exposure to restraint stress. The left panel represents birds that were injected with DEX and ACTH (A) and right panel birds injected with Ringer's physiological solution (B). Each circle denotes an individual and arrows indicate the time of injections.

2.5. POPs determination in plasma

POPs were analyzed from whole blood of 27 birds injected with DEX and ACTH, for three individuals blood volumes were too small for POP measurements. Analyses were performed at the Norwegian Institute for Air Research (NILU) in Tromsø and the following compounds were search for: the PCBs (CB-28, -52, -99, -101, -105, -118, -138, -153, -180, -183, -187 and -194), and the organochlorine pesticides (OCPs: *o,p'* DDT, *p,p'* DDT, *p,p'*DDE, *o,p'* DDE, *o,p'* DDD, *p,p'* DDD, α-, β-, γ-HCH, HCB, *trans*-, *cis*-chlordane, oxychlordane, *trans*-, *cis*-nonachlor and mirex). To a blood sample of 0.5 to 1.5 ml, an internal standard solution was added (^{13}C-labelled compounds from Cambridge Isotope Laboratories: Woburn, MA, USA). The sample was extracted twice with 6 ml of n-hexane, after denaturation with ethanol and a saturated solution of ammonium sulphate in water. Matrix removal on florisil columns, separation on an Agilent Technology 7890 GC and detection on an Agilent Technology 5975C MSD were performed as described by Herzke et al. (2009). The limit for detection (LoD) was threefold the signal-to-noise ratio, and for the compounds investigated the limit ranged from 1.1 to 632.7 pg/g wet weights (ww). For validation of the results, blanks (clean and empty glass tubes treated like a sample, 3 in total) were run for every 10 samples, while standard reference material (3 in total, 1589a human serum from NIST) also was run for every 10 samples. The accuracy of the method was between 70 and 108%. For further investigations, concentrations below LoD were assigned LoD value, and only compounds detected in at least 70% of the individuals were included into the statistical analyses (Noël et al. 2009). In incubating male kittiwakes the following PCBs (CB-28, -99, -105, -118, -138, -153, -180, -183 and -187) and OCPs (*p,p'*-DDE, HCB, oxychlordane, *trans*-, *cis*-nonachlor and mirex) were detected in at least 70 % of the individuals (means ± SD and ranges are given in **Table 1**).

Contaminants	Mean (median)	SD	Min	Max
Hg	1.80 (1.96)	0.45	0.82	2.96
PCB 28	224 (200)	84	50	478
PCB 99	927 (740)	566	410	2700
PCB 105	323 (267)	222	135	1163
PCB 118	1124 (930)	731	500	3870
PCB 138	3931 (3120)	2865	1550	14750
PCB 153	4968 (3820)	3824	1800	20280
PCB 180	2143 (1550)	1648	730	8490
PCB 183	434 (325)	336	148	1759
PCB 187	756 (591)	519	321	2529
ΣPCBs	14831 (11827)	10694	5801	55951
HCB	2977 (2700)	872	1850	6400
oxy-Chlordane	801.6 (667)	385	359	2120
trans-Nonachlor	38.9 (35)	21	6	98
cis-Nonachlor	35 (32)	16	5	71
Mirex	388 (352)	198	167	959
p,p'-DDE	1265 (1177)	697	306	3506
ΣOCPs	4241 (3700)	1413	2495	9616

Table 1: Concentrations of red blood cells' Hg (µg/g dw) and whole blood persistent organic pollutants (pg/g ww) in incubating male black-legged kittiwakes.

2.6. Hg determination in red blood cells

Total Hg was measured for the 34 individuals at LIENSs (La Rochelle), 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 analyzed 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 with recoveries of 98 to 102%). Hg concentrations are expressed in µg/g dry weight (dw).

2.7. Statistics

All statistical analyses were performed using R 2.13.1 (R Development Core Team 2008). To validate the effects of DEX and ACTH injections on CORT release we used generalised linear mixed models (GLMM) with bird identity as a random effect (dependent variable: 'CORT'; independent factors 'Time' and 'Treatment'). Then we tested whether CORT would vary following handling stress and injections (controls, DEX, ACTH). We used generalised linear models (GLM) with a normal/binomial error distribution and an identity/ logit link function, respectively, to test our biological assumptions. We summed POPs as follows: ΣPCBs (n = 9 congeners) and ΣOCPs (n=6 congeners). First, we tested for an effect of 'sampling date' and 'scaled mass index' on contaminants 'ΣPCBs, ΣOCPs and Hg' and on absolute concentration of '[$CORT_{BL}$], [$CORT_{30min}$], [$CORT_{45min}$] and [$CORT_{60min}$]'. Second, we tested for relationships between contaminants 'ΣPCBs, ΣOCPs and Hg' and '[$CORT_{BL}$], $CORT_{SI}$, $CORT_{DEX}$ and $CORT_{ACTH}$'. Finally, we tested if 'hatching date' and 'hatching success' were related to 'scaled mass index' and contaminants 'ΣPCBs, ΣOCPs and Hg'. Diagnostic plots were used to assess whether the data sufficiently met the assumptions of the linear model, and dependent continuous variables were log-10 transformed when necessary. Values are mean ± SD.

3. Results

3.1. CORT stress series

CORT concentrations were significantly related to the time of blood sampling, the treatment and their interaction (GLMM, time: $F_{3,96}$=340.5, P<0.001; treatment: $F_{1,32}$=6.3, P=0.017; time × treatment: $F_{3,96}$=12.15, P<0.001). Following the capture-restraint protocol, CORT concentrations significantly increased (GLMM, $F_{1,29}$=449.3, P<0.001), after the DEX injection CORT concentration significantly decreased over 15 minutes (GLMM, $F_{1,29}$=160.0, P<0.001). Lastly, ACTH injection did not result in a significant increase of CORT within ~15min of action (GLMM, $F_{1,29}$=1.7, P=0.208). However we observed a large inter-individual variation: some individuals may not respond to ACTH injection, in other ones CORT would increase sharply and in others CORT would even decrease (**Fig.1A**). In control birds CORT significantly increased following the capture-restraint protocol (GLMM, $F_{1,3}$=78.5, P=0.003) then remained steady (GLMM, $F_{1,3}$<2.4, P>0.220, for all tests **Fig. 1B**). Incubating male kittiwakes were heavier than the average body mass used for DEX and ACTH dissolutions (427.2 ± 31g, range: 360-490). However $CORT_{DEX}$ and $CORT_{ACTH}$ were not related to body mass (GLM, $F_{1,28}$<0.7, P>0.400).

3.2. Effects of sampling date and scaled mass index on contaminants and hormones

Absolute concentrations of [$CORT_{BL}$], [$CORT_{30min}$], [$CORT_{45min}$] and [$CORT_{60min}$] were not related to sampling date nor to scaled mass index (GLM, $F_{1,28}$<1.3, P>0.269 for all tests). Hg, ΣPCBs or ΣOCPs were not related to sampling date (Hg: GLM, $F_{1,32}$=0.6, P=0.428 and ΣPCBs and ΣOCPs: GLM, $F_{1,25}$<0.2, P>0.674 for all tests). Hg concentrations were not related to scaled mass index (GLM, $F_{1,32}$=0.1, P=0.757), however ΣPCBs and ΣOCPs increased with decreasing scaled mass index (GLM, $F_{1,25}$>4.3, P<0.0480 for all tests).

3.3. Effects of contaminants on HPA activity

Dependent variable	Independent variable	Intercept	Estimate	Df	F	P-value
A) [$CORT_{BL}$]	Hg	2.07	-0.33	1,28	0.81	0.376
	ΣPCBs	1.73	0.02	1,25	0.01	0.913
	ΣOCPs	6.40	-0.54	1,25	2.19	0.152
B) $CORT_{SI}$	Hg	3.32	-0.03	1,28	0.02	0.882
	ΣPCBs	3.89	-0.06	1,25	0.34	0.562
	ΣOCPs	3.55	0.03	1,25	0.02	0.885
C) $CORT_{DEX}$	Hg	-9.81	3.52	1,28	2.38	0.134
	ΣPCBs	-10.07	0.22	1,25	0.03	0.867
	ΣOCPs	-26.12	2.18	1,25	0.76	0.391
D) $CORT_{ACTH}$	Hg	2.89	-3.55	1,28	1.83	0.187
	ΣPCBs	**-27.10**	**2.96**	**1,25**	**4.68**	**0.040**
	ΣOCPs	-36.12	4.45	1,25	2.64	0.117

Table 2: Modeling the relationships between contaminants (Hg, ΣPCBs and ΣOCPs) and A) [$CORT_{BL}$], B) $CORT_{SI}$, C) $CORT_{DEX}$ and D) $CORT_{ACTH}$ in incubating male black-legged kittiwakes.

[$CORT_{BL}$], $CORT_{SI}$ and $CORT_{DEX}$ (**Fig. 2A)** were not related to contaminants (Hg, ΣPCBs, ΣOCPs, **Table 2**). $CORT_{ACTH}$ was not related to Hg or ΣOCPs. However there was a significant and positive relationship between $CORT_{ACTH}$ and ΣPCBs (**Fig. 2B**, **Table 2**): individuals with high PCB burden showed a stronger CORT release following ATCH challenge than less contaminated ones.

3.4. Hatching dates and hatching success in relation to Hg, PCBs and OCPs

Hatching date was not related to scaled mass index (GLM, $F_{1,23}$=0.2, P=0.663) but was positively related to ΣPCBs (GLM, $F_{1,17}$=16.3, P<0.001; **Fig. 3**) and ΣOCPs (GLM, $F_{1,17}$=9.2, P=0.008). The relationship between hatching date and Hg was only close to statistical significance (GLM, $F_{1,23}$=3.4, P=0.076). Hatching success was not related to Hg, ΣPCBs and ΣOCPs (GLM, χ^2<0.2, P>0.635). However, scaled mass index tended to be lower in

individuals with no chicks that hatched (GLM, χ^2=3.7, P=0.055).

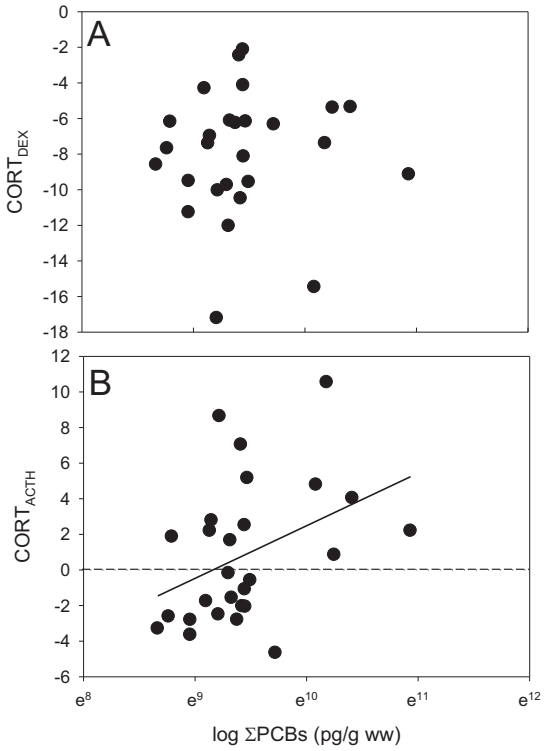


Figure 2: In incubating male black-legged kittiwakes, $CORT_{DEX}$ (upper panel **A**), was not related to blood ΣPCBs (pg/g ww, log-natural transformed). Whereas $CORT_{ACTH}$ (lower panel **B**), was stronger in birds with the higher concentrations of blood ΣPCBs (pg/g^1 ww, log-natural transformed) and lower in the less polluted ones. ACTH injection elicited an increase of CORT in male kittiwakes above dashed line and a decrease of CORT in individuals below.

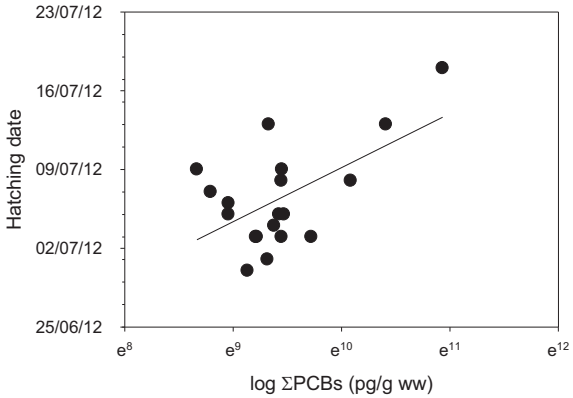


Figure 3: In male black-legged kittiwakes increasing blood ΣPCBs (pg/g ww, log-natural transformed) delayed the hatching of the first egg laid.

4. Discussion

4.1. Contaminants and HPA activity

The aim of this study was to test whether the exacerbated adrenocortical response in the most PCB-contaminated kittiwakes (Tartu et al. 2014) was connected to a higher adrenal activity. In this study we also investigated which type of contaminant (Hg, PCBs or OCPs) would modify the adrenal activity. Contrary to what has been reported in other studies on kittiwakes from the same breeding colony, we did not find any relationship between contaminants, $CORT_{BL}$ and $CORT_{SI}$ (Nordstad et al. 2012; Tartu et al. 2014). $CORT_{DEX}$ was not related to contaminants. This may indicate that environmental contaminants do not alter the functioning of glucocorticoid-receptors from the hypothalamus or the pituitary. According to our prediction, the adrenal responsiveness ($CORT_{ACTH}$) was increased in the most PCB-contaminated kittiwakes. Administration of a standardized dose of ACTH is an alternative approach to measure the stress response that is specifically due to variation in the sensitivity of the adrenal cortex to ACTH. Response to exogenous ACTH may also provide a more accurate measure of maximum glucocorticoid production than response to restraint stress (Wada et al., 2007; Schmidt et al. 2012). However in some birds ACTH injection was not effective and CORT levels even decreased in the less PCB-contaminated birds. These results are surprising but could be the consequence of a too short time of action of ACTH in the present study. Indeed, the time necessary to elicit a maximal CORT release post-ACTH injection was 45min (e.g. Schmidt et al. 2012) and our experimental kittiwakes were only exposed for 15min. If this hypothesis could be verified this would support the fact the activity adrenals of the most PCB-contaminated males is exacerbated. An exacerbated adrenal activity, which may result from increased number of ACTH-receptors (ACTH-R) on the adrenals. In mammals, ACTH is one of the few

polypeptide hormones having a positive trophic effect on its own receptors (Beuschlein et al. 2001; Penhoat et al. 1989). Although there is no evidence for such a relationship in birds, we suggest that it act similar to what is observed in mammals. Thus, the exacerbated adrenal activity in the most PCB contaminated kittiwakes may be the consequence of an excess of ACTH input to adrenals. This also suggests that PCBs may stimulate ACTH release and/or that PCBs may mimic ACTH and bind to ACTH-R, which in that case would mobilize more ACTH-R from the adrenals of the most contaminated individuals. In experimental studies, direct effects of POPs on ACTH-R have already been described: PCB126 can increase ACTH-R levels (Li and Wang, 2005), and a pesticide, the methyl thiophanate, could mimic ACTH and directly activate ACTH-R from the adrenals (De Falco et al. 2007). This and other recent findings (Nordstad et al. 2012; Tartu et al. 2014, Tartu et al. unpublished data) shows that PCBs and not OCPs may specifically interact with CORT in seabirds and that PCBs may increase the number of ACTH-R on the adrenals. This study also provides evidence that Hg is not related to the adrenocortical response or to the adrenal activity, but rather interferes with other endocrine mechanisms, such as pituitary hormones (luteinizing hormone and prolactin, Tartu et al. 2013; Tartu et al. unpublished data). Relationships between CORT and PCBs could also be breeding stage-dependent or environment-dependent. Indeed, in the here present study [$CORT_{BL}$] or $CORT_{SI}$ were not related to PBCs and these relationships could depend of body-condition. In the present study, ΣPCBs and ΣOCPs significantly increased with decreasing body-condition as depicted by scaled mass index. Since POPs are stored into fatty tissues it is not surprising that male kittiwakes with poor body reserves show higher concentrations of PCBs and OCPs in their plasma. Such relationships have been observed in several bird species such as kittiwakes (Henriksen et al. 1996; Tartu et al. 2014), glaucous gulls *Larus hyperboreus* (Sagerup et al. 2009), common eiders *Somateria mollissima* (Bustnes et al. 2010; 2012), sparrowhawks *Accipiter nisus* (Bogan and Newton, 1977) and white-tailed eagle *Haliaeetus albicilla* (Kenntner et al. 2003). Body-condition in birds predicts a wide range of fitness related traits such as incubation pattern, breeding success or survival (see Labocha and Hayes, 2012 for a review). Also, kittiwakes with lower body-condition have higher concentrations of organochlorine contaminants in their blood. As a result these contaminants will therefore be more available for sensitive vital organs as brain, kidneys and liver (Fuglei et al. 2007; Henriksen et al. 1996). Specifically it is possible that the most PCB-contaminated male kittiwakes would be more sensitive to environmental stress (exacerbated adrenocortical response) and thus would be less able to maintain parental investment than less polluted individuals.

4.2. Fitness consequences of Hg, PCBs and OCPs

In our study, we observed a positive relationship between ΣPCBs, ΣOCPs and hatching date. The first egg of the most contaminated male kittiwakes hatched later in the season. The same pattern has been found in female south polar skuas *Catharacta maccormicki* (Bustnes et al. 2007). A longer incubation period could be the result of a non-optimal egg temperature (Hepp et al. 2006; Verboven et al. 2009) thus an impaired incubating behaviour. An experimental study conducted on American kestrels *Falco sparverius,* reported that PCB administration resulted in longer incubation periods and altered incubation behaviours in males especially (Fisher et al. 2006). In the study conducted by Fisher et al. (2006) there was an asynchrony between the incubation bouts realized by females and males, the eggs were left unattended for a longer period and eggs given inadequate heating may

took longer to hatch (Fisher et al. 2006). In free-ranging species, such as glaucous gulls, the proportion of time absent from the nest site when not incubating and the number of absences were related to blood PCB (Bustnes et al. 2001), and still in glaucous gulls, the most contaminated individuals were less able to maintain an optimal nest temperature (Verboven et al. 2009). Consequently the relationship observed in male kittiwakes could result from longer or more frequent recesses during the incubation period as a consequence of high PCBs and OCPs burden. However, the incubation duration generally observed in kittiwakes averages 27 days ± 2-3 days, Coulson 2011), consequently, the observed relationship is more likely to originate from a variation in the onset of mating. It is therefore possible that PCBs or OCPs disrupt some endocrine mechanisms involved in the onset of mating, resulting in a delayed egg-laying date in the most contaminated individuals. Contrary to our prediction, the most contaminated male kittiwakes did not show a lower hatching success. However, in case of poor foraging conditions when CORT secretion is stimulated (Kitaysky et al., 1999; Goutte et al. 2014), it is possible that the most PCB-contaminated male kittiwakes would be more sensitive to environmental stress and would be less able to properly incubate their eggs than less polluted ones. More generally an exacerbated adrenal responsiveness to stress, as depicted by the CORT response to ACTH, often mirrors poor fitness related traits as poor parental investment (Angelier et al. 2009; Bókony et al. 2009; Goutte et al. 2011; Lendvai et al. 2007) or lower adult survival (Blas et al. 2007; Goutte et al. 2010b; Romero 2012). Thus, although most legacy POPs show decreasing trend in Arctic seabirds (e.g. Helgason 2011), the prevalence of PCBs could make individuals more susceptible to other environmental stressors such as ongoing climate change (Jenssen 2006).

Acknowledgments: This project was supported by Institut Polaire Français (IPEV project 330 to O. Chastel), Agence National de la Recherche (ANR project PolarTop to O. Chastel), the Norwegian Research Council and the FRAM Centre (COPOL to GW Gabrielsen & JO Bustnes) and Svalbard Science forum (Arctic field grant 1786 to S Tartu). The authors thank C. Parenteau, C. Trouvé, S. Dano, for their excellent technical assistance in hormones assays and molecular sexing and the NILU staff for their assistance during the chemical analyses.

References

Amaral Mendes, J.J., 2002. The endocrine disrupters: a major medical challenge. Food and Chemical Toxicology 40, 781–788. doi:10.1016/S0278-6915(02)00018-2

Angelier, F., Clément-Chastel, C., Welcker, J., Gabrielsen, G.W., Chastel, O., 2009a. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. Functional Ecology 23, 784–793. doi:10.1111/j.1365-2435.2009.01545.x

Angelier, F., Moe, B., Blanc, S., Chastel, O., 2009b. What Factors Drive Prolactin and Corticosterone Responses to Stress in a Long-Lived Bird Species (Snow Petrel Pagodroma nivea)? Physiological and Biochemical Zoology 82, 590–602. doi:10.1086/592846

Angelier, F., Wingfield, J.C., Weimerskirch, H., Chastel, O., 2010. Hormonal correlates of individual quality in a long-lived bird: a test of the "corticosterone–fitness hypothesis". Biol. Lett. 6, 846–849. doi:10.1098/rsbl.2010.0376

Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1994. Gender and Seasonal Differences in the Adrenocortical Response to ACTH Challenge in an Arctic Passerine, Zonotrichia leucophrys gambelii. General and Comparative Endocrinology 94, 33–43. doi:10.1006/gcen.1994.1057

Beuschlein, F., Fassnacht, M., Klink, A., Allolio, B., Reincke, M., 2001. ACTH-receptor expression, regulation and role in adrenocortial tumor formation. Eur J Endocrinol 144, 199–206. doi:10.1530/eje.0.1440199

Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R., Marchant, T.A., 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. PNAS 104, 8880–8884. doi:10.1073/pnas.0700232104

Bogan, J.A., Newton, I., 1977. Redistribution of DDE in sparrowhawks during starvation. Bull. Environ. Contam. Toxicol. 18, 317–321. doi:10.1007/BF01683425

Bókony, V., Lendvai, Á.Z., Liker, A., Angelier, F., Wingfield, J.C., Chastel, O., 2009. Stress Response and the Value of Reproduction: Are Birds Prudent Parents? The American Naturalist 173, 589–598. doi:10.1086/597610

Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009. Do baseline glucocorticoids predict fitness? Trends in Ecology & Evolution 24, 634–642. doi:10.1016/j.tree.2009.04.013

Braune, B.M., 2007. Temporal trends of organochlorines and mercury in seabird eggs from the Canadian Arctic, 1975–2003. Environmental Pollution 148, 599–613. doi:10.1016/j.envpol.2006.11.024

Breuner, C.W., Patterson, S.H., Hahn, T.P., 2008. In search of relationships between the acute adrenocortical response and fitness. General and Comparative Endocrinology 157, 288–295. doi:10.1016/j.ygcen.2008.05.017

Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C., Caurant, F., 2006. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: Influence of geographical origin and feeding ecology. Science of The Total Environment 368, 585–596. doi:10.1016/j.scitotenv.2006.01.038

Bustnes, J. O., Bakken, V., Erikstad, K. E., Mehlum, F., Skaare, J. U., 2001. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. Journal of Applied Ecology 38, 791–801. doi:10.1046/j.1365-2664.2001.00633.x

Bustnes, J.O., Erikstad, K.E., Hanssen, S.A., Tveraa, T., Folstad, I., Skaare, J.U., 2006. Anti-parasite treatment removes negative effects of environmental pollutants on reproduction in an Arctic seabird. Proc. R. Soc. B 273, 3117–3122. doi:10.1098/rspb.2006.3687

Bustnes, J.O., Tveraa, T., Varpe, Ø., Henden, J.A., Skaare, J.U., 2007. Reproductive performance and organochlorine pollutants in an Antarctic marine top predator: The south polar skua. Environment International 33, 911–918. doi:10.1016/j.envint.2007.04.010

Bustnes, J.O., Moe, B., Herzke, D., Hanssen, S.A., Nordstad, T., Sagerup, K., Gabrielsen, G.W., Borgå, K., 2010. Strongly increasing blood concentrations of lipid-soluble organochlorines in high arctic common eiders during incubation fast. Chemosphere 79, 320–325. doi:10.1016/j.chemosphere.2010.01.026

Bustnes, J.O., Moe, B., Hanssen, S.A., Herzke, D., Fenstad, A.A., Nordstad, T., Borgå, K., Gabrielsen, G.W., 2012. Temporal Dynamics of Circulating Persistent Organic Pollutants in a Fasting Seabird under Different Environmental Conditions. Environ. Sci. Technol. 46, 10287–10294. doi:10.1021/es301746j

Colborn, T., vom Saal, F.S., Soto, A.M., 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101, 378–384.

Coulson, J.C., 2011. The Kittiwake. A&C Black. - 320 p.

De Falco, M., Sciarrillo, R., Capaldo, A., Russo, T., Gay, F., Valiante, S., Varano, L., Laforgia, V., 2007. The Effects of the Fungicide Methyl Thiophanate on Adrenal Gland Morphophysiology of the Lizard, Podarcis sicula. Arch Environ Contam Toxicol 53, 241–248. doi:10.1007/s00244-006-0204-2

Dickens, M.J., Earle, K.A., Romero, L.M., 2009. Initial transference of wild birds to captivity alters stress physiology. General and Comparative Endocrinology 160, 76–83. doi:10.1016/j.ygcen.2008.10.023

Fisher, S.A., Bortolotti, G.R., Fernie, K.J., Bird, D.M., Smits, J.E., 2006. Behavioral variation and its consequences during incubation for American kestrels exposed to polychlorinated biphenyls. Ecotoxicology and Environmental Safety 63, 226–235. doi:10.1016/j.ecoenv.2005.07.021

Franceschini, M.D., Lane, O.P., Evers, D.C., Reed, J.M., Hoskins, B., Romero, L.M., 2009. The corticosterone stress response and mercury contamination in free-living tree swallows, Tachycineta bicolor. Ecotoxicology 18, 514–521. doi:10.1007/s10646-009-0309-2

Fuglei, E., Bustnes, J.O., Hop, H., Mørk, T., Björnfoth, H., van Bavel, B., 2007. Environmental contaminants in arctic foxes (Alopex lagopus) in Svalbard: Relationships with feeding ecology and body condition. Environmental Pollution

146, 128–138. doi:10.1016/j.envpol.2006.06.036

Gabrielsen, G.W., 2007. Levels and effects of persistent organic pollutants in arctic animals, in: Ørbæk, D.J.B., Kallenborn, D.R., Tombre, D.I., Hegseth, D.E.N., Falk-Petersen, D.S., Hoel, D.A.H. (Eds.), Arctic Alpine Ecosystems and People in a Changing Environment. Springer Berlin Heidelberg, pp. 377–412.

Giesy, J.P., Feyk, L.A., Jones, P.D., Kannan, K., Sanderson, T., 2003. Review of the effects of endocrine-disrupting chemicals in birds. Pure and Applied Chemistry 75, 2287–2303. doi:10.1351/pac200375112287

Goutte, A., Angelier, F., Welcker, J., Moe, B., Clément-Chastel, C., Gabrielsen, G.W., Bech, C., Chastel, O., 2010. Long-term survival effect of corticosterone manipulation in Black-legged kittiwakes. General and Comparative Endocrinology 167, 246–251. doi:10.1016/j.ygcen.2010.03.018

Goutte, A., Clément-Chastel, C., Moe, B., Bech, C., Gabrielsen, G.W., Chastel, O., 2011a. Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes. J Exp Biol 214, 2005–2013. doi:10.1242/jeb.051979

Goutte, A., Antoine, É., Chastel, O., 2011b. Experimentally delayed hatching triggers a magnified stress response in a long-lived bird. Hormones and Behavior 59, 167–173. doi:10.1016/j.yhbeh.2010.11.004

Goutte, A., Angelier, F., Bech, C., Clément-Chastel, C., Dell'Omo, G., Gabrielsen, G.W., Lendvai, Á.Z., Moe, B., Noreen, E., Pinaud, D., 2014. Annual variation in the timing of breeding, pre-breeding foraging areas and corticosterone levels in an Arctic population of black-legged kittiwakes. Mar Ecol Prog Ser 496, 233–247.

Helgason, L.B., Sagerup, K., Gabrielsen, G.W. 2011. Temporal trends and contaminant profiles of persistent organic pollutants (POPs) in seabird eggs from Northern Norway and Svalbard. Loganathan, B.G., Lam, P.K.S. (eds.). Global Contamination Trends of Persistent Organic Chemicals. CRC Press.

Henriksen, E.O., Gabrielsen, G.W., Skaare, J.U., 1996. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (Rissa tridactyla), in relation to mobilization of body-lipids associated with reproduction. Environmental Pollution 92, 27–37. doi:10.1016/0269-7491(95)00087-9

Hepp, G.R., Kennamer, R.A., Johnson, M.H., 2006. Maternal effects in Wood Ducks: incubation temperature influences incubation period and neonate phenotype. Functional Ecology 20, 308–314. doi:10.1111/j.1365-2435.2006.01108.x

Herring, G., Ackerman, J.T., Herzog, M.P., 2012. Mercury Exposure May Suppress Baseline Corticosterone Levels in Juvenile Birds. Environ. Sci. Technol. 46, 6339–6346. doi:10.1021/es300668c

Herzke, D., Nygård, T., Berger, U., Huber, S., Røv, N., 2009. Perfluorinated and other persistent halogenated organic compounds in European shag (Phalacrocorax aristotelis) and common eider (Somateria mollissima) from Norway: A suburban to remote pollutant gradient. Science of The Total Environment 408, 340–348. doi:10.1016/j.scitotenv.2009.08.048

Hinson, J.P., Raven, P.W., 2006. Effects of endocrine-disrupting chemicals on adrenal function. Best Practice & Research Clinical Endocrinology & Metabolism 20, 111–120. doi:10.1016/j.beem.2005.09.006

Jenssen, B.M., 2005. Endocrine-Disrupting Chemicals and Climate Change: A Worst-Case Combination for Arctic Marine Mammals and Seabirds? Environmental Health Perspectives 114, 76–80. doi:10.1289/ehp.8057

Kenntner, N., Krone, O., Oehme, G., Heidecke, D., Tataruch, F., 2003. Organochlorine contaminants in body tissue of free-ranging white-tailed eagles from northern regions of Germany. Environmental Toxicology and Chemistry 22, 1457–1464. doi:10.1002/etc.5620220706

Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 1999. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. Functional Ecology 13, 577–584. doi:10.1046/j.1365-2435.1999.00352.x

Labocha, M.K., Hayes, J.P., 2012. Morphometric indices of body condition in birds: a review. J Ornithol 153, 1–22. doi:10.1007/s10336-011-0706-1

Lendvai, Á.Z., Giraudeau, M., Chastel, O., 2007. Reproduction and modulation of the stress response: an experimental test in the house sparrow. Proc. R. Soc. B 274, 391–397. doi:10.1098/rspb.2006.3735

Li, L.A., Wang, P. W., 2005. PCB126 Induces Differential Changes in Androgen, Cortisol, and Aldosterone Biosynthesis in Human Adrenocortical H295R Cells. Toxicol. Sci. 85, 530–540. doi:10.1093/toxsci/kfi105

Lindström, J., 1999. Early development and fitness in birds and mammals. Trends in Ecology & Evolution 14, 343–348. doi:10.1016/S0169-5347(99)01639-0

Lormée, H., Jouventin, P., Trouve, C., Chastel, O., 2003. Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies Sula sula. Ibis 145, 212–219. doi:10.1046/j.1474-919X.2003.00106.x

Mallory, M.L., Braune, B.M., 2012. Tracking contaminants in seabirds of Arctic Canada: Temporal and spatial insights. Marine Pollution Bulletin 64, 1475–1484. doi:10.1016/j.marpolbul.2012.05.012

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Hormones and behavior 43, 2–15.

Noël, M., Barrett-Lennard, L., Guinet, C., Dangerfield, N., Ross, P.S., 2009. Persistent organic pollutants (POPs) in killer whales (Orcinus orca) from the Crozet Archipelago, southern Indian Ocean. Marine Environmental Research 68, 196–202. doi:10.1016/j.marenvres.2009.06.009

Nordstad, T., Moe, B., Bustnes, J.O., Bech, C., Chastel, O., Goutte, A., Sagerup, K., Trouvé, C., Herzke, D., Gabrielsen, G.W., 2012. Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (Rissa tridactyla) across their breeding cycle. Environmental Pollution 164, 219–226. doi:10.1016/j.envpol.2012.01.044

Odermatt, A., Gumy, C., 2008. Glucocorticoid and mineralocorticoid action: Why should we consider influences by environmental chemicals? Biochemical Pharmacology 76, 1184–1193. doi:10.1016/j.bcp.2008.07.019

Oskam, I., Ropstad, E., Lie, E., Derocher, A., Wiig, Ø., Dahl, E., Larsen, S., Skaare, J.U., 2004. Organochlorines Affect the Steroid Hormone Cortisol in Free-Ranging Polar Bears (Ursus maritimus) at Svalbard, Norway. Journal of Toxicology and Environmental Health, Part A 67, 959–977. doi:10.1080/15287390490443731

Ottinger, M.A., Carro, T., Bohannon, M., Baltos, L., Marcell, A.M., McKernan, M., Dean, K.M., Lavoie, E., Abdelnabi, M., 2013. Assessing effects of environmental chemicals on neuroendocrine systems: potential mechanisms and functional outcomes. Gen. Comp. Endocrinol. 190, 194–202. doi:10.1016/j.ygcen.2013.06.004

Parkinson, A., Ogilvie, B.W., 2008. Biotransformation of xenobiotics, in: Casarett and Doull's Toxicology: The Basic Science of Poisons. C.D. Klaassen, McGraw Hill, USA.

Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118, 1883–1891. doi:10.1111/j.1600-0706.2009.17643.x

Penhoat, A., Jaillard, C., Saez, J.M., 1989. Corticotropin positively regulates its own receptors and cAMP response in cultured bovine adrenal cells. PNAS 86, 4978–4981.

Preston, J.R., Hoffman, G.R., 2008. Gene toxicology, in: Casarett and Doull's Toxicology: The Basic Science of Poisons. C.D. Klaassen, McGraw Hill, USA.

Rich, E.L., Romero, L.M., 2005. Exposure to chronic stress downregulates corticosterone responses to acute stressors. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 288, R1628–R1636. doi:10.1152/ajpregu.00484.2004

Romero, L.M., 2012. Using the reactive scope model to understand why stress physiology predicts survival during starvation in Galápagos marine iguanas. General and Comparative Endocrinology 176, 296–299. doi:10.1016/j.ygcen.2011.11.004

Sagerup, K., Helgason, L.B., Polder, A., Strøm, H., Josefsen, T.D., Skåre, J.U., Gabrielsen, G.W., 2009. Persistent organic pollutants and mercury in dead and dying glaucous gulls (Larus hyperboreus) at Bjørnøya (Svalbard). Science of The Total Environment 407, 6009–6016. doi:10.1016/j.scitotenv.2009.08.020

Savinova, T.N., Polder, A., Gabrielsen, G.W., Skaare, J.U., 1995. Chlorinated hydrocarbons in seabirds from the Barents Sea area. Science of The Total Environment 160–161, 497–504. doi:10.1016/0048-9697(95)04383-C

Schmidt, K.L., Furlonger, A.A., Lapierre, J.M., MacDougall-Shackleton, E.A., MacDougall-Shackleton, S.A., 2012. Regulation of the HPA axis is related to song complexity and measures of phenotypic quality in song sparrows. Hormones and Behavior 61, 652–659. doi:10.1016/j.yhbeh.2012.02.027

Schultner J., B. Moe, O. Chastel, C. Bech, A.S. Kitaysky (2014) Migration and stress during reproduction govern telomere dynamics in a seabird. Biology Letters 10: 20130889

Tan, S.W., Meiller, J.C., Mahaffey, K.R., 2009. The endocrine effects of mercury in

humans and wildlife. Critical Reviews in Toxicology 39, 228–269. doi:10.1080/10408440802233259

Tartu, S., Goutte, A., Bustamante, P., Angelier, F., Moe, B., Clément-Chastel, C., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2013. To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biology Letters 9. doi:10.1098/rsbl.2013.0317

Tartu, S., Angelier, F., Herzke, D., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2014. The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. Science of The Total Environment 476–477, 553–560. doi:10.1016/j.scitotenv.2014.01.060

Tyler, C.R., Jobling, S., Sumpter, J.P., 1998. Endocrine Disruption in Wildlife: A Critical Review of the Evidence. Critical Reviews in Toxicology 28, 319–361. doi:10.1080/10408449891344236

Verboven, N., Verreault, J., Letcher, R.J., Gabrielsen, G.W., Evans, N.P., 2009. Nest temperature and parental behaviour of Arctic-breeding glaucous gulls exposed to persistent organic pollutants. Animal Behaviour 77, 411–418. doi:10.1016/j.anbehav.2008.10.022

Verboven, N., Verreault, J., Letcher, R.J., Gabrielsen, G.W., Evans, N.P., 2010. Adrenocortical function of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. General and Comparative Endocrinology 166, 25–32. doi:10.1016/j.ygcen.2009.11.013

Verreault, J., Gabrielsen, G.W., Bustnes, J.O., 2010. The Svalbard Glaucous Gull as Bioindicator Species in the European Arctic: Insight from 35 Years of Contaminants Research, in: Whitacre, D.M. (Ed.), Reviews of Environmental Contamination and Toxicology Volume 205, Reviews of Environmental Contamination and Toxicology. Springer New York, pp. 77–116.

Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambré, C., Tarazona, J.V., Brandt, I., Vethaak, A.D., 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. CRC Critical Reviews in Toxicology 30, 71–133.

Wada, H., Cristol, D.A., McNabb, F.M.A., Hopkins, W.A., 2009. Suppressed Adrenocortical Responses and Thyroid Hormone Levels in Birds near a Mercury-Contaminated River. Environ. Sci. Technol. 43, 6031–6038. doi:10.1021/es803707f

Wada, H., Hahn, T.P., Breuner, C.W., 2007. Development of stress reactivity in white-crowned sparrow nestlings: Total corticosterone response increases with age, while free corticosterone response remains low. General and Comparative Endocrinology 150, 405–413. doi:10.1016/j.ygcen.2006.10.002

Wayland, M., Gilchrist, H.G., Marchant, T., Keating, J., Smits, J.E., 2002. Immune Function, Stress Response, and Body Condition in Arctic-Breeding Common Eiders in Relation to Cadmium, Mercury, and Selenium Concentrations. Environmental Research 90, 47–60. doi:10.1006/enrs.2002.4384

Weimerskirch, H., Lallemand, J., Martin, J., 2005. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. Journal of Animal Ecology 74, 285–291. doi:10.1111/j.1365-2656.2005.00922.x

Wingfield, J., 1994. Modulation of the adrenocortical response to stress in birds. Perspectives in comparative endocrinology 520–528.

Wingfield, J.C., 2013. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. Funct Ecol 27, 37–44. doi:10.1111/1365-2435.12039

Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and Resistance to Stress: When and How. Journal of Neuroendocrinology 15, 711–724. doi:10.1046/j.1365-2826.2003.01033.x

Wingfield, J.C., Kelley, J.P., Angelier, F., 2011. What are extreme environmental conditions and how do organisms cope with them? Current Zoology 57.

PAPER VIII

Endocrine and fitness correlates of long-chain perfluorinated carboxylates exposure in Arctic breeding black-legged kittiwakes.

Environmental Science & Technology

Article

pubs.acs.org/est

Endocrine and Fitness Correlates of Long-Chain Perfluorinated Carboxylates Exposure in Arctic Breeding Black-Legged Kittiwakes

Sabrina Tartu,*[,†] Geir W. Gabrielsen,[‡] Pierre Blévin,[†] Hugh Ellis,[§] Jan Ove Bustnes,[∥] Dorte Herzke,[⊥] and Olivier Chastel[†]

[†]Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372-ULR CNRS, Villiers-en-Bois F-79360, France

[‡]Norwegian Polar Research Institute, FRAM – High North Research on Climate and the Environment, N-9296 Tromsø, Norway

[§]Department of Biology, University of San Diego, San Diego, California 92110, United States

[∥]Norwegian Institute for Nature Research, FRAM – High North Research Centre on Climate and the Environment, N-9296 Tromsø, Norway

[⊥]Norwegian Institute for Air Research, FRAM – High North Research Centre on Climate and the Environment, N-9296 Tromsø, Norway

Supporting Information

ABSTRACT: Increasing levels of poly- and perfluorinated alkyl substances (PFASs) have recently been described in Arctic biota. These emerging substances are of concern given their resistance to degradation and metabolization. Some studies have reported endocrine disrupting effects for some PFASs. However, there is a gap of knowledge on the potential relationships between PFASs and hormones mediating the life-history trade-off between reproduction and survival, such as glucocorticoids. The aims of this study were to (1) describe the concentrations of plasma perfluoroalkyl sulfonates and perfluoroalkyl carboxylates in Svalbard black-legged kittiwakes (*Rissa tridactyla*) in relation to gender and body-condition, (2) explore the relationships between PFASs and corticosterone (the major glucocorticoid in birds), and (3) assess the consequences of PFAS exposure for reproductive success. Perfluorononanoate was positively related to body-condition in male kittiwakes; perfluorotridecanoate and perfluorotetradecanoate to decreased baseline corticosterone in both sexes; and perfluorododecanoate was related to lower hatching success. These results underline the importance of considering each compound separately when investigating the hazardous effects of PFASs on wildlife.

INTRODUCTION

While most attention was directed toward the endocrine disrupting effects of legacy persistent organic pollutants (POPs),[1−4] the so-called emerging POPs came into focus in the late 90s. Many emerging POPs are not regulated and comprise a wide array of everyday life products,[5] but ecotoxicological data on these compounds are lacking.[6] Among them, poly- and perfluorinated alkyl substances (PFASs) are used as surface-active agents in a multitude of manufactured and consumer products (e.g., fire-fighting foam and impregnation agent for carpets, papers, and textiles). PFASs are particularly alarming, because of their special properties: they are thermally and chemically stable, have no route of degradation and cannot be metabolized by vertebrates under normal environmental conditions[7] which makes them extremely persistent in the environment. Moreover, PFASs consist of perfluorinated carbon chains that are hydrophobic and lipophobic, so they can accumulate in the blood, liver and kidney.[8,9] Nowadays, human exposure to PFASs measured in the blood reaches the highest values observed by an exogenous chemical.[9] The occurrence of PFASs have been described in polar region such as the Arctic[10] and concentrations of some PFASs tend to increase over time in several Arctic mammal and seabird species.[11−13] In Arctic regions long-chained perfluoroalkyl carboxylic acids (PFCAs) are prevalent[11] and acute toxicity of PFCAs increases with chain length.[9] With regard to the potential endocrine disrupting properties of PFASs, laboratory studies have shown that some PFAS possess estrogenic, androgenic and thyroid-like activity.[5,9,14,15] However, to date very few studies have investigated the relationships between hormones and PFAS in free living species.[16] Furthermore, the possible influence of PFAS on some major endocrine axes has only been investigated in a few studies. This is especially the case for the hypothalamo-pituitary-adrenal (HPA) axis. The HPA axis plays an important role in mediating the life-history trade-off between reproduction and survival across the release of stress hormones such as glucocorticoids.[17] However, little is known about the disruption of PFASs on glucocorticoids. The release of glucocorticoid hormones (cortisol, corticosterone: CORT) during stressful events triggers physiological and behavioral adjustments that shift

Received: July 8, 2014
Revised: October 1, 2014
Accepted: October 22, 2014

ACS Publications © XXXX American Chemical Society

Table 1. PFASs Mean Concentrations ± Standard Deviation (pg/mL ww), LOD and LOQ in the plasma of female and male chick-rearing adult kittiwakes

				females					males				
	n° C	LOD	LOQ	N > LOD	mean		SD	range	N > LOD	mean		SD	range
Perfluoroalkyl Sulfonates (PFSA)													
PFBS	4	18.5	55.5	0	nd				0				
PFHxS	6	10.7	32.0	5				[<10.7; 216]	4				[<10.7; 130]
PFOSlin	8	704	2111	10	9299	±	2611	[6804; 13581]	10	10 233	±	2685	[7002; 15183]
Perfluoroalkyl carboxylates (PFCA)													
PFBA	4	918	2754	0	nd				0				
PFPA	5	36.2	109	0	nd				0				
PFHxA	6	6.2	18	0	nd				0				
PFHpA	7	91.2	274	0	nd				0				
PFOA	8	26.5	80	2				[<26.5; 122]	2				[<26.5; 167]
PFNA	9	40.9	123	10	967	±	704	[805; 3047]	10	1241	±	547	[787; 2593]
PFDcA	10	61.9	186	10	1705	±	464	[1301; 2764]	10	2162	±	528	[1233; 3123]
PFUnA	11	83.0	249	10	10 449	±	2636	[7712; 16618]	10	11 413	±	2808	[7853; 17546]
PFDoA	12	109	327	10	2188	±	709	[1472; 4014]	10	2658	±	662	[1893; 3815]
PFTrA	13	360	1079	10	12 960	±	7330	[4495; 29735]	10	18 156	±	4022	[11217; 23055]
PFTeA	14	235	706	10	1167	±	840	[289; 3258]	9	1798	±	532	[<235; 2712]
∑7PFASs					47 947	±	11 213	[29172; 66048]		41 339	±	11 967	[24336; 71204]

energy investment away from reproduction and redirects it toward self-preservation and hence survival.[17,18] CORT is very likely to mediate parental effort and parental investment in birds[19,20] and any disruption of this hormone may alter the ability of an individual to adjust reproductive decisions to environmental conditions.[21,22] Because of increasing prevalence of PFASs in the environment, especially of the most toxic long-chained PFCAs in the Arctic, and because of the pivotal role of the HPA axis, PFASs should therefore become the focus of interest as emerging endocrine disruptors for wildlife. There is also very limited information on the potential negative impact of PFASs on fitness (e.g., reproductive success, survival) of free-living organisms.[23−25] Arctic seabirds are top predators particularly at risk given the biomagnification properties of some PFASs along the trophic web.[26] The aims of this paper are to (1) describe the concentrations of plasma PFSAs (perfluoroalkyl sulfonic acids) and PFCAs in an Arctic seabird species in relation to gender and body-condition, (2) explore their relationships with the HPA axis, and especially with plasma CORT concentrations, (3) assess the consequences of PFAS exposure on fitness traits like reproductive success. To do so, we investigated these relationships in chick-rearing adult black-legged kittiwakes (*Rissa tridactyla*) which in Svalbard are known to be exposed to PFASs.[16]

EXPERIMENTAL SECTION

Study Area and Sampling Collection. 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, RIS 2961). The study was conducted at Kongsfjorden, Svalbard (78°54′N, 12°13′E) from July 12th to July 26th 2012 during the chick-rearing period. Twenty birds (10 males and 10 females), were caught on 20 different nests with a noose at the end of a 5 m fishing rod. A first blood sample (ca. 0.3 mL) was collected immediately after capture, from the alar vein with a 1 mL heparinised syringe and a 25-gauge needle to assess baseline CORT concentrations. Bleeding time (i.e., time elapsed from capture to the end of the first blood sample: 2 min 27s ± 31s (SD), on average) did not affect CORT concentrations (GLM, $F_{1,18} = 0.39$, $p = 0.538$). Eighteen kittiwakes (10 males and 8 females) were then placed into a cloth bag and a second blood sample (ca. 2.5 mL) was collected from the alar vein at 30 min from capture (30 min 13 s ± 1 min 02 s) to assess stress-induced CORT and PFAS concentrations. 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. Birds were weighed to the nearest 2 g using a Pesola spring balance, and their skull length (head+bill) was measured to the nearest 0.5 mm with a sliding calliper. For each bird we calculated its scaled mass index[27] as a measure of body-condition. Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observation and then released. Prior to the beginning of the sampling period, using a mirror at the end of an 8 m fishing rod, we checked the whole plot (ca. 117 nests) every 2 days to monitor the clutch size, the number of chicks that hatched (thereafter 'hatching success') and those that reached at least 12 days of age per active nest (thereafter called "breeding success"). All birds studied and sampled had a clutch of two eggs.

Molecular Sexing and Hormone Assay. Blood samples were centrifuged and stored at −20 °C until used respectively in hormone assays or molecular sexing, at the Centre d'Etudes Biologiques de Chizé (CEBC). The sex was determined by polymerase chain reaction (PCR) amplification of part of two highly conserved genes (CHD) present on the sex chromosomes at the Centre d'Etudes Biologiques de Chizé (CEBC).[28] Plasma concentrations of CORT were determined by radioimmunoassay[29] at the CEBC. The lowest detectable concentration for CORT was 1.05 ng/mL. Only one assay was performed and the intra-assay coefficient of variation was 6.7% ($N = 5$ duplicates).

Chemical Analyses. Analyses for perfluorinated compounds in plasma samples were performed at the Norwegian Institute of Air Research (NILU, Tromsø, Norway). We searched for 14 PFASs: perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), linear perfluorooctanesulfonate (PFOSlin), perfluorobutanoate (PFBA), perfluoropentanoate (PFPA), perfluorohexanoate (PFHxA), perfluorohepta-

ACS Publications © XXXX American Chemical Society

noate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDcA), perfluoroundecanoate (PFUnA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTrA), and perfluorotetradecanoate (PFTeA). The method was described in detail along with instrumental settings in previous studies.[30,31] In short, a sample (0.5 mL) spiked with internal standards was extracted in acetonitrile (1 mL) by repeated sonication and vortexing. The supernatant was cleaned up using ENVI-Carb graphitized carbon absorbent and glacial acetic acid. Extracts were analyzed by UPLC/MS/MS. Recovery of the internal standards ranged between 45% and 120%. The deviation of the target concentrations in the SRMs (NIST Human serum 1958) were within the laboratory's accepted deviation from target concentrations (<40%) (n = 4). All blanks contained concentrations below the instrument detection limits (three times chromatographic noise). For each compound, limit of detection (LOD) and limit of quantification (LOQ) are given in Table 1.

Statistical analyses. For statistics, only samples with concentrations over the analyte-specific LODs and detected in more than 70% of the samples were included. All statistical analyses were performed using R 2.13.1 and generalized linear models (GLM) with a normal/binomial error distribution and an identity/logit link function were used to test our biological assumptions. First, we tested the effects of "sex" on "body-condition", "CORT" and "PFASs". Second we checked for relationships between "PFASs" and "CORT". Third, we tested if PFAS were related to body-condition in males and females separately. Finally, we tested whether "CORT" and "PFASs" affected "hatching success" and 'breeding success'. Since only one bird had a null hatching success and thus a null breeding success it was removed from statistical analyses, we thus performed analyses with a binomial response, hatching success (1 or 2 eggs have hatched) and breeding success (1 or 2 chicks have reached 12 days of age) . Model selection was performed by a step-down approach starting from the global model including all the independent variables, these latter were log-10 transformed when necessary and statistical significance was fixed to $\alpha < 0.05$. In all models we tested the effect of each compound separately.

RESULTS AND DISCUSSION

Compounds and levels of PFASs. Fourteen PFASs were analyzed of which six (PFOSlin, PFNA, PFDcA, PFUnA, PFDoA, PFTrA) were detected in the 20 captured kittiwakes and PFTeA was detected in 19 kittiwakes. PFHxS and PFOA were detected in 9 and 4 birds, respectively (Table 1). They were thus excluded from statistical analyses. The dominating compound was PFTrA closely followed by PFUnA and PFOSlin then by decreasing order PFDoA > PFDcA > PFTeA > PFNA (Table 1). Most studies on PFASs have concentrated on PFOS and PFOA, as they are often the most present compounds in vertebrates.[32] As a consequence there is limited information available on the toxicological effects and risk of PFCAs with longer chains than PFOA.[32,33] Contrary to kittiwake chicks, where PFOSlin was the dominant compound,[16] in adult chick-rearing kittiwakes, odd numbered longer chained PFCAs (C11 and C13) were the dominant fluorinated compounds. This difference in PFASs profile could originate from PFOS regulations taken in 2009 by the Stockholm Convention on POPs, indeed the study on chicks[16] occurred 6 years before the present one. Another explanation could be a diet difference between chicks and adults.

Hormones and PFASs in Relation to Sex and Body-Condition. CORT concentrations (baseline and stress-induced) and PFASs were not related to sex (GLM, $F < 2.7$, $p > 0.11$, SI) however for the PFCAs with longer chains (i.e., PFDoA, PFTrA, and PFTeA), plasma concentrations tended to be higher in males than in females ($F < 3.88$, $p > 0.064$, SI). Body-condition was higher in males than in females (GLM, $F_{1,18} = 38.7$, $p < 0.001$). CORT concentrations were not related to body-condition in males or in females ($F < 3.01$, $p > 0.133$). PFNA was positively related to body-condition in males only (GLM, $F_{1,8} = 7.19$, $p = 0.028$; Figure 1) and no relationships

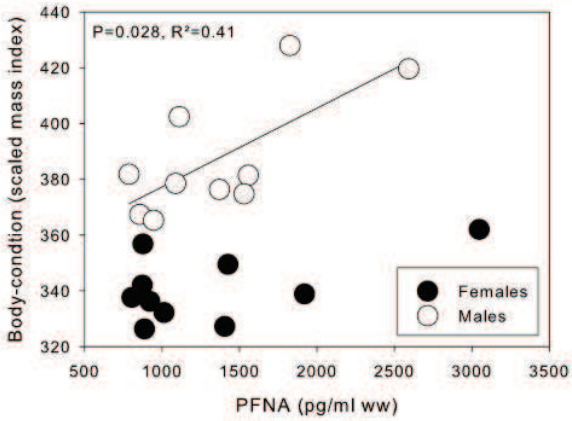


Figure 1. In male black-legged kittiwakes (open circles), body-condition (scaled mass index) was positively related to plasma PFNA concentrations. A relationship not found in females (closed circles). The solid line refers to a statistically significant linear regression.

were found between the other PFASs and body-condition in males or females ($F < 2.43$, $p > 0.158$, SI). In the results presented here, male kittiwakes with higher concentrations of PFNA were in better body- condition. Body-condition as measured by scaled mass index is a reliable predictor of body fat and proteins[27] and PFASs have high affinity for proteins.[8] The positive relationship observed between PFNA and body-condition in male kittiwakes could be related to the structural resemblance of PFASs to fatty acids and their capability to bind to nuclear receptors which play a key role in lipid metabolism and adipogenesis.[34,35] Activation is greater as carbon backbone length increases, and carboxylates (PFOA and PFNA) have higher activation properties.[35] In humans, PFNA can be associated with increased cholesterol and adiponectin concentrations[36,37] and PFOA levels correlate with body weight but also insulin and leptin concentrations.[38] All these hormones are strongly associated with obesity and food intake; it is possible that similar mechanisms to those reported in humans could occur in birds. However, we should be cautious in interpreting this result, as we have no evidence that PFNA disrupts lipid metabolism in birds, and the reason why no relationship was found in females remains unexplained. It may result from the ability of females to transfer elevated amounts of PFASs into their eggs[13,39] however in the present study PFNA concentrations were not lower in females.

Relationships between PFASs and Hormones. In adult chick-rearing kittiwakes baseline CORT concentrations were negatively related to PFTrA and PFTeA (Figure 2, Table 2). No relationships were found with the other PFASs, and no

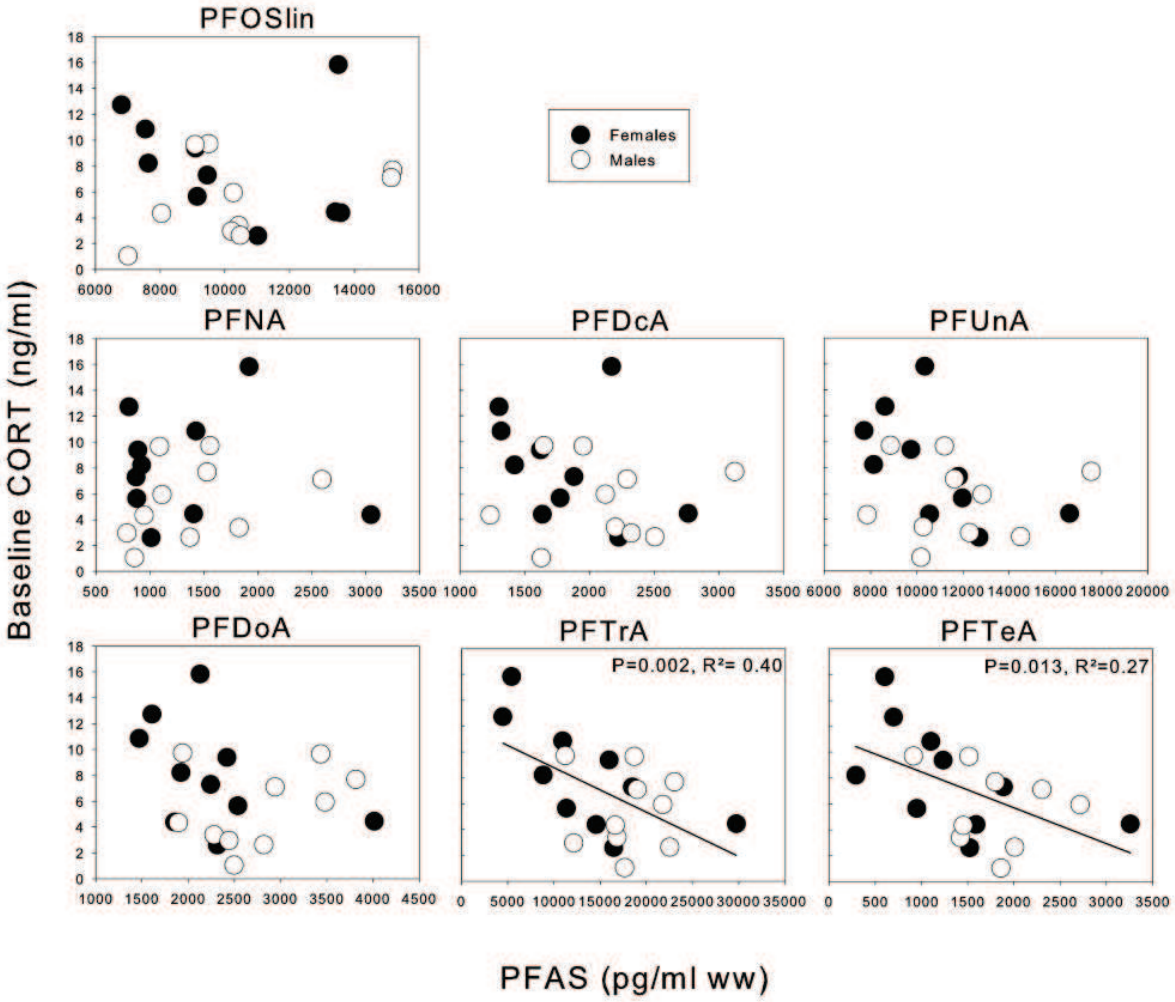


Figure 2. Baseline CORT concentrations in relation to seven PFASs. Baseline CORT decreased with increasing plasma PFTrA and PFTeA concentrations, in male (open circles) and female (closed circles) chick-rearing black-legged kittiwakes. The solid line refers to a statistically significant linear regression.

relationship was found between PFASs and stress-induced CORT concentrations (Table 2, SI). This negative relationship between PFTrA, PFTeA, and CORT could be the result of several mechanisms: a negative feedback due to other hormones, hormone displacement due to high protein affinity, or a disruption of the HPA axis that has resulted in a lower ability to secrete proper baseline CORT. Some experimental studies have reported effects of PFCAs on CORT: for example in PFNA treated mice, the group receiving the higher dose had increased concentrations of both ACTH and cortisol.[40] However, in kittiwakes PFNA concentrations were not related to CORT. One possible interpretation to explain these relationships between CORT, PFTrA, and PFTeA comes from the ability of PFASs to displace hormones by binding to proteins.[8] Because of their high affinity for proteins, it has been suggested that PFASs could easily bind to transport proteins and cause hormone displacement.[8] CORT is protein dependent to ensure its specific role by binding to CORT-binding-globulin (CBG).[41] This binding of CORT to CBG may serve as a tissue buffer against potentially deleterious effects of elevated circulating CORT.[41,42] Measuring the quantity of CBG and the number of glucocorticoid receptors (GR) could inform on the effective activity of CORT in kittiwakes contaminated by PFASs. Indeed, the observed decrease of baseline CORT concentrations with increasing PFTrA and PFTeA in kittiwakes could be a response to an increase of GR and/or a decrease of CBG. Indeed, the action of CBG is supposed to make CORT inactive,[43] so if most of the CBG are bounded with PFTrA and PFTeA, more CORT will remain free and active. Very high levels of active CORT could have an important impact on health, thus a decrease in the production of CORT from the adrenals may be an adaptation to keep free CORT concentration within the normal physiological ranges. It has been suggested[8] that "*given the current environmental concentrations of PFOS, it was unlikely that PFOS would cause displacement of hormones from serum proteins in wildlife*"; indeed though no relationships between PFOS and CORT were found in the present study, the relationships with longer chain PFCAs

dx.doi.org/10.1021/es503297n | Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Table 2. Modelling the Relationship Between PFASs and (A) Baseline and (B) Stress-Induced CORT Concentrations in Chick-Rearing Black-Legged Kittiwakes

dependent variable	independent variable	SS	Df	F	Pr(>F)
(A) baseline CORT	linear PFOS	0.6	1.18	0.0	0.838
	PFNA	2.9	1.18	0.2	0.668
	PFDcA	18.6	1.18	1.3	0.266
	PFUnA	36.3	1.18	2.8	0.114
	PFDoA	23.8	1.18	1.7	0.207
	PFTrA	117.2	1.18	13.5	0.002
	PFTeA	80.1	1.17	7.7	0.013
(B) stress-induced CORT	linear PFOS	0.5	1.16	0.0	0.905
	PFNA	2.5	1.16	0.1	0.795
	PFDcA	0.5	1.16	0.0	0.909
	PFUnA	0.6	1.16	0.0	0.899
	PFDoA	1.9	1.16	0.1	0.819
	PFTrA	1.0	1.16	0.0	0.868
	PFTeA	46.1	1.15	1.3	0.267

(PFTrA and PFTeA) are still of concern. These results should be interpreted cautiously and would greatly benefit from experimental support. It would thus be interesting to measure free CORT and GR in relation to PFTrA and PFTeA. Another interesting point is that the observed pattern in the present study is the opposite of what has been found in previous studies with regard to legacy POPs and CORT in Arctic seabird species.[44−46] In these latter studies, baseline[44,45] or stress-induced[46] CORT increased with increasing legacy POPs. The lower concentrations of CORT in relation to PFTrA and PFTeA, could have interfered with the adaptive weight loss observed in seabirds and consequently with chick feeding.[47,48] However, in the present study PFTrA and PFTeA were not related to body-condition or reproductive success.

Relationships between PFASs and Reproductive Traits. Hatching success and breeding success were not related to baseline or stress-induced CORT concentrations, or with the interactions with sex ($\chi^2 < 1.11$, $p > 0.29$). Hatching success was significantly lower in birds with higher concentrations of PFDoA (GLM, PFDoA: $\chi^2 = 4.2$, $p = 0.040$; sex: $\chi^2 = 0.4$, $p = 0.528$ PFDoA × sex: GLM, $\chi^2 = 0.1$, $p = 0.72$; Figure 3, SI) and was significantly related to the interaction between PFTeA and sex (PFTeA × sex: GLM, $\chi^2 = 4.0$, $p = 0.045$, SI). However, when analyzing males and females separately, the negative relationship between hatching success and PFTeA was only close to statistical significance in males (GLM, $\chi^2 = 3.4$, $p = 0.064$) and no relationship was found in females (GLM, $\chi^2 = 0.6$, $p = 0.455$). All the other compounds were unrelated to hatching success ($p > 0.35$ for all tests, SI). Breeding success was not related to PFASs ($p > 0.07$ for all tests, SI). In this study, male and female kittiwakes bearing the higher concentrations of PFDoA were more likely to hatch one egg instead of two in a two eggs clutch. In mammals, some effects of PFDoA on reproduction and development have been observed. For example, in male rats fed PFDoA lower spermatid and spermatozoa counts were observed in reproductive organs and in female rats PFDoA administration resulted in death or in the delivery of dead pups.[49] Relationships between PFASs and fitness have rarely been investigated for wildlife. In tree swallows *Tachycineta bicolor*, a similar apparent reproductive impairment was observed as in the present study, although the associated PFASs differed: PFOS concentration measured directly in eggs was negatively associated with hatching success, with PFOS concentration ≥150 ng/g.[24,25] In lesser black-backed gulls *Larus fuscus* no relationships were found between PFASs and life-history traits.[23] However, in lesser black-backed gulls, whole blood concentrations for long chain PFCAs were slightly lower than in kittiwakes' plasma (1.4 ng/g versus 2.5 ng/g, respectively, for PFDoA). The relationships between PFDoA and hatching success could either be the result of nonviable embryos or less efficient incubation behavior. In oviparous vertebrates, females transfer a large amount of PFASs to their eggs[39] which may result in nonviable embryos for the most contaminated females. However, in the present study high PFDoA concentrations measured in male kittiwakes were also related to lower hatching success. Consequently the lower hatching success observed in birds with the higher concentrations of PFDoA, is more likely to result from disrupted incubating behavior. Regarding legacy POPs, an experimental study conducted on American kestrels *Falco sparverius*, reported that PCB administration resulted in altered incubation behaviors:[50] the incubation bouts realized by female and male American kestrels were not synchronized and the eggs were left unattended for longer periods.[50] In free-ranging glaucous gulls *Larus hyperboreus*, the proportion of time absent from the nest site when not incubating and the number of absences were related to blood PCB,[51] and the most contaminated glaucous gulls were less able to maintain an optimal nest temperature.[52] However, we have no evidence for such effects of PFDoA. Another possible explanation that could relate PFDoA to hatching success would be a disruption of the hormonal control of brood patches. Brood patches are highly vascularized featherless patches placed on the ventral surface of both male and female birds when biparental care is provided.[53] These patches enable the egg to be kept at an optimal temperature: if incubation patches are too small, one egg at least would probably be less exposed to parental heat. The feather loss and vascularization of those patches are under hormonal control, particularly through a synergetic association of prolactin and estrogen.[53] In rats, PFDoA administration reduces serum estradiol concentration in males and the expression of estrogen receptors in the ovaries of females.[54−56] If PFDoA reduces estradiol expression in kittiwakes, this could lead to reduced brood patches, less efficient incubation and nonviable embryos. Again, further studies are needed to test

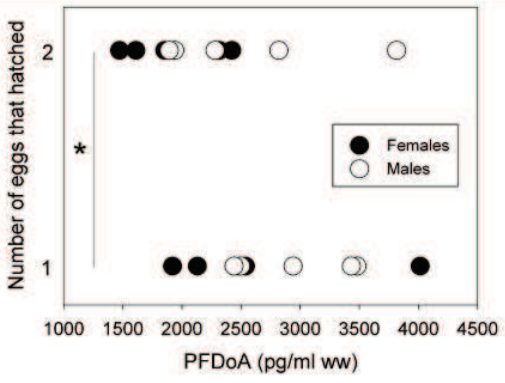


Figure 3. Hatching success (1 or 2 eggs have hatched) was lower in black-legged kittiwakes with high concentrations of PFDoA (*: $p = 0.040$) open circles denote males and closed circles denote females.

dx.doi.org/10.1021/es503297n | Environ. Sci. Technol. XXXX, XXX, XXX−XXX

these hypotheses, such as measurement of estrogen and monitoring incubation behavior in relation to PFDoA. Another, important point is that we did not measure PFASs in the partners of our focal birds. Because kittiwakes provide biparental care to the brood, measuring PFASs in the partner would provide important data to explain the observed interaction between PFDoA and hatching success.

This study underlines the importance of considering each PFAS separately and their relationships with sex. To the best of our knowledge this is the first study which shows relationships between long chain PFCAs (PFNA, PFDoA, PFTrA, and PFTeA), body-condition, baseline CORT concentrations and hatching success in a free-ranging seabird. Most toxicity studies of PFASs have concentrated on PFOS and PFOA, hence limited information is available on the toxicological effects and risk of other PFASs.[33] Additionally, PFCAs show dramatic increasing trends in Arctic seabird eggs,[13,57] given their hazardous effects on hormones and fitness related traits, more studies are needed. The small amount of data available makes interpreting the statistical results difficult; additionally this study is correlational; it is thus difficult to draw conclusions on the causality of these relationships, some of them could be the result of unmeasured chemicals or parameters that could confound these associations.

■ ASSOCIATED CONTENT

Supporting Information

Figures depicting the nonsignificant relationships between PFASs, body-condition and hatching success are given in Supporting Information. Additionally, we also included tables with statistics concerning the relationships between (1) sex, PFASs, CORT and body-condition, (2) PFASs and body-condition, (3) PFASs and reproductive traits. This material is available free of charge via the Internet at http://pubs.acs.org/.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: tartu.sabrina@gmail.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project was supported by Institut Polaire Français (IPEV project 330 to O. Chastel), Agence National de la Recherche (ANR project PolarTop to O. Chastel), INSU- EC2CO 2014 (to O. Chastel) and COPOL (GW Gabrielsen & JO Bustnes). This study was approved by the French and Norwegian Ethic committees and by the Governor of Svalbard. The authors thank C. Parenteau, C. Trouvé, S. Dano, for their excellent technical assistance in hormones assays and molecular sexing, Linda Hanssen and the staff of the NILU laboratory in Tromsø for their assistance during the chemical analyses and P. Duncan for his help with a previous version of the paper.

■ REFERENCES

(1) Colborn, T.; vom Saal, F. S.; Soto, A. M. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Perspect* **1993**, *101*, 378−384.

(2) Guillette, L. J.; Crain, D. A.; Rooney, A. A.; Pickford, D. B. Organization versus activation: The role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. *Environ. Health Perspect* **1995**, *103*, 157−164.

(3) Jimènez, B. Environmental effects of endocrine disruptors and current methodologies for assessing wildlife health effects. *TrAC, Trends Anal. Chem.* **1997**, *16*, 596−606.

(4) Tyler, C. R.; Jobling, S.; Sumpter, J. P. Endocrine disruption in wildlife: A critical review of the evidence. *Crit. Rev. Toxicol.* **1998**, *28*, 319−361.

(5) la Farré, M.; Pérez, S.; Kantiani, L.; Barceló, D. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. *TrAC, Trends Anal. Chem.* **2008**, *27*, 991−1007.

(6) Petrovic, M.; Eljarrat, E.; Alda, M. J. L. de; Barceló, D. Endocrine disrupting compounds and other emerging contaminants in the environment: A survey on new monitoring strategies and occurrence data. *Anal Bioanal Chem.* **2004**, *378*, 549−562.

(7) Muir, D. C. G.; de Wit, C. A. Trends of legacy and new persistent organic pollutants in the circumpolar arctic: Overview, conclusions, and recommendations. *Sci. Tot. Environ.* **2010**, *408*, 3044−3051.

(8) Jones, P. D.; Hu, W.; De Coen, W.; Newsted, J. L.; Giesy, J. P. Binding of perfluorinated fatty acids to serum proteins. *Environ. Toxicol. Chem.* **2003**, *22*, 2639−2649.

(9) Jensen, A. A.; Leffers, H. Emerging endocrine disrupters: perfluoroalkylated substances. *Int. J. Androl.* **2008**, *31*, 161−169.

(10) Haukås, M.; Berger, U.; Hop, H.; Gulliksen, B.; Gabrielsen, G. W. Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. *Environ. Pollut.* **2007**, *148*, 360−371.

(11) Butt, C. M.; Mabury, S. A.; Muir; Braune, B. M. Prevalence of long-chained perfluorinated carboxylates in seabirds from the Canadian Arctic between 1975 and 2004. *Environ. Sci. Technol.* **2007**, *41*, 3521−3528.

(12) Butt, C. M.; Berger, U.; Bossi, R.; Tomy, G. T. Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Sci. Tot. Environ.* **2010**, *408*, 2936−2965.

(13) Helgason, L. B., Sagerup, K., Gabrielsen, G. W. Temporal trends and contaminant profiles of persistent organic pollutants (POPs) in seabird eggs from Northern Norway and Svalbard. In *Global Contamination Trends of Persistent Organic Chemicals*; Loganathan, B. G., Lam, P. K. S., Eds.; CRC Press, 2011.

(14) Liu, C.; Du, Y.; Zhou, B. Evaluation of estrogenic activities and mechanism of action of perfluorinated chemicals determined by vitellogenin induction in primary cultured tilapia hepatocytes. *Aquat. Toxicol.* **2007**, *85*, 267−277.

(15) Benninghoff, A. D.; Bisson, W. H.; Koch, D. C.; Ehresman, D. J.; Kolluri, S. K.; Williams, D. E. Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro. *Toxicol. Sci.* **2011**, *120*, 42−58.

(16) Nøst, T. H.; Helgason, L. B.; Harju, M.; Heimstad, E. S.; Gabrielsen, G. W.; Jenssen, B. M. Halogenated organic contaminants and their correlations with circulating thyroid hormones in developing Arctic seabirds. *Sci. Tot. Environ.* **2012**, *414*, 248−256.

(17) Wingfield, J. C.; Sapolsky, R. M. Reproduction and resistance to stress: When and how. *J. Neuroendocrinol.* **2003**, *15*, 711−724.

(18) Ricklefs, R. E.; Wikelski, M. The physiology/life-history nexus. *Trends Ecol. Evol.* **2002**, *17*, 462−468.

(19) Lendvai, Á. Z.; Giraudeau, M.; Chastel, O. Reproduction and modulation of the stress response: An experimental test in the house sparrow. *Proc. R. Soc. B* **2007**, *274*, 391−397.

(20) Angelier, F.; Clément-Chastel, C.; Welcker, J.; Gabrielsen, G. W.; Chastel, O. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. *Funct. Ecol.* **2009**, *23*, 784−793.

(21) Jenssen, B. M. Endocrine-disrupting chemicals and climate change: A worst-case combination for Arctic marine mammals and seabirds? *Environ. Health Perspect.* **2005**, *114*, 76−80.

(22) Tartu, S.; Goutte, A.; Bustamante, P.; Angelier, F.; Moe, B.; Clément-Chastel, C.; Bech, C.; Gabrielsen, G. W.; Bustnes, J. O.; Chastel, O. To breed or not to breed: Endocrine response to mercury contamination by an Arctic seabird. *Biol. Lett.* **2013**, *9*, 20130317.

(23) Bustnes, J. O.; Erikstad, K. E.; Lorentsen, S.-H.; Herzke, D. Perfluorinated and chlorinated pollutants as predictors of demographic parameters in an endangered seabird. *Environ. Pollut.* **2008**, *156*, 417−424.
(24) Custer, C. M.; Custer, T. W.; Schoenfuss, H. L.; Poganski, B. H.; Solem, L. Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. *Reprod. Toxicol.* **2012**, *33*, 556−562.
(25) Custer, C. M.; Custer, T. W.; Dummer, P. M.; Etterson, M. A.; Thogmartin, W. E.; Wu, Q.; Kannan, K.; Trowbridge, A.; McKann, P. C. Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA. *Arch. Environ. Contam. Toxicol.* **2014**, *66*, 120−138.
(26) Kelly, B. C.; Ikonomou, M. G.; Blair, J. D.; Surridge, B.; Hoover, D.; Grace, R.; Gobas, F. A. P. C. Perfluoroalkyl contaminants in an Arctic marine food web: Trophic magnification and wildlife exposure. *Environ. Sci. Technol.* **2009**, *43*, 4037−4043.
(27) Peig, J.; Green, A. J. New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos* **2009**, *118*, 1883−1891.
(28) Weimerskirch, H.; Lallemand, J.; Martin, J. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. *J. Anim. Ecol.* **2005**, *74*, 285−291.
(29) Lormée, H.; Jouventin, P.; Trouve, C.; Chastel, O. Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies *Sula sula*. *Ibis* **2003**, *145*, 212−219.
(30) Powley, C. R.; George, S. W.; Ryan, T. W.; Buck, R. C. Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. *Anal. Chem.* **2005**, *77*, 6353−6358.
(31) Rylander, C.; Phi, D. T.; Odland, J. Ø.; Sandanger, T. M. Perfluorinated compounds in delivering women from south central Vietnam. *J. Environ. Monit.* **2009**, *11*, 2002−2008.
(32) Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* **2007**, *99*, 366−394.
(33) Martin, J. W.; Smithwick, M. M.; Braune, B. M.; Hoekstra, P. F.; Muir, D. C. G.; Mabury, S. A. Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environ. Sci. Technol.* **2004**, *38*, 373−380.
(34) Vanden Heuvel, J. P. V.; Thompson, J. T.; Frame, S. R.; Gillies, P. J. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: A comparison of human, mouse, and rat peroxisome proliferator-activated receptor-α, -β, and -γ, liver X receptor-β, and retinoid X receptor-α. *Toxicol. Sci.* **2006**, *92*, 476−489.
(35) Wolf, C. J.; Takacs, M. L.; Schmid, J. E.; Lau, C.; Abbott, B. D. Activation of mouse and human peroxisome proliferator−activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. *Toxicol. Sci.* **2008**, *106*, 162−171.
(36) Nelson, J. W.; Hatch, E. E.; Webster, T. F. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. *Environ. Health Perspect.* **2009**, *118*, 197−202.
(37) Lin, C.-Y.; Wen, L.-L.; Lin, L.-Y.; Wen, T.-W.; Lien, G.-W.; Chen, C.-Y.; Hsu, S. H.; Chien, K.-L.; Sung, F.-C.; Chen, P.-C.; et al. Associations between levels of serum perfluorinated chemicals and adiponectin in a young hypertension cohort in Taiwan. *Environ. Sci. Technol.* **2011**, *45*, 10691−10698.
(38) Halldorsson, T. I.; Rytter, D.; Haug, L. S.; Bech, B. H.; Danielsen, I.; Becher, G.; Henriksen, T. B.; Olsen, S. F. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: A prospective cohort study. *Environ. Health Perspect* **2012**, *120*, 668−673.
(39) Gebbink, W. A.; Letcher, R. J. Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls. *Environ. Pollut.* **2012**, *162*, 40−47.
(40) Fang, X.; Zhang, L.; Feng, Y.; Zhao, Y.; Dai, J. Immunotoxic effects of perfluorononanoic acid on BALB/c mice. *Toxicol. Sci.* **2008**, *105*, 312−321.
(41) Breuner, C. W. Stress and reproduction in birds. *Horm. Reprod. Vertebr.* **2011**, *4*, 129−151.
(42) Breuner, C. W.; Orchinik, M. Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J. Endocrinol* **2002**, *175*, 99−112.
(43) Malisch, J. L.; Breuner, C. W. Steroid-binding proteins and free steroids in birds. *Mol. Cell. Endocrinol.* **2010**, *316*, 42−52.
(44) Verboven, N.; Verreault, J.; Letcher, R. J.; Gabrielsen, G. W.; Evans, N. P. Adrenocortical function of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. *Gen. Comp. Endrocrinol.* **2010**, *166*, 25−32.
(45) Nordstad, T.; Moe, B.; Bustnes, J. O.; Bech, C.; Chastel, O.; Goutte, A.; Sagerup, K.; Trouvé, C.; Herzke, D.; Gabrielsen, G. W. Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (*Rissa tridactyla*) across their breeding cycle. *Environ. Pollut.* **2012**, *164*, 219−226.
(46) Tartu, S.; Angelier, F.; Herzke, D.; Moe, B.; Bech, C.; Gabrielsen, G. W.; Bustnes, J. O.; Chastel, O. The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. *Sci. Tot. Environ.* **2014**, *476*−*477*, 553−560.
(47) Moe, B.; Langseth, I.; Fyhn, M.; Gabrielsen, G. W.; Bech, C. Changes in body condition in breeding kittiwakes *Rissa tridactyla*. *J. Avian Biol.* **2002**, *33*, 225−234.
(48) Angelier, F.; Clément-Chastel, C.; Gabrielsen, G. W.; Chastel, O. Corticosterone and time−activity budget: An experiment with black-legged kittiwakes. *Horm. Behav.* **2007**, *52*, 482−491.
(49) Kato, H.; Fujii, S.; Takahashi, M.; Matsumoto, M.; Hirata-Koizumi, M.; Ono, A.; Hirose, A. Repeated dose and reproductive/developmental toxicity of perfluorododecanoic acid in rats. *Environ. Toxicol.* **2014**, DOI: 10.1002/tox.21996.
(50) Fisher, S. A.; Bortolotti, G. R.; Fernie, K. J.; Bird, D. M.; Smits, J. E. Behavioral variation and its consequences during incubation for American kestrels exposed to polychlorinated biphenyls. *Ecotoxicol. Environ. Saf.* **2006**, *63*, 226−235.
(51) Bustnes, J. O.; Bakken, V.; Erikstad, K. E.; Mehlum, F.; Skaare, J. U. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. *J. Appl. Ecol.* **2001**, *38*, 791−801.
(52) Verboven, N.; Verreault, J.; Letcher, R. J.; Gabrielsen, G. W.; Evans, N. P. Nest temperature and parental behaviour of Arctic-breeding glaucous gulls exposed to persistent organic pollutants. *Anim. Behav.* **2009**, *77*, 411−418.
(53) Bailey, R. E. *Incubation Patch of Passerine Birds*. Doctoral dissertation, University of California, Berkeley, 1951.
(54) Shi, Z.; Zhang, H.; Liu, Y.; Xu, M.; Dai, J. Alterations in gene expression and testosterone synthesis in the testes of male rats exposed to perfluorododecanoic acid. *Toxicol. Sci.* **2007**, *98*, 206−215.
(55) Shi, Z.; Ding, L.; Zhang, H.; Feng, Y.; Xu, M.; Dai, J. Chronic exposure to perfluorododecanoic acid disrupts testicular steroidogenesis and the expression of related genes in male rats. *Toxicol. Lett.* **2009**, *188*, 192−200.
(56) Shi, Z.; Zhang, H.; Ding, L.; Feng, Y.; Xu, M.; Dai, J. The effect of perfluorododecanonic acid on endocrine status, sex hormones and expression of steroidogenic genes in pubertal female rats. *Reprod. Toxicol.* **2009**, *27*, 352−359.
(57) Braune, B. M.; Letcher, R. J. Perfluorinated sulfonate and carboxylate compounds in eggs of seabirds breeding in the Canadian Arctic: Temporal trends (1975−2011) and interspecies comparison. *Environ. Sci. Technol.* **2013**, *47*, 616−624.

APPENDIX

Blévin, P., **Tartu, S.,** Angelier, F., Leclaire, S., Bustnes, J. O., Moe, B., Herzke D., Gabrielsen G.W. & Chastel, O. (2014). Integument colouration in relation to persistent organic pollutants and body condition in arctic breeding black-legged kittiwakes. *Science of the Total Environment*, *470*, 248-254.

Science of the Total Environment 470–471 (2014) 248–254

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Integument colouration in relation to persistent organic pollutants and body condition in arctic breeding black-legged kittiwakes (*Rissa tridactyla*)

Pierre Blévin [a,*], Sabrina Tartu [a], Frédéric Angelier [a], Sarah Leclaire [b], Jan Ove Bustnes [c], Børge Moe [c], Dorte Herzke [d], Geir Wing Gabrielsen [e], Olivier Chastel [a,**]

[a] Centre d'Etudes Biologiques de Chizé (CEBC-CNRS), UPR 1934-CNRS, F-79360 France
[b] Université Toulouse 3 Paul Sabatier, ENFA, UMR 5174 EDB (Laboratoire Évolution & Diversité Biologique; CNRS), 118 route de Narbonne, F-31062 Toulouse, France
[c] Norwegian Institute for Nature Research, NINA, Fram centre, NO-9296 Tromsø, Norway
[d] Norwegian Institute for Air Research, NILU, Fram centre, NO-9296 Tromsø, Norway
[e] Norwegian Polar Research Institute, Fram centre, NO-9296 Tromsø, Norway

HIGHLIGHTS

- We studied the relationships between POPs burden and integument colouration in an artic seabird.
- Saturation of eye-ring, gapes and tongue was negatively related to POPs' burden.
- Individuals with a better body condition displayed more orange gapes and tongue.
- POPs could affect the amount of carotenoid coloration in this arctic seabird.

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form 15 September 2013
Accepted 16 September 2013
Available online 18 October 2013

Editor: Frank Riget

Keywords:
Arctic
Seabird
Carotenoid
Contamination
Pesticide
PCB

ABSTRACT

Vertebrates cannot synthetize carotenoids de novo but have to acquire them through their diet. In birds, carotenoids are responsible for the yellow to red colouration of many secondary sexual traits. They are also involved in physiological functions such as immunostimulation and immunoregulation. Consequently, carotenoid-based colouration is very often considered as a reliable signal for health and foraging abilities. Although a few studies have suggested that carotenoid-based coloured traits could be sensitive to environmental pollution such as persistent organic pollutants (POPs) contamination, the relationships between pollutants and colouration remain unclear. Here, we examined the relationships between the colouration of carotenoid-based integuments and individual POP levels in pre-laying female black-legged kittiwakes from very high latitudes. In this area, these arctic seabirds are exposed to high POPs contamination. Additionally, we investigated the relationships between colouration and body condition, a frequently used index of individual quality. We found a negative relationship between POP levels and several components of integument colouration: saturation of eye-ring, gapes and tongue, suggesting that POPs could disrupt colouration of labile integuments in female kittiwakes. In addition, we found that females in better body condition displayed more orange and brighter gapes and tongue than females in poor body condition. These results demonstrate that hue and brightness are sensitive to the current health and nutritional status of female kittiwakes. Overall, our study shows that carotenoid-based colour integuments can be affected by several environmental-driven variables.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Many animals exhibit elaborate ornamental traits such as colourful skin, feathers and cuticles that evolved as quality signals. Those signals can have an impact on the fitness of an individual by influencing the behaviour of mates or opponents (Andersson, 1994; Møller et al., 2000). Carotenoids represent one of the central components of colour signals used in animal communication, and thus are highly involved in social behaviours of many species (Møller et al., 2000; Olson and Owen, 1998). In birds, carotenoids are responsible for the yellow to red colouration of many secondary sexual traits (Brush, 1990). Mate choice studies have shown that the most preferred individuals are often those expressing greater carotenoid pigmentation in sexual signals (Amundsen and Forsgren, 2001; review in Hill, 2006). Although the antioxidant property of carotenoids appears to be controversial for birds

* Correspondence to: P. Blévin, CEBC, CNRS UPR 1934, BP 14, 79360 Villiers-en-Bois, France. Tel.: +33 549096111.
** Correspondence to: O. Chastel, CEBC, CNRS UPR 1934, BP 14, 79360 Villiers-en-Bois, France. Tel.: +33 549097837.
E-mail addresses: blevin.pierre@gmail.com (P. Blévin), chastel@cebc.cnrs.fr (O. Chastel).

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.09.049

(Costantini and Møller, 2008; Hartley and Kennedy, 2004; Krinsky, 2001), they are involved in other physiological functions such as immunostimulation and immunoregulation (Blount et al., 2003; Chew and Park, 2004; Faivre et al., 2003; review in Møller et al., 2000). Thereby, they enhance T- and B-lymphocyte proliferative responses, stimulate effector T-cell function, enhance macrophage and T-cell capacities, increase the population of specific lymphocyte subpopulations and stimulate the production of various cytokines and interleukins (Bendich, 1989; Chew, 1993). They also maintain the structural integrity of immune cells by removing free radical molecules that are produced through normal cellular activity, and also through environmental stressors (Chew, 1996). Consequently, carotenoids promote survival (immunity, antioxidant capacity) suggesting that a trade-off may exist between allocations of carotenoids towards sexual ornaments signalling versus physiological functions for self-maintenance (Eraud et al., 2007; Pérez et al., 2010a; Von Schantz et al., 1999). It is widely assumed that condition-dependence is a common feature of sexual displays (Kristiansen et al., 2006; Martinez-Padilla et al., 2007; Mougeot et al., 2006, 2007; Pérez-Rodríguez and Viñuela, 2008; Velando et al., 2006). This implies that healthy individuals should require fewer carotenoids for immune defences and could therefore allocate more of this limited resource to enhance sexual signals, thereby indicating of a high-quality mate. Several studies have already highlighted some correlational evidences between carotenoid-based colouration and body condition, a frequently used index of individual quality (Birkhead et al., 1998; Bustnes et al., 2007; Massaro et al., 2003; Mougeot et al., 2006, 2007; Pérez-Rodríguez and Viñuela, 2008; Pérez et al., 2010b). As birds cannot synthetize carotenoids de novo, they have to acquire them through their diet and thus, carotenoid pigmentation depends on the quality and/or quantity of food ingested (Goodwin, 1986). Consequently, carotenoid-based colouration can be considered as a reliable signal of health and foraging abilities (Olson and Owens, 1998).

In addition to this effect of body condition on carotenoid-based colouration, a few studies have suggested that environmental pollution could also affect, and more precisely disrupt, the expression of avian colouration (Eeva et al., 1998; Pérez et al., 2010a). For example, persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and pesticides appear to reduce the expression of carotenoid-based colouration. Thus, captive American kestrels (*Falco sparverius*) exposed to an enriched-PCB diet showed a disruption of both plasma carotenoid concentration and colouration of ceres and lores (Bortolotti et al., 2003). However, this effect of POPs on colouration does not seem equivocal since, Bustnes et al. (2007) did not find any relationships between POP levels and integuments' colouration in free-living great black-backed gulls (*Larus marinus*). Thereby, this discrepancy emphasizes the importance of conducting further studies on the potential deleterious impacts of POPs contamination on carotenoid-based colouration.

The black-legged kittiwake *Rissa tridactyla* is a long-lived and monogamous seabird. Males are bigger than females (Helfenstein et al., 2004; Jodice et al., 2000) but no sexual chromatic dimorphisms are found (Doutrelant et al., 2013; Leclaire et al., 2011a). Both sexes show intense carotenoid-based colouration during the breeding season (Doutrelant et al., 2013; Leclaire et al., 2011a), including the red eye-ring, red/orange gapes, orange tongue and yellow bill. Recent studies have shown that these integuments could reflect individual quality in both sexes (Doutrelant et al., 2013; Leclaire, 2010; Leclaire et al., 2011a,b). In the Arctic, black-legged kittiwakes are exposed to POPs which are known to act as endocrine disruptors and to have a negative impact on reproductive performances (Bustnes et al., 2003, 2008; Helberg et al., 2005; Nordstad et al., 2012). Black-legged kittiwakes are therefore excellent models to investigate the relationships between POPs and carotenoid-based colouration in free-living birds. In that context, the specific aims of the present study were to evaluate the potential correlates of individual POP levels on integument carotenoid-based colouration (eye-ring, gapes, tongue and bill) in pre-laying female kittiwakes from Svalbard. We predicted that females bearing high POP levels would show a reduced expression of integument colouration. In addition, we also examined the correlates between body condition and integument colouration since body condition could reflect individual quality in this species. According to previous studies (Doutrelant et al., 2013; Leclaire, 2010; Leclaire et al., 2011a,b), we predicted that females with a better body condition would display the most colourful integuments.

2. Materials and methods

2.1. Study area and sample collection

Fieldwork was carried out in 2011 from May 21st to June 7th in a colony of black-legged kittiwakes at Kongsfjorden, (Krykkjefjellet, 78°54′ N, 12°13′E), Svalbard. POP analyses were conducted only for females, thus males were not included in this study. Individuals ($n = 28$) were caught on their nest with a noose at the end of a 5 m fishing rod during the pre-laying period (i.e. the courtship and mating period). Females were attending the colony, on their nest on cliffs at a height of 5–10 m during the pre-laying period (i.e. before egg-laying). Birds were individually marked with white PVC plastic bands engraved with a three-letter code and fixed to the bird's tarsus. Thus, kittiwakes could be identified from a distance without perturbation. At capture, blood samples (2.5 mL) were collected from the alar vein using a heparinized syringe and a 25G needle for the determination of blood POP concentrations and molecular sexing. Then, birds were weighted to the nearest 2 g with a Pesola spring balance and skull length (head + bill) was measured with an accuracy of 0.1 mm using a calliper. Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observations and were released. Using a mirror at the end of an 8 m fishing rod, we checked the whole plot every two days to monitor the subsequent reproductive status of the sampled females (pre-laying breeders were the birds that laid at least one egg after the sampling period). Blood samples were stored at −20 °C until subsequent analyses. Sex was determined at the Centre d'Etudes Biologiques de Chizé (CEBC), by polymerase chain reaction (PCR), as detailed in Weimerskirch et al. (2005).

2.2. POPs' analyses

POPs were analysed from whole blood at the Norwegian Institute for Air Research (NILU) in Tromsø. The following compounds were analysed: the PCBs (CB-28, -52, -99, -101, -105, -118, -128, -138, -153, -180, -183, -187 and -194), and the pesticides (p,p-DDE, α-, β-, γ-HCH, HCB, oxychlordane, trans-, cis-chlordane, trans-, cis-nonachlor). Congeners detected in less than 70% of the samples were removed from the data set (Noël et al., 2009). Thereby, those remaining for further investigations were the PCBs (CB-99, -105, -118, -128, -138, -153, -180, -183, -187 and -194), and the pesticides (p,p-DDE, HCB, oxychlordane, trans-chlordane, trans-, cis-nonachlor). To a blood total sample of 0.5 to 1.5 mL, a 100 μL internal standard solution was added (^{13}C-labelled compounds from Cambridge Isotope Laboratories: Woburn, MA, USA). The sample was extracted twice with 6 mL of n-hexane. after denaturation with ethanol and a saturated solution of ammonium sulphate in water. Matrix removal on florisil columns, separation on an Agilent Technology 7890 GC and detection on an Agilent Technology 5975C MSD were performed as described by Herzke et al. (2009). The limit for detection was threefold the signal-to-noise ratio, and for the compounds investigated the limit ranged from 0.4 to 122 $pg \cdot g^{-1}$ wet weights (ww). For validation of the results, blanks (clean and empty glass tubes treated like a sample, 3 in total) were run for every 10 samples, while standard reference material (3 in total, 1589a human serum from NIST) was run for every 10 samples. The accuracy of the method was within the 70 and 108% range.

2.3. Colour measurements

Integument colouration was measured from digital photographs as detailed in Montgomerie (2006). Pictures were taken at a standard

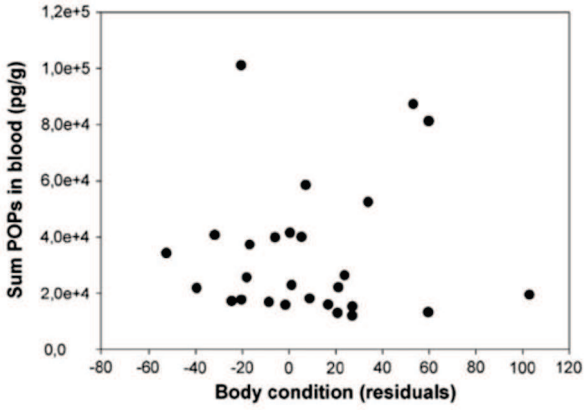


Fig. 1. Relationship between ΣPOPs (pg·g^{-1} ww) in blood and body condition of pre-laying female black-legged kittiwakes.

distance of approximately 40 cm using a digital camera (Olympus U770sw, s770sw) with flash. For each photograph, the same colour swatch was placed next to the bird to standardize subsequent measurements. Prior to photograph analysis, low quality pictures (due to ambient lighting variations) were removed from the data set, thus individuals used for one given integument can partially be different for another one. All pictures were analysed using Adobe Photoshop v 12.0. The average components of red (R), green (G) and blue (B) were recorded within the whole area of the eye-ring and in a standardized selected area for the gapes, tongue and bill. Each component was assessed 3 times to ensure a good repeatability of the measurement (relative standard deviation of <5%, in all cases). RGB system was then converted into hue (H), saturation (S) and brightness (B). The HSB values of each integument were corrected according to the HSB values of the colour swatch. This system is by far the most commonly reported tristimulus colour variables measured in the study of bird colouration and is extensively commented in literature (Montgomerie, 2006).

Such human-oriented model presents some inaccuracies and is only an approximation since gulls and other birds have a tetrachromatic vision and can perceive UV light (Cuthill, 2006; Hastad et al., 2009).

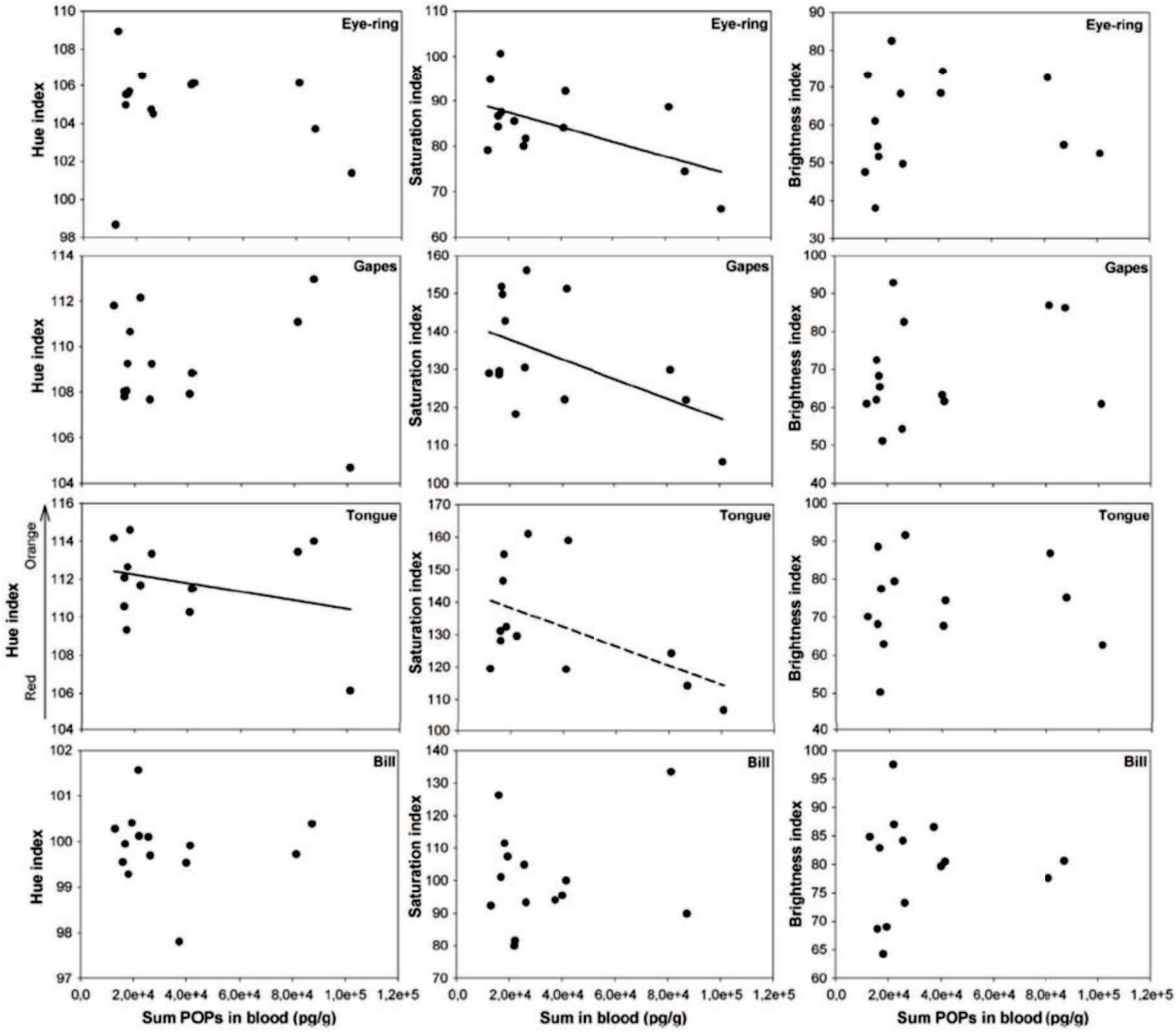


Fig. 2. Relationships between colouration parameters (hue, saturation, brightness) and ΣPOPs (pg·g^{-1} ww) in blood of pre-laying female black-legged kittiwakes for all integuments. Solid line represents significant relationship ($P < 0.05$) and dashed line represents marginally significant relationship ($P < 0.1$).

However, this method has already been investigated on black-legged kittiwakes (Leclaire, 2010; Leclaire et al., 2011a,b) and information obtained from digital pictures is still very useful as it reveals patterns and effects of biological meaning (Alonzo-Alvarez et al., 2004; Bortolotti et al., 2003; Kilner, 1997; Leclaire, 2010; Leclaire et al., 2011a,b; Massaro et al., 2003; Mougeot et al., 2007; Pérez-Rodríguez and Viñuela, 2008; Pérez et al., 2010b). Furthermore, using photography rather than spectrometry might be advantageous because digital photography is a much easier method for quantifying colouration of wet, hard to reach, and irregular surfaces such as gapes, irises and bills (Montgomerie, 2006).

2.4. Statistical analyses

Statistical tests were performed using R 2.14.1 (R Core Team, 2012). ΣPCB and ΣPesticides were highly significant and positively related (Pearson correlation, $r = 0.907$, $t = 10.756$, $P < 0.001$, $n = 27$). Consequently, continuous explanatory variables were defined as follow: body condition (i.e. the residuals of the regression of body mass against skull length) and ΣPOP concentrations (the sum of PCB and pesticides). We first tested the relationship between these two variables using a Pearson correlation. Then, the influence of POPs' contamination and body condition on colouration parameters was investigated with General Linear Models (GLMs). HSB values of each integument as independent variables were log transformed and models were constructed with a normal distribution and an identity link function. Explanatory variables were both included simultaneously in each model. Diagnostic plots were then assessed to test whether the data sufficiently met the assumption of the linear model. A significance level of $\alpha < 0.05$ was used for all tests.

3. Results

Individual POPs' concentration ranged from 1.21×10^4 to 1.01×10^5 pg·g^{-1} ww in whole blood. Body condition was not related to ΣPOP levels (Pearson correlation: $r = 0.06$, $t = 0.306$, $P = 0.766$, $n = 27$, Fig. 1). Saturation of eye-ring and gapes decreased significantly with ΣPOPs and a similar relationship, although not statistically significant was found between tongue's saturation and ΣPOPs (Fig. 2; Table 1), i.e. the most contaminated individuals were those displaying a reduced saturation of their labile integuments. Hue of tongue was negatively related to ΣPOPs but the relation seems to be driven by the presence of an outlier (Fig. 2; Table 1). Besides, hue and brightness were not related to ΣPOPs for all integuments (GLMs: all P-values > 0.315, Fig. 2; Table 1). Hue of gapes and tongue significantly increased with increasing body condition, i.e. individuals with a better condition displayed more orange gapes and tongue (Fig. 3; Table 1). We found a significant increase of brightness of the gapes with increasing body condition, and a similar trend, although not statistically significant was found between the brightness of the tongue and body condition i.e. individuals in better body condition displayed brighter gapes and tongue (Fig. 3; Table 1). By contrast, brightness of the bill decreased in birds with higher body condition (Fig. 3; Table 1). No significant relationships were found between body condition and saturation for all integuments (GLMs: all P-values > 0.116) (Fig. 3; Table 1).

4. Discussion

The results of this study first indicated a negative relationship between POP levels and saturation of labile integuments (i.e. eye-ring, gapes and tongue). This suggests that POPs could affect integument carotenoid-based colouration of kittiwakes. Secondly, body condition was positively related to hue and brightness for gapes and tongue implying that these colour parameters are sensitive to current nutritional conditions and health of individuals, as previously found in this species (Doutrelant et al., 2013; Leclaire, 2010; Leclaire et al., 2011a,b).

Table 1
Effects of ΣPOPs and body condition on colour parameters (hue, saturation and brightness) of carotenoid-based integuments in pre-laying female black-legged kittiwakes.

Independent variables		Dependent variables	*df*	*F*	P-value
Hue	Eye-ring	ΣPOPs	1,11	0.600	0.455
		Body condition	1,11	0.008	0.929
	Gapes	ΣPOPs	1,11	2.678	0.130
		Body condition	1,11	18.681	**0.001**
	Tongue	**ΣPOPs**	1,10	6.431	**0.030**
		Body condition	1,10	15.197	**0.003**
	Bill	ΣPOPs	1,11	0.098	0.761
		Body condition	1,11	0.143	0.713
Saturation	Eye-ring	**ΣPOPs**	1,11	6.978	**0.023**
		Body condition	1,11	0.522	0.485
	Gapes	**ΣPOPs**	1,11	5.019	**0.047**
		Body condition	1,11	0.256	0.623
	Tongue	ΣPOPs	1,10	4.140	0.070
		Body condition	1,10	0.006	0.940
	Bill	ΣPOPs	1,11	0.021	0.888
		Body condition	1,11	1.396	0.262
Brightness	Eye-ring	ΣPOPs	1,11	0.087	0.774
		Body condition	1,11	0.001	0.977
	Gapes	ΣPOPs	1,11	0.233	0.639
		Body condition	1,11	7.080	**0.022**
	Tongue	ΣPOPs	1,10	0.091	0.769
		Body condition	1,10	3.503	0.091
	Bill	ΣPOPs	1,11	1.333	0.273
		Body condition	1,11	6.355	**0.028**

Significant variables are in bold.

4.1. POPs and integument colouration

We found that POP levels negatively affected saturation of labile integuments. This colour parameter is usually assumed as a proxy of the amount of carotenoids present in tissues (Montgomerie, 2006) when colour is produced by only one pigment. However, integuments' colouration of kittiwakes results from a mix of different carotenoid species (Doutrelant et al., 2013) and, therefore, a same saturation can be obtained from mix of carotenoid species at different concentrations. This also means that equivalent amounts of carotenoids may produce different saturations depending on the exact composition of the mix of carotenoids. Consequently, POP levels could affect saturation either by decreasing the amount of pigments and/or by modifying the carotenoid species' composition present in integuments. Thereby, our study provides evidences that POPs' contamination can affect integument carotenoid-based colouration. This is consistent with previous work since Pérez et al. (2010a) showed that organic compounds negatively influence the red bill spot size of adult yellow-legged gulls (*Larus michahellis*) during the courtship period. Moreover, Bortolotti et al. (2003) found that colouration of ceres and lores was disrupted by an enriched-PCB diet in captive American kestrels: exposed males were duller than controls, and juveniles of both sexes were brighter in winter. By contrast, Bustnes et al. (2007) did not find any relationship between POP levels and integuments' colouration in adult breeding great black-backed gulls (*Larus marinus*). Consequently, relationships between POPs and colouration seem to be complex and future studies including further parameters such as period, sex or stage might provide clearer information.

Carotenoids are thought to promote survival (immunity, antioxidant capacity) and might be mobilised to overcome the harmful effects of POPs' ingestion on immunity (Bustnes et al., 2004; e.g. Pérez et al., 2010a; Sagerup et al., 2009) at the expense of coloured sexual signals. Under this scenario, female kittiwakes with the highest POP levels could allocate preferentially their carotenoids towards protective physiological functions (immunity, antioxidant capacity) whereas female kittiwakes with the lowest POP levels could allocate preferentially the available carotenoids towards sexual signalling. These results are thus consistent with the existence of a trade-off between allocations of carotenoids towards sexual ornaments signalling versus physiological functions for detoxification processes (Pérez et al., 2010a). However, we did not

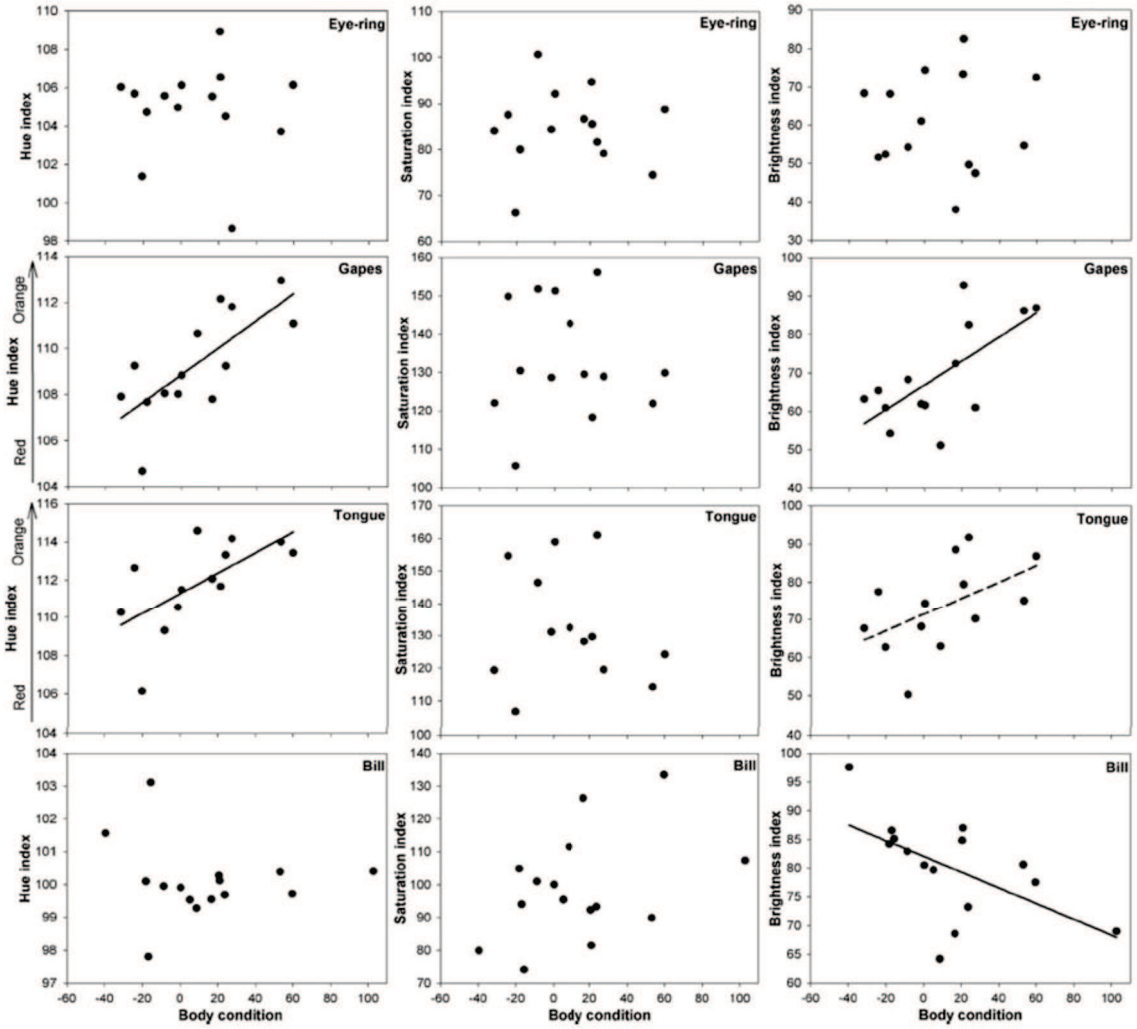


Fig. 3. Relationships between colouration parameters (hue, saturation, brightness) and body condition (residuals) of pre-laying female black-legged kittiwakes for all integuments. Solid line represents significant relationship ($P < 0.05$) and dashed line represents marginally significant relationship ($P < 0.1$).

perform any physiological analysis in our study and, thus, the existence of this trade-off could only be confirmed by coupling integument and plasma carotenoid measurements and POP levels in future studies.

4.2. POPs and body condition

Inter-individual variations in POP levels potentially originated from different foraging behaviours. Indeed, variations in POP levels between birds could be related to the foraging areas used by kittiwakes, i.e. during the pre-laying period, birds forage in oceanic and coastal areas (GPS tracking: Goutte et al., unpublished data). It could also be related to the type of prey ingested, i.e. contaminant levels increase with trophic position according to the biomagnification process (Kelly et al., 2007). Besides, POPs' contamination is usually negatively related to the body condition (Bustnes et al., 2010; Henriksen et al., 1996; Kenntner et al., 2003; Nordstad et al., 2012); organic pollutants are lipophilic and if body fat reserves are low, POPs can be redistributed in internal tissues through the bloodstream (Fuglei et al., 2007). The lack of relationship between POP levels and body condition in our study may be related to an overall sufficient body condition of pre-laying females avoiding a redistribution of POPs in internal tissues.

4.3. Body condition and integument colouration

We reported that body condition was positively related to gapes and tongue hue and brightness, suggesting a beneficial effect of the current condition of kittiwakes on colouration. These results are consistent with the literature since Doutrelant et al. (2013) have shown that kittiwakes displayed brighter and more orange gapes when in better body condition. Animals are thought to absorb carotenoids and other dietary lipids (e.g. fats, oil) through the gut lining via passive diffusion (Parker, 1996; but see During et al., 2002). By doing so, they mix carotenoids with bile salts and fatty acids to form micelles that migrate through the intestinal mucosa and are incorporated into chylomicrons to be secreted into lymph. Then, these micelles enter into the blood where they are transported via lipoproteins (Furr and Clack, 1997). During poor nutritional conditions, the amount of lipids and lipoproteins is reduced (Alonzo-Alvarez and Ferrer, 2001) and this may reduce the extraction yield of carotenoids from food (Solomons and Bulux, 1993), which in turn results in a reduction of circulating carotenoids and, ultimately, in a reduced transfer of carotenoids into integuments. Consequently lower hue and brightness may be related either to poor individual foraging efficiencies (birds in poor body condition ingesting less and/or low

quality food), either to poor environmental quality and thus, to nutritional conditions (e.g. Leclaire, 2010). In addition, metabolic pathways may also be condition-dependent. Before being deposited into integuments, ingested carotenoids may be reduced through the activation of metabolic pathways that may depend on the birds' body condition (Hill, 2000; McGraw et al., 2005). Finally, the relationship between body condition and integument colouration might be related to the current physiological condition of individuals, i.e. the most colourful kittiwakes are those with the best immunological status. This implies that healthy individuals should require fewer carotenoids for immune defences and could therefore allocate more carotenoids to enhance colouration (Pérez et al., 2010a; Pérez-Rodríguez and Viñuela, 2008). However, this hypothesis could only be confirmed by measuring carotenoids in integuments and plasma coupled to the measure of immunological parameters.

Each relationship between POP levels and saturation was very similar among labile integuments. Similarly, relationships between body condition and colouration parameters (hue and brightness) for gapes and tongue suggest that POPs and body condition affect in the same way each labile integuments colouration. Contrary to fleshy integuments with rapid colour changes, e.g. 48 h for the skin of the blue-footed booby *Sulax nebouxi* (Velando et al., 2006), the bill is a keratinized structure and the turnover of carotenoids deposited in the bill is obviously slower (Pérez-Rodríguez and Viñuela, 2008). Moreover, body condition and POP levels of kittiwakes from the studied colony are known to vary rapidly through the breeding season (Moe et al., 2002; Nordstad et al., 2012). Therefore, the faster turnover of eye-ring, gapes and tongue colouration compared to that of bill may explain why we only observed relationships between the current condition of birds (POPs' level and body condition) and labile integuments colourations.

5. Conclusion

The present study provides the first evidence of a potential effect of individual POP levels on integument carotenoid-based colouration of black-legged kittiwakes. In addition, it also shows that body condition can explain integument colouration in this species. However, and importantly, body condition and POPs' burden do not seem to act on the same component of integument colouration. Consequently, our results suggest that, in female black-legged kittiwakes, carotenoid-based colour integuments may be sensitive to several independent pressures, such as POPs' contamination, nutritional conditions and current health of individuals.

Acknowledgements

This project was supported by French Polar Institute (IPEV, program 330, O. Chastel) and by Agence Nationale de la Recherche (ANR PolarTop, O. Chastel). The authors thank J. Colucci for his help with photography processing, A. Goutte for comments that improved an earlier draft of the manuscript and two anonymous referees for their very useful comments.

References

Alonso-Alvarez C, Ferrer M. A biochemical study of fasting, subfeeding, and recovery processes in yellow-legged gulls. Physiol Biochem Zool 2001;74:703–13.
Alonso-Alvarez C, Bertrand S, Devevey G, Gaillard M, Prost J, Faivre B, et al. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. Am Nat 2004;164:651–9.
Amundsen T, Forsgren E. Male mate choice selects for female coloration in a fish. Proc Natl Acad Sci 2001;98:13155–60.
Andersson MB. Sexual selection. Princeton: Princeton University Press; 1994.
Bendich A. Carotenoids and the immune response. J Nutr 1989;119:112–5.
Birkhead TR, Fletcher F, Pellatt EJ. Sexual selection in the zebra finch *Taeniopygia guttata*: condition, sex traits and immune capacity. Behav Ecol Sociobiol 1998;44:179–91.
Blount JD, Metcalfe NB, Birkhead TR, Surai PF. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science 2003;300:125–7.
Bortolotti GR, Fernie KJ, Smits JE. Carotenoid concentration and coloration of American Kestrels (*Falco sparverius*) disrupted by experimental exposure to PCBs. Funct Ecol 2003;17:651–7.
Brush AH. Metabolism of carotenoid pigments in birds. FASEB J 1990;4:2969–77.
Bustnes JO, Erikstad KE, Skaare JU, Bakken V, Mehlum F. Ecological effects of organochlorine pollutants in the arctic: a study of the glaucous gull. Ecol Appl 2003;13:504–15.
Bustnes JO, Hanssen SA, Folstad I, Erikstad KE, Hasselquist D, Skaare JU. Immune function and organochlorine pollutants in arctic breeding glaucous gulls. Arch Environ Contam Toxicol 2004;47:530–41.
Bustnes JO, Kristiansen KO, Helberg M. Immune status, carotenoid coloration, and wing feather growth in relation to organochlorine pollutants in great black-backed gulls. Arch Environ Contam Toxicol 2007;53:96–102.
Bustnes JO, Erikstad KE, Lorentsen S-H, Herzke D. Perfluorinated and chlorinated pollutants as predictors of demographic parameters in an endangered seabird. Environ Pollut 2008;156:417–24.
Bustnes JO, Moe B, Herzke D, Hanssen SA, Nordstad T, Sagerup K, et al. Strongly increasing blood concentrations of lipid-soluble organochlorines in high arctic common eiders during incubation fast. Chemosphere 2010;79:320–5.
Chew BP. Role of carotenoids in the immune-response. J Dairy Sci 1993;76:2804–11.
Chew BP. Importance of antioxidant vitamins in immunity and health in animals. Anim Feed Sci Technol 1996;59:103–14.
Chew BP, Park JS. Carotenoid action on the immune response. J Nutr 2004;134:257S–61S.
Costantini D, Møller AP. Carotenoids are minor antioxidants for birds. Funct Ecol 2008;22:367–70.
Cuthill IC. Color perception. In: Hill GE, McGraw, editors. Bird coloration: mechanisms and measurements. Cambridge, Massachusetts: Harvard University Press; 2006. p. 3–40.
Doutrelant C, Grégoire A, Gomez D, Staszewski V, Arnoux E, Tveraa T, et al. Colouration in Atlantic puffins and blacklegged kittiwakes: monochromatism and links to body condition in both sexes. J Avian Biol 2013;44:451–60.
During A, Hussain MM, Morel DW, Harrison EH. Carotenoid uptake and secretion by CaCo-2 cells β-carotene isomer selectivity and carotenoid interactions. J Lipid Res 2002;43:1086–95.
Eeva T, Lehikoinen E, Rönkä M. Air pollution fades the plumage of the Great Tit. Funct Ecol 1998;12:607–12.
Eraud C, Devevey G, Gaillard M, Prost J, Sorci G, Faivre B. Environmental stress affects the expression of a carotenoid-based sexual trait in male zebra finches. J Exp Biol 2007;210:3571–8.
Faivre B, Grégoire A, Préault M, Cézilly F, Sorci G. Immune activation rapidly mirrored in a secondary sexual trait. Science 2003;300:103.
Fuglei E, Bustnes JO, Hop H, Mørk T, Björnfoth H, van Bavel B. Environmental contaminants in arctic foxed (*Alopex lagopus*) in Svalbard: relationships with feeding ecology and body condition. Environ Pollut 2007;146:128–38.
Furr HC, Clark RM. Intestinal absorption and tissue distribution of carotenoids. J Nutr Biochem 1997;8:364–77.
Goodwin TW. Metabolism, nutrition, and function of carotenoids. Annu Rev Nutr 1986;6:273–97.
Hartley RC, Kennedy MW. Are carotenoids a red herring in sexual display? Trends Ecol Evol 2004;19:353–4.
Håstad O, Partridge JC, Ödeen A. Ultraviolet photopigment sensitivity and ocular media transmittance in gulls, with an evolutionary perspective. J Comp Physiol A 2009;195:585–90.
Helberg M, Bustnes JO, Erikstad KE, Kristiansen KO, Skaare JU. Relationships between reproductive performance and organochlorine contaminants in great black-backed gulls (*Larus marinus*). Environ Pollut 2005;134:475–83.
Helfenstein F, Danchin E, Wagner RH. Assortative mating and sexual size dimorphism in black-legged Kittiwakes. Waterbirds 2004;27:350–4.
Henriksen EO, Gabrielsen GW, Skaare JU. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (*Rissa tridactyla*), in relation to mobilization of body-lipids associated with reproduction. Environ Pollut 1996;92:27–37.
Herzke D, Nygård T, Berger U, Huber S, Røv N. Perfluorinated and other persistent halogenated organic compounds in European shag (*Phalacrocorax aristotelis*) and common eider (*Somateria mollissima*) from Norway: a suburban to remote pollutant gradient. Sci Total Environ 2009;408:340–8.
Hill GE. Energetic constraints on expression of carotenoid-based plumage coloration. J Avian Biol 2000;31:559–66.
Hill GE. Female mate choice for ornamental coloration. In: Hill GE, McGraw, editors. Bird coloration: function and evolution. Cambridge, Massachussetts: Harvard University Press; 2006. p. 137–200.
Jodice PGR, Lanctot RB, Gill VA, Roby DD, Hatch SA. Sexing adult black-legged kittiwakes by DNA, behavior, and morphology. Waterbirds 2000:405–15.
Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FAPC. Food web-specific biomagnification of persistent organic pollutants. Science 2007;317:236–9.
Kenntner N, Krone O, Oehme G, Heidecke D, Tataruch F. Organochlorine contaminants in body tissue of free-ranging white-tailed eagles from northern regions of Germany. Environ Toxicol Chem 2003;22:1457–64.
Kilner R. Mouth colour is a reliable signal of need in begging canary nestlings. Proceedings of the Royal Society of London, 264. Series B: Biological Sciences; 1997. p. 963–8.
Krinsky NI. Carotenoids as antioxidants. Nutrition 2001;17:815–7.
Kristiansen KO, Bustnes JO, Folstad I, Helberg M. Carotenoid coloration in great black-backed gull *Larus marinus* reflects individual quality. J Avian Biol 2006;37:6–12.
Leclaire S. Signaux sexuels, choix du partenaire et investissement parental chez la mouette tridactyle *Rissa tridactyla*. Université de Toulouse, Université Toulouse III-Paul Sabatier; 2010.

Leclaire S, Bourret V, Wagner RH, Hatch SA, Helfenstein F, Chastel O, et al. Behavioral and physiological responses to male handicap in chick-rearing black-legged kittiwakes. Behav Ecol 2011a;22:1156–65.
Leclaire S, White J, Arnoux E, Faivre B, Vetter N, Hatch SA, et al. Integument coloration signals reproductive success, heterozygosity, and antioxidant levels in chick-rearing black-legged kittiwakes. Naturwissenschaften 2011b;98:773–82.
Martínez-Padilla J, Mougeot F, Pérez-Rodríguez L, Bortolotti GR. Nematode parasites reduce carotenoid-based signalling in male red grouse. Biol Lett 2007;3:161–4.
Massaro M, Davis LS, Darby JT. Carotenoid-derived ornaments reflect parental quality in male and female yellow-eyed penguins (*Megadyptes antipodes*). Behav Ecol Sociobiol 2003;55:169–75.
McGraw KJ, Hill GE, Parker RS. The physiological costs of being colourful: nutritional control of carotenoid utilization in the American goldfinch, *Carduelis tristis*. Anim Behav 2005;69:653–60.
Moe B, Langseth I, Fyhn M, Gabrielsen GW, Bech C. Changes in body condition in breeding kittiwakes *Rissa tridactyla*. J Avian Biol 2002;33:225–34.
Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, et al. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? Avian Poult Biol Rev 2000;11:137–59.
Montgomerie R. Analyzing colors. In: Hill GE, McGraw, editors. Bird coloration: mechanisms and measurements. Cambridge, Massachusetts: Harvard University Press; 2006. p. 90–147.
Mougeot F, Martínez-Padilla J, Pérez-Rodríguez L, Bortolotti GR. Carotenoid-based colouration and ultraviolet reflectance of the sexual ornaments of grouse. Behav Ecol Sociobiol 2006;61:741–51.
Mougeot F, Pérez-Rodríguez L, Martínez-Padilla J, Leckie F, Redpath SM. Parasites, testosterone and honest carotenoid-based signalling of health. Funct Ecol 2007;21:886–98.
Noël M, Barrett-Lennard L, Guinet C, Dangerfield N, Ross PS. Persistent organic pollutants (POPs) in killer whales (*Orcinus orca*) from the Crozet Archipelago, southern Indian Ocean. Mar Environ Res 2009;68:196–202.
Nordstad T, Moe B, Bustnes JO, Bech C, Chastel O, Goutte A, et al. Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (*Rissa tridactyla*) across their breeding cycle. Environ Pollut 2012;164:219–26.
Olson VA, Owens IPF. Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol Evol 1998;13:510–4.
Parker RS. Absorption, metabolism, and transport of carotenoids. FASEB J 1996;10:542–51.
Pérez C, Lores M, Velando A. Oil pollution increases plasma antioxidants but reduces coloration in a seabird. Oecologia 2010a;163:875–84.
Pérez C, Munilla I, López-Alonso M, Velando A. Sublethal effects on seabirds after the Prestige oil-spill are mirrored in sexual signals. Biol Lett 2010b;6:33–5.
Pérez-Rodríguez L, Viñuela J. Carotenoid-based bill and eye ring coloration as honest signals of condition: an experimental test in the red-legged partridge (*Alectoris rufa*). Naturwissenschaften 2008;95:821–30.
R core team. A language and environment for statistical computing3-900051-07-0; 2012 [URL http://www.R-project.org/.].
Sagerup K, Larsen HJS, Skaare JU, Johansen GM, Gabrielsen GW. The toxic effects of multiple persistent organic pollutant exposures on the post-hatch immunity maturation of glaucous gulls. J Toxicol Environ Health A Curr Issues 2009;72:14,870–83.
Solomons NW, Bulux J. Effects of nutritional status on carotene uptake and bioconversiona. Ann N Y Acad Sci 1993;691:96–109.
Velando A, Beamonte-Barrientos R, Torres R. Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. Oecologia 2006;149:535–42.
Von Schantz T, Bensch S, Grahn M, Hasselquist D, Wittzell H. Good genes, oxidative stress and condition-dependent sexual signals. Proceedings of the Royal Society of London, 266. Series B: Biological Sciences; 1999. p. 1–12.
Weimerskirch H, Lallemand J, Martin J. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. J Anim Ecol 2005;74:285–91.