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# Maternal transfer and physiological consequences of per- and polyfluoroalkyl substances in seabird eggs

William Jouanneau

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LA ROCHELLE UNIVERSITY

DOCTORAL SCHOOL

-  
618

Centre d'Etudes Biologiques de Chizé

DOCTORAL THESIS

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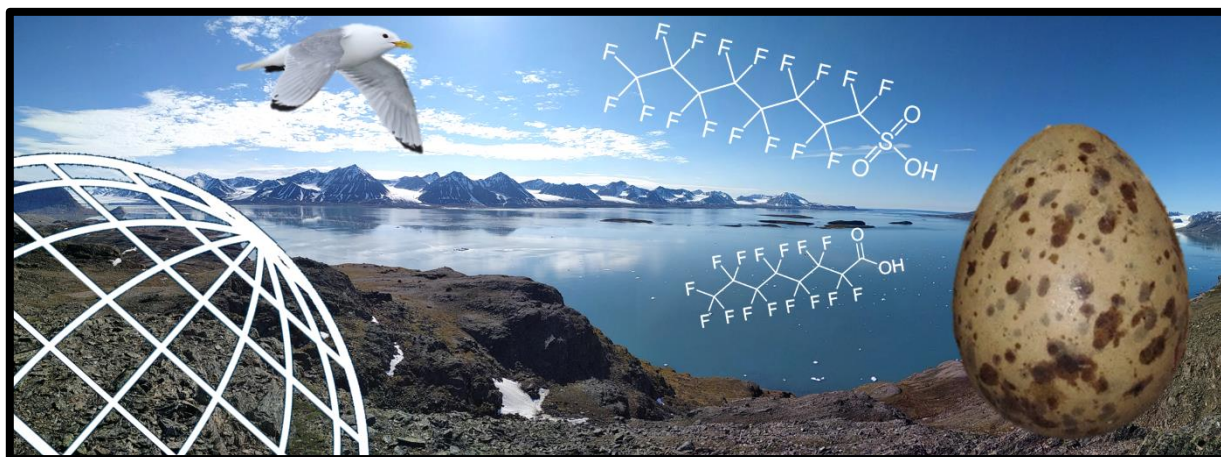
**William JOUANNEAU**

Defended on November 21<sup>st</sup> 2022

Submitted for the degree of doctor of La Rochelle University

Specialty: Environmental and Population Biology, Ecology

## **Maternal transfer and physiological consequences of per- and polyfluoroalkyl substances in seabird eggs**



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LA ROCHELLE UNIVERSITE

ECOLE DOCTORALE

-  
618

Centre d'Etudes Biologiques de Chizé

THÈSE

présentée par :

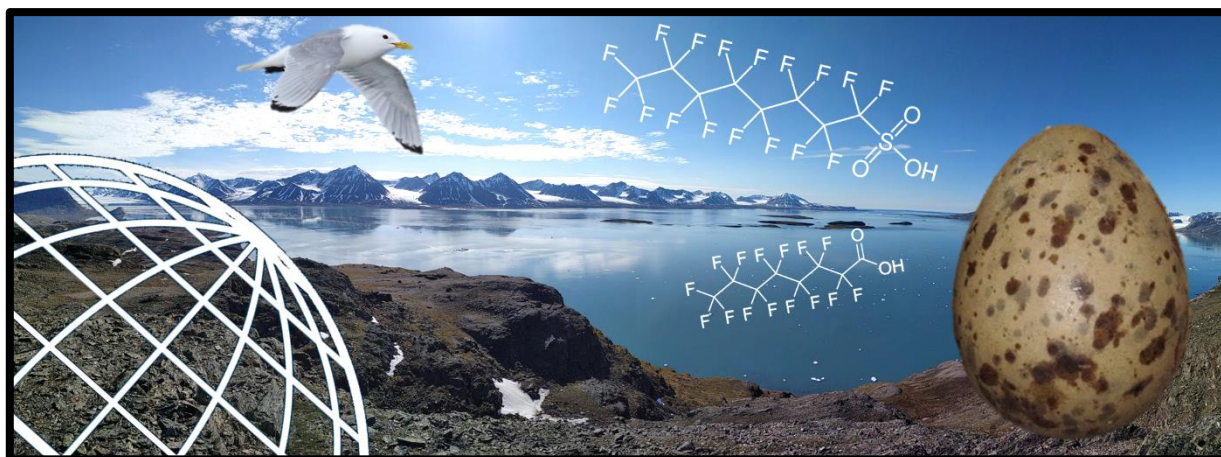
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Soutenu le 21 novembre 2022

pour l'obtention du grade de Docteur de La Rochelle Université

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## **Transfert maternel et conséquences physiologiques des substances per- et polyfluoroalkylées dans les œufs d'oiseaux marins**



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**The French Polar Institute (IPEV)**

**The Norwegian Polar Institute (NPI)**

**This doctoral thesis was based on collaborative works conducted in different laboratories**

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*Glaucous gulls (Larus hyperboreus) chicks waiting for their parents to return to the nest*

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*Ice floes from glacier calving in Kongsfjord*

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# PREFACE



*Purple sandpiper (Calidris maritima) performing a threat display*

## I. PUBLICATIONS

### a. Publications included in this PhD thesis

This manuscript is based on four main articles (one published, one in review and two in preparation) provided in **Chapters I to IV** and referred to in the text with capital letters as followed (**Paper A, Paper B, Paper C and Paper D**).

### Paper A

**Jouanneau W.**; Léandri-Breton D-J.; Corbeau A.; Herzke D.; Moe B.; Nikiforov V. A.; Gabrielsen G.W.; Chastel O. A Bad Start in Life? Maternal Transfer of Legacy and Emerging Poly- and Perfluoroalkyl Substances to Eggs in an Arctic Seabird. *Environmental Science and Technology*. **2022**, 56, 10, 6091-6102.

[DOI 10.1021/acs.est.1c03773](https://doi.org/10.1021/acs.est.1c03773)

### Paper B

**Jouanneau W.**; Léandri-Breton D-J.; Herzke D.; Moe B.; Nikiforov V. A.; Pallud M.; Parenteau C.; Gabrielsen G. W.; Chastel O. Does contaminant exposure disrupt maternal hormones deposition? A study on per- and polyfluoroalkyl substances in an Arctic seabird. Submitted to *Science of the Total Environment*.

### Paper C

**Jouanneau W.**; Blévin P.; Angelier F.; Dhainaut P.; Herzke D.; Léandri-Breton D-J.; Moe B.; Nikiforov V. A.; Ribout C.; Gabrielsen G. W.; Chastel O. Is in ovo exposure to per- and polyfluoroalkyl substances affecting telomere length in embryos of an Arctic seabird? In prep. for *Chemosphere*.

### Paper D

**Jouanneau W.**; Boulinier T.; Angelier F.; Budzinski H.; Labadie P.; Lemesle P.; Lourdaïs O.; Herzke D.; Nikiforov V. A.; Gabrielsen G. W.; Chastel O. Occurrence and developmental toxicity of 7:3 fluorotelomer carboxylic acid in yellow-legged gulls. In prep. for *Environmental Toxicology and Chemistry*.

**b. Other publications in connection with this PhD thesis**

- Léandri-Breton D.-J.; Tarroux A.; Elliott J. E.; Legagneux P.; Angelier F.; Blévin P.; Bråthen V. S.; Fauchald P.; Goutte A.; **Jouanneau W.**; Tartu S.; Moe B.; Chastel O. Long-term tracking of an Arctic-breeding seabird indicates high fidelity for pelagic wintering areas. *Marine Ecology Progress Series*. **2021**, 676, 205-218.  
[DOI 10.3354/meps13798](https://doi.org/10.3354/meps13798)

**c. Other publications conducted during the course of this PhD**

- **Jouanneau W.**; Sebastiano M.; Rozen-Rechels D.; Harris S. M.; Blévin P.; Angelier F.; Brischoux F.; Gernigon J.; Lemesle J.-C.; Robin F.; Cherel Y.; Bustamante P.; Chastel O. Blood mercury concentrations in four sympatric gull species from South Western France: insights from stable isotopes and biologging. *Environmental Pollution*. **2022**, 308, 119619.  
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- Sebastiano M.; **Jouanneau W.**; Blévin P.; Angelier F.; Parenteau C.; Gernigon J.; Lemesle J.C.; Robin F.; Pardon P.; Burzinski H.; Labadie P.; Chastel O. High levels of fluoroalkyl substances and potential disruption of thyroid hormones in three gull species from South Western France. *Science of the Total Environment*. **2020**, 765, 144611.  
[DOI 10.1016/j.scitotenv.2020.144611](https://doi.org/10.1016/j.scitotenv.2020.144611)
- Sauser C.; Angelier F.; Blévin P.; Chastel O.; Gabrielsen G. W.; **Jouanneau W.**; Kato A.; Moe B.; Ramírez F.; Tartu S.; Descamps S. Demographic responses of Arctic seabirds to spring sea ice variations. Submitted to *Frontiers in Ecology and Evolution*.
- Lemesle P.; **Jouanneau W.**; Legroux N.; Pischuitta R.; Ward A.; Cherel Y.; Bustamante P.; Chastel O. Dietary exposure to mercury in a French black-legged kittiwake colony: ecotoxicological risks? In prep. for *Chemosphere*.
- Sebastiano M.; **Jouanneau W.**; Blévin P.; Angelier F.; Parenteau C.; Gernigon J.; Lemesle J.C.; Robin F.; Pardon P.; Burzinski H.; Labadie P.; Chastel O. One more piece of evidence that PFAS potentially disrupt thyroid hormones: clues from seabird chicks from South Western France. In prep. for *Environmental Research*.



## II. COMMUNICATION

### a. Conferences

- **CEBC's PhD candidate seminar 2021** – June 3 – Villiers-en-Bois, France. **Oral** [French]
  
- **Society of Environmental Toxicology and Chemistry (SETAC) North America 2021** – November 14-18 – Portland, Oregon, USA. *Maternal transfer of legacy and emerging poly- and perfluoroalkyl substances to eggs in an Arctic seabird*. Jouanneau W.; Léandri-Breton D-J.; Herzke D.; Moe B.; Nikiforov V. A.; Pallud M.; Parenteau C.; Gabrielsen G. W.; Chastel O. **Oral** [English]
  
- **5<sup>th</sup> animal ecophysiology seminar “CEPA<sup>5</sup>” 2021** – November 2-4 – Montpellier, France. *Maternal transfer of contaminants and endocrine disruption in an Arctic seabird*. Jouanneau W.; Léandri-Breton D-J.; Herzke D.; Moe B.; Nikiforov V. A.; Pallud M.; Parenteau C.; Gabrielsen G. W.; Chastel O. **Oral** [English]
  
- **La Rochelle University's PhD candidate seminar 2021** – September 23-24 – La Rochelle, France. **Oral** [French] **and Poster**  
*Award: « Best poster awarded by the scientific committee »*
  
- **16<sup>th</sup> science days on polar research of the French Committee for Arctic and Antarctic Research (CNFRAA) 2020** – September 22-23 – La Rochelle, France. *Transfert maternel de contaminants et perturbation endocrine chez un oiseau marin Arctique*. Jouanneau W.; Léandri-Breton D-J.; Herzke D.; Moe B.; Nikiforov V. A.; Pallud M.; Parenteau C.; Gabrielsen G. W.; Chastel O. **Oral** [French]  
*Award : « Best presentation »*
  
- **4<sup>th</sup> animal ecophysiology seminar “CEPA<sup>4</sup>” 2019** – October 28-30 – Nantes, France. *Exposition au mercure chez trois espèces de goélands de la côte Atlantique en lien avec les isotopes stables du carbone, de l'azote et du soufre*. Jouanneau W.; Sebastiano M.; Rozen-Rechels D.; Harris S. M.; Blévin P.; Angelier F.; Brischoux F.; Gernigon J.; Lemesle J-C.; Robin F.; Cherel Y.; Bustamante P.; Chastel O. **Oral** [French]

**b. Public communication**

- **Science Festival 2021** – October 8-9 – Zoodyssée, Villiers-en-Bois, France. *Contaminants in polar seabirds, exposure and consequences*. Presentations for schoolchild and families.

**III. SUPERVISION OF TRAINEES**

- Co-supervision of a Master student (Prescillia Lemesle) – 6 months in 2021 – *Dietary exposure to mercury in a French black-legged kittiwake colony: ecotoxicological risks?*
- Training of 10 Master students during fieldwork sessions (2019 and 2021) for bird handling, banding, morphometric, blood sampling, global location sensor (GLS) and global positioning system (GPS) loggers setting and deployments.

**IV. TRAINING COURSES**

**a. Courses**

- LaTeX for beginners (**2021**) – La Rochelle University, France
- Applied statistics with R: modelling (**2020**) – CEBC, France

**b. Certifications**

- Certification for Use of Animals in Research for researchers (**2021**), National Museum of Natural History, Paris, France
- Certification for Use of Animals in Research for field assistants (**2020**) – Norwegian Institute for Nature Research, Trondheim, Norway
- Work-place first aider (**2020 & 2022**), CEBC, Villiers-en-Bois, France
- Coastal (**2019**) and offshore (**2020**) boat driving license (used for fieldwork)

## V. FUNDINGS & FINANCIAL SUPPORT

To successfully complete this PhD, I received funding received from different organizations:

- La Rochelle University: 3-years PhD grant, mobility grant (3000€), training grant (700€)
- French National Agency for Research (ANR): Projects ILETOP (PI: P. Bustamante) and ToxSeaBird (PI: O. Chastel)
- French Polar Institute (IPEV): Program 330 [ORNITHO-ENDOCRINO] (PI: O. Chastel)
- The Research Council of Norway: Arctic Field Grant to D-J. Léandri-Breton

## VI. COLLABORATIVE NETWORK & PERSONAL IMPLICATION

This PhD work is part of the IPEV Program 330 (see **boxed text**). It relies on a large network of multidisciplinary researchers, engineers and fieldwork technicians, but also PhD candidates and master students from French, Norwegian and Canadian research institutions mainly. The Covid-19 certainly affected the course of this PhD, preventing for instance the planned team including myself to conduct fieldwork during the 2020 seabirds breeding season in Svalbard. However, this network showed its resilience and efficiency and managed to conduct the most important planned experiments in the field (done by P. Blévin and P. Dhainaut) and to carry the laboratory analyses as fast as possible despite the challenging context, preserving the work of two PhD candidates (D-J. Léandri-Breton and W. Jouanneau).

Supported by this network, during my PhD I organized three field sessions in Ny-Ålesund, Svalbard (together with O. Chastel and D-J Léandri-Breton) and travelled there two times (2 months in 2019 and 2 months in 2021) for data collection. I have also been received two times in NPI and NILU (1 month in 2020 and 2 months in 2022) in Tromsø, Norway as a visiting PhD candidate to conduct PFAS measurements in the samples collected during fieldwork.

I have also been involved in hormones analyses at the CEBC and have been trained on measuring steroid and thyroid hormones and organized a sampling campaign in the Parc National des Calanques of Marseille, France where I was received for fieldwork in 2021.

## Preface

This PhD thesis is based on data collected on kittiwakes during the 2019 and 2020 fieldwork seasons and on yellow-legged gull in 2021.

### **IPEV Program 330 [ORNITHO-ENDOCRINO]**

*Physiological and demographic consequences of the exposure to contaminants in Arctic seabirds*



PI: Olivier CHASTEL



Conducted in the Norwegian Arctic and supported from the French Polar Institute (IPEV), in collaboration with the Norwegian Polar Institute (NPI), the Norwegian Institute for Nature Research (NINA) and the Norwegian Institute for Air Research (NILU), the **program 330** is a long-term study running since the beginning of the 2000s. It aims at investigating how the high concentrations of contaminants accumulating in the Arctic may affect physiological, behavioral, reproductive and demographic parameters in seabird species breeding there. Focusing on persistent organic pollutants and mercury, it enabled to highlight the adverse effects of these compounds in dozens of publications within its framework.

In addition to the data collected for my studies, I help collect diverse samples and deploy various tags including GLS and GPS for several studies that investigate the ecology, ecotoxicology or ecophysiology of birds. I did not use these data in my PhD, although some of them were collected in the framework of D-J. Léandri-Breton's PhD and will result in studies which I be co-author or lead-author. I also had the opportunity to collaborate with diverse institutions to conduct side projects on urban nesting gulls from Boulogne-sur-Mer, France, and diverse gull species from the National Natural Reserve of Ile de Ré, France.



*Panoramic view on Kongsfjord, Svalbard*

## VII. GLOSSARY OF ACRONYMS

### Contaminants

ADONA	Dodecafluoro-3H-4,8-dioxanonoate	PFDoA	Perfluorododecanoic acid
DDT	Dichlorodiphenyltrichloroethane	PFECA	Per- and polyfluoroalkyl ether carboxylic acid
Ether-PFAS	Fluoroalkylether substances	PFEcHS	Perfluoroethylcyclohexane sulfonate
FTCA	Fluorotelomer carboxylic acid	PFHxS	Perfluorohexanesulfonic acid
FTOH	Fluorotelomer alcohol	PFNA	Perfluorononanoic acid
FTS	Fluorotelomer sulfonic acid	PFOA	Perfluorooctanoic acid
F-53B	Chlorinated polyfluorinated ether sulfonate	PFOS	Perfluorooctanesulfonic acid
GenX	Hexafluoropropylene oxide dimer acid	PFPS	Perfluoropentanesulfonic acid
PBDE	Polybromodiphényléther	PFSA	Perfluoroalkyl sulfonic acids
PCB	Polychlorinated biphenyl	PFTeA	Perfluorotetradecanoic acid
PFAS	Per- and polyfluoroalkyl substances	PFTriA	Perfluorotridecanoic acid
PFBS	Perfluorobutanesulfonic acid	PFUnA	Perfluoroundecanoic acid
PFCA	Perfluoroalkyl carboxylic acids	POPs	Persistent Organic Pollutants
PFDCa	Perfluorodecanoic acid	$\alpha$ -HBCD	$\alpha$ -hexabromocyclododecane

### Laboratories and institutions

CEBC	Centre d'Etudes Biologiques de Chizé
CEFE	Centre d'Ecologie Fonctionnelle et Evolutive
CNRS	Centre National de la Recherche Scientifique
IPEV	Institut Polaire Français Paul-Emile Victor
IUCN	International Union for Conservation of Nature
LRU	La Rochelle Université
NILU	Norwegian Institute for Air Research
NINA	Norwegian Institute for Nature Research
NPI	Norwegian Polar Institute

### Others

Ab	Antibody
AFFF	Aqueous film-forming foam
CO <sub>2</sub>	Carbon dioxide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDC	Endocrine Disrupting Chemical
ELISA	Enzyme Linked ImmunoSorbent Assay

### Hormones

AND	Androstenedione
CORT	Corticosterone
DHT	Dihydrotestosterone
T	Testosterone
T <sub>3</sub>	Triiodothyronine
T <sub>4</sub>	Thyroxine
GLS	Global Location Sensor
GPS	Global Positioning System
O <sub>2</sub>	Dioxygen
PCR	Polymerase Chain Reaction
qPCR	quantitative Polymerase Chain Reaction
RBC	Red Blood Cells
RIA	Radio-immunoassay
RMR	Resting Metabolic Rate
SD	Standard Deviation
TEWL	Total Evaporative Water Loss

# GENERAL

# INTRODUCTION



*Ivory gull (Pagophila eburnea) cleaning its feathers*

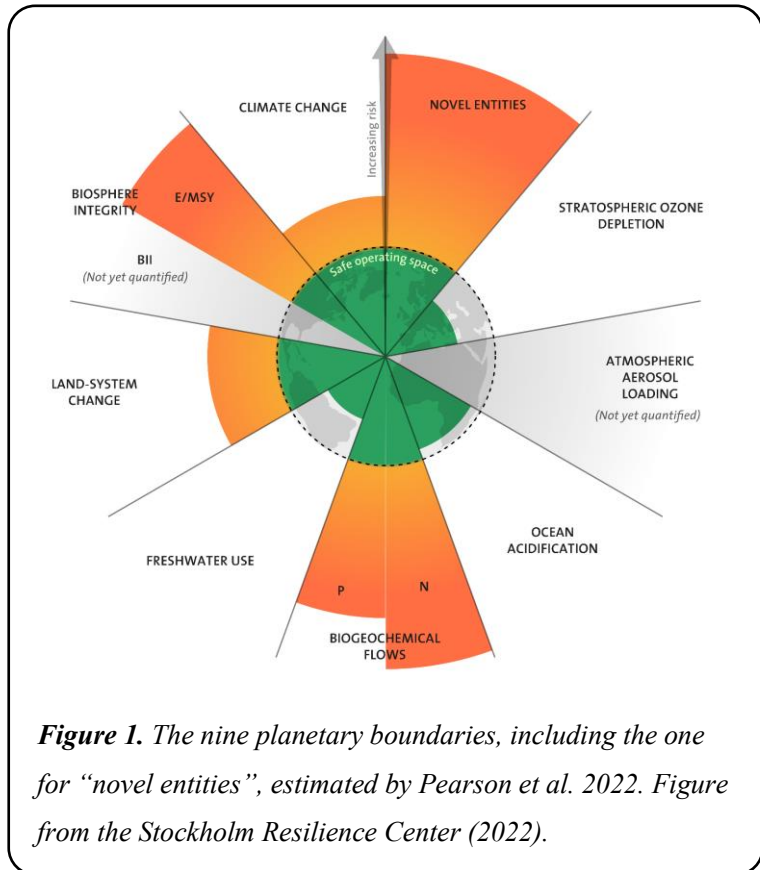
## I. ANTHROPOGENIC POLLUTION

### a. Global context

The impact of Human activities on the environment is changing Earth more rapidly than we understand it, compromising ecosystems and species survival (Steffen *et al.* 2011; Vitousek *et al.* 1997). Anthropogenic pollution is a form of pollution produced directly by human activities rather than by natural processes such as wildfires or volcanic activities. Along with habitat loss, climate change, overexploitation and invasive species, anthropogenic pollution jeopardizes, among others, more than a quarter of all mammal species, 13 % of all bird species and 41 % of amphibians, altogether threatening more than 41 000 animals and plants species with extinction (IUCN, 2022, <https://www.iucnredlist.org/>; Tilman *et al.* 2017; Whitmee *et al.* 2015).

If the earlier forms of anthropogenic pollution likely date back to Prehistory (Rieuwerts 2015), it has only massively increased to the levels we know today since the industrial revolution (Steffen *et al.* 2011). Anthropogenic pollution is considered as the ninth planetary boundary (Rockström *et al.* 2009), namely one of the environmental limits within which humanity may safely operate. Renamed the “Novel entities” boundary in 2015, it encapsulates all “new substances, new forms of existing

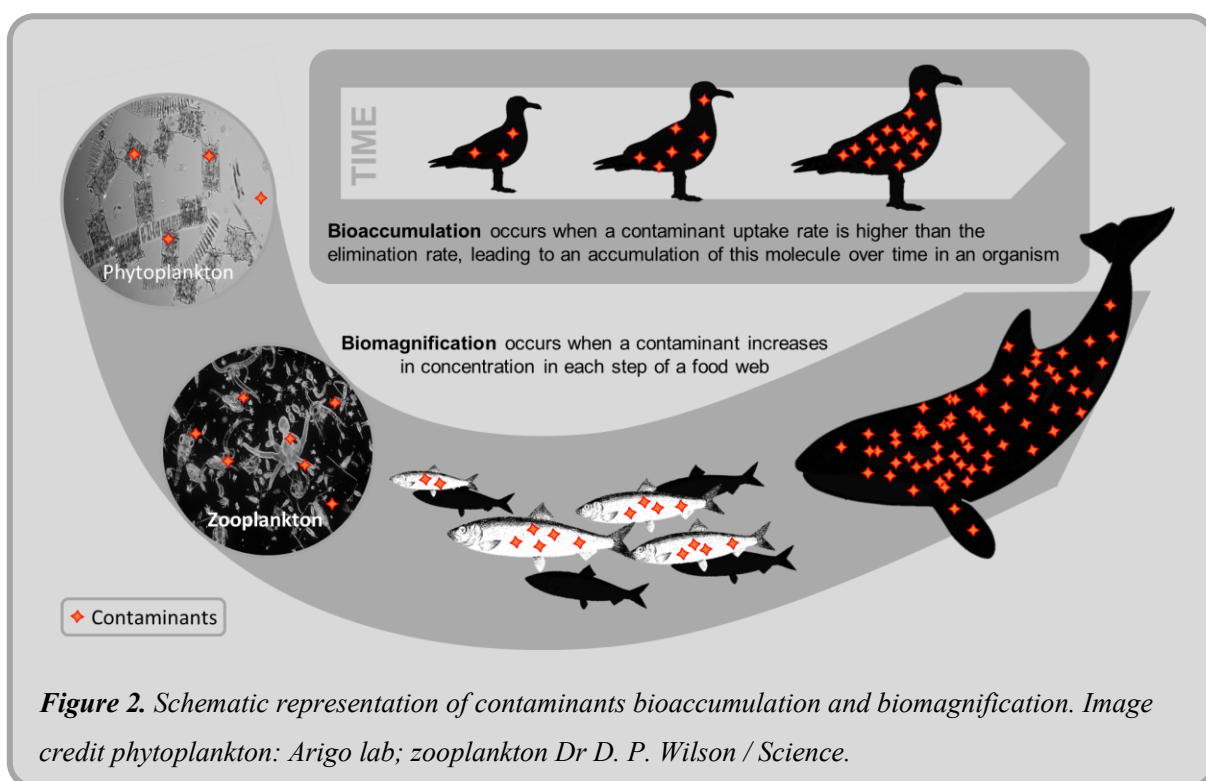
substances, and modified life forms that have the potential for unwanted geophysical and/or biological effects” (Steffen *et al.* 2015). A recent study considered this virtual threshold exceeded based on the much greater rate of production and emissions of novel chemical entities, compared to the societies’ ability to conduct safety related risk potentials assessments and monitoring for humans and ecosystems (**Figure 1**; Persson *et al.* 2022). In addition to increasing



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anthropogenic emissions of natural elements (e.g., heavy metals, sulfur dioxide or radon), the 20<sup>th</sup> century saw the emergence of thousands of synthetic chemicals through the rapid progress of industrial chemistry. Currently, over 350 000 substances are registered for commercial production and use while around 400 million tons of chemicals are produced yearly on a global scale, ending up for a large part in the environment, biota and human body (Wang *et al.* 2020).

Within synthetic chemicals, persistent organic pollutants (POPs) received increasing attention in the last decades for their worldwide distribution, even reaching some of the most remote environments on Earth (Jamieson *et al.* 2017). These compounds bioaccumulate in organisms and biomagnify along trophic webs (**Figure 2**), leading long-lived top predators to often exhibit very high concentrations (Connell, 1988). Such levels may affect wildlife in numerous ways, including impaired development, physiology, behavior, immunology, endocrinology and reproductive processes, through teratogenic actions, altering individual fitness and survival and ultimately impacting population trends and ecosystems (El-Shahawi *et al.* 2010; Hao *et al.* 2021; Johnson *et al.* 2013). Some of these findings led to the Stockholm Convention on Persistent Organic Pollutants in 2001, an international treaty aiming at eliminating or restricting the production and use of 12 POPs (e.g., chlorinated pesticides such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs) or brominated flame retardants), the so-called “the dirty dozen”, currently ratified by 185 parties.



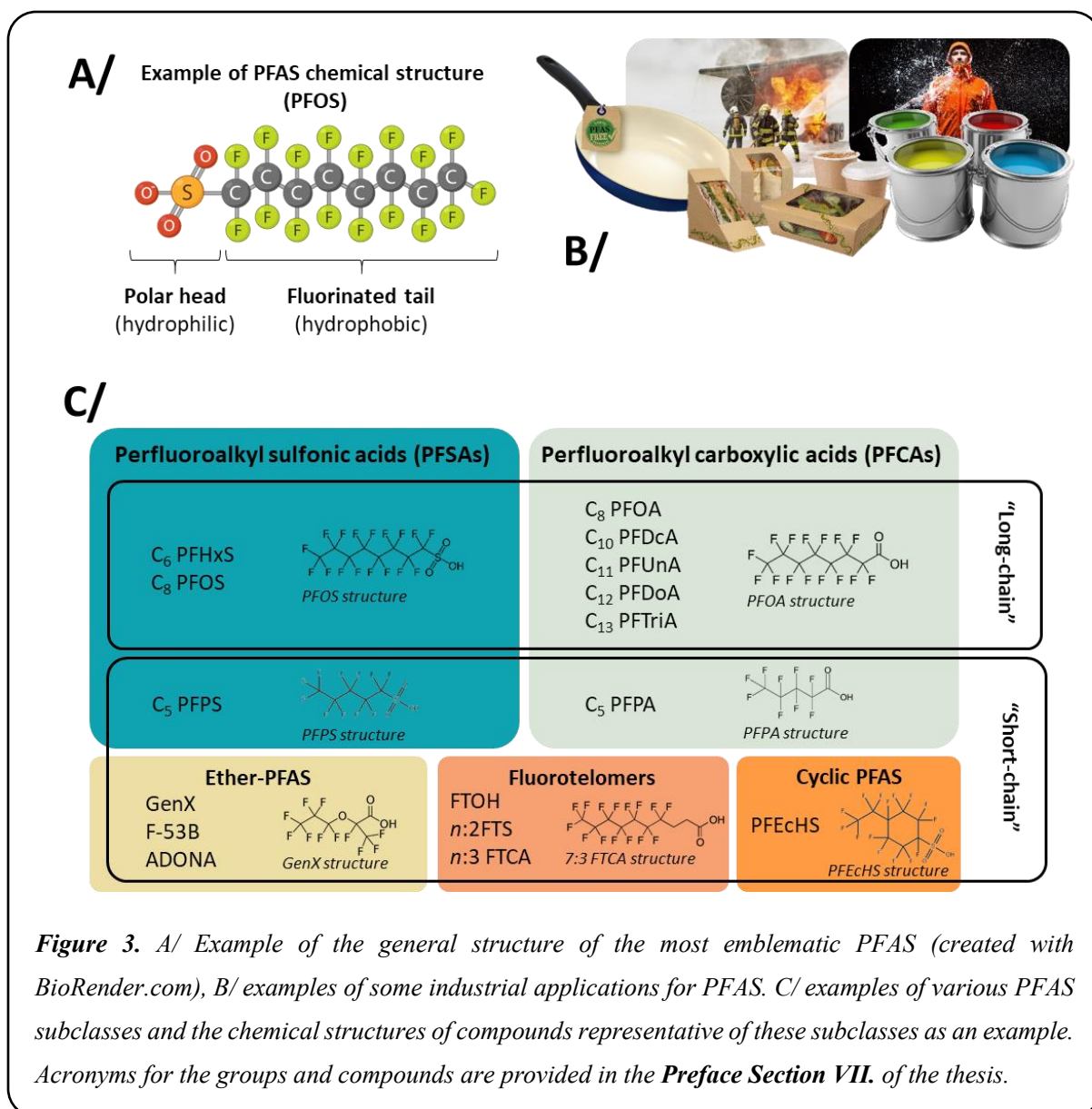
**Figure 2.** Schematic representation of contaminants bioaccumulation and biomagnification. Image credit phytoplankton: Arigo lab; zooplankton Dr D. P. Wilson / Science.



### **b. Per- and polyfluoroalkyl substances**

Among the immense group of organic pollutants, per- and polyfluoroalkyl substances (PFAS) represent a family of thousands of individual synthetic chemicals. PFAS were first produced in the 1950s and are widely used since (Evich *et al.* 2022). Defined in 2021 by the Organisation for Economic Cooperation and Development: “*PFAS are fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/C/Br/I atom attached to it)*” (Figure 3; Wang *et al.* 2021b). Carbon-fluorine being one of the most stable chemical bonds known in nature, PFAS are commonly nicknamed “forever chemicals” owing to their extreme chemical and thermal stability. Some PFAS are amphiphilic (i.e., possessing both hydrophilic and lipophilic properties), consequently having highly useful surfactant properties used in a myriad of industrial processes and consumer products. Their main applications include, among others, electro-plating, food packages, grease- and waterproof textiles, non-stick cookware, medical devices and aqueous film-forming foams (AFFFs) used in airport fire extinguishers (Glüge *et al.* 2020).

PFAS may be released in the environment at each step of their life cycle from production to disposal. Volatile precursors and recalcitrant final compounds manufactured by the producers may travel long distances through atmospheric and oceanic currents (Evich *et al.* 2022), even reaching polar areas (Garnett *et al.* 2022; Muir *et al.* 2019). PFAS can then potentially be taken up by biota through exposure in drinking water or their diet. Some of these substances are characterized by high bioaccumulation or biomagnifying potential. Although in use for more than seven decades, the global extent of PFAS has been detected in the environment and wildlife only relatively recently in the early 2000s. First for perfluorooctane sulfonic acid (PFOS) in wildlife (Giesy and Kannan 2001), then for perfluorooctanoic acid (PFOA) in human blood (Hansen *et al.* 2001). Consequently, the major PFAS producers phased-out the production of PFOS, PFOA and related products straight afterwards. Some PFAS were then added to the list of the POPs by the Stockholm Convention: in the 2009 amendment, PFOS and its salts were included in Annex B restricting their production and use, and in 2019 PFOA was included in Annex A aiming towards its elimination, followed by perfluorohexanesulfonic acid (PFHxS) in 2022 (United Nations Environment Programme 2022).



Over the last 20 years, the scientific knowledge about PFAS has increased rapidly and thousands of articles are currently published on PFASs every year. The study of long-chain perfluoroalkyl sulfonic acids (PFSAs;  $C_nF_{2n+1}SO_3H$ ,  $n \geq 6$ ), including PFOS, and perfluoroalkyl carboxylic acids (PFCAs;  $C_nF_{2n+1}COOH$ ,  $n \geq 7$ ), including PFOA, represents most of the publications (Pelch *et al.* 2022), since these compounds were initially shown to be more bioaccumulative and toxic for living organisms. The current understanding of biological impacts of PFAS were evaluated in laboratory studies on mainly invertebrates, fish, birds and mammals. While these studies found numerous impacts on growth and development, immunity, endocrinology, metabolism, reproduction and survival (Ankley *et al.* 2021), the mechanism-based linkages between exposure and effects are still to be established. It is much less examined

## General introduction

how PFAS affect organisms' health in free-living animals. In general, studies in wildlife focused on measuring PFAS occurrence and patterns in tissues, through exploring their impacts on a range of biomarkers including biochemical and immune parameters or endpoints related to reproductive success, and all have been affected by PFAS (Ankley *et al.* 2021; Dietz *et al.* 2019). These historical compounds are now considered as “legacy” PFAS, as opposed to “emerging PFAS” which consists in some of the legacy compounds precursors (e.g., fluorotelomer alcohols (FTOH) and their intermediate breakdown products), or shorter-chain PFAS (e.g., perfluoroethylcyclohexane sulfonate (PFECyHS) or ether-PFAS; **Figure 3**) that have been synthesized more recently as an alternative to the first ones' drawback.

The development and manufacturing of emerging PFAS remains ongoing, despite recent evidence of their occurrence in the environment and presence in wildlife tissue (Chen *et al.* 2021; Munoz *et al.* 2019). Actually, exposure assessments focusing primarily on long-chain substances may severely underestimate overall exposure to fluorinated compounds in wildlife (Herzke *et al.* 2022). Some emerging PFAS remain largely uncharacterized in terms of risk for wildlife (the chemical structure of some of them still being unknown). Recent studies investigating emerging PFAS reported a weaker bioaccumulation and biomagnification potential (Kempisty *et al.* 2018; Ren *et al.* 2022), but this is still debated for some of them (Munoz *et al.* 2019; Zhang *et al.* 2022). Although emerging PFAS have been suggested to be less toxic than legacy PFAS (Sonne *et al.* 2021), some emerging PFAS may be even more toxic than the legacy compounds they replace (Espartero *et al.* 2022; Mahoney *et al.* 2022; Wang *et al.* 2019). Moreover, some emerging PFAS may even have stronger abilities for long-range transport than legacy compounds (Feng *et al.* 2018).

Despite being entirely manufactured, little is known about the extent of PFAS production and the places where they are used (Ng *et al.* 2021). It is estimated that the historical yearly production of PFAS was about a megaton worldwide at the end of the 2010s (Evich *et al.* 2022), but due to a continuous input of new molecules, scientists struggle to keep the pace for monitoring their environmental sources, chemistry, fate, transport and toxicological implications for living organisms (Guelfo *et al.* 2021). Although recent promising findings may pave the way for future remediation techniques for PFAS (Krause *et al.* 2022; Trang *et al.* 2022), to date, most of the PFAS synthesized are not destroyed since this requires substantial energy inputs. Because of their massive production and long-range transport, PFAS are now found in detectable concentrations in almost the entire human population (Fenton *et al.* 2021). Moreover, a recent study demonstrated that PFAS in rainwater on a global scale often greatly

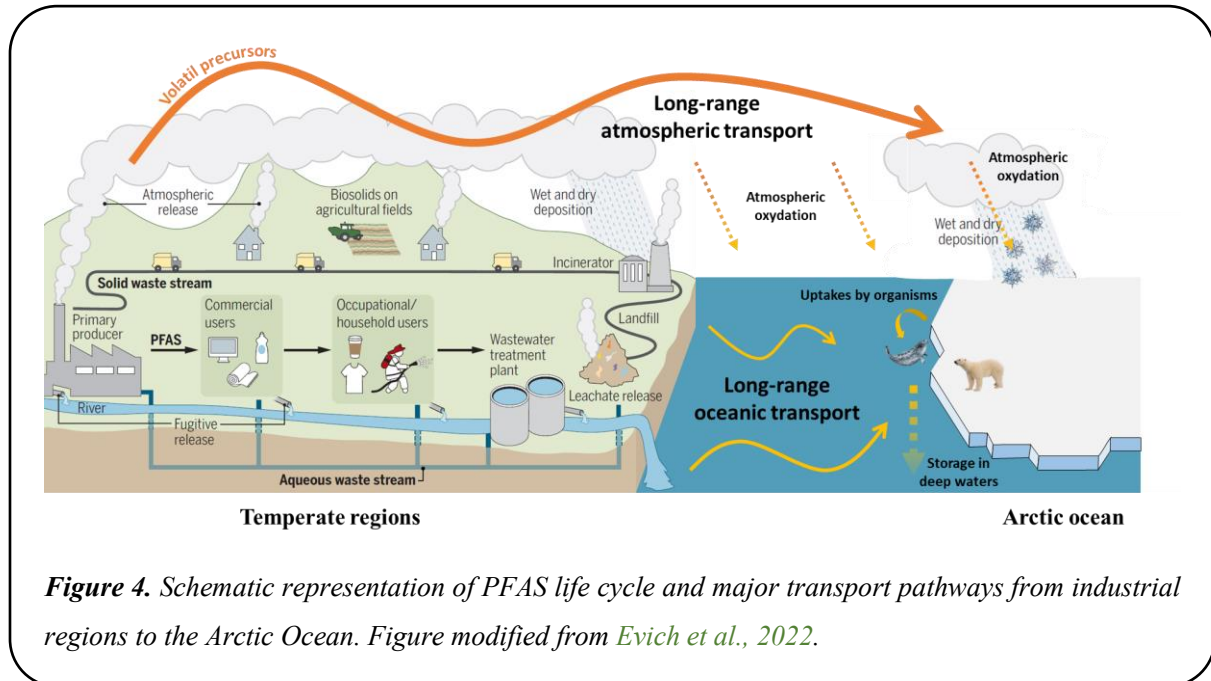
exceeds the US and European advisory thresholds for drinking and surface water, making it unsuitable for human consumption, even in the most remote regions (Cousins *et al.* 2022). Some members of the scientific community call for an urgent ban of all fluorinated organic substances (Sonne *et al.* 2022), or at the very least limit their production to those demonstrated to be of first importance for health, safety and functioning of society, introducing the concept of “essentiality” for PFAS (Blum *et al.* 2015; Cousins *et al.* 2019; 2021). However, to date, due to the numerous societal benefits provided by PFAS and their relatively low production cost, there are no known alternatives to the myriad of industrial applications.

### c. PFAS in the Arctic

The High Arctic is a polar region, largely marine, characterized by year-round harsh climatic conditions. Although this region is remote and situated away from industry, human activities are responsible for a wide range of pressure on Arctic ecosystems. These threats include rapid climate change, overfishing and anthropogenic pollution, making Arctic ecosystems highly vulnerable (Townhill *et al.* 2022). It is now well identified that the major atmospheric, oceanic, and riverine pathways result in the long-range transport of many contaminants from European, Asian and North American source regions to the Arctic (Bergmann *et al.* 2022; Burkow and Kallenborn 2000; Kirk *et al.* 2012). Significant efforts have been made to estimate the occurrence, fate and toxicity of legacy POPs and methylmercury (MeHg) on Arctic wildlife (Albert *et al.* 2019; Dietz *et al.* 2019; Letcher *et al.* 2010), but many understudied emerging contaminants reach the Arctic (Vorkamp and Riget 2014), and have unexplored consequences on the local wildlife (Sonne *et al.* 2021).

It is the case of PFAS, and more particularly emerging compounds. PFAS precursors are massively transported to the Arctic by atmospheric currents primarily, although marine pathways are also major input routes for final products (Figure 4. ; Joerss *et al.* 2020; Yeung *et al.* 2017). Some compounds are even present in higher concentrations in Arctic waters than in lower latitudes waters influenced by industrial sources (Garnett *et al.* 2021). In air, water and biota samples from the Arctic, legacy PFAS show inconsistent temporal trends since the 2010s despite global efforts in the regulation of long-chain PFAS that started in the early 2000s (Jouanneau *et al.* 2020; Muir *et al.* 2019; Muir and Miaz 2021; Routti *et al.* 2017; Wong *et al.* 2021). Compared to legacy compounds, emerging PFAS have received less attention, probably due to their extremely large diversity and relatively recent appearance, but also most likely as they were supposed to be less harmful to humans and biota. Nonetheless, recent studies reported

the presence of numerous emerging PFAS in Arctic marine fauna (Barrett *et al.* 2021; Gebbink *et al.* 2016; Spaan *et al.* 2020).



**Figure 4.** Schematic representation of PFAS life cycle and major transport pathways from industrial regions to the Arctic Ocean. Figure modified from *Evich et al., 2022*.

#### d. Seabirds as bioindicators of local anthropogenic pollution

Bioindicators are used to screen the quality of the environment, at a specific moment or over time. They include biological processes, species or communities. They are widely used to evaluate environmental contamination, especially for contaminants that bioaccumulate. Behavioral, chemical or physiological information may be collected to provide cues on alterations in the environment. Many different microorganisms, plants and animal species are used as bioindicators of environmental pollution (Zaghloul *et al.* 2020).

Regarding local anthropogenic pollution, colonial seabirds are of particular interest as bioindicators since they present numerous benefits. Foraging in a wide spectrum of marine habitats and in a broad range of trophic levels, they can provide information about a large variety of environments. Recent progress in biologging, including the use of miniaturized geolocators (Yoda 2018), now offers the opportunity to finely identify foraging marine grounds of many arctic seabirds in relation to contaminant uptake (Albert *et al.* 2021; Jouanneau *et al.* 2022 [Appendix A]). Their high or even apex position in most marine trophic webs also make them ideal to monitor biomagnifying contaminants in marine ecosystems (Elliott and Elliott

2013; Sebastiano *et al.* 2020b [**Appendix B**]). Moreover, they are long-lived organisms, which makes them particularly prone to bioaccumulation. They are relatively easy to sample, and many species are philopatric, coming back to the same nest year after year facilitating long-term studies (e.g., Tartu *et al.* 2022). Moreover, living in dense colonies, a large number of samples may easily be collected. Arctic seabirds are therefore widely used in ecotoxicological studies investigating health consequences of exposure to contaminants in wild population (e.g., Chastel *et al.* 2022).

A handful of studies investigated the occurrence and health consequences of PFAS in Arctic breeding seabirds. They found relative high legacy PFAS concentrations in various seabird species including the ivory gull (*Pagophila eburnea*; Lucia *et al.* 2017), the white-tailed eagle (*Haliaeetus albicilla*; Jouanneau *et al.* 2020; Sletten *et al.* 2016), the thick-billed murre (*Uria lomvia*; Choy *et al.* 2022), the black-legged kittiwake (*Rissa tridactyla*; Ask *et al.* 2021; Blévin *et al.* 2017a; 2017b; 2018; 2020; Costantini *et al.* 2019; 2022; Nøst *et al.* 2012; Tartu *et al.* 2014), the northern fulmar (*Fulmarus glacialis*; Braune *et al.* 2011; Nøst *et al.* 2012) and the glaucous gull (*Larus hyperboreus*; Melnes *et al.* 2017; Sebastiano *et al.* 2020a; Verreault *et al.* 2005). In seabirds, PFAS contamination was found to be related to a wide range of endpoints including oxidative stress, metabolism, endocrine system, carotenoid-base ornamentation, telomere length, eggshell thickness and hatching success (reviewed in Ankley *et al.* 2021; Dietz *et al.* 2019).

## II. THE AVIAN EGG

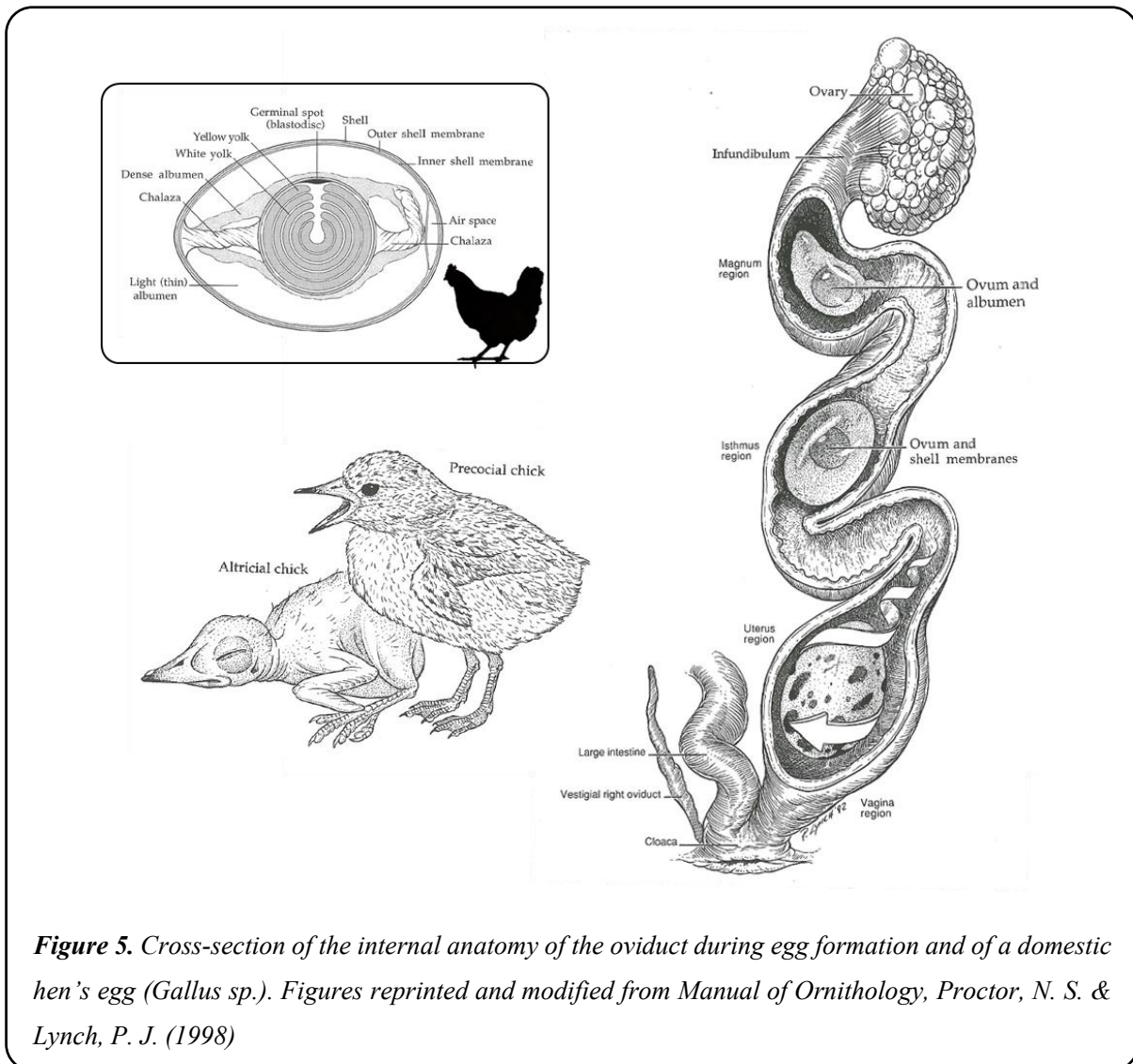
### a. Embryo's development

Once fertilized and laid, the avian egg is a chemical and mechanical powerhouse functioning as an incubation chamber. The egg contains all essential nutrients and substances necessary for the development of the embryo during the incubation period. Other required compounds as water vapor and O<sub>2</sub> or waste as CO<sub>2</sub> may move through pores across the eggshell. The yolk consists mainly of lipids and proteins, as well as vitamins, minerals and carbohydrates acting as a nutrient reservoir for the embryo, and the albumen serves as a protective coat and as a source of water, as well as proteins (Johnston 1983).

In most bird species, only the left side of the reproductive system develops into the functional organs (**Figure 5**). Follicles are gathered in the ovary, the mature oocyte (i.e., egg yolk) will travel to the cloaca through the oviduct, receiving successive coats of albumen along

## General introduction

the way. After the eggshell membrane is secreted, the solid shell is added in the uterus, where pigment glands also add color and pattern to it. The egg is then held in the vagina until egg-laying. The entire process from yolk growth to egg-laying varies in length among bird species. Yolk formation duration has been estimated in several seabird species to last between eight days and one month (Astheimer and Grau 1990).



The embryo is formed from a fertilized blastoderm on the yolk surface. Through the developmental period, the embryo will grow and develop into a fully formed chick using substances from the yolk, the albumen and the shell. Before hatching, the embryo will invaginate the rest of the yolk as a reserve. In the last hours, the pre-hatchling will shift from respiring via the chorioallantois, a vascular fetal membrane, to breathing with its lungs by piercing through the egg air cell. Using a specialized structure on its beak called the “egg-

tooth”, it will then break through the egg shell and hatch (Sturkie's Avian Physiology 2021). Most seabirds are semi-precocial, in other words, chicks are rapidly able to thermoregulate but are nutritionally dependent on their parents for an extended period. This has the consequence of generating a relatively long period of embryonic growth.

### **b. PFAS in seabird eggs**

During egg synthesis, in addition to essential substances for the development of the embryos, female seabirds passively depurate to their eggs a significant part of many of the contaminants they absorb. Therefore, as for adults, seabird eggs have been proposed as excellent samplers of the local environments for contaminants, including PFAS (Bianchini *et al.* 2022; Morganti *et al.* 2021). Sampling eggs can thus provide the same benefit as sampling adult tissues while being a cost-effective, easier and less invasive way of sampling the environment for bioaccumulating contaminants. Bird eggs furthermore tend to represent contaminant uptakes by the female a few days prior to laying, therefore, close to the breeding site (Mallory and Braune 2012). For all previous reasons, seabird eggs are often used to examine spatial and temporal trends of environmental contamination.

A wide range of contaminants have been found in seabird eggs, for instance, legacy POPs including polychlorinated biphenyls (PCBs) and chlorinated pesticides, mercury and recently plastic contaminants (Bianchini *et al.* 2022). The implicit mechanisms behind maternal contaminant deposition in eggs are still largely unclear, but may be tightly linked to contaminants physico-chemical properties (Verreault *et al.* 2006). This produces variation in maternal transfer efficiency among chemicals. For instance, several major contaminant classes in egg yolk (e.g., PCBs, DDT, polybrominated diphenyl ethers (PBDEs)) were found to correlate with plasma concentrations in females glaucous gulls, but concentrations of  $\alpha$ -hexabromocyclododecane ( $\alpha$ -HBCD) did not (Verboven *et al.* 2009). Correspondingly, the maternal transfer of most heavy metals is low due to the ovary acting as a barrier for these compounds (Klein *et al.* 2012). On top of female contamination and transfer mechanisms, egg characteristics (e.g., egg size, egg mass, rank of the egg in the laying order, number of eggs in clutch) are known to be relevant factors influencing egg concentrations of organic contaminants (Verreault *et al.* 2006).

If the maternal transfer of highly lipophilic legacy POPs has been investigated in seabird eggs, it has been much less studied for PFAS, which are more prone to bind to proteins.



Moreover, legacy PFAS have been widely investigated in seabird eggs (Bertolero *et al.* 2015; Braune and Letcher 2013; Colomer-Vidal *et al.* 2022; Faxneld *et al.* 2016; Gebbink *et al.* 2009; 2011; Gebbink and Letcher 2012; Huber *et al.* 2015; Jang *et al.* 2022; Lucia *et al.* 2015; Miljeteig *et al.* 2009; 2012; Miller *et al.* 2015; Pereira *et al.* 2021; Su *et al.* 2017; van der Schyff *et al.* 2020; Verreault *et al.* 2005; 2007; Vicente *et al.* 2012; 2015; Wilkinson *et al.* 2022), but very few studies examined PFAS of emerging concern. Recently though, some emerging PFAS were detected in a large proportion of black-tailed gull (*Larus crassirostris*) and bald eagle (*Haliaeetus leucocephalus*) eggs in South-Korea and the US, respectively (Wang *et al.* 2021b; Wu *et al.* 2020). Although emerging PFAS are increasingly detected in the Arctic environment and biota, no information about emerging PFAS in Arctic seabirds is available to date

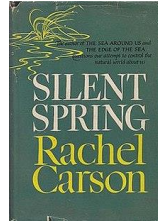
### **c. Contaminants in seabird eggs: consequences**

Early-life development is a very sensitive period for birds and any disruption could lead to life-long consequences for the exposed individual. A wealth of correlative and experimental studies investigated the consequences of a developmental exposure to diverse contaminants in wild birds, and numerous effects were found on a large range of endpoints at both short and long terms. In an effort to evaluate their toxicity for wildlife, PFAS received an increasing interest in recent years and some experimental studies investigated how they affect bird development. It was suggested that embryos exposed to PFAS during their development may have altered the endocrine system, lipid metabolism, heart rate, organs development, gene expression and embryos' pipping success and survival (Briels *et al.* 2018; Cassone *et al.* 2012a; 2012b; Geng *et al.* 2019; Jacobsen *et al.* 2018; Mattsson *et al.* 2015; 2019; Molina *et al.* 2006; O'Brien *et al.* 2009; Parolini *et al.* 2016; Strömquist *et al.* 2012). However, the vast majority of these studies were conducted in the laboratory on chicken (*Gallus gallus domesticus*) only, and injected PFAS concentrations were often much higher than environmental levels found in biota, making it difficult to draw conclusions and create toxicity thresholds for eggs of wild bird populations. Besides, most studies have focused on PFOS and PFOA, but other PFAS including other PFCAs received less attention. Nonetheless, this is urgently needed since many top predators are known to bear high concentrations of long-chain PFCAs (Chen *et al.* 2021). Moreover, the consequences of embryo exposure to emerging PFAS have been overlooked and deserve more attention given the increasing body of evidence showing high concentrations of some of these compounds in Arctic biota in recent years.

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Female contamination may also indirectly affect their offsprings, and lead to an additive effect of both the female's and egg's contamination on the growing organism. One of the most infamous examples is the one of Dichlorodiphenyltrichloroethane (DDT) in birds of prey. This organic insecticide was massively used in the mid-20<sup>th</sup> century as a means of eradicating insect-borne diseases Malaria and Typhus. In a world convinced of their merits, one of the first voices that raised against synthetic pesticides was that of Rachel Carson (see **boxed text**) in her book *Silent Spring* (Carson 1962). It was later discovered that DDT and its breakdown products caused eggshell thinning leading to massive population declines in numerous North American and European birds of prey, since the eggshells were unable to support the weight of the incubating bird, and eggs were breaking in the nest (Hickey and Anderson 1968; Peakall 1993). To a lesser extent, PFAS were also associated with thinner eggshells in a wild population of great tits (*Parus major*; Groffen *et al.* 2019), but not in ivory gulls (Miljeteig *et al.* 2012). Numerous contaminants including PFAS are considered or suspected to be endocrine disrupting chemicals (EDCs; Metcalfe *et al.* 2022). Because of their chemical structure, EDCs may interfere with hormones synthesis, metabolism and actions. It has been extensively studied how maternally transferred EDCs may directly affect the endocrine system in both adult birds and embryos (Marlatt *et al.* 2021). A disruption of maternal hormone transfer (see **boxed text** on maternal effects) by EDCs, called “transgenerational endocrine disruption”, has been suggested in birds and may have severe consequences on the adaptation of the future offspring to its environment. Nonetheless, it has received little investigation so far and the outcomes are still unclear (Cortinovis *et al.* 2008; French *et al.* 2001; Ruuskanen *et al.* 2019; Verboven *et al.* 2008). Moreover, although PFAS are well identified EDCs, transgenerational endocrine disruption have not been examined to date.

### Rachel Carson's *Silent Spring*



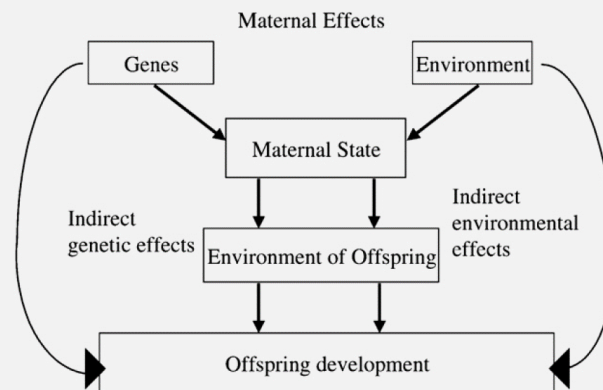
Rachel Carson (1907 – 1964) was an American biologist. Her book *Silent Spring* published in 1962 raised an unprecedented awareness of the harm caused by the unregulated use of pesticides, and contributed to initiate the environmental movement in the Western World. The creation of the Environmental Protection Agency (EPA) in 1970 in the US, and the consecutive ban of several POPs, were additional aftermaths of the commotion caused by Carson's book.

## Maternal effects

Hormones are pleiotropic molecules produced by specialized cells of specific organs of the endocrine system after a stimulus and transported via blood circulation to act on specific receptors on target organs. They have a communication function and therefore act as chemical messengers to transmit information or orders in the whole organism. Hormones contribute to numerous processes including reproduction, homeostasis and cellular differentiation, as well as in the regulation of the main chronobiological rhythms.

A maternal deposition of hormones in the egg during its synthesis was long suspected but first discovered in the early 1990s, when steroids were detected in the yolk of freshly laid canary (*Serinus canaria*) and zebra finch (*Poephila guttata*) eggs (Schwabl 1993). Since then, thyroid hormones, catecholamines, melatonin, growth hormones, insulin, glucagon and leptin of maternal origin have also been measured in eggs (von Engelhardt and Groothuis 2011). Hormones levels in females are highly dependent of environmental conditions and their own state. In variable but predictable environments, hormone levels in avian eggs are assumed to be a mechanism by which information experienced in one generation may be transmitted to the next, as a means to enable an optimized phenotype of the offspring according to prevailing environmental conditions. Maternal hormones may thus act as adaptive maternal effects, although this is still debated (Williams and Groothuis 2015).

Maternal effects represent the fact that an organism's phenotype is not only determined by its genotype and the environment it experiences, but also by its mother's genotype and environment (**Figure 6**). It has been suggested that maternal effects are essential for the evolution of adaptive responses to environmental heterogeneity in the context of natural selection, by generating phenotypic plasticity for the offspring (Moore *et al.* 2019). An adaptive maternal effect should therefore benefit the offspring and thus the mother, and help maximize their fitness (Mousseau and Fox 1998). But maternal effects may also strategically result in females favoring themselves at the expense of their offspring (Marshall and Uller 2007), especially in long-lived species in which adult survival is often preferred to offspring in case of poor circumstances.

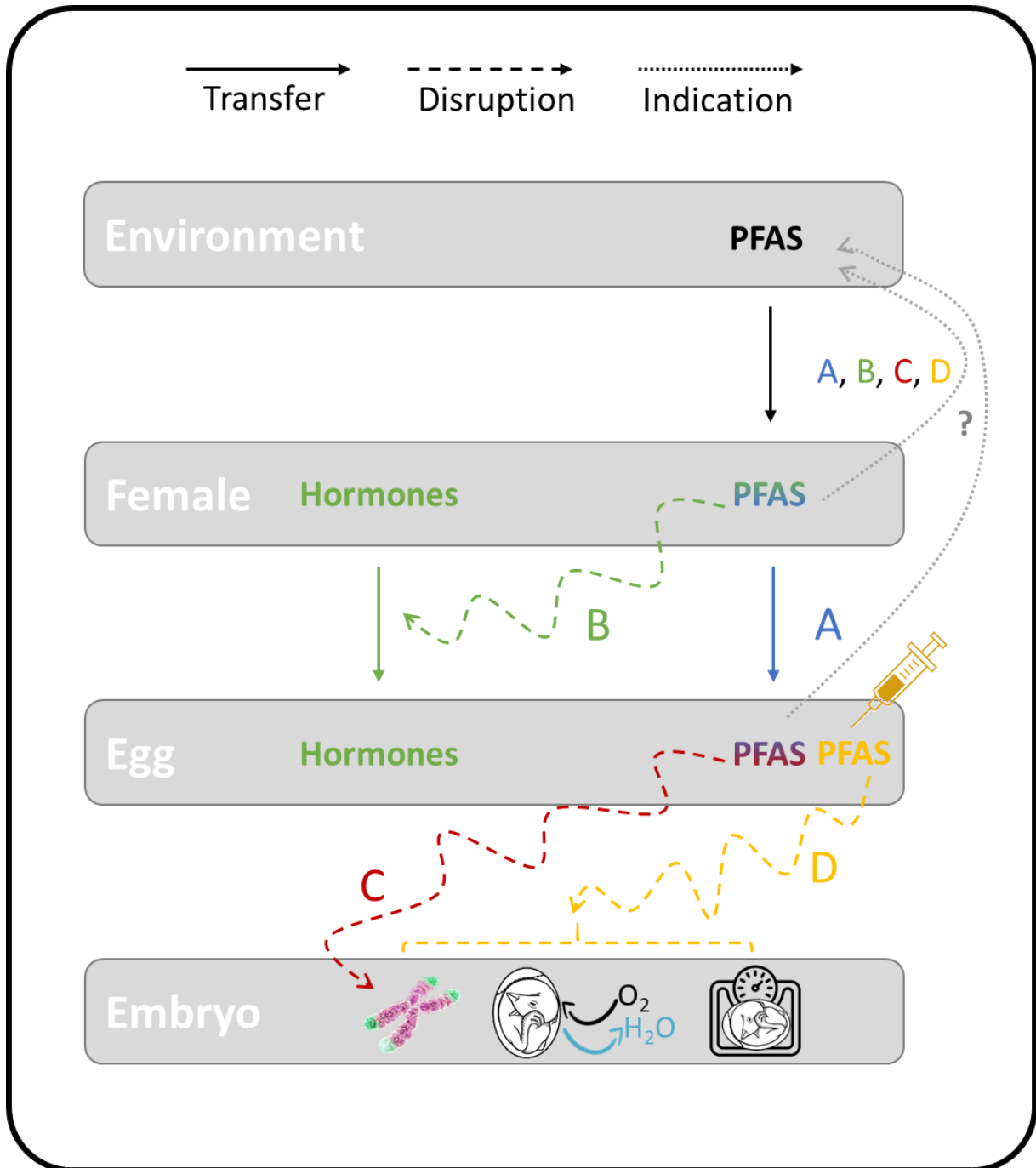


**Figure 6.** Representation of maternal effects: genetic and environmental maternal effects interaction with offspring development. Reprinted from Groothuis *et al.* 2005.

### III. THESIS OBJECTIVES

As aforementioned, PFAS emissions are not regulated in their vast majority, and emerging compounds were increasingly manufactured in recent years. This has resulted in a critical need to assess the occurrence of legacy and emerging compounds in wildlife, in order to better understand their distribution and fate in biota. Arctic seabirds have been found to bear PFAS in large concentrations, which may affect their fitness and survival. Even so it is yet unexplored if PFAS can impact the fate of embryos during development, despite the fact that they are known to be transferred to seabird eggs. Yet, this is urgently needed since disruptions experienced during the developmental phase may have deleterious repercussions for an organism throughout its entire life. This is even more required for emerging PFAS, found in an increasing number of individuals in the Arctic, but of unknown toxicity. In that context, this PhD thesis addresses the potential disruption of maternal effects by environmental contaminants and specifically focuses on legacy and emerging PFAS occurrence in seabird eggs and their consequences for the development of the embryos (**Figure 7**). This thesis mainly focuses on an Arctic seabird, the black-legged kittiwake (**Papers A, B and C**), but I also experimentally explore the consequences of *in ovo* exposure to PFAS in a temperate seabird, the yellow-legged gull (*Larus michahellis*; **Paper D**). The main objectives of the thesis are:

- 1) To describe the concentrations of legacy and emerging PFAS in prelaying females of an Arctic breeding seabird and their eggs, to identify the main compounds absorbed locally and investigate some of the mechanisms regulating the maternal transfer of PFAS.
- 2) To explore the potential for maternal PFAS to affect hormone-driven maternal effects, by investigating the relationship between PFAS in prelaying females and steroids, glucocorticoids and thyroid hormones in their freshly laid eggs.
- 3) Since PFAS may affect aging via reduced telomere length, and since telomere length at birth may have strong consequences at adulthood, I investigated if PFAS deposited in eggs may impair aging during embryonic development via telomere length, using a unique study design.
- 4) Using an experimental design, I tested the consequences of a developmental exposure to an emerging PFAS increasingly found in Arctic biota, in embryos of a seabird species, using a combination of endpoints known to be affected by PFAS in adults.



*Figure 7. Schematic representation of the global structure of this thesis.*



# MATERIALS

# &

# METHODS



*Arctic fox (Vulpes lagopus) with a GPS collar*



## I. FIELDWORK

### a. Study Models and study sites

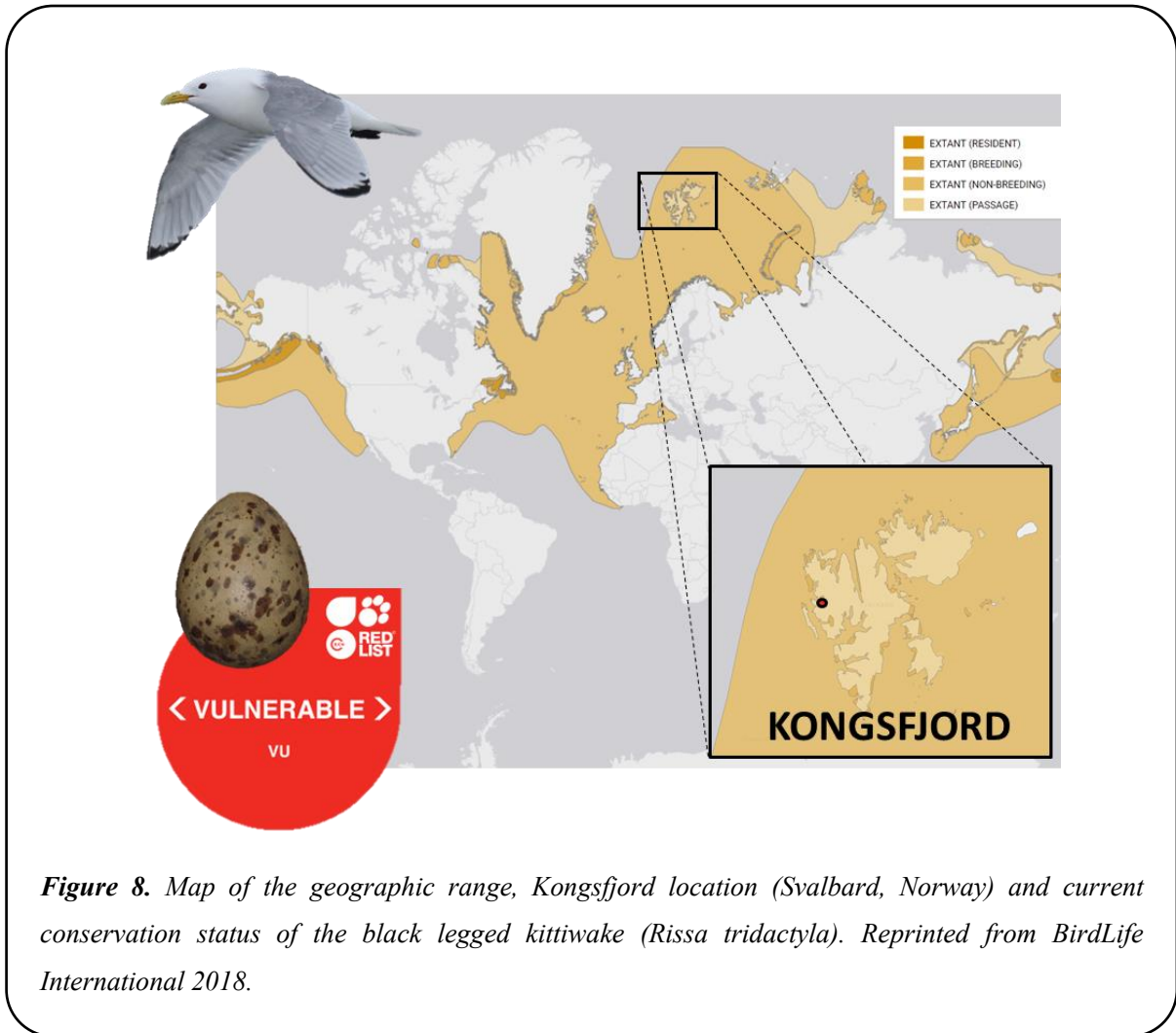
To fulfill my PhD thesis objectives (described in the **Introduction section III.**), I mainly focused on two seabird species:

- The black-legged kittiwake breeding in Arctic Norway for the first three objectives. The samples were obtained during two two-months long fieldwork seasons conducted in Svalbard, Norway in 2019 and 2020.
- The yellow-legged gull, breeding in a temperate region of Southern France, for the fourth issue. All samples were collected in two fieldwork sessions in 2021.



#### **The black-legged kittiwake**

The black-legged kittiwake (hereafter “kittiwake”) is the most abundant gull species in the world with a global breeding population estimated to be about 4 000 000 pairs (Conservation of Arctic Flora and Fauna (CAFF) 2020). This small, long-lived, migratory seabird has a circumpolar distribution in the Northern hemisphere, breeding in dense colonies on steep cliffs. Their breeding range goes from boreal areas to the High Arctic, as far as the 80th parallel north (**Figure 8**). Subject to anthropogenic threats (Doyle *et al.* 2020), the global population has decreased by 40 % since 1975 (BirdLife International 2019; Descamps *et al.* 2017), leading its conservation status to be revised to “vulnerable” in 2017 on the global IUCN Red List of Threatened Species (BirdLife International 2018). The diet of these surface-feeders changes throughout the year: strictly pelagic during the non-breeding season and spending most of their time foraging offshore, while foraging on both pelagic and coastal environments during the breeding period, from the immediate vicinities of the colony to hundreds of kilometers away (Goutte *et al.* 2014). Kittiwakes feed mainly on small aquatic invertebrates and fish during summer (Barrett 1996; Stempniewicz *et al.* 2021), but their winter prey are difficult to identify due to their offshore remoteness. Birds from the northernmost colonies have an extensive use of tidal-glacier fronts to feed during the breeding season (Bertrand *et al.* 2021). As many gull species, kittiwakes lay each year between one and three eggs, but most commonly two, that parents incubate for approximately 27 days (Coulson 2011).



Most of my work was conducted on kittiwakes breeding in a colony situated in Svalbard, a High-Arctic Norwegian archipelago located between mainland Norway and the North Pole. The colony called “Krykkjefjellet” (78°53'48"N 12°11'43"E) is settled near the scientific village of Ny-Ålesund in Kongsfjord (**Figure 8**), a large fjord on the western side of Svalbard. This colony is among the northernmost kittiwake colonies and is subject to a long-term monitoring through the IPEV program 330. Approximately 175 nests are monitored yearly. Individuals from this colony leave for the fall migration in October, travelling through considerable distance to the North-West Atlantic where they stay during the winter. The spring migration then leads kittiwakes to arrive at the colony by mid-April (Léandri-Breton *et al.* 2021 [**Appendix C**]). Kittiwakes are philopatric and usually come back to the same nest with the same partner. After building their nest and mating during the prelaying period, eggs will be laid around early-June. Hatching then occurs in June-July, and chicks are reared for approximately 40 days before fledging.

## Materials & methods

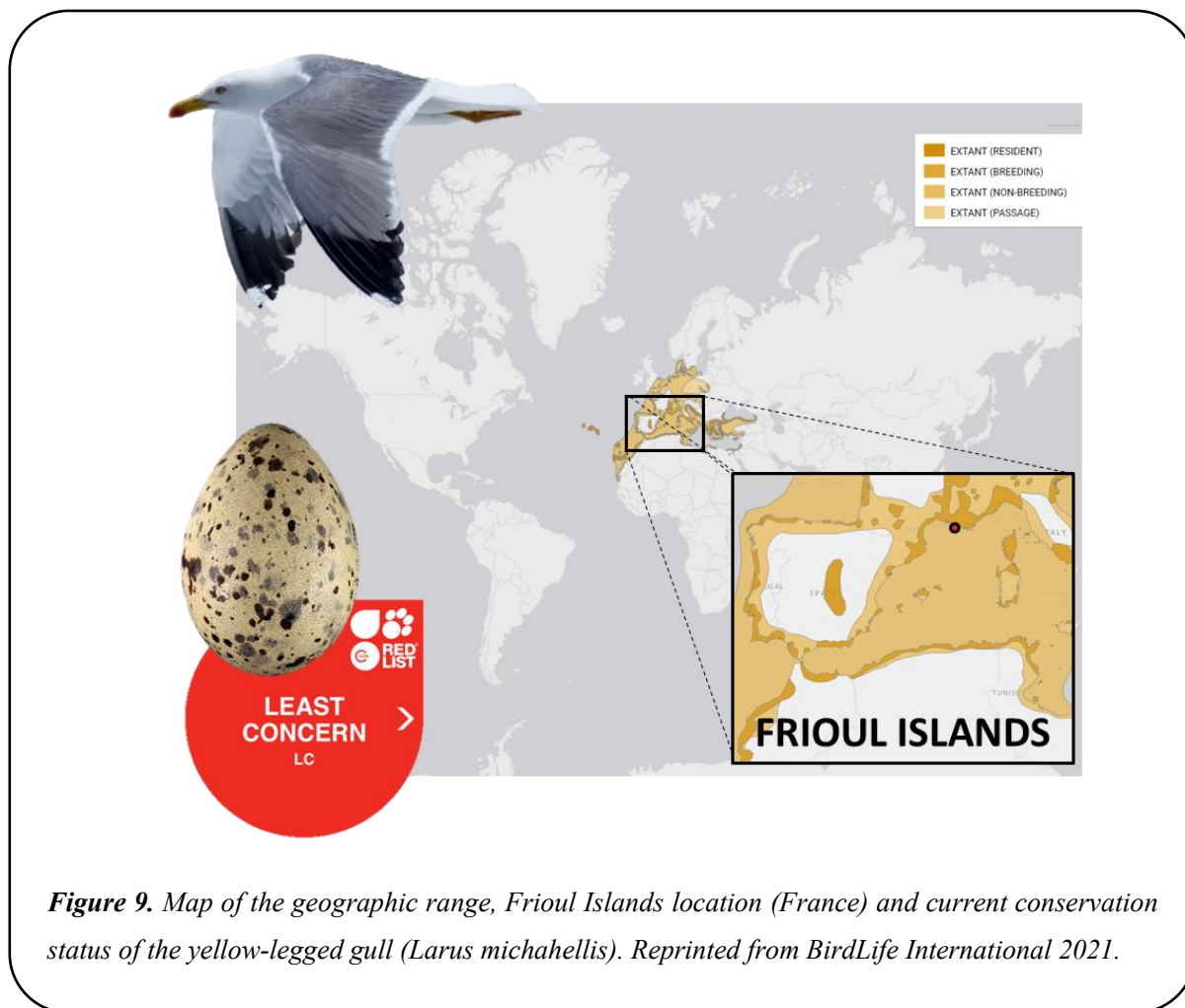
Previous studies showed that adults from Kongsfjord colony exhibit relatively high blood plasma concentrations of legacy PFAS, leading to adverse consequences on telomere length, oxidative stress, endocrinology, incubation and fitness (Ask *et al.* 2021; Blévin *et al.* 2017b; 2018; 2020; Costantini *et al.* 2019; Tartu *et al.* 2014). On this basis, the kittiwake is therefore an appropriate study model to investigate the maternal transfer of legacy and emerging PFAS in their eggs and their impacts on the offspring development.



### **The yellow-legged gull**

For conservation status issue, experimental investigation of the consequences of *in ovo* exposure to PFAS could not be conducted on Svalbard kittiwake, we chose to conduct this experiment on yellow-legged gull eggs which were collected as part of gull sterilization campaign.

The yellow-legged gull is a large gull that can be found in Europe, North Africa and the Middle East, with a breeding range centered on the Mediterranean Sea (BirdLife International 2021). Through its generalist diet, yellow-legged gull populations benefited from its cohabitation with humans during the 20<sup>th</sup> century (Duhem *et al.* 2008). The European population is now estimated around 400 000 - 500 000 pairs and appears to be increasing, justifying the “Least Concern” conservation status assign to the species (BirdLife International 2021). Most birds of this species are sedentary, although some dispersive individuals may migrate to the coast of Western Africa during winter. The yellow-legged gull is a central-place forager during the breeding period as are most seabirds. It forages on both terrestrial and marine environments and has an extremely diverse diet that includes invertebrates, fish, small mammals, birds and bird eggs among others (BirdLife International 2021; Moreno *et al.* 2009). Nevertheless, it also largely relies on anthropogenic wastes and resources such as open landfills, crop residuals, fishery discards or livestock food and carcasses, making the yellow-legged gull particularly bound to human activities (Parra-Torres *et al.* 2020; Ramirez *et al.* 2020). Mature individuals usually lay three eggs around the end of March or April on a nest often built directly on the ground, although they may nest on building roofs as well. The incubation lasts 27 to 31 days and the offspring fledge after approximately 40 days.



**Figure 9.** Map of the geographic range, Frioul Islands location (France) and current conservation status of the yellow-legged gull (*Larus michahellis*). Reprinted from BirdLife International 2021.

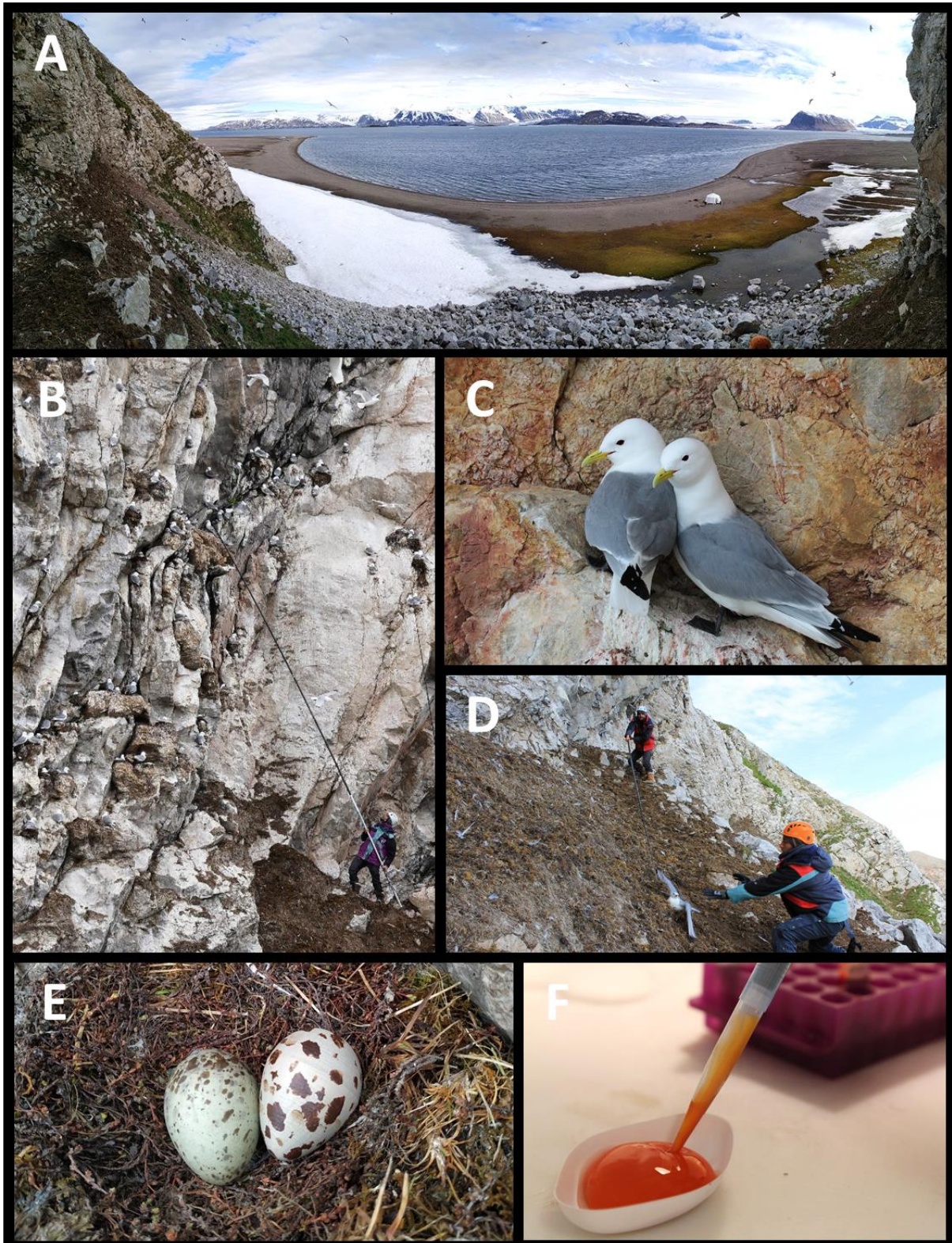
In this thesis, I used yellow-legged gull eggs from a colony situated on the Frioul Islands (**Figure 9**), in front of the Southern France city of Marseille, to estimate local seabirds' exposure. Although the Mediterranean Sea has a low PFAS concentration on average compared to other seas worldwide (Muir and Miaz 2021), some contamination hotspot led to extremely high concentrations in seabirds locally (Morganti *et al.* 2021). Concentrations of PFOS exceeding the European Environmental Quality Standard were found in seawater from the bay of Marseille, as well as large concentrations of PFCAs (Schmidt *et al.* 2019), most likely due to large inputs from the Rhône River (Munoz *et al.* 2015) and from surface currents (Brumovsky *et al.* 2016). However, there is no evidence about the occurrence of PFAS in local seabird species. A few studies investigated legacy PFAS concentrations in yellow-legged gulls from Spain and Italy and their eggs (Bertolero *et al.* 2015; Parolini *et al.* 2020; Vicente *et al.* 2012), but levels of emerging PFAS are largely unexplored in the Mediterranean region. Consequently, I used yellow-legged gull eggs as bioindicators of local environment contamination, to examine the exposure of local seabirds to legacy and emerging PFAS. I also used yellow-legged gull

eggs from the same colony in an experimental study investigating the impact on the embryonic development of an emerging PFAS increasingly found in biota worldwide.

### **b. Adults handling and sampling**

In this thesis framework, I aimed at sampling kittiwake females before they start egg formation (i.e., during the prelaying period). They were sampled  $17.9 \pm 8$  days (mean  $\pm$  standard deviation) before laying, which roughly corresponds to the moment kittiwakes start growing the egg yolk (Astheimer and Grau 1990). This enabled me to obtain PFAS steady state representing local contamination before depurating in eggs, as well as the concentration of females' circulating hormones at the time of maternal transfer in the yolk. Prelaying adults were captured on their nest using a nylon loop at the end of a fishing rod and deposited on the ground, where a second person caught the bird by hand (**Figure 10**). Immediately (i.e., less than 3 min after the bird was removed from the nest), we proceeded to a first blood sample (1 mL) in the alar vein using a heparinized 1 mL syringe and a 25G needle. After this initial blood sample, we took a subsequent blood sample (2 mL) in the alar vein using a 2 mL syringe and a 25G needle. Birds were then individually identified using a metal band (supplied by the Stavanger Museum) and a plastic ring with a three-digit code to enable identification while keeping distance. We then measured the tarsus, wing and skull (head and bill) length to the nearest 0.1 mm using a sliding caliper and weighted the birds to the nearest 5g using a spring balance dynamometer. The birds were finally released after checking if bleeding stopped from the sampled veins and marking their head using a non-permanent marker to avoid recapture by mistake.

At the end of each day in the field, I centrifuged both blood samples, to separate plasma from red blood cells (RBCs) in individual microtubes, and kept them frozen ( $-20$  °C) until assays. Later, I used plasma from the first blood sample to determine baseline hormones concentration, especially for corticosterone that increases with stress short after the capture (Romero and Romero 2002). Plasma from the second blood sample was used for PFAS measurements and RBCs to identify the sex of the bird. Additional information on fieldwork manipulation of adult kittiwake including sample-size can be found in **papers A and B**.



**Figure 10.** A/ Panoramic view on Kongsfjord from Krykkjefjellet colony, B/ a capture of an adult kittiwake using a fishing rod, C/ a kittiwake pair on its nest, D/ an adult kittiwake is being captured, E/ a real kittiwake egg (left) and a dummy-egg (right) in a nest, F/ an egg yolk being sampled in the laboratory.

**c. Kittiwake eggs handling and incubation**

During fieldwork, I daily monitored nests of previously sampled birds using a mirror at the end of a long pole to determine reproductive outputs and to ensure collecting eggs as fresh as possible (i.e., less than 24h after laying), in order to get PFAS and maternal hormone concentrations before the embryo may uptake them. In these nests, I collected the first-laid egg and immediately replaced it with a plastic dummy-egg in order to avoid potential nest desertion (**Figure 11**). When the second egg was laid in these nests, I collected it together with the dummy one. All collected eggs were immediately labeled, stored in a protective case and brought back to the laboratory in Ny-Ålesund. The protocols were different in 2019 and 2020:

- During the 2019 fieldwork season, at the end of each sampling day, I opened both first- and second-laid eggs to separate yolk and albumen. In brief, after cutting the top of the egg with scissors, I separated yolk from albumen using a separator slotted spoon to avoid breaking the yolk and mixing it with albumen. I then sucked up the yolk using a 1 000 µL pipette by stabbing through the yolk membrane with a bevel-cut tip. I then stored albumen and yolk, frozen (-20 °C) in individual microtubes until assays. I cleaned all tools before and after eggs opening and sampling using clear water and 70 % ethanol. Yolk were later used for analyses as almost all PFAS and hormones are transferred to the yolk and very little is found in albumen (Gebbinck and Letcher 2012; Schwabl 1993).
- In 2020, only second-laid eggs were opened, first-laid eggs were randomly deposited in an incubator (Ovation 28 Ex, Brinsea, Titusville, USA) until incubation day 20 when embryo were euthanized and kept frozen (-20 °C). Embryos' liver were then sampled before analysis for telomere assay.

Additional information about fieldwork and laboratory operations for kittiwake eggs and embryos can be found in **paper A, B and C**.



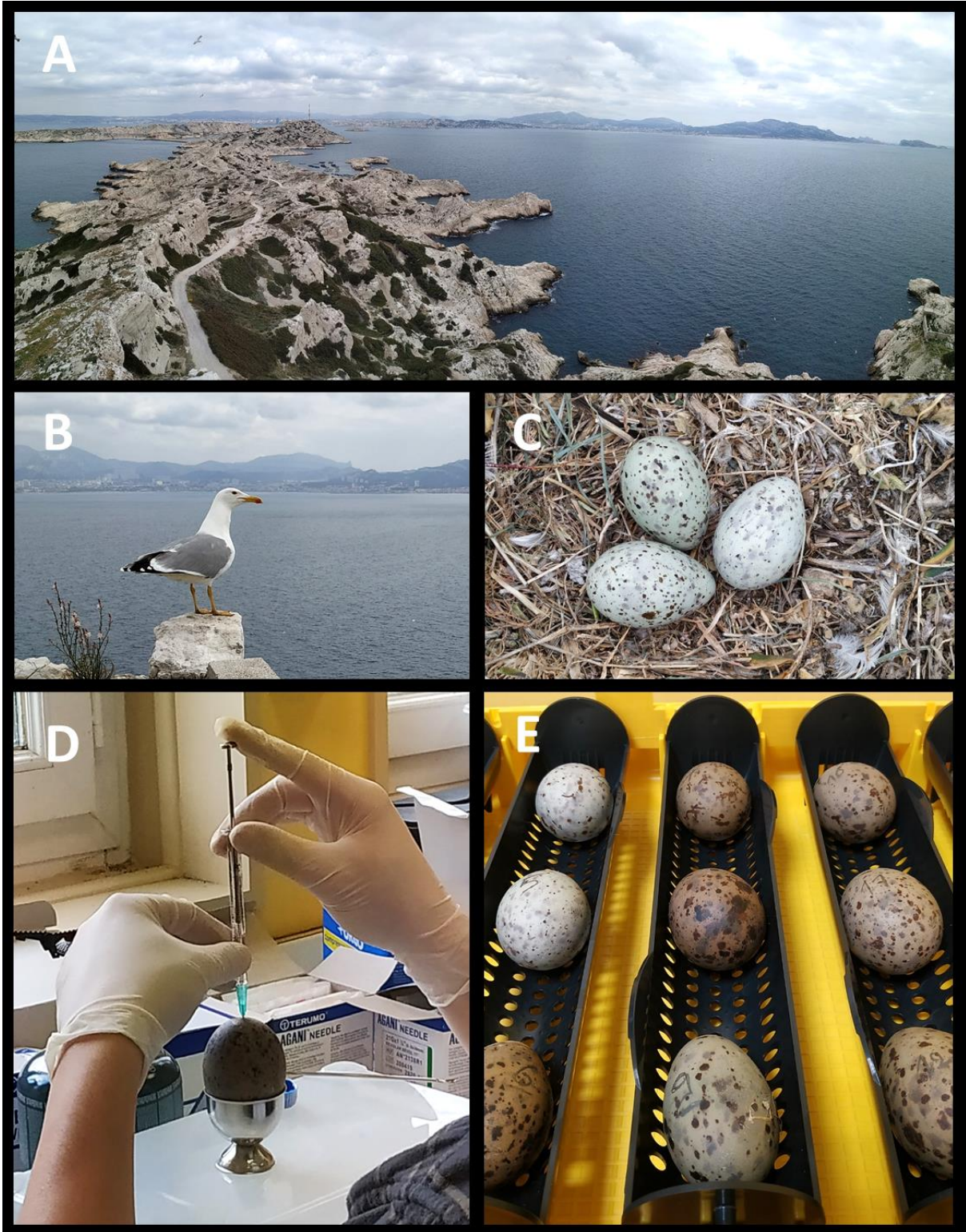
**Figure 11.** D-J. Léandri-Breton climbing on a ladder to collect an egg in the black-legged kittiwake colony from Kongsfjord, Svalbard

#### **d. Yellow-legged gull eggs handling, PFAS injection and incubation**

I selected yellow-legged gull eggs for the experimental study based on egg buoyancy in saltwater to select and sample only freshly laid eggs (i.e., those that lied at the bottom of the container with no buoyancy; Van Paassen *et al.* 1984). I removed a single egg per nest to obtain independent data and to reduce the impact on the adults' breeding success (**Figure 12**). I took them back in protective cases to the Centre D'Etudes Biologiques de Chizé (CEBC, Villiers-en-Bois, France). After being randomly allocated to the control or treatment group, I injected either a control solution containing dimethyl sulfoxide (DMSO) only, or a treatment solution containing DMSO and a known concentration of an emerging PFAS increasingly found in Arctic biota but of unknown toxicity, 7:3 fluorotelomer carboxylic acid (7:3 FTCA). I then deposited the eggs in a random order in the incubators. On incubation day 23, I measured O<sub>2</sub> consumption and Total Evaporative Water Loss (TEWL) of the embryos in a metabolic chamber. A few days before expected hatching, on incubation day 24, eggs were opened and embryos euthanized. By proceeding as such, I ensured that the exposure to PFAS lasted for almost the entire incubation period. Immediately, I sampled the liver, prone to accumulate PFAS in seabirds (Verreault *et al.* 2005), and kept it frozen (-20 °C) until telomere analyses.

Yolks from yellow-legged gull egg of the same colony and year for PFAS measurements were kindly provided by Dr. Thierry Boulinier (Centre d'Ecologie Fonctionnelle et Evolutive; CEFE, Montpellier, France). Additional information on injected solutions manufacture, yellow-legged gull egg injection and incubation and embryo treatment can be found in **paper D**.





**Figure 12.** *A/ Panoramic view on the Frioul Islands in the Bay of Marseille, B/ an adult of yellow-legged gull, C/ a full-size clutch of yellow-legged gull, D/ injection in eggs of the solutions for the experimental study, E/ yellow-legged gull eggs in an artificial incubator.*

### e. Animal well-being

Maximizing animal well-being during fieldwork manipulation is essential both ethically and to ensure the scientific validity of the data obtained. Bird stress due to repeated handling have been associated to short-term effects on physiology, but also long-term energy expenditure, survival, reproduction and behavioral consequences (Watson *et al.* 2016). Other researches were planned on adult kittiwakes during my stay in Kongsfjord. Therefore, to limit the recapture event as well as the number of birds handled, we aimed at optimizing the protocols to use as few birds as possible. This strategy also helped in collecting numerous information on single individuals, which may increase the scientific quality of the studies. We also aimed at limiting to less than 15 minutes the duration between the bird capture and release, to reduce our impact on bird stress (Huber *et al.* 2021). As recommended by the *Guidelines to the use of wild birds in research* (Fair *et al.* 2010), we limited blood samples to 3 mL in total, which represents less than 1 % of the total bird weight (adult kittiwake ~400 g), although health hazard may still be caused by <1 % blood samples. I completed two courses during this PhD project to be trained with the latest ethical recommendations and regulations in Norway and in France. All experiments in this present PhD thesis were conducted in accordance to European, French and Norwegian laws for ethic and handling of wild birds.

## II. LABORATORY ANALYSES

All analyses described below have been conducted in internationally renowned laboratory following tried and tested protocols including quality controls.

### a. Per- and polyfluoroalkyl substances assays

PFAS accumulate preferentially in plasma and liver in seabirds (Verreault *et al.* 2005). PFCAs in plasma reflect recent uptakes, but PFSAs, including PFOS, have a much longer half-life in this tissue and may therefore represent a larger accumulation period (Tarazona *et al.* 2015; Yeung *et al.* 2009; Yoo *et al.* 2009), most likely due to a lower excretion rate (Zhang *et al.* 2013). Plasma is thus a relevant tissue to estimates local exposure to PFCAs and may additionally represent input acquired during the migration or even on the wintering grounds for some PFSAs. In some seabird species, it has been estimated that the egg yolk is largely built from exogenous nutrients representing inputs from the breeding grounds (Bond and Diamond 2010). Moreover, although undertaking long-distance migration, the kittiwake is believe to be

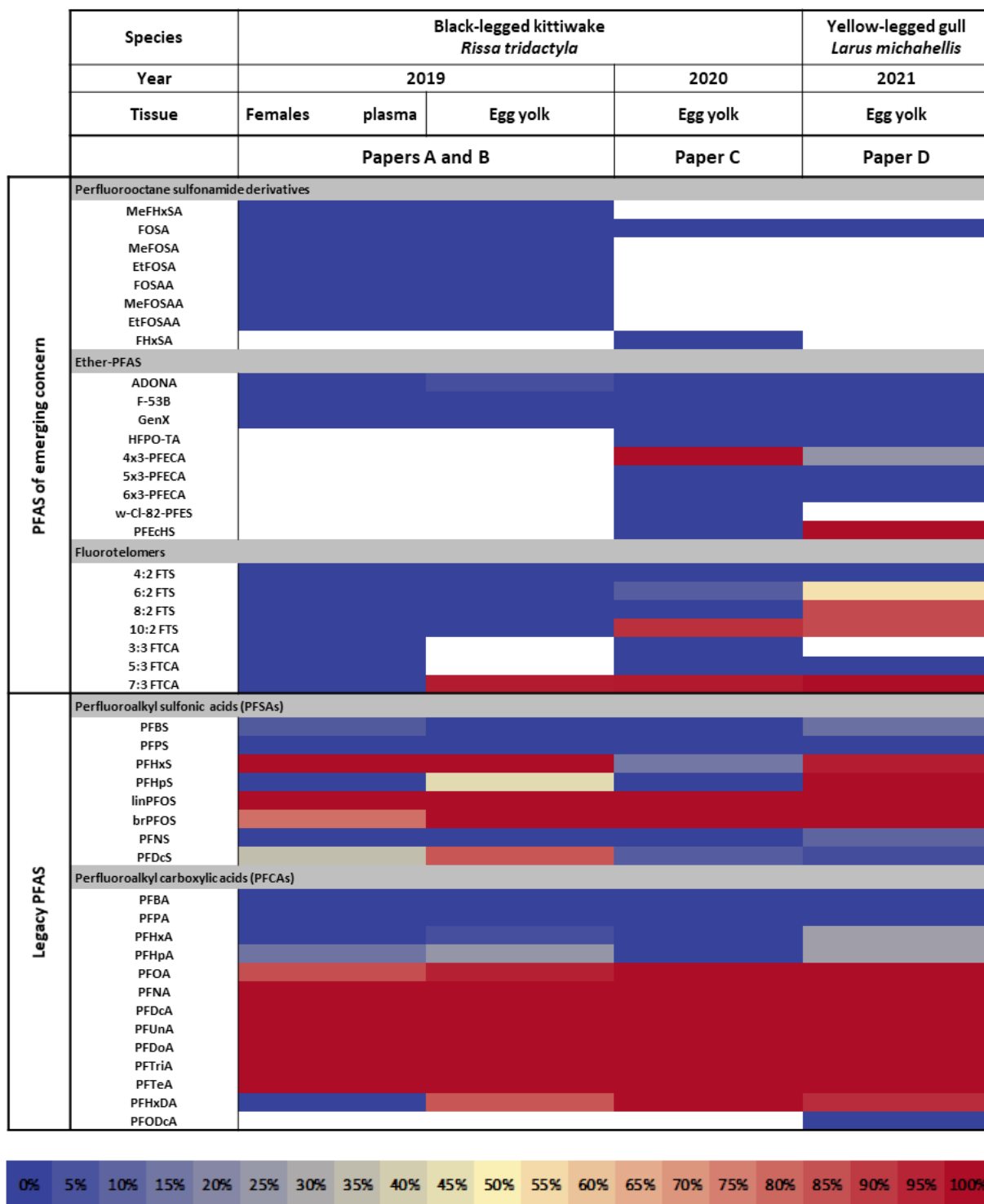
## Materials & methods

an income breeder, using mainly exogenous resources to build their eggs (Guzzo *et al.* 2014). Kittiwake egg yolk should therefore primarily represent local intakes and are a relevant medium to be used as a bioindicator of PFAS contamination in Svalbard.

PFAS were measured in blood plasma of adult kittiwake and yolk of kittiwake and yellow-legged gull eggs at the Norwegian Institute for Air Research (NILU, Tromsø, Norway). First, an extraction in methanol was realized as a purification procedure to remove matrix components and optimize PFAS quantification. Briefly, a sample (plasma: 0.20 mL, yolk: 0.2 g) was spiked with internal standards (20 µL) and mixed with 0.8 mL of methanol then vortexed. After three sessions of sonication (10 min) and vortexing, I centrifuged 5 minutes for sedimentation. Then, 0.7 mL of the supernatant was vortexed thoroughly with graphitized carbon (25 mg) and acetic acid (50 µL). After one last centrifugation of 10 minutes, I transferred 0.5 mL of the supernatant to a vial and added recovery standards (20 µL). Then, PFAS were quantified by ultrahigh-performance liquid chromatography triple-quadrupole mass spectrometry (UHPLC–MS/MS). A detailed protocol as well as the quality control results per study can be found in **papers A, B, C and D**. The targeted compounds were both legacy PFAS and PFAS of emerging concerns (**Figure 13**).

In my thesis, since there is no clear definition, I called “emerging” PFAS all compounds not considered as “legacy” PFAS. Legacy PFAS are generally constituted by the most common PFASs and PFCAs, most of them being long-chain compounds (**Figure 13**). Therefore, I included in the emerging PFAS group: some alternatives to legacy PFAS (i.e., created to fill the gap of the regulated PFAS) and some precursors of the reluctant long-chain PFCAs and PFASs. Nonetheless, all compounds included in the emerging PFAS group are not necessarily created recently, some of them may even have a well identify toxicity and bioaccumulation potential and could in many respects be considered as “legacy” PFAS.

## Materials & methods



**Figure 13.** Heatmap of the detection percentage among samples (i.e., above the limit of detection) of the different PFAS tested per year, species and tissue. A white cell means that the specific compounds was not measured. NB: one compound may have different limits of detection among years.

### **b. Hormone assays**

Hormones were measured in blood plasma of adult kittiwake and yolk of their eggs at the CEBC, using both Radio-immunoassay (RIA) or Enzyme Linked ImmunoSorbent Assay (ELISA) methods after extracting the samples. We measured total levels of steroids (dihydrotestosterone (DHT), androstenedione (AND), testosterone (T), and corticosterone (CORT)) and thyroid hormones (triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>)) of maternal origin in both plasma and yolk.

Quantification using RIA is based on the competitive binding to a specific antibody for the hormone to measure (cold hormone) and a radiolabelled homologue in known concentration (hot hormone). The antibody being in limited concentration, both cold and hot hormones cannot all bound to form antibody-antigen complexes. The free-fraction and the bound-fraction are then separated. Finally, the concentration of the hormone to measure is precisely evaluated by quantifying the radioactivity from the bound-fraction of the hot hormone. Contrarily to RIA, the ELISA method does not require the use of radioactively labelled materials and is not based on a competitive binding with an antibody. Instead, the hormone of interest is mixed with an antibody linked to an enzyme, and the unbound fraction is then removed. The enzyme substrate is subsequently added, which emit a detectable and quantifiable signal while reacting with the enzyme.

After extractions, CORT and T were assayed using RIA. In brief, 100 µL of extract were incubated overnight at 4 °C with 4000 cpm of the appropriate H<sup>3</sup>-steroid (Perkin Elmer, US) and polyclonal antiserum (Ab). The two anti-corticosterone Ab (one for plasma and one for yolk) were supplied by Merck, Sigma Aldrich, France. The bound fraction was then separated from the free fraction by addition of dextran-coated charcoal and the activity was counted on a Tri-Carb 2810TR scintillation counter (Perkin Elmer, US). AND and DHT were assayed with commercial ELISA kits. After extractions, total T<sub>3</sub> and T<sub>4</sub> were measured with RIA method, 25 µL of yolk extract or plasma were incubated overnight at 4 °C with 10 000 cpm of the appropriate I<sup>125</sup>-steroid (Perkin Elmer, US) and polyclonal rabbit antiserum (Sigma Aldrich, France). The bound fraction was then separated from free fraction by addition of a polyclonal sheep antiserum against rabbit antiserum and the activity was counted on a Gamma Wizard 2470 counter (Perkin Elmer, US). A detailed protocol of the analyses and quality controls can be found in **paper B**.

### **c. Telomere length measurements**

Absolute telomere length was measured in the CEBC on the liver of kittiwake and yellow-legged gull embryos using real-time quantitative polymerase chain reaction (qPCR). In brief, after digesting liver tissues, extracting the DNA and evaluating its quality, we used universal telomere primers (TELO) and selected and amplified the control single-copy recombination activating gene 1 (RAG1) using specific primers. We ran all samples and quality-control samples on a single 96-well plate, using concentrations of 800 and 300 nM for telomere and control gene primers respectively. We added an eight-point standard range in triplicate to ensure a good amplification efficiency of the reactions. All samples were run in several replicates together with blanks. Samples with an averaged cycle threshold SD > 0.2 between replicates were repeated. We then calculated the relative telomere length. A detailed protocol of the analyses can be found in **papers C and D**.

### **d. Embryo respirometry and egg evaporative water loss measurements**

I investigated respiratory exchanges and TEWL at the CEBC at the end of the incubation of yellow-legged gull embryos from the experimental study, in order to estimate embryos O<sub>2</sub> consumption as a proxy of their resting metabolic rate (RMR). A detailed protocol of the analyses can be found in **paper D**.

### **e. Molecular sex determination**

I identified adult and embryos sex to ensure that adult birds were females, and since male and female seabirds may answer to PFAS exposure in different ways (Blévin *et al.* 2017b; Choy *et al.* 2022; Melnes *et al.* 2017; Sebastiano *et al.* 2020a). Molecular sexing, performed at the CEBC, was obtained from RBCs by polymerase chain reaction (PCR) amplification of part of two highly conserved genes present on sexual chromosomes on adults and embryos, following Fridolfsson and Ellegren (1999).



# CHAPTER I

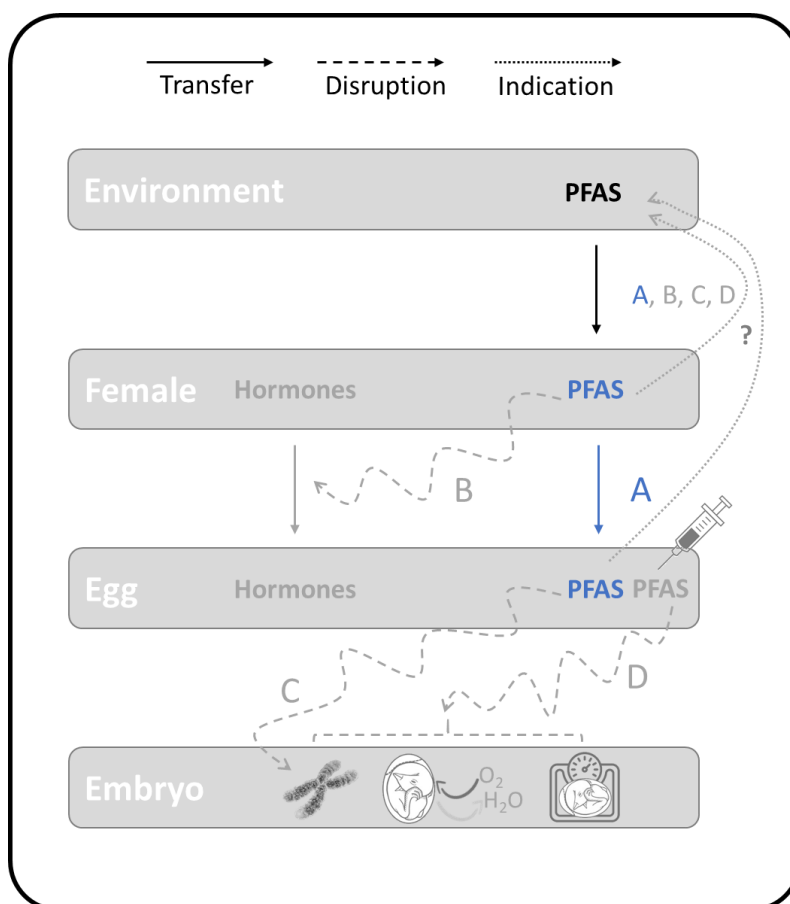


*Black-legged kittiwake (Rissa tridactyla) defending her spot*



## Chapter I

Understanding how PFAS are transferred in eggs is essential in the process of evaluating their impacts during embryonic development. This first chapter aimed at investigating the extent and some of the main drivers of the maternal transfer of PFAS in kittiwake eggs (**Figure 14**).



**Figure 14.** Schematic representation of Chapter I goals included in the global structure of this thesis.

### We found:

- A linear correlation between females and their eggs for most PFAS.
- Higher concentrations of PFAS in first- than in second-laid eggs.
- An increasing efficiency of the maternal transfer with increasing PFAS chain-length.
- Some emerging PFAS in eggs, including ADONA, observed for the first-time in biota, and 7:3 FTCA, increasingly discovered in aquatic organisms in recent years.
- High concentrations of PFAS in eggs, making them particularly relevant bioindicators of local pollution to low concentration chemicals such as emerging PFAS.

# Paper A

A bad start in life? Maternal transfer of legacy and emerging poly- and perfluoroalkyl substances to eggs in an Arctic seabird

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# A bad start in life? Maternal transfer of legacy and emerging poly- and perfluoroalkyl substances to eggs in an Arctic seabird

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## ABSTRACT

In birds, maternal transfer is a major exposure route for several contaminants, including poly- and perfluoroalkyl substances (PFAS). Little is known, however, about the extent of the transfer of the different PFAS compounds to the eggs, especially for alternative fluorinated compounds. In the present study we measured legacy and emerging PFAS, including GenX, ADONA and F-53B, in the plasma of pre-laying black-legged kittiwake females breeding in Svalbard and the yolk of their eggs. We aimed to 1/ describe the contaminant levels and patterns in both females and eggs, and 2/ investigate the maternal transfer, i.e. biological variables and the relationship between the females and their eggs for each compound. Contamination of both females and eggs were dominated by linPFOS then PFUnA or PFTriA. We notably found 7:3 fluorotelomer carboxylic acid - a precursor of long-chain carboxylates - in 84% of the egg yolks, and provide the first documented finding of ADONA in wildlife. Emerging compounds were all below the detection limit in female plasma. There was a linear association between females and eggs for most of the PFAS. Analyses of maternal transfer ratios in females and eggs suggest that the transfer is increasing with PFAS carbon chain length, therefore the longest chain perfluoroalkyl carboxylic acids (PFCAs) were preferentially transferred to the eggs. The mean  $\sum_{\text{PFAS}}$  in the second-laid eggs was 73% of that in the first-laid eggs. Additional effort on assessing the outcome of maternal transfers on avian development physiology is essential, especially for PFCAs and emerging fluorinated compounds which are under-represented in experimental studies.

**Key-word:** black-legged kittiwake, *Rissa tridactyla*, top predator, Svalbard, PFAS, emerging contaminants

## INTRODUCTION

Poly- and perfluoroalkyl substances (PFAS) are synthetic chemicals widespread globally.<sup>1</sup> Since the 1950s, thousands of compounds were developed in this family and used in a multitude of manufactured products (firefighting foams, waterproof clothing, non-stick cookware, coatings, food packaging, personal care products, dental floss, electronics, metal plating and even pesticides) owing to their extremely stable chemical structure and their surfactant properties. After they have been revealed as ubiquitous and toxic for organisms in the early 2000s, some of the historical most noxious compounds such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were phased out by the main manufacturers and recommended for elimination by the Stockholm Convention on Persistent Organic Pollutants.<sup>2-5</sup>

Some of the PFAS bioaccumulate in organisms and biomagnify along trophic chains, particularly in aquatic ecosystems.<sup>6, 7</sup> As a consequence, high trophic level aquatic organisms including seabirds often have elevated tissue concentrations.<sup>1, 8</sup> Oceanic and atmospheric transport can contribute to the long-range dispersal of PFAS, their precursors and breakdown products<sup>9-15</sup> which are found in wildlife tissues worldwide, including remote areas like the Arctic.<sup>8, 16</sup>

In birds, during egg formation, the female transfers various substances required to enable and sustain the development of the embryo. However, in addition to essential compounds such as water, minerals, proteins, lipids, vitamins, antibodies or hormones, some contaminants may also be transferred. Persistent organic pollutants (POPs) have been shown to be transferred from mothers to eggs due to their physicochemical properties, via either lipids (e.g. polychlorinated biphenyls (PCBs), pesticides) or proteins (PFAS).<sup>17</sup> Therefore, the avian egg is considered as a relevant monitoring tool for a selection of organic contaminants exposure in many species, as its composition directly reflects that of maternal tissues.<sup>18</sup> However, the few field studies concurrently comparing variability in, for instance, PCBs and organochlorine pesticides concentrations and composition in both eggs and mother tissues, showed that organic contaminants transfer can be influenced by a combination of biological factors (ex: egg size, egg mass, egg laying order, number of eggs in clutch, age of the female) and by the physicochemical properties of contaminants.<sup>17</sup> Despite PFAS being studied for almost two decades, their concentrations in egg have been poorly documented in wildlife, and very few studies evaluated maternal transfers in birds by concurrently measuring PFAS in the eggs and

the mother in the field.<sup>19-23</sup> Although fluorinated compounds are transferred at a lower degree than lipophilic contaminants, they can still be found at high concentrations in eggs.<sup>19, 24-26</sup> Once accumulated into females, PFAS tend to bind to proteins (especially very low-density lipoproteins) synthesized in the liver, these being then transferred via blood to the ovary and finally to the eggs and the carbon-chain length has been suggested to be an important driver of PFAS transfer efficiency.<sup>27-29</sup>

In humans and in laboratory models, PFAS can cause cancer, affect immunocompetence and disrupt the endocrine system.<sup>30</sup> Regarding developing avian embryos, some PFAS compounds were found to be responsible for a lower heart rate, an enlarged liver, thyroid hormones and immune system disruption, as well as a lower hatching success and a lower survival.<sup>31-37</sup> However, the consequences of PFAS exposure remain poorly investigated in wildlife. Recent studies on kittiwakes and other seabirds of the Kongsfjord area suggest that exposure to long-chain perfluorinated carboxylic acids (PFCAs) can be associated with physiological and fitness impairments.<sup>38-43</sup> PFAS cover thousands of substances, the vast majority are not regulated yet and some are increasingly detected in biota.<sup>44</sup> Furthermore, development and manufacturing of alternatives to the legacy PFAS remains largely uncharacterized in terms of risks, despite recent evidences of their environmental occurrence in wildlife tissues.<sup>45, 46</sup> Alternative fluorinated compounds are now continuously introduced to the market and despite many of them being still unidentified, some are now known as bioaccumulative.<sup>47</sup> As an example, emerging fluorinated chemicals such as F-53B, ADONA or HFPO-DA (GenX) and short-chained precursors are part of these alternative PFAS which have been very scarcely screened for in wildlife before.<sup>48</sup> To the best of our knowledge, to date only three studies detected emerging PFAS in avian eggs.<sup>49-51</sup>

In the present study we aimed to: 1/ describe concentration and composition of emerging and legacy PFAS in the egg yolk of an Arctic-breeding population of black-legged kittiwakes (*Rissa tridactyla*) facing significant PFAS exposure<sup>43</sup>; 2/ investigate the maternal transfer, i.e. the relationship between emerging and legacy PFAS found in the eggs yolk with those found in the plasma of the corresponding females sampled during the pre-laying stage (i.e. before egg-laying) as well as the effects of biological variable (laying date, clutch size, laying order, female condition). We expect strong correlations of contaminant concentrations between females and their eggs with highly contaminated adults laying highly contaminated eggs, but a decrease in eggs contamination with laying order.<sup>52</sup> We also hypothesize that variations in the transfer of

PFAS to the egg should be influenced by the physicochemical properties of the different PFAS compounds, especially the carbon chain length.

## MATERIALS AND METHODS

### Sample collection

The study was conducted in a Black-legged kittiwake colony (“Krykkjefjellet” in Kongsfjorden, Svalbard, 78°53'48"N 12°11'43"E) from May to June 2019. On fourteen nests, adult females of black-legged kittiwake (hereafter “kittiwakes”) were captured a few days before expected laying using a noose at the end of a fishing rod. Females of each nest ( $n = 14$ ) were individually identified using a numbered metal ring (Stavanger Museum) and a three digits plastic ring to allow individual identification from a distance. Molecular sexing of the adults was performed at the Centre d’Etude Biologiques de Chizé (CEBC), following Fridolfsson and Ellegren (1999).<sup>53</sup> Immediately after capture, a 2 mL blood sample was taken from the alar vein using a heparinized 2.5 mL syringe and a 25G needle. Blood was then transferred in a 2 mL Eppendorf tube and stored in a cooler. At the end of each day, the samples were centrifuged for 10 minutes. Both tubes of red blood cells (RBC, for molecular sexing) and plasma (for PFAS) were stored in a -20 °C freezer until analyses. The nests corresponding to the sampled birds were then monitored daily using a mirror attached to an 8 m rod. As soon as the first egg was laid, it was collected ( $n = 14$ ) and replaced by a plastic dummy-egg in order to avoid nest desertion. Similarly, the second-laid egg was collected ( $n = 11$ ) the day it was laid and the dummy-egg was removed. If they were no second-laid egg after 10 days, the dummy-egg was retrieved. All egg samples were processed in the lab immediately. The eggs were measured (height and diameter) with an electronic caliper and weighted to the nearest 0.01g. Eggs were opened and the yolk and the albumen were separated and stored in 2 mL Eppendorf tubes at -20°C in a freezer until assayed. All eggs were collected less than 24h after they were laid, and thus were at a similar development stage, avoiding contaminants to be absorbed by the growing embryo and allowing a comparison between eggs. Sample size was limited by authorization to collect eggs and by the number of accessible nests in the colony. The sampling of birds and eggs was approved by the Governor of Svalbard and by the Norwegian Animal Research Authority (NARA, permit number 19970).

## Poly and perfluoroalkyl substances extraction and analysis

We used a method described by Sletten *et al.* (2016) adapted from Powley *et al.* (2005) to measure PFAS in females' plasma.<sup>54, 55</sup> For yolk samples (~0.20 g), after mixing methanol and the samples spiked with internal standards (listed in the SI), an additional initial step consisting in thoroughly mixing the solution using zirconium beads was added. This step ensured that yolk was completely homogenized with the solvent. PFAS concentrations were only measured in yolk as this is where the most part of these contaminants are located in bird eggs.<sup>21, 29</sup> Quantification was conducted by ultrahigh-performance liquid chromatography triple-quadrupole mass spectrometry (UHPLC–MS/MS). The chromatograms were quantified with LCQuan software (version 2.6, Thermo Fisher Scientific Inc., Waltham, MA, USA), applying the isotopic dilution method. An eight-point calibration curve with a concentration range from 0.02 to 10.0 pg  $\mu\text{L}^{-1}$  was used. The following “legacy” PFAS were screened in both plasma and yolk: perfluorooctane sulfonamide (FOSA), perfluorobutanesulfonic acid (PFBS), perfluoropentanesulfonic acid (PFPS), perfluorohexanesulfonic acid (PFHxS), perfluoroheptanesulfonic acid (PFHpS), branched perfluorooctanesulfonic acid (brPFOS), linear perfluorooctanesulfonic acid (linPFOS), perfluorononanesulfonic acid (PFNS), perfluorodecanesulfonic acid (PFDcS), perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDcA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriA), perfluorotetradecanoic acid (PFTeA), perfluorohexadecanoic acid (PFHxDA) and methyl perfluorohexane sulphonamide (MeFHxSA). Additionally, PFAS of emerging concern were measured: methylperfluorooctane sulfonamide (MeFOSA), ethylperfluorooctane sulphonamide (EtFOSA), perfluorooctanesulfonamidoacetic acid (FOSAA), methyl perfluorooctane sulfonamidoacetic acid (MeFOSAA), ethyl perfluorooctane sulfonamidoacetic acid (EtFOSAA), 4:2 fluorotelomer sulfonic acid (4:2 FTS), 6:2 fluorotelomer sulfonic acid (6:2 FTS), 8:2 fluorotelomer sulfonic acid (8:2 FTS), 10:2 fluorotelomer sulfonic acid (10:2 FTS), 3:3 fluorotelomer carboxylic acid (3:3 FTCA), 5:3 fluorotelomer carboxylic acid (5:3 FTCA), 7:3 fluorotelomer carboxylic acid (7:3 FTCA), a mixture of 6:2 and 8:2 chlorinated polyfluorinated ether sulfonate (F-53B), dodecafluoro-3H-4,8-dioxanonanoate (ADONA) and hexafluoropropylene oxide dimer acid (GenX), see Supporting Information (SI), Table S1. All concentrations are expressed in ng  $\text{g}^{-1}$  wet weight (ww).



## Quality control

To guarantee the quality and control for reproducibility and precision of the PFAS analyses method, one blank and a standard reference material (human serum AM-S-Y-1908 INSPQ within the Arctic Monitoring and Assessment Program ring test) were concurrently analyzed every 15 samples. The determined concentrations varied between 78 and 125 % of the target value. The recovery of the  $^{13}\text{C}$  labelled internal standard was calculated by adding a recovery standard at the end of the sample processing to every sample. The recoveries of all internal standard compounds varied between 66 and 120 %. Limit of detection (LOD) was defined as three times the signal to noise ratio for the specific matrix, or in the case of detection in the blanks as the sum of the average of the blank level and 3 times standard deviation. LOD varied depending on the compounds and ranged from 0.004 to 0.500 ng g<sup>-1</sup> for the PFAS.

## Statistical analyses

Among PFAS, only compounds detected in >70 % of both pre-laying females plasma and eggs yolk were included in analyses to increase statistics robustness. For each included PFAS, values <LOD were set to half of the LOD of the specific compound.

First, we tested for each PFAS the relationship between concentrations in eggs and the effects of the following biological variables: the laying date, the egg mass and the rank of the egg in the clutch by laying order (egg number: 1 or 2) as well as both interactions between the laying date or the egg mass and the egg number, using linear mixed effect models (LMMs: lme function from “nlme” R-package, v.3.1.147).<sup>56</sup> The females body condition (residuals of the linear correlation between the skull length and the mass) was not included as an explanatory variable in the model as it was correlated to the laying date (LM:  $t=-4.054$ ,  $p<0.001$ ).

Second, LMMs were built to test the maternal transfer efficiency of each individual PFCAs. The maternal transfer ratio, calculated as *concentration in egg/concentration in female* for each compound, was used as the response variable and log-transformed to meet the assumptions of linear models. The number of carbons in the PFCAs chain, the egg number as well as the interaction between these two variables were used as predictors. For these models, all PFAS concentrations lower than LOD were removed from the dataset to avoid outliers in ratios ( $n=15$ ).

Third, we investigated for each PFAS the effect of the concentration in females plasma and the egg number on the concentration in eggs using LMMs.

Each model of the three steps were then ranked and rescaled according to the Akaike's Information Criterion for small sample size (AICc): in a set of models from the full to the null model, the model with the lowest AICc was selected for all steps independently. If the  $\Delta$ AICc with the next ranked model was  $<2$ , the most parsimonious was chosen.<sup>57, 58</sup> The nest number was used as a random intercept in all models. The normality of residuals was verified through inspection of diagnosis plots (residuals vs fitted values and Q-Q plots). For all models a significance level of  $\alpha < 0.05$  was used. All statistical analyses were performed using R (v.4.0.0; R Core Team, 2020).<sup>59</sup>

## RESULTS AND DISCUSSION

### PFAS concentrations in females and their eggs

In the studied population, kittiwakes arrived in the colony in early April after migrating from pelagic areas of the western North-Atlantic Ocean.<sup>60</sup> During the two-months pre-laying period that follows migration, females feed mostly on small fish and crustaceans in the marine system near the colony before laying a clutch of usually two eggs.<sup>61-63</sup> Only 3 nests were observed to have single egg clutches in the present study. Females having a single egg clutch and females having a two eggs clutch had PFAS concentrations of similar magnitude in plasma (respectively 8.46-35.4 and 12.4-89.4 ng g<sup>-1</sup> ww). Therefore they were both considered the same way in analyses.

The following legacy PFAS were detected in  $>70\%$  of both females plasma and eggs yolk and therefore included in analyses: PFHxS, brPFOS, linPFOS, PFOA, PFNA, PFDcA, PFUnA, PFDcA, PFDoA, PFTriA, PFTeA (SI Table S1). Among legacy compounds, both PFDcS and PFHxDA were above LOD in 84% of the eggs. However, in maternal plasma PFDcS and PFHxDA were above LOD in only 24% and 0% of the samples, respectively. All other legacy PFAS were not detected in maternal plasma nor in eggs. None of the emerging PFAS were detected in the maternal plasma. In contrast, 7:3 FTCA was detected in 84% of the eggs whereas ADONA was only detected in a single egg (SI Table S1). None of the other emerging PFAS investigated were detected in the yolk. PFDcS found in  $<70\%$  of the females and PFHxDA, 7:3 FTCA and ADONA found in none of the females were therefore excluded from statistical analyses, but descriptive statistics of the compounds in eggs are provided in SI Table S2.

**Table 1.** Descriptive statistics (mean  $\pm$  standard deviation *SD*, median and range *min-max*) for PFAS concentrations (ng g<sup>-1</sup> ww) in plasma and yolk of black-legged kittiwakes from Svalbard.

	Prelaying females (n=14)			First laid eggs (n=14)			Second laid eggs (n=11)		
	Mean $\pm$ <i>SD</i>	Median	Min-max	Mean $\pm$ <i>SD</i>	Median	Min-max	Mean $\pm$ <i>SD</i>	Median	Min-max
PFHxS	0.23 $\pm$ 0.09	0.21	0.11 - 0.40	0.26 $\pm$ 0.14	0.26	0.08 - 0.57	0.22 $\pm$ 0.09	0.20	0.08 - 0.40
brPFOS	0.75 $\pm$ 0.49	0.86	0.028 - 1.38	3.00 $\pm$ 1.01	3.12	1.44 - 4.45	1.93 $\pm$ 0.77	1.93	0.88 - 3.10
linPFOS	10.8 $\pm$ 4.70	11.3	3.14 - 17.42	28.6 $\pm$ 10.3	25.9	17.0 - 50.7	21.3 $\pm$ 7.90	19.6	11.8 - 34.7
PFOA	0.17 $\pm$ 0.13	0.14	0.04 - 0.57	0.23 $\pm$ 0.12	0.22	0.04 - 0.47	0.21 $\pm$ 0.08	0.19	0.13 - 0.41
PFNA	1.06 $\pm$ 0.61	1.07	0.19 - 2.44	1.98 $\pm$ 1.07	1.75	0.85 - 3.64	1.74 $\pm$ 0.80	1.48	0.71 - 2.98
PFDeA	1.71 $\pm$ 1.56	1.56	0.40 - 3.26	3.63 $\pm$ 1.88	3.07	1.73 - 6.83	3.07 $\pm$ 1.38	2.60	1.19 - 5.20
PFUnA	6.88 $\pm$ 3.22	7.42	1.40 - 12.4	17.9 $\pm$ 5.78	16.8	10.5 - 28.6	14.0 $\pm$ 4.55	13.4	7.33 - 20.8
PFDoA	1.78 $\pm$ 0.98	1.78	0.02 - 3.76	6.07 $\pm$ 1.46	5.90	4.36 - 9.41	4.40 $\pm$ 1.19	4.55	2.84 - 6.62
PFTriA	7.80 $\pm$ 3.35	7.93	1.81 - 13.9	33.5 $\pm$ 6.45	33.8	22.5 - 50.8	23.0 $\pm$ 5.65	22.3	14.7 - 35.2
PFTeA	1.44 $\pm$ 0.60	1.53	0.471 - 2.63	6.74 $\pm$ 1.55	7.07	4.36 - 10.1	4.60 $\pm$ 1.38	4.71	2.72 - 7.00
$\Sigma$ PFASs	11.77 $\pm$ 5.03	12.5	3.37 - 18.9	31.9 $\pm$ 11.1	28.4	19.5 - 55.2	23.4 $\pm$ 8.48	22.1	12.9 - 37.8
$\Sigma$ PFCAs	20.73 $\pm$ 8.85	21.8	5.09 - 36.5	70.1 $\pm$ 15.4	67.5	48.0 - 109	51.2 $\pm$ 13.3	48.2	33.8 - 78.4
$\Sigma$ PFASs	32.5 $\pm$ 13.5	33.5	8.46 - 51.9	102 $\pm$ 25.9	98.2	68.5 - 164	74.5 $\pm$ 21.4	71.1	46.6 - 116

The most abundant PFAS found in maternal plasma were linPFOS (mean  $\pm$  *SD*: 10.8  $\pm$  4.70 ng g<sup>-1</sup> ww), followed by PFTriA (7.80  $\pm$  3.35 ng g<sup>-1</sup> ww) and PFUnA (6.88  $\pm$  3.22 ng g<sup>-1</sup> ww, Table 1). Together, these three PFAS represented 78.6% of the  $\Sigma$ PFAS. In both eggs, PFTriA was dominant (28.9  $\pm$  7.99 ng g<sup>-1</sup> ww), followed by linPFOS (25.4  $\pm$  9.89 ng g<sup>-1</sup> ww) and PFUnA (16.2  $\pm$  5.54 ng g<sup>-1</sup> ww), representing 78.4% of the  $\Sigma$ PFAS. For example, incubating glaucous gulls (*Larus hyperboreus*) sampled in the same area showed dominating linPFOS, then PFUnA and PFTriA in both plasma and eggs.<sup>38, 64</sup> Odd-chain PFCAs (e.g. C<sub>9</sub>, C<sub>11</sub>, C<sub>13</sub>) being more abundant than even-numbered chain is a frequent pattern in seabirds and their eggs, this is believed to be linked to the degradation process of precursor compounds such as fluorotelomer alcohols (FTOH), as well as a selective bioaccumulation.<sup>21, 27, 44, 65-67</sup>

PFCAs in plasma reflect recent dietary uptake due to their efficient elimination from birds' bodies.<sup>68</sup> In contrast, PFOS and other PFSAs show much longer half-lives in plasma, suggesting that blood acts as a significant reservoir for them. Thus, occurrence of PFCAs may represent more recent and local inputs (marine foraging area close to Svalbard), whereas PFSAs would rather represent contamination before birds enter the Svalbard areas to breed (winter and

early spring in the West Atlantic).<sup>69</sup> Moreover, plasma samples may represent a snapshot of recent exposure to PFAS while egg concentrations integrate a longer period of time of egg formation, rather reflecting recent days and weeks.<sup>23</sup> In some cases even revealing the exposure at the wintering grounds, hampering direct comparisons of PFAS patterns in plasma and eggs. Kittiwakes in Svalbard nonetheless arrive on the breeding grounds by mid-April and females of the present study were caught on the 4<sup>th</sup> of June  $\pm$  8 days (mean  $\pm$  SD).<sup>60</sup> Before they laid, they were thus feeding locally for a longer time than the length of most PFAS clearance time measured in eggs of PFAS fed hens (*Gallus gallus*).<sup>23,70</sup> Despite different metabolisms and egg-laying pattern (and thus excretion) between these two species, PFAS concentrations of kittiwakes plasma and egg yolks could therefore mainly represent local contamination.

### Emerging PFAS in females and their eggs

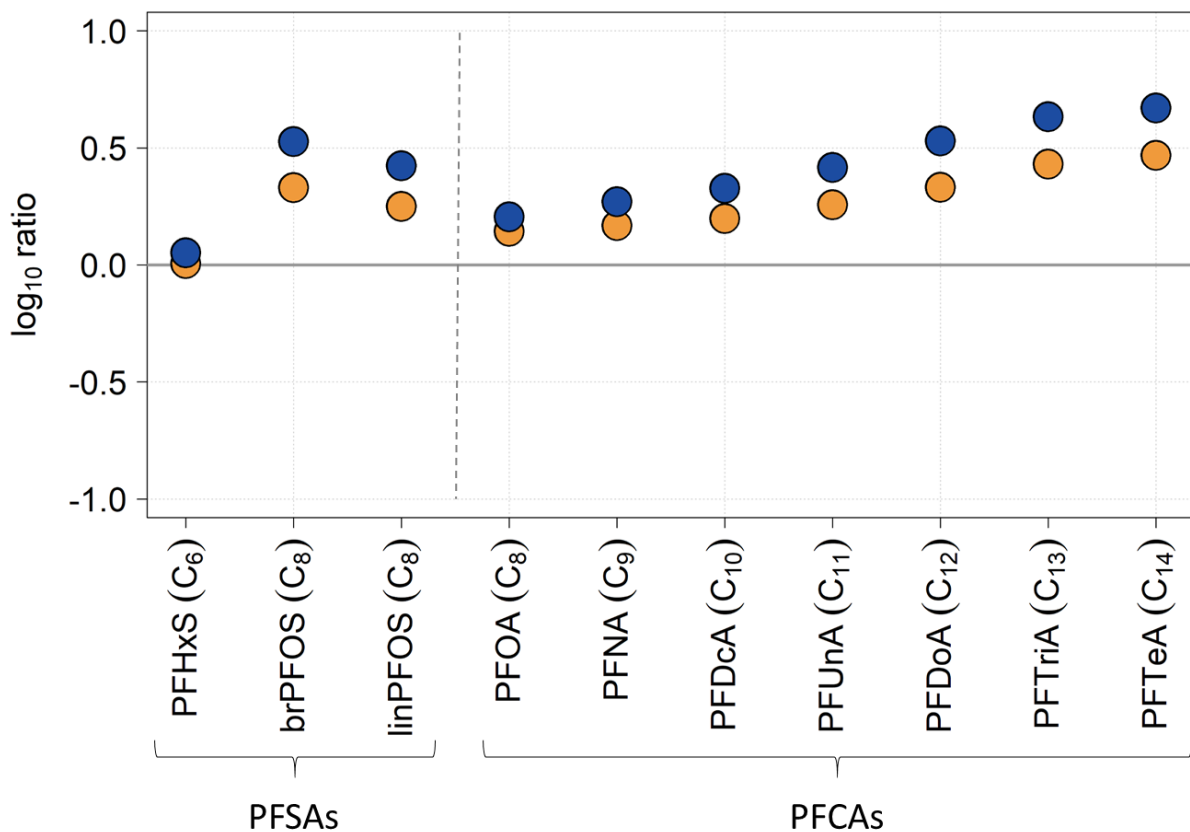
A broad number of shorter-chain alternatives, supposedly less bioaccumulative and toxic than long-chain persistent PFAS, have been synthesized since the 2000's when C<sub>8</sub> and related PFAS were phased out or regulated in North America and in Europe.<sup>71,72</sup> Even so, some of them have been found in biota, sometimes with higher levels of bioaccumulation and toxicity than the long-chain PFAS they replaced.<sup>45,48,73-79</sup> In the present study, we measured 7:3 FTCA above LOD in 84% of the eggs (mean  $\pm$  SD: 0.21  $\pm$  0.09 ng g<sup>-1</sup> ww; range: 0.10 – 0.40 ng g<sup>-1</sup> ww). To the best of our knowledge, this is the first report of this compound in seabird eggs, and the second for Arctic top predators.<sup>46</sup> 7:3 FTCA is an intermediate environmental degradation product from fluorotelomer alcohols (FTOH), whose final degradation products are PFCAs.<sup>80</sup> It has been recently detected in human tissues and wildlife.<sup>46,81-87</sup> In eggs of osprey (*Pandion haliaetus*), tawny owl (*Strix aluco*) and common kestrel (*Falco tinnunculus*), this compound ranged from <0.24 (LOD) to 2.7 ng g<sup>-1</sup> much higher concentrations than those found in kittiwake eggs reported here.<sup>78</sup> Literature regarding fluorotelomer toxicity is scarce and even non-existent for avian species, but some were found to be potentially more toxic than long-chain PFAS.<sup>88-90</sup> Among emerging PFAS, ADONA was detected in a single egg at a concentration of 0.11 ng g<sup>-1</sup> ww, which is the first documented finding for this compound in wildlife (chromatograms for ADONA measurements are provided in SI Figure S1). To the best of our knowledge, this compound was detected only twice before in biota, in blood of humans living close to a production plant using ADONA in Germany and in human milk from women in China.<sup>91,92</sup> Therefore we recommend additional investigations for this compound in bird eggs. Other fluorinated alternatives such as F-53B and GenX were not detected in any of the

samples, F-53B was very recently discovered in herring gull (*Larus argentatus*) eggs from Germany (despite not being officially used in Europe) and, along with GenX, in 100% of black-tailed gull (*Larus crassirostris*) eggs from South Korea with an increasing trend over time.<sup>50, 51</sup> None of the analyzed emerging PFAS were found in females plasma in the present study, which means that kittiwakes foraging grounds are not important sources. However, PFAS accumulation in a specific tissue dependent on their physicochemical characteristics.<sup>21</sup> Thus, although we detected 7:3 FTCA and ADONA in eggs yolk and not in maternal plasma, these compounds may be present in other organs of the birds. Another hypothesis for the presence of emerging PFAS in yolk but not in females' plasma could be a very high transfer efficiency of these compounds since some emerging PFAS (including ADONA) have been found to bind to human and rats liver proteins at least as strongly as PFOA or PFOS.<sup>93</sup> A significant transfer of precursors such as FTOH in eggs could also be a reason for the detection of 7:3 FTCA in eggs but not in females, however this is highly speculative as these compounds were not measured in the present study. Adults blood plasma is frequently used in birds to describe the extent of local contamination to a wide range of pollutants.<sup>94</sup> However, in our study this tissue shows its limitation as a tool to document the contamination of the environment, as all emerging compounds investigated and detected in egg yolks could not be detected in any of the plasma samples. For future studies, we suggest systematic screening for emerging PFAS in biota to increase the overview of the exposure to these compounds and we suggest that the use of eggs would be more pertinent rather than adults plasma. Toxicological studies are also strongly needed to evaluate the threats of the alternative fluorinated compounds in biota.

### **Relationship between biological factors and PFAS concentrations in eggs**

The variables “laying date” and “egg mass” were excluded from all best models explaining PFAS variations in eggs (SI Table S3). Therefore, developing embryos from eggs laid early or late in the season should have a similar exposure to PFAS. Moreover, juvenile survival is known to be affected by egg size (and therefore egg mass) in various avian species,<sup>95</sup> however egg mass was unrelated to PFAS contamination in our study. Relationship between laying date and PFAS concentrations in eggs is not commonly studied, but in great tits (*Parus major*) a negative and linear correlation was found between PFOS concentration of eggs and the laying date but no relationship with any of the other PFAS was found.<sup>96</sup> In the present study, the absence of relationship between the laying date and any of the PFAS concentrations in eggs might be due to the highly synchronized and time-limited laying period during which significant

variations of PFAS concentrations in Svalbard water would be unlikely. In the same study on great tits, a positive and linear relationship was also noted between PFOS concentration and the egg mass, which was presumably attributed to the lipoprotein content of the eggs, heavier eggs having a higher lipoprotein content, yet the result of the present study do not support this hypothesis. All selected PFAS, but PFHxS and PFOA (null model selected), were in higher concentration in first- than in second-laid eggs (all  $t < -2.57$ , all  $p < 0.03$ ; Figure 2; SI Table S3). This difference in PFAS concentrations between eggs of the same clutch is generally observed in gulls, but it is not always the case in other birds with larger clutches.<sup>29, 52, 96</sup> As a consequence, toxicological impairments on hatchability and survival would be more frequent for the first-laid egg, which could be critical for small clutch size species, including kittiwake. Decreasing body contaminant burden along the laying sequence is the main hypothesis to explain the decreasing concentrations in eggs. In species with larger clutch sizes, daily exogenous intakes or food shortage during laying could be of higher importance, leading to variations in blood circulating PFAS and thus in eggs along the laying sequence. In kittiwakes, the similar concentrations observed in both eggs for PFHxS and PFOA might be the consequence of low concentrations of these two compounds in maternal plasma, resulting in a lower transfer efficiency to eggs.



**Figure 1.** Maternal transfer ratios ( $\log_{10}$  ratios) of PFAS in black-legged kittiwakes from Svalbard. The PFASs and PFCAs are ordered by carbon chain length (given in parentheses). Ratios are calculated as  $\log_{10}(\text{mean concentration in eggs}/\text{mean concentration in plasma})$  for first- and second-laid eggs. Blue and orange colors are used for first- and second-laid eggs respectively. The grey horizontal solid line ( $\log_{10}$  ratio = 0) stand for a 1:1 relationship between female and eggs.

### PFAS transfer ratios between females and their eggs

Maternal transfer ratios (MTRs) were all higher than 0, which means that eggs are an important excretion route for all measured PFAS compounds in kittiwakes (Figure 1). The model selection showed that both the length of the PFAS carbon chain, the egg number and their interaction were significant predictors of the MTRs for PFCAs (SI Table S4). MTRs were increasing with the carbon chain length both for first- and second-laid eggs ( $t=12.5$ ,  $p<0.001$  and  $t=6.87$ ,  $p<0.001$  respectively; Figure 1). This suggests a preferential transfer of PFCAs with the longest chains. Similar findings that those of the present study were documented for PFCAs maternal transfer measured between females liver and eggs yolk in herring and common guillemots (*Uria aalge*) respectively from North America and the Baltic Sea.<sup>21, 27</sup> This pattern may originate from a selective binding of the longest chain length PFCAs to proteins and a subsequent facilitated transfer to the egg. Such a selective binding have been revealed in *in-vitro* studies with a variable affinity of PFAS to bovine serum albumin depending on the carbon-chain length.<sup>97</sup> Moreover, long-chain PFAS generally have greater bioaccumulation potential than short-chain homologues, they are thus more likely to be transferred to the eggs.<sup>6</sup> However, MTRs of PFCAs had no clear trends with the compounds chain length in herring gulls of southern Norway.<sup>19</sup> A linear relationship between MTRs and PFCAs chain length depict the absence of compounds-specific transfer mechanisms. However, a leveling off of the curve on the extremes (see Figure 1) could be the sign of additional transfer mechanisms for PFOA (C<sub>8</sub>) and a transfer kinetic barrier for PFTeA (C<sub>14</sub>).

The extent of the MTRs increase with chain length was different between first- and second-laid eggs ( $t=-3.25$ ,  $p<0.01$ ; Figure 1), leading to an increasing difference in the MTRs with PFCAs carbon chain length between both eggs. Consequently, second-laid eggs received a lower proportion of females plasma contaminants than first-laid eggs for the compounds with

the longest chains. Such a difference could be a consequence of the preferential transfer of the longest chain PFCAs, an increasing transfer of PFCAs with the chain length would lead to a higher depletion of these compounds in females and therefore a lower transfer in their second-laid eggs.

Facilitated transfer of PFCAs with the longest carbon chains is concerning as it could potentially induce adverse effects for the development of the embryo, as toxicity is known to be dependent on the carbon chain length.<sup>41, 98, 99</sup> However, the majority of the studies on the effects of PFAS exposure on the embryo focused on PFOS and PFOA. These studies revealed that PFOS and PFOA can impair hatchability and disrupt embryos' physiology, but at higher concentrations than those measured in the present study.<sup>31, 32, 34-36, 100-102</sup> Studies on the consequences of long-chain PFCAs *in ovo* exposure on birds' embryo are needed, especially as long chain PFCAs are widely detected in wildlife, despite decreasing concentrations in the last few years.<sup>65, 103-107</sup>

Among PFSAs, PFHxS MTR was lower than those of brPFOS and linPFOS (LMM:  $t=-10.4$ ,  $p<0.001$  and  $t=-8.56$ ,  $p<0.001$  respectively), suggesting, as for PFCAs, an increasing transfer with carbon chain length. PFOS and C<sub>11</sub>-C<sub>14</sub> PFCAs have also been found to be more lipophilic than other short-chain PFAS, representing a large proportion of the  $\sum_{\text{PFAS}}$  in the fat of marine mammals.<sup>108</sup> Seabirds egg yolk being constituted of 20 to 30% lipids,<sup>109</sup> the maternal transfer of PFAS could be both lipid- and protein-driven. Concerning PFOS, brPFOS was significantly more transferred to the eggs than linPFOS (LMM:  $t=-2.50$ ,  $p=0.02$ ) despite a similar carbon chain length for these isomers. This may be a consequence of different transfer mechanisms for the branched and linear forms. However, contrarily to linPFOS, brPFOS is a mixture of different isomers and not all of them are covered by the labeled standards used for analyses, potentially leading to lower analytical precision in the measurements of brPFOS concentrations.

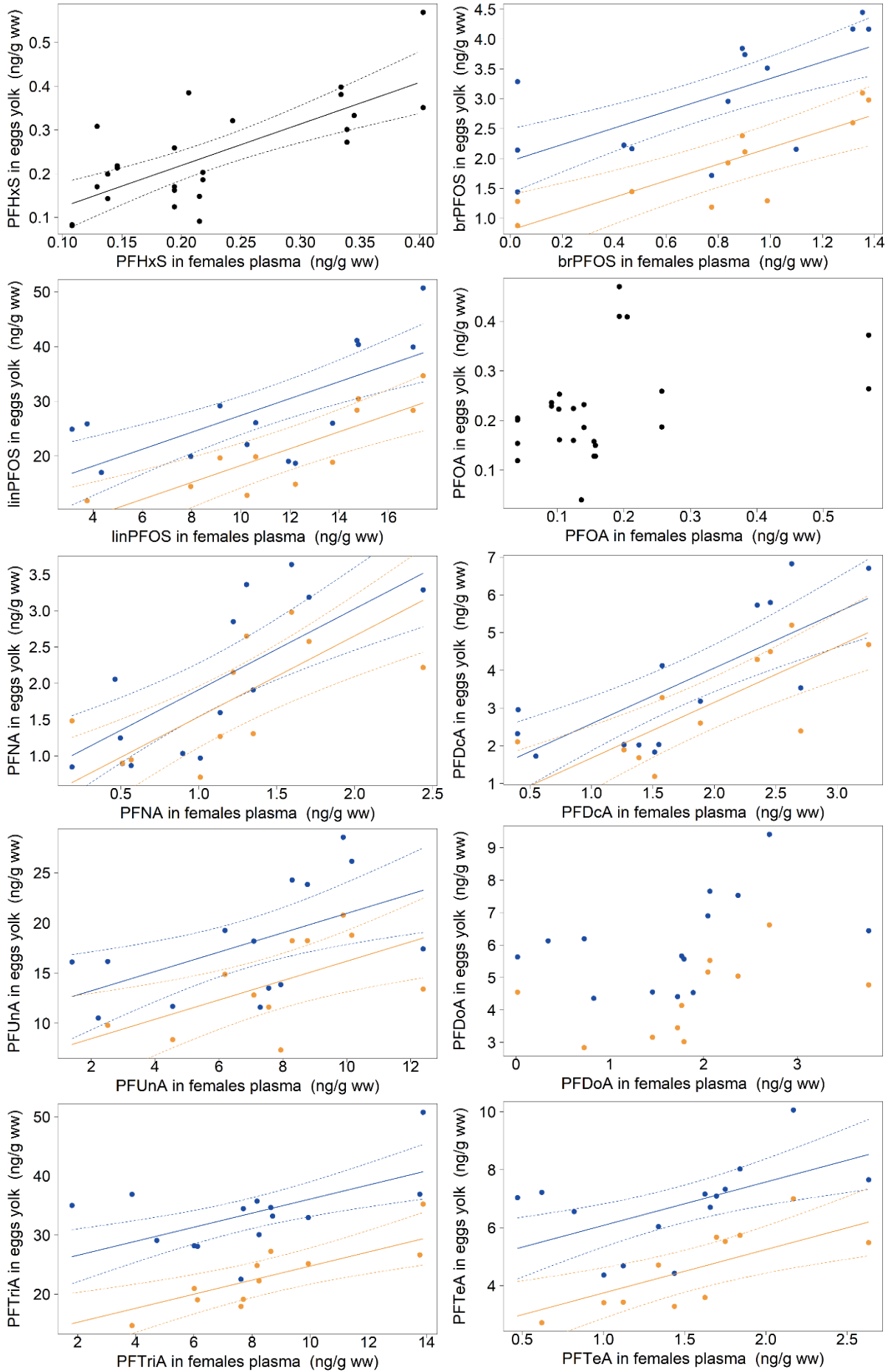
PFAS are bound to proteins (including serum albumin, fatty acid binding proteins and organic anion transporters), which affects their distribution, bioaccumulation and excretion.<sup>110</sup> In marine mammals, the trophic magnification factors of individual PFAS were found to have a significant relationship with their protein-water partition coefficients ( $\text{LogK}_{\text{PW}}$ ).<sup>111</sup> Therefore, PFASs transfer is affected by their binding affinity to proteins. In hooded seals (*Cystophora cristata*) and humans, the transfer efficiency of PFCAs presented a U-shape with a decreasing transfer proficiency from C<sub>8</sub> to C<sub>10</sub> and an increasing from C<sub>10</sub> to C<sub>13</sub> and that of PFSAs was decreasing with compounds chain length.<sup>112-114</sup> The relationship between MTRs and PFASs



chain length described in the present study is different. However, this U-shape trend in mammals has been described as the integrated result of opposite trends in the transfer from maternal blood to placenta and from placenta to cord blood.<sup>113</sup> Similar trends than those of the present study were found in the transfer of PFASs and PFCAs from maternal blood to placenta, this has been described as the consequence of a high water content in blood and a higher protein and lipid content in the placenta. As a consequence, short chain PFASs with greater water solubility and less capacity to bind with proteins were less transferred to placenta. Similar transfer mechanisms between females blood and eggs yolk could lead to the MTRs pattern observed in kittiwakes.

Investigating lipid and protein content of female plasma and egg yolk in future studies would help in understanding the mechanisms behind maternal transfer of PFAS. Sampling females before and after egg-laying as well as males, would also be interesting in order to evaluate if females are depleted in the longest chain PFCAs after laying and compared to males.

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**Figure 2.** PFAS concentrations (ng g<sup>-1</sup> ww) relationship between females plasma and yolks of first- and second-laid eggs of black-legged kittiwakes from Svalbard. The solid line refers to a statistically significant linear maternal transfer (see Table 2), with dotted lines representing 95% confidence intervals and no line representing no significant relationship. Blue and orange colors are used for first- and second-laid eggs respectively when concentrations are significantly different between them, black is used if the trends are similar between egg numbers.

**Table 2.** Factors affecting each PFAS concentrations in black-legged kittiwakes' eggs from Svalbard, estimated by mixed linear regression models. Significant *p*-values are bolded.

Parameter	Estimate	SE	<i>t</i> -value	<i>p</i> -value
<b>PFHxS (R<sup>2</sup>m: 0.52; R<sup>2</sup>C: 0.72)</b>				
Female plasma	0.94	0.22	4.35	<0.001
<b>brPFOS (R<sup>2</sup>m: 0.59; R<sup>2</sup>C: 0.84)</b>				
Female plasma	1.25	0.36	3.51	<0.01
Egg number (2)	-1.18	0.18	-6.68	<0.001
<b>linPFOS (R<sup>2</sup>m: 0.55; R<sup>2</sup>C: 0.93)</b>				
Female plasma	1.45	0.39	3.74	<0.01
Egg number (2)	-9.52	1.14	-8.34	<0.001
<b>PFOA (Null model)</b>				
<b>PFNA (R<sup>2</sup>m: 0.48; R<sup>2</sup>C: 0.88)</b>				
Female plasma	1.10	0.30	3.64	<0.01
Egg number (2)	-0.38	0.14	-2.68	<b>0.023</b>
<b>PFDCa (R<sup>2</sup>m: 0.54; R<sup>2</sup>C: 0.93)</b>				
Female plasma	1.43	0.35	4.13	<0.01
Egg number (2)	-0.93	0.19	-5.02	<0.001
<b>PFUnA (R<sup>2</sup>m: 0.38; R<sup>2</sup>C: 0.95)</b>				
Female plasma	0.96	0.39	2.42	<b>0.032</b>
Egg number (2)	-5.29	0.54	-9.86	<0.001
<b>PFDoA (R<sup>2</sup>m: 0.34; R<sup>2</sup>C: 0.90)</b>				
Egg number (2)	-1.92	0.23	-8.52	<0.001
<b>PFTriA (R<sup>2</sup>m: 0.61; R<sup>2</sup>C: 0.79)</b>				
Female plasma	1.09	0.37	2.98	<b>0.012</b>
Egg number (2)	-1.10	1.50	-7.36	<0.001
<b>PFTeA (R<sup>2</sup>m: 0.53; R<sup>2</sup>C: 0.79)</b>				
Female plasma	1.34	0.52	2.59	<b>0.024</b>
Egg number (2)	-2.21	0.34	-6.60	<0.001

**Relationship between PFAS concentrations in females and their eggs**

In this study, eggs laid by the sampled females were collected, enabling the study of the actual profile of the maternal transfer of PFAS. Among PFAS, a positive and linear association between yolks and female plasma was observed for the following compounds: PFHxS, brPFOS, linPFOS, PFNA, PFDcA, PFUnA, PFTriA and PFTeA (all  $t > 2.42$ , all  $p < 0.032$ , SI Table S5, Table 2 and Figure 2). A similar linear relationship between females and eggs was documented in tree swallows (*Tachycineta bicolor*) for PFOS, in great tits for PFOS and PFOA, as well as for PFHxS, PFOS and PFOA in hens.<sup>20, 23, 32</sup> PFAS have been found to have relatively strong binding abilities to yolk proteins (low-density lipoprotein, high-density lipoprotein and vitellin proteins).<sup>115</sup> All yolk proteins (except immunoglobulins) being synthesized in the liver, it makes it the entry point for PFAS in egg yolks.<sup>116</sup> A linear relationship between females and eggs for most of the PFAS as found in the present study suggests that the efficiency of PFAS transfer mechanisms are not dependent on the concentration in females' plasma. These linear relationships observed between females' plasma and eggs yolk therefore cannot explain the differences found in the PFAS patterns presented above. The affinity of the different PFAS for proteins varies to a large extent, suggesting binding site-specific interactions and facilitated transport of some compounds.

However, PFAS are not always linearly transferred as found between females and eggs for PFHxA in hens and PFCAs (except PFOA) in great tits.<sup>20, 23</sup> A comparable absence of relationship was found for PFOA and PFDoA in the present study. At low levels, the transfer dynamic of contaminants could be different. This could have been the case for PFOA, for which we found the lowest concentrations in both the females and the eggs. However, this was not the case for PFHxS which was also in very low concentrations in both females and eggs. Additional studies are needed to reveal whether the non-linear transfer of PFOA and PFDoA stems from real biological mechanisms or is due to low statistical resolution caused by the low sample size. The linear relationship between both eggs and females for most PFAS validates the use of eggs to assess the female's exposure in kittiwakes, however, we emphasized the relative importance of sampling eggs of similar laying numbers to avoid biased estimation due to the decrease in contamination with the laying-sequence.

To conclude, there was no measurable effects of the maternal plasma concentrations of PFAS on the efficiency of the transfer mechanisms into the yolk. However, the physicochemical characteristics of the different compounds (carbon chain length in particular) seem to affect the transfer, with a likely facilitated transportation of the longest chain PFCAs into the yolk. Effects

of these long chain compounds on the development of seabird embryos are thus crucial to investigate as development represents a critical period to contaminants exposure. As highly contaminated females lay highly contaminated eggs, kittiwake embryos might be at strong risk as they are impacted twice by the female exposure to contaminants: 1/ via impaired incubation and chick-rearing behaviors and 2/ via maternal transfer to the egg with impaired embryonic and early days physiology and development. Therefore, for further studies, estimating the outcomes of the maternal transfer of PFAS on avian development physiology is essential to assess if this leads nestlings to a bad start in life, especially for emerging fluorinated compounds which are under-represented in experimental studies.

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## Supporting Information

# A bad start in life? Maternal transfer of mercury, legacy and emerging poly- and perfluoroalkyl substances in an Arctic seabird

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**Internal standards used in PFAS analyses**

Perfluoro-n-[2,3,4-<sup>13</sup>C<sub>3</sub>]butanoic acid

Perfluoro-n-[<sup>13</sup>C<sub>5</sub>]pentanoic acid

Perfluoro-n-[1,2,3,4,6-<sup>13</sup>C<sub>5</sub>]hexanoic acid

Perfluoro-n-[1,2,3,4-<sup>13</sup>C<sub>4</sub>]heptanoic acid

Perfluoro-n-[<sup>13</sup>C<sub>8</sub>]octanoic acid

Perfluoro-n-[<sup>13</sup>C<sub>9</sub>]nonanoic acid

Perfluoro-n-[1,2,3,4,5,6-<sup>13</sup>C<sub>6</sub>]decanoic acid

Perfluoro-n-[1,2,3,4,5,6,7-<sup>13</sup>C<sub>7</sub>]undecanoic acid

Perfluoro-n-[1,2-<sup>13</sup>C<sub>2</sub>]dodecanoic acid

Perfluoro-n-[1,2-<sup>13</sup>C<sub>2</sub>]tetradecanoic acid

Sodium perfluoro-1-[2,3,4-<sup>13</sup>C<sub>3</sub>]butanesulfonate

Sodium perfluoro-1-[1,2,3-<sup>13</sup>C<sub>3</sub>]hexanesulfonate

Sodium perfluoro-1-[<sup>13</sup>C<sub>8</sub>]octanesulfonate

Perfluoro-1-[<sup>13</sup>C<sub>8</sub>]octanesulfonamide

Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-<sup>13</sup>C<sub>2</sub>]-octane sulfonate

Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-<sup>13</sup>C<sub>2</sub>]-decane sulfonate

**Table S1.** List of targeted poly- and perfluoroalkyl substances (PFAS): their groups, abbreviations, chemical names, detection rates in females and eggs of black-legged kittiwakes, limits of detection in plasma and yolk and descriptive statistics (mean  $\pm$  standard deviation *SD*; values <LOD not replaced by ½ LOD). Selected PFAS (i.e. detected in more than 70% of both samples and therefore used in analyses) are stressed in bold. PFAS considered as legacy compounds are in black, those considered as emerging are in grey.

Group	Abbreviation	Chemical name	Detection rate		Limit of detection (ng g <sup>-1</sup> ww)	Mean $\pm$ <i>SD</i>	
			Females	Eggs		Females	Eggs
Perfluoroalkane sulfonic acids	PFBS	perfluorobutanesulfonic acid	0.0%	0.0%	0.10	na	na
	PFPS	perfluoropentanesulfonic acid	0.0%	0.0%	0.05	na	na
	<b>PFHxS</b>	<b>perfluorohexanesulfonic acid</b>	<b>100.0%</b>	<b>100.0%</b>	<b>0.05</b>	<b>0.23 <math>\pm</math> 0.09</b>	<b>0.24 <math>\pm</math> 0.12</b>
	PFHpS	perfluoroheptanesulfonic acid	0.0%	44.0%	0.08	na	0.10 $\pm$ 0.03
	<b>brPFOS</b>	<b>branched perfluorooctanesulfonic acid</b>	<b>85.7%</b>	<b>100.0%</b>	<b>0.06</b>	<b>0.95 <math>\pm</math> 0.32</b>	<b>2.53 <math>\pm</math> 1.04</b>
	<b>linPFOS</b>	<b>linear perfluorooctanesulfonic acid</b>	<b>100.0%</b>	<b>100.0%</b>	<b>0.072</b>	<b>10.8 <math>\pm</math> 4.70</b>	<b>25.4 <math>\pm</math> 9.88</b>
	PFNS	perfluorononanesulfonic acid	0.0%	0.0%	0.06	na	na
	PFDCS	perfluorodecanesulfonic acid	23.8%	84.0%	0.10	0.12 $\pm$ 0.02	0.28 $\pm$ 0.14
Perfluoroalkyl carboxylic acids	PFBA	perfluorobutanoic acid	0.0%	0.0%	0.08	na	na
	PFPA	perfluoropentanoic acid	0.0%	0.0%	0.08	na	na
	PFHxA	perfluorohexanoic acid	0.0%	4.0%	0.08	na	0.08 $\pm$ na
	PFHpA	perfluoroheptanoic acid	9.5%	24.0%	0.04	0.06 $\pm$ 0.00	0.07 $\pm$ 0.02
	<b>PFOA</b>	<b>perfluorooctanoic acid</b>	<b>71.4%</b>	<b>96.0%</b>	<b>0.08</b>	<b>0.19 <math>\pm</math> 0.13</b>	<b>0.23 <math>\pm</math> 0.10</b>
	<b>PFNA</b>	<b>perfluorononanoic acid</b>	<b>100.0%</b>	<b>100.0%</b>	<b>0.03</b>	<b>1.06 <math>\pm</math> 0.61</b>	<b>1.88 <math>\pm</math> 0.95</b>
	<b>PFDA</b>	<b>perfluorodecanoic acid</b>	<b>100.0%</b>	<b>100.0%</b>	<b>0.03</b>	<b>1.71 <math>\pm</math> 0.89</b>	<b>3.39 <math>\pm</math> 1.67</b>
	<b>PFUnA</b>	<b>perfluoroundecanoic acid</b>	<b>100.0%</b>	<b>100.0%</b>	<b>0.06</b>	<b>6.88 <math>\pm</math> 3.21</b>	<b>16.2 <math>\pm</math> 5.53</b>
	<b>PFDoA</b>	<b>perfluorododecanoic acid</b>	<b>95.2%</b>	<b>100.0%</b>	<b>0.03</b>	<b>1.81 <math>\pm</math> 0.89</b>	<b>5.33 <math>\pm</math> 1.57</b>
	<b>PFTriA</b>	<b>perfluorotridecanoic acid</b>	<b>100.0%</b>	<b>100.0%</b>	<b>0.07</b>	<b>7.80 <math>\pm</math> 3.35</b>	<b>28.9 <math>\pm</math> 7.99</b>
	<b>PFTeA</b>	<b>perfluorotetradecanoic acid</b>	<b>100.0%</b>	<b>100.0%</b>	<b>0.08</b>	<b>0.14 <math>\pm</math> 0.60</b>	<b>5.80 <math>\pm</math> 1.80</b>
	PFHxDA	perfluorohexadecanoic acid	0.0%	84.0%	0.12	na	0.24 $\pm$ 0.08



Chapter I – Paper A: Supporting Information

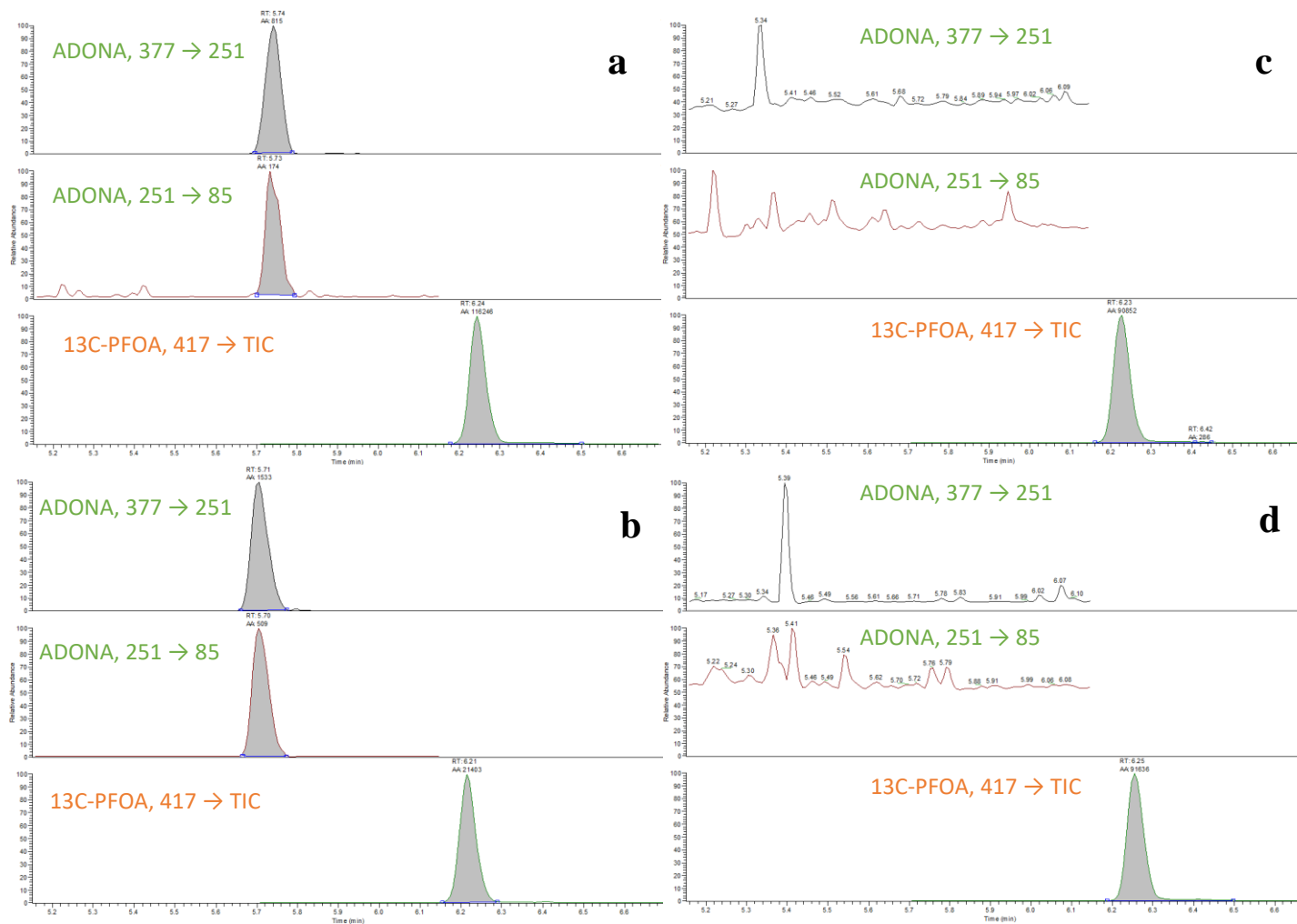
Perfluoroalkane sulfonamido substances	MeFHxSA	methyl perfluorohexane sulphonamide	0.0%	0.0%	0.05	na	na
	FOSA	perfluorooctane sulfonamide	0.0%	0.0%	0.05	na	na
	MeFOSA	methylperfluorooctane sulfonamide	0.0%	0.0%	0.10	na	na
	EtFOSA	ethylperfluorooctane sulphonamide	0.0%	0.0%	0.10	na	na
	FOSAA	perfluorooctanesulfonamidoacetic acid	0.0%	0.0%	0.10	na	na
	MeFOSAA	methyl perfluorooctane sulfonamidoacetic acid	0.0%	0.0%	0.10	na	na
	EtFOSAA	ethyl perfluorooctane sulfonamidoacetic acid	0.0%	0.0%	0.10	na	na
fluorotelo mer sulfonic acid	4:2 FTS	4:2 fluorotelomer sulfonic acid	0.0%	0.0%	0.05	na	na
	6:2 FTS	6:2 fluorotelomer sulfonic acid	0.0%	0.0%	0.05	na	na
	8:2 FTS	8:2 fluorotelomer sulfonic acid	0.0%	0.0%	0.10	na	na
	10:2 FTS	10:2 fluorotelomer sulfonic acid	0.0%	0.0%	1.00	na	na
fluoro elomer carbox ylic	3:3 FTCA	3:3 fluorotelomer carboxylic acid	0.0%	0.0%	0.50	na	na
	5:3 FTCA	5:3 fluorotelomer carboxylic acid	0.0%	0.0%	0.10	na	na
	7:3 FTCA	7:3 fluorotelomer carboxylic acid	0.0%	84.0%	0.10	na	0.21 ± 0.09
Fluoro alkylet her compo	F-53B	6:2 and 8:2 chlorinated polyfluorinated ether sulfonate	0.0%	0.0%	0.10	na	na
	ADONA	dodecafluoro-3H-4,8-dioxanonanoate	0.0%	4.0%	0.10	na	0.11 ± na
	GenX	hexafluoropropylene oxide dimer acid	0.0%	0.0%	1.00	na	na

**Table S2.** Descriptive statistics (mean  $\pm$  standard deviation *SD*, median and range *min-max*) for PFAS detected in more than 70% of the samples concentrations in eggs only (ng g<sup>-1</sup> ww) in plasma and egg yolk and albumen of black-legged kittiwakes from Svalbard.

	<b>First laid eggs</b>			<b>Second laid eggs</b>		
	<b>Mean <math>\pm</math> SD</b>	<b>Median</b>	<b>Min-max</b>	<b>Mean <math>\pm</math> SD</b>	<b>Median</b>	<b>Min-max</b>
PFDcS	0.32 $\pm$ 0.15	0.26	0.13 - 0.64	0.22 $\pm$ 0.08	0.2	0.11 - 0.37
PFHxDA	0.29 $\pm$ 0.07	0.29	0.16 - 0.38	0.19 $\pm$ 0.04	0.2	0.13 - 0.24
7:3 FTCA	0.25 $\pm$ 0.09	0.23	0.11 - 0.42	0.16 $\pm$ 0.05	0.15	0.10 - 0.25

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**Figure S1.** Chromatograms of the sample (a), the standard (b), the blank (c) and the standard reference material (d) for the measurement of ADONA in black-legged kittiwake eggs; two ion traces are provided for ADONA and one for the internal standard (<sup>13</sup>C-PFOA).



**Table S3.** Model selections for the relationship between PFAS and biological variables in black-legged kittiwake eggs, based on the lowest second-order Akaike’s Information Criterion corrected for small sample sizes (AICc); the predicting variables were the laying date, the egg mass, the rank of the eggs in the laying order (Egg number) as well as both interactions between the laying date or the egg mass and the egg number. The most parsimonious model is given in bold. AICcwt: Akaike’s weight;  $\Delta$ AICc: difference between the model with the smallest AICc-value and the model of interest (*i*).

## PFHxS

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod13</b>						<b>3</b>	<b>-37.53</b>	<b>0.26</b>	<b>0.00</b>
mod10	X					4	-37.24	0.22	0.29
mod5	X		X	X		6	-37.12	0.21	0.41
mod12			X			4	-35.22	0.08	2.31
mod11		X				4	-34.82	0.07	2.71
mod8	X		X			5	-34.23	0.05	3.30
mod7	X	X				5	-34.09	0.05	3.44
mod2	X	X	X	X		7	-33.53	0.03	4.00
mod9		X	X			5	-32.07	0.02	5.46
mod4	X	X	X			6	-30.74	0.01	6.79
mod1	X	X	X	X	X	8	-29.54	0.00	7.99
mod6		X	X		X	6	-28.56	0.00	8.97
mod3	X	X	X		X	7	-26.81	0.00	10.71

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**brPFOS**

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod12</b>			X			<b>4</b>	<b>62.22</b>	<b>0.59</b>	<b>0.00</b>
mod8	X		X			5	64.79	0.16	2.57
mod9		X	X			5	65.05	0.14	2.83
mod5	X		X	X		6	67.60	0.04	5.37
mod4	X	X	X			6	68.18	0.03	5.96
mod6		X	X		X	6	68.55	0.02	6.32
mod2	X	X	X	X		7	71.25	0.01	9.03
mod3	X	X	X		X	7	72.09	0.00	9.87
mod1	X	X	X	X	X	8	75.57	0.00	13.35
mod7	X	X				5	76.97	0.00	14.75
mod13						3	78.49	0.00	16.27
mod11		X				4	80.52	0.00	18.30
mod10	X					4	80.64	0.00	18.42

**linPFOS**

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod12</b>			<b>X</b>			<b>4</b>	<b>170.51</b>	<b>0.54</b>	<b>0.00</b>
mod8	X		X			5	172.15	0.24	1.64
mod9		X	X			5	173.67	0.11	3.16
mod4	X	X	X			6	175.64	0.04	5.13
mod5	X		X	X		6	175.65	0.04	5.14
mod6		X	X		X	6	177.17	0.02	6.66
mod3	X	X	X		X	7	179.56	0.01	9.04
mod2	X	X	X	X		7	179.56	0.01	9.05
mod1	X	X	X	X	X	8	183.96	0.00	13.45
mod13						3	188.70	0.00	18.19
mod7	X	X				5	189.17	0.00	18.65
mod11		X				4	189.45	0.00	18.94
mod10	X					4	190.43	0.00	19.92

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## PFOA

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod13</b>						<b>3</b>	<b>-46.46</b>	<b>0.23</b>	<b>0.00</b>
mod4	X	X	X			6	-46.39	0.22	0.06
mod12			X			4	-45.16	0.12	1.30
mod8	X		X			5	-44.78	0.10	1.68
mod9		X	X			5	-44.65	0.09	1.81
mod11		X				4	-43.92	0.06	2.54
mod10	X					4	-43.66	0.06	2.80
mod2	X	X	X	X		7	-42.53	0.03	3.93
mod3	X	X	X		X	7	-42.47	0.03	3.98
mod5	X		X	X		6	-41.28	0.02	5.18
mod6		X	X		X	6	-41.17	0.02	5.29
mod7	X	X				5	-40.82	0.01	5.64
mod1	X	X	X	X	X	8	-38.13	0.00	8.33

## PFNA

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod12</b>			X			<b>4</b>	<b>60.17</b>	<b>0.41</b>	<b>0.00</b>
mod13						3	62.66	0.12	2.49
mod8	X		X			5	62.85	0.11	2.68
mod9		X	X			5	63.30	0.09	3.14
mod11		X				4	63.48	0.08	3.31
mod10	X					4	63.81	0.07	3.65
mod5	X		X	X		6	64.39	0.05	4.22
mod7	X	X				5	65.35	0.03	5.18
mod4	X	X	X			6	66.33	0.02	6.17
mod6		X	X		X	6	66.54	0.02	6.37
mod2	X	X	X	X		7	68.30	0.01	8.14
mod3	X	X	X		X	7	70.04	0.00	9.87
mod1	X	X	X	X	X	8	71.96	0.00	11.79

## Chapter I – Paper A: Supporting Information

**PFDcA**

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod12</b>			<b>X</b>			<b>4</b>	<b>82.43</b>	<b>0.54</b>	<b>0.00</b>
mod9		X	X			5	85.26	0.13	2.84
mod8	X		X			5	85.32	0.13	2.89
mod5	X		X	X		6	86.51	0.07	4.09
mod10	X					4	88.61	0.02	6.18
mod4	X	X	X			6	88.62	0.02	6.19
mod6		X	X		X	6	88.72	0.02	6.30
mod7	X	X				5	89.03	0.02	6.60
mod11		X				4	89.93	0.01	7.50
mod2	X	X	X	X		7	90.23	0.01	7.80
mod13						3	92.19	0.00	9.76
mod3	X	X	X		X	7	92.49	0.00	10.07
mod1	X	X	X	X	X	8	94.27	0.00	11.85

**PFUnA**

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod12</b>			<b>X</b>			<b>4</b>	<b>138.50</b>	<b>0.43</b>	<b>0.00</b>
mod8	X		X			5	139.42	0.27	0.92
mod9		X	X			5	140.80	0.14	2.30
mod4	X	X	X			6	142.39	0.06	3.89
mod5	X		X	X		6	142.84	0.05	4.34
mod6		X	X		X	6	144.31	0.02	5.81
mod2	X	X	X	X		7	146.17	0.01	7.67
mod3	X	X	X		X	7	146.30	0.01	7.80
mod1	X	X	X	X	X	8	150.55	0.00	12.05
mod7	X	X				5	156.77	0.00	18.27
mod11		X				4	158.30	0.00	19.80
mod13						3	159.50	0.00	21.00
mod10	X					4	160.91	0.00	22.41

## Chapter I – Paper A: Supporting Information

**PFD<sub>o</sub>A**

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AIC <sub>cwt</sub>	ΔAICc
mod8	X		X			5	78.49	0.51	0.00
<b>mod12</b>			<b>X</b>			<b>4</b>	<b>80.36</b>	<b>0.20</b>	<b>1.86</b>
mod5	X		X	X		6	81.47	0.11	2.97
mod4	X	X	X			6	81.99	0.09	3.49
mod9		X	X			5	83.45	0.04	4.96
mod3	X	X	X		X	7	84.97	0.02	6.47
mod2	X	X	X	X		7	85.38	0.02	6.89
mod6		X	X		X	6	86.03	0.01	7.53
mod1	X	X	X	X	X	8	88.38	0.00	9.88
mod13						3	99.39	0.00	20.89
mod11		X				4	101.16	0.00	22.66
mod10	X					4	102.08	0.00	23.58
mod7	X	X				5	104.21	0.00	25.71

**PFTriA**

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AIC <sub>cwt</sub>	ΔAICc
mod8	X		X			5	161.79	0.37	0.00
<b>mod12</b>			<b>X</b>			<b>4</b>	<b>162.14</b>	<b>0.31</b>	<b>0.35</b>
mod5	X		X	X		6	164.45	0.10	2.65
mod9		X	X			5	164.73	0.09	2.94
mod4	X	X	X			6	165.12	0.07	3.33
mod6		X	X		X	6	167.57	0.02	5.78
mod2	X	X	X	X		7	168.12	0.02	6.32
mod3	X	X	X		X	7	168.39	0.01	6.60
mod1	X	X	X	X	X	8	171.35	0.00	9.56
mod13						3	180.97	0.00	19.18
mod11		X				4	182.00	0.00	20.21
mod10	X					4	183.82	0.00	22.03
mod7	X	X				5	184.66	0.00	22.87



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**PFTeA**

	Laying date	Egg mass	Egg number	Laying date : Egg number	Egg mass : Egg number	K	AICc	AICcwt	ΔAICc
<b>mod12</b>			<b>X</b>			<b>4</b>	<b>89.20</b>	<b>0.44</b>	<b>0.00</b>
mod8	X		X			5	90.17	0.27	0.97
mod9		X	X			5	92.15	0.10	2.95
mod5	X		X	X		6	92.52	0.08	3.32
mod4	X	X	X			6	93.64	0.05	4.44
mod6		X	X		X	6	94.83	0.03	5.63
mod2	X	X	X	X		7	96.37	0.01	7.17
mod3	X	X	X		X	7	96.73	0.01	7.54
mod1	X	X	X	X	X	8	99.18	0.00	9.98
mod13						3	106.09	0.00	16.89
mod11		X				4	106.72	0.00	17.52
mod10	X					4	108.61	0.00	19.41
mod7	X	X				5	108.64	0.00	19.44

**Table S4.** Model selections for maternal transfer ratios of PFAS in black-legged kittiwakes, based on the lowest second-order Akaike’s Information Criterion corrected for small sample sizes (AICc); the predicting variables were the carbon chain length of PFCAs compounds, the rank of the eggs in the laying order (Egg number) as well as the interaction between these variables; see Table S3 for additional information.

	Carbon chain length	Egg number	Carbon chain length : Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod1</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>6</b>	<b>130.12</b>	<b>0.98</b>	<b>0.00</b>
mod2	X	X		5	138.39	0.02	8.27
mod3	X			4	165.48	0.00	35.36
mod4		X		4	254.96	0.00	124.84
mod5				3	267.96	0.00	137.85

**Table S5.** Model selections for maternal transfer of PFAS in black-legged kittiwakes, based on the lowest second-order Akaike's Information Criterion corrected for small sample sizes (AICc); the predicting variables were the female contamination and the number of the eggs in the laying order (Egg number); see Table S3 for additional information.

<b>PFHxS</b>						
	<b>Females contamination</b>	<b>Egg number</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>	<b>X</b>		<b>4</b>	<b>-44.55</b>	<b>0.77</b>	<b>0.00</b>
mod1	X	X	5	-45.12	0.23	2.43
mod4			3	-37.53	0.01	10.03
mod3		X	4	-35.22	0.00	12.33

<b>brPFOS</b>						
	<b>Females contamination</b>	<b>Egg number</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod1</b>	<b>X</b>	<b>X</b>	<b>5</b>	<b>56.07</b>	<b>0.96</b>	<b>0.00</b>
mod3		X	4	62.22	0.04	6.15
mod2	X		4	71.59	0.00	15.52
mod4			3	78.49	0.00	22.42

<b>linPFOS</b>						
	<b>Females contamination</b>	<b>Egg number</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod1</b>	<b>X</b>	<b>X</b>	<b>5</b>	<b>162.91</b>	<b>0.98</b>	<b>0.00</b>
mod3		X	4	170.51	0.02	7.60
mod2	X		4	182.11	0.00	19.20
mod4			3	188.70	0.00	25.79

<b>PFOA</b>						
	<b>Females contamination</b>	<b>Egg number</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod4</b>			<b>3</b>	<b>-46.46</b>	<b>0.35</b>	<b>0.00</b>
mod2	X		4	-46.34	0.33	0.12
mod3		X	4	-45.16	0.18	1.30
mod1	X	X	5	-44.76	0.15	1.70

<b>PFNA</b>						
	<b>Females contamination</b>	<b>Egg number</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod1</b>	<b>X</b>	<b>X</b>	<b>5</b>	<b>52.93</b>	<b>0.78</b>	<b>0.00</b>
mod2	X		4	55.76	0.19	2.84
mod3		X	4	60.17	0.02	7.24
mod4	X	X	3	62.66	0.01	9.73

**PFDcA**

	Females contamination	Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod1</b>	<b>X</b>	<b>X</b>	<b>5</b>	<b>73.19</b>	<b>0.99</b>	<b>0.00</b>
mod3		X	4	82.43	0.01	9.23
mod2	X		4	83.05	0.00	10.76
mod4			3	92.19	0.00	19.00

**PFUnA**

	Females contamination	Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod1</b>	<b>X</b>	<b>X</b>	<b>5</b>	<b>136.09</b>	<b>0.77</b>	<b>0.00</b>
mod3		X	4	138.50	0.23	2.41
mod2	X		4	158.19	0.00	22.10
mod4			3	159.50	0.00	23.41

**PFDoA**

	Females contamination	Egg number	K	AICc	AICcwt	$\Delta$ AICc
mod1	X	X	5	80.30	0.51	0.00
<b>mod3</b>		<b>X</b>	<b>4</b>	<b>80.36</b>	<b>0.49</b>	<b>0.06</b>
mod4			3	99.39	0.00	19.09
mod2	X		4	99.77	0.00	19.48

**PFTriA**

	Females contamination	Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod1</b>	<b>X</b>	<b>X</b>	<b>5</b>	<b>157.85</b>	<b>0.90</b>	<b>0.00</b>
mod3		X	4	162.14	0.10	4.29
mod2	X		4	179.73	0.00	21.88
mod4			3	180.97	0.00	23.12

**PFTeA**

	Females contamination	Egg number	K	AICc	AICcwt	$\Delta$ AICc
<b>mod1</b>	<b>X</b>	<b>X</b>	<b>5</b>	<b>86.33</b>	<b>0.81</b>	<b>0.00</b>
mod3		X	4	89.20	0.19	2.87
mod2	X		4	104.74	0.00	18.41
mod4			3	106.09	0.00	19.77



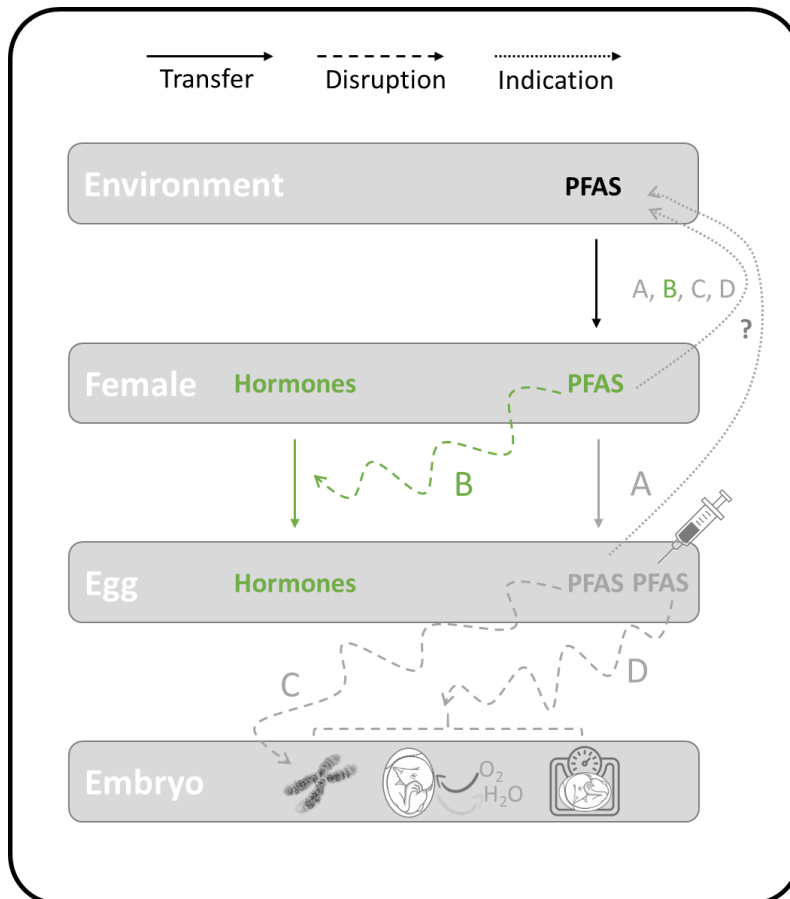
# CHAPTER II



*Young bearded seal (Erignathus barbatus) resting on an ice floe*

## Chapter II

In birds, hormone-mediated maternal effects are suggested to be first-order mechanisms shaping the phenotype of the future offspring to match the environmental conditions at hatching. Any disruption of the maternal deposition of hormones in eggs may therefore have long-term adverse consequences on offspring fitness. In this chapter, we examined the outcomes of kittiwake females circulating PFAS, known endocrine disrupting chemicals, on the maternal hormonal transfer in their eggs (**Figure 15**).



*Figure 15. Schematic representation of Chapter II goals included in the global structure of this thesis.*

### We found

- No relationship between hormone concentrations in females and their eggs
- A positive correlation between some long-chain PFCAs in females and testosterone deposited in eggs.

# Paper B

Does contaminant exposure disrupt maternal hormones  
deposition? A study on per- and polyfluoroalkyl  
substances in an Arctic seabird

**Jouanneau, W.,** Léandri-Breton, D-J., Herzke, D., Moe, B., Nikiforov, V. A., Pallud, M.,  
Parenteau, C., Gabrielsen, G. W., Chastel, O.

**Under review in *Science of the Total Environment***



# Does contaminant exposure disrupt maternal hormones deposition? A study on per- and polyfluoroalkyl substances in an Arctic seabird

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## ABSTRACT

Maternal effects are thought to be essential tools for females to modulate offspring development. The selective deposition of avian maternal hormones could therefore allow females to strategically adjust the phenotype of their offspring to the environmental situation encountered. However, at the time of egg formation, several contaminants are also transferred to the egg, including per- and polyfluoroalkyl substances (PFAS) which are ubiquitous organic contaminants with endocrine disrupting properties. It is, however, unknown if they can disrupt maternal hormone deposition. In this study we explored relationships between female PFAS burden and maternal deposition in the eggs of steroids (dihydrotestosterone, androstenedione and testosterone), glucocorticoids (corticosterone) and thyroid hormones (triiodothyronine and thyroxine) in a population of Arctic-breeding black-legged kittiwake (*Rissa tridactyla*). Egg yolk hormone levels were unrelated to female hormone plasma levels. Second-laid eggs had significantly lower concentrations of androstenedione than first-laid eggs. Triiodothyronine yolk levels were decreasing with increasing egg mass but increasing with increasing females' body condition. Testosterone was the only transferred yolk hormone correlated to maternal PFAS burden: specifically, we found a positive correlation between testosterone in yolks and circulating maternal perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDCa) and perfluoroundecanoic acid (PFUnA) in first-laid eggs. This correlative study provides a first insight on the potential of some long-chain perfluoroalkyl acids to disrupt maternal hormones deposition in egg and raises the question of the consequences of increased testosterone deposition on the developing embryo.

Key-word: Black-legged kittiwake, PFAS, Maternal effect, Corticosterone, Testosterone, Thyroid hormones.

## INTRODUCTION

Freshly laid avian eggs contain, in addition to basic resources, substantial levels of maternal hormones (Groothuis *et al.* 2005; Schwabl 1993). The amount of maternal hormones deposited in the egg yolk is known to be influenced by environmental factors (e.g., abiotic conditions (Lessells *et al.* 2016), food availability (Verboven *et al.* 2003) and social interactions (Pilz and Smith 2004)), the offspring characteristics (e.g., sex of the embryo (Müller *et al.* 2002), laying date (Tomita *et al.* 2011), position of the egg in the laying order (Schwabl 1993), composition of the egg (Groothuis *et al.* 2006)) and the state of the mother bird (Tschirren *et al.* 2009; e.g., her age, physical condition, past experience, genetic background) or the male quality (Rutkowska *et al.* 2020). Although mainly explored for steroids, experimental prenatal exposure to maternal hormones *in ovo* have shown to impact hatching date and offspring growth (von Engelhardt *et al.* 2006), metabolism (Tobler *et al.* 2007), immune system (Navara *et al.* 2005), sex-ratio (Rubolini *et al.* 2006), behavior (Possenti *et al.* 2018) and survival (Saino *et al.* 2005). Maternal hormones, in interaction with the offspring and maternal genotype, are believed to behave as messengers enabling adaptive maternal effects to prepare the offspring to prevailing environmental conditions (Mousseau and Fox 1998; von Engelhardt and Groothuis 2011). Independent regulation between circulating and transferred maternal hormones is an essential prerequisite for hormones to be adaptive regulators of offspring development. Although very little is known about transfer mechanisms, the literature suggests that such a separate control over deposition and response may at least be possible (Della Costa *et al.* 2020; Groothuis and Schwabl 2008; Kumar *et al.* 2019; Okuliarova *et al.* 2018). Being the results of a coevolutionary process between maternal transfer and offspring responses to these substances, such intergenerational effects may either have beneficial or even detrimental outcomes for the mother and/or the offspring (Hsu *et al.* 2020a; Marshall and Uller 2007). Indeed, maternal effects may increase offspring fitness, but also reduce it to increase maternal survival in unprofitable conditions. Maternal hormones and offspring's response should therefore match an optimal trade-off for a given situation.

In that context, any disruption of the transfer of maternal hormones may be detrimental not only to the offspring but also to the siblings and the parents (Bebbington and Groothuis 2021; Groothuis *et al.* 2019). In birds, endocrine pathways are known to be affected by various environmental contaminants (trace elements, organic compounds; Tan *et al.* 2009; Tyler *et al.* 1998), which are transferred to the yolk (Verreault *et al.* 2006), and thus potentially disrupting maternal effects (Giesy *et al.* 2003; Marlatt *et al.* 2021; Metcalfe *et al.* 2022). The effects of

contaminants on maternal hormones deposition has been however seldom investigated: legacy persistent organic pollutants (POPs: organochlorines, brominated flame retardants) altered maternal steroids deposition in glaucous gulls (*Larus hyperboreus*) eggs (Verboven *et al.* 2008), but not in common tern (*Sterna hirundo*; French 2001) and great tits (*Parus major*; Ruuskanen *et al.* 2019).

There is a clear need for further investigations on the effects of contaminants on maternal hormones deposition. Notably since most of the work has focussed on steroids, and not on other hormones transferred to the eggs (e.g., glucocorticoids and thyroid hormones; von Engelhardt and Groothuis 2011). Moreover, the impact of some contaminants of growing concern on maternal endocrine effects have not been yet investigated. Among them are per- and polyfluoroalkyl substances (PFAS), a family of synthetic compounds used as surface-active agents in a multitude of manufactured products: firefighting foams, waterproof clothing, non-stick cookware, coatings, food packaging; Buck *et al.* 2011). PFAS can be transferred to the yolk (Bertolero *et al.* 2015; Gebbink and Letcher 2012; Jouanneau *et al.* 2022). However, despite the known PFAS endocrine disrupting capabilities (Chen *et al.* 2019; Coperchini *et al.* 2020; Di Nisio *et al.* 2020; Di Nisio *et al.* 2019; Jensen and Leffers 2008; Rickard *et al.* 2022; Shi *et al.* 2019), to date our knowledge on that matter remains extremely limited in wildlife. Indeed, some few correlative studies on birds suggests that PFAS may affect concentrations of thyroid hormones, corticosterone and prolactin, potentially leading to effects on physiology, parental investment and incubation behaviours (Ask *et al.* 2021; Blévin *et al.* 2020; Mortensen *et al.* 2020; Nøst *et al.* 2012; Sebastiano *et al.* 2020b; Tartu *et al.* 2014). The disrupting mechanisms are not well identified, but likewise other endocrine-disrupting compounds, PFAS may cause an alteration of the synthesis and breakdown, the release, transport and binding or elimination of endogenous hormones (Metcalf *et al.* 2022). Specifically, some studies showed that some PFAS are, for example, particularly prone to bind to hormones transport proteins (Jones *et al.* 2003; Kar *et al.* 2017; Mortensen *et al.* 2020). Thus, regarding maternal effects, any disruption of the formation of maternal hormones or competitive binding by PFAS to transport proteins may lead to the deterioration of maternal hormones transfer and consequently to the transmission of maladaptive information to the offspring.

The aim of the present study is to investigate maternal hormone deposition in eggs of Arctic-breeding black-legged kittiwake (*Rissa tridactyla*; hereafter “kittiwake”), known to be dietary exposed to PFAS in the wild (Blévin *et al.* 2017a; Jouanneau *et al.* 2022; Tartu *et al.* 2014). Specifically, we studied the relationship between female PFAS burden, and yolk

maternal glucocorticoids (corticosterone), steroids (testosterone, dihydrotestosterone and androstenedione), and thyroid hormones (triiodothyronine and thyroxine), all known to be transferred to the eggs and to play a key role in embryo development (Groothuis *et al.* 2005; Groothuis and Schwabl 2002; 2008; Ruuskanen *et al.* 2016; Sarraude *et al.* 2020a; Sarraude *et al.* 2020c). By sampling females during the pre-laying stage (i.e., during egg formation), and their eggs as soon as they were laid, we provide a valuable insight of the relationship between maternal circulating and maternally deposited hormones, and shed light on the potential impacts of maternal PFAS on maternal transfer. Given the established endocrine disrupting properties of PFAS, we hypothesized a possible alteration of the maternal hormone's deposition, via a disrupted transfer of some hormones in eggs in the most PFAS-contaminated females.

## MATERIAL AND METHODS

### **Study area and sample collection**

We carried out the field study between May and June 2019 in a kittiwake colony in Kongsfjorden, Svalbard (see Jouanneau *et al.* 2022 for further information on the field operations). Briefly, females from 14 nests were captured using a noose at the end of a fishing rod, during the pre-laying period (i.e., at the time of egg formation), and immediately sampled for 2 mL of blood from the alar vein. Skull length (head + bill) was measured with an accuracy of 0.1 mm using a calliper and birds were weighted to the nearest 2 g with a Pesola spring balance. The sex of individuals was confirmed by molecular sexing from red blood cells following Fridolfsson and Ellegren (1999). At the end of each day, we separated plasma and red blood cells via centrifugation and kept both samples frozen (-20 °C) until analyses. In this species, females usually lay a two-egg clutch (Moe *et al.* 2009). We monitored those nests daily and collected first- ( $n = 14$ ) and second-laid eggs ( $n = 11$ ) less than 24h after laying. Hormones measured in freshly laid eggs, as we did in the present study, are entirely synthesised by the females before being transferred to the eggs (von Engelhardt and Groothuis 2011). We weighted the eggs in the laboratory to the nearest 0.01 g, then separated yolk and albumen into microtubes and kept both samples frozen until analyses. Field operations were approved by the Governor of Svalbard and by the Norwegian Animal Research Authority (NARA, permit number 19970).

### **Per- and polyfluoroalkyl substances analysis**

We adapted a method from Powley *et al.* (2005), contaminants analysis method and concentrations have been extensively described in a previous study on PFAS maternal transfer (Jouanneau *et al.* 2022). Only compounds detected in more than 70 % of the females were kept for further investigations (see supplementary information (SI), Table S1 for a list of all measured compounds and detection percentages). Consequently, only the following compounds were kept for statistical analyses: perfluorohexanesulfonic acid (PFHxS), branched perfluorooctanesulfonic acid (brPFOS), linear perfluorooctanesulfonic acid (linPFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDCa), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriA) and perfluorotetradecanoic acid (PFTeA). For all included PFAS, values lower than the limit of detection (LOD) were set to half of the LOD of the specific compound. We provided all concentrations in ng g<sup>-1</sup> wet weight (ww).

### **Hormones analyses**

We measured the following steroid and thyroid hormones in female plasma and egg yolk: dihydrotestosterone (DHT), androstenedione (AND), testosterone (T), corticosterone (CORT), triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>). Extraction of the plasma and yolk samples is detailed for all hormones in the SI.

#### **- Steroid hormones (DHT, AND, T and CORT)**

The four hormones were assayed with similar procedures: Radio-immunoassay (RIA) or Enzyme Linked ImmunoSorbent Assay (ELISA) methods. CORT and T were measured with RIA method, 100 µL of extract were incubated overnight at 4 °C with 4000 cpm of the appropriate H<sup>3</sup>-steroid (Perkin Elmer, US) and polyclonal antiserum (Ab). Anti-testosterone Ab was provided by Dr. Picaper (Nuclear medicine, CHU La Source, Orléans, France). The two anti-corticosterone Ab (one for plasma and one for yolk) were supplied by Merck, Sigma Aldrich, France. The bound fraction was then separated from the free fraction by addition of dextran-coated charcoal and the activity was counted on a Tri-Carb 2810TR scintillation counter (Perkin Elmer, US). AND and DHT were assayed with commercial ELISA kits supplied by IBL International (AND and DHT in yolk, ref. DB 52161 and DB 52021 respectively), Abnova (AND in plasma, ref. KA 1898) and Demeditec (DHT in plasma, ref. DE 2330).

- **Thyroid hormones (T<sub>3</sub> and T<sub>4</sub>)**

Total T<sub>3</sub> and T<sub>4</sub> were measured with RIA method, 25 µL of yolk extract or plasma were incubated overnight at 4 °C with 10 000 cpm of the appropriate I<sup>125</sup>-steroid (Perkin Elmer, US) and polyclonal rabbit antiserum (Sigma Aldrich, France). The bound fraction was then separated from free fraction by addition of a polyclonal sheep antiserum against rabbit antiserum and the activity was counted on a Gamma Wizard 2470 counter (Perkin Elmer, US).

**Quality control**

We previously validated RIA and ELISA hormones assays on kittiwakes plasma and yolk. We diluted all extracts in the appropriate assay buffer, their displacement regression lines were all parallel to the standard ones. To guarantee the quality and control for reproducibility and precision of the radioimmunoassays, hormone standards, blanks, total activity and non-specific activity were concurrently analysed. We ran all samples in duplicate in one or two runs. Additional information on the hormone analyses quality control can be found in SI, minimal detectable and intra-assay variation for plasma and yolk can be found in SI, Table S2. Samples below the LOD ( $n = 6$ ) were replaced with a value equal to half of the LOD of the specific hormone. We provided all plasma concentrations in ng mL<sup>-1</sup>, and yolk concentrations in ng g<sup>-1</sup> ww. Bleeding time was kept as short as possible (mean ± SD: 02 min 09 sec ± 36 sec) and was not related to baseline CORT levels in plasma (Linear model:  $F_{1, 12} = 0.15, p = 0.70$ ).

**Statistical analyses**

**Preliminary analyses**

All statistical analyses were performed using R (v. 4.0.0; R Core Team, 2020). We tested the normality of residuals and visually inspected diagnostic plots for each models, to check whether the data met the linear model assumptions (Zuur *et al.* 2009). First, we investigated the relationship between each hormone or PFAS in females' plasma and the sampling date using linear models (LM). If the relationship was significant ( $p < 0.05$ ) we used the residuals of the LM instead of the specific hormone or PFAS in the following analyses. This enabled us to obtain comparable individuals by minimizing the variance due to the sampling time. T<sub>3</sub> and PFTeA were significantly decreasing with time in females (LM:  $F_{1, 12} = 7.06, p = 0.02$  and  $F_{1, 12} = 6.56, p = 0.03$  respectively) and residuals of the LM were consequently used in subsequent analyses.

### **Factors influencing hormones maternal transfer**

We used linear mixed-effect models (LMMs, package “nlme”) to investigate the effect of females and eggs characteristics on the concentrations of each transferred hormone independently (i.e., one model per hormone). The full models included the maternal circulating concentration for the specific hormone, the female’s scaled mass index (a proxy of the body condition of the female calculated following Peig and Green (2009)), the egg mass (used as a proxy of egg quality), the rank of the egg in the clutch by laying order (egg rank: 1 or 2) and the laying date of the first-laid eggs as fixed factors. For all full LMMs, we built a set of models ranging from the full to the null model with all possible combination of predictors and we selected the best model according to the Akaike’s Information Criterion for small sample size (AICc; i.e., the most parsimonious one among those with a  $\Delta\text{AICc} < 2$ ; package “AICcmodavg”). In all LMMs, the nest ID was used as a random variable to account for the relation between both eggs of a clutch and therefore avoid pseudo-replication.

### **Relationship between PFAS concentration in females and maternal hormones in their eggs.**

We assessed the effect of each maternal circulating PFAS on the transfer of each maternal hormone in eggs using LMs. As females were sampled before they laid their first egg, and since females may excrete a significant amount of PFAS during the laying process, we only included first-laid eggs in these analyses to avoid biased results. We built one model per hormone. For each PFAS independently, the full model included the specific PFAS as fixed factor as well as any variables identified as having a significant impact on the specific maternal hormone concentrations in the yolk in section 2.4.2. AICc selection was also performed on the full models, although only two models were compared: the full model and the full model without the PFAS variable.

## **RESULTS**

We provide the descriptive statistics for hormone concentration in females’ plasma and egg yolk as well as PFAS concentrations in females’ plasma in Table 1 & Table 2.



### **Factors influencing hormones maternal transfer**

Egg yolk hormone levels were unrelated to female hormone plasma levels (SI Table S3). First-laid eggs had significantly lower AND concentrations than second-laid eggs (SI Table S3 & Table S4; mean of first- and second-laid eggs respectively: 855 and 1030 ng g<sup>-1</sup>; confidence interval (CI) of the difference: [93.1 – 218]). We also found decreasing T<sub>3</sub> and T<sub>3</sub>/T<sub>4</sub> ratio with egg mass (CI of the slope: [-0.021 – -0.001] and [-0.282 – 0.079] respectively; SI Table S4 & Figure 1), but increasing T<sub>3</sub> and T<sub>3</sub>/T<sub>4</sub> ratio with female's scaled mass index (CI of the slope: [0.000 – 0.002] and [0.001 – 0.019] respectively; SI Table S4 & Figure 1). No other investigated egg or female characteristics affected maternal hormones deposition (SI Table S4).

### **Females PFAS contamination and maternal hormones deposition**

Yolk concentration of CORT, AND, DHT, T<sub>3</sub> and T<sub>4</sub>, as well as T<sub>3</sub>/T<sub>4</sub> ratios were not related to maternal PFAS burden in first-laid eggs (SI Table S5). The model selection showed that T was the only maternally transferred hormone related to certain female PFAS, PFNA, PFDcA and PFUnA (SI Table S5). Specifically, we found increasing T in eggs with increasing plasma circulating maternal concentrations of these three compounds with comparable strength even with different range of concentration found (PFNA:  $F_{1, 12} = 5.88$ ;  $p = 0.032$ ; CI of the slope: [0.41 – 7.75], PFDcA:  $F_{1, 12} = 5.87$ ;  $p = 0.032$ ; CI of the slope: [0.28 – 5.27], and PFUnA:  $F_{1, 12} = 6.27$ ;  $p = 0.028$ ; CI of the slope: [0.10 – 1.47]; SI Table S6 and Figure 2).

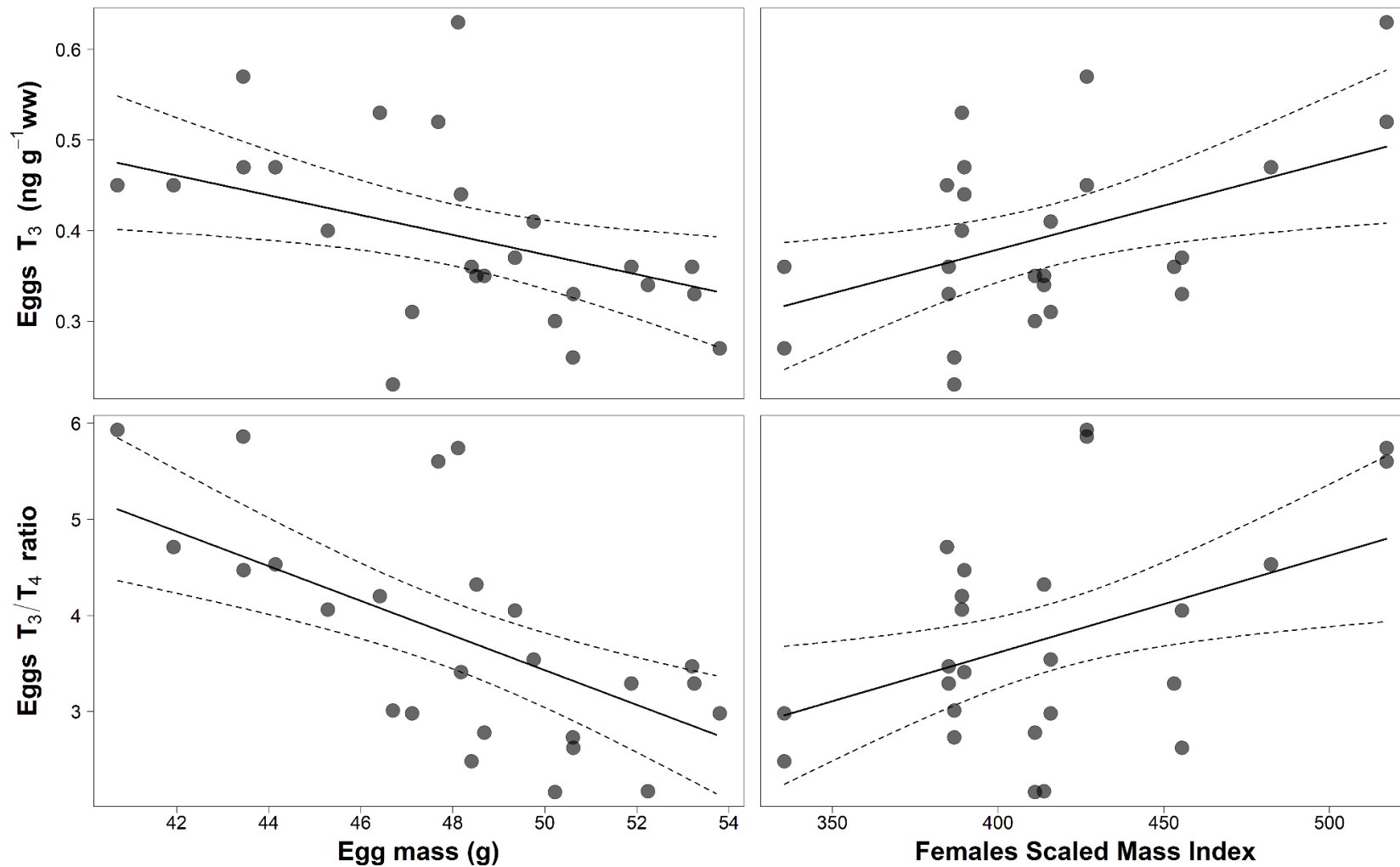
**Table 1.** Descriptive statistics (mean  $\pm$  standard deviation *SD*, median and range *min-max*) for hormones concentrations in plasma (ng mL<sup>-1</sup>) and yolk (ng g<sup>-1</sup> ww) of black-legged kittiwakes from Svalbard, samples measured below the limit of detection (LOD) included as half of the specific LOD. Dihydrotestosterone (DHT), androstenedione (AND), corticosterone (CORT), testosterone (T), triiodothyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>) and the ratio between T<sub>3</sub> and T<sub>4</sub> (T<sub>3</sub>/T<sub>4</sub>).

	Pre-laying females			Eggs yolk		
	Mean $\pm$ SD	Median	Min-max	Mean $\pm$ SD	Median	Min-max
DHT	83.5 $\pm$ 47.1	78.8	< LOD - 200	2 649 $\pm$ 856	2 603	1 419 - 4 317
AND	0.21 $\pm$ 0.17	0.16	< LOD - 0.53	932 $\pm$ 219	920	573 - 1 523
T	0.52 $\pm$ 0.43	0.33	< LOD - 1.46	16.2 $\pm$ 4.72	15.9	9.59 - 28.8
CORT	11.2 $\pm$ 7.68	7.66	4.43 - 29.0	5.37 $\pm$ 1.34	5.15	3.16 - 8.42
T <sub>3</sub>	1.04 $\pm$ 0.58	1.17	0.17 - 1.87	0.39 $\pm$ 0.10	0.36	0.23 - 0.63
T <sub>4</sub>	47.6 $\pm$ 13.8	45.1	26.8 - 78.7	10.8 $\pm$ 2.04	10.4	7.59 - 15.5
T <sub>3</sub> /T <sub>4</sub>	2.21 $\pm$ 1.33	2.14	0.48 - 4.85	3.78 $\pm$ 1.15	3.47	2.16 - 5.93

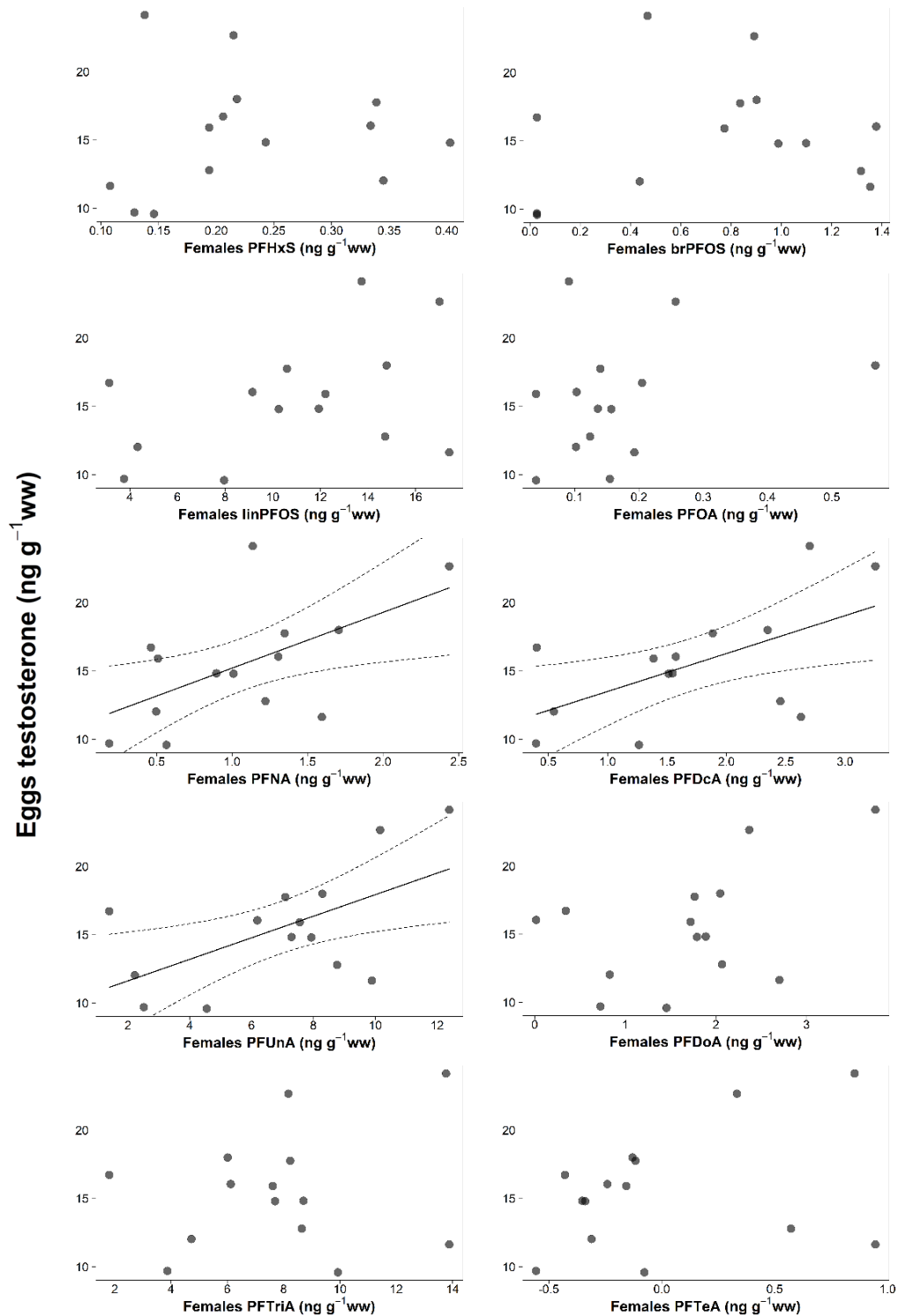
**Table 2.** Descriptive statistics (mean  $\pm$  standard deviation *SD*, median and range *min-max*) for PFAS concentrations (ng g<sup>-1</sup> ww) in plasma of female black-legged kittiwakes from Svalbard. In bold, the compounds associated with maternal T in eggs yolk.

	Mean $\pm$ SD	Median	Min-max
PFHxS	0.23 $\pm$ 0.09	0.21	0.11 - 0.40
brPFOS	0.75 $\pm$ 0.49	0.86	0.03 - 1.38
linPFOS	10.8 $\pm$ 4.70	11.3	3.14 - 17.42
PFOA	0.17 $\pm$ 0.13	0.14	0.04 - 0.57
<b>PFNA</b>	<b>1.06 <math>\pm</math> 0.61</b>	<b>1.07</b>	<b>0.19 - 2.44</b>
<b>PFDoA</b>	<b>1.71 <math>\pm</math> 1.56</b>	<b>1.56</b>	<b>0.40 - 3.26</b>
<b>PFUnA</b>	<b>6.88 <math>\pm</math> 3.22</b>	<b>7.42</b>	<b>1.40 - 12.4</b>
PFDoA	1.78 $\pm$ 0.98	1.78	0.02 - 3.76
PFTriA	7.80 $\pm$ 3.35	7.93	1.81 - 13.9
PFTeA	1.44 $\pm$ 0.60	1.53	0.471 - 2.63

**Figure 1.** Yolk maternal hormone concentrations relationship with total egg mass, in eggs of black-legged kittiwake from Svalbard for triiodothyronine ( $T_3$ ) and the ratio triiodothyronine/thyroxine ( $T_3/T_4$ ). The solid line refers to a statistically significant relationship (see SI Table S4), with dotted lines representing 95% confidence intervals.



**Figure 2.** Yolk- maternal testosterone concentration relationships with circulating PFAS in plasma of black-legged kittiwake from Svalbard. The solid line refers to a statistically significant relationship (see SI Table S6), with dotted lines representing 95% confidence intervals and no line representing no significant relationship. Females PFTeA was detrended by females sampling date (see statistical analyses section).



## DISCUSSION

We investigated maternally deposited concentrations of androgens, glucocorticoids and thyroid hormones in egg yolk of black-legged kittiwake in relation with the female's and eggs characteristics, and the potential disrupting effect of PFAS on maternal hormone deposition. We found that egg yolk hormone levels were unrelated to female hormone plasma levels at the time of sampling, but that laying order, egg mass and the female's scaled mass index appeared to influence yolk concentration of some of the studied hormones. We observed an increased maternal T deposition in the first-laid egg in females bearing high levels of PFNA, PFDCa or PFUnA.

### **Factors influencing hormones maternal transfer**

Differential deposition of androgens along the laying sequence – as observed for AND in the present study – have been described as a mean to influence the outcome of sibling rivalry in various avian species implementing brood reduction, ultimately maximising females' reproductive outputs (Eising *et al.* 2001; Muriel *et al.* 2019; Poisbleau *et al.* 2011). Study on black-legged kittiwakes have reported higher T and AND in second-laid eggs, the amount deposited likely being determined by a complex trade-off between offspring and mother fitness (Benowitz-Fredericks *et al.* 2013; Gasparini *et al.* 2007; Müller *et al.* 2012; Vallarino *et al.* 2011). Higher androgen concentrations enhance embryo growth and therefore reduce the detrimental effect of hatching asynchrony for the junior egg, however, at the expense of potential long-term costs for the offspring (Groothuis *et al.* 2005). The outcome of such a differential androgen deposition largely depends on females and environmental conditions (Benowitz-Fredericks *et al.* 2013; Gasparini *et al.* 2007; but see Addison *et al.* 2008). Interestingly, contrarily to AND, we did not observe any difference in the deposition of T, DHT or CORT in first- and second-laid eggs in the present study, despite the fact that they are all products of the same metabolic pathway.

Recent studies showed that maternally deposited thyroid hormones were associated with environmental factors including food availability and temperature experienced during the pre-laying stage (Hsu *et al.* 2022; Ruuskanen and Hsu 2018). Their effects are still unclear but seem to occur mainly during the embryological development period and largely act on offspring growth and positively impact hatching success and telomere length during early postnatal period (Hsu *et al.* 2019; Ruuskanen *et al.* 2016; Sarraude *et al.* 2020a; Sarraude *et al.* 2020b;

Sarraude *et al.* 2020c; Stier *et al.* 2020). The increasing concentration of maternal T<sub>3</sub> (but not T<sub>4</sub>) deposited with increasing females scaled mass index found in the present study may illustrate that females in good condition can invest more in their offspring than those in lower condition. However, heavier eggs – which have been identified in many species as eggs of higher quality, leading to a higher survival probability of the offspring (Williams 1994) – received lower T<sub>3</sub> concentrations (but not T<sub>4</sub>) than lighter eggs, although we investigated concentrations and not total deposited amount in eggs. Data on yolk thyroid hormones are very limited for birds at the moment and the factors driving thyroid hormone deposition in eggs are still unclear and need further clarifications (Hsu *et al.* 2020b).

Other factors including adult features (e.g., age, past experience, phenotype and genetic (Tschirren *et al.* 2009; von Engelhardt and Groothuis 2011)), and the environment experienced before or during egg laying by females (e.g., food quality and availability (Gasparini *et al.* 2007; Verboven *et al.* 2003) or social interactions (Bentz *et al.* 2016)) were also proven important predictors of maternal hormones deposition in eggs. These data were not available in the present study and would be interesting to include in further integrative studies. That being said, breeding occurred extremely late in 2019 (laying date median in the whole colony in 2019 = 25<sup>th</sup> of June, 10 days later than usual average median laying date for the same population; Keogan *et al.* 2022), likely due to specific environmental conditions, the maternal hormones deposition may thus have been largely impacted by these particular conditions. Therefore, we do recommend additional investigations in various environmental conditions, as well as experimental studies, to better identify the drivers of maternal hormone deposition in eggs.

### **Females PFAS contamination and maternal hormones deposition**

Very few studies explored the relationship between endocrine-disrupting chemicals (EDCs) and maternal transferred hormones in avian or non-avian oviparous species, and to the best of our knowledge, only two studies found a correlation (Hamlin *et al.* 2010; Verboven *et al.* 2008), when others could not demonstrate any clear tendency (French *et al.* 2001; Johnston *et al.* 2005; Ruuskanen *et al.* 2019). Several emerging and legacy PFAS are suspected or identified EDCs (Coperchini *et al.* 2020; Johnson *et al.* 2021), and recent studies on seabirds and raptors have reported association between glucocorticoids, thyroid hormones and some long-chain perfluoroalkyl acids (Ask *et al.* 2021; Choy *et al.* 2022; Melnes *et al.* 2017; Sebastiano *et al.* 2020a; Sun *et al.* 2021; Tartu *et al.* 2014). Regarding maternal hormones

transfer to the eggs, our study shows that PFNA (C<sub>9</sub>), PFDCa (C<sub>10</sub>) and PFUnA (C<sub>11</sub>) concentrations in females are positively correlated with maternal T concentration in eggs. No significant correlations could be found for the perfluoroalkyl carboxylic acids (PFCAs) with longer carbon chains (C<sub>12</sub> – C<sub>14</sub>), even though they were present at comparable concentrations. Long-chain PFCAs ( $\geq$ C<sub>8</sub>) have very similar physico-chemical characteristics (Thackray *et al.* 2020), we could therefore expect a similar mode of action for them, and so, an additive effect when adding up the concentrations. However, no correlation was found between the sum of long-chain PFCAs (C<sub>8</sub> – C<sub>14</sub>) and T (LM:  $F_{1, 12} = 3.24$ ;  $p = 0.097$ ; CI of the slope: [-0.05 – 0.50]). Although we cannot exclude that the observed relationships for C<sub>9</sub> – C<sub>11</sub> PFCAs, may have been induced by unidentified, correlated factors, our results suggest that at least some long-chain PFCAs may cause a disruption of the maternal hormones concentrations in eggs.

A hypothesis behind this relationship may include disturbance of T biosynthesis or breakdown mechanisms by PFAS. Among hormones, if androgens are mainly formed in the theca interna cells of the follicles wall, thyroid hormones and corticosterone are synthesized by distant glands (thyroid and adrenal glands, respectively) of the mother bird (Groothuis and Schwabl 2008). All hormones are then transferred to the oocyte via blood, although the mechanisms are still largely unexplored (von Engelhardt and Groothuis 2011). For instance, a disrupting effect of PFAS on the steroidogenesis may happen as steroids are produced from cholesterol, which concentration in blood is known to be affected by PFAS in vertebrates (Geiger *et al.* 2014; Geng *et al.* 2019; Jacobsen *et al.* 2018; Lin *et al.* 2019; Roth *et al.* 2021). However, we would expect a similar disruption in females' plasma, which was not the case (LM for PFNA:  $F_{1, 12} = 1.79$ ;  $p = 0.206$ ; CI of the slope: [-0.67 – 0.16], PFDCa:  $F_{1, 12} = 1.17$ ;  $p = 0.30$ ; CI of the slope: [-0.43 – 0.15], and PFUnA:  $F_{1, 12} = 0.73$ ;  $p = 0.41$ ; CI of the slope: [-0.11 – 0.05]). A disruption of the steroidogenesis may also happen at the enzymatic level. AND is metabolized to T by the 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD) which is known to be inhibited by PFAS in humans and rats (Zhu *et al.* 2020). However, this reduced the production of T and thus cannot explain the higher levels of T observed in eggs of females with higher PFAS concentration. Another hypothesis could involve gonadotrophic hormones such as luteinizing hormone (LH). PFAS are known to affect LH in humans (Raymer *et al.* 2012), but this hormone was found essential to control T deposition in Japanese quail (*Coturnix japonica*; Okuliarova *et al.* 2018). We did not measure LH in females and thus cannot explore this assumption, but this may be a valuable lead to explore in further studies.

Maternal hormone transfer mechanisms may also be affected. Unlike other vertebrates, birds do not produce any transport protein with high affinity to steroids as the sex hormone-binding globulin (Wingfield *et al.* 1984). Instead, the corticosteroid-binding globulin which has a high affinity for CORT, may also transport androgens (Lin *et al.* 2021; Vashchenko *et al.* 2016). Lipoproteins and albumin may bind to all steroids as well, with a low affinity (Malisch and Breuner 2010; McNabb and Morgan 1997). PFAS in blood are bound to albumin, fatty-acid binding proteins and organic anion transporters (Ng and Hungerbuhler 2014). Therefore, a competition for blood transporters, including albumin, between PFAS and maternal T during the process of maternal transfer to egg should result in a negative relationship between PFAS in females and deposited T in eggs, but we observe an opposite trend. Also, a displacement of hormones fixed to albumin by PFAS have been previously described as unlikely at environmental concentrations (Jones *et al.* 2003). An alternative explanation can be offered on the basis of the molecular structure of the observed PFCAs. In kittiwakes, PFAS are linearly positively correlated between females and their eggs for most compounds, with the transfer efficiency depending on their physico-chemical characteristics caused by the increasing perfluorinated carbon chain length (Jouanneau *et al.* 2022). Therefore, we can predict that the disrupting activity of PFAS in general and PFCAs with medium long chain length (C<sub>9</sub> – C<sub>11</sub>) on T will vary according to the PFAS carbon chain length. In our study, the sample size was relatively low, which may limit the relationship between T and some of the PFAS. Further studies with larger sample size would be essential to investigate the aforementioned hypotheses.

Overall, our study provides another evidence that certain long-chain PFCAs are the most associated with effects in this kittiwakes' population (Blévin *et al.* 2017a; Blévin *et al.* 2020; Blévin *et al.* 2017b; Costantini *et al.* 2019; Tartu *et al.* 2014), and that it is essential to investigate how single compound affects physiological markers, in addition to how they act together (Sebastiano *et al.* 2020a). However, correlative studies in wild population need to be interpreted with caution as the observed relationship between C<sub>9</sub> to C<sub>11</sub> PFCAs and T may be the consequence of alternative and unknown correlated contextual cues. Maternal yolk hormone concentrations are related to egg quality in some species, therefore in this wild population, females feeding on higher trophic level could accumulate more PFAS and invest more in reproduction by laying eggs with higher T concentrations.

A large body of literature describes the consequences of high T concentrations in eggs. In semi-precocial seabirds, elevated yolk T was related to various development factors on offspring of seabirds from the same family (impaired sex-ratio (Rubolini *et al.* 2006), growth



and body mass (Eising *et al.* 2001; Parolini *et al.* 2017; Rubolini *et al.* 2006), increased begging (Boncoraglio *et al.* 2006), competitiveness (Müller *et al.* 2009) and survival (Eising and Groothuis 2003), decreased hatching time (Eising *et al.* 2001), immune response (Muller *et al.* 2005) and telomere length (Parolini *et al.* 2019)). In kittiwake more specifically, experimentally elevated T in eggs led to an increased aggressiveness and dominance in the clutch (Müller *et al.* 2013; Müller *et al.* 2012). Most of the studies cited above investigate the effects of experimentally elevated T, which often exceed T concentrations observed in wild populations. Embryos *in natura* may buffer maternal T through inactivation pathways, which could at least reduce the impact of an elevated deposition of maternal T (Campbell *et al.* 2020). A disruption of maternal deposited T may impact the embryo at different levels, inducing a mismatch between the environment experienced early and later in life by the offspring and its phenotype, which may ultimately affect its survival and fitness. Moreover, in kittiwakes, the most PFAS contaminated females laid the most contaminated eggs (Jouanneau *et al.* 2022), therefore the disrupted deposition of maternal T and the high concentrations of PFAS in females and eggs may have additive consequences on the embryo (known to be particularly sensitive to EDCs; Hamlin and Guillette 2011) through disruptions of the maternal adaptive effect, of embryo development and of the female's behavior during incubation and chick-rearing periods.

In the present study, we found no effect of PFAS on thyroid hormones (THs) deposition in eggs, despite these contaminants are known to positively correlate with circulating THs in seabirds including kittiwakes and some birds of prey (Ask *et al.* 2021; Choy *et al.* 2022; Sebastiano *et al.* 2020b; Sun *et al.* 2021). Mechanisms affected were explored in humans and birds and may include interaction with THs receptors and binding proteins, with the thyroid peroxidase enzyme activity or with thyroid-stimulating hormone (Fenton *et al.* 2021; Kar *et al.* 2017; Mortensen *et al.* 2020). In birds, maternal THs were found to impact several parameters in embryogenesis development and in post-hatch performance and fitness (Darras 2019; Hsu *et al.* 2019; Sarraude *et al.* 2020a; Sarraude *et al.* 2020c; Stier *et al.* 2020). Therefore, the impact of PFAS may be limited on these endpoints during kittiwake embryo development, at least at the concentrations we measured in the present study. However, transmitted PFAS may impact THs later during development (Mattsson *et al.* 2019).

### CONCLUSION

This study identified some of the main factors driving the deposition of several major maternal hormones in eggs of the black-legged kittiwake. It also produced a first insight on the relationship between females PFAS burden in plasma and maternal transferred hormones in egg yolk. None of the investigated thyroid (T<sub>3</sub> and T<sub>4</sub>) and glucocorticoid (CORT) hormones deposition seemed to be affected by females' contamination. Similar results were observed for steroid hormones including AND and DHT, but we observed a positive relationship between some long-chain PFAS and T deposited in eggs. Although our results do not provide evidence for a causal relationship between females PFAS contamination and maternal T in eggs, we suggested that the transfer mechanism may be similar for some PFAS and T, which may eventually affect maternal adaptive effects driven by T deposition.

Nonetheless, maternal hormones deposition depends on a complex combination of various intrinsic and extrinsic factors, including environmental conditions. The specific context in 2019, leading to a very late breeding may thus have affected maternal hormones transfer in a specific manner. We strongly recommend additional investigations among species and time, as well as experimental studies to draw strong conclusions on the main effects of PFAS on hormone deposition in avian eggs.

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**Supporting Information**

Does contaminant exposure disrupt maternal hormones deposition? A study of per- and polyfluoroalkyl substances in an Arctic seabird

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### Internal standards used in PFAS analyses

Perfluoro-n-[2,3,4-<sup>13</sup>C<sub>3</sub>]butanoic acid

Perfluoro-n-[<sup>13</sup>C<sub>5</sub>]pentanoic acid

Perfluoro-n-[1,2,3,4,6-<sup>13</sup>C<sub>5</sub>]hexanoic acid

Perfluoro-n-[1,2,3,4-<sup>13</sup>C<sub>4</sub>]heptanoic acid

Perfluoro-n-[<sup>13</sup>C<sub>8</sub>]octanoic acid

Perfluoro-n-[<sup>13</sup>C<sub>9</sub>]nonanoic acid

Perfluoro-n-[1,2,3,4,5,6-<sup>13</sup>C<sub>6</sub>]decanoic acid

Perfluoro-n-[1,2,3,4,5,6,7-<sup>13</sup>C<sub>7</sub>]undecanoic acid

Perfluoro-n-[1,2-<sup>13</sup>C<sub>2</sub>]dodecanoic acid

Perfluoro-n-[1,2-<sup>13</sup>C<sub>2</sub>]tetradecanoic acid

Sodium perfluoro-1-[2,3,4-<sup>13</sup>C<sub>3</sub>]butanesulfonate

Sodium perfluoro-1-[1,2,3-<sup>13</sup>C<sub>3</sub>]hexanesulfonate

Sodium perfluoro-1-[<sup>13</sup>C<sub>8</sub>]octanesulfonate

Perfluoro-1-[<sup>13</sup>C<sub>8</sub>]octanesulfonamide

Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-<sup>13</sup>C<sub>2</sub>]-octane sulfonate

Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-<sup>13</sup>C<sub>2</sub>]-decane sulfonate

**Table S1.** List of targeted per- and polyfluoroalkyl substances (PFAS): their groups, abbreviations, chemical names, detection rates in plasma of black-legged kittiwakes females, limits of detection and descriptive statistics (mean  $\pm$  standard deviation SD; values <LOD not replaced by  $\frac{1}{2}$  LOD). Selected PFAS (i.e. detected in more than 70% of both samples and therefore used in analyses) are stressed in bold.

Groupe	Abbreviation	Chemical name	Detection rate	Limit of detection (ng g <sup>-1</sup> ww)	Mean $\pm$ SD
Perfluoroalkane sulfonic acids	PFBS	perfluorobutanesulfonic acid	0.0%	0.10	na
	PFPS	perfluoropentanesulfonic acid	0.0%	0.05	na
	<b>PFHxS</b>	<b>perfluorohexanesulfonic acid</b>	<b>100.0%</b>	<b>0.05</b>	<b>0.23 <math>\pm</math> 0.09</b>
	PFHpS	perfluoroheptanesulfonic acid	0.0%	0.08	na
	<b>brPFOS</b>	<b>branched perfluorooctanesulfonic acid</b>	<b>85.7%</b>	<b>0.06</b>	<b>0.95 <math>\pm</math> 0.32</b>
	<b>linPFOS</b>	<b>linear perfluorooctanesulfonic acid</b>	<b>100.0%</b>	<b>0.072</b>	<b>10.8 <math>\pm</math> 4.70</b>
	PFNS	perfluorononanesulfonic acid	0.0%	0.06	na
	PFDCS	perfluorodecanesulfonic acid	23.8%	0.10	0.12 $\pm$ 0.02
Perfluoroalkyl carboxylic acids	PFBA	perfluorobutanoic acid	0.0%	0.08	na
	PFPA	perfluoropentanoic acid	0.0%	0.08	na
	PFHxA	perfluorohexanoic acid	0.0%	0.08	na
	PFHpA	perfluoroheptanoic acid	9.5%	0.04	0.06 $\pm$ 0.00
	<b>PFOA</b>	<b>perfluorooctanoic acid</b>	<b>71.4%</b>	<b>0.08</b>	<b>0.19 <math>\pm</math> 0.13</b>
	<b>PFNA</b>	<b>perfluorononanoic acid</b>	<b>100.0%</b>	<b>0.03</b>	<b>1.06 <math>\pm</math> 0.61</b>
	<b>PFDA</b>	<b>perfluorodecanoic acid</b>	<b>100.0%</b>	<b>0.03</b>	<b>1.71 <math>\pm</math> 0.89</b>
	<b>PFUnA</b>	<b>perfluoroundecanoic acid</b>	<b>100.0%</b>	<b>0.06</b>	<b>6.88 <math>\pm</math> 3.21</b>
	<b>PFDoA</b>	<b>perfluorododecanoic acid</b>	<b>95.2%</b>	<b>0.03</b>	<b>1.81 <math>\pm</math> 0.89</b>
	<b>PFTriA</b>	<b>perfluorotridecanoic acid</b>	<b>100.0%</b>	<b>0.07</b>	<b>7.80 <math>\pm</math> 3.35</b>
	<b>PFTeA</b>	<b>perfluorotetradecanoic acid</b>	<b>100.0%</b>	<b>0.08</b>	<b>0.14 <math>\pm</math> 0.60</b>
PFHxDA	perfluorohexadecanoic acid	0.0%	0.12	na	

## Hormones analyses

### - Steroid hormones (DHT, AND, T and CORT)

For extraction, 100 mg of yolk were homogenised in 1.0 mL of distilled water with three glass beads, using a vortex. Steroids were extracted by adding 3 mL of diethyl-ether to 300  $\mu$ L of the yolk mixture or 50  $\mu$ L of plasma, vortexing and centrifuging (5 minutes at 2000 rpm, at 4 °C). The diethyl-ether phase containing steroids was decanted and poured off after snap freezing the tube. This was done twice for yolk. The solvent was then evaporated at 37 °C. The dried extracts were re-dissolved in phosphate buffer (0.01 M pH 7.4).

Cross-reactions of testosterone antiserum were as follows: androsterone (63 %), progesterone (1.45 %), 17- $\beta$ -estradiol (0.176 %), corticosterone (0.41 %), estrone (0.03 %), aldosterone (< 0.01 %), cortisone (< 0.01 %). Cross-reactions of yolk corticosterone antiserum were as follows: 11-dehydrocorticosterone (0,67 %), deoxycorticosterone (1,5 %), 18-hydroxy-deoxycorticosterone (< 0,01 %), cortisone (< 0,01 %), cortisone (< 0,01 %), progesterone (0,004 %), aldosterone (0.2 %).

Cross-reactions of plasma corticosterone antiserum were as follows : progesterone (12 %), deoxycorticosterone (7 %), 17-hydroxyprogesterone (4.5 %), cortisol (2 %), Compound S (1.3 %), testosterone (0.01 %), dehydroandrosterone (0.01 %), androstenedione (0.01 %), androsterone (0.01 %), estrone (0.01 %).

Cross-reactions of A4 antiserum (IBL) were as follows: androstenedione (100 %), DHEA (1.8 %), testosterone (0.2%), estrone (<0.1 %), estradiol (<0.1 %), progesterone (<0.1 %), 17-OH-progesterone (<0.1 %), 5 $\alpha$ -DHT (<0.1 %), cortisol (<0.01 %), DHEA-S (<0.01 %).

Cross-reactions of A4 antiserum (Demeditec) were as follows: androstenedione (100 %), androsterone (<0.01 %), aldosterone (< 0.01 %), cortisol (0.2 %), dihydroandrosterone (<0.01 %), dihydroepiandrosterone (0.01 %), estriol (1.8 %), 16-epiestriol (<0.01 %), estradiol (<0.1 %), estriol-3-glucuronide (<0.01 %), estriol-16-glucuronide (<0.01 %), estriol-16-sulfate (<0.01 %), estrone (<0.01 %), 17-a-pregnenolone (<0.01 %), 17-OH-pregnenolone (0.3 %), progesterone (<0.01 %), testosterone (0.01 %).

Cross-reactions of DHT antiserum (IBL and Demeditec) were as follows: dihydrotestosterone (100 %), testosterone (8.7 %), 5- $\beta$ - dihydrotestosterone (2.0 %), androstenedione (0.2 %).

- **Thyroid hormones (T<sub>3</sub> and T<sub>4</sub>)**

Thyroid hormones have been extracted from yolk following the protocol used by Boertje and colleagues (Boertje *et al.* 2019) with slightly modifications. Thyroids hormones were extracted in 500 mg of yolk with 2.0 mL of methanol, using a vortex (glass beads) and a sonication bath. After centrifugation 10 min at 3100 rpm at 20 °C, supernatant was decanted. This step has been replicated once and supernatant was decanted into a separate tube. Then, we added 5.0 mL of chloroform, 0.5 mL of ammonium hydroxide and 0.5 mL of water to each tube, centrifuged, collected the upper phase (combining both upper phases, from the same samples, into the same tube) and dried the extract at 60 °C with nitrogen. Precipitate was purified with 1.0 mL of ammonium hydroxide and 1.0 mL of water. After centrifugation, supernatant was decanted into a new tube to which we added 1.0 mL of chloroform. After centrifugation, we collected the upper phase and dried the purified extract. We resuspended the precipitate in 150 µL of phosphate buffer solution and stored the samples at -20 °C.

Cross-reactions of T<sub>3</sub> antiserum were as follows: Diiodo-L-Tyrosine (< 0.01 %), Monoiodo-L-Tyrosine (< 0.01 %), L-Thyroxine (0.2 %), Triiodo D-Thyroacetic Acid (6.0 %). Cross-reactions of T<sub>4</sub> antiserum were as follows: Diiodo-L-Tyrosine (<0.01%), Monoiodo-L-Tyrosine (< 0.01 %), Triiodothyronine (4.0 %).



**Table S2.** Minimal detectable (females plasma: pg mL<sup>-1</sup>; egg yolk: pg g<sup>-1</sup>), intra-, inter-assay, and number of samples measured lower than LOD for androstanolone (AND), androstenedione ( $\Delta^4$ ), corticosterone (CORT), testosterone (T), triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>) analyses in female plasma and egg yolk of black-legged kittiwakes from Svalbard.

	Minimum detectable		intra-assay precision		inter-assay precision		<LOD	
	female plasma	egg yolk	female plasma	egg yolk	female plasma	egg yolk	female plasma	egg yolk
<b>DHT</b>	7.23	0.03	4.78	4.78	NA	NA	1	0
<b>AND</b>	20	0.16	7.13	7.28	8.37	NA	1	0
<b>CORT</b>	280	1.03	9.80	9.80	10.07	10.07	0	0
<b>T</b>	300	1.20	3.71	3.71	NA	NA	4	0
<b>T<sub>3</sub></b>	70	0.04	8.52	11.23	16.31	NA	0	0
<b>T<sub>4</sub></b>	602	0.18	9.14	9.14	NA	NA	0	0

**Table S3.** Model selections for the relationship between maternal transferred hormones in black-legged kittiwake eggs and female and egg characteristics, based on the lowest second-order Akaike's Information Criterion corrected for small sample sizes (AICc); the predicting variables were the maternal circulating hormones concentration for the specific hormone (Circ. hormone), the scaled mass index (SMI) of the females, the egg mass, the rank of the eggs in the laying order (Egg rank) and the laying date of the first-laid egg of the clutch (Laying date). The most parsimonious model is given in bold. AICcwt: Akaike's weight;  $\Delta$ AICc: difference between the model with the smallest AICc-value and the model of interest. Dihydrotestosterone (DHT), androstenedione (AND), corticosterone (CORT), testosterone (T), triiodothyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>) and the ratio between T<sub>3</sub> and T<sub>4</sub> (T<sub>3</sub>/ T<sub>4</sub>).

<b>CORT</b>									
	Circ. Hormone	SMI	Egg mass	Egg number	Laying date	K	AICc	AICcwt	$\Delta$ AICc
<b>mod32</b>						<b>3</b>	<b>91.610</b>	<b>0.210</b>	<b>0.000</b>
mod30		X				4	91.780	0.190	0.180
mod28				X		4	93.990	0.060	2.390
mod31	X					4	94.010	0.060	2.400
mod16					X	4	94.230	0.060	2.630
mod26		X				5	94.370	0.050	2.760
mod29			X			4	94.450	0.050	2.840
mod22	X	X				5	94.450	0.050	2.850
mod14		X			X	5	94.720	0.040	3.110
mod25		X	X			5	94.770	0.040	3.160
mod24	X			X		5	96.770	0.020	5.160
mod12				X	X	5	96.820	0.020	5.220
mod27	X		X			5	96.980	0.010	5.380
mod15	X				X	5	97.000	0.010	5.390
mod23			X	X		5	97.040	0.010	5.440
mod13			X		X	5	97.350	0.010	5.740
mod18	X	X	X			6	97.370	0.010	5.770
mod21		X	X	X		6	97.470	0.010	5.870
mod19	X	X		X		6	97.490	0.010	5.880
mod10		X		X	X	6	97.540	0.010	5.940
mod6	X	X			X	6	97.810	0.010	6.200
mod9		X	X		X	6	97.970	0.010	6.360
mod20	X		X	X		6	99.930	0.000	8.330
mod8	X			X	X	6	100.020	0.000	8.410
mod7			X	X	X	6	100.110	0.000	8.500
mod11	X		X		X	6	100.270	0.000	8.670
mod17	X	X	X	X		7	100.510	0.000	8.910
mod5		X	X	X	X	7	100.840	0.000	9.240
mod2	X	X	X		X	7	101.030	0.000	9.420
mod3	X	X		X	X	7	101.150	0.000	9.540
mod4	X		X	X	X	7	103.440	0.000	11.830
mod1	X	X	X	X	X	8	104.430	0.000	12.820

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**T**

	Circ. Hormone	SMI	Egg mass	Egg number	Laying date	K	AICc	AICcwt	ΔAICc
<b>mod32</b>						<b>3</b>	<b>152.190</b>	<b>0.210</b>	<b>0.000</b>
mod16					X	4	153.370	0.120	1.180
mod28				X		4	153.670	0.100	1.480
mod30		X				4	154.020	0.090	1.820
mod31	X					4	154.770	0.060	2.570
mod29			X			4	154.970	0.050	2.780
mod12				X	X	5	155.370	0.040	3.170
mod14		X			X	5	155.390	0.040	3.190
mod26		X				5	155.920	0.030	3.730
mod23			X	X		5	156.220	0.030	4.020
mod15	X				X	5	156.360	0.030	4.170
mod24	X			X		5	156.500	0.020	4.310
mod13			X		X	5	156.520	0.020	4.320
mod22	X	X				5	156.880	0.020	4.690
mod25		X	X			5	157.120	0.020	4.930
mod27	X		X			5	157.670	0.010	5.470
mod10		X		X	X	6	157.870	0.010	5.680
mod7			X	X	X	6	158.550	0.010	6.360
mod8	X			X	X	6	158.670	0.010	6.470
mod6	X	X			X	6	158.730	0.010	6.530
mod20	X		X	X		6	158.860	0.010	6.660
mod9		X	X		X	6	158.890	0.010	6.700
mod21		X	X	X		6	158.970	0.010	6.780
mod19	X	X		X		6	159.100	0.010	6.900
mod11	X		X		X	6	159.790	0.000	7.600
mod18	X	X	X			6	160.190	0.000	8.000
mod3	X	X		X	X	7	161.590	0.000	9.390
mod5	X	X	X			7	161.590	0.000	9.400
mod4	X	X		X	X	7	161.920	0.000	9.730
mod17	X	X	X	X		7	162.080	0.000	9.890
mod2	X	X	X		X	7	162.600	0.000	10.410
mod1	X	X	X	X	X	8	165.520	0.000	13.330

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**DHT**

	Circ. Hormone	SMI	Egg mass	Egg number	Laying date	K	AICc	AICcwt	$\Delta$ AICc
<b>mod32</b>						<b>3</b>	<b>411.740</b>	<b>0.250</b>	<b>0.000</b>
mod30		X				4	412.590	0.160	0.860
mod16					X	4	414.220	0.070	2.480
mod28				X		4	414.340	0.070	2.610
mod31	X					4	414.400	0.070	2.660
mod29			X			4	414.470	0.060	2.740
mod14		X			X	5	415.330	0.040	3.590
mod22	X	X				5	415.500	0.040	3.760
mod25		X	X			5	415.530	0.040	3.790
mod26		X				5	415.590	0.040	3.850
mod12				X	X	5	417.170	0.020	5.440
mod13			X		X	5	417.180	0.020	5.440
mod24	X			X		5	417.320	0.020	5.580
mod15	X				X	5	417.320	0.020	5.580
mod23			X	X		5	417.470	0.010	5.740
mod27	X		X			5	417.510	0.010	5.770
mod9		X	X		X	6	418.490	0.010	6.750
mod10		X		X	X	6	418.720	0.010	6.990
mod6	X	X			X	6	418.750	0.010	7.010
mod19	X	X		X		6	418.860	0.010	7.120
mod18	X	X	X			6	418.890	0.010	7.150
mod21		X	X	X		6	418.970	0.010	7.240
mod7			X	X	X	6	420.600	0.000	8.860
mod8	X			X	X	6	420.630	0.000	8.890
mod11	X		X		X	6	420.680	0.000	8.940
mod20	X		X	X		6	420.830	0.000	9.090
mod5	X	X	X			7	422.400	0.000	10.660
mod2	X	X	X		X	7	422.400	0.000	10.670
mod3	X	X		X	X	7	422.560	0.000	10.820
mod17	X	X	X	X		7	422.730	0.000	11.000
mod4	X	X		X	X	7	424.500	0.000	12.770
mod1	X	X	X	X	X	8	426.800	0.000	15.060

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**AND**

	Circ. Hormone	SMI	Egg mass	Egg number	Laying date	K	AICc	AICcwt	$\Delta$ AICc
<b>mod28</b>				<b>X</b>		<b>4</b>	<b>341.370</b>	<b>0.420</b>	<b>0.000</b>
mod26		X				5	344.460	0.090	3.080
mod24	X			X		5	344.460	0.090	3.080
mod12				X	X	5	344.490	0.090	3.120
mod23			X	X		5	344.520	0.090	3.150
mod32						3	345.870	0.040	4.490
mod8	X			X	X	6	347.900	0.020	6.530
mod19	X	X		X		6	347.910	0.020	6.540
mod20	X		X	X		6	347.920	0.020	6.550
mod10		X		X	X	6	347.930	0.020	6.550
mod21		X	X	X		6	347.940	0.020	6.570
mod29			X			4	347.970	0.020	6.590
mod7			X	X	X	6	347.980	0.020	6.610
mod30		X				4	348.420	0.010	7.050
mod31	X					4	348.480	0.010	7.110
mod16					X	4	348.550	0.010	7.170
mod27	X		X			5	350.480	0.000	9.110
mod25		X	X			5	350.730	0.000	9.360
mod13			X		X	5	350.760	0.000	9.390
mod15	X				X	5	351.330	0.000	9.960
mod14		X			X	5	351.400	0.000	10.030
mod22	X	X				5	351.420	0.000	10.050
mod4	X	X		X	X	7	351.730	0.000	10.350
mod3	X	X		X	X	7	351.770	0.000	10.400
mod17	X	X	X	X		7	351.780	0.000	10.410
mod5	X	X	X			7	351.810	0.000	10.440
mod11	X		X		X	6	353.210	0.000	11.840
mod18	X	X	X			6	353.740	0.000	12.370
mod9		X	X		X	6	353.870	0.000	12.500
mod6	X	X			X	6	354.640	0.000	13.270
mod1	X	X	X	X	X	8	356.080	0.000	14.710
mod2	X	X	X		X	7	356.910	0.000	15.540

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**T<sub>3</sub>**

	Circ. Hormone	SMI	Egg mass	Egg number	Laying date	K	AICc	AICcwt	ΔAICc
<b>mod21</b>		<b>X</b>	<b>X</b>	<b>X</b>		<b>6</b>	<b>-50.640</b>	<b>0.200</b>	<b>0.000</b>
mod25		X	X			5	-49.750	0.130	0.890
mod9		X	X		X	6	-49.600	0.120	1.040
mod23			X	X		5	-48.980	0.090	1.650
mod5	X	X	X			7	-48.850	0.080	1.780
mod30		X				4	-47.670	0.050	2.970
mod14		X			X	5	-47.590	0.040	3.050
mod29			X			4	-46.790	0.030	3.850
mod17	X	X	X	X		7	-46.770	0.030	3.870
mod7			X	X	X	6	-46.670	0.030	3.970
mod18	X	X	X			6	-46.250	0.020	4.390
mod32						3	-45.830	0.020	4.810
mod2	X	X	X		X	7	-45.830	0.020	4.810
mod20	X		X	X		6	-45.730	0.020	4.900
mod13			X		X	5	-45.680	0.020	4.960
mod26		X				5	-45.410	0.010	5.230
mod16					X	4	-45.130	0.010	5.500
mod10		X		X	X	6	-44.700	0.010	5.930
mod1	X	X	X	X	X	8	-44.620	0.010	6.020
mod22	X	X				5	-44.570	0.010	6.070
mod27	X		X			5	-44.270	0.010	6.370
mod6	X	X			X	6	-44.080	0.010	6.560
mod28				X		4	-44.060	0.010	6.580
mod31	X					4	-43.870	0.010	6.770
mod4	X	X		X	X	7	-42.950	0.000	7.690
mod12				X	X	5	-42.880	0.000	7.760
mod15	X				X	5	-42.650	0.000	7.990
mod11	X		X		X	6	-42.640	0.000	8.000
mod19	X	X		X		6	-41.950	0.000	8.690
mod24	X			X		5	-41.720	0.000	8.920
mod3	X	X		X	X	7	-40.790	0.000	9.850
mod8	X			X	X	6	-39.990	0.000	10.650

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T<sub>4</sub>

	Circ. Hormone	SMI	Egg mass	Egg number	Laying date	K	AICc	AICcwt	ΔAICc
<b>mod13</b>			X		X	<b>5</b>	<b>111.980</b>	<b>0.100</b>	<b>0.000</b>
mod28				X		4	112.120	0.090	0.140
mod29			X			4	112.530	0.070	0.540
mod32						3	112.650	0.070	0.670
mod8	X			X	X	6	112.740	0.070	0.760
mod24	X			X		5	113.110	0.060	1.130
mod23			X	X		5	113.320	0.050	1.340
mod11	X		X		X	6	113.440	0.050	1.460
mod15	X				X	5	113.470	0.050	1.480
mod16					X	4	113.480	0.050	1.500
mod12				X	X	5	113.500	0.050	1.520
mod7			X	X	X	6	113.670	0.040	1.680
mod31	X					4	114.120	0.030	2.140
mod4	X	X		X	X	7	114.610	0.030	2.620
mod26		X				5	114.800	0.020	2.820
mod27	X		X			5	115.090	0.020	3.100
mod30		X				4	115.230	0.020	3.250
mod9		X	X		X	6	115.420	0.020	3.430
mod20	X		X	X		6	115.570	0.020	3.590
mod25		X	X			5	115.590	0.020	3.610
mod19	X	X		X		6	116.300	0.010	4.310
mod14		X			X	5	116.340	0.010	4.360
mod3	X	X		X	X	7	116.370	0.010	4.390
mod10		X		X	X	6	116.500	0.010	4.520
mod21		X	X	X		6	116.590	0.010	4.610
mod6	X	X			X	6	116.830	0.010	4.850
mod22	X	X				5	117.120	0.010	5.130
mod2	X	X	X		X	7	117.340	0.010	5.350
mod5	X	X	X			7	117.390	0.010	5.410
mod18	X	X	X			6	118.530	0.000	6.550
mod1	X	X	X	X	X	8	118.900	0.000	6.910
mod17	X	X	X	X		7	119.300	0.000	7.320

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 $T_3/T_4$ 

	Circ. Hormone	SMI	Egg mass	Egg number	Laying date	K	AICc	AICcwt	$\Delta$ AICc
<b>mod25</b>		<b>X</b>	<b>X</b>			<b>5</b>	<b>65.220</b>	<b>0.470</b>	<b>0.000</b>
mod18	X	X	X			6	68.020	0.120	2.810
mod29			X			4	68.330	0.100	3.110
mod9		X	X		X	6	68.470	0.090	3.250
mod21		X	X	X		6	68.720	0.080	3.510
mod13			X		X	5	71.310	0.020	6.100
mod23			X	X		5	71.410	0.020	6.190
mod27	X		X			5	71.480	0.020	6.270
mod2	X	X	X		X	7	71.640	0.020	6.420
mod17	X	X	X	X		7	71.940	0.020	6.730
mod5	X	X	X			7	72.390	0.010	7.170
mod7			X	X	X	6	74.770	0.000	9.560
mod11	X		X		X	6	74.820	0.000	9.600
mod20	X		X	X		6	74.910	0.000	9.700
mod30		X				4	75.120	0.000	9.910
mod26		X				5	76.010	0.000	10.790
mod1	X	X	X	X	X	8	76.050	0.000	10.830
mod32				<b>X</b>		3	76.430	0.000	11.210
mod28		X			X	4	77.170	0.000	11.960
mod14	X	X				5	77.570	0.000	12.350
mod22		X		X	X	5	77.840	0.000	12.630
mod10		X		X	X	6	78.610	0.000	13.400
mod4	X	X		X	X	7	78.700	0.000	13.480
mod16					X	4	78.720	0.000	13.500
mod19		X		X	X	6	79.200	0.000	13.990
mod31	X					4	79.280	0.000	14.060
mod12				X	X	5	79.620	0.000	14.400
mod24	X			X		5	80.310	0.000	15.090
mod6	X	X			X	6	80.560	0.000	15.350
mod15	X				X	5	81.870	0.000	16.660
mod3	X	X		X	X	7	82.160	0.000	16.940
mod8	X			X	X	6	83.110	0.000	17.900



**Table S4.** Factors affecting each maternal transmitted hormones concentrations in black-legged kittiwakes' eggs from Svalbard, estimated by mixed linear regression models. Dihydrotestosterone (DHT), androstenedione (AND), corticosterone (CORT), testosterone (T), triiodothyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>) and the ratio between T<sub>3</sub> and T<sub>4</sub> (T<sub>3</sub>/ T<sub>4</sub>).

Parameter	Estimate	SE	t-value	p-value
<b><u>CORT (Null model)</u></b>				
-				
<b><u>T (Null model)</u></b>				
-				
<b><u>DHT (Null model)</u></b>				
-				
<b><u>AND (R<sup>2</sup>m<sup>a</sup>: 0.17; R<sup>2</sup>c<sup>b</sup>: 0.59)</u></b>				
Egg rank (2)	182.188	59.136	3.081	0.012
<b><u>T<sub>3</sub> (R<sup>2</sup>m: 0.41; R<sup>2</sup>c: 0.62)</u></b>				
SMI	0.000	0.000	2.487	0.029
Egg mass	-0.011	0.005	-2.406	0.037
<b><u>T<sub>4</sub> (Null model)</u></b>				
-				
<b><u>T<sub>3</sub>/T<sub>4</sub> (R<sup>2</sup>m: 0.55; R<sup>2</sup>c: 0.74)</u></b>				
SMI	0.010	0.004	2.542	0.026
Egg mass	-0.181	0.046	-3.964	0.003

<sup>a</sup> R<sup>2</sup>m: Marginal coefficient of determination, i.e. variance explained by the fixed effects.

<sup>b</sup> R<sup>2</sup>c: Conditional coefficient of determination, i.e. variance explained by the entire model, including both fixed and random effects.

**Table S5.** Model selections for the relationship between maternal transferred hormones in eggs and maternal circulating PFAS in plasma of black-legged kittiwake from Svalbard, based on the lowest second-order Akaike's Information Criterion corrected for small sample sizes (AICc); for each investigated hormone, the predicting variables were the maternal circulating PFAS concentration for the specific PFAS (Circ. PFAS) and those selected from the biological variables (see Table S3) for each hormone independently. The most parsimonious model is given in bold. *AICcwt*: Akaike's weight;  $\Delta$ AICc: difference between the model with the smallest AICc-value and the model of interest. Dihydrotestosterone (DHT), androstenedione (AND), corticosterone (CORT), testosterone (T), triiodothyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>) and the ratio between T<sub>3</sub> and T<sub>4</sub> (T<sub>3</sub>/T<sub>4</sub>).

CORT						T					
PFHxS	Circ. PFAS	K	AICc	AICcwt	$\Delta$ AICc	PFHxS	Circ. PFAS	K	AICc	AICcwt	$\Delta$ AICc
<b>mod2</b>		<b>2</b>	<b>53.770</b>	<b>0.820</b>	<b>0.000</b>	<b>mod2</b>		<b>2</b>	<b>84.770</b>	<b>0.830</b>	<b>0.000</b>
mod1	X	3	56.820	0.180	3.050	mod1	X	3	87.970	0.170	3.200
brPFOS						brPFOS					
PFHxS	Circ. PFAS	K	AICc	AICcwt	$\Delta$ AICc	PFHxS	Circ. PFAS	K	AICc	AICcwt	$\Delta$ AICc
<b>mod2</b>		<b>2</b>	<b>53.770</b>	<b>0.840</b>	<b>0.000</b>	<b>mod2</b>		<b>2</b>	<b>84.770</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	3	57.070	0.160	3.310	mod1	X	3	87.790	0.180	3.020
linPFOS						linPFOS					
PFHxS	Circ. PFAS	K	AICc	AICcwt	$\Delta$ AICc	PFHxS	Circ. PFAS	K	AICc	AICcwt	$\Delta$ AICc
<b>mod2</b>		<b>2</b>	<b>91.610</b>	<b>0.790</b>	<b>0.000</b>	<b>mod2</b>		<b>2</b>	<b>84.770</b>	<b>0.560</b>	<b>0.000</b>
mod1	X	3	94.290	0.210	2.690	mod1	X	3	85.240	0.440	0.470
PFOA						PFOA					
PFHxS	Circ. PFAS	K	AICc	AICcwt	$\Delta$ AICc	PFHxS	Circ. PFAS	K	AICc	AICcwt	$\Delta$ AICc
<b>mod2</b>		<b>3</b>	<b>53.770</b>	<b>0.830</b>	<b>0.000</b>	<b>mod2</b>		<b>2</b>	<b>84.770</b>	<b>0.760</b>	<b>0.000</b>
mod1	X	4	56.920	0.170	3.160	mod1	X	3	87.020	0.240	2.250

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**PFNA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>53.770</b>	<b>0.830</b>	<b>0.000</b>
mod1	X	3	56.890	0.170	3.130

**PFDoA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>53.770</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	3	56.780	0.180	3.010

**PFUnA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>53.770</b>	<b>0.800</b>	<b>0.000</b>
mod1	X	3	56.580	0.200	2.810

**PFDoA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>53.770</b>	<b>0.800</b>	<b>0.000</b>
mod1	X	3	56.480	0.200	2.710

**PFTriA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>53.770</b>	<b>0.790</b>	<b>0.000</b>
mod1	X	3	56.430	0.210	2.670

**PFTeA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>53.770</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	3	56.730	0.180	2.970

**PFNA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod1</b>	<b>X</b>	<b>3</b>	<b>82.500</b>	<b>0.760</b>	<b>0.000</b>
mod2		2	84.770	0.240	2.270

**PFDoA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod1</b>	<b>X</b>	<b>3</b>	<b>82.500</b>	<b>0.760</b>	<b>0.000</b>
mod2		2	84.770	0.240	2.270

**PFUnA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod1</b>	<b>X</b>	<b>3</b>	<b>82.200</b>	<b>0.780</b>	<b>0.000</b>
mod2		2	84.770	0.220	2.580

**PFDoA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
mod1	X	3	84.510	0.530	0.000
<b>mod2</b>		<b>2</b>	<b>84.770</b>	<b>0.470</b>	<b>0.260</b>

**PFTriA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>84.770</b>	<b>0.800</b>	<b>0.000</b>
mod1	X	3	87.530	0.200	2.760

**PFTeA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>84.770</b>	<b>0.720</b>	<b>0.000</b>
mod1	X	3	86.630	0.280	1.860

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DHT

PFHxS	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>229.830</b>	<b>0.830</b>	<b>0.000</b>
mod1	X	3	232.960	0.170	3.130

brPFOS

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>229.830</b>	<b>0.830</b>	<b>0.000</b>
mod1	X	3	233.060	0.170	3.230

linPFOS

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>229.830</b>	<b>0.770</b>	<b>0.000</b>
mod1	X	3	232.220	0.230	2.390

PFOA

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>229.830</b>	<b>0.790</b>	<b>0.000</b>
mod1	X	3	232.490	0.210	2.660

PFNA

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>229.830</b>	<b>0.740</b>	<b>0.000</b>
mod1	X	3	231.950	0.260	2.120

PFDcA

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>229.830</b>	<b>0.690</b>	<b>0.000</b>
mod1	X	3	231.420	0.310	1.600

AND

PFHxS	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.740</b>	<b>0.000</b>
mod1	X	3	193.160	0.260	2.110

brPFOS

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.840</b>	<b>0.000</b>
mod1	X	3	194.360	0.160	3.310

linPFOS

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.840</b>	<b>0.000</b>
mod1	X	3	194.340	0.160	3.300

PFOA

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.810</b>	<b>0.000</b>
mod1	X	3	193.900	0.190	2.850

PFNA

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.760</b>	<b>0.000</b>
mod1	X	3	193.410	0.240	2.360

PFDcA

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	3	194.020	0.180	2.980

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**PUnA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>229.830</b>	<b>0.540</b>	<b>0.000</b>
mod1	X	3	230.130	0.460	0.310

**PFDaA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>3</b>	<b>229.520</b>	<b>0.540</b>	<b>0.000</b>
mod1	X	2	229.830	0.460	0.300

**PFTriA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>229.830</b>	<b>0.800</b>	<b>0.000</b>
mod1	X	3	232.610	0.200	2.780

**PFTeA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>229.830</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	3	232.800	0.180	2.970

**PUnA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	3	194.070	0.180	3.020

**PFDaA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.840</b>	<b>0.000</b>
mod1	X	3	194.300	0.160	3.250

**PFTriA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.830</b>	<b>0.000</b>
mod1	X	3	194.260	0.170	3.210

**PFTeA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>191.050</b>	<b>0.840</b>	<b>0.000</b>
mod1	X	3	194.310	0.160	3.270

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T <sub>3</sub>							
PFHxS	Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc
mod2		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.890</b>	<b>0.000</b>
mod1	X	X	X	5	-19.420	0.110	4.210
brPFOS							
Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc	
mod2		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	X	X	5	-20.530	0.180	3.100
linPFOS							
Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc	
mod2		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.870</b>	<b>0.000</b>
mod1	X	X	X	5	-19.910	0.130	3.720
PFOA							
Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc	
mod2		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.930</b>	<b>0.000</b>
mod1	X	X	X	5	-18.580	0.070	5.050
PFNA							
Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc	
mod2		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.910</b>	<b>0.000</b>
mod1	X	X	X	5	-19.110	0.090	4.520
PFDcA							
Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc	
mod2		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.890</b>	<b>0.000</b>
mod1	X	X	X	5	-19.440	0.110	4.190

T <sub>4</sub>					
PFHxS	Circ. PFAS	K	AICc	AICcwt	ΔAICc
mod2		<b>2</b>	<b>61.360</b>	<b>0.700</b>	<b>0.000</b>
mod1	X	3	63.070	0.300	1.720
brPFOS					
Circ. PFAS	K	AICc	AICcwt	ΔAICc	
mod2		<b>2</b>	<b>61.360</b>	<b>0.840</b>	<b>0.000</b>
mod1	X	3	64.660	0.160	3.300
linPFOS					
Circ. PFAS	K	AICc	AICcwt	ΔAICc	
mod2		<b>2</b>	<b>61.360</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	3	64.420	0.180	3.060
PFOA					
Circ. PFAS	K	AICc	AICcwt	ΔAICc	
mod2		<b>2</b>	<b>61.360</b>	<b>0.840</b>	<b>0.000</b>
mod1	X	3	64.630	0.160	3.280
PFNA					
Circ. PFAS	K	AICc	AICcwt	ΔAICc	
mod2		<b>2</b>	<b>61.360</b>	<b>0.680</b>	<b>0.000</b>
mod1	X	3	<b>62.880</b>	<b>0.320</b>	<b>1.520</b>
PFDcA					
Circ. PFAS	K	AICc	AICcwt	ΔAICc	
mod2		<b>2</b>	<b>61.360</b>	<b>0.790</b>	<b>0.000</b>
mod1	X	3	<b>64.010</b>	<b>0.210</b>	<b>2.650</b>

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**PFUnA**

	Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.910</b>	<b>0.000</b>
mod1	X	X	X	5	-19.020	0.090	4.610

**PFD0A**

	Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.920</b>	<b>0.000</b>
mod1	X	X	X	5	-18.650	0.080	4.980

**PFTriA**

	Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.930</b>	<b>0.000</b>
mod1	X	X	X	5	-18.600	0.070	5.030

**PFTeA**

	Circ. PFAS	SMI	Egg mass	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>-23.630</b>	<b>0.920</b>	<b>0.000</b>
mod1	X	X	X	5	-18.610	0.080	5.020

**PFUnA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>61.360</b>	<b>0.840</b>	<b>0.000</b>
mod1	X	3	64.670	0.160	3.310

**PFD0A**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>61.360</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	3	<b>64.440</b>	<b>0.180</b>	<b>3.080</b>

**PFTriA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>61.360</b>	<b>0.840</b>	<b>0.000</b>
mod1	X	3	64.640	0.160	3.280

**PFTeA**

	Circ. PFAS	K	AICc	AICcwt	ΔAICc
<b>mod2</b>		<b>2</b>	<b>61.360</b>	<b>0.830</b>	<b>0.000</b>
mod1	X	3	64.500	0.170	3.150

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T<sub>3</sub>/T<sub>4</sub>**PFHxS**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.820</b>	<b>0.000</b>
mod1	X	X	X	5	46.160	0.180	3.020

**brPFOS**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.800</b>	<b>0.000</b>
mod1	X	X	X	5	45.880	0.200	2.740

**linPFOS**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.860</b>	<b>0.000</b>
mod1	X	X	X	5	46.770	0.140	3.630

**PFOA**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.920</b>	<b>0.000</b>
mod1	X	X	X	5	47.970	0.080	4.820

**PFNA**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.930</b>	<b>0.000</b>
mod1	X	X	X	5	48.180	0.070	5.040

**PFDeA**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.910</b>	<b>0.000</b>
mod1	X	X	X	5	47.780	0.090	4.630



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**PUnA**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.870</b>	<b>0.000</b>
mod1	X	X	X	5	47.020	0.130	3.880

**PFDaA**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.910</b>	<b>0.000</b>
mod1	X	X	X	5	47.890	0.090	4.750

**PFTriA**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.930</b>	<b>0.000</b>
mod1	X	X	X	5	48.190	0.070	5.040

**PFTeA**

	<b>Circ. PFAS</b>	<b>SMI</b>	<b>Egg mass</b>	<b>K</b>	<b>AICc</b>	<b>AICcwt</b>	<b>ΔAICc</b>
<b>mod2</b>		<b>X</b>	<b>X</b>	<b>4</b>	<b>43.140</b>	<b>0.920</b>	<b>0.000</b>
mod1	X	X	X	5	48.160	0.080	5.020

**Table S6.** Maternal circulating PFAS affecting maternal transmitted testosterone (T) concentrations in black-legged kittiwakes' eggs from Svalbard, estimated by mixed linear regression models.

<b>Parameter</b>	<b>Estimate</b>	<b>SE</b>	<b>t-value</b>	<b>p-value</b>
<b><u>T (R<sup>2</sup>: 0.27)</u></b>				
PFNA	4.082	1.684	2.425	0.032
<b><u>T (R<sup>2</sup>: 0.27)</u></b>				
PFDCa	2.773	1.144	2.424	0.032
<b><u>T (R<sup>2</sup>: 0.29)</u></b>				
PFUnA	0.788	0.315	2.504	0.028

## REFERENCES

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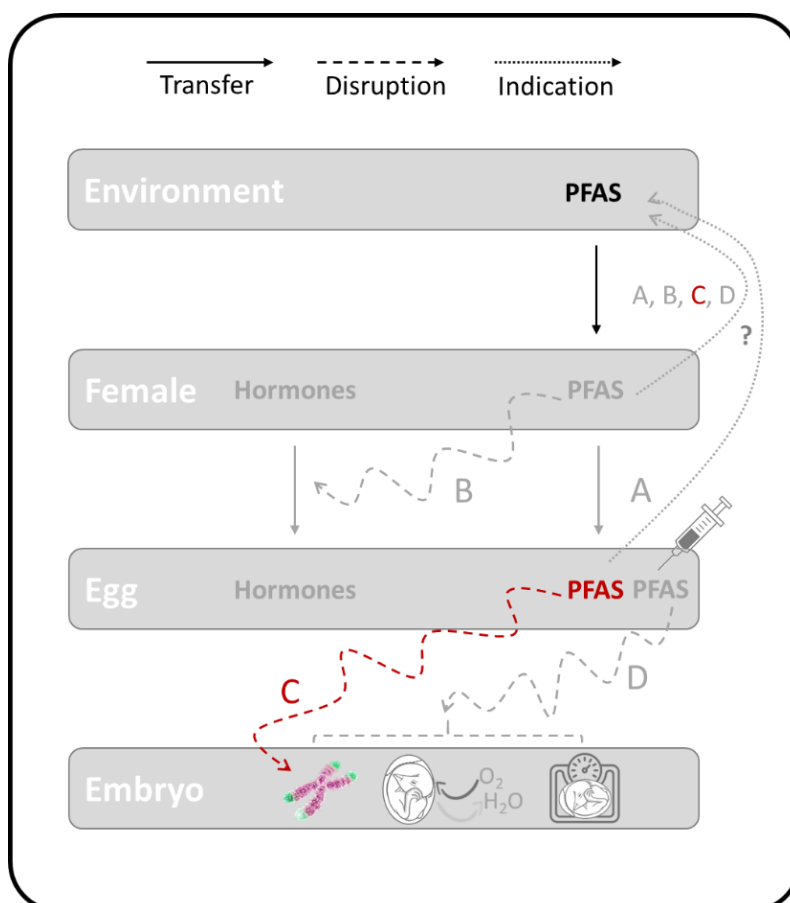
# CHAPTER III



*Snow bunting (Plectrophenax nivalis)*

## Chapter III

PFAS are known to alter numerous health markers in adult wild birds, however, although they are known to be transferred in high concentrations in eggs, their impact during embryonic development is still largely unexplored. In this chapter, we investigated the relationship between the concentrations of maternal deposited PFAS in kittiwake eggs and embryos' telomere length, a biomarker of aging, at the end of the development (**Figure 16**).



**Figure 16.** Schematic representation of Chapter III goals included in the global structure of this thesis.

### We found:

- No significant relationship between maternal deposited PFAS concentrations and embryos' telomere length.
- Some emerging PFAS in eggs yolk, including some fluorotelomers and a per- and polyfluoroalkyl ether carboxylic acid (PFECA).

# Paper C

Is in ovo exposure to per- and polyfluoroalkyl  
substances affecting telomere length in embryos of an  
Arctic seabird?

**Jouanneau, W.**, Blévin, P., Angelier, F., Dhainaut, P., Herzke, D., Léandri-Breton, D-J.,  
Moe, B., Nikiforov, V. A., Ribout, C., Gabrielsen, G. W., Chastel, O.

**In prep.** for *Chemosphere*

# Is *in ovo* exposure to per- and polyfluoroalkyl substances affecting telomere length in embryos of an Arctic seabird?

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## ABSTRACT

At the time of egg formation, crucial substances are provided to the embryo via maternal transfer. However, several contaminants are transferred to the egg as well. This can have significant consequences for the growing organism, since conditions experienced during development can have negative aftermaths later in life. Per- and polyfluoroalkyl substances (PFAS) are synthetic ubiquitous toxic contaminants which can be transferred to the avian egg, but little is known about the consequences for the developing embryo in wildlife. In eggs of black-legged kittiwakes (*Rissa tridactyla*) nesting in Svalbard, we investigated yolk concentrations of legacy and emerging PFAS. In addition to perfluoroalkylated sulfonates (PFSA) and long-chain perfluoroalkyl carboxylates (PFCA), we notably reported the occurrence of *n*:2 and *n*:3 fluorotelomeres, as well as HPFO-TeA, a per- and polyfluoroalkyl ether carboxylic acid (PFECA). Within clutches, maternal deposition was highly repeatable in eggs for brPFOS and C<sub>8</sub> to C<sub>11</sub> perfluoroalkyl carboxylic acids (PFCAs) but not for emerging PFAS. We thus considered the concentration of these compounds in the second-laid egg as a proxy of the concentration in the first-laid egg, which was artificially incubated for 20 days. At the end of the development, we explored yolk PFAS consequences for the embryo, by investigating telomeres length, a biomarker of health and lifespan suspected to be disrupted by PFAS. We found no clear relationship between yolk PFAS in eggs and embryo's telomere length. We suggest that the maternal transfer of legacy PFAS in this Svalbard's kittiwake population has low consequences on embryos telomere length.

Key-word: Black-legged kittiwake, PFAS, Pollutants, Maternal effects, embryogenesis, aging.



## INTRODUCTION

In vertebrates, many crucial substances needed to produce a fully functioning organism are provided to the embryo by maternal transfer. However, deleterious compounds as contaminants may also be transferred concurrently (Hamlin and Guillette, 2011). Embryos are particularly sensitive to contaminants exposure, as development is a critical step shaping individuals long-term fitness (Baos *et al.* 2012). Among the endpoints potentially affected by contaminants, telomeres length is considered as a proxy of individuals' health and fitness. Telomeres are highly conserved sequences of DNA repeated at chromosomal ends providing protection to maintain genomic integrity (O'Sullivan and Karlseder, 2010). Telomere attrition is a natural phenomenon occurring at every division in normal wholesome somatic cells, acting as a major barrier to tumorigenesis by interrupting cellular growth and inducing apoptosis or senescence in long-lived vertebrates (Gomes *et al.* 2010; Srinivas *et al.* 2020). Their protective function may however fade when they shorten to a critical length, in some rare cases leading some cells to a malignant phenotype or other age-related diseases (Karlseder, 2020; Rossiello *et al.* 2022). On this basis, telomere length is a recognized biomarker of the individual quality, reproductive lifespan expectancy and survival in vertebrates (Wilbourn *et al.* 2018; Angelier *et al.* 2019). The rate of telomere erosion may be impacted by environmental stressors, leading to accelerated biological ageing of the cells, ultimately causing adverse effects to the organisms (Monaghan and Haussmann, 2006). This is of particular importance during vertebrates' development, since early life telomere shortening is believed to have strong negative consequences in adulthood (Marasco *et al.* 2022).

Among the environmental stressors, some contaminants have been identified as being able to affect telomere length in vertebrates (Louzon *et al.* 2019). Exposure to metallic trace elements, legacy persistent organic pollutants, radiations, light and air pollution may have impacts on vertebrates telomere length (reviewed in (Salmon and Burraco, 2022)). However, one class of contaminants has been seldom studied with regards to an alteration of telomere length: Per- and polyfluoroalkyl substances (PFAS) consist of numerous man-made chemicals used in a wide range of everyday products. PFAS are known to be ubiquitous and persistent (Ankley *et al.* 2021), and several of them can bioaccumulate in organisms and biomagnify along trophic web, leading top predators to exhibit high concentrations even in remote locations as the Arctic (Butt *et al.* 2010). Our knowledge on the impact of PFAS on telomere is very limited in wildlife and restricted to birds, two studies on adult seabirds suggested a positive impact of PFAS burden on telomere elongation over time (telomere dynamic; Blévin *et al.* 2017a;

Sebastiano *et al.* 2020). In human adults, some studies also concluded on a potential positive (Huang *et al.* 2019; Clarity *et al.* 2021), or negative (Vriens *et al.* 2019) effect of PFAS on telomere length. Altogether, these results suggest mechanisms by which these contaminants may affect carcinogenesis and other adverse health outcomes. However, when focusing on early-life, recent studies showed contrasting results in the relationship between PFAS and telomere length in human new-borns and white-tailed eagles (*Haliaeetus albicilla*) nestlings (Sletten *et al.* 2016; Liu *et al.* 2018a; Eick *et al.* 2021; Pan *et al.* 2022).

Due to the great consequences of developmental conditions on the individual telomere length and fitness in adulthood (Hall *et al.* 2004; Boonekamp *et al.* 2014; Gorenjak *et al.* 2020; Stier *et al.* 2020; Salmon *et al.* 2021), it is essential to better understand the outcomes of early-life exposure to contaminants on telomere length disruption. Since avian embryos grow in eggs with low exchanges with the external environment, the initial maternal deposition of contaminants represents a relatively stable exposure for the embryo over time. In this study, we examined the association between some legacy and emerging PFAS deposited in egg yolk and the absolute telomere length of embryos of Arctic-breeding Black-legged kittiwake (*Rissa tridactyla*; hereafter “kittiwake”) known to be exposed to PFAS (Tartu *et al.* 2014; Blévin *et al.* 2017a; Costantini *et al.* 2019), and to transfer PFAS to their eggs (Jouanneau *et al.* 2022). To do so, we first explored the repeatability in the deposition of PFAS in eggs by females and, if applicable, considered concentrations of the second-laid egg as a proxy of the PFAS concentration of the first-laid which was artificially incubated. We then measured liver telomere length on 20 days old embryos. We hypothesize that high PFAS concentrations deposited in the eggs by the mother could affect embryos telomere length.

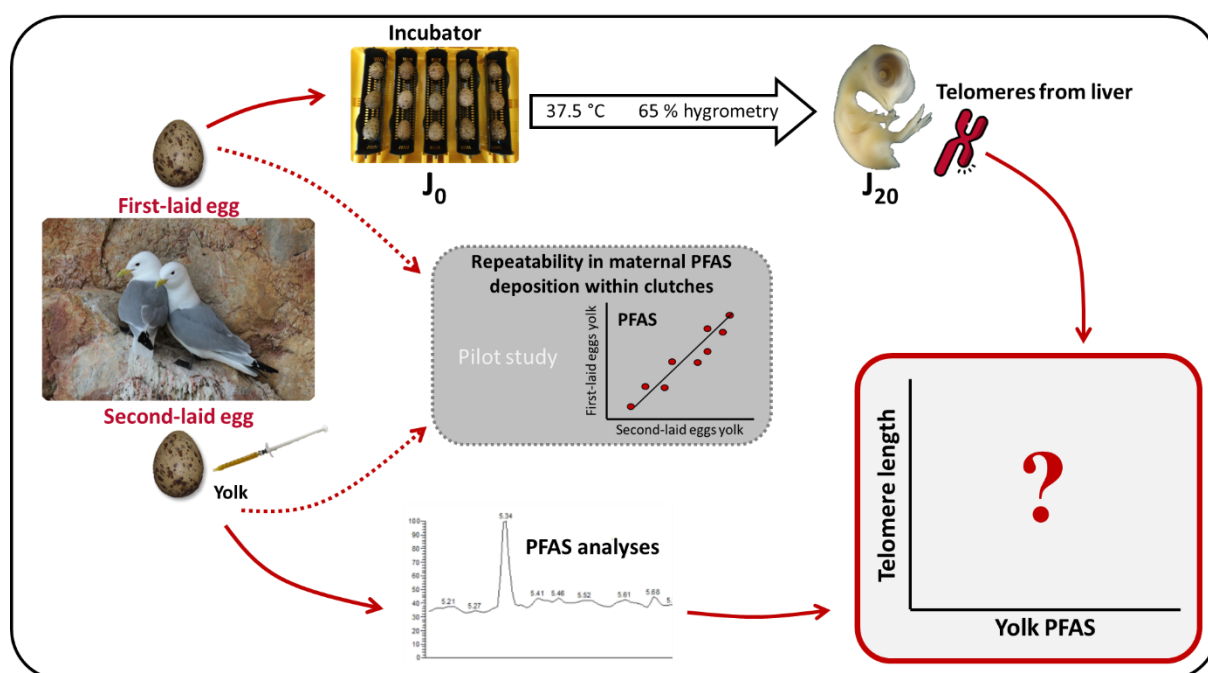
## METHODS

### **Experimental design and sample collection**

We aimed at investigating the correlation between PFAS deposited by maternal transfer in the egg and embryo’s absolute telomere length. However, in an egg, measuring both yolk PFAS at laying and embryo’s telomere at the end of the incubation is challenging since yolk biopsy may affect hatching success and could therefore lead to biased results (Rubolini *et al.* 2006). Since PFAS yolk burden are known to be correlated between eggs of the same clutch in great tit (*Parus major*) and Audouin’s gulls (*Ichthyaetus audouinii*) (Vicente *et al.* 2015; Lasters

*et al.* 2019), PFAS concentration in an egg may be a good proxy of the concentration in the other ones in clutches. Therefore, it would make sense to explore the relationship between PFAS of an egg yolk and telomere length of the embryo from another egg from the same clutch to investigate the impact of maternal PFAS on embryos telomeres. In that context, in kittiwakes we conducted our analysis in two steps: 1) a pilot study to explore the repeatability in the maternal deposition of PFAS between eggs of similar clutches, to ensure that second-laid eggs PFAS concentrations may be used as a proxy of those of the first-laid eggs; 2) using the compounds found to have a high repeatability in their transfer within clutches in the first step, we explored their relationship with telomere length in kittiwake embryos at the end of the incubation period (Figure 1).

**Figure 1.** Schematic representation of the experimental design conducted on black-legged kittiwake first- and second-laid eggs from Svalbard.



The pilot study was conducted in 2019 in eggs from a kittiwake colony in Kongsfjorden, Svalbard (78°53'48"N 12°11'43"E). In this species, females usually lay a two-egg clutch, both hatching after an incubation period of ~27 days (Coulson and White, 1958; Moe *et al.* 2009). We used a subset of the same eggs ( $n = 11$  full clutches) from of a previous publication by Jouanneau *et al.* (2021) in which the field protocol and PFAS analyses are described. In brief, we monitored occupied nests daily and collected first- ( $n = 11$ ) and second-laid eggs ( $n = 11$ ) as soon as they were laid (< 24h), to ensure a precise estimation of the laying date. When a first-



and Tel2b: 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'; RAG1: Rag1F: 5'-GGGTCCTCTGATAGCCGAAA-3' and Rag1R: 5'-CATCATAACCTGTACCCCGGA-3'). We ran all samples and quality-control samples on a single 96-well plate, using concentrations of 800 and 300 nM for telomere and control gene primers respectively. We added an eight-point standard range (from 0.31 to 40 ng) in triplicate to ensure a good amplification efficiency of the reactions. All samples were run in five replicates together with blanks. Samples with an averaged cycle threshold SD > 0.2 between replicates were repeated. Coefficient of variability were 0.62 % (RAG1) and 1.85 % (TELO), and amplification efficiency was within the acceptable range for both RAG1 and telomere (RAG1: 101 %; Telomere: 93.5 %). We then calculated the relative telomere length (expressed as the  $T/S$  ratio) as the number of telomere copies ( $T$ ) relative to the single copy gene ( $S$ ).

### **Per- and polyfluoroalkyl substances analysis**

We adapted a method from Powley *et al.* (2005) described in Sletten *et al.* (2016) and adjusted for egg yolk (Jouanneau *et al.* 2022) to investigate PFAS concentrations in eggs of 2019 and 2020.

In egg yolk from second-laid eggs in 2020, we analysed a total of 42 PFAS and kept compounds detected in more than 70 % of the eggs for further investigations (SI Table S1). Consequently, a total of 13 compounds were kept for statistical analyses, some legacy PFAS: branched perfluorooctanesulfonic acid (brPFOS), linear perfluorooctanesulfonic acid (linPFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDoA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriA), perfluorotetradecanoic acid (PFTeA) and Perfluorohexadecanoic acid (PFHxDA), and some emerging compounds, two fluorotelomers: 10:2 Fluorotelomer sulfonic acid (10:2 FTS), 7:3 Fluorotelomer carboxylic acid (7:3 FTCA), and one short-chain PFAS related to HFPO-DA (GenX): Perfluoro-2,5,8-trimethyl-3,6,9-trioxadecanoic acid (HPFO-TeA or 3333-PFECA). For all included PFAS, values lower than the LOD were set to half of the LOD of the specific compound. We provided all concentrations in  $\text{ng g}^{-1}$  wet weight (ww).

The PFAS measured in first- and second-laid eggs for the pilot study in 2019 were not entirely similar to those of the 2020 experiment (Jouanneau *et al.* 2022). The following compounds were investigated and found above the limit of detection (LOD) in more than 70 %

of the egg sampled in both studies: ten legacy PFAS: branched perfluorooctanesulfonic acid (brPFOS), linear perfluorooctanesulfonic acid (linPFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDcA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriA), perfluorotetradecanoic acid (PFTeA) and Perfluorohexadecanoic acid (PFHxDA), and one emerging compounds: 7:3 Fluorotelomer carboxylic acid (7:3 FTCA).

## Statistical analyses

All analyses were performed using R software (v.4.0.0 ((2020), 2020)). For all models below, the model fit was inspected by using residual diagnostic plots.

First, for the pilot study, we investigate the repeatability in females' PFAS deposition in their eggs, focusing on compounds found in more than 70 % of the samples both in 2019 and 2020 (i.e. brPFOS, linPFOS, PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTriA, PFTeA, PFHxDA and 7.3 FTCA). To this end, we investigated the relationship between both eggs within clutches in 2019 using repeatability analyses (Nakagawa and Schielzeth, 2010; Stoffel *et al.* 2019), estimated from linear mixed-effects models (LMM) fitted by restricted maximum likelihood (REML). We included the female identify as a random factor and ran a bootstrap of 1000 iterations for each model. Yolk PFAS concentrations presented a similar pattern and were within the same ranges in 2019 and 2020 (SI Figure S1), therefore, we hypothesize that the repeatability in the maternal deposition within clutch was similar in both years for the investigated compounds. For the following analyses, we considered only PFAS found to have a significant repeatability in their deposition within clutch: linPFOS, PFOA, PFNA, PFDcA and PFUnA (see results section).

In a second step, on eggs sampled in 2020, we explored the relationship between telomere length of embryos from first-laid eggs and the maternal deposited PFAS concentrations in second-laid eggs (used as a proxy of first-laid eggs contamination). As some of the selected PFAS were highly correlated (SI Table S2 and Figure S2), we performed a principal component analysis (PCA; “FactoMineR” R package, version 2.3) to reduce the number of explanatory variables to a few representative ones. We retained the two first principal components (PCs; eigenvalues: 3.65 and 1.20 respectively) as they explained a high proportion of the total variance (97 % in total; SI Table S3), and since all 5 PFAS significantly contributed to at least one PC (SI Figure S3) and were well represented by each PCs (SI Figure S4). In a

second phase, we investigated the relationship between telomere length and PFAS using a linear model (LM) and including the two selected PCs and the egg mass at laying (to control for egg quality and indirectly embryos' quality as this may influence their telomere length (Whittingham *et al.* 2007; Stier *et al.* 2020)) as predictors. Telomere length was not different between males and females (LM:  $F_{1, 9} = 0.0003$ ,  $p = 0.986$ ) and the variable *Sex* was consequently not included as a predictor. To select the best predictors and therefore the best model, we ranked and rescaled a set of models from the full to the null model according to the Akaike's Information Criterion corrected for small sample size (AICc; (Buckland *et al.* 1997; Burnham and Anderson, 2004)). We selected the model with the lowest AICc, if the  $\Delta$ AICc with the next ranked model was  $<2$ , the most parsimonious was chosen.

Although examining each contaminant separately is not recommended, since testing many models can potentially increase the type I error, this method has been commonly applied in the analyses of PFAS data since it is very often found that adverse effects may be correlated to a single or few compounds in wildlife (Tartu *et al.* 2014; Blévin *et al.* 2018, 2020; Sebastiano *et al.* 2020). To examine this, we also explored the relationship between telomere length and each of the PFAS in independent LMs, using the specific PFAS as well as the egg mass at laying to account for the potential effect of single PFAS. The best model using the AICc.

## RESULTS

We found a significant repeatability in the deposition of PFAS between first- and second-laid eggs from 2019 for the following compounds: linPFOS (repeatability:  $r \pm se = 0.51 \pm 0.22$ ;  $p = 0.045$ ; Figure 2 and SI Figure S5), PFOA ( $r \pm se = 0.76 \pm 0.15$ ;  $p < 0.01$ ), PFNA ( $r \pm se = 0.83 \pm 0.12$ ;  $p < 0.001$ ), PFDcA ( $r \pm se = 0.80 \pm 0.13$ ;  $p < 0.001$ ) and PFUnA ( $r \pm se = 0.53 \pm 0.21$ ;  $p = 0.041$ ). But repeatability was relatively low for brPFOS ( $r \pm se = 0.30 \pm 0.23$ ;  $p = 0.194$ ; Figure 2 and SI Figure S5), PFDcA ( $r \pm se = 0.20 \pm 0.22$ ;  $p = 0.308$ ), PFTriA ( $r \pm se = 0.01 \pm 0.17$ ;  $p = 1$ ), PFTeA ( $r \pm se = 0.19 \pm 0.22$ ;  $p = 0.312$ ), PFHxDA ( $r \pm se = 0 \pm 0.20$ ;  $p = 1$ ) and 7:3 FTCA ( $r \pm se = 0 \pm 0.20$ ;  $p = 1$ ). We therefore only used linPFOS, PFOA, PFNA, PFDcA and PFUnA in further analyses on data obtain in 2020.

Descriptive statistics of PFAS concentrations measured in second-laid eggs from 2020 are provided in Table 1. The two PCs extracted from the PCA (Figure 3) indicated that high PC<sub>1</sub> scores reflected high concentrations of linPFOS (loading: 0.51; SI Table S3), PFDcA (0.51) and PFUnA (0.51). High PC<sub>2</sub> scores reflected high concentrations of PFOA (0.78) and PFNA

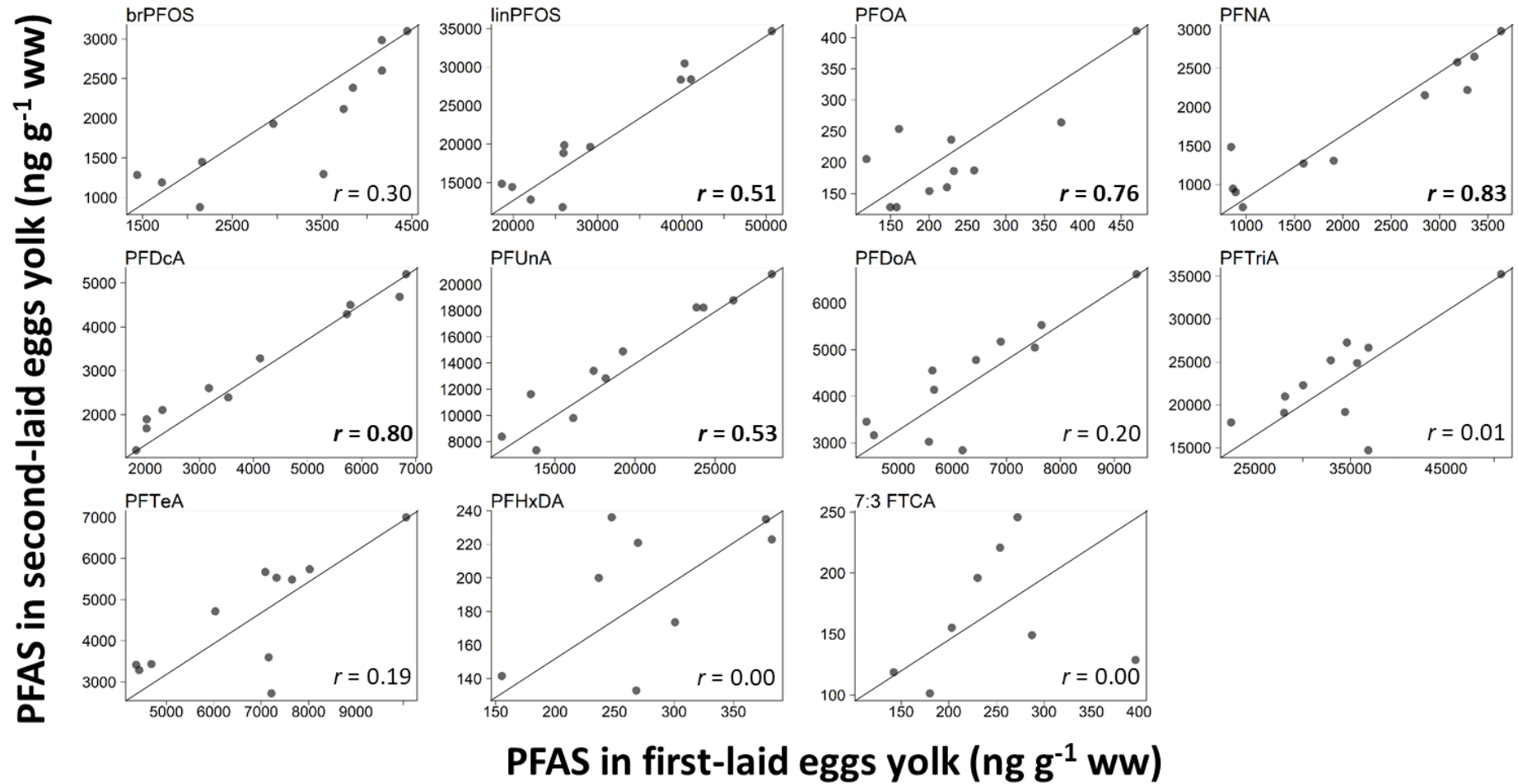
(0.60). However, the model selection showed that none of the two PCs was significantly correlated with telomere length in embryos (SI Table S4). The model selection ran on independent models per PFAS also showed that none of the independent PFAS was significantly correlated with telomere length (Figure 4 and SI Table S5).

**Table 1.** Descriptive statistics (mean  $\pm$  standard deviation *SD*, median and range *min-max*) for PFAS concentrations (ng g<sup>-1</sup> ww) in second-laid eggs yolk of black-legged kittiwakes from Svalbard.

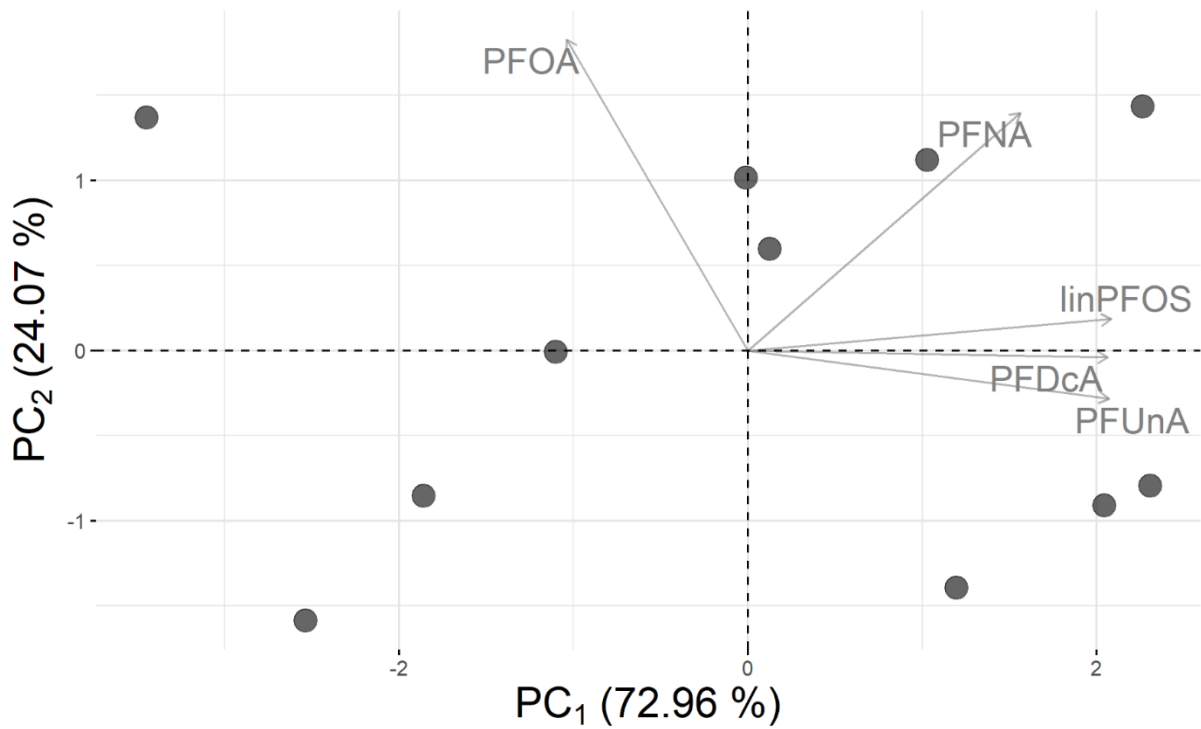
<b>Second-laid eggs (<i>n</i>=11)</b>			
	<b>Mean <math>\pm</math> SD</b>	<b>Median</b>	<b>Min-max</b>
brPFOS	1.54 $\pm$ 0.33	1.61	0.98 - 2.08
linPFOS	20.6 $\pm$ 3.02	21.2	15.6 - 24.8
PFOA	0.16 $\pm$ 0.04	0.17	0.10 - 0.24
PFNA	3.35 $\pm$ 0.62	3.45	2.29 - 4.36
PFDCa	4.23 $\pm$ 0.88	4.36	2.82 - 5.39
PFUnA	18.8 $\pm$ 4.29	19.0	10.8 - 24.07
PFDoA	4.38 $\pm$ 0.95	4.54	2.94 - 5.54
PFTriA	24.3 $\pm$ 4.36	24.8	15.8 - 30.2
PFTeA	7.05 $\pm$ 1.54	7.47	4.71 - 9.23
PFHxDA	0.30 $\pm$ 0.08	0.3	0.21 - 0.45
10:2 FTS	0.28 $\pm$ 0.17	0.29	0.05 - 0.51
7:3 FTCA	0.21 $\pm$ 0.08	0.21	0.06 - 0.34
HPFO-TeA	0.16 $\pm$ 0.04	0.16	0.11 - 0.25



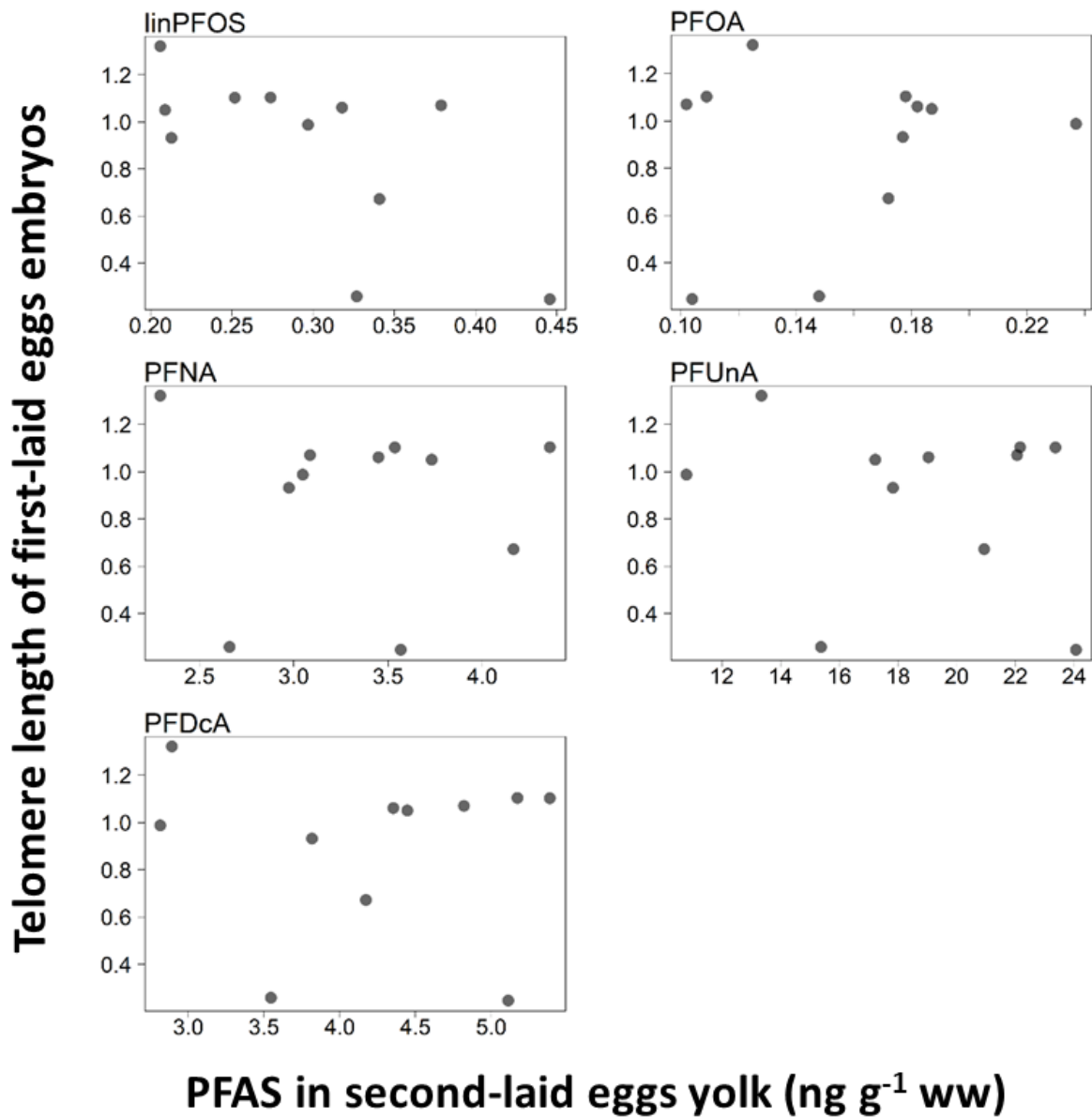
Figure 2. Repeatability ( $r$ ) in the maternal deposition of PFAS within clutch of black-legged kittiwakes from Svalbard. Bolded  $r$  are significant.



**Figure 3.** Graphical display of the principal components analysis for the first and second principal components ( $PC_1$  and  $PC_2$ ). Each arrow represents a single PFAS with the direction representing where the contaminants load in the principal component space, and the length representing the quality of the representation by the principal components. Each point represent the coordinates of a second-laid egg in the PCs space.



**Figure 4.** Relationship between telomere length in liver from first-laid eggs embryos after 20 days of incubation, and PFAS measured in second- laid eggs yolk (as a proxy of the maternal deposition in first-laid eggs). No relationship were significant. PFAS used are those found to be deposited with high repeatability within clutches.



## DISCUSSION

### **Intra-clutch PFAS relationship**

Repeatability in the deposition of PFAS along the laying sequence has not been widely studied previously: A study on seabirds (Audouin's gulls) showed that PFOS was correlated among eggs within a clutch (Vicente *et al.* 2015), and a second one on great tits (*Parus major*) found that some long-chain perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) were correlated between some of the eggs but not all, within a clutch (Lasters *et al.* 2019). Such differences between species may be due to the differences in their life history traits and foraging strategies. Gulls laying fewer eggs and being exposed to higher concentrations of PFAS due to their aquatic diet of higher trophic position, may transfer a relatively low proportion of their body burden in each egg. On the opposite, small passerines, largely depending on exogenous substances to synthesize their eggs may transfer a larger proportion of their PFAS burden in their eggs. Therefore, the PFAS input during the interval between two eggs synthesis, could largely contribute to the deposited concentrations in eggs leading to variations in eggs concentration within a clutch. Contrarily to correlative studies, as done in the two studies cited above, repeatability analysis enables an estimation of the degree of stability in the maternal deposition of PFAS within clutches.

We expected a high repeatability in the deposition of PFAS within clutches, since PFAS in yolks are highly correlated with circulating concentrations in females' plasma for most PFAS (Jouanneau *et al.* 2022), but only linPFOS and C<sub>8</sub> to C<sub>11</sub> PFCAs exhibited a high repeatability between first- and second-laid eggs. If the low repeatability in the deposition of PFHxDA and 7:3 FTCA in eggs may potentially be explained by their relative low abundance in the yolk, it is surprising that brPFOS and C<sub>12</sub> to C<sub>14</sub> PFCAs, present in elevated concentrations, does not show a high repeatability between egg yolks. Nonetheless, considering the large proportion of the longest-chain PFCAs being transferred in eggs during oogenesis (Jouanneau *et al.* 2022), females may be depleted in this compound before laying their second egg, which may explain the absence of repeatability in the deposition within clutches. This result shows that although there may be a correlation in PFAS concentrations within a clutch, as found in Audouin's gulls and great tits, there is a relatively important residual yet unexplained variation in the deposition of some PFAS by females.

Although, these results were obtained on a limited number of samples collected in a single year and further investigations may be necessary to corroborate these results, we

hypothesized that the repeatability in the maternal deposition of PFAS was similar in 2019 and 2020. As specified, to ensure that second-laid eggs provide a decent estimation of first-laid eggs PFAS concentrations, we only used the compounds found to be deposited with a high repeatability in eggs within clutches in the telomere analyses. However, as second-laid eggs have lower PFAS concentration than first-laid eggs in black-legged kittiwakes (Jouanneau *et al.* 2022), we were not able to directly estimate the consequences of specific concentrations of PFAS on telomere length.

### **PFAS pattern and occurrence**

Among all PFAS deposited in eggs in 2020, PFTriA was the most abundant, followed by linPFOS and PFUnA. Among PFCAs, this pattern with odd dominating over even-chain is commonly observed in seabirds from this region, including kittiwakes (Blévin *et al.* 2017a; Blévin *et al.* 2017b; Blévin *et al.* 2018; Ask *et al.* 2021; Sebastiano *et al.* 2020). Overall, concentrations of a similar range were observed in kittiwake eggs sampled from the same population in 2019, indicating a relatively stable concentration at medium term (Jouanneau *et al.* 2022). However, an elevated concentration of long-chain PFCAs ( $\geq C_8$  (Buck *et al.* 2011)) is of concern as these compounds are among the most toxic ones for seabirds (Blévin *et al.* 2017b; Ask *et al.* 2021; Sebastiano *et al.* 2020). Nonetheless, in kittiwakes and bird embryos in general, PFAS toxicity has been little studied at environmental concentrations, and it is difficult to estimate if the investigated population may be at risk or not. Among PFCAs, PFHxDA, a long-chain PFAS ( $C_{16}$ ) seldom investigated or detected in vertebrates, was found for the second time in kittiwake eggs (Jouanneau *et al.* 2022), but it has never been reported in adults. Although being one of the PFCAs with the longest carbon chain, and therefore prone to a high accumulation in the organism (Ng and Hungerbuhler, 2014), especially in eggs (Jouanneau *et al.* 2022), its relatively low concentration suggests a low exposure of adults in the environment. Nonetheless, the toxicity of PFHxDA has been rarely investigated. *In vitro*, PFHxDA was found to alter vascular permeability, through degradation of adherents' junction in human endothelial cells (Liu *et al.* 2018b) and could potentially disrupt thyroid receptors (Ren *et al.* 2015). In laboratory rats (*Rattus norvegicus*), PFHxDA caused hepatotoxicity (Hirata-Koizumi *et al.* 2015), although a lower toxicity risk was suggested for this compound compared to other long-chain PFAS in this species (Bil *et al.* 2021). PFAS maternal transfer and adverse effects generally increase with their chain length (Ankley *et al.* 2021; Jouanneau *et al.* 2022), we

recommend increasing the effort in monitoring the occurrence and effects of all long-chain PFCAs in wildlife.

Aside from legacy PFAS, we observed emerging PFAS in the form of two fluorotelomers, namely 10:2 FTS and 7:3 FTCA, as well as HPFO-TeA, a polyfluoroalkyl ether carboxylic acid (PFECA), in egg yolk. Apart from the fact that most of them eventually degrade to long-chain PFAS (Buck *et al.* 2011), little is known about fluorotelomers occurrence and toxicity. Nevertheless, some compounds have been suggested as being more toxic than long-chain PFCAs (Phillips *et al.* 2007). Among fluorotelomers, 7:3 FTCA an intermediate product of fluorotelomer alcohol degradation, has been recently increasingly found in marine mammals in the Arctic and was also measure in kittiwake eggs of the same population in 2019 (Schultes *et al.* 2020; Spaan *et al.* 2020; Barrett *et al.* 2021; Jouanneau *et al.* 2022). PFECA are also increasingly reported in surface water worldwide (Joerss *et al.* 2020; Muir and Miaz, 2021) and in biota in the Arctic (Schultes *et al.* 2020; Spaan *et al.* 2020), but little is known about HPFO-TeA. This compound is a derivative from HPFO-DA (GenX), a relatively recent emerging PFAS, used as an alternative to long-chain compounds, and suggested to have a low developmental toxicity, as well as a low bioaccumulation potential (Munoz *et al.* 2019; Gaballah *et al.* 2020). The fact that HPFO-TeA is found in most kittiwake eggs may indicate either a strong bioaccumulation potential or a strong occurrence of this compound or precursors in the local environment.

### **PFAS relationship with absolute telomere length**

The absence of relationship between embryos telomere length and the concentration of maternal PFAS deposited in the yolk represented by the two PCs or each of the PFAS independently, suggests that the investigated PFAS of this study have no strong effects on the telomeres of the developing embryo of this kittiwake population. Overall, in the literature, effects of PFAS on telomere length were unclear. Previous studies on embryo development showed inconsistent results (either positive, negative or no relationships) in human newborns (Liu *et al.* 2018a; Eick *et al.* 2021; Pan *et al.* 2022). In birds, no relationship between PFAS and absolute telomere length was found in white-tailed eagle (*Haliaeetus albicilla*) nestlings nor in adults of glaucous gulls (Sebastiano *et al.* 2020) or kittiwakes from the same population than those of the present study (Blévin *et al.* 2017a). However, in the same studies on glaucous gulls and kittiwakes, PFAS were related to telomere elongation over time (Blévin *et al.* 2017a;

Sebastiano *et al.* 2020), and it was hypothesized that PFAS may stimulate self-maintenance mechanisms in adults. As for telomere length, telomere dynamic have previously been identified as a more reliable marker of biological age than absolute telomere length (Barrett *et al.* 2013). Nonetheless, the studies cited above are correlative, and a causal link between exposure to PFAS and telomere length was not investigated. By conducting a study on embryos incubated in similar conditions, we could liberate from confounding effects including the age or the individual variation in incubation effort, which are known to affect telomere length (Dupont *et al.* 2018). However, we found similar results than the previously cited studies on adult birds, with no effect of PFAS on absolute telomere length, although an effect on telomere dynamic may still be possible in embryos and need further investigation. Nonetheless, in kittiwake embryos, the development period lasts 27 days. The exposure period may thus be too short to induce significant change in telomere length, especially since we euthanized the embryos after three quarters (20 days) of the full incubation duration. However, assuming that PFAS may alter telomere length, PFAS concentrations may also be lower than specific toxicity thresholds to impair embryos' telomere length in eggs of the present study.

Telomere length has been suggested to be affected by different factors including parents' age and quality, oxidative stress, metabolic rate and growth or high hormone concentrations (Dupont *et al.* 2018; Monaghan and Ozanne, 2018; Noguera *et al.* 2018; Vedder *et al.* 2018; Criscuolo *et al.* 2020; Stier *et al.* 2020; Salmon and Burraco, 2022). These parameters were not measured or available in our study, and it would be critical to integrate in further studies evaluating the potential impact of PFAS on telomere length in bird embryos.

## CONCLUSION

PFAS have been previously found to affect telomere length in adult kittiwakes, yet, during embryonic development this may have a fundamental influence on individuals' long-term fitness and survival. In this study, we investigated the relationship between maternal PFAS deposited by females in the yolk during egg formation and embryos telomere length at the end of the incubation period. We found no overall association between kittiwake yolk PFAS and embryos telomere length, and therefore suggest that PFAS do not significantly affect telomere length during the development at environmental concentrations in Svalbard. However, we recommend further experimental assessments to corroborate these findings, especially on long-chain PFCAs and emerging PFAS, still largely under investigated.

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**Supporting Information**

Is *in ovo* exposure to per- and polyfluoroalkyl substances affecting telomere length in embryos of an Arctic seabird?

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**Table S1.** List of targeted poly- and perfluoroalkyl substances (PFAS): their groups, abbreviations, chemical names, detection rates in egg yolk of black-legged kittiwakes and limits of detection. Selected PFAS (i.e. detected in more than 70% of the samples and therefore used in analyses) are stressed in bold. **Table S1.** List of targeted poly- and perfluoroalkyl substances (PFAS): their groups, abbreviations, chemical names, detection rates in egg yolk of black-legged kittiwakes and limits of detection. Selected PFAS (i.e. detected in more than 70% of the samples and therefore used in analyses) are stressed in bold.

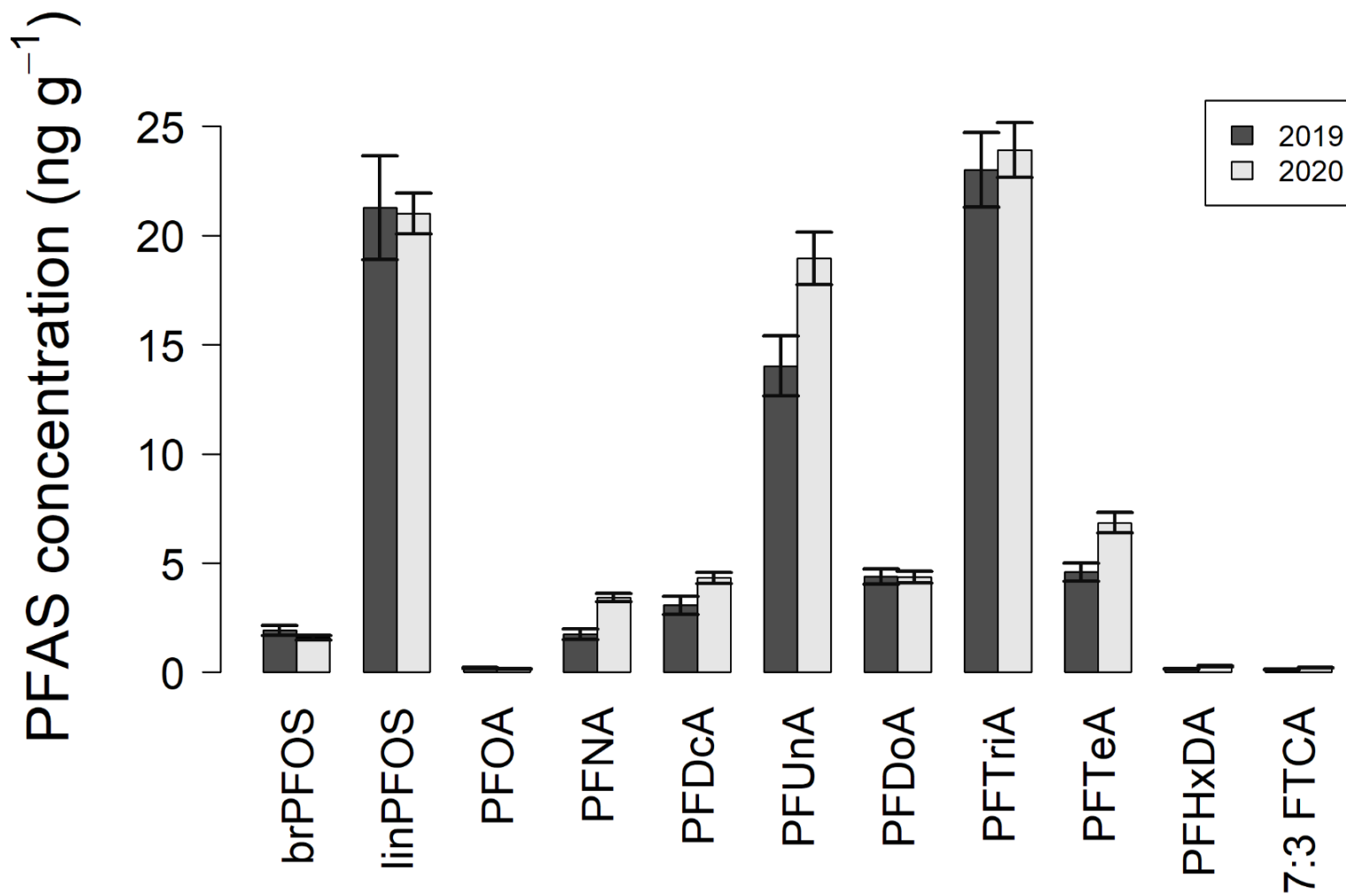
Group	Abbreviation	Chemical name	Eggs yolk	Limit of detection (pg g <sup>-1</sup> ww)
Perfluoroalkane sulfonic acids	PFBS	perfluorobutanesulfonic acid	0.0%	100
	PFPS	perfluoropentanesulfonic acid	0.0%	100
	PFHxS	perfluorohexanesulfonic acid	15.4%	100
	PFHpS	perfluoroheptanesulfonic acid	0.0%	100
	<b>brPFOS</b>	<b>branched perfluorooctanesulfonic acid</b>	<b>100.0%</b>	<b>250</b>
	<b>linPFOS</b>	<b>linear perfluorooctanesulfonic acid</b>	<b>100.0%</b>	<b>100</b>
	PFNS	perfluorononanesulfonic acid	0.0%	200
	PFDCS	perfluorodecanesulfonic acid	7.7%	300
Perfluoroalkyl carboxylic acids	PFBA	perfluorobutanoic acid	0.0%	400
	PFPA	perfluoropentanoic acid	0.0%	200
	PFHxA	perfluorohexanoic acid	0.0%	200.0
	PFHpA	perfluoroheptanoic acid	0.0%	50.0
	<b>PFOA</b>	<b>perfluorooctanoic acid</b>	<b>100.0%</b>	<b>60.0</b>
	<b>PFNA</b>	<b>perfluorononanoic acid</b>	<b>100.0%</b>	<b>100.0</b>
	<b>PFDA</b>	<b>perfluorodecanoic acid</b>	<b>100.0%</b>	<b>100.0</b>
	<b>PFUnA</b>	<b>perfluoroundecanoic acid</b>	<b>100.0%</b>	<b>100.0</b>
	<b>PFDoA</b>	<b>perfluorododecanoic acid</b>	<b>100.0%</b>	<b>100.0</b>
	<b>PFTriA</b>	<b>perfluorotridecanoic acid</b>	<b>100.0%</b>	<b>100.0</b>
	<b>PFTeA</b>	<b>perfluorotetradecanoic acid</b>	<b>100.0%</b>	<b>100</b>
<b>PFHxDA</b>	<b>perfluorohexadecanoic acid</b>	<b>92.3%</b>	<b>100</b>	

## Chapter III – Paper C: Supporting Information

Perfluoroalkane sulfonamido	FOSA	perfluorooctane sulfonamide	0.0%	100
	4:2 FTS	4:2 fluorotelomer sulfonic acid	0.0%	100
Fluorotelomer sulfonic acid	6:2 FTS	6:2 fluorotelomer sulfonic acid	7.7%	100
	8:2 FTS	8:2 fluorotelomer sulfonic acid	0.0%	200
	<b>10:2 FTS</b>	<b>10:2 fluorotelomer sulfonic acid</b>	<b>92.3%</b>	<b>100</b>
Fluorotelomer carboxylic acid	5:3 FTCA	5:3 fluorotelomer carboxylic acid	0.0%	100
	<b>7:3 FTCA</b>	<b>7:3 fluorotelomer carboxylic acid</b>	<b>84.6%</b>	<b>120</b>
	GenX or 33-PFECA (HPFO-DA)		0.0%	500
	333-PFECA (HFPO-TA)		0.0%	75
	<b>3333-PFECA (HPFO-TeA)</b>		<b>100.0%</b>	<b>75</b>
	33333-PFECA (5x3-PFECA)		0.0%	200
	333333-PFECA (6x3-PFECA)		0.0%	200
	Furan-PFECA		0.0%	100
	122-PFECA		0.0%	100
Fluoroalkylether compounds	Iodo-25-PFECA		0.0%	100
	F-53B (w-Cl-62-PFES)		0.0%	100
	F53 (62-PFES)		0.0%	200
	w-Cl-42-PFES		0.0%	200
	w-Cl-82-PFES		0.0%	100
	w-Cl-10:2-PFES		0.0%	200
	PFEcHS		0.0%	250
	FHxSA		0.0%	200



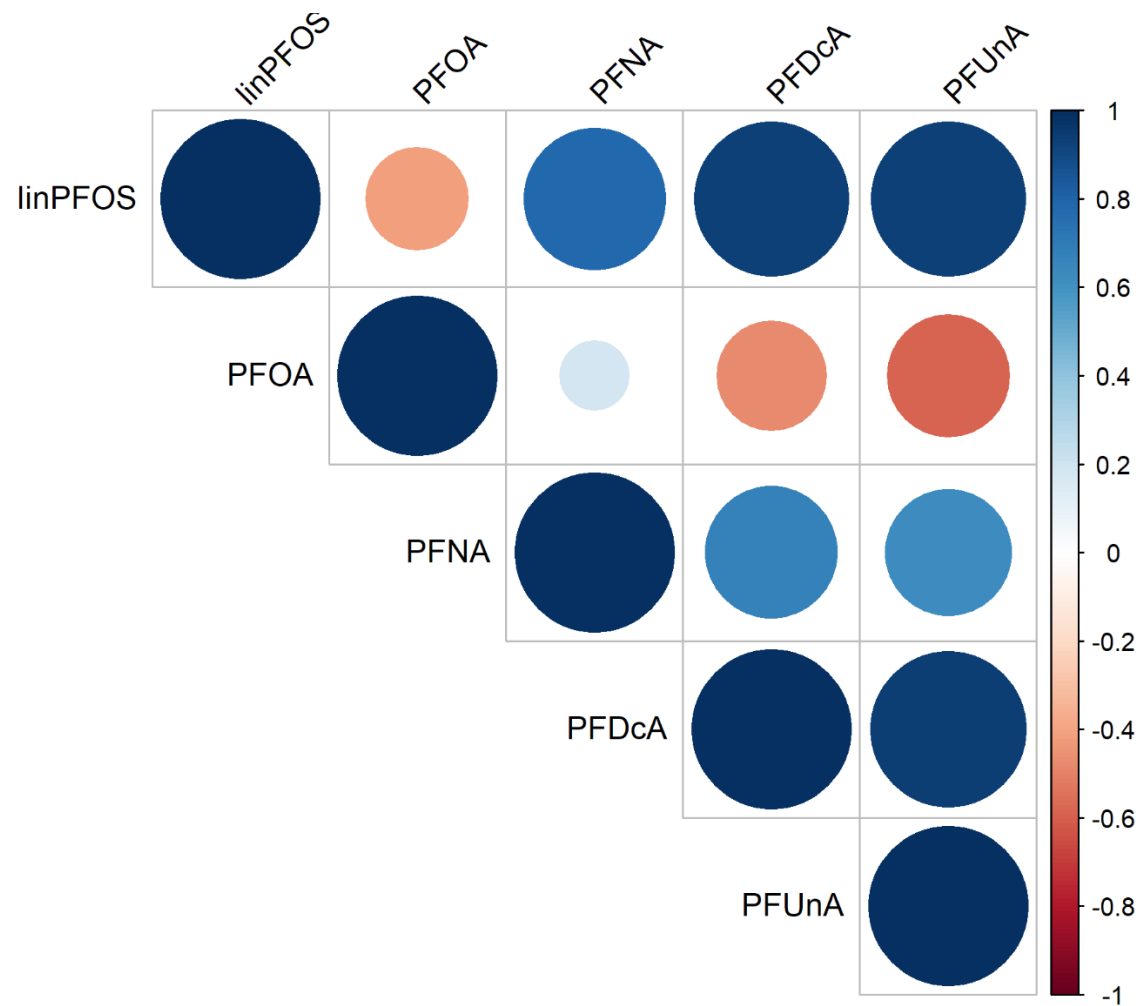
**Figure S1.** Mean  $\pm$  standard error of the concentrations of PFAS (expressed as  $\text{ng g}^{-1}$  of yolk ww) in egg yolks of black-legged kittiwake from Svalbard sampled in 2019 and 2020.



**Table S2.** Correlation matrix among PFAS included in statistical analyses: Pearson's correlation coefficient (top-table) and *p*-values (bottom-table), significant correlations are stressed in bold.

	<b>linPFOS</b>	<b>PFOA</b>	<b>PFNA</b>	<b>PFDCa</b>	<b>PFUnA</b>
<b>linPFOS</b>	1.00	-0.41	<b>0.78</b>	<b>0.94</b>	<b>0.93</b>
<b>PFOA</b>	-0.41	1.00	0.19	-0.47	-0.58
<b>PFNA</b>	<b>0.78</b>	0.19	1.00	<b>0.68</b>	<b>0.63</b>
<b>PFDCa</b>	<b>0.94</b>	-0.47	<b>0.68</b>	1.00	<b>0.95</b>
<b>PFUnA</b>	<b>0.93</b>	-0.58	<b>0.63</b>	<b>0.95</b>	1.00
	<b>linPFOS</b>	<b>PFOA</b>	<b>PFNA</b>	<b>PFDCa</b>	<b>PFUnA</b>
<b>linPFOS</b>		0.207	<b>0.004</b>	<b>0.000</b>	<b>0.000</b>
<b>PFOA</b>	0.207		0.579	0.142	0.059
<b>PFNA</b>	<b>0.004</b>	0.579		<b>0.021</b>	<b>0.039</b>
<b>PFDCa</b>	<b>0.000</b>	0.142	<b>0.021</b>		<b>0.000</b>
<b>PFUnA</b>	<b>0.000</b>	0.059	<b>0.039</b>	<b>0.000</b>	

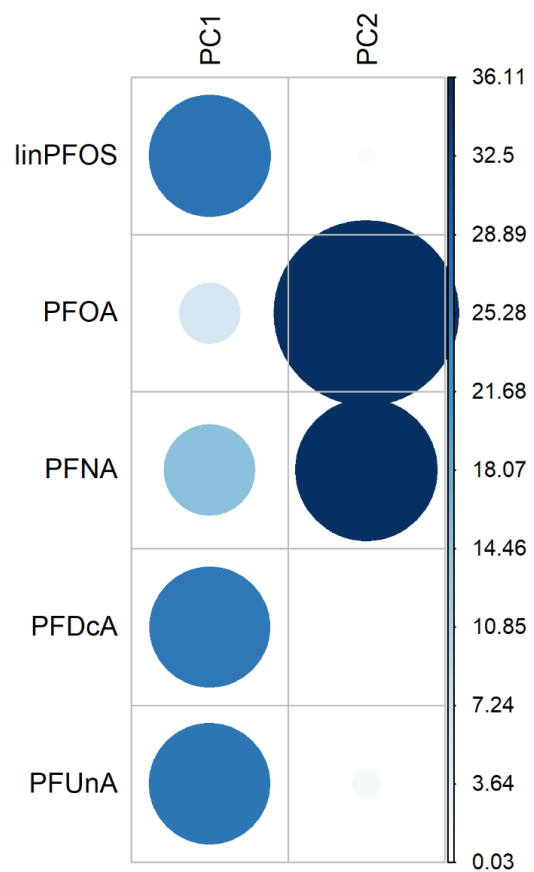
**Figure S2.** Graphical display of the correlation matrix among PFAS variables included in the statistical analyses. Colored circles indicate a positive (blue) or negative (red) correlation, and the size and color intensity correspond to the strength of the correlation.



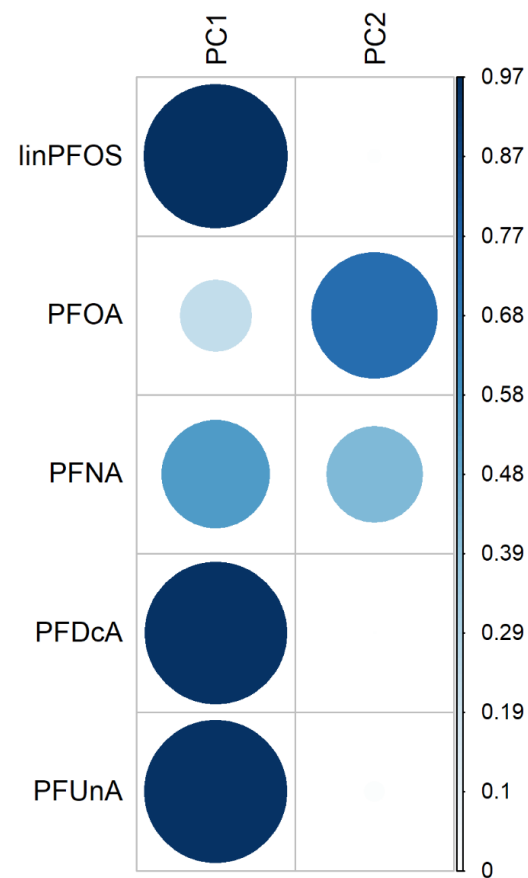
**Table S3.** Principal components analyses outputs (loadings and cumulative variance) for each PFAS variable on each of the two first principal components (PCs). Large loadings (positive or negative) indicate a strong relationship the specific PC. The sign of a loading indicates whether a variable and a PC are positively or negatively correlated. Values about the cut-off for important loading (0.447; calculated as  $\sqrt{\frac{1}{x}}$ , with  $x$  being the number of PFAS used for the PCA) are in bold.

	<b>PC<sub>1</sub></b>	<b>PC<sub>2</sub></b>
<b>linPFOS</b>	<b>0.982</b>	0.087
<b>PFOA</b>	<b>-0.490</b>	<b>0.862</b>
<b>PFNA</b>	<b>0.736</b>	<b>0.659</b>
<b>PFDCa</b>	<b>0.973</b>	-0.019
<b>PFUnA</b>	<b>0.977</b>	-0.134
<b>Cumulative variance explained</b>	72.957	97.022

**Figure S3.** Graphical representation of variable contributions in the determination of each of the first two principal components. The size of the circle and the color intensity indicate the strength of the contribution.

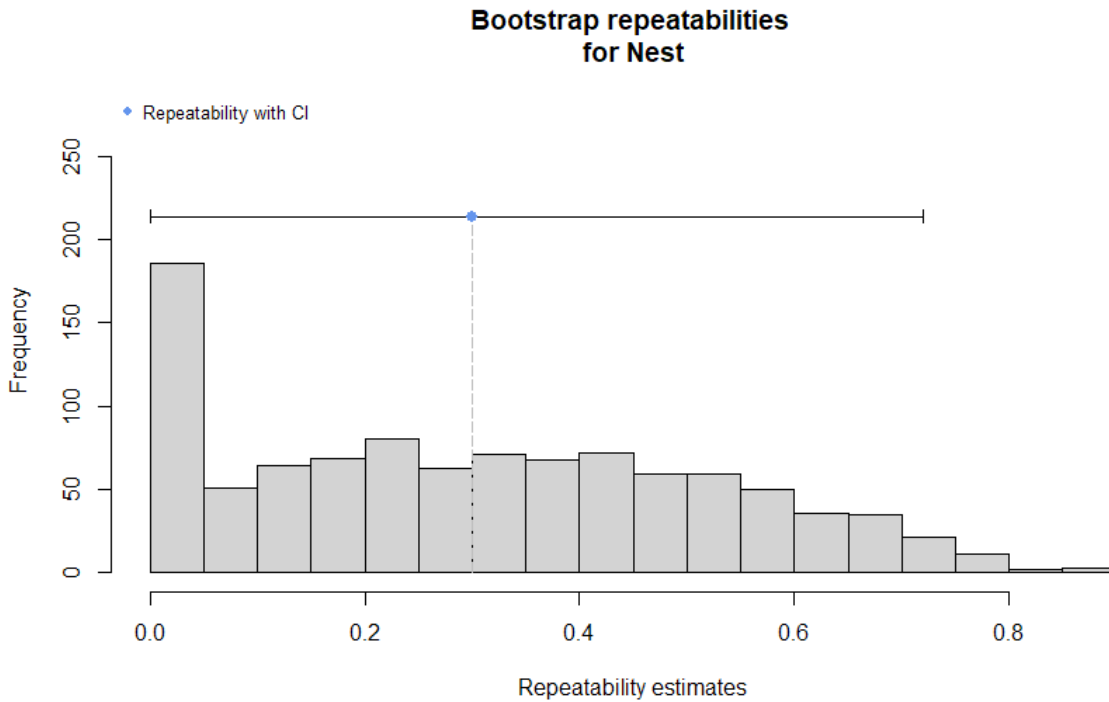


**Figure S4.** Graphical representation of quality of the variable representation in each of the first two principal components. The size of the circle and the color intensity indicate the quality.

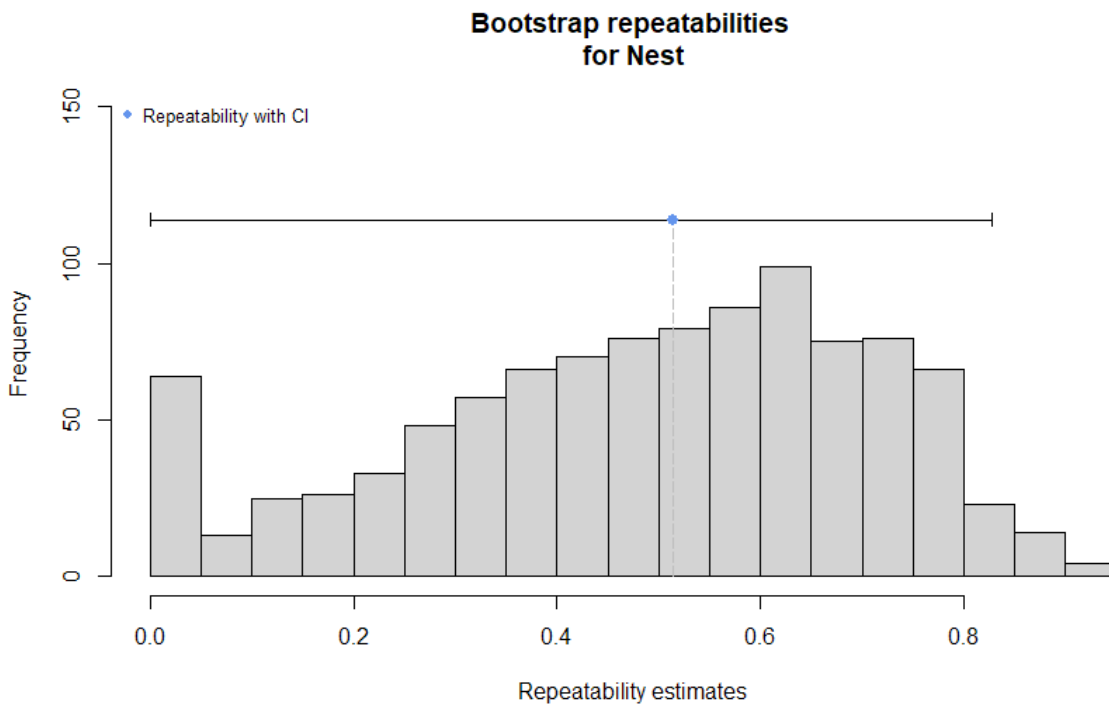


**Figure S5.** Distribution of the parametric bootstrap samples from the repeatability analyses, along with the point estimate and the limits of the confidence interval.

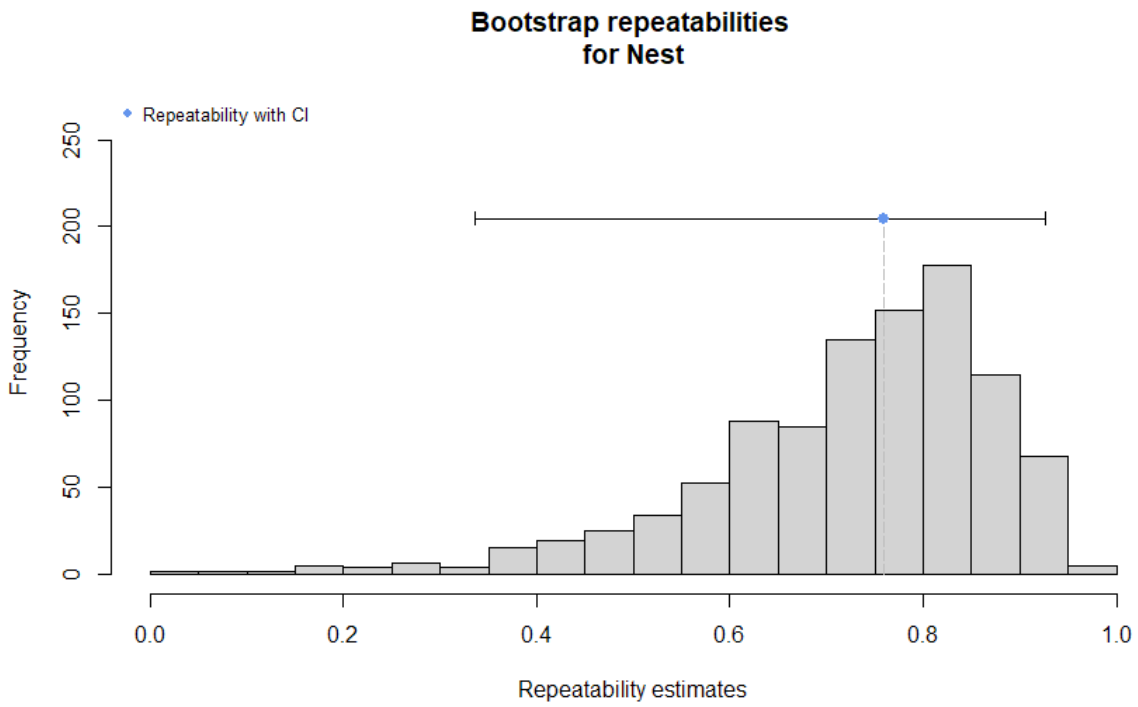
**brPFOS**



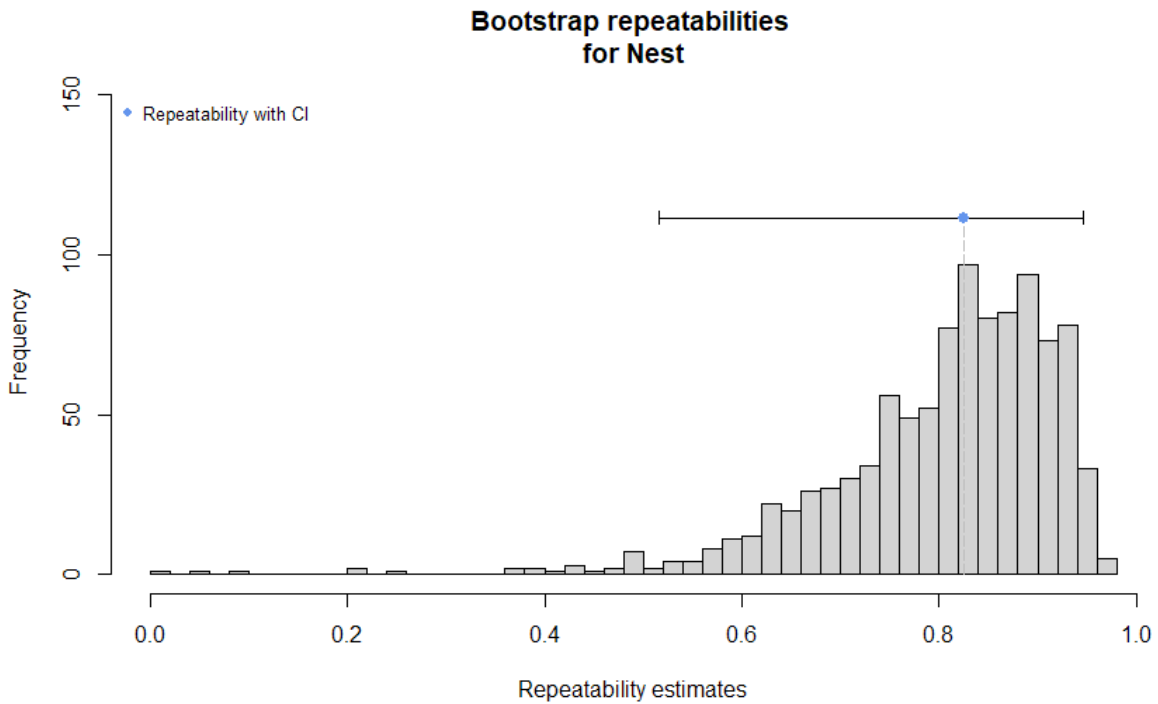
**linPFOS**



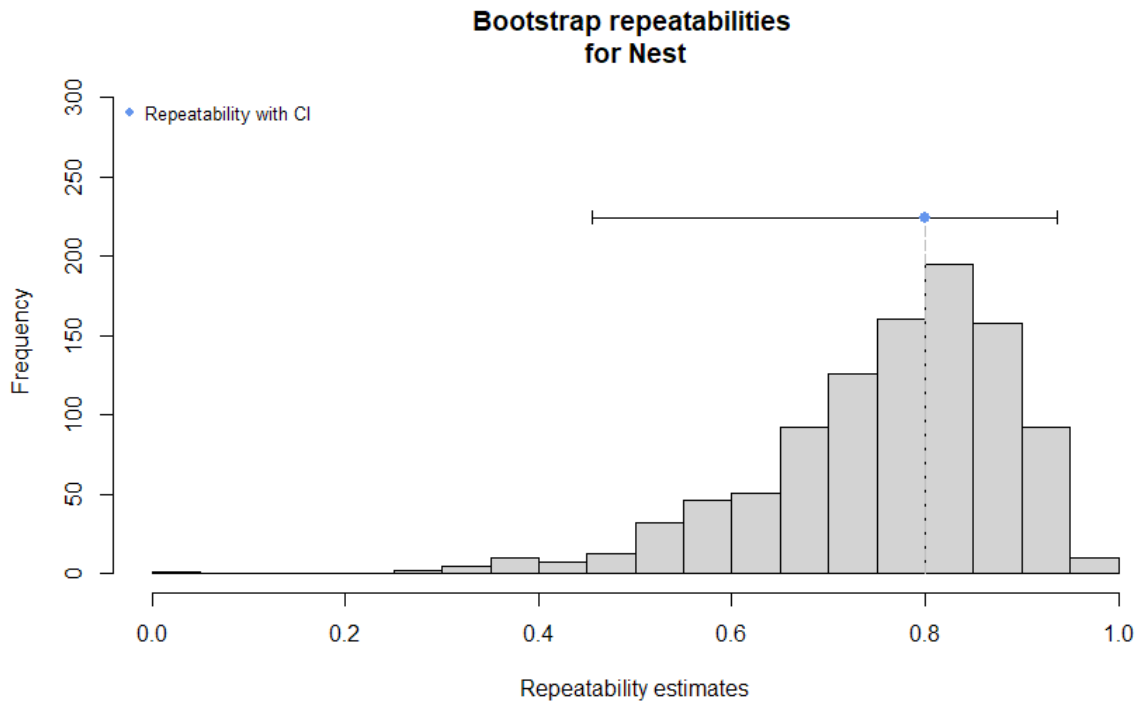
**PFOA**



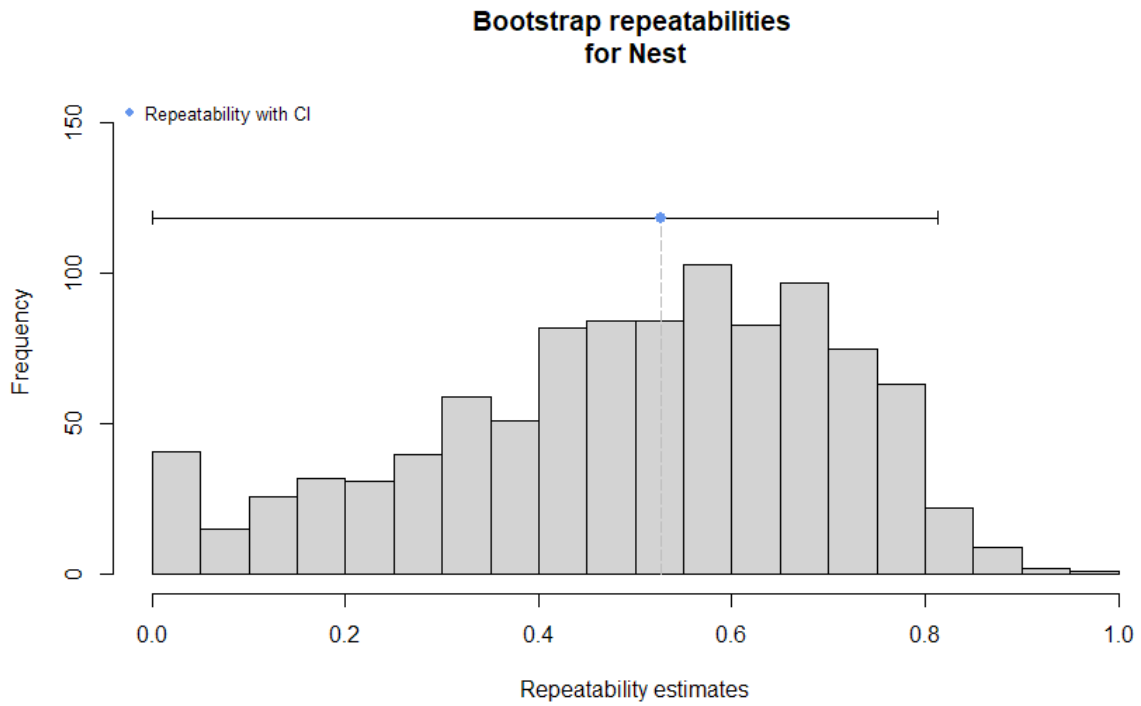
**PFNA**



**PFDcA**

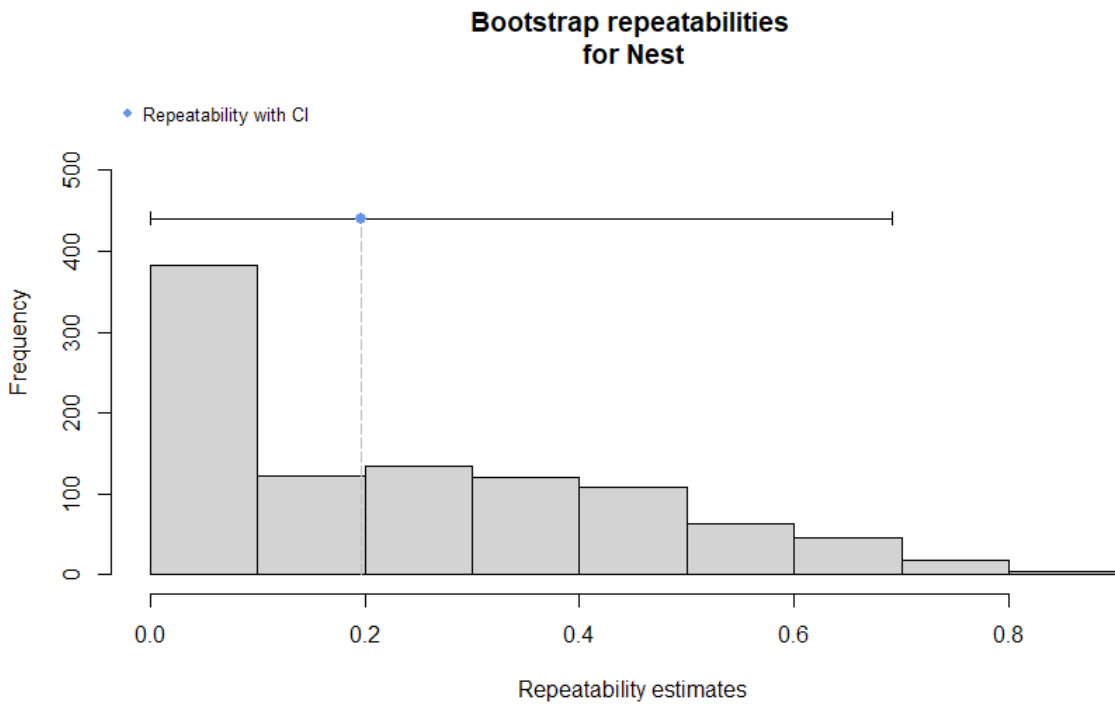


**PFU<sub>n</sub>A**

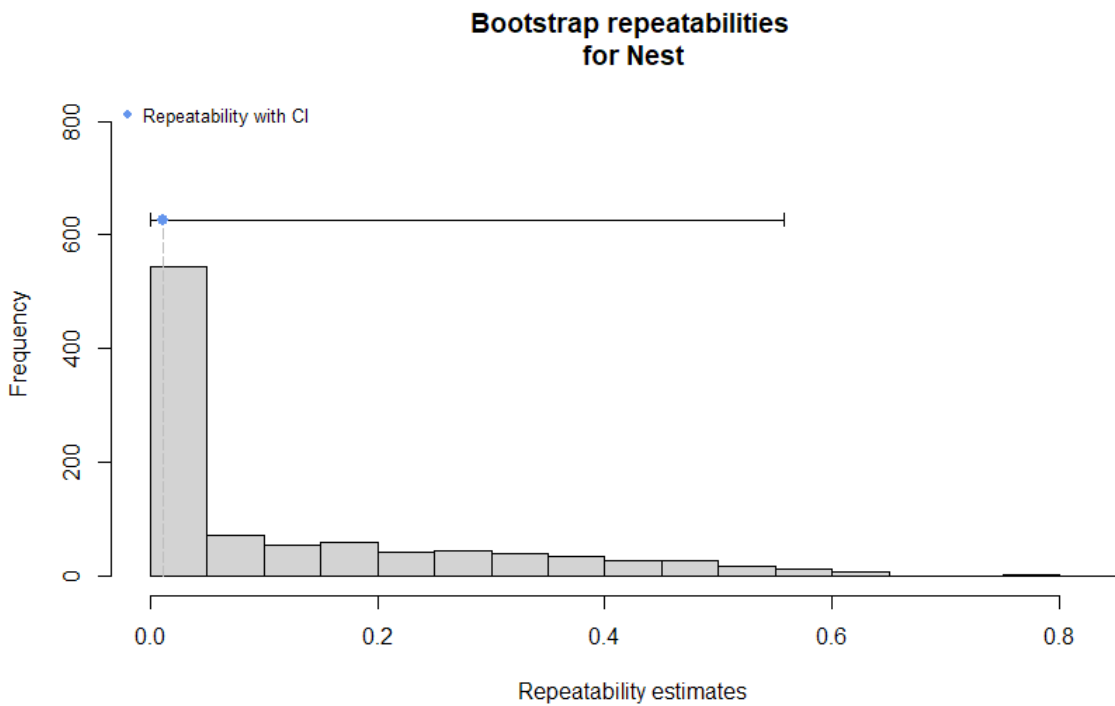




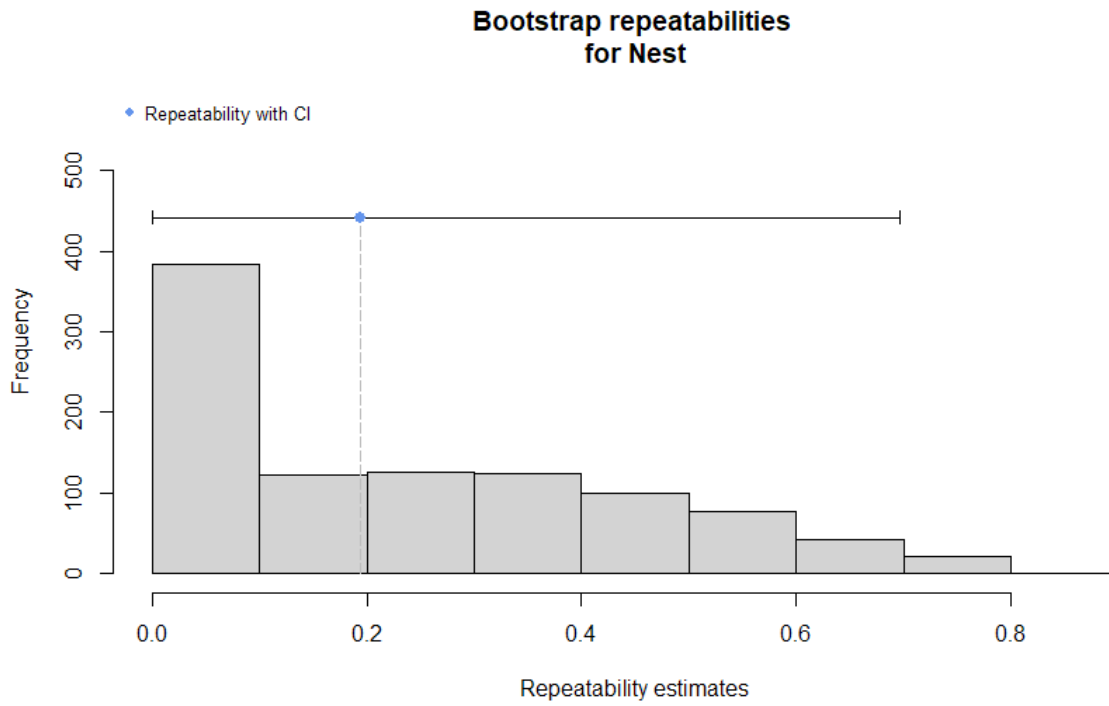
**PFD<sub>oA</sub>**



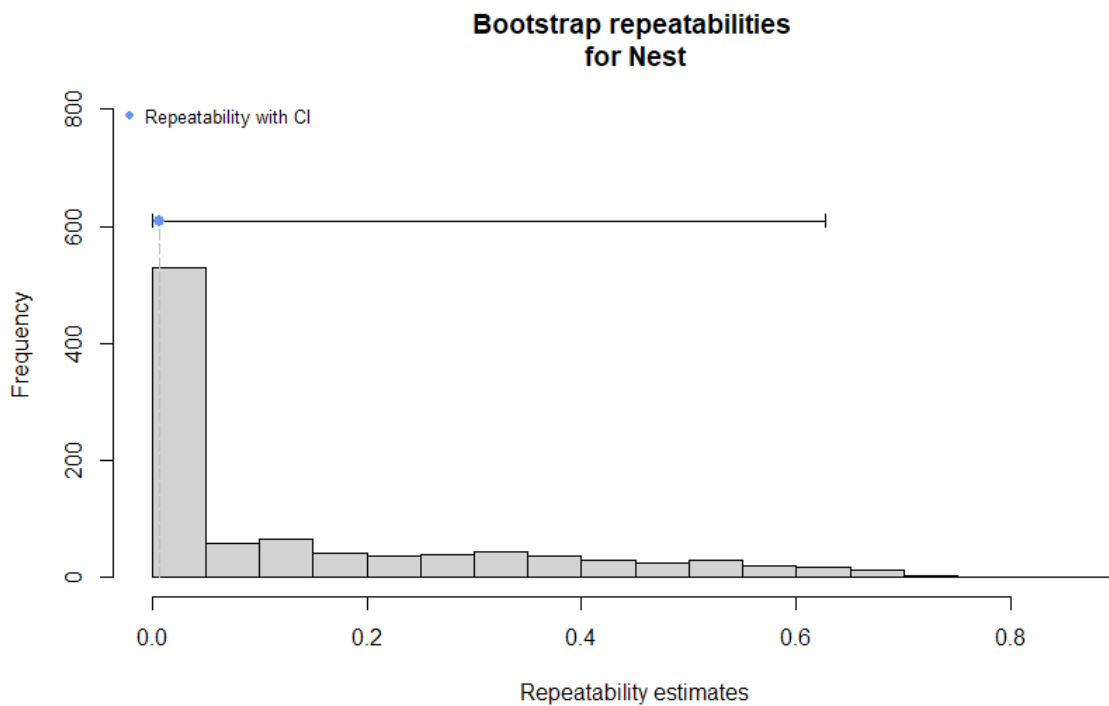
**PFTriA**



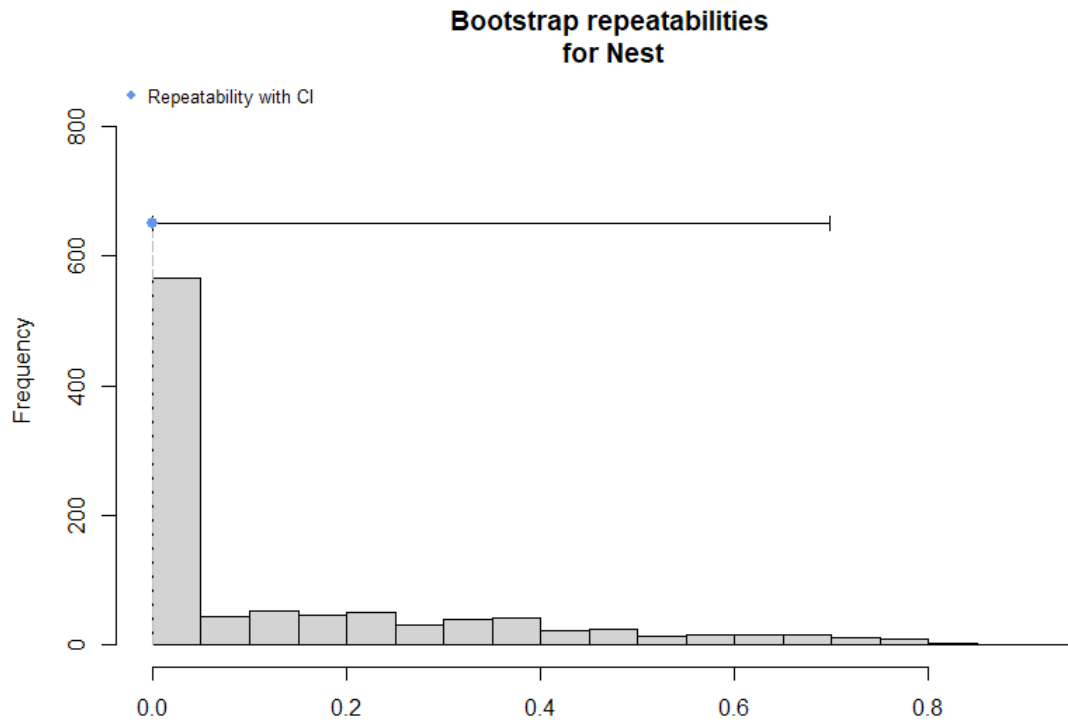
**PFTeA**



**PFHxDA**



7:3 FTCA



**Table S4.** Model selections for the relationship between telomere length in liver of embryos of black-legged kittiwake and maternal PFAS deposited in egg yolk. The selection is based on the lowest second-order Akaike’s Information Criterion corrected for small sample sizes (AICc); the predicting variables were the two first principal components (PC<sub>1</sub> and PC<sub>2</sub>) of a principal components analyses realized on all PFAS selected from the first step of the analyses, as well as the egg mass at laying. The most parsimonious model is given in bold. AICc<sub>wt</sub>: Akaike’s weight;  $\Delta$ AICc: difference between the model with the smallest AICc-value and the model of interest.

	PC <sub>1</sub>	PC <sub>2</sub>	Egg mass at laying	K	AICc	AICc <sub>wt</sub>	$\Delta$ AICc
<b>mod8</b>				<b>2</b>	<b>12.690</b>	<b>0.670</b>	<b>0.000</b>
mod7	X			3	16.320	0.110	3.630
mod6		X		3	16.530	0.100	3.840
mod5			X	3	16.590	0.100	3.900
mod3	X		X	4	21.530	0.010	8.840
mod2	X	X		4	21.560	0.010	8.870
mod4		X	X	4	21.740	0.010	9.050
mod1	X	X	X	5	28.860	0.000	16.170

**Table S5.** Model selections for the relationship between telomere length in liver of embryos of black-legged kittiwake and each maternal PFAS deposited in egg yolk and found to have a highly repeated deposition within clutches (see main manuscript). The selection is based on the lowest second-order Akaike's Information Criterion corrected for small sample sizes (AICc); the predicting variables were the concentration of the specific investigated PFAS and the egg mass at laying. Additional information can be found in Table S4.

	linPFOS	Egg mass at laying	K	AICc	AICcwt	$\Delta$ AICc
<b>mod4</b>			<b>2</b>	<b>12.690</b>	<b>0.770</b>	<b>0.000</b>
mod3		X	3	16.590	0.110	3.900
mod2	X		3	16.600	0.110	3.910
mod1	X	X	4	21.800	0.010	9.110

	PFOA	Egg mass at laying	K	AICc	AICcwt	$\Delta$ AICc
<b>mod4</b>			<b>2</b>	<b>12.690</b>	<b>0.760</b>	<b>0.000</b>
mod2	X		3	16.310	0.120	3.620
mod3		X	3	16.590	0.110	3.900
mod1	X	X	4	21.530	0.010	8.840

	PFNA	Egg mass at laying	K	AICc	AICcwt	$\Delta$ AICc
<b>mod4</b>			<b>2</b>	<b>12.690</b>	<b>0.770</b>	<b>0.000</b>
mod3		X	3	16.590	0.110	3.900
mod2	X		3	16.600	0.110	3.910
mod1	X	X	4	21.810	0.010	9.120

	PFDCa	Egg mass at laying	K	AICc	AICcwt	$\Delta$ AICc
<b>mod4</b>			<b>2</b>	<b>12.690</b>	<b>0.770</b>	<b>0.000</b>
mod2	X		3	16.550	0.110	3.860
mod3		X	3	16.590	0.110	3.900
mod1	X	X	4	21.760	0.010	9.060

	PFUnA	Egg mass at laying	K	AICc	AICcwt	$\Delta$ AICc
<b>mod4</b>			<b>2</b>	<b>12.690</b>	<b>0.750</b>	<b>0.000</b>
mod2	X		3	16.230	0.130	3.540
mod3		X	3	16.590	0.110	3.900
mod1	X	X	4	21.440	0.010	8.750

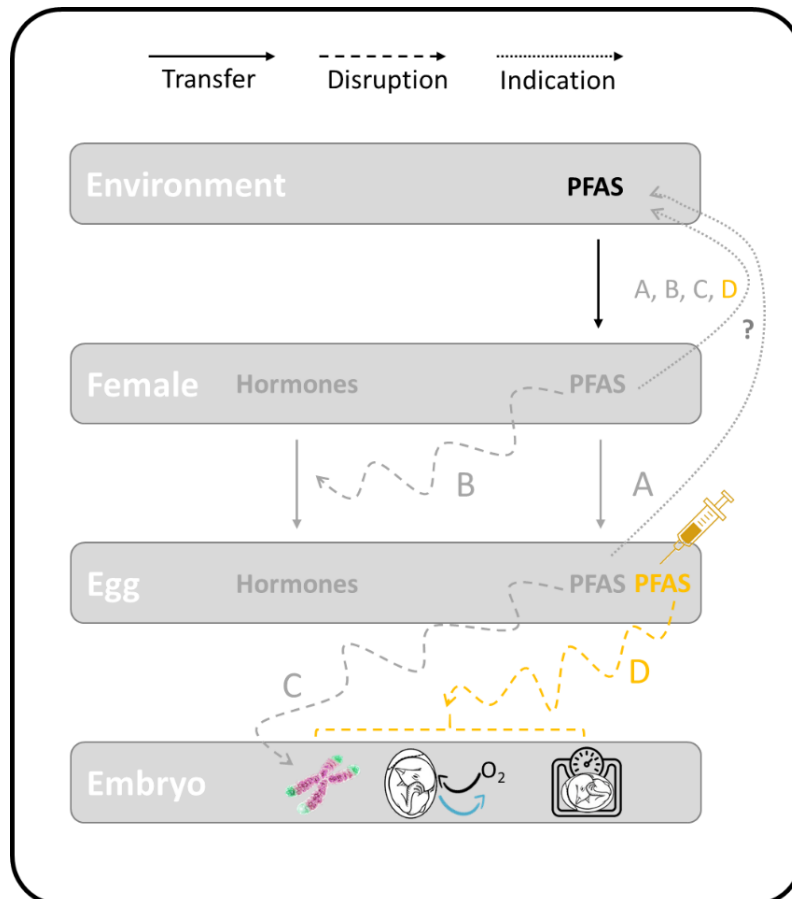
# CHAPTER IV



*Long-tailed skua (Stercorarius longicaudus) in flight*

## Chapter IV

Since the early 2000s emerging PFAS are increasingly produced and emitted in the environment worldwide as replacements for legacy PFAS. Nonetheless, their toxicity is far less documented than that of the latter. In this chapter, we examined the consequences on yellow-legged gull embryonic development of an experimental *in ovo* exposure to an emerging PFAS, 7:3 FTCA, recently found in high concentrations in numerous species and in kittiwake eggs (**Figure 17**).



**Figure 17.** Schematic representation of Chapter IV investigations included in the global structure of this thesis.

### We found:

- No effect of 7:3 FTCA on embryos telomere length, metabolism and on eggs parameters including mass loss along incubation and shell thickness.
- Similar concentrations of legacy PFAS compared to other Mediterranean colonies.
- Occurrence of  $n:2$  and  $n:3$  fluorotelomers, as well as one PFECA and perfluoroethylcyclohexane sulphonate (PFEC<sub>6</sub>HS), in yellow-legged gull eggs.

# Paper D

## Occurrence and developmental toxicity of 7:3 fluorotelomer carboxylic acid in yellow-legged gulls

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**In prep.** for *Environmental Toxicology and Chemistry*



# Occurrence and developmental toxicity of 7:3 fluorotelomer carboxylic acid in yellow-legged gulls

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## ABSTRACT

More than 70 years of industrial production of per- and polyfluoroalkyl substances (PFAS) have resulted in their ubiquitous presence in the environment on a global scale, although sources, transport and fate generate contrasted occurrence in the environment. Gulls eggs are excellent bioindicators of environmental pollution, especially for PFAS known to bioaccumulate in organisms and to be deposited in bird eggs by maternal transfer. Using yellow-legged gull (*Larus michahellis*) eggs, we investigated the occurrence of more than 30 PFAS, including the most common ones (i.e. legacy PFAS) as well as their alternatives (i.e. emerging PFAS) in the Bay of Marseille, the second largest city in France. We found PFAS concentrations of average similar to other yellow-legged gull eggs sampled in the Mediterranean Sea, although lower than those sampled close to PFAS hotspots. Due to their high biomagnifying potential and toxicity, top predators are vulnerable to the exposure to some PFAS. If health consequences have been evaluated for some legacy PFAS, this is still largely unexplored for emerging compounds although they are increasingly found in biota. The developmental period is a particularly crucial stage during a bird life, since any disruption may result in long-term affections. Therefore, in a second phase, we experimentally investigated the effect of *in ovo* exposure to an emerging PFAS on embryos of yellow-legged gulls from the same population. We injected in freshly laid eggs 7:3 fluorotelomer carboxylic acid (7:3 FTCA), a compound abundantly found in biota including seabirds and their eggs in recent years, and examined the consequences on eggs parameters and embryos development. We did not found any differences between the treated and the control eggs and suggest that 7:3 FTCA is not putting yellow-legged gulls at risk in the environmental concentrations found in the Bay of Marseille.

**Key-word:** PFAS, 7:3 FTCA, Egg injection, Metabolism, Growth, Telomere

## INTRODUCTION

Poly- and perfluoroalkyl substances (PFAS) are man-made chemicals in use since the 1950's. Their high chemical and thermal stability as well as both their hydrophobic and lipophobic nature make them useful in a wide variety of industrial processes and consumer products (Buck *et al.* 2011; Herzke *et al.* 2012). As a consequence of the widespread use of PFASs, some of the main compounds were detected as globally distributed in humans and wildlife in the early 2000's (Giesy and Kannan 2001; Hansen *et al.* 2001). Rapidly, a large body of evidence have characterized “long-chain” perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) as persistent and bioaccumulative, but also as a potential concern for human health and the environment (McCarthy *et al.* 2017; Ankley *et al.* 2021). As a consequence, a decade ago, some of the main producers decided to phase-out their production of major legacy PFAS. Since, correlations between concentrations of legacy PFAS and multiple health indicators have been observed in experimental and correlative studies in vertebrates (Ankley *et al.* 2021; Metcalfe *et al.* 2022; Rickard *et al.* 2022). Therefore, in a will to reduce the production and use of legacy long-chain PFAS, manufacturers synthesized a myriad of alternative short-chain molecules, despite a knowledge gap in their fate and effects on wildlife (Munoz *et al.* 2019; Wang *et al.* 2019; Brase *et al.* 2021; Pelch *et al.* 2022).

In birds, all essential substances for embryo development are deposited in the growing oocyte by maternal transfer. Nonetheless, various contaminants including PFAS may concomitantly be transferred into the egg (Vinas *et al.* 2020; Wu *et al.* 2020; Pereira *et al.* 2021; Wang *et al.* 2021b; Jouanneau *et al.* 2022). Although PFAS are known to affect numerous factors including physiology (metabolism and telomere length), behavior and ultimately survival and reproduction in adult birds (Tartu *et al.* 2014; Blévin *et al.* 2017a; Blévin *et al.* 2017b; Costantini *et al.* 2019; Ask *et al.* 2021; Blévin *et al.* 2020; Sebastiano *et al.* 2020a; Sebastiano *et al.* 2020b; Custer 2021), their effects have been relatively scarcely studied during embryo development despite it may be critical in shaping individuals long-term fitness (Lindström 1999; Baos *et al.* 2012). In particular, about fifteen experimental studies focused on estimating legacy PFAS developmental toxicity on bird embryos and found disrupting effects on cognitive performance, heart rate, lipid metabolism, liver, immune and endocrine functions, growth and survival (Molina *et al.* 2006; O'Brien *et al.* 2009; Pinkas *et al.* 2010; Cassone *et al.* 2012a; Cassone *et al.* 2012b; Strömquist *et al.* 2012; Jiang *et al.* 2013; Mattsson *et al.* 2015; Norden *et al.* 2016; Parolini *et al.* 2016; Briels *et al.* 2018; Jacobsen *et al.* 2018; Geng *et al.* 2019; Mattsson *et al.* 2019; Dennis *et al.* 2020). However, only a handful investigated

environmental concentrations. Moreover, most of them focused on perfluorooctanesulfonic acid (PFOS), very few on PFCAs and a single one investigated an emerging alternative to legacy compounds: F-53B. Yet, due to their increasing production by manufacturers, emerging PFAS detection is rising in recent years in the environment and biota (Gebbinck *et al.* 2016; Gebbinck *et al.* 2017; Munoz *et al.* 2019; Awad *et al.* 2020), and some of them have recently been found to be at least as toxic as legacy PFAS (Gomis *et al.* 2018). Among these compounds, 7:3 fluorotelomer carboxylic acid (7:3 FTCA) is of particular interest as it has been recently increasingly discovered in humans and numerous marine invertebrate, mammal, fish and bird species worldwide (Powley *et al.* 2008; Peng *et al.* 2010; Guruge *et al.* 2011; Loi *et al.* 2011; Nyberg *et al.* 2018; Schultes *et al.* 2020; Spaan *et al.* 2020; Barrett *et al.* 2021; Meng *et al.* 2021). 7:3FTCA has a high bioaccumulation potential, and is relatively lipophilic (Xie *et al.* 2020), which most likely is the reason for its finding in large proportions of terrestrial and seabirds' eggs in Northern Europe (Eriksson *et al.* 2016; Jouanneau *et al.* 2022). 7:3 FTCA is one of the several degradation products of fluorotelomer alcohols (FTOHs), which ultimately transform in PFCAs, although the final compounds are still unclear (Fasano *et al.* 2006; Fasano *et al.* 2009; Butt *et al.* 2010; D'Eon and Mabury 2011; Xie *et al.* 2020). These intermediates are short-lived, yet, some of them including FTCAs, may have a much higher toxicity than long-chain legacy PFCAs. In particular, some FTCAs were identified as up to more than 10 000 times more toxic than PFCAs to aquatic invertebrates and plants (Phillips *et al.* 2007, 2010; Mitchell *et al.* 2011; Ankley *et al.* 2021). However, in vertebrates, no studies were conducted to evaluate FTCAs toxicity.

The yellow-legged gull (*larus michahellis*) is a large colonial seabird breeding primarily in the Mediterranean Sea. This generalist gull feeds on small fish and marine organisms but also relies for a large part on anthropogenic food resources including trawling discards or open landfills (Duhem *et al.* 2008; Ramirez *et al.* 2020). Yellow-legged gulls are considered as excellent indicators of the environment contamination (Vinas *et al.* 2020; Zorrozuza *et al.* 2020), as seabirds eggs in particular for emerging PFAS (Wang *et al.* 2021b; Jouanneau *et al.* 2022). In this species, elevated concentrations of legacy PFAS have been measured in eggs from colonies in Northeast Italy (Parolini *et al.* 2020) and Spain (Vicente *et al.* 2012; Bertolero *et al.* 2015; Colomer-Vidal *et al.* 2022), eggs from colonies closer to urbanized regions being the most contaminated. However, local concentration of emerging compounds is still largely unknown in the Mediterranean Sea and its wildlife in general, although this almost closed sea

has heavily industrialized coasts and receives important river inputs from largely anthropized regions.

In the present study, we assessed 1) the concentrations of legacy and emerging PFAS in egg yolks of yellow-legged gulls breeding in the vicinities of Marseille, the second biggest city in France, and 2) the impact on diverse health physiological markers (respiratory exchanges, telomere length, embryo's mass) of an experimental exposure to an environmental concentration of the emerging compound 7:3 FTCA on yellow-legged gull embryos during development. Due to the highly urbanized and industrialized surroundings, we expect high levels of both legacy and emerging PFAS in eggs. We also hypothesized adverse effects of 7:3 FTCA on embryos physiology during development, with disrupted telomeres length and resting metabolism for individuals experimentally exposed compared to those of the control group.

## MATERIALS AND METHODS

### **Study area and sampling**

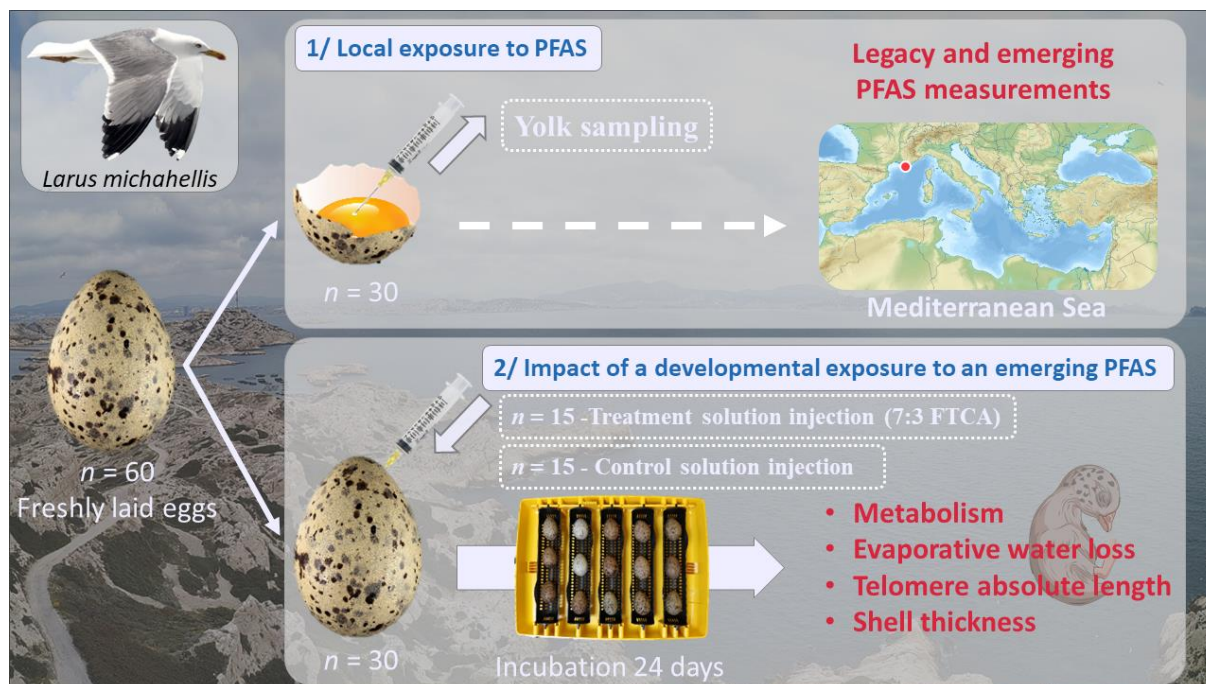
All needed sampling permits have been obtained from the Direction Départementale des Territoires et de la Mer des Bouches-du-Rhône and the Parc National des Calanques, and the study was conducted according to the appropriate French regulations and protocols. The work was conducted at the Iles du Frioul (Parc National des Calanques, Marseille, France) in March 2021. During the peak egg laying period, one freshly-laid egg was collected from 60 different yellow-legged gull nests. Egg floatability is commonly used to estimate the incubation stage of bird eggs (Van Paassen *et al.* 1984), we used this method to select the last-laid egg and only sampled it if it showed no floatability (i.e., freshly-laid egg). We considered the sampling day as the start of the incubation period (embryonic day 0: E<sub>0</sub>). On 30 eggs (first set, Figure 1), we separated albumen and yolk and kept them frozen separately until PFAS assay, to investigate local embryos exposure to legacy and emerging compounds. The 30 other eggs (second set, Figure 1) were brought back to the laboratory using a protective case before injection. As the majority of PFAS in bird eggs is found in the yolk and not in the albumen, we measured PFAS and injected the solutions in the egg yolk (Gebbink and Letcher 2012).

***In ovo* exposure to 7:3 FTCA**

In the lab at E<sub>1</sub>, we randomly assigned eggs from the second set to two groups and injected each group with either a control solution (control eggs;  $n = 15$ ) or a PFAS solution containing 7:3 FTCA (treatment eggs;  $n = 15$ ). We weighted the eggs to the nearest 0.001g before injection. We purchased 7:3 fluorotelomer carboxylic acid (7:3 FTCA or FHpPA; CAS no. 812-70-4; >98 % purity; lot no. FHpPA1217) diluted in methanol (1.2 mL x 50  $\mu\text{g mL}^{-1}$ ) from Wellington laboratories (Guelph, Canada). To prepare the injection solution containing PFAS, we thoroughly vortexed and evaporated methanol from 1 mL of the initial 7:3 FTCA solution at 60°C under constant nitrogen flow. We then added 1mL of dimethyl sulfoxide (DMSO;  $\geq 99.5$  % purity; Sigma Aldrich, St. Louis, USA) to the vial and sonicated it for 15 minutes. After agitating for 1h30 the solution was diluted in 249 mL of DMSO to obtain a final concentration of 2  $\text{mg L}^{-1}$  of 7:3 FTCA in DMSO. After 2 min agitation, the solution was transferred to injection vials and autoclaved. We proceeded the same for the control injection solution, but used 1 mL methanol instead of the PFAS initial solution. Both solutions were then stored frozen until injection. DMSO is a commonly used carrier solvent in ecotoxicological studies and did not cause any toxicity issues during embryo development in chickens (*Gallus gallus domesticus*; O'Brien 2009). Both solutions were kept at room temperature for 30 min before injection. We worked next to a Bunsen burner and used sterile gloves, changed before each injection, to keep the environment sterile. We located the air cell and the yolk using an egg-candler. After cleaning a small area of the shell above the air cell with 70 % ethanol and an antiseptic using a disposable compress, we used a disposable needle to drill through the eggshell. We exposed embryos to 8 ng of 7:3 FTCA per gram of yolk. To obtain this final concentration, we vortexed and injected a volume ( $85 \pm 8 \mu\text{L}$ ) of the injected solution (control or PFAS) in eggs, based on full eggs weight (1  $\mu\text{L}$  per gram egg, yolk mass  $\approx$  total mass/4; Rubolini *et al.* (2006); Rubolini *et al.* (2011)), using two Hamilton 250  $\mu\text{L}$  syringe (Hamilton company, Reno, USA) and 21G disposable needles. We then sealed the hole in the shell using melted beeswax. After wax dried, we randomly assigned eggs of the two groups to two similar incubators (Ovation 56 Ex, Brinsea, Titusville, USA), 15 eggs in each. The temperature of the incubators was set to 38°C, with 55% humidity, the eggs were incubated horizontally and automatically turned every hour. We controlled the egg mass at E<sub>10</sub>, E<sub>20</sub> and E<sub>24</sub> of incubation. At E<sub>24</sub> (i.e., two days before expected hatching; Rubolini 2005), eggs were opened by cutting around the bottom of the air cell and embryos were euthanized. The exposure to PFAS therefore covered most of the embryonic developmental period. We immediately weighted the embryo

to the nearest 0.001 g, collected the liver and kept it frozen in a microtube, until telomere analysis.

**Figure 1.** Schematic representation of the experimental design conducted on yellow-legged gull eggs from Frioul Islands (Marseille, France).



### Respiratory exchanges

On eggs of the second set, at E<sub>23</sub>, we measured oxygen consumption rate ( $\dot{V}O_2$ ; mL h<sup>-1</sup>), used as a proxy for resting metabolic rate (RMR), and the total evaporative water loss (TEWL; mg h<sup>-1</sup>). We randomized egg order for this experiment. We used an eight-channel closed-system respirometer (FlowBar-8, Sable Systems, Las Vegas, USA), to provide constant influx ( $250 \pm 1$  mL min<sup>-1</sup>) to each test chamber. As hearing abilities is already developed in embryo at this development stage (Rumpf and Tzschentke 2010), we used a silent climatic room to ensure that embryos stayed calm. Ambient temperature was set to 35 °C. We calibrated the O<sub>2</sub> analyzer before each measurement session using outside air (O<sub>2</sub> proportion = 20.95 %) for 1h30. Each measurement channel corresponding to a test chamber (0.5 L), we sequentially measured oxygen consumption of seven eggs and used an empty one to provide the baseline for each batch. The expelled air of each test chamber was sequentially sampled using a gas flow switcher (RM-Multiplexer, Sable Systems, Las Vegas, USA), following three repetitions of 600 s measurements of each channel, starting with the empty chamber. Water was extracted from

air using Drierite (W. A. Hammond Drierite, Xenia, USA) before dried air was sent to the gas analyzer (O<sub>2</sub>: FC10A, CO<sub>2</sub>: CA10; Sable System, Las Vegas, USA). We finally estimated bird  $\dot{V}O_2$  using equations adapted to our setup (Powolny *et al.* 2016). At the end of the experiment, we returned the eggs to their initial place in the incubators.

### **Telomere analyses**

We carried out telomere analyses on liver samples using real-time quantitative polymerase chain reaction (qPCR; BioRad CFX 96; Bio-Rad, USA), with recombination activating gene 1 (RAG1) as a single-copy reference gene (McLennan *et al.* 2019). This method have already been validated for birds (Sebastiano *et al.* 2020a). In brief, we extracted DNA from liver samples using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). We then checked DNA quality and concentration using an optical density spectrophotometer (Nanodrop, DN-1000, Thermo Fisher Scientific, Waltham, USA), and by running samples on a gel (Nussey *et al.* 2014). We ran each samples in five replicates, using 5 ng of DNA per reaction, in a single 96-well plate together with internal standards (RAG1 and TELO) and blanks. Each sample was randomly distributed in the plate. Telomere primers (TELO: Tel1b: 5'-CGGTTTGTGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTT-3' and Tel2b: 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3') were used at a concentration of 800 nM, while RAG1 S3 primers (Rag1F: 5'-GGGTCCTCTGATAGCCGAAA-3' and Rag1R: 5'-CATCATAACCTGTACCCCGGA-3') were used at a concentration of 300 nM. A reference curve was prepared (TELO and RAG1: 0.625 to 20 ng  $\mu\text{L}^{-1}$ ), and a six-point standard curve (40 to 0.31 ng) from a pooled sample was included in triplicate in the plate to control for the amplifying efficiency of the qPCR. Coefficient of variability were 0.40 % (RAG1) and 0.93 % (TELO) and efficiency was within the acceptable range for both RAG1 and telomere (RAG1: 95.70 %; Telomere: 104.3 %). The relative telomere length (expressed as the  $T/S$  ratio) was calculated as the number of telomere copies ( $T$ ) relative to the single copy gene ( $S$ ).

### **PFAS analyses**

To measure PFAS in yolk samples from the first egg set, we adapted a method from Powley *et al.* (2005) described in Sletten *et al.* (2016) and adjusted for egg yolk (Jouanneau *et al.* 2022). Briefly, sample (~0.20 g of yolk) were extracted in methanol, and matrix was removed using graphitized carbon and glacial acetic acid. We also measured PFAS in both the



treatment and the control injected solutions, to ensure that 1/ the final concentration of 7:3 FTCA matched  $2 \text{ mg L}^{-1}$  in the treatment solution, as required, 2/ the control solution was exempted from 7:3 FTCA, and 3/ both solutions were free from all other PFAS. No extraction was needed, therefore, we diluted both solutions ( $20 \text{ }\mu\text{L}$ ) in methanol ( $60\mu\text{L}$ ). Duplicates were made for both solutions. Internal PFAS standards ( $20 \text{ }\mu\text{L}$ ) were added at the beginning of the process of every samples, as well as recoveries ( $20 \text{ }\mu\text{L}$ ) and the end, to calculate recovery of the  $^{13}\text{C}$  labeled internal standards.

We then conducted the quantification by ultrahigh-performance liquid chromatography triple-quadrupole mass spectrometry (UHPLC – MS/MS; Thermo Fischer Scientific, Waltham, USA). Chromatograms were then quantified using LCQuan software (Thermo Fischer Scientific, Waltham, USA), by applying the isotopic dilution method. An eight-point calibration curve with a concentration range from  $0.02$  to  $10.0 \text{ pg }\mu\text{L}^{-1}$  was used. We screened for a total of 34 PFAS (14 considered as emergent and 20 legacy; see supporting information (SI) Table S1). All concentrations are expressed in  $\text{ng g}^{-1}$  wet weight (ww). One blank and a standard reference material (human serum AM-S-Y-2108 INSPQ; AMAP ring test) were concurrently analyzed every 13 samples (and after the four solution samples) to ensure analyses quality.

The limit of detection (LOD) was defined as three times the signal-to-noise ratio for the specific matrix, or in the case of detection in the blanks as the sum of the average of the blank level and 3 times standard deviation. LOD varied depending on the compounds and ranged from 3 to  $250 \text{ ng g}^{-1}$  ww (SI Table S1). For egg yolk samples in further analyses, for each included PFAS, values  $< \text{LOD}$  were set to half of the LOD of the specific compound.

We measured a final concentration of (mean  $\pm$  SD of the duplicates)  $2.19 \pm 0.08 \text{ mg L}^{-1}$  of 7:3 FTCA in the injected treatment solution, but none of the other PFAS were above the LOD. We therefore injected a final concentrations of 7:3 FTCA of  $8 \text{ ng g}^{-1}$  ww in egg yolk. This, therefore, do not mimic an environmental exposure but may still provide valuable information on the effects of this compound on the embryo development. In the control solution, we measured no PFAS above the LOD, except 7:3 FTCA at  $0.5 \pm 0.2 \text{ }\mu\text{g L}^{-1}$ . This is 1 000 times less than in the treatment solution, we consequently added in control eggs a concentration of 10 times less 7:3 FTCA than the average concentration found in yellow legged gull eggs (see results section), which may thus be considered as negligible.

### **Statistical analyses**

Statistical analysis was performed using R (version 4.0.0). Most likely due to the mechanic action of the syringe in the egg during the injection, although no non-injected control group was made, only 40 % ( $n = 6$ ) of the embryos from the control group, and 53 % ( $n = 8$ ) of those of the treatment group, survived until E<sub>24</sub>. On the embryos that were alive at E<sub>24</sub>, we investigated the difference between both the treatment and control groups, for embryos' mass, liver telomere length,  $\dot{V}O_2$  and TEWL, as well as on eggs' mass loss between E<sub>0</sub> and E<sub>24</sub>. There was no difference for any of the investigated factors between both incubators, and therefore, all eggs were considered the same way in further analyses (see SI for detailed statistics). Embryo's mass had a relatively large range (min – max: 24.6 – 59.1 g), but no difference between experimental and control groups (linear model (LM):  $F_{1, 12} = 0.06$ ,  $p = 0.816$ ). The large variability in mass may therefore indicate a difference in age among embryos (Ricklefs 2010), likely due to a lack of precision of the method to select fresh eggs. We consequently explored the relationship of each investigated factors with embryos' mass in order to identify an eventual effect of the age on these factors.  $\dot{V}O_2$  and TEWL were positively correlated with embryo's mass (LMs:  $F_{1, 12} = 36.7$ ,  $p < 0.001$  and  $F_{1, 12} = 21.2$ ,  $p < 0.001$ , respectively; SI Figures S1 & S2). We, therefore, normalized  $\dot{V}O_2$  and TEWL by the embryo mass (by using the residuals of the LMs in further analyses) to reject the potential confounding effect of this variable. Differences between the treatment and control groups were finally tested using LMs for each investigated factors, using the status (treatment or control) and the egg mass at E<sub>0</sub> (as a control for egg quality; Williams 1994) as explanatory variables. We did not investigated the interaction of the treatment and the sex of the embryos since this would result in a weak statistical power due to the already low sample size. For each models, we visually check if the data sufficiently met the linear model assumptions using histograms of the residuals and plot of residuals vs fitted (Zuur 2007; SI Figure S3). An effect was considered as significant when  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

### Baseline PFAS concentrations in eggs

Among the 34 targeted compounds, PFHpS, brPFOS, linPFOS, C<sub>8</sub> to C<sub>14</sub> PFCAs and perfluoroethylcyclohexane sulphonate (PFEC<sub>6</sub>HS) were detected in 100 % of the eggs sampled. PFHxS (96.7 %), PFHxDA (93.3 %), 8:2 FTS (86.7 %), 10:2 FTS (86.7 %) and 7:3 FTCA (86.7 %) were also frequently detected and PFBS (13.3 %), PFNS (10 %), PFDcS (3.3 %), PFHxA (26.7), PFHpA (26.7 %), 6:2 FTS (53.3 %) and HPFO-TeA (23.3 %) were occasionally

measured above LOD (SI Table S1). linPFOS was by far the dominant compound measured in egg yolk (Table 1 & Figure 2), accounting for 61 % of the  $\sum_{\text{PFAS}}$ , followed by PFTriA, PFTeA and PFDoA together representing 10 % of the  $\sum_{\text{PFAS}}$ . This may reflect a high concentration of PFOS in adults, potentially due to a strong occurrence in local prey, intensified by the longer half-life in organisms of PFOS compared to other long-chain PFAS (Yoo *et al.* 2009; Tarazona *et al.* 2015). In the literature, PFOS in wild bird eggs was mostly measured in homogenized eggs content and not in the yolk, which makes the comparison to other studies difficult. Eggs yolk contains most of the PFAS burden due to their high lipid and protein content (Gebbinck and Letcher 2012), therefore using homogenized eggs to measure PFAS provides diluted concentrations. In the Mediterranean Sea, PFAS were however measured in the yolk of yellow-legged gull eggs from Northern Italy, PFOS burden was found similar and PFCAs in slightly higher concentrations than those of the present study (Table 1), in particular, PFOA was 4 times higher in the Italian colony (Parolini *et al.* 2020). This high concentration in PFOA is believed to be caused by a local contamination hotspot due to a fluoropolymer plant. Yellow-legged gull eggs contamination to PFAS has also been investigated in Spain, and, although on homogenized eggs, PFOS was found in higher (Bertolero *et al.* 2015) or in similar or lower concentration on average than those of the present study (Vicente *et al.* 2012; Colomer-Vidal *et al.* 2022). In these studies, PFOS concentrations were highly different among colonies, suggesting that the contamination is highly tied to the location, proving the usefulness of eggs as bioindicators of local environmental contamination to PFAS. Concerning the local abiotic environment in the Bay of Marseille, PFOS was recently found to exceed the European Environmental Quality Standard in seawater and high concentrations of PFCAs were highlighted (Schmidt *et al.* 2019). The coast around the city of Marseille is largely industrialized, which may contribute to the relatively high concentrations measured in water and eggs locally. However, large inputs from the Rhône River, as well as surface currents may also cause such local contamination (Munoz *et al.* 2015; Brumovsky *et al.* 2016).

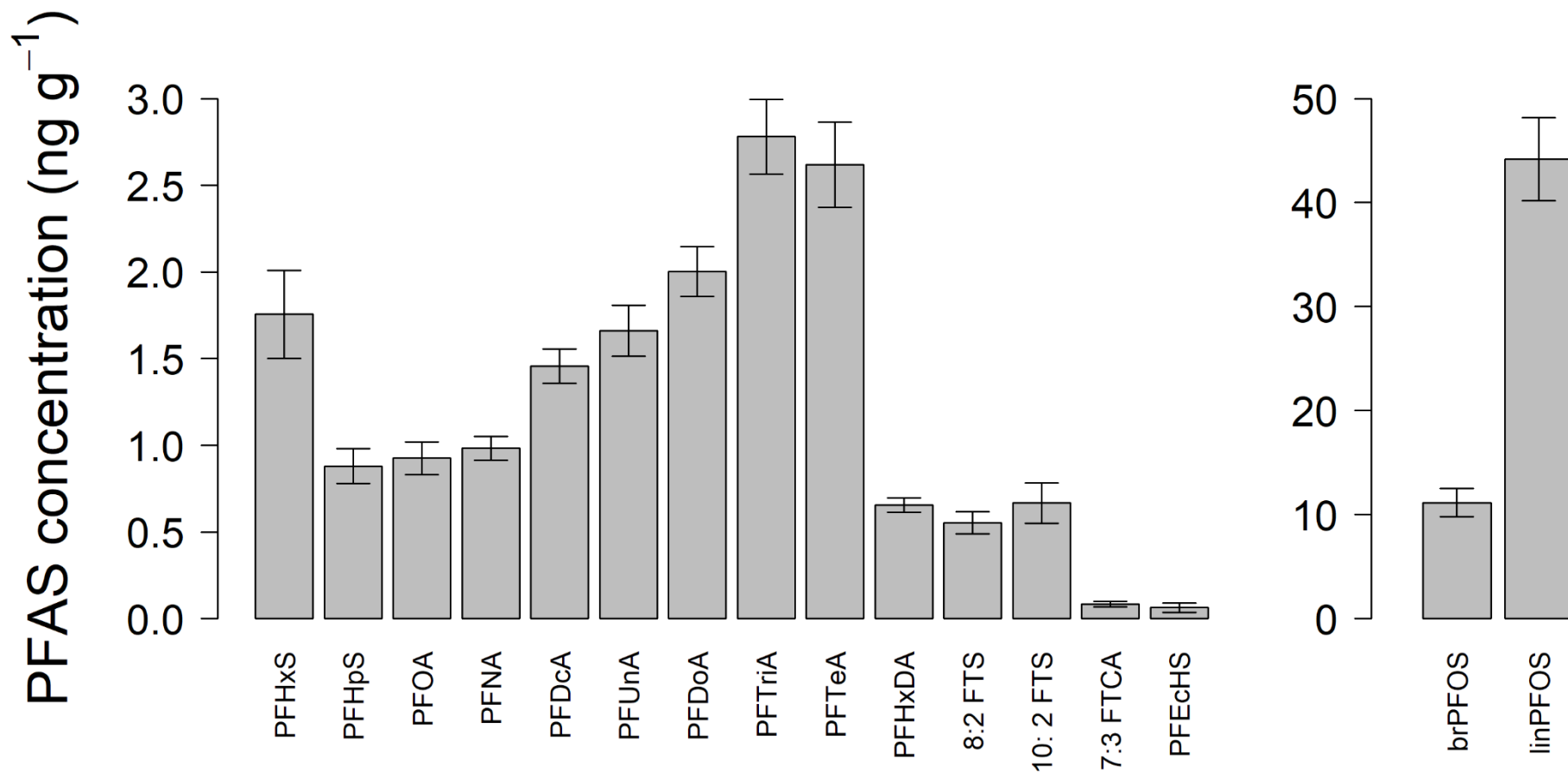
There were few experimental studies investigating legacy PFAS toxicity during development in birds. Most of them focused on PFOS and PFOA in the domestic chicken (*Gallus gallus domesticus*) and found several impacts on immunological, morphological, and neurological endpoints and embryo's survival, however at much higher dose than those measured in the present study (Peden-Adams *et al.* 2009; Jiang *et al.* 2013; Mattsson *et al.* 2015; Norden *et al.* 2016). Some studies nonetheless investigated the effect of concentrations of PFOS relatively close to those of the present study, and related reduced hatchability, lower hatch rate

or impaired lipid metabolism (Molina *et al.* 2006; Briels *et al.* 2018; Geng *et al.* 2019). However, in yellow-legged gull embryos specifically, there were no significant effects of PFOS on morphological endpoints or oxidative and genetic damages, in similar concentrations than those found in our study (Parolini *et al.* 2016). Concerning other PFAS, the toxicity of PFHxS and PFHxA was also examined in chicken and found numerous adverse effects of PFHxS, but not PFHxA, nonetheless at much higher concentrations than in yellow-legged gull eggs of the present study (Cassone *et al.* 2012a; Cassone *et al.* 2012b). A mixture of all long-chain PFASs and PFCAs, in similar or lower concentrations than those we measured in eggs' yolk, were also found to affect immune and endocrine systems as well as gene expression (Mattsson *et al.* 2019).

**Table 1.** Descriptive statistics (Mean  $\pm$  Standard Deviation: SD, Median and Range: Min–Max) for PFAS concentrations ( $\text{ng g}^{-1}$  ww) in egg yolk of yellow-legged gulls from the Frioul Islands (Marseille, France).

		<b>Mean <math>\pm</math> SD</b>	<b>Median</b>	<b>Min-max</b>
Legacy PFAS	PFHxS	1.76 $\pm$ 1.40	1.33	0.03 – 4.94
	PFHpS	0.88 $\pm$ 0.55	0.8	0.19 – 2.67
	brPFOS	11.2 $\pm$ 7.38	9.55	1.70 – 31.9
	linPFOS	44.2 $\pm$ 21.9	47.20	15.4 – 87.3
	PFOA	0.93 $\pm$ 0.51	0.72	0.29 – 2.63
	PFNA	0.98 $\pm$ 0.38	0.9	0.48 – 2.07
	PFDCa	1.46 $\pm$ 0.54	1.30	0.67 – 2.44
	PFUnA	1.66 $\pm$ 0.81	1.4	0.57 – 4.18
	PFDoA	2.00 $\pm$ 0.78	1.89	0.90 – 3.73
	PFTriA	2.78 $\pm$ 1.18	2.70	1.08 – 5.54
	PFTeA	2.62 $\pm$ 1.34	2.53	0.95 – 6.81
	PFHxDA	0.66 $\pm$ 0.22	0.64	0.08 – 1.37
Emerging PFAS	8:2 FTS	0.55 $\pm$ 0.36	0.53	0.05 – 1.42
	10:2 FTS	0.67 $\pm$ 0.64	0.46	0.05 – 2.36
	7:3 FTCA	0.08 $\pm$ 0.09	0.05	0.01 – 0.47
	PFEcHS	0.06 $\pm$ 0.15	0.03	0.01 – 0.85

**Figure 2.** Mean  $\pm$  Standard error of the concentrations of emerging and legacy PFAS (expressed as  $\text{ng g}^{-1}$  of yolk ww) in egg yolks of yellow-legged gulls from the Frioul Islands, Marseille, France.



Among PFCAs, long-chain PFAS were transferred to the eggs to a greater extent than short-chain homologs, this is not surprising as bioaccumulation potential and maternal transfer efficiency are known to increase with the carbon chain length (Conder *et al.* 2008; Jouanneau *et al.* 2022). This may be the cause of the observed pattern showing an increasing concentration of PFCAs with the carbon-chain length (Table 1 & Figure 2). A similar pattern has been observed for PFCAs in herring gulls (*Larus argentatus*) from the Great Lakes region in the US (Gebbinck and Letcher 2012). But not in yellow-legged gull egg yolks from Northern Italy, although the special conditions with an important local hotspot may highly influence this pattern (Parolini *et al.* 2020).

Regarding emerging PFAS, the fluorotelomers sulfonic acids (FTSs) including 6:2; 8:2 and 10:2 FTS are scarcely found in biota, but they were only targeted relatively recently (Ankley *et al.* 2021). Although found in a several fishes, birds and mammals species (Androulakakis *et al.* 2022), 6:2 FTS was suggested to have little bioaccumulation potential (Yeung and Mabury 2013). It was nonetheless measured above LOD in several eggs yolk of the present study, together with its 8:2 and 10:2 homologues (SI Tables S1 & S2). 6:2 FTS was present in much larger concentrations in eggs of the Bay of Marseille than in eggs yolk from Antarctic petrels (*Thalassoica Antarctica*; Alfaro Garcia 2022), but in lower concentration than in bald eagle (*Haliaeetus leucocephalus*) eggs from the Great Lakes region (Wu *et al.* 2020). 6:2 FTS was also recently found in significant concentrations in wide PFAS screening studies on marine mammals from various places of the Northern hemisphere (Spaan *et al.* 2020; Wang *et al.* 2021a). FTSs are a major component of aqueous film-forming foams among others (AFFFs; Shaw 2019; Yeung 2013). AFFFs are massively used in airports for firefighting trainings and FTSs may be emitted in the environment during these events, it is however unknown if local airports are significant sources for these contaminants in the Bay of Marseille. Another fluorotelomer, 7:3 FTCA, is a fluorotelomers carboxylic acid seldom screened for but increasingly found, especially in Arctic biota (Guruge *et al.* 2011; Muir *et al.* 2019; Schultes *et al.* 2020; Spaan *et al.* 2020; Barrett *et al.* 2021). It has been previously described in eggs of both terrestrial and marine birds (Eriksson *et al.* 2016; Jouanneau *et al.* 2022). Its toxicity, as those of other fluorotelomers, is largely unknown in vertebrates (Pelch *et al.* 2022), although FTCAs were suggested to be more toxic than long-chain PFCAs (Phillips *et al.* 2007; Mitchell *et al.* 2011). We examined this question on yellow-legged gull embryos in the second part of the present study.

Among emerging PFAS, PFEcHS, an alternative to PFOS used in airplanes, was found in 100 % of the egg samples with an average concentration of  $0.06 \text{ ng g}^{-1} \text{ ww}$ . This compound was recently detected in abiotic matrices and within various media in the northern hemisphere and considered to have a global distribution (Mahoney *et al.* 2022). This compound was suggested to have a relatively low toxicity potential, although this was based on only two studies, PFEcHS was therefore identified as top priority in the need for future research on toxicity assessment (Mahoney *et al.* 2022). Other emerging alternatives to legacy PFAS, including the fluoroalkylether substances (ether-PFAS: Gen-X, ADONA and F-53B), were not detected in any samples (SI Table S1). If ADONA was, to the best of our knowledge, found only once in biota, in a single egg yolk of black-legged kittiwakes (Jouanneau 2022), Gen-X and F-53B were previously detected in all black-tailed gull (*Larus crassirostris*) eggs sampled on different spots of the coast of Korea, suggesting that they can bioaccumulate (Wang *et al.* 2021b). In fact for F-53B, a high bioaccumulation potential has been suggested and this compound have been found in biota in several previous studies (Munoz *et al.* 2019). This indicate that the Bay of Marseille is probably not a hotspot for these emerging contaminants, although the LOD for Gen-X was quite high ( $250 \text{ ng g}^{-1} \text{ ww}$ ; SI Table S1).

### **Consequences of a developmental experimental exposure to 7:3 FTCA**

Telomere length was the only investigated variable related to egg mass, with increasing telomere length measured in embryos from heavier eggs (LM:  $t = 2.601$ ,  $p = 0.025$ ; SI Table S3). One of the underlying reasons may be that heavier eggs contain more resources for the embryos and could therefore be considered of higher quality (Whittingham *et al.* 2007), and may enhance embryonic telomere maintenance machinery during development. The comparisons of the embryos' mass, telomere length, egg mass loss,  $\dot{V}O_2$  and TEWL showed no differences between treatment and control groups (LMs: all  $p \geq 0.056$  Table 2 & SI Table S3).

In previous studies,  $n:2$  FTCAs have been suggested to have a much higher acute and chronic toxicity than long-chain PFCAs in some species of invertebrates and plant (Phillips *et al.* 2007, 2010; Mitchell *et al.* 2011). This was corroborated on vertebrates in a study on zebrafish (*Danio rerio*) embryos, in which exposure to 6:2 FTCA increased mortality at lower concentrations than PFOA and caused embryonic malformations, lowered heart rate and reduced hemoglobin content (Shi *et al.* 2017). However,  $n:3$  FTCAs were not investigated in these studies. It is unknown if  $n:2$  and  $n:3$  FTCAs may affect yellow-legged gull embryos, but

our study suggests that 7:3 FTCA, at the concentration used in the present study, does not present any risk on the investigated parameters. We may hypothesize that 7:3 FTCA's toxicity is relatively low in vertebrates, and should not affect organisms' survival in a significant way at concentrations currently observed in bird eggs, this may explain why seabirds and marine mammals may be found with relatively high concentrations of this substance (Schultes *et al.* 2020; Spaan *et al.* 2020; Barrett *et al.* 2021; Szabo *et al.* 2021; Wang *et al.* 2021a; Jouanneau *et al.* 2022). Nonetheless, these compounds are metabolic intermediates in the degradation from FTOHs to long-chain PFCAs (Xie *et al.* 2020), which toxicity has been demonstrated in wild birds (Tartu *et al.* 2014; Blévin *et al.* 2017a; Blévin *et al.* 2017b; Blévin *et al.* 2020; Sebastiano *et al.* 2020a; Sebastiano *et al.* 2020b).

Despite 7:3 FTCA did not seem to affect the investigated variables, there was a non-significant tendency for a difference in egg mass loss along incubation between treated and control groups, treatment eggs losing more mass daily than control ones on average (LM:  $t = -2.132$ ,  $p = 0.056$ ; Tables 2 & SI Table S3). Egg mass is slowly decreasing along incubation due to water loss (Ar and Rahn 1980). An increased rate of mass loss is therefore due to an increased evaporative water loss (Rahn 1984), that may be caused by different factors. These factors may include a disrupted eggshell structure integrity, such as eggshell thickness and porosity (Şahan *et al.* 2003), or an increased embryonic metabolism, resulting in a higher water evaporation (Ar and Rahn 1985). Some legacy PFAS were previously found to positively impact metabolism in adults of black-legged kittiwake (Blévin *et al.* 2017b), and to be related to thinner eggshells in a population of great tits (*Parus major*) living close to a fluoropolymers plant (Groffen *et al.* 2019), although this was not the case in the yellow-legged gull and the ivory gull (*Pagophila eburnea*) eggs at lower concentrations (Miljeteig *et al.* 2012; Vicente *et al.* 2012). Nonetheless, in the present study we did not observe any difference between both treated and control groups in terms of TEWL or RMR. The tendency for a higher mass loss in 7:3 FTCA treated eggs may therefore be due to a low sample size, increasing the weight of single influential points on the relationship. However, since disrupted mass loss along incubation may alter embryo survival (Soliman *et al.* 1994), we recommend further investigations to conclude on the effect of fluorotelomers including 7:3 FTCA on egg mass loss.

Our study suggests no effects of 7:3 FTCA at the exposure concentration during embryo's development on the investigated endpoints. Similarly, 7:3 FTCA measured in the present study in egg yolks of yellow-legged gull did not exceed the concentration experimentally tested here, which suggests that the population in Frioul Islands should not suffer



from the embryonic exposure to this compound. There were, however, a few limiting factors to our study, the major one being the low final number of individuals included in the statistical analyses, due to the low survival of embryos in both groups after egg puncture. Although egg injection was conducted in a sterile environment, injection in the yolk are very invasive and may have had reduced the survival. Bird eggs have been generally exposed to PFAS via injections in the albumen or the air cell in previous experimental studies (Molina *et al.* 2006; O'Brien *et al.* 2009; Norden *et al.* 2016; Parolini *et al.* 2016; Jacobsen *et al.* 2018; Mattsson *et al.* 2019). However, PFAS are found in yolk in bird eggs (Gebbinck and Letcher 2012), and therefore we considered it more relevant to inject PFAS in the yolk to mimic the exposure in the wild. Low survival, may also be linked to the relatively high amount of injected solutions in eggs ( $85 \pm 8 \mu\text{L}$ ), as this is approximately twice the amount injected in previous studies cited above ( $\sim 50 \mu\text{L}$ ), however, the injected solutions concentration in eggs was similar in the present experiment ( $1 \mu\text{L g}^{-1}$  of egg). Another limitation is due to the large variance in embryos mass at E<sub>24</sub>, highlighting a large variance in embryos age when we sampled and injected the eggs, and which may have impacted our results. The method used to select fresh eggs by examining their buoyancy in water may be relatively imprecise to detect variations of incubation time in the early incubation period.

## CONCLUSION

We found relatively high levels of linPFOS, increasing concentrations of PFCAs with the carbon chain length, and the occurrence of a few emerging PFAS in eggs of yellow-legged gulls from a colony in the Frioul Islands in Southern France. In general, legacy PFAS concentrations were close to levels experimentally found to affect health parameters in chicken, although if this may affect seabirds is unclear. Our study also suggests that 7:3 FTCA does not impact development at the concentrations observed in yellow-legged gull eggs in the Frioul Islands. However, this study is only a first step in exploring the health consequences of an increasingly found emerging PFAS, investigating the effect of different concentrations as well as the effect of other emerging PFAS would be crucial in concluding about PFAS alternatives' potential to disrupt embryos' development in seabirds.

**Table 2.** Descriptive statistics for telomere length,  $\dot{V}O_2$ , total evaporative water loss (TEWL), embryo's mass, egg mass loss from E<sub>0</sub> to E<sub>24</sub> and eggshell thickness, in yellow-legged gull eggs injected with a control or a treatment solution containing 7.3 FTCA. Outputs (estimate [95 % confidence interval]) of the linear models exploring the differences between eggs from the control and treatment eggs for the different studied variables are also provided. The star (\*) indicates variables that were first corrected for embryos' mass.

	Control eggs ( <i>n</i> = 6)			Treatment eggs ( <i>n</i> = 8)			Linear model's estimate [confidence interval]
	Mean ± SD	Median	Min – max	Mean ± SD	Median	Min – max	
<b>Telomere length</b>	1.05 ± 0.13	1.1	0.86 – 1.17	1.03 ± 0.20	1.08	0.65 – 1.23	-0.06 [-0.23 – 0.11]
<b><math>\dot{V}O_2</math> (mL h<sup>-1</sup>)</b>	26.3 ± 5.24	26.4	18.2 – 32.8	27.7 ± 10.7	28.6	13.5 – 48.1	2.32 [-3.01 – 7.64]*
<b>TEWL (mg h<sup>-1</sup>)</b>	47.8 ± 19.2	46.9	28.0 – 74.2	40.3 ± 20.6	32.1	22.8 – 74.7	-3.08 [-15.9 – 9.73]*
<b>Embryo's mass (g)</b>	41.5 ± 6.29	36.4	35.5 – 52.3	40.1 ± 13.0	39.3	24.6 – 54.1	-0.19 [-13.2 – 12.8]
<b>Egg mass loss (g per day)</b>	-0.31 ± 0.05	-0.31	-0.39 – -0.24	-0.40 ± 0.11	-0.37	-0.62 – -0.25	-0.10 [-0.21 – 0.00]
<b>Eggshell thickness (mm)</b>	0.34 ± 0.02	0.33	0.31 – 0.37	0.33 ± 0.01	0.33	0.30 – 0.35	-0.00 [-0.03 – 0.02]

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## Supporting Information

# Occurrence and developmental toxicity of 7:3 fluorotelomer carboxylic acid in yellow-legged gulls

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**Table S1.** List of targeted per- and polyfluoroalkyl substances (PFAS): their groups, abbreviations, chemical names, detection rates in egg yolk of yellow-legged gulls from Frioul Islands and limits of detection. Those found in more than 70 % of the samples are stressed in bold.

Group	Abbreviation	Chemical name	Detection rate	Limit of detection (pg g <sup>-1</sup> ww)
Perfluoroalkane sulfonic acids	PFBS	perfluorobutanesulfonic acid	13.3%	50
	PFPS	perfluoropentanesulfonic acid	0.0%	100
	<b>PFHxS</b>	<b>perfluorohexanesulfonic acid</b>	<b>96.7%</b>	<b>50</b>
	<b>PFHpS</b>	<b>perfluoroheptanesulfonic acid</b>	<b>100.0%</b>	<b>100</b>
	<b>brPFOS</b>	<b>branched perfluorooctanesulfonic acid</b>	<b>100.0%</b>	
	<b>linPFOS</b>	<b>linear perfluorooctanesulfonic acid</b>	<b>100.0%</b>	
	PFNS	perfluorononanesulfonic acid	10.0%	100
	PFDCS	perfluorodecanesulfonic acid	3.3%	100
Perfluoroalkyl carboxylic acids	PFHxA	perfluorohexanoic acid	26.7%	100
	PFHpA	perfluoroheptanoic acid	26.7%	50
	<b>PFOA</b>	<b>perfluorooctanoic acid</b>	<b>100.0%</b>	<b>20</b>
	<b>PFNA</b>	<b>perfluorononanoic acid</b>	<b>100.0%</b>	<b>100</b>
	<b>PFDCa</b>	<b>perfluorodecanoic acid</b>	<b>100.0%</b>	<b>100</b>
	<b>PFUnA</b>	<b>perfluoroundecanoic acid</b>	<b>100.0%</b>	<b>100</b>
	<b>PFDoA</b>	<b>perfluorododecanoic acid</b>	<b>100.0%</b>	<b>100</b>
	<b>PFTriA</b>	<b>perfluorotridecanoic acid</b>	<b>100.0%</b>	<b>100</b>
	<b>PFTeA</b>	<b>perfluorotetradecanoic acid</b>	<b>100.0%</b>	<b>100</b>
	<b>PFHxDA</b>	<b>perfluorohexadecanoic acid</b>	<b>93.3%</b>	<b>100</b>
	PFODcA	perfluorooctadecanoic acid	0.0%	150

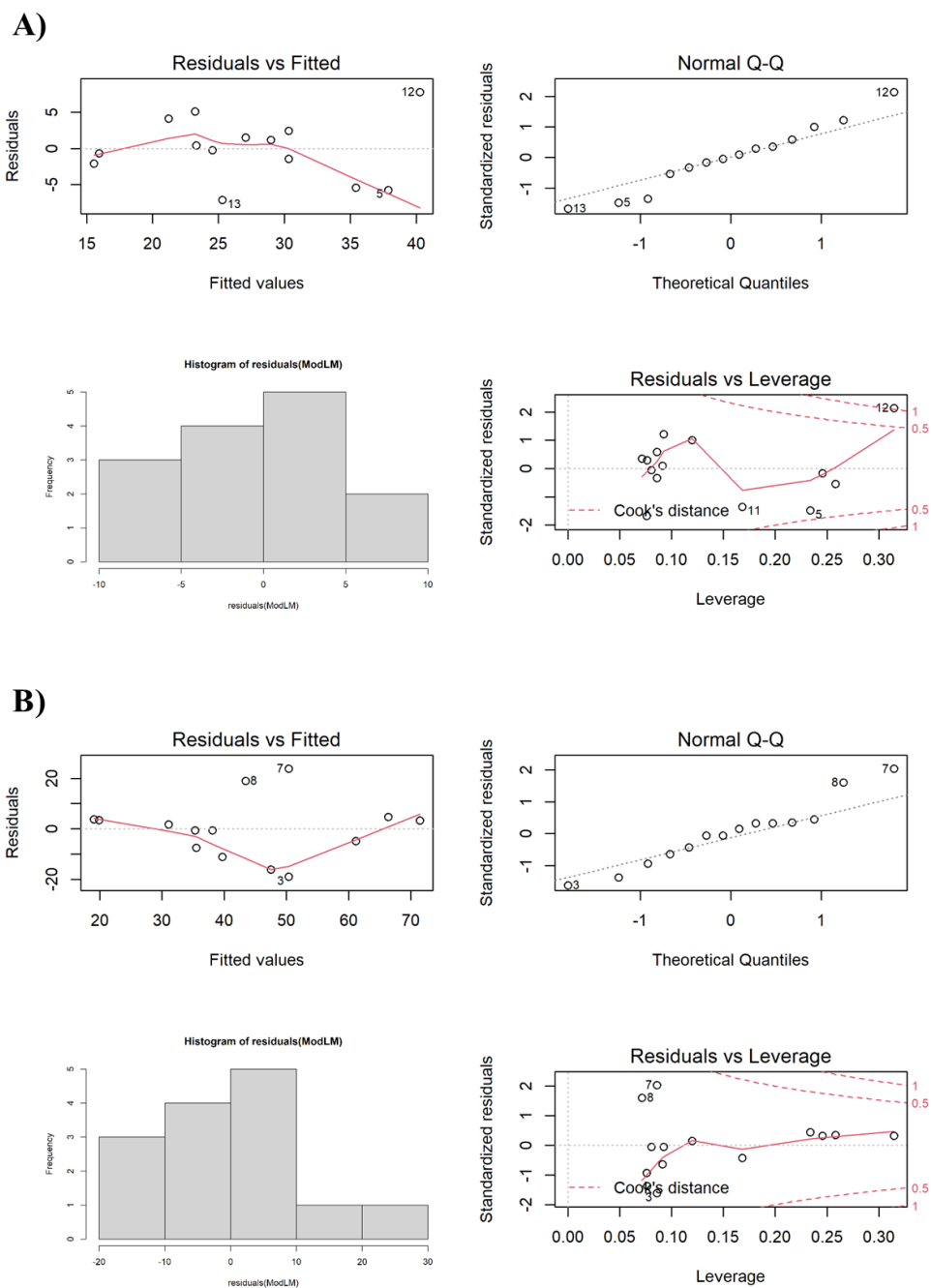
## Chapter IV – Paper D: Supporting Information

Perfluoroalkane sulfonamido substances	FOSA	perfluorooctane sulfonamide	0.0%	100
Fluorotelomer sulfonic acid	4:2 FTS	4:2 fluorotelomer sulfonic acid	0.0%	100
	6:2 FTS	6:2 fluorotelomer sulfonic acid	53.3%	100
	<b>8:2 FTS</b>	<b>8:2 fluorotelomer sulfonic acid</b>	<b>86.7%</b>	<b>100</b>
	<b>10:2 FTS</b>	<b>10:2 fluorotelomer sulfonic acid</b>	<b>86.7%</b>	<b>100</b>
Fluorotelomer carboxylic acid	5:3 FTCA	5:3 fluorotelomer carboxylic acid	0.0%	25
	<b>7:3 FTCA</b>	<b>7:3 fluorotelomer carboxylic acid</b>	<b>86.7%</b>	<b>15</b>
Fluoroalkylether compounds	GenX or 33-PFECA (HPFO-DA)		0.0%	250
	333-PFECA (HFPO-TA)		0.0%	250
	3333-PFECA (HPFO-TeA)		23.3%	5
	33333-PFECA (5x3-PFECA)		0.0%	50
	333333-PFECA (6x3-PFECA)		0.0%	25
	ADONA (3H-133-PFECA)		0.0%	3
	F-53B (w-Cl-62-PFES)		0.0%	3
	<b>PFEcHS</b>		<b>100.0%</b>	<b>10</b>

### **Differences between incubators**

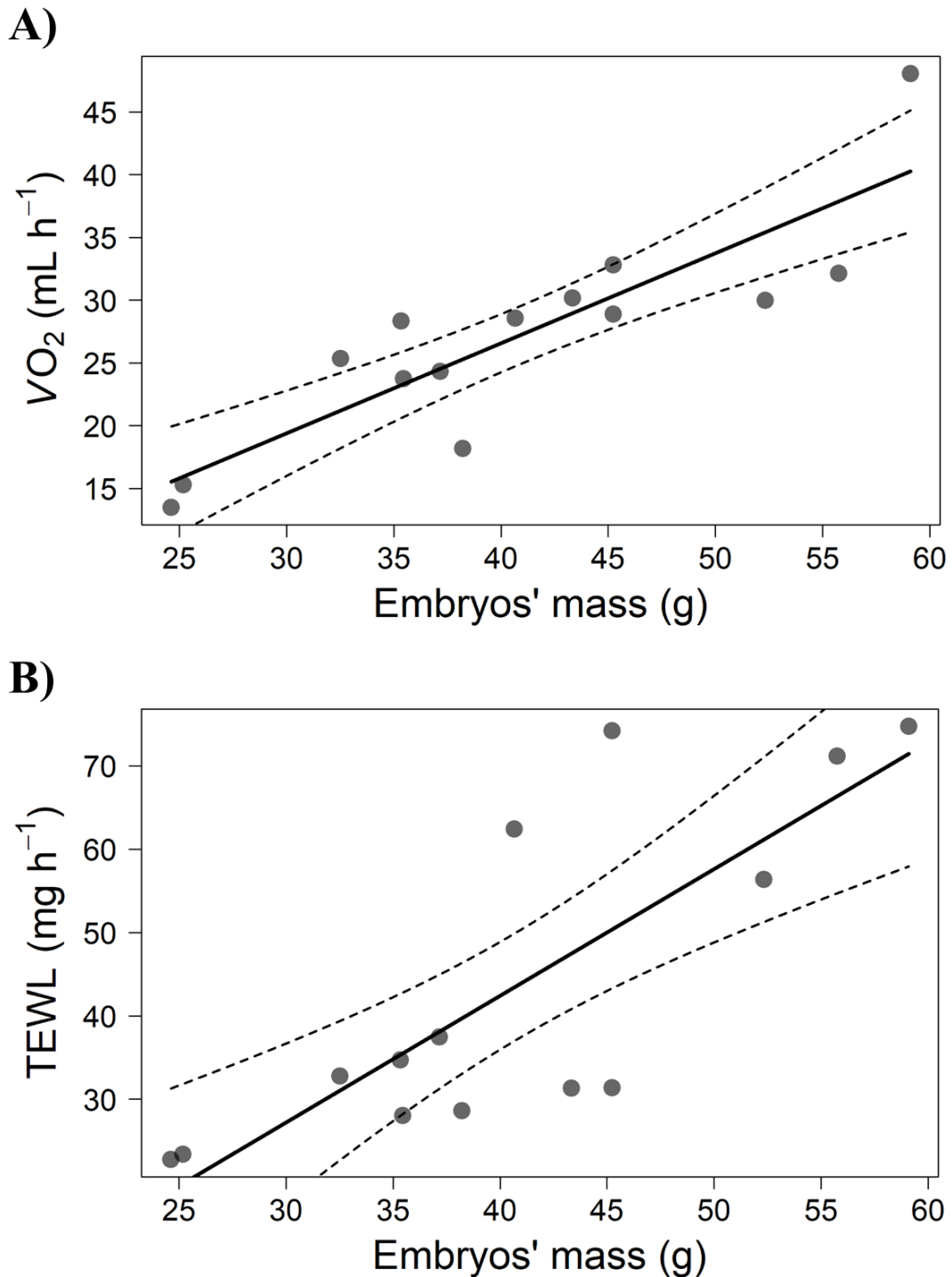
We compared each of the investigated factors between both incubators using linear models to ensure that the incubation conditions were not different and that eggs from both incubators could be considered the same way in further analyses. We found no difference between incubators for Eggs' mass at E<sub>24</sub> ( $F_{1, 12} = 0.167, p = 0.690$ ), Embryos' mass ( $F_{1, 12} = 0.021, p = 0.888$ ), Telomere length ( $F_{1, 12} = 0.164, p = 0.692$ ), Eggshell thickness ( $F_{1, 12} = 0.006, p = 0.938$ ), Eggs' mass loss ( $F_{1, 12} = 0.064, p = 0.805$ ),  $VO_2$  ( $F_{1, 12} = 0.0002, p = 0.989$ ), and total evaporative water loss ( $F_{1, 12} = 0.476, p = 0.503$ ). For each models, we visually check if the data sufficiently met the linear model assumptions using histograms of the residuals and plot of residuals vs fitted (Zuur 2007).

**Figure S1.** Diagnostic plots of the linear models of: A) the  $\dot{V}O_2$  against the embryo mass; B) the total evaporative water loss against the embryo mass. Plots represent: 1) top-left: “Residuals versus fitted data”, to check if there is evidence of increasing unexplained variance at increasing predicted values; 2) top-right: “Normal Q-Q”, which shows ordered residuals against quartiles of the normal distribution; 3) bottom-left: “Histogram of the residuals”, to estimate if residuals follow a normal distribution; 4) bottom-right: “Residuals versus leverage”, leverage measures the potential for each data point to influence the overall model fit.



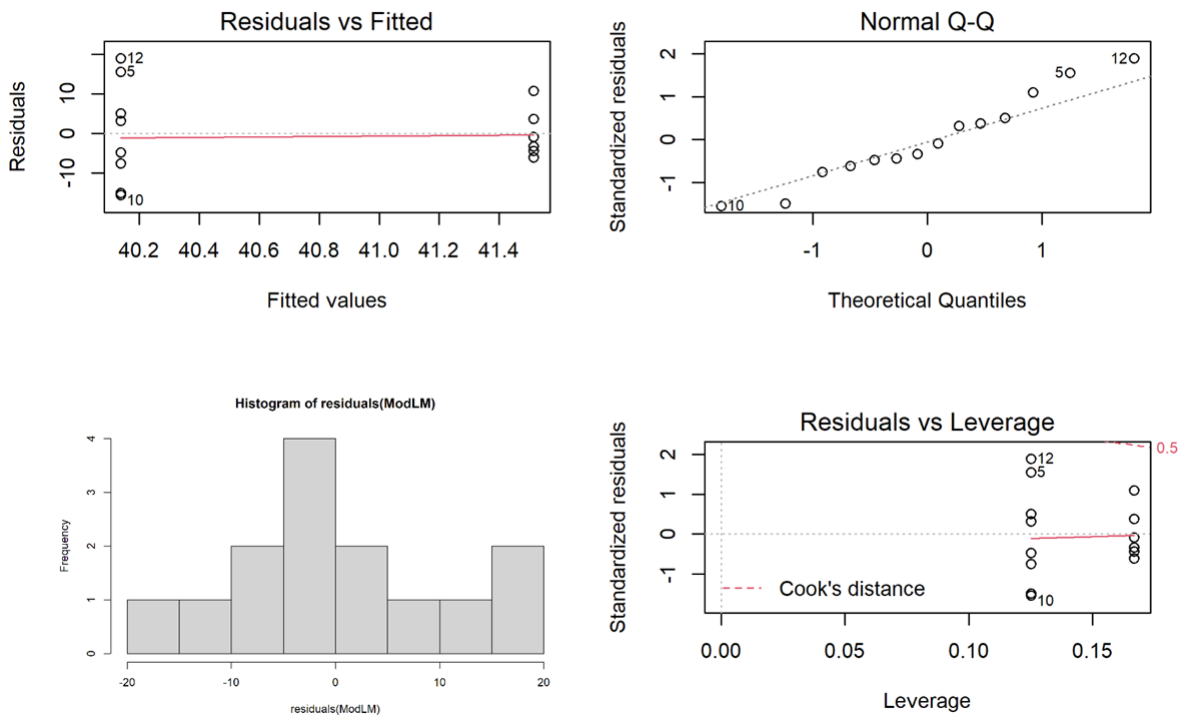


**Figure S2.** In eggs of yellow-legged gulls from the Frioul Islands: A) Relationship between  $VO_2$  at E<sub>23</sub> and embryos' mass at E<sub>24</sub>; and B) Relationship between total evaporative water loss (TEWL) at E<sub>23</sub> and embryos' mass at E<sub>24</sub>. The solid line refers to a statistically significant linear maternal transfer (see main manuscript), with dotted lines representing 95% confidence intervals.

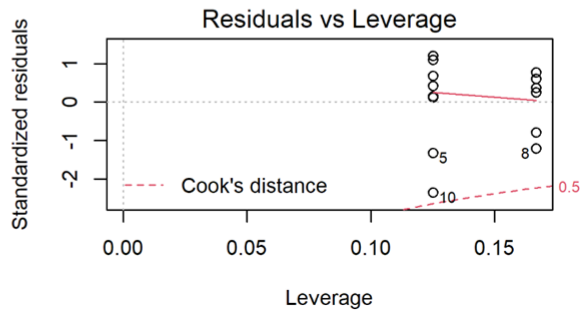
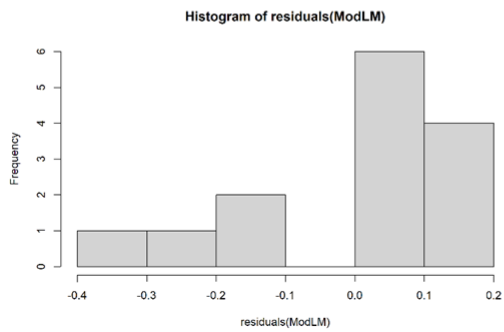
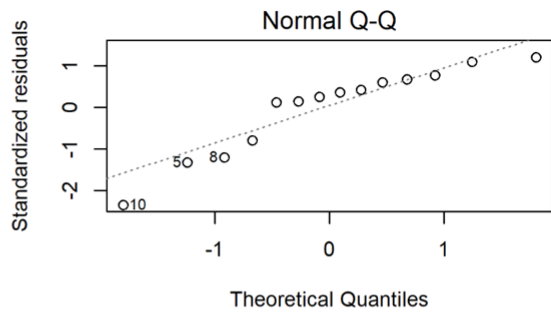
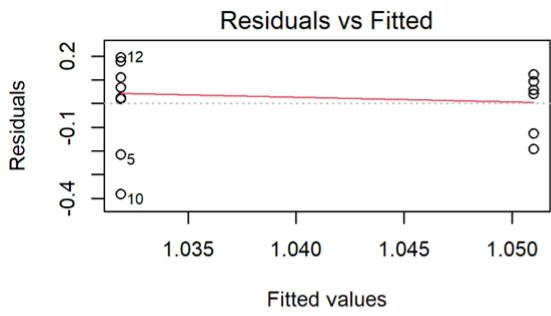


**Figure S3.** Diagnostic plots of the linear models of: A) the embryos' mass against the status (“treatment” or “control”); B) the liver telomere length against the status; C) the  $\dot{V}O_2$  (corrected for embryo's mass, see main manuscript) against the status; D) the total evaporative water loss (TEWL; corrected for embryo's mass, see main manuscript) against the status; E) the eggshell thickness against the status; and F) the eggs' mass loss between  $E_0$  and  $E_{24}$  against the status. See Figure S1 for additional information.

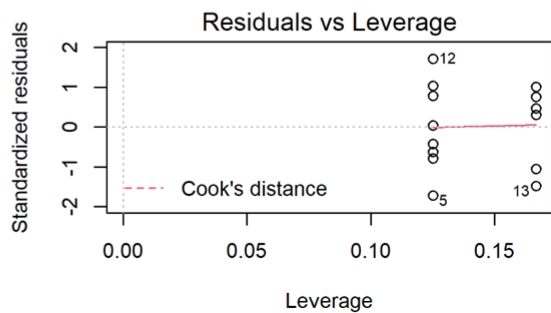
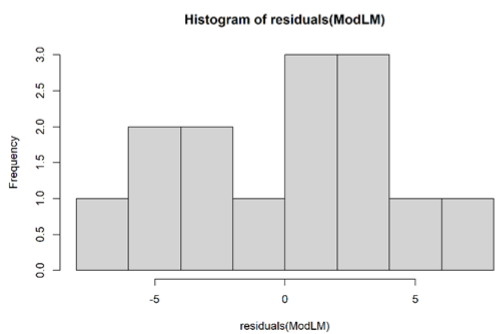
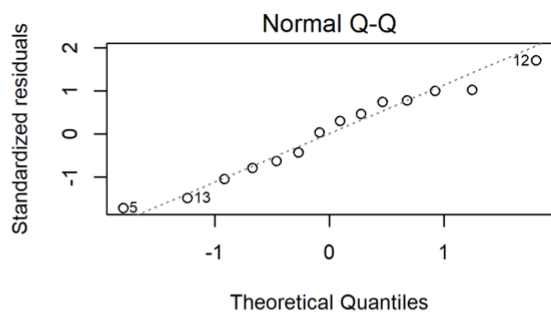
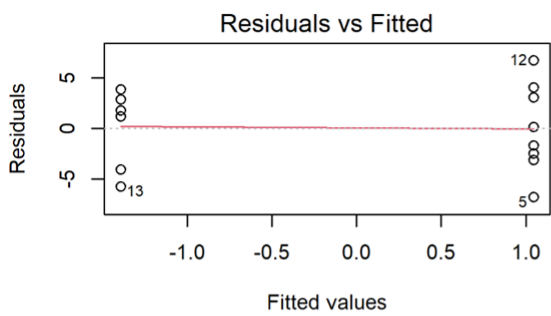
A)



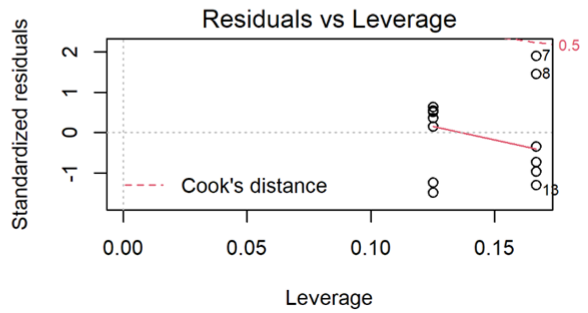
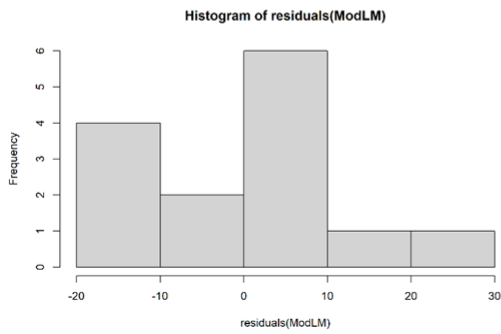
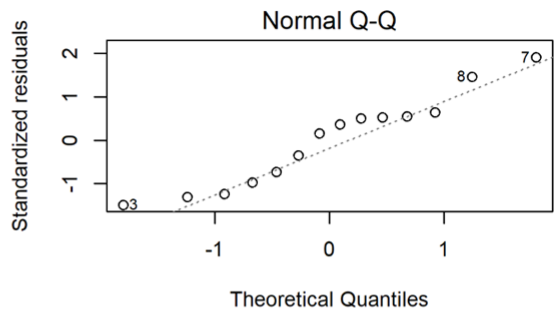
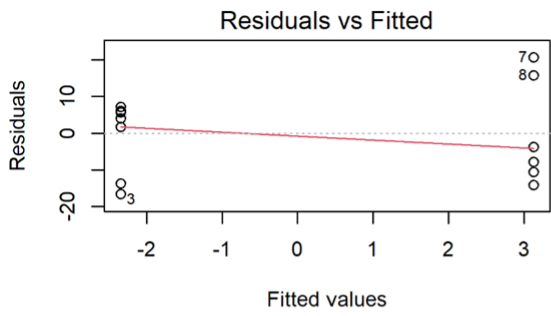
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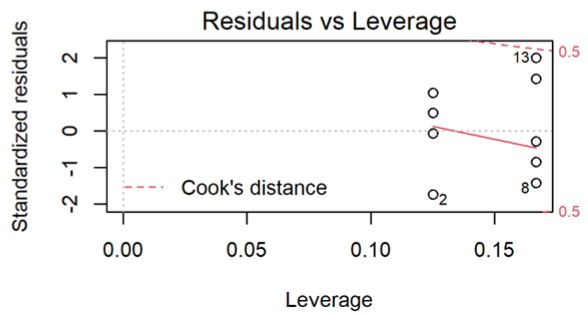
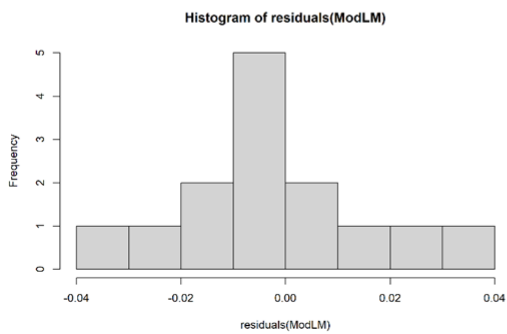
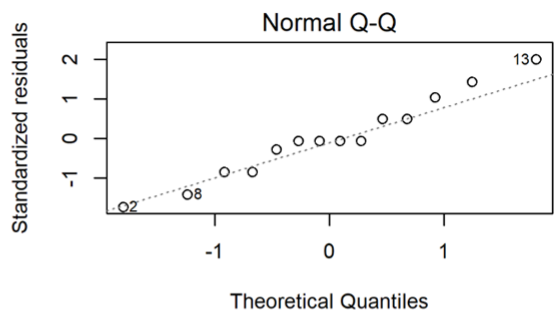
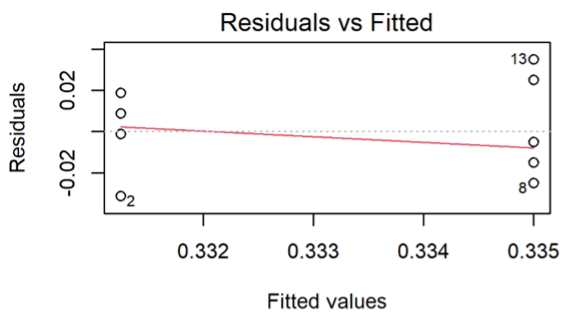
**C)**



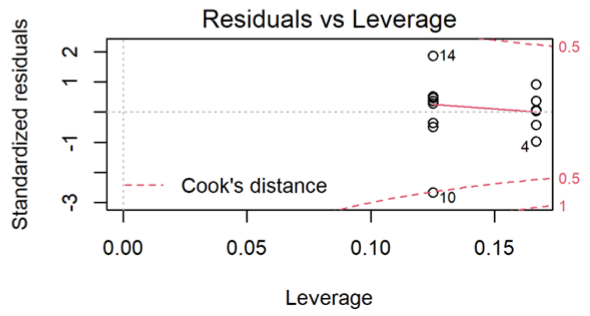
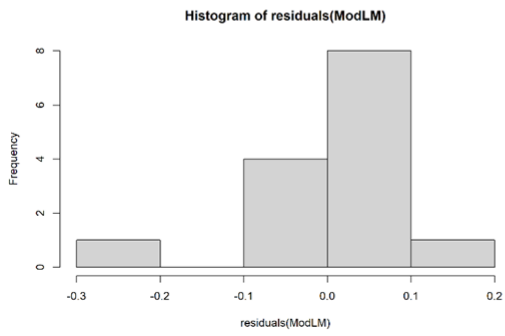
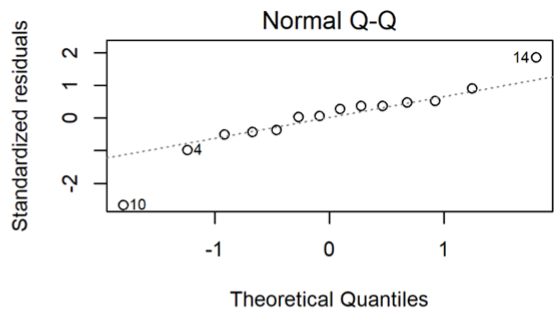
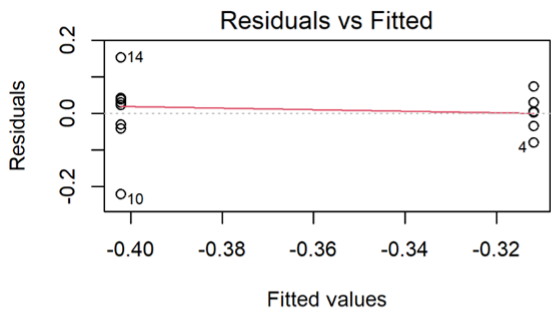
**D)**



**E)**



**F)**



**Table S2.** Descriptive statistics (Mean  $\pm$  Standard Deviation SD, Median and Range Min–Max) of concentrations ( $\text{ng g}^{-1}$  ww) of PFAS detected in less than 70 % of the compounds (see Table S1) in egg yolk of yellow-legged gulls from Frioul Islands (Marseille, France). A single egg was measured above LOD for PFDCS. The calculation of the descriptive statistics for these compounds only includes the samples measured above LOD.

		<b>Mean <math>\pm</math> SD</b>	<b>Median</b>	<b>Min-max</b>
Legacy compounds	PFBS	0.11 $\pm$ 0.00	0.11	0.11 – 0.11
	PFNS	0.27 $\pm$ 0.03	0.3	0.25 – 0.30
	PFDCS	5.72 $\pm$ NA	5.72	NA
	PFHxA	0.20 $\pm$ 0.13	0.17	0.12 – 0.52
	PFHpA	0.41 $\pm$ 0.29	0.30	0.19 – 0.91
Emerging compounds	6.2 FTS	2.22 $\pm$ 4.21	0.45	0.15 – 16.0
	HPFO-TeA	0.01 $\pm$ 0.00	0.01	0.01 – 0.01

**Table S3.** Model outputs of the differences in the telomere length, the  $V\text{O}_2$ , the total evaporative water loss (TEWL), the embryo's mass, the egg mass loss from  $E_0$  to  $E_{24}$  and the eggshell thickness, between control and treatment eggs and their relationship with freshly laid egg mass, estimated by lineal models. Significant  $p$ -values are bolded. The star (\*) indicates variables that have been corrected for embryo's mass initially (see main manuscript).

Parameter	Estimate	SE	$t$ -value	$p$ -value
<b><u>Telomere length (<math>F_{2,11}</math>: 3.41; <math>p</math> = 0.07; <math>R^2</math>: 0.27)</u></b>				
Status (treatment)	-0.060	0.079	-0.757	0.465
Egg mass $E_0$	0.011	0.004	2.601	<b>0.025</b>
<b><u><math>V\text{O}_2^*</math> (<math>F_{2,11}</math>: 0.56; <math>p</math> = 0.59; <math>R^2</math>: -0.07)</u></b>				
Status (treatment)	2.316	2.419	0.958	0.359
Egg mass $E_0$	0.034	0.134	0.257	0.802
<b><u>TEWL* (<math>F_{2,11}</math>: 2.64; <math>p</math> = 0.12; <math>R^2</math>: 0.20)</u></b>				
Status (treatment)	-3.076	5.816	-0.529	0.607
Egg mass $E_0$	-0.672	0.322	-2.088	0.061
<b><u>Embryo's mass (<math>F_{2,11}</math>: 0.55; <math>p</math> = 0.59; <math>R^2</math>: -0.08)</u></b>				
Status (treatment)	-0.190	5.905	-0.032	0.975
Egg mass $E_0$	-0.333	0.327	-1.018	0.331
<b><u>Egg mass loss (<math>F_{2,11}</math>: 2.59; <math>p</math> = 0.12; <math>R^2</math>: 0.20)</u></b>				
Status (treatment)	-0.102	0.048	-2.132	0.056
Egg mass $E_0$	0.003	0.003	1.202	0.255
<b><u>Eggshell thickness (<math>F_{2,11}</math>: 0.47; <math>p</math> = 0.64; <math>R^2</math>: -0.09)</u></b>				
Status (treatment)	-0.006	0.011	-0.529	0.608
Egg mass $E_0$	0.001	0.001	0.898	0.388

## REFERENCE

Zuur A, Ieno EN, Smith GM. 2007. *Analyzing ecological data*. Spring

# GENERAL DISCUSSION



*Black-legged kittiwakes (Rissa tridactyla)*



## I. Major outcomes of this doctoral thesis

Using an arctic seabird, the black-legged kittiwake as study model, I provided important new insights on the causes and consequences of maternal PFAS transfer (summarized in **Figure 18**). Specifically, I could demonstrate that:

1/ Contamination of both females and eggs were dominated by legacy PFAS, including linPFOS, PFUnA and PFTriA and that the longest chain perfluoroalkyl carboxylic acids (PFCAs) were preferentially transferred to the eggs, especially to the first laid egg (**Paper A**).

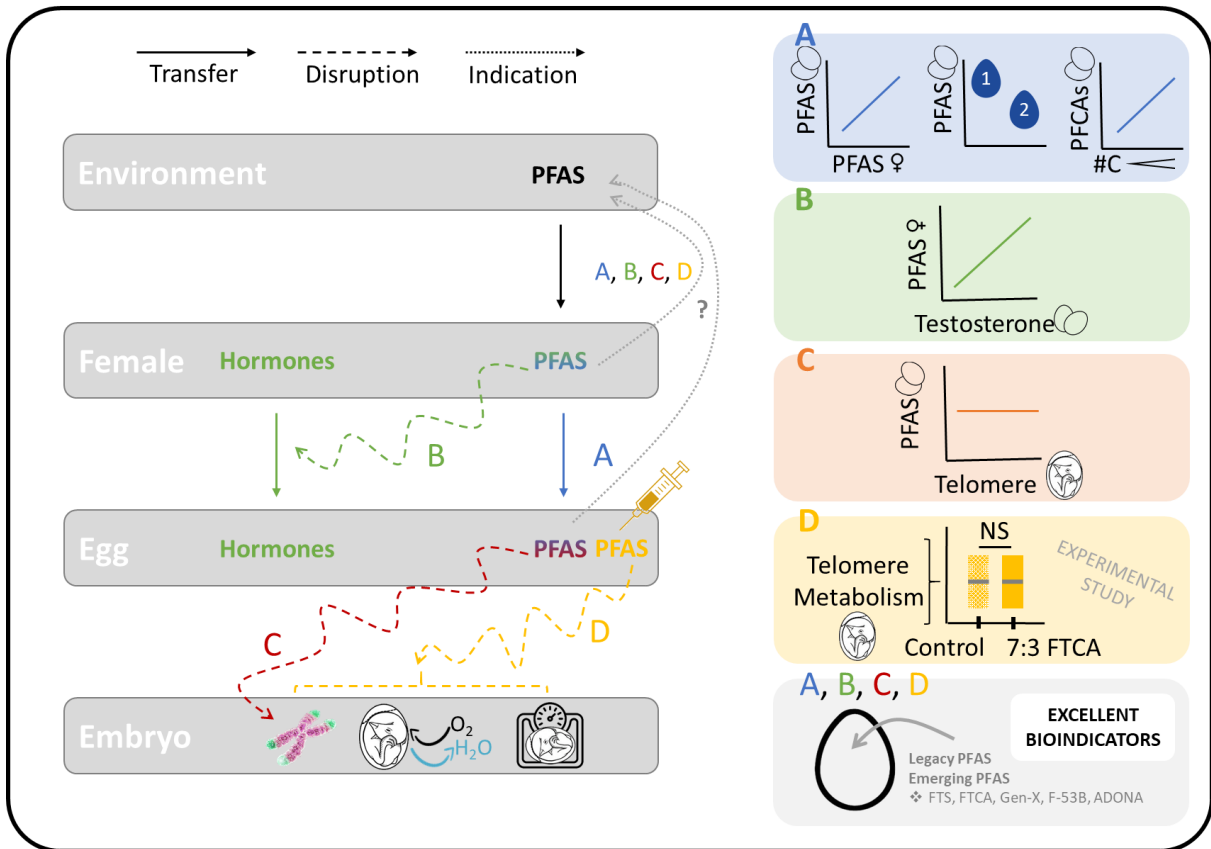
2) Several emerging PFAS were well present in the egg yolk. I notably found 7:3 FTCA, a precursor of long-chain carboxylate in 84% of the egg yolks, and provided new evidences of the occurrence of PFEcHS, 10:2 FTS and 3333-PFECA in the yolk of an arctic seabird as well as the first documented finding of ADONA in wildlife. When tested, these emerging PFAS were all below the detection limit in female plasma, suggesting that the yolk is a more relevant tissue to detect and monitor emerging compounds (**Paper A**).

3) Within clutches, maternal deposition was highly repeatable in eggs for brPFOS and C<sub>8</sub> to C<sub>11</sub> perfluoroalkyl carboxylic acids (PFCAs) but not for emerging PFAS (**Paper C**).

4) Some long-chain PFCAs (PFNA, PFDcA and PFUnA) have the potential to disrupt and actually increase maternal testosterone deposition in egg. Maternal transfer of other hormones (glucocorticoids and thyroid hormones) was unaffected by PFAS (**Paper B**).

5) Embryos telomeres length, a biomarker of health and lifespan, was unaffected by yolk levels of brPFOS and C<sub>8</sub> to C<sub>11</sub> PFCAs (**Paper C**).

6) Experimental exposure to 7:3 FTCA in yellow-legged gull eggs did not impact embryos telomere length and metabolism. This is the first toxicity assessment for this emerging compound in wildlife (**Paper D**).



**Figure 18.** Schematic representation of the global structure of this thesis and the main results associated to each step.

In this section, I discuss the novelty of the results presented in **Chapters I to IV** in the framework of the global knowledge on PFAS occurrence and impact on seabirds, I also discuss the strengths and limitations of my studies. Finally, I propose some avenues for the research on how PFAS may affect seabird embryos.

## II. Legacy PFAS in seabird eggs, an overview

### a. Levels and patterns

For historical reasons, legacy PFAS (i.e., the most produced and supposedly harmful compounds) are often the only PFAS considered in biomonitoring studies although they may represent only a small number of all fluorinated compounds present in organisms (Koch *et al.* 2021; Pelch *et al.* 2022). They are consequently very precisely measured in numerous species worldwide including seabirds, and many of their adverse effects were characterized. When investigating the levels and consequences of PFAS on biota, all legacy PFAS should, therefore, be included in the list of screened substances.

## General Discussion

Overall, during my PhD I found two highly different patterns between eggs of kittiwakes and yellow-legged gulls for legacy PFAS. LinPFOS, PFUnA and PFTriA were prevailing in fairly similar concentrations in kittiwakes, but linPFOS were largely dominating (i.e., between 4 and 20 times higher than the other PFAS) in yellow-legged gulls (**Papers A, C and D; Figure 19**). The dominance of PFOS in eggs is observed in almost all terrestrial and aquatic bird species worldwide (Alfaro Garcia *et al.* 2022; Custer *et al.* 2014; Eriksson *et al.* 2016; Groffen *et al.* 2017; Herzke *et al.* 2022; Jang *et al.* 2022; Norden *et al.* 2013; Parolini *et al.* 2020; Pereira *et al.* 2021; van der Schyff *et al.* 2020; Vorkamp *et al.* 2019; Wilkinson *et al.* 2022; Wu *et al.* 2020; but see Braune and Letcher, 2013; Wang *et al.* 2021), and echoes the prevalence of this compound in wild birds blood and organs (Custer *et al.* 2014; Groffen *et al.* 2019; Roscales *et al.* 2019). The main reason explaining its overwhelming presence is that PFOS is one of the first and most popular PFAS created (see **boxed text**). The high affinity of PFOS for protein-rich tissues may also accentuate the concentration of PFOS in liver (Gebbinck and Letcher 2012), and therefore in eggs since several of the liver proteins are transferred in the growing oocyte (Vezina *et al.* 2003). Nonetheless, I did not account for the egg protein and lipid content in the study of the maternal transfer in kittiwakes (**Paper A**) although this may play a major role in the final concentration of contaminants in eggs (Bargar *et al.* 2001; Verreault *et al.* 2006), and could be particularly useful when comparing inter-specific levels.

### Perfluorooctanesulfonic acid (PFOS)

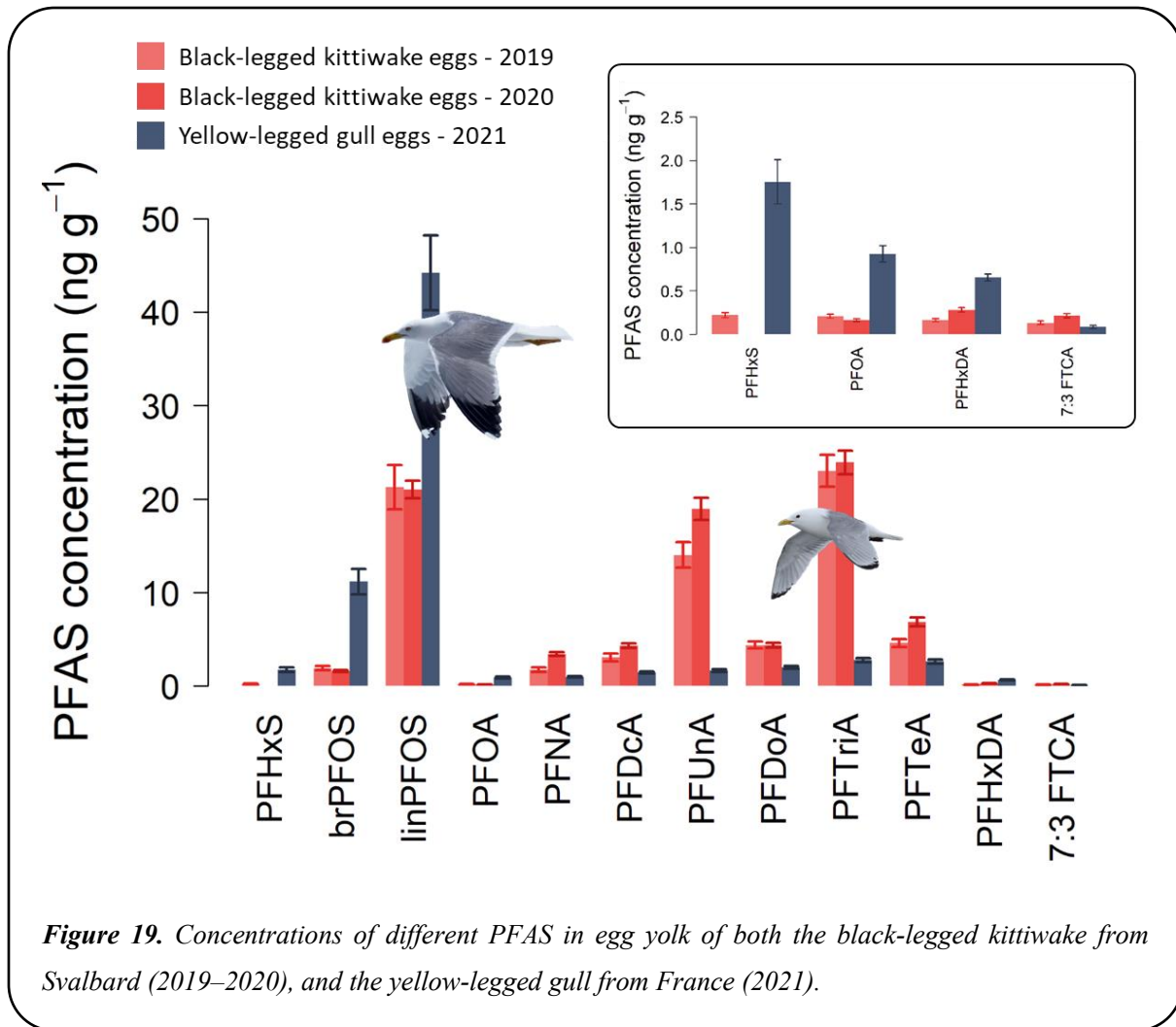
First synthesized at the end of the 1940s by the 3M Company, its production and use increased until the 1990s owing to its unique chemical attributes that made it useful in numerous industrial applications. It is only 50 years later that, after its global distribution was revealed, this compound was regulated in the US and in Europe and subsequently added to the POPs list under the Stockholm Convention. During this period, it was estimated that the total historical production of PFOS equivalents reached ~100 000 tons (Paul *et al.* 2009), with an annual production of around 4500 tons of PFOS-related products at its production peak, compared to an estimated emissions of C<sub>4</sub> – C<sub>14</sub> PFCAs of around 70 to 800 tons per year globally at the same period (Wang *et al.* 2014). Emissions have largely decrease since, but PFOS is still produced in some countries including China (Chen *et al.* 2009). Nonetheless, the levels observed recently in biota demonstrate how persistent this compound is.

## General Discussion

Compared to the several studies documenting legacy PFAS in seabird eggs, yellow-legged gulls had relatively similar concentrations of PFOS to other colonies from the Mediterranean Sea, and kittiwake were fairly more contaminated than those from Northern Norway and Canada [**Appendix D**]. In the context of using eggs as bioindicators of local environmental contamination, interestingly, when compared to other species from the Laridae family [**Appendix D**], the highest concentrations of PFOS were found in species nesting next to lakes in North America and Sweden or in the neighborhood of a fluoro-chemical plant (Letcher *et al.* 2015; Lopez-Antia *et al.* 2017; Norden *et al.* 2013; Su *et al.* 2017). For example, PFOS median in herring gull eggs from Lake Vänern in Sweden was 423 ng g<sup>-1</sup> wet weight (Norden *et al.* 2013). Enclosed waters receiving contaminant inputs from urbanized surroundings may explain such high concentrations compared to the constantly renewed waters of large seas. Nonetheless, all studies examining PFAS concentration in seabird eggs provided inconstant information (median, mean or range in whole eggs or yolk only), moreover, the rank of the egg within the clutch is rarely controlled, even though this parameter is a major parameter to explain PFAS deposition in eggs (**Paper A**). Thus, for a systematic use of eggs as bioindicators, there is an urgent need to homogenize sampling protocols.

Regarding PFCAs, kittiwake eggs exhibited similar levels of PFUnA and PFTriA to PFOS, suggesting a high accumulation of these compounds in the Norwegian Arctic (**Papers A and C**). The pattern observed in kittiwake yolks, with dominating odd-numbered over even-numbered long-chain PFCAs, is often found in the different tissues of other seabird species including their eggs (Alfaro Garcia *et al.* 2022; Braune and Letcher 2013; Herzke *et al.* 2022; Jang *et al.* 2022), although not in all (Custer *et al.* 2014; Gebbink and Letcher 2012; Norden *et al.* 2013; Wilkinson *et al.* 2022). The origin of this predominance is still unclear, Herzke *et al.* (2022) suggested that the differences in yolk PFAS patterns may be related to species-specific PFAS toxicokinetic, habitat, or diet.

In this PhD thesis, I provided new insights on how PFAS may be transferred from mother to eggs, which is essential in the process of evaluating their toxicity for embryos. We limited potential bias by collecting females before laying as well as their own freshly-laid eggs. However, I also identified several limiting factors that should be taken into account in future studies, including the egg protein and lipid content or females parameters such as their age or metabolic rate (Bertolero *et al.* 2015).



### b. Legacy PFAS, what consequences?

PFAS toxicity generally increases with the chain-length within a PFAS group (Ankley *et al.* 2021), therefore, the high concentration of long-chain PFCAs found in kittiwakes is concerning. Moreover, these compounds, along with PFOS to a lesser extent, are very often associated with various disrupted endpoints in seabirds (**Table 1**), suggesting that these species might be particularly sensitive to long-chain PFCAs. Considering the very low concentrations of PFCAs in yellow-legged gull (**Figure 19**), it may be regarded as relatively safe compared to kittiwakes. Even bearing in mind that we did not control for egg rank within the clutch in yellow-legged gull despite it may greatly affect PFAS levels (**Paper A**). However, studies on the effect of PFAS during the development are scarce in birds, and they experimentally tested the effect of PFOS on the domestic chicken in the overwhelming majority.

Since PFAS are known EDCs (Marlatt *et al.* 2021; **Table 1**), we examined their potential impact on the maternal transfer of hormones, suggested to be an essential adaptive mechanism

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to control offspring phenotype (von Engelhardt and Groothuis 2011). Studying a wild species exposed to high concentrations of various PFAS *in natura*, we could test the potential impact of many of these contaminants. We conducted the first study investigating the effect of PFAS on the maternal transfer of hormones in birds (**Paper B**) and showed that females long-chain PFCAs (C<sub>9</sub> to C<sub>11</sub>) may potentially disrupt the maternal transfer of testosterone in eggs. Elevated maternal testosterone in seabird eggs, including kittiwakes, is known to enhance offspring aggressiveness and competitiveness (Müller *et al.* 2009a; Müller *et al.* 2012, 2013), however with counterparts including lowered immune response and telomere length and impaired growth (Eising *et al.* 2001; Muller *et al.* 2005; Parolini *et al.* 2019). Several experimental studies also provided cues that may illustrate the potential long-term consequences of a disrupting effect on testosterone deposition in avian eggs (Hsu *et al.* 2016; Matson *et al.* 2016; Müller *et al.* 2009b; Ruuskanen *et al.* 2012a; 2012b; 2012c; Schweitzer *et al.* 2013). Nonetheless, most of these studies were experimental and some of the injected levels of testosterone were much higher than what is usually measured in bird eggs. Additional studies are needed to conclude on 1/ if elevated levels of testosterone in eggs really stem from a disruption of the maternal transfer by PFAS, since our study was correlative and confounding factors, such as female quality may interfere, and 2/ how may elevated testosterone in eggs affect kittiwake at both short and long term. On free-ranging birds, this study must be repeated on different environmental conditions since the egg is “multi-variate”, meaning that several maternally transferred substances may have additive, synergetic or antagonistic effects and consequently, affect the embryo in many different ways (Torres *et al.* 2019).

Recently, telomere shortening has been suggested as a short- and long-term cost of elevated testosterone exposure during development, in livers of yellow-legged gulls chicks (Parolini *et al.* 2019), and in adults males of dark-eyed juncos (*Junco hyemalis carolinensis*; Heidinger *et al.* 2021). Telomeres are biomarkers of health and lifespan, and any disruptions experienced during development may be conserved through life and have strong consequences at adulthood (Marasco *et al.* 2022). Considering this, we therefore expected shortened telomeres in embryos from eggs receiving a high deposition of testosterone (i.e., those laid by highly contaminated females and therefore containing high PFAS concentrations; **Papers A and B**). Previous studies investigating the relationship PFAS/telomere length during early-life were scarce and limited to Human newborns to the best of my knowledge. In these studies; contrasting but significant correlations between PFAS and telomere length were found (Eick *et al.* 2021; Liu *et al.* 2018; Pan *et al.* 2022). To explore our hypothesis that high levels of PFAS leading to high concentrations of testosterone in eggs may affect telomere length, and since no

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studies explored the effect of PFAS on telomere during the development in birds, we chose to investigate the relationship between PFAS and telomere length in kittiwakes.

Overall, exploring the effect of maternal contaminants on embryos during the development is challenging since it requires to sample the egg as soon as it is laid while keeping the embryo alive. One option could be to sample a small part of the yolk using a needle (Stebbins *et al.* 2009), but this may impact survival, consequently biasing the final results. Moreover, only small amounts of yolk can be taken with this method and this cannot be done for PFAS which require a relatively high volume for assays. We thus took advantage of the fact that some PFAS were found to be deposited with a significant repeatability within clutches (**Paper C**), to use first-laid eggs as a proxy of the second one. Nonetheless, not all PFAS were deposited with a high repeatability and therefore many could not be included in the study of how they may affect telomere length. Moreover, we could not test the hypothesis of a crossed-relationship between yolk PFAS, yolk testosterone and embryos telomere length since we did not find a significant relationship in the deposition of testosterone among eggs within a clutch (**Paper B**).

We did not find any suggestions that PFAS in eggs may alter offspring telomere length during the development (**Paper C**). To the best of my knowledge, three previous studies investigated the consequences of PFAS exposure on telomere length in wild birds including kittiwakes, finding elongating telomeres with increasing PFAS loading in adults plasma, but no relationship with absolute telomere length in adults and chicks (Blévin *et al.* 2017a; Sebastiano *et al.* 2020a; Sletten *et al.* 2016). Telomere dynamic may therefore be more representative of PFAS adverse effects than telomere length. Additionally, it has also been suggested as a better indicator of health consequences (Boonekamp *et al.* 2014). Nonetheless, collecting at least two samples at different time of the development is required to test this assumption, and this would be challenging to conduct on embryos. It is nonetheless easily doable on hatchlings. Notably, testosterone actions on telomere length was only measurable during a few days at hatching in yellow-legged gulls (Noguera and Velando 2022), which suggest that we may have sampled embryos too early to observe such effect.

The sample size was limited in this experiment due to the conservation status of the kittiwake. However, despite species such as the domestic chicken or quails (Galliformes) are often use in experimental studies since they are easy to breed, I believe that the results obtained could not be relevant for seabirds since they have very different physiology and life-history traits.

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**Table 1.** Summary of correlative studies investigating PFAS relationship (positive (+) or negative (-)) with several biomarkers of health in adults of different seabird species, the black-legged kittiwake (*Rissa tridactyla*; BLK), the glaucous gull (*Larus hyperboreus*; GG), the lesser black-backed gull (*Larus fuscus*; LBBG), the great black-backed gull (*Larus marinus*; GBBG), the thick-billed murre (*Uria lomvia*; TBM). Investigated variables include corticosterone (CORT), prolactine (PRL), total or free triiodothyronine (TT<sub>3</sub> and FT<sub>3</sub> respectively), total or free thyroxine (TT<sub>4</sub> and FT<sub>4</sub> respectively). ♂ or ♀ is indicated if relationship is found for males or females respectively, if none the relationship is valid for both. Studies indicated with a \* were conducted on chicks.

Variables	PFAS	Association	Species	References
<b>Endocrine disruption</b>				
CORT	C <sub>13</sub> –C <sub>14</sub> PFCAs	-	BLK	Tartu <i>et al.</i> 2014
PRL (♀)	C <sub>8</sub> PFSA, C <sub>9</sub> , C <sub>11</sub> PFCAs	+	BLK	Blévin <i>et al.</i> 2020
TT <sub>4</sub>	C <sub>7</sub> –C <sub>8</sub> PFSAs, C <sub>9</sub> PFCA	+	BLK	Nøst <i>et al.</i> 2012*
TT <sub>4</sub> (♂)	C <sub>8</sub> PFSA, C <sub>10</sub> PFCA	+	BLK	Ask <i>et al.</i> 2021
FT <sub>4</sub>	C <sub>7</sub> –C <sub>8</sub> PFSAs, C <sub>9</sub> PFCA		BLK	Nøst <i>et al.</i> 2012*
TT <sub>3</sub>	C <sub>8</sub> PFSA, C <sub>11</sub> –C <sub>14</sub> PFCAs	+	GBBG	Sebastiano <i>et al.</i> 2020b [Appendix B]
TT <sub>3</sub>	C <sub>6</sub> PFSA	-	GBBG	Sebastiano <i>et al.</i> 2020b [Appendix B]
TT <sub>3</sub> (♂)	C <sub>8</sub> PFSA, C <sub>12</sub> , C <sub>14</sub> PFCAs	-	TBM	Choy <i>et al.</i> 2022
TT <sub>3</sub> (♀)	C <sub>12</sub> –C <sub>14</sub> PFCAs	+	BLK	Ask <i>et al.</i> 2021
FT <sub>3</sub>	C <sub>8</sub> PFSA	+	GG	Melnes <i>et al.</i> 2017
FT <sub>3</sub>	C <sub>8</sub> PFSA, C <sub>9</sub> , C <sub>10</sub> , C <sub>12</sub> , C <sub>14</sub> PFCAs	+	TBM	Choy <i>et al.</i> 2022
<b>DNA damage</b>				
Telomere dynamic	C <sub>8</sub> PFSA, C <sub>9</sub> –C <sub>13</sub> PFCAs	+	GG	Blévin <i>et al.</i> 2017a
Telomere erosion	C <sub>9</sub> , C <sub>14</sub> PFCAs	-	GG	Sebastiano <i>et al.</i> 2020a
<b>Oxidative status</b>				
Oxidative damages	C <sub>12</sub> –C <sub>14</sub> PFCAs	+	BLK	Costantini <i>et al.</i> 2019
Antioxidants	C <sub>8</sub> PFSA, C <sub>11</sub> , C <sub>12</sub> , C <sub>14</sub> PFCAs	-	BLK	Costantini <i>et al.</i> 2019
<b>Energy expenditure</b>				
Metabolic rate (♀)	C <sub>13</sub> PFCA	+	BLK	Blévin <i>et al.</i> 2017b
<b>Behavior</b>				
Egg-turning angular change (♂)	C <sub>9</sub> PFCA	+	BLK	Blévin <i>et al.</i> 2020
Egg-turning angular change (♀)	C <sub>8</sub> PFSA, C <sub>9</sub> , C <sub>11</sub> , C <sub>13</sub> PFCAs	+	BLK	Blévin <i>et al.</i> 2020
Egg-turning frequency (♂)	C <sub>8</sub> PFSAs, C <sub>9</sub> PFCA	+	BLK	Blévin <i>et al.</i> 2020
Egg-turning frequency (♀)	C <sub>8</sub> PFSAs, C <sub>9</sub> PFCA	+	BLK	Blévin <i>et al.</i> 2020
<b>Reproductive outputs</b>				
Hatching success	C <sub>12</sub> PFCAs	-	BLK	Tartu <i>et al.</i> 2014
<b>Phenotypic traits</b>				
Body mass	C <sub>12</sub> –C <sub>14</sub> PFCAs	-	TBM	Choy <i>et al.</i> 2022
Body condition (♂)	C <sub>9</sub> , C <sub>10</sub> PFCAs	-	LBBG	Sebastiano <i>et al.</i> 2020b [Appendix B]
Body condition (♂)	C <sub>9</sub> PFCA	+	BLK	Tartu <i>et al.</i> 2014
Body condition (♂)	C <sub>13</sub> –C <sub>14</sub> PFCAs	+	BLK	Ask <i>et al.</i> 2021
Body condition (♀)	C <sub>6</sub> –C <sub>8</sub> PFSAs, C <sub>9</sub> , C <sub>10</sub> PFCAs	-	GBBG	Sebastiano <i>et al.</i> 2020b [Appendix B]
Integument coloration (♂)	C <sub>8</sub> PFSA, C <sub>9</sub> –C <sub>12</sub> PFCAs	-(chroma), +(brightness)	BLK	Costantini <i>et al.</i> 2022
Carotenoids (♂)	C <sub>8</sub> PFSA, C <sub>9</sub> –C <sub>14</sub> PFCAs	+	BLK	Costantini <i>et al.</i> 2022
<b>Survival</b>				
Re-sighting probability (♂)	C <sub>6</sub> PFSA, C <sub>8</sub> PFCA	+	GG	Sebastiano <i>et al.</i> 2020a



### III. Emerging PFAS in seabird eggs, an overview

#### a. Levels and patterns

Novel fluorinated substances, part of the emerging PFAS, are synthesized by PFAS manufacturers to replace legacy compounds in the aim of creating chemicals that degrade faster (for example by adding an oxygen atom in the fluorinated carbon chain), or with lower accumulation potential in Humans and wildlife. Nonetheless, some emerging PFAS were found in high concentrations in biota and some of them even had a toxicity equivalent to that of other most harmful legacy compounds (Munoz *et al.* 2019).

Regarding their occurrence in both species, several compounds were present in a large proportion of the eggs, however with differences between the two species (**Papers A, C and D**). For example, among fluorotelomers, 6:2 to 10:2 fluorotelomer sulfonic acids (FTS) were well represented in yellow-legged gull eggs but only 10:2 FTS was found in kittiwake eggs, when 7:3 FTCA was measured in more than 85 % of the eggs of both species. 7:3 FTCA was measured in relatively similar concentrations in kittiwake and yellow-legged gull eggs on average (**Figure 19**). F-53B and GenX were not found above the limit of detection, but 3333-PFECA (a compounds from GenX family) was found in kittiwake eggs only. Quite unexpectedly, since its bioaccumulation potential was supposedly low (Munoz *et al.* 2019), ADONA was detected in one kittiwake egg. This is the only mention of this ether-PFAS in wildlife to date. The cyclic analog of PFOS perfluoroethylcyclohexane sulfonate (PFEC<sub>6</sub>HS) was detected in yellow-legged gull eggs only but in many of them. Some of these substances including PFEC<sub>6</sub>HS and 7:3 FTCA were only detected in high concentrations in top predators relatively recently and this raised questions about their implications for wildlife (Chen *et al.* 2021; Herzke *et al.* 2022; Mahoney *et al.* 2022; Muir *et al.* 2019; Nakayama *et al.* 2019; Wang *et al.* 2021a). Various hypothesis may be ventured to explain the difference in occurrence between kittiwakes and yellow-legged gulls, including a spatial difference in the distribution of these compounds, different toxicokinetics of PFAS in both species, or difference in dietary habits. Some of these emerging PFAS were detected for the first time in seabirds.

PFAS is a family of thousands of different chemical compounds, but little is known about many of them in terms of chemical structure. Therefore, there is a lack of authentic standards needed to tackle the complete suite of possible PFAS in organisms using today's analytical methodologies. Recently however, new methodological and analytical methods enable the determination of PFAS as groups rather than individual compounds, for example, the total fluorine (TF) and the extractable organofluorine (EOF) provides an estimation of the

total amount of fluorine present in a sample (Cousins *et al.* 2020; De Silva *et al.* 2021). EOF was estimated in eggs of kittiwake from Svalbard and other species (Herzke *et al.* 2022), despite kittiwakes were found to produce some of the most contaminated eggs to legacy PFASs, their EOF concentrations were one of the lowest (Herzke *et al.* 2022). This highlights how important the background contamination to fluorinated compounds in wildlife may be, despite it is not taken into account in ecotoxicological studies.

In this thesis, I contributed to the effort in mapping emerging PFAS occurrence worldwide. More than only their occurrence, information is lacking about their transport abilities and their fate in the environment. Providing measurements of emerging PFAS in egg yolks of kittiwakes is therefore contributing to increase our knowledge about these substances. We also showed in **Paper A** that emerging PFAS are more easily detectable in eggs than in females due to the concentration process in the yolk during maternal transfer. I therefore claim that seabird eggs may be a better sampler of the environmental contamination than adult birds, for species with an appropriate conservation status. Moreover, they are easily collectable and the process is less invasive than blood sampling. On the other hand, a minimal number of information, including the rank within the clutch or the laying date, is required to enable a correct use of eggs as bioindicators of environmental pollution.

### **b. Emerging PFAS, what consequences?**

To the best of my knowledge, to date there is a single study investigating the consequences of an emerging PFAS on an avian species (F-53B; Briels *et al.* 2018). We discovered 7:3 FTCA in a large proportion of the eggs, meaning that it is widely distributed in relatively high concentration in the environment. The only toxicological assessment conducted on aquatic invertebrates and plants for FTCAs revealed that some of them may be up to more than 10 000 times more toxic than PFCAs (Ankley *et al.* 2021; Mitchell *et al.* 2011; Phillips *et al.* 2007; 2010). In **paper C**, we could not evaluate the consequences of 7:3 FTCA on embryos telomere length since it was not deposited in eggs with a high repeatability within clutches. We therefore conducted the first assessment of 7:3 FTCA toxicity in vertebrates. We experimentally tested its consequences on yellow-legged gull embryos metabolism and telomere length, two biomarkers of growth and health known to be affected in adults (**Table 1**). However, we did not find any significant effects of this compound despite a high injected amount in the egg yolk (**Paper D**). The presence of 7:3 FTCA in eggs of both species may nonetheless add a significant risk to the embryos since it also degrades to PFCAs (see **boxed text**).

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Considering the results of this experiment and the concentrations of 7:3 FTCA *in ovo* on the field, we may suggest that 7:3 FTCA should be relatively safe during the development for the investigated populations of yellow-legged gull and kittiwakes at the exposed concentrations. However, there were several potential limitations that could skew the results of this experimental study. First, we exposed yellow-legged gull embryos to a much higher (more than twentyfold) concentration of 7:3 FTCA than what was observed *in natura* in kittiwakes and yellow-legged gulls (**Paper A, C and D**). Although we found no effects of 7:3 FTCA which strongly suggest that lower concentrations should also be safe, we know that organisms answer to contaminants are not necessarily linear and that low concentrations may even have very different effects (Sebastiano *et al.* 2022). Second, the buoyancy method to select freshly laid eggs may lack precision in the first days after laying, therefore, we may have selected embryos of different age, biasing the measurements of metabolism and telomere length at the end of the experiment. This is suggested by the large range of embryos mass on the individuals alive at the end of the experiment (**Paper D**). Third, we collected eggs from nests with different clutch size, yet we know that yolk PFAS and other substances including carotenoids, vitamins or hormones is highly related to the rank of the egg within the clutch, and this may interfere with how 7:3 FTCA may affect telomere length (**Paper A**; Rubolini *et al.* 2011). Fourth, one of the strengths of our study was the common garden study design, which eliminated the influence of parental quality and potential physiological and behavioral disruption by their own contaminant burden during incubation. Nonetheless, artificial incubation is rarely as efficient as natural incubation and embryos had a low survival rate during the experiment (Klimstra *et al.* 2009), therefore we were limited in terms of sample-size. Another option would have been cross-fostering eggs among sampled nests, although we opted for artificial incubation since cross-fostering eggs would have been difficult to implement in the field. Finally, as for **Paper C**, the consequences of a disruption of telomere length may be visible only later after hatching.

Although this is the first study investigating 7:3 FTCA toxicity in birds, it can be considered as a pilot study since numerous limitations prevented us to draw clear conclusions for seabirds. We are still at an early stage of exploring how emerging PFAS may impact wild birds during development, despite the strong implications they may have. During this PhD I focused on investigating on a single compound, since we identified it as the main potential threat to the investigated seabirds among emerging PFAS. Nonetheless there are several other emerging compounds that could be investigated due to their relative high concentrations in kittiwakes and yellow-legged gull eggs. For instance, PFEcHS found in 100 % of the yellow-legged gull eggs sampled or 3333-PFECA in all kittiwake eggs, as well as the FTSs especially

10:2 FTS present in many eggs of both species in large concentrations. Their toxicity is largely overlooked so far and no studies have been conducted on large species including birds or mammals (Field and Seow 2017; Mahoney *et al.* 2022).

### **7:3 fluorotelomer carboxylic acid (7:3 FTCA)**

This compound is produced from the *in vivo* and abiotic degradation of FTOHs in the environment (Butt *et al.* 2014). The atmospheric lifetimes of some of the shorter chain length FTOHs suggests that transport to remote regions is highly likely (Ellis *et al.* 2004), which may explain the slightly higher levels found in kittiwake eggs (**Figure 19**). FTCAs notably ultimately degrade to PFCAs and may thus be contributing to the higher concentrations of PFCAs measured in kittiwake eggs (Dinglasan *et al.* 2004; Wang *et al.* 2014). In addition to my studies, this PFAS was recently detected in various marine mammals and bird species in Japan, China, Norway, Greenland and Canada, revealing its global occurrence (Eriksson *et al.* 2016; Guruge *et al.* 2011; Schultes *et al.* 2020; Spaan *et al.* 2020; Wang *et al.* 2021a). Before 2010 it was detected only once in the Arctic, in ringed seals (*Phoca hispida*; Butt *et al.* 2010). Nonetheless, FTOHs are produced since the 1970s, and it is unclear if the increasing mention of 7:3 FTCA in biota is due to a real increase of this substance in the environment or an increasing effort in screening for emerging PFAS in general.

## **IV. Conclusion and perspectives**

Altogether, these results contribute to the effort to better identify some of the key drivers of the maternal transfer of PFAS in bird eggs as well as the consequences for developing embryos. We demonstrated that eggs could be an even better sampler of the environment than adult birds, since they accumulate high concentrations of all compounds including emerging PFAS, not detected in females. Some emerging compounds were found for the first time in avian eggs and this work contributed to the essential global effort in documenting their occurrence. We found that maternal PFAS may disrupt the transfer of testosterone in the yolk, which may cause important adverse effects for the embryo. However, causality cannot be established with certainty in this correlative study. Despite eggs exhibited high concentrations of PFAS, our studies also suggested that they have a relatively weak direct impact on embryos

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during the development. The work conducted in this thesis provides a first insight on PFAS toxicity during the development in free-ranging seabirds, but additional studies will be needed to assess if kittiwakes are at risk. Moreover, this work identified several parameters that constituted limitations to our studies, highlighting the difficulty to conduct ecotoxicological studies on wild species. Experimental work should thus be conducted to validate these results, especially with *in vitro* experiments which are more ethically advisable and enable larger sample size.

In this thesis, I could only scratch the surface of the main factors driving PFAS concentrations in seabird eggs or how these contaminants may affect embryos development in birds, especially for emerging PFAS. Many questions remain to be explored and require additional studies to be resolved. I propose below some avenues to go further towards these questions.

It is largely unknown where kittiwakes accumulate their contaminants. We believe that PFAS in eggs represent inputs accumulated on the breeding grounds mainly, although they most likely also originate in a significant part from absorption during winter. Examining carbon and nitrogen stable isotopes in egg yolk may help in identifying where most PFAS are recruited. Moreover, we found that kittiwakes have a strong individual fidelity for their migration routes and to the wintering grounds (Léandri-Breton *et al.* 2020 [Appendix C]), and they are also known to have a high individual fidelity to specific tidal glacier fronts in Svalbard during summer (Bertrand *et al.* 2021). During my PhD, we deployed GLS and GPS on females, took blood samples and sampled one of their eggs, in the future we plan to map the spatial distribution of legacy and emerging PFAS and identify PFAS hotspots in the visited areas.

In seabirds, in addition to the examined parameters in this thesis, other physiological endpoints are known or suggested to be affected by PFAS. These include oxidative status or DNA integrity among others (Costantini *et al.* 2019; Haarr *et al.* 2018; Keilen *et al.* 2022). Investigating these parameters in embryos exposed to high concentrations of PFAS is particularly interesting since elevated concentrations of testosterone have been found to be related to increased oxidative damages and to reduced DNA damage repair efficiency (Alonso-Alvarez *et al.* 2007; Treidel *et al.* 2013). Since both may impact telomere length (von Zglinicki 2002), it would also complement our studies from **Papers C and D**. Moreover, in the yellow-legged gull, maternal testosterone in egg increased growth and telomerase activity (an enzyme involved in telomeres repair), and affected postnatal telomere length (Noguera and Velando 2022). Telomerase has also been suggested as a target substance for PFAS, leading to altered

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telomere repair (Clarity *et al.* 2021; Sebastiano *et al.* 2020a), although this hypothesis still needs to be tested. Finally, PFAS are suspected to alter other health parameters which may have strong adverse effects for future chicks at hatching, including immune status and lipid metabolism (Jacobsen *et al.* 2018; Peden-Adams *et al.* 2009). Information is also lacking on the long term consequences of a developmental exposure to toxic compounds. In the case of seabirds, this is challenging to evaluate since they are long-lived and many environmental factors may skew the results of adverse effects experienced in early-life. However, it is totally worth considering in a relatively philopatric species as the black-legged kittiwake.

In addition, PFAS only represent a small percentage of the mixture of contaminants transmitted to the eggs (Bianchini *et al.* 2022). Although my thesis focused on PFAS since they are known to be highly toxic for wildlife, other contaminants may be important to monitor in terms of occurrence in eggs and consequences for the embryos. Some compounds were identified as emerging threats for Arctic wildlife and may be important to investigate, they include flame retardants, polycyclic aromatic hydrocarbons (PAHs), siloxanes, phthalates or UV stabilizers, among others (Sonne *et al.* 2021). This is particularly relevant since several of these compounds were found in bird eggs (Allen *et al.* 2021; Huber *et al.* 2015; Smythe *et al.* 2020; Vinas *et al.* 2020). The studied kittiwake population is exposed to a broad mixture of several contaminants including legacy POPs and mercury (Goutte *et al.* 2015), we may also investigate if these contaminants interact altogether and how this “cocktail effect” could affect the developing embryo.

Finally, one major issue concerning ecotoxicology in the Arctic is to estimate how global climate change may influence contaminants occurrence and species response. Arctic regions are experiencing some of the fastest changes on the planet due to a high rate rising in average temperatures (Comiso and Hall 2014). There, sea ice melting and changes in oceanic waters circulation may affect the deposition of contaminants including PFAS (AMAP, 2021; Borgå *et al.* 2022; Ma *et al.* 2016). For instance in kittiwakes from Svalbard, rising temperature caused higher inputs of water from the Atlantic in Kongsfjord during the mid-2010s, which was responsible for a shift in kittiwake diet (Vihtakari *et al.* 2018), and this was suggested as a major driver of their contamination to mercury on the last 20 years (Tartu *et al.* 2022). Moreover, predictability and recurrence in environmental conditions is required in the evolution of adaptations. In the context of the climate change, abilities of organisms to adapt to environmental alterations are therefore of prime importance, but contaminants may alter these response via impairment of the endocrine system (Esparza *et al.* 2022). Any disruption of adaptive maternal effects by PFASs may consequently cause additional stress to offspring

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(Jenssen 2006), altering survival and ultimately population trends. Predictive studies on how climate change may affect PFAS deposition in eggs and how this may alter the adaptive response of the future offspring are therefore of high interest.

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*Black-legged kittiwakes (Rissa tridactyla) on their nests*



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# APPENDIX A



*Young Arctic foxes (Vulpes lagopus) walking out of the den*

Blood mercury concentrations in four sympatric gull  
species from South Western France: Insights from stable  
isotopes and biologging

**Jouanneau, W.**, Sebastiano, M., Rozen-Rechels, D., Harris, S. M., Blévin, P., Angelier, F.,  
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## Blood mercury concentrations in four sympatric gull species from South Western France: Insights from stable isotopes and biologging<sup>☆</sup>

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### ABSTRACT

Mercury (Hg) is a toxic trace element widely distributed in the environment, which particularly accumulates in top predators, including seabirds. Among seabirds, large gulls (*Larus* sp) are generalist feeders, foraging in both terrestrial and marine habitats, making them relevant bioindicators of local coastal Hg contamination. In the present study, we reported blood Hg concentrations in adults and chicks of four different gull species breeding on the French Atlantic coast: the European herring gull (*Larus argentatus*), the Lesser black-backed gull (*L. fuscus*), the Great black-backed gull (*L. marinus*) and the Yellow-legged gull (*L. michahellis*). We also investigated the potential role of foraging ecology in shaping Hg contamination across species, using the unique combination of three dietary tracers (carbon, nitrogen and sulfur stable isotopes) and biologging (GPS tracking). A high concentration of Hg was associated with high trophic position and a marine diet in gulls, which was corroborated by birds' space use strategy during foraging trips. Adults of all four species reached Hg concentrations above reported toxicity thresholds. Specifically, adults of Great black-backed gulls had a high trophic marine specialized diet and significantly higher Hg concentrations than the three other species. Blood Hg was 4–7 times higher in adults than in chicks, although chicks of all species received mainly marine and high trophic position prey, which is expected to be the cause of blood Hg concentrations of toxic concern. By using both stable isotopes and GPS tracking, the present study provides compelling insights on the main feeding habits driving Hg contamination in a seabird assemblage feeding in complex coastal environments.

### CRedit authorship contribution statement

**William Jouanneau:** Conceptualization, Investigation, Formal analysis, Writing - Original Draft. **Manrico Sebastiano:** Investigation, Writing - Review & Editing. **David Rozen-Rechels:** Conceptualization, Formal analysis, Writing - Review & Editing. **Stephanie M. Harris:** Formal analysis, Writing - Review & Editing. **Pierre Blévin:**

Investigation, Writing - Review & Editing. **Frédéric Angelier:** Investigation, Writing - Review & Editing. **François Brischoux:** Investigation, Writing - Review & Editing. **Julien Gernigon:** Resources, Investigation. **Jean-Christophe Lemesle:** Resources, Investigation. **Frédéric Robin:** Resources, Investigation, Writing - Review & Editing. **Yves Cherel:** Conceptualization, Writing - Review & Editing. **Paco Bustamante:** Resources, Investigation, Writing - Review & Editing. **Olivier Chastel:**

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## 1. Introduction

Mercury (Hg) is a non-essential trace element released by both natural and anthropogenic processes (Pirrone et al., 2010). Organic Hg (mostly methylmercury; MeHg) is the main bioavailable and toxic form for living organisms (Diez, 2008; Thompson and Furness, 1989). MeHg is incorporated in organisms mainly via ingestion of contaminated food (Eagles-Smith et al., 2018). In birds, accumulation of MeHg has been associated with a wide range of behavioral and physiological detrimental consequences, impacting individual fitness, and ultimately, population dynamics (Tan et al., 2009; Whitney and Cristol, 2017). Due to a relatively long biological half-life in tissues (Stickel et al., 1977), Hg tends to bioaccumulate in organisms over time and biomagnify up trophic food webs, leading long-lived top predators to exhibit elevated Hg concentrations (Atwell et al., 1998; Cherel et al., 2018; Evers et al., 2005). In coastal environments, seabirds are therefore considered as excellent sentinels of the local contamination (Burger and Gochfeld, 2004; Furness and Camphuysen, 1997; Monteiro and Furness, 1995).

Seabirds show a large interspecific variation in foraging strategies (Ceia and Ramos, 2015), leading to varied degrees of Hg exposure among species (Carravieri et al., 2014b; Monteiro et al., 1998; Stewart et al., 1997). Large gulls of the *Larus* genus are omnivorous and opportunistic feeders. *Larus* spp. are known to exploit both marine and terrestrial habitats, and can scavenge from multiple anthropogenic sources including waste dumps, landfill sites and fishing discards, as well as predated upon eggs or chicks (Buckley, 1990; Mudge and Fems, 1982; Ramos et al., 2009). Assessing Hg concentrations in sympatric *Larus* species with differences in foraging ecology thus enables to have an overview of the local environmental contamination, particularly in complex coastal habitats (Binkowski et al., 2020). Additionally, in seabirds, individual variation in foraging behavior appears to be highly prevalent, with individuals differing in their resource use, habitat selection, and their fidelity to foraging sites (Ceia and Ramos, 2015; Phillips et al., 2017). Such variation in foraging behavior may be consistent over time, and linked to individuals' characteristics, such as their age or sex (Bolnick et al., 2003). As Hg exposure largely varies with feeding strategies and habitat use, this variability shapes intraspecific differences in Hg contamination (Bustamante et al., 2016; Ceia and Ramos, 2015; Stewart et al., 1997), with consequences for individual fitness. Characterizing foraging ecology at the individual level is therefore essential to understand patterns of Hg contamination within and among species.

Tracking devices, including Global Positioning System (GPS) loggers, enable fine scale monitoring of an animal's movements, which facilitates a direct characterization of its habitat use (Burger and Shaffer, 2008). Furthermore, recently developed analytical tools - including hidden Markov models (HMMs) - facilitate behavioral classification of GPS tracking data (Langrock et al., 2012), enabling the identification of the precise locations and habitats in which animals are foraging. The analysis of stable isotopes - used as a proxy for the trophic niche (Newsome et al., 2007) - can also represent an effective tool to define the foraging habitats (carbon:  $\delta^{13}\text{C}$  and sulfur:  $\delta^{34}\text{S}$ ) and trophic positions (nitrogen:  $\delta^{15}\text{N}$ ) of seabirds (Hobson et al., 1994; Kelly, 2000; Lott et al., 2003). A marine diet is characterized by higher  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values, as opposed to lower levels indicating terrestrial food intakes (Chisholm et al., 1982; Hobson, 1987; MacAvoy et al., 2000; Schoeninger and DeNiro, 1984). More specifically,  $\delta^{34}\text{S}$  is preferentially used to differentiate marine vs terrestrial foraging habitats, since it does not present a stepwise enrichment within the food chain, and therefore shows a greater discriminating power than  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Hobson, 1999; McCutchan Jr et al., 2003). This characteristic makes  $\delta^{34}\text{S}$  particularly interesting to depict seabirds' use of coastal habitats. Therefore, the use of GPS trackers combined with isotopic

measurements enables a holistic overview of birds' feeding strategies with respect to multiple available foraging habitats, as well as a high discriminant power (Bracey et al., 2021; Caron-Beaudoin et al., 2013; Ceia et al., 2018; Mendes et al., 2018).

In France, Hg contamination of seabirds has been largely documented in overseas departments and territories (e.g., French Guiana, Sebastiano et al., 2016; Scattered Islands, Kojadinovic et al., 2007a; Réunion Island, Kojadinovic et al., 2007b; 2007c) and French Southern and Antarctic Territories (e.g., Blévin et al., 2013; Carravieri et al., 2014a, 2014b; 2016; Goutte et al., 2014a, 2014b; Tartu et al., 2014, 2015a). Although the coasts of metropolitan France host large numbers of breeding seabirds (e.g., 174 000 pairs, Cadiou, 2011), data on Hg concentrations in seabird tissues are scarce and limited to chicks of four gull species (*Larus argentatus*, *Larus fuscus*, *Larus marinus* and *Larus michahellis*) from the Southern Bay of Biscay and the English Channel (Binkowski et al., 2020; Zorrozua et al., 2020). In these studies, high blood Hg concentrations were associated with a diet of marine origin and of high trophic position. Yet, our knowledge on Hg concentrations in other potentially contaminated areas remains very limited.

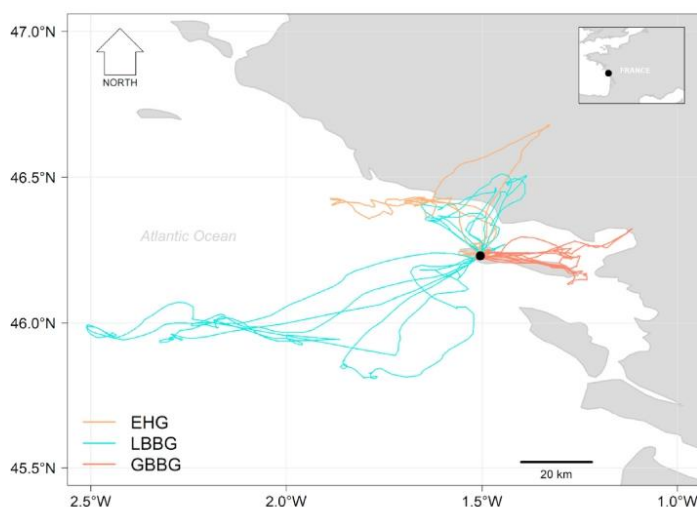
In the present study, we investigated Hg concentrations in adults and chicks of four gull species from a South Western France colony: Herring gulls *Larus argentatus argentatus*, Lesser black-backed gulls *L. fuscus graellsii*, Great black-backed gulls *L. marinus* and Yellow-legged gulls *L. michahellis*. We were particularly interested to i) describe Hg concentrations of the species during the breeding period; ii) identify the factors driving inter- and intraspecific Hg variations among adults and chicks - in relation to their different feeding habitats inferred from blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  stable isotopes, and in relation to their individuals characteristics as age and sex; and iii) explore whether fine-scale habitat partitioning using GPS tracking further elucidates the potential variation in adult Hg contamination. Due to the known differences in foraging ecology among the four species, as well as the individual differences within species, we expected a high inter- and intraspecific variation in Hg concentrations. We further predicted that i) individuals feeding at higher trophic positions (indicated by higher  $\delta^{15}\text{N}$  values) or foraging in marine habitats (indicated by both high  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values, as well as GPS tracking) would exhibit higher Hg concentrations; and that ii) these patterns would persist both between and within species.

## 2. Materials and methods

### 2.1. Field methodology

In the breeding seasons of 2016–2019, we sampled gulls breeding in "Lilleau des Niges" National Nature reserve, Ile de Ré, South Western France (46°13'53" N, 1°30'22" W; Fig. 1): European herring gulls (EHG, approx. 400 pairs), Lesser black-backed gulls (LBBG, approx. 400 pairs), Great black-backed gulls (GBBG, approx. 200 pairs), and Yellow-legged gull (YLG, approx. 100 pairs). Adult birds were sampled in May ( $n = 140$ ), and chicks in July ( $n = 107$ ). All individuals were captured and sampled only once over the four years. Annual sample sizes for adults and chicks of each species can be found in the Supporting Information (SI) Table S1. Adults incubating a full-size clutch (i.e., 2 to 3 eggs) were caught on their nests, using a trap placed over the eggs. Chicks of approximately 1 month of age were caught by hand, within the colony. From an early age, gull chicks are developed enough to explore their environment and do not stay in their nest, therefore we were unable to assign relatedness among chicks and adults. Skull and tarsus were measured using a caliper ( $\pm 0.1$  mm). Wing length was also measured using a ruler ( $\pm 1$  mm), and birds were weighted using a Pesola spring balance ( $\pm 5$  g). Blood samples (2 mL, <1% of the body mass of LBBG, the smallest species in this study) were taken from the brachial vein of adults and chicks using heparinized syringes. Blood was transferred into a 2 mL Eppendorf® tube and immediately stored in a cool box. At the end of each sampling day, whole blood was centrifuged and red blood cells (hereafter "blood") were stored at  $-20$  °C until laboratory analyses.

# Appendix A



**Fig. 1.** Map of the Ile de Ré and surroundings, France, visited by the adults of four species of gulls from the “Lilleau des Niges” colony during their breeding period; European herring gulls (EHG), Lesser black-backed gulls (LBBG), Great black-backed gulls (GBBG) and Yellow-legged gulls (YLG). The studied colony is represented by the black point. YLG were not tracked and so are not presented, but were included in analyses of Hg and stable isotopes. Tracks presented here are a random selection of 5 trips per species, see SI Fig. S1 for maps of all tracks per species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Blood samples were then used to measure Hg and stable isotopes and for molecular sexing.

## 2.2. Mercury analysis

The analysis of total Hg in lyophilized blood was carried out using an Altec AMA 254 spectrophotometer following Bustamante et al. (2006). Each sample (mass: ~0.5 mg dry weight, dw) was analysed twice and Hg concentrations were then averaged. For each individual, the relative standard deviation was <10%. Accuracy was checked at the beginning and the end of each measurement session using a certified reference material (2016–2018: DOLT-5 dogfish liver - Hg certified concentration (mean ± SD):  $0.44 \pm 0.18 \mu\text{g g}^{-1}$  dw; 2019: TORT-3 lobster hepatopancreas - Hg certified concentration:  $0.29 \pm 0.02 \mu\text{g g}^{-1}$  dw; obtained from NRC, Canada). Recoveries were respectively  $0.422 \pm 0.005$  and  $0.318 \pm 0.014 \mu\text{g g}^{-1}$  dw. Blanks were also run before and after each set of samples. The limit of detection of the AMA was 0.1 ng. Hg concentrations are expressed as  $\mu\text{g g}^{-1}$  dw. In several bird species, MeHg represents most of the total Hg in blood (Renado et al., 2018; Rimmer et al., 2005). Chicks accumulate Hg from hatching, being fed by their parents from local food items. For this reason, they are relevant bioindicators of the local environment contamination (Binkowski et al., 2020; Blévin et al., 2013). By contrast to chicks, using adult gulls as bioindicators of their local environment during breeding is still debated as the half-life of Hg in avian tissues is 1–3 months, and contaminants acquired during migration might still be present in blood (Bearhop et al., 2000; Monteiro and Furness, 2001; Stickel et al., 1977). However, all species used in the present study were on the breeding grounds more than 3 months before sampling, therefore blood Hg should mainly represent local contamination.

## 2.3. Stable isotope analysis

Sub-samples of blood were weighed (mean ± SD:  $0.29 \pm 0.06$  mg dw for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ;  $0.71 \pm 0.05$  mg dw for  $\delta^{34}\text{S}$ ) with a microbalance and then packed into tin containers for combustion. Relative abundances of carbon, nitrogen and sulfur isotopes were measured using a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled with an elemental analyzer (carbon and nitrogen: Thermo Scientific Flash EA 1112; sulfur: Thermo Scientific Flash IRMS EA IsoLink). The

delta ( $\delta$ ) notation relative to Vienna PeeDee Belemnite, atmospheric  $\text{N}_2$  and Vienna Cañon Diablo troilite for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  respectively, was used to present isotopic results. Accuracy was checked by replicate measurements of the internal laboratory standard, and analytical precision was <0.10‰ for  $\delta^{13}\text{C}$ , <0.15‰ for  $\delta^{15}\text{N}$  and <0.20‰ for  $\delta^{34}\text{S}$  values. The stable isotopes values were computed as  $\delta X = \left( \frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right) \times 1000$ , where X stands for  $^{13}\text{C}$ ,  $^{15}\text{N}$  or  $^{34}\text{S}$  and R being the ratio  $^{15}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$  or  $^{34}\text{S}/^{32}\text{S}$ . Turnover time for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in blood is 11–30 days (Barquete et al., 2013; Hobson and Clark, 1992, 1993), therefore stable isotopes values are representative of the breeding period in adults.

## 2.4. GPS tracking analysis

A subset ( $n = 43$ ) of the captured adults (18 EHG, 18 LBBG and 7 GBBG, see SI Table S1) were equipped with GPS-UHF loggers (HARRIER, Ecotone®) in 2017 and 2018. Among the 43 GPS equipped birds, 17 EHG, 18 LBBG and 6 GBBG were blood sampled and included in Hg and stable isotopes analyses. The total mass of the logger and the harness (used for attachment) weighted on average 13 g (i.e., <2% of the body mass of LBBG, the smallest species in this study).

GPS loggers were set to record locations every 5 min. For each tracked individual, locations were filtered to the period between the day after blood sampling to the last day before either nest failure or egg hatching had been recorded (Fig. 1 & SI Fig. S1 & Table S2). Foraging sites were identified from GPS tracks by assigning a behavioral state to each location using Hidden Markov Models (hereafter “HMMs”, Franke et al., 2004) fitted using the moveHMM R package (Michélot et al., 2016). Additional information on the spatial data treatment and the behavioral classification can be found in the SI. The habitat for each of these locations was assigned as either “pelagic”; “coastal” (i.e., lagoons, estuaries, coastal salt marshes, intertidal flats), or “terrestrial” (all others) based on the CORINE Land Cover dataset (CORINE Land Cover; Feranec et al., 2016). Ratios of pelagic vs coastal and marine (i.e., pelagic and coastal) vs terrestrial habitats used by each individual were then calculated.

For an overview of the feeding behaviors leading to the observed Hg concentrations in the tracked species, we estimated birds’ foraging site

fidelity. As well as giving important insight into the degree of specialization in birds' uses of foraging habitat, this measure of site fidelity served to test whether individuals' foraging behavior in the period after blood sampling was likely to be representative of their behavior prior to blood sampling (see SI). Individual foraging site fidelity was assessed following the randomization procedure of Harris et al. (2020). For each foraging site, the distance to a randomly selected site from the same individual during another trip (within-individual comparison), as well as to a randomly selected site from each other individual of the same species (between-individual comparisons) was measured. The site fidelity index corresponds to the proportion of between-individual sites that were farther from the focal site than the within-individual site. This index is bounded between 0 and 1, with high values representing high site fidelity (an individual forages more closely to its own foraging sites than to the sites of other individuals), and low values representing low site fidelity (an individual forages more closely to the foraging sites of other individuals than to its own foraging sites). Additional information can be found in the SI.

### 2.5. Molecular sexing

All adults and chicks ( $n = 247$ ) were sexed using blood samples by polymerase chain reaction (PCR) amplification of parts of two highly conserved genes (CHD) of sex chromosomes, following Fridolfsson and Ellegren (1999).

### 2.6. Statistical analyses

Statistical analyses were performed using R 4.0.0 (R Core Team, 2020). One YLG chick was removed from the dataset due to outliers in  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values. In all species, adults had much higher Hg concentrations than chicks, and therefore chicks and adults were treated separately.

Firstly, relationships between Hg concentrations and diet (inferred by stable isotope values) were investigated. As  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  were positively correlated in adults (Pearson correlation  $>0.70$ ,  $p < 0.001$  for all relationships) and partially correlated in chicks (correlation: C-N = 0.72,  $p < 0.001$ ; S-C = -0.15; S-N = -0.18, both  $p > 0.05$ ), principal components analyses ("FactoMineR" R package, version 2.3; Lé et al., 2008) were performed on the three isotopes to reduce the number of explanatory variables. Principal components (PCs) which explained more than 80% of the total variance were retained. In adults, principal components analysis reduced  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  to one PC (PC<sub>1</sub>) explaining 83.6% of the variance and equally influenced by  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  (SI Fig. S2 & Table S3). High PC<sub>1</sub> scores reflected high  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ . In chicks, principal components analyses reduced  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  to two PCs (PC<sub>1</sub> and PC<sub>2</sub>) explaining 92.0% of the variance (SI Fig. S2 & Table S3). High PC<sub>1</sub> scores indicated high  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, and high PC<sub>2</sub> scores indicated high  $\delta^{34}\text{S}$  values. The isotopic niches, which are essential for an overview of each species' local feeding habits, were investigated, and the foraging ecology of each species were illustrated and described in the SI. Generalized least square (GLS) models were then used to investigate the effects of the selected PCs on Hg ("nlme" R package, version 3.1-151; Pinheiro et al., 2017). For that, using Hg as the response variable, we included as explanatory covariates the selected PCs and the species. Year was not included as a predictor in the models to improve statistical power, and also because four years of sampling was insufficient to evaluate temporal trends in Hg concentration. We used GLS models instead of linear models (LM) due to heteroscedasticity in Hg concentrations among species (see SI for further information). For both adults and chicks, after running GLS models, Tukey post-hoc pairwise comparison tests were performed to quantify the differences in Hg concentrations among species ("emmeans" R package, version 1.7.1-1; Lenth et al., 2021). Although performed on only three variables, PCA enabled the inclusion of ecological information provided by the three stable isotopes rather than exploring the

effect of each stable isotopes in independent models (similar conclusions were obtained using both methods, see SI).

Secondly, as Hg levels were found to be highly variable among species, we ran one LM per species to investigate differences between the sexes. With Hg as the response variable, we included the sex as an explanatory variable. This model was not fitted for adults of YLG as only a single female was sampled. In previous studies, chick age has been found to be a key predictor of Hg contamination, as blood Hg strongly fluctuates during chick growth, reaching highest concentrations when feathers stop growing (Spalding et al., 2000). Morphology is commonly used as a proxy of chick age, and we therefore also included the skull length (head and bill) in the LMs for chicks as an additional explanatory variable. Where Hg differed significantly between sexes, an additional LM was built to investigate whether this was linked to sex differences in diet, with the selected PCs fitted as the response and sex as an explanatory variable.

Finally, in GPS-tracked adults, the impact of the use of marine or terrestrial habitats on Hg concentrations was investigated using a GLS model: Hg was fitted as the response variable, and the proportional use of marine vs terrestrial habitat and species (to control for the variation among species) were included as explanatory covariates. A similar model was built to investigate the impact of the use of coastal or pelagic habitats on Hg concentrations, including coastal vs pelagic habitats and species (to control for the variation among species) as explanatory covariates. Before modelling, two individuals that were tracked but not blood sampled were removed from the dataset.

For all models, a set of candidate models including all possible combinations of the predictors, ranging from the full model (that includes each of the stated variables) to the null model was built. In each case, the best candidate model – i.e., the one with the lowest second-order Akaike's Information Criterion value for small sample size (AICc; "AICcmodavg" R package, version 2.2-2; Mazerolle, 2017), or the most parsimonious model among those with a  $\Delta\text{AICc} \leq 2$  – was retained for inference. Homogeneity and normality of the residuals were visually checked using plots of residuals vs fitted values and histograms of the residuals (Zuur et al., 2007). In post-hoc tests, an  $\alpha < 0.05$  threshold was used to assess the significance of the tests.

## 3. Results

### 3.1. Mercury in relation to feeding habits as inferred by stable isotopes

In chicks, Hg concentrations ranged from  $0.27 \mu\text{g g}^{-1}$  dw in EHG to  $3.86 \mu\text{g g}^{-1}$  dw in GBBG (Table 1). Model selection indicated that PC<sub>1</sub> and species were significant predictors of Hg concentrations, but not PC<sub>2</sub> (SI Table S4). Post-hoc comparison test showed that GBBG had the highest Hg concentrations, followed by YLG that was not significantly different from EHG and LBBG, but LBBG had lower Hg concentrations than EHG (Tables 1 and 2, Fig. 2). Chicks also exhibited a positive relationship between Hg concentration and PC<sub>1</sub> (estimate  $\pm$  SE =  $0.09 \pm 0.03$ ,  $t = 3.49$ ,  $p < 0.001$ ; Table 2 & Fig. 3). Sex was not retained as a significant predictor of Hg concentration for any species, but skull size (as a proxy for chick age) was included in the best model for GBBG only (SI Table S5), with Hg significantly increasing with skull size in this species ( $F_{1, 26} = 6.51$ ,  $p = 0.017$ ; estimate  $\pm$  SE =  $0.04 \pm 0.02$ ).

In adults, individual blood Hg concentrations ranged from  $0.89 \mu\text{g g}^{-1}$  dw in EHG to  $21.5 \mu\text{g g}^{-1}$  dw in GBBG (Table 1). Model selection showed that PC<sub>1</sub> and species were significant predictors of Hg concentrations in adult gulls (SI Table S4). Post-hoc comparison test indicated that GBBG had the highest Hg concentrations, while lower concentrations were found in YLG, LBBG and EHG (not significantly different among them; Tables 1 and 2, Fig. 2). Adults also showed a significant and positive association between Hg concentration and PC<sub>1</sub> (estimate  $\pm$  SE =  $0.55 \pm 0.06$ ,  $t = 9.25$ ,  $p < 0.001$ ; Table 2 & Fig. 3). Sex was retained for GBBG only (SI Table S5), with males having higher Hg concentrations than females in adults of this species ( $F_{1, 35} = 19.2$ ,  $p < 0.001$ ;

# Appendix A

**Table 1**  
Hg concentrations ( $\mu\text{g g}^{-1}$  dw) and carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ) and sulfur ( $\delta^{34}\text{S}$ ) stable isotope values (‰) measured in blood of adults and chicks of four gull species from the Ile de Ré, France: sample size (n), mean  $\pm$  standard deviation (SD), median and range (min-max). For adults, Hg values are presented separately for females (♀) and males (♂).

	n	Hg		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$	
		Mean $\pm$ SD	Min/max	Mean $\pm$ SD	Min/max	Mean $\pm$ SD	Min/max	Mean $\pm$ SD	Min/max
<b>Adults</b>									
EHG ( <i>Larus argentatus</i> )	44	2.29 $\pm$ 1.06	0.89/5.01	-19.4 $\pm$ 1.93	-22.9/-15.5	12.7 $\pm$ 1.82	9.76/16.0	13.1 $\pm$ 2.23	8.72/17.0
♀	23	2.05 $\pm$ 0.96	0.90/4.31	-	-	-	-	-	-
♂	21	2.55 $\pm$ 1.12	0.89/5.01	-	-	-	-	-	-
LBBG ( <i>Larus fuscus</i> )	54	3.08 $\pm$ 1.31	0.95/6.42	-19.1 $\pm$ 1.34	-22.6/-17.6	13.8 $\pm$ 1.30	10.9/15.8	15.9 $\pm$ 2.37	9.90/18.6
♀	27	2.82 $\pm$ 1.03	0.98/4.78	-	-	-	-	-	-
♂	27	3.35 $\pm$ 1.52	0.95/6.42	-	-	-	-	-	-
GBBG ( <i>Larus marinus</i> )	37	12.5 $\pm$ 3.93	6.97/21.5	-16.8 $\pm$ 0.83	-19.7/-14.7	16.4 $\pm$ 0.41	15.8/17.8	16.9 $\pm$ 1.07	14.6/18.7
♀	19	10.3 $\pm$ 2.20	6.97/13.8	-	-	-	-	-	-
♂	18	14.9 $\pm$ 4.00	7.26/21.5	-	-	-	-	-	-
YLG ( <i>Larus michahellis</i> )	5	6.00 $\pm$ 2.72	2.25/9.63	-19.4 $\pm$ 3.51	-25.2/-16.7	15.1 $\pm$ 1.32	12.76/15.91	12.6 $\pm$ 4.66	5.87/16.7
♀	1	2.25 $\pm$ NA	2.25/2.25	-	-	-	-	-	-
♂	4	6.94 $\pm$ 2.00	5.26/9.63	-	-	-	-	-	-
<b>Chicks</b>									
EHG ( <i>Larus argentatus</i> )	30	0.68 $\pm$ 0.25	0.27/1.19	-18.3 $\pm$ 0.66	-19.9/-17.1	13.8 $\pm$ 0.75	12.1/15.0	16.8 $\pm$ 1.21	14.0/18.7
LBBG ( <i>Larus fuscus</i> )	37	0.58 $\pm$ 0.15	0.35/0.94	-18.3 $\pm$ 0.27	-18.8/-17.7	14.5 $\pm$ 0.41	13.7/15.2	18.4 $\pm$ 0.93	16.7/19.8
GBBG ( <i>Larus marinus</i> )	28	1.91 $\pm$ 0.76	0.81/3.86	-17.1 $\pm$ 0.77	-19.33/-16.7	15.6 $\pm$ 0.48	14.0/16.6	16.5 $\pm$ 1.32	12.8/18.6
YLG ( <i>Larus michahellis</i> )	11	0.90 $\pm$ 0.34	0.48/1.75	-18.0 $\pm$ 0.61	-19.37/-17.42	14.9 $\pm$ 1.07	12.4/16.1	16.5 $\pm$ 1.46	14.07/18.4

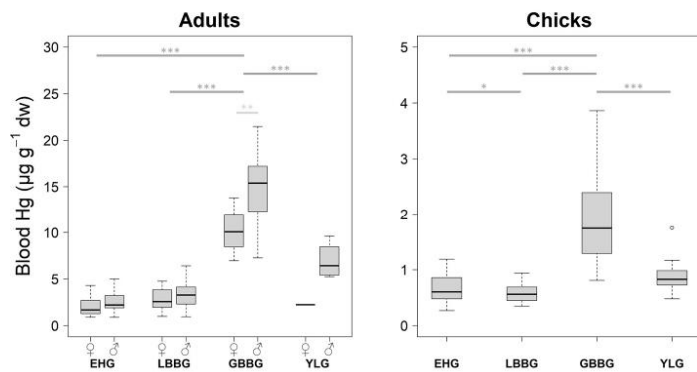
**Table 2**  
Outputs of linear models examining the variables affecting Hg concentration in four gull species from the Ile de Ré, France: European herring gulls (EHG), Lesser black-backed gulls (LBBG), Great black-backed gulls (GBBG) and Yellow-legged gulls (YLG). Significant *p*-values are in bold.

Parameter	Estimate	SE	t-value	p-value
<b>Adults</b>				
PC <sub>1</sub>	0.55	0.06	9.25	<0.001
EHG - LBBG	0.10	0.19	0.56	0.943
EHG - GBBG	8.56	0.67	12.70	<0.001
EHG - YLG	2.65	0.79	3.38	0.089
LBBG - GBBG	8.45	0.66	12.79	<0.001
LBBG - YLG	2.55	0.79	3.22	0.099
GBBG - YLG	-5.90	1.02	-5.78	<0.001
<b>Chicks</b>				
PC <sub>1</sub>	0.09	0.03	3.49	<0.001
EHG - LBBG	-0.13	0.05	-2.72	<b>0.043</b>
EHG - GBBG	1.01	0.16	6.33	<0.001
EHG - YLG	0.11	0.12	0.93	0.792
LBBG - GBBG	1.14	0.15	7.45	<0.001
LBBG - YLG	0.24	0.11	2.16	0.189
GBBG - YLG	-0.90	0.18	-5.05	<0.001

estimate  $\pm$  SE = 4.62  $\pm$  1.05). However, sex was not retained as a significant predictor of stable isotopes in GBBG (PC<sub>1</sub>; SI Table S6).

### 3.2. Mercury in relation to habitats use as inferred by GPS tracking

From GPS tracked adults, a total of 1069 foraging trips from 43 individuals were recorded (612 in EHG, 304 in LBBG and 154 in GBBG). The three tracked species showed different patterns of habitat use: GBBG forage predominantly at sea and showed low inter-individual variation (average use of marine habitats  $\pm$  SD: 0.93  $\pm$  0.05%), while EHG (0.63  $\pm$  0.23%) and LBBG (0.78  $\pm$  0.17%) individuals were more spread on a gradient from strictly terrestrial to strictly marine (Fig. 1 & SI Fig. S3). The use of marine habitats in EHG and GBBG was mainly coastal (proportion of coastal habitats use  $\pm$  SD: 0.58  $\pm$  0.25 and 0.90  $\pm$  0.07 respectively), while LBBG used pelagic and coastal habitats (proportion of coastal habitats use  $\pm$  SD: 0.23  $\pm$  0.16; Fig. 1 & SI Fig. S3). Model selection indicated that the proportional use of marine vs terrestrial habitats and the species were significant predictors of Hg variations (SI Table S7), Hg concentrations increased with the use of marine habitats in all three species (estimate  $\pm$  SE = 2.59  $\pm$  0.99,  $t = 2.62$ ,  $p = 0.01$ ; Fig. 4). Conversely, the coastal vs pelagic gradient was not a significant predictor of Hg variations in the three gull species. (Table S7). GBBG,



**Fig. 2.** Blood concentrations of Hg in adults and chicks of four gull species from the Ile de Ré, France; European herring gulls (EHG), Lesser black-backed gulls (LBBG), Great black-backed gulls (GBBG) and Yellow-legged gulls (YLG). For adults (left), Hg values are shown separately for females (♀) and males (♂). Interspecific (dark-grey) and between sex (light grey) statistically significant differences are indicated by the asterisk: \*, \*\*, \*\*\*; indicating a *p*-value < 0.05, <0.01, and <0.001, respectively. Values are median, 25th and 75th percentiles and range.

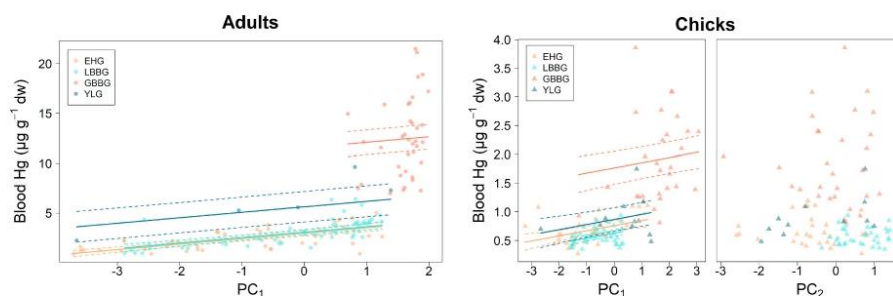


Fig. 3. Relationship between blood Hg concentrations and PC<sub>1</sub> ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values in adults and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  in chicks) or PC<sub>2</sub> ( $\delta^{34}\text{S}$  values in chicks) of four gull species from Ile de Ré, France: European herring gulls (EHG), Lesser black-backed gulls (LBBG), Great black-backed gulls (GBBG) and Yellow-legged gulls (YLG). For each species, the solid line refers to the fitted models obtained and dotted lines represent 95% confident intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

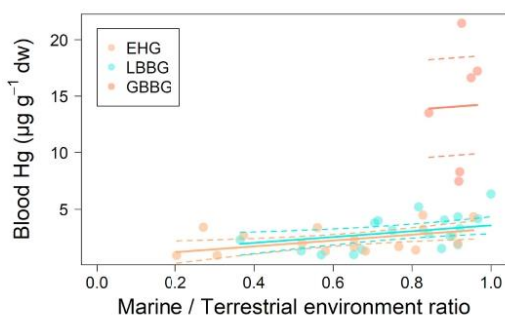


Fig. 4. Relationship between blood Hg concentrations and the proportion of foraging trips in marine (1: exclusively marine) vs terrestrial (0: exclusively terrestrial) habitats obtain by GPS tracking in adults of three gull species from Ile de Ré, France: European herring gulls (EHG), Lesser black-backed gulls (LBBG) and Great black-backed gulls (GBBG). For each species, the solid line refers to the fitted models obtained and dotted lines represent 95% confident intervals.

EHG and LBBG exhibited a relatively high fidelity to foraging sites (fidelity index  $\pm$  SD =  $0.71 \pm 0.18$ ;  $0.59 \pm 0.13$  and  $0.72 \pm 0.13$  respectively).

#### 4. Discussion

Blood Hg concentrations of four sympatric gull species were investigated in relation to their foraging ecology inferred from stable isotopes  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  blood values in adults and chicks, and from GPS tracking of adult birds. High Hg concentrations were related to stable isotopes values representative of a high trophic position food, mainly of marine origin. Foraging movements tracked during breeding corroborated the relationship between Hg and the birds' foraging strategies with relation to terrestrial vs marine habitat use. All species showed foraging site fidelity, indicating a degree of specialization in their use of foraging sites, and thus high potential for consistent individual differences in contaminant level to emerge. This led some adult individuals to exceed reported toxicity thresholds in all four species, particularly in GBBG, showing extremely high levels of Hg. These findings are also concerning for chicks, which predominantly receive marine food from their parents, and are therefore likely to accumulate high concentrations of Hg. Together, these results support the use of sympatric species of gulls as bioindicators of contamination in complex coastal habitats.

#### 4.1. Mercury concentrations

LBBG and GBBG chicks had blood Hg concentrations similar to those from the French coast of the English Channel, although EHG chicks had higher Hg concentrations, even compared to the colonies from the Seine estuary, one of the most polluted rivers in Europe (Binkowski et al., 2020; Cossa et al., 2002). Toxicological benchmarks are usually assessed for adult birds, meaning that chicks Hg concentrations can only be compared to published thresholds from adults with caution. However, much lower blood Hg concentrations have been associated with an impaired physiological condition in LBBG chicks (lower metabolism markers, increased oxidative stress and increased anaerobic metabolism; Santos et al., 2020). In the present study, all four species showed similar Hg levels between sexes, likely because sex difference in contaminant exposure is usually visible later in life. However, in GBBG we detected increased levels of Hg with skull size (a proxy of chick age), but this result was not found in other species. Blood Hg is known to increase with the chicks age when most of the feathers have grown (Ackerman et al., 2011). This relationship may be easier to detect in GBBG as they grow larger, thus their among individual variation in size is higher than the other investigated species.

Hg concentrations in adults ranged from similar (EHG and LBBG) to much higher (YLG and GBBG) than in gulls from the Baltic Sea including EHG and GBBG, or Brown skuas (*Stercorarius antarcticus lonnbergi*) from three colonies of the Southern Ocean (Mills et al., 2022; Szumilo-Pilarska et al., 2017). Notably, we found GBBG to have average blood Hg concentrations similar to those of the Wandering albatross (*Diomedea exulans*; Anderson et al., 2009), the highest trophic position seabird in the Southern Ocean (Cherel et al., 2017) and one of the species with the highest known Hg levels worldwide (Blévin et al., 2013; Carravieri et al., 2014a, 2014b; Cherel et al., 2018). Such Hg concentrations are known to significantly impact reproduction, including breeding success in various bird species (Goutte et al., 2015; Tartu et al., 2013, 2014; 2015b; Ackerman et al., 2016). As no physiological endpoints were measured in the present study, it is not possible to directly draw conclusions on the impact of Hg concentrations of this population. Other factors may also contribute to a reduced toxicity of Hg. For instance, selenium – which was not measured in the present study – is an essential trace element which may protect birds against Hg toxicity (Dietz et al., 2000; Manceau et al., 2021). However, Hg is known to affect almost all aspects of avian physiology and life history traits (Sebastiano et al., 2022; Tartu et al., 2015a; Whitney and Cristol, 2017). We therefore emphasize further work to investigate whether the reported high blood Hg concentrations are detrimental to the different species of birds in the region. Similarly to what we observed in GBBG, higher concentrations of Hg in males than in females were previously found in other bird species (Ackerman et al.,

2007; Burgess et al., 2005; La Sala et al., 2011). Interestingly, incubating GBBGs males and females were not found to differ in terms of stable isotopes concentration, suggesting diet is not the main driver of this difference. A possible explanation may be represented by the fact that female gulls deposit Hg into their eggs therefore lowering their circulating contaminants (Lewis et al., 1993).

#### 4.2. Mercury concentrations in relation to foraging ecology

The positive relationship between blood Hg concentrations and  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values observed in gull chicks is in agreement with previous studies on the same four species in the English Channel (Binkowski et al., 2020) and the southern Bay of Biscay (Zorrozuza et al., 2020). However, blood  $\delta^{34}\text{S}$  was not found to be an important driver of Hg contamination at Ile de Ré, contrary to chicks in the English Channel (Binkowski et al., 2020) and Spain (Ramos et al., 2013). In the present study, chick isotopic values were in the highest range values of those of their parents for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ , meaning that they predominantly received food at a higher trophic position and of marine origin. A diet shift after hatching – reflecting different food requirement before and after hatching – has previously been described in EHG and other species of gulls (Annett and Pierotti, 1989; Bukacinska et al., 1996; Pierotti and Annett, 1987). This specialized chick diet of low marine prey diversity, provided by the parents, may explain why the terrestrial vs marine gradient is not a good predictor of Hg variations in chicks.

Isotopic data showed that, similarly to chicks, blood Hg contamination was higher in adult gulls feeding on marine than terrestrial items. This is consistent with previous studies investigating the relationship between Hg and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  in coastal seabirds and their prey (Gongora et al., 2018; Peterson et al., 2017). These patterns likely reflect the biomagnifying characteristics of Hg along food webs, with longer and more complex food webs in the marine environment (Beldowska and Falkowska, 2016; Morel et al., 1998), as well as that MeHg is mainly produced in the ocean (Chen et al., 2008). Conversely, some previous studies did not report such relationship in other aquatic birds (Einoder et al., 2018; Soldatini et al., 2020), however, the association between Hg and  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  is complex and largely depends on the species or their environments. Different environments likely differ in their  $\delta^{15}\text{N}$  baseline (Elliott et al., 2021), thus precluding comparing directly consumers'  $\delta^{15}\text{N}$  values as reflecting their trophic position. This is likely the case of gulls from Ile de Ré, meaning that any blood  $\delta^{15}\text{N}$  difference at the species or individual level must be interpreted with caution, especially if their  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values indicate foraging in different habitats.

In the present study, EHG, LBBG and YLG showed important inter-individual variations in blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values, that characterize populations of generalist species made of specialist individuals (Jaeger et al., 2009). In contrast, GBBG exhibited narrower ranges for all isotopic values, representative of a specialist species. GBBG's isotopic values were in the highest range of those of the other gull species, that characterize a diet based on marine items ( $\delta^{34}\text{S}$ : +15 to +19‰), whereas EHG, LBBG and YLG individuals showed a diet of mixed terrestrial, intertidal and marine sources (Nehlich, 2015; Rees et al., 1978). The very high Hg concentrations found in GBBG are therefore most likely related to its marine diet. These isotopic results are supported by the analyses of foraging trips in EHG, LBBG and GBBG, as the use of a marine habitats was related to higher blood Hg concentrations. In other species of coastal gulls equipped with tracking devices, including the American Herring gull (*Larus smithsonianus*) and the Western gull (*Larus occidentalis*), a similar pattern was recently observed, with individuals mainly foraging in marine environments exhibiting the highest Hg concentrations (Clatterbuck et al., 2021; Thorne et al., 2021). EHG showed higher use of coastal foraging sites but similar Hg concentrations compared to the more pelagic LBBG. The coastal specialists GBBG also presented the highest Hg concentrations, further suggesting that "marine vs terrestrial" may represent a better gradient than "coastal vs pelagic" to explain Hg variations in gull species. As  $\delta^{34}\text{S}$  highly

discriminates between terrestrial and marine habitats (McCutchan Jr et al., 2003), we further stress the importance of the use of  $\delta^{34}\text{S}$  as relevant index of foraging habitats.

Nevertheless, Figs. 3 and 4 reveal a weaker relationship between blood Hg and  $\text{PC}_1$  in GBBGs. It was however not possible to include the interaction between  $\text{PC}_1$  and species in the models to study if this relationship was different among species, due to the relatively different range in  $\text{PC}_1$  and Hg values between GBBG and the other species. In chicks, the additive effect of maternal Hg initially transferred in the egg may contribute to blur the relationship in all species.

Surprisingly, the range of Hg concentrations seems to be inversely correlated to the isotopic niche width in adults, i.e., with the foraging habitat diversity. In the specialist GBBG, high fidelity to foraging sites may explain the high inter-individual variation in Hg concentration despite a narrow isotopic niche, i.e., birds feeding on a similar trophic position prey but specialized in areas of different contamination intensities (river estuarine, salt-marshes, intertidal area, open sea, ...; Mitchell and Gilmour, 2008), generating individual variation in Hg exposure. EHG and LBBG also exhibited a relatively high fidelity to foraging sites, but the lower range of Hg concentrations compared to GBBG suggests that the foraging areas provided by movement analyses are important to understand the sources of Hg in specialist species.

#### 5. Conclusion

In the present study, we have highlighted the need to refine ecotoxicological studies with stable isotope analyses, as the trophic position (using  $\delta^{15}\text{N}$  as a proxy) and the feeding habitat ( $\delta^{34}\text{S}$ ) were important drivers of blood Hg contamination. We also point out that not only the use of GPS data highly reflects the results on Hg concentrations and habitat selection through isotopic analyses, but it may further provides fundamental insights on inter-individual contamination through habitat fidelity. We argue for the concomitant use of both GPS and the stable isotopes method to characterize the sources of contaminants in seabirds.

Our results also call for a potential toxicological risk in both chicks and adults. As chicks receive high trophic position prey of marine origin from their parents, such diet could lead to Hg concentrations of concern during their development. In adults, GBBG showed particularly high concentrations of Hg due to its almost exclusively marine food. Nonetheless, several adults of all four species might be at risk as they exceeded previously reported Hg toxicity thresholds. It is therefore fundamental to further investigate the potential detrimental effects of Hg exposure on physiological and life-history traits of the studied species.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2022.119619>.

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# Appendix A

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# APPENDIX B



*Arctic tern (Sterna paradisaea) in flight*

High levels of fluoroalkyl substances and potential  
disruption of thyroid hormones in three gull species  
from South Western France

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## High levels of fluoroalkyl substances and potential disruption of thyroid hormones in three gull species from South Western France



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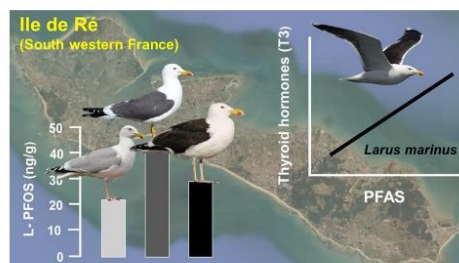
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### HIGHLIGHTS

- PFAS from three gull species from South western France were investigated.
- Positive relationships were found between exposure to PFAS and thyroid hormones.
- Great black-backed gulls document an increasing trend of plasma PFAS concentration.
- PFAS were negatively associated with the body condition of two of the studied species.
- Results on thyroid hormones and body condition were related to the sex of the birds.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Per- and poly-fluoroalkyl substances (PFAS) raised increasing concerns over the past years due to their persistence and global distribution. Understanding their occurrence in the environment and their disruptive effect on the physiology of humans and wildlife remains a major challenge in ecotoxicological studies. Here, we investigate the occurrence of several carboxylic and sulfonic PFAS in 105 individuals of three seabird species (27 great black-backed gull *Larus marinus*; 44 lesser black-backed gull *Larus fuscus graellsii*; and 34 European herring gull *Larus argentatus*) from South western France. We further estimated the relationship between plasma concentrations of PFAS and i) the body condition of the birds and ii) plasma concentrations of thyroid hormone triiodothyronine (TT3). We found that great and lesser black-backed gulls from South Western France are exposed to PFAS levels comparable to highly contaminated species from other geographical areas, although major emission sources (i.e. related to industrial activities) are absent in the region. We additionally found that PFAS are negatively associated with the body condition of the birds in two of the studied species, and that these results are sex-dependent. Finally, we found positive associations between exposure to PFAS and TT3 in the great black-backed gull, suggesting a potential disrupting mechanism of PFAS exposure. Although only three years of data have been collected, we investigated PFAS trend over the study period, and found that great black-backed gulls document an increasing trend of plasma PFAS concentration from 2016 to 2018. Because PFAS might have detrimental effects on birds, French seabird populations should be monitored since an increase of PFAS exposure may impact on population viability both in the short- and long-term.

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## 1. Introduction

Per- and poly-fluoroalkyl substances (PFAS) raised increasing concerns over the past years due to their persistence and global distribution. Because of their high thermal and chemical stability, these synthetic substances have found an application in the manufacturing industry, mostly used as surfactants and additives (Buck et al., 2011), and have been widely produced over the past 50 years (Wang et al., 2017). Being extremely persistent in the environment, and due to their long-range transport via atmospheric and oceanic currents, they have been detected worldwide (Giesy and Kannan, 2001). Several studies have found PFAS to accumulate into living organisms (including invertebrates, fishes, amphibians, mammals, and birds) and to biomagnify through food webs (Kannan et al., 2005; Kelly et al., 2009; Simonnet-Laprade et al., 2019), and to date, PFAS exposure represents a global threat to human health and wildlife (Sunderland et al., 2019). Documenting their occurrence in the environment and understanding their disruptive effect on the physiology of humans and wildlife remains a major challenge.

Seabirds are long-lived apex predators generally exposed to high levels of environmental contaminants (Elliott and Elliott, 2013; Furness and Camphuysen, 1997), thus they prove particularly valuable to investigate PFAS accumulation in marine food webs especially in northern areas. High levels of PFOS have been found in plasma samples of several seabird species from the Arctic including ivory gulls *Pagophila eburnea* (average concentration of 31 ng/g, Lucia et al., 2017); glaucous gulls *Larus hyperboreus* (average concentration of 47 ng/g, Melnes et al., 2017; and of 134 ng/g, Verreault et al., 2005); in black-legged kittiwakes *Rissa tridactyla* from Svalbard (average concentration of 10.2 ng/g, Tartu et al., 2014); and in European shags *Phalacrocorax aristotelis* from Isle of May in Scotland (average concentration of 251 ng/g in females and 163 ng/g in males, Carravieri et al., 2020). High levels of PFOS were also found in egg samples of the European shag and common eider *Somateria mollissima* from Norway (average concentration of 36.8 ng/g and 37.4 ng/g, respectively, Herzke et al., 2009); in whole blood of the endangered lesser black backed gull *Larus fuscus* from Norway (average concentration of 33.5 ng/g, Bustnes et al., 2008a); and in several other seabird species. However, much work has been devoted to seabirds from the Arctic regions (i.e. considered a sink for environmental contaminants; Barrie et al., 1992; Braune et al., 2014; Wong et al., 2018), or in highly contaminated areas (e.g. China; Xie et al., 2013), while fewer studies have focused on areas with not-known sources of PFAS (i.e. Antarctica, Munoz et al., 2017b). In France, most studies examining PFAS occurrence and exposure in aquatic ecosystems focused on water, sediments, invertebrates, and fishes (Couderc et al., 2015; Fernandes et al., 2018; Munoz et al., 2019; Simonnet-Laprade et al., 2019). However, to the extent of our knowledge, no studies have been carried out on top predators including birds in this area, which may be exposed to concentrations of concern. It is therefore crucial to investigate PFAS exposure in French seabirds to document PFAS occurrence in marine biota and to provide early warning of its effects on their health status.

Over the past few years, there has been an increased body of evidence showing that PFAS may i) impact on adipogenesis thus with body condition (Tartu et al., 2014), and ii) disrupt several physiological traits of seabirds. For instance, previous work found that PFAS exposure is associated with lower levels of the stress hormone corticosterone (Tartu et al., 2014), higher oxidative stress (Costantini et al., 2019), longer telomeres (Blévin et al., 2017a; Sebastiano et al., 2020), and a higher metabolic rate (Blévin et al., 2017b). Further studies found PFAS to be associated with higher levels of the parental hormone prolactin and altered incubation behaviours (Blévin et al., 2020), lower hatching success (Tartu et al., 2014), and a higher survival rate (Sebastiano et al., 2020). Specifically, one way through which PFAS may impact on organism function is by disrupting hormonal mechanisms. Previous work provided evidence that PFAS have a strong affinity for proteins and are known to bind to the thyroid hormone transport protein transthyretin

(Ren et al., 2016; Weiss et al., 2009). In birds, the hypothalamic-pituitary-thyroid (HPT) axis controls the secretion of the thyroid hormone thyroxine (T<sub>4</sub>), which is then converted to triiodothyronine (T<sub>3</sub>), the active form of T<sub>4</sub> (McNabb, 2007). Although Blévin et al. (2017b) found no association between PFAS exposure and thyroid hormones in adult black-legged kittiwakes, Braune et al. (2011) found a significant positive correlation between total triiodothyronine (T<sub>3</sub>) levels and hepatic concentrations of PFAS in northern fulmars. Nøst et al. (2012) also found a positive association between PFAS levels and total thyroxine (T<sub>4</sub>) in black-legged kittiwake and northern fulmar chicks, suggesting that PFAS may potentially act through an endocrine disrupting mechanism. More recently, Melnes et al. (2017) found that PFAS were positively associated with free triiodothyronine (FT<sub>3</sub>) in the glaucous gull. To date, further work is needed to understand the relationship between PFAS exposure and thyroid functioning in birds, especially considering that in birds, T<sub>3</sub> and T<sub>4</sub> are involved in a multitude of physiological pathways (McNabb, 2007). A disruption of thyroid hormone levels may be detrimental to development, behaviour, and reproduction (McNabb, 2007).

The Lilleau des Niges Natural Reserve is an important site for breeding, wintering, and migration of several bird species. It is located north of Ile de Ré, an island off the west coast of France, in front of La Rochelle, in the Bay of Biscay. By hosting several seabird species during the breeding season, this island offers a unique opportunity to investigate the occurrence of PFAS in a French seabird community. Although most previous studies have been carried out on a single species (Blévin et al., 2017b; Costantini et al., 2019; Melnes et al., 2017; Tartu et al., 2014), investigating several species simultaneously and from the same geographical area can help to better understand the mechanisms of exposure to PFAS and the potential physiological consequences of PFAS contamination. For instance, the Herring gull *Larus argentatus*, the lesser black-backed gull *Larus fuscus graellsii*, and the great black-backed gull *Larus marinus*, which breed sympatrically on the island, are characterized by different foraging and migratory strategies, thus potentially exposed to different concentrations of PFAS. The aims of this study were to i) assess to which extent French seabirds are contaminated by PFAS; ii) investigate the relationship between exposure to PFAS and body condition; and iii) determine the association between exposure to PFAS and plasma thyroid hormone T<sub>3</sub> concentration in the three above mentioned seabirds from Ile de Ré. Data on PFAS occurrence and their potential adverse effects in seabirds from France are not yet available. To date, we are not aware of known point sources of PFAS in the region. But considering that diverse important rivers may discharge PFAS near the study area (Simonnet-Laprade et al., 2019), and that PFAS may reach and accumulate in remote areas due to their long-range oceanic and atmospheric transport (Munoz et al., 2019), we expect comparable PFAS concentrations with seabirds from the Arctic. In addition, if PFAS have a stimulating effect on thyroid hormone production as found in previous work (DeWitt, 2015; Liu et al., 2011; Nøst et al., 2012), we expect a positive association between PFAS and the concentration of thyroid hormones. Furthermore, although some PFAS are listed as POPs by the Stockholm Convention and their production has subsequently been reduced over the past years, studies investigating temporal trends of PFAS in tissues of wildlife are limited. Although our data have been solely collected over three years of study, we further aim to describe the temporal variation in blood concentration of PFAS from 2016 to 2018 in local seabirds.

## 2. Materials and methods

### 2.1. Sampling

Field work was performed in 2016, 2017, and 2018 at the Lilleau des Niges Natural Reserve (46° 13' 53" N, -1° 30' 22" W), managed by the Ligue pour la Protection des Oiseaux (LPO) located on the North side of Ile de Ré, France, as a part of a monitoring program for PFAS in the

region. A total of 108 breeding adult birds from three species were captured during the incubation stage on their nests using a nest trap. Because out of the 108 observations, three were coming from the same individuals sampled at different years, one or the other observation was randomly excluded to perform statistical analyses. Therefore, the final dataset included a total of 105 birds (European herring gull,  $n = 9$  in 2016,  $n = 16$  in 2017, and  $n = 9$  in 2018; lesser black-backed gull,  $n = 11$  in 2016,  $n = 17$  in 2017, and  $n = 16$  in 2018; great black-backed gull,  $n = 9$  in 2016,  $n = 7$  in 2017, and  $n = 11$  in 2018). After capture, 2 mL of blood was collected from the alar vein using a heparinized syringe and a 25 gauge needle. Blood was kept in a cold container and centrifuged for 10 min at  $8000 \times g$  at  $20^\circ\text{C}$  at the laboratory within a few hours after collection; plasma and red blood cells were kept frozen at  $-20^\circ\text{C}$  until laboratory analyses. Skull and tarsus were measured with an accuracy of 0.1 mm using a caliper. Wing length was also measured with an accuracy of 1 mm using a ruler, and birds were weighted to the nearest 5 g using a Pesola spring balance. Birds were sexed from red blood cells by polymerase chain reaction amplification (PCR) of part of two highly conserved genes (CHD) of sexual chromosomes. Briefly, DNA was extracted from erythrocytes and the sex was determined by molecular sexing based on PCR amplification of the CHD gene as described in Fridolfsson and Ellegren (1999). Amplification was performed in 20  $\mu\text{L}$  final volume with a Eppendorf Mastercycler using 0.5 U Taq DNA polymerase, 200  $\mu\text{M}$  dNTPs, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM  $\text{MgCl}_2$  and 0.4  $\mu\text{M}$  of primers 2550F (5'-GTTACTGATTCGCTACGAGA-3') and 2718R (5'-ATTGAAATGATCCAGTCTTG-3'). Female birds may deposit a significant amount of PFAS into their eggs. Therefore, to minimize the variation due to PFAS deposition in eggs, we have only sampled individuals having either two (17/105, 16% of birds) or three eggs (87/105, 83% of birds) except one sampled females that only laid one egg (1/105, 1%). Preliminary statistical analyses were carried out to test whether females with two (8/52, 15% of females) or three eggs (43/52, 83%) had different concentrations of PFAS. However, linear models showed that for any PFAS, concentrations were similar between females that laid two or three eggs (all  $t < 1.14$ , all  $P > 0.26$ ), thus clutch size was not further included in the statistical analyses.

## 2.2. PFAS analyses

A total of 14 PFAS were analysed in each plasma sample, including eight carboxylates: branched- (Br-PFOA) and linear-perfluorooctanoate (L-PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTTrDA), perfluorotetradecanoate (PFTeDA); and six sulfonates: perfluorohexanesulfonate (PFHxS), branched- (Br-PFHpS) and linear-perfluoroheptasulfonate (L-PFHpS), branched perfluorooctanesulfonate (Br-PFOS), linear perfluorooctanesulfonate (L-PFOS), and perfluorooctanesulfonamide (FOSA). Analytical standards of native PFAS along with a series of  $^{13}\text{C}$ ,  $^{18}\text{O}$  or D mass-labelled internal standards used for quantification purposes were supplied by Wellington laboratories. All reagents were analytical grade or equivalent (see Munoz et al. (2017b) for full details). Briefly, in a 2 mL polypropylene Eppendorf tubes, a 25  $\mu\text{L}$  aliquot of plasma was weighed ( $\sim 25$  mg) and internal standards (ISs) were subsequently added under gravimetric control ( $\sim 15$  mg of a 1  $\mu\text{g}/\mu\text{L}$  IS mixture prepared in methanol). Following protein precipitation with 100  $\mu\text{L}$  of acetonitrile (ACN), extracts were centrifuged for 10 min at  $24,000 \times g$  at  $20^\circ\text{C}$ . The supernatant was then transferred to 2 mL polypropylene centrifuge tubes (0.22  $\mu\text{m}$  nylon filter). After centrifugation for 3 min at  $7000 \times g$  at  $20^\circ\text{C}$ , extracts were transferred to 2 mL auto sampler glass vials and diluted with 675  $\mu\text{L}$  of HPLC-water. Extracts were briefly vortexed and then processed using an Agilent Technologies (Massy, France) on-line SPE platform which comprises a standard auto sampler (1260 Infinity ALS), a quaternary pump (1260 Infinity Quaternary Pump VL), a switch valve (1200 2 Position/6 Port Valve) and an on-line SPE column support (1200 6 Position Selection Valve), which

were all automatically controlled via the Acquisition module of the Agilent Mass Hunter software as previously done (Munoz et al., 2017b). HPLC-water aliquots were run between each seabird plasma sample to eliminate any cross-contamination. Note that on-line extraction was performed with Waters Oasis HLB on-Line SPE columns ( $2 \times 10$  mm,  $\text{dp} = 25\text{--}35$   $\mu\text{m}$ ) while analyte separation was carried out using an Agilent C<sub>18</sub> Poroshell analytical column ( $2.1 \times 100$  mm, 2.7  $\mu\text{m}$ ).

## 2.3. Quality assurance/quality control (QA/QC)

When analytes were detected in blanks, blank correction was performed and a limit of reporting (LOR) was defined as three times the maximum blank signal divided by the average mass of plasma used for analysis. A limit of detection (LOD) was also defined as the concentration yielding a signal to noise ratio of 3 in spiked plasma samples. Because laboratory analyses were performed in different years, a unique left-censoring threshold was set for each analyte, i.e. the maximum between LORs and LODs, all years combined. For those PFAS with concentrations below this threshold in less than 30% of samples, left-censored data were arbitrarily replaced with  $\frac{1}{2} \times \text{LOR}$  or  $\text{LOD}$  to enable statistical analyses. Therefore, ten PFAS (PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, PFTeDA, PFHxS, L-PFHpS (hereafter PFHpS), Br-PFOS, and L-PFOS) could be further investigated (i.e. other analytes were excluded from statistical analyses). LORs, LODs and detection frequencies are presented in the supplementary information (Table S1). For each sample batch (20 samples), several QA/QC points were assessed by analyzing: i) two procedural blanks consisting of 25  $\mu\text{L}$  of HPLC-water that went through the entire analytical procedure; ii) one human serum standard reference material (NIST SRM 1957, trueness assessment); iii) replicate spiked chicken plasma samples (target analytes added jointly with mass-labelled ISs at the beginning of the preparation procedure at 2 ng/g each, accuracy assessment); and iv) HPLC-water samples spiked at 2 ng/g, accuracy assessment) as previously described (Munoz et al., 2017b). Procedural blanks showed very limited contamination. The analysis of NIST SRM 1957 gave satisfactory results, i.e. within the specified uncertainty interval. For those compounds with a reference concentration (i.e. PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFHxS and PFOS), levels deviated between 2 and 20% from the reference concentration (except for FOSA, which deviated 36%).

## 2.4. Thyroid hormone analyses

TT3 was determined by radioimmunoassay. Briefly, 25  $\mu\text{L}$  of plasma was incubated for 24 h at  $4^\circ\text{C}$  with a known concentration (10,000 cpm) of T3 marked with the radioisotope Iodine-125 ( $\text{T}_3\text{-}^{125}\text{I}$ , Perkin Elmer, US, reference: NEX110X100UC) and an antibody *Ab* (polyclonal rabbit antiserum, Sigma-Aldrich, US, reference: T-2777). Because *Ab* is available in a limited concentration, T3 and  $\text{T}_3\text{-}^{125}\text{I}$  compete for *Ab*, to which they bind. Therefore, after incubation, there is a bound fraction (T3 and  $\text{T}_3\text{-}^{125}\text{I}$  bound to *Ab*) and a free fraction (T3 and  $\text{T}_3\text{-}^{125}\text{I}$  unbound to *Ab*), which are separated by adding a sheep anti-rabbit antibody (whole anti-serum anti rabbit IgG produced in sheep), incubated for 12 h at  $4^\circ\text{C}$  followed by centrifugation at  $4300 \times g$  at  $18\text{--}20^\circ\text{C}$  for 45 min. The bound fraction is then counted with a wizard 2 gamma counter (Perkin Elmer, US). Pooled plasma of diverse gull samples were serially diluted and produced a dose-response curve parallel to the T3 standard curve. The lowest TT3 detectable concentration was 0.07 ng/ml (LOD). Samples below this limit ( $n = 3$ ) were replaced with a value equal to  $\frac{1}{2} \times \text{LOD}$  to enable statistical analyses. All samples were run in duplicates. Samples that had a coefficient of variation above 15% and could not be done in triplicates due to low plasma volume were not included in statistical analyses ( $n = 11$ ). An additional measurement of TT3 was excluded from statistical analyses since it was considered an outlier (the measurement exceeded the mean  $\pm 3$  times the standard deviation and was highly influential in statistical analyses). Therefore, for a total of 58 samples ( $n = 6$  in 2016 and  $n = 12$  in 2017 for the European herring gull;  $n = 10$  in 2016 and  $n = 14$  in

# Appendix B

2017 in lesser black-backed gull; n = 8 in 2016 and n = 8 in 2017 in great black-backed gull), both TT3 and PFAS data were available. The intra-assay coefficient of variation was 9.73%, while the inter-assay coefficient of variation amounted to 15.13%.

## 2.5. Statistical analyses

After PFAS data were log-transformed to reduce the influence of extreme values (see below), linear models were used to investigate differences in PFAS concentrations among species and between genders (in samples collected from 2016 to 2018). In each model, each PFAS was considered as a dependent variable while the factors *Species*, *Sex*, and their interaction were considered as predictors. To simultaneously investigate species specific temporal trends in PFAS exposure from 2016 to 2018, the *Year*, the factor *Species*, and their interaction were also included in the model as explanatory variables. We used a similar model to test the difference in body condition between sexes. Briefly, the body condition has been calculated using the body mass adjusted by a linear body measurement (i.e. skull length) using the formula described in Peig and Green (2009).

Linear models were additionally used to study the association between TT3 and PFAS (in samples collected in 2016 and 2017). In these models, a three-way interaction between PFAS, the factors *Species* and *Sex*, was used to investigate sex-related responses to PFAS exposure. These models additionally included the *Year* (as a factor, to control for

the temporal variation in TT3 and PFAS), and *body condition* (as a covariate, to control for the individual condition of birds). A similar model was built to test the association between the body condition and PFAS in all samples collected from 2016 to 2018.

All PFAS concentrations (except PFTeDA, which was normally distributed and assumptions listed below were respected without data transformation), were log-transformed when testing for time trends and when testing for inter-species and between sex differences. All PFAS concentrations were log-transformed when testing for the association between either TT3 or body condition and PFAS. Data transformation was done to meet model assumptions as homoscedasticity and normality of residuals, further confirmed by visually inspecting Q-Q plots. All data transformation and violation of models' assumptions are reported throughout the manuscript. Statistical significance was set to  $\alpha = 0.05$  and 95% confidence intervals were used during data processing and data visualization. All statistical analyses were performed using R version 3.5.2.

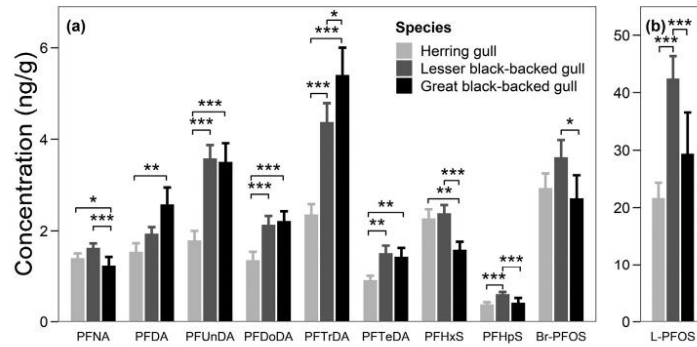
## 3. Results

PFAS used in statistical analyses were detected in all samples (Table S1) and their concentrations are summarized in Table 1 and Fig. 1. L-PFOS was the most abundant, followed by the odd-chain carboxylates PFTTrDA and PFUnDA. Linear models showed statistically significant differences among species for all carboxylic and sulfonic PFAS (all  $F > 5.15$ , all  $P < 0.01$ ), and all statistical outputs and post-hoc

**Table 1**  
PFAS concentration (ng/g of ww) in females and males of the three seabird species from Ile de Re. P-values refer to the difference in PFAS between females and males. Significant P-values are bolded. SE refers to standard errors.

Herring gull <i>Larus argentatus</i>					
	Females (n = 18)		Males (n = 16)		P-value
	Mean ± SE	Median (range)	Mean ± SE	Median (range)	
PFNA	1.16 ± 0.11	1.06 (0.36, 2.16)	1.68 ± 0.17	1.63 (0.67, 2.85)	0.07
PFDA	1.16 ± 0.19	0.94 (0.38, 4.01)	1.98 ± 0.30	1.79 (0.69, 6.04)	<b>0.01</b>
PFUnDA	1.23 ± 0.17	1.01 (0.47, 3.45)	2.43 ± 0.34	2.17 (0.61, 6.49)	<b>&lt;0.001</b>
PFDoDA	0.95 ± 0.16	0.9 (0.3, 3.22)	1.82 ± 0.32	1.56 (0.55, 6.17)	<b>&lt;0.001</b>
PFTTrDA	1.76 ± 0.27	1.42 (0.59, 5.25)	3.03 ± 0.29	3.05 (1.04, 4.81)	<b>&lt;0.001</b>
PFTeDA	0.61 ± 0.07	0.61 (0.25, 1.23)	1.23 ± 0.16	1.03 (0.33, 2.8)	<b>0.04</b>
PFHxS	2.26 ± 0.21	2.16 (0.88, 3.5)	2.29 ± 0.36	1.89 (0.79, 6.92)	0.99
PFHpS	0.27 ± 0.02	0.25 (0.11, 0.5)	0.49 ± 0.09	0.38 (0.11, 1.63)	<b>0.045</b>
Br-PFOS	2.03 ± 0.34	1.71 (0.86, 6.8)	3.95 ± 0.45	3.75 (1.07, 7.9)	<b>&lt;0.001</b>
L-PFOS	13.82 ± 2.0	11.64 (5.57, 37.04)	30.44 ± 4.31	28.44 (6.6, 62.52)	<b>&lt;0.001</b>
Lesser black-backed gull <i>Larus fuscus graellsii</i>					
	Females (n = 20)		Males (n = 24)		P-value
	Mean ± SE	Median (range)	Mean ± SE	Median (range)	
PFNA	1.14 ± 0.09	1.11 (0.46, 1.83)	2.04 ± 0.1	2.06 (0.95, 3.36)	<b>&lt;0.001</b>
PFDA	1.1 ± 0.08	1.15 (0.54, 1.64)	2.63 ± 0.15	2.58 (1.17, 4.16)	<b>&lt;0.001</b>
PFUnDA	1.88 ± 0.16	1.69 (0.66, 3.28)	5 ± 0.29	4.89 (1.98, 8.7)	<b>&lt;0.001</b>
PFDoDA	1.02 ± 0.07	1.02 (0.39, 1.87)	3.06 ± 0.2	2.86 (1.75, 5.52)	<b>&lt;0.001</b>
PFTTrDA	1.97 ± 0.12	2.06 (0.66, 2.91)	6.38 ± 0.44	5.74 (3.53, 11.66)	<b>&lt;0.001</b>
PFTeDA	0.66 ± 0.07	0.61 (0.13, 1.39)	2.23 ± 0.2	1.87 (1.1, 4.35)	<b>&lt;0.001</b>
PFHxS	2.18 ± 0.25	1.95 (1.12, 6.01)	2.56 ± 0.26	2.24 (1.08, 5.56)	0.94
PFHpS	0.37 ± 0.03	0.35 (0.16, 0.82)	0.79 ± 0.07	0.69 (0.34, 1.84)	<b>&lt;0.001</b>
Br-PFOS	1.86 ± 0.19	1.96 (0.58, 3.15)	5.06 ± 0.5	4.41 (2.38, 13.67)	<b>&lt;0.001</b>
L-PFOS	21.09 ± 2.06	22.76 (5.74, 34.49)	60.23 ± 4.44	54.68 (26.51, 119.69)	<b>&lt;0.001</b>
Great black-backed gull <i>Larus marinus</i>					
	Females (n = 14)		Males (n = 13)		P-value
	Mean ± SE	Median (range)	Mean ± SE	Median (range)	
PFNA	0.59 ± 0.06	0.59 (0.24, 0.95)	1.94 ± 0.29	1.72 (0.89, 4.85)	<b>&lt;0.001</b>
PFDA	1.41 ± 0.23	1.13 (0.32, 3.55)	3.83 ± 0.58	3.34 (1.52, 7.2)	<b>&lt;0.001</b>
PFUnDA	2.42 ± 0.35	2.23 (0.49, 4.93)	4.67 ± 0.65	4.08 (2.62, 11.46)	<b>0.01</b>
PFDoDA	1.69 ± 0.24	1.63 (0.32, 3.12)	2.79 ± 0.29	2.37 (1.68, 5)	<b>0.04</b>
PFTTrDA	4.04 ± 0.72	3.55 (0.68, 9.61)	6.87 ± 0.83	5.79 (3.85, 14.39)	<b>0.02</b>
PFTeDA	0.96 ± 0.17	0.92 (0.13, 2.4)	1.94 ± 0.32	1.42 (0.91, 4.17)	<b>0.03</b>
PFHxS	1.2 ± 0.11	1.16 (0.62, 2.06)	2.01 ± 0.32	1.58 (0.93, 5.33)	0.16
PFHpS	0.2 ± 0.03	0.15 (0.09, 0.47)	0.63 ± 0.21	0.42 (0.22, 3.01)	<b>&lt;0.001</b>
Br-PFOS	1.32 ± 0.17	1.1 (0.39, 2.53)	4.21 ± 0.89	3.21 (1.85, 13.01)	<b>&lt;0.001</b>
L-PFOS	13.45 ± 1.52	10.53 (5.18, 25.92)	46.55 ± 13.74	27.62 (16.71, 194.72)	<b>&lt;0.001</b>

# Appendix B



**Fig. 1.** Plasma concentrations of PFAS (expressed as ng/g of ww) in the three seabird species from Ile de Re. Statistically significant differences are indicated by the asterisk; \*, \*\*, \*\*\*, indicate a *P*-value < 0.05, < 0.01, and < 0.001, respectively.

differences can be found in Table S2. Among carboxylates, PFNA levels were higher in herring gulls and lesser black-backed gulls than great black-backed gulls (both  $P < 0.05$ , Fig. 1). PFDA levels were higher in great black-backed gulls than herring gulls ( $P < 0.01$ , Fig. 1). PFUnA, PFDoDA, PFTrDA, and PFTeDA levels were higher in great and lesser black-backed gulls than herring gulls (all  $P < 0.01$ , Fig. 1), and PFTrDA levels were also higher in great than lesser black-backed gulls ( $P < 0.05$ , Fig. 1). Among sulfonic acids, PFHxS was higher in both lesser black-backed and herring gulls than great black-backed gulls (both  $P < 0.01$ , Fig. 1). PFHpS and L-PFOS were highest in lesser black-backed gulls (all  $P < 0.001$ , Fig. 1), and Br-PFOS was higher in lesser than great black-backed gulls ( $P < 0.05$ , Fig. 1).

All carboxylates showed significantly higher concentrations in males than in females for all species (all  $P < 0.05$ , except for PFNA in the herring gull, for which  $P = 0.07$ ; Table 1). Among sulfonates, PFHxS showed similar concentrations between females and males in all species (all  $P > 0.16$ , Table 1), while PFHpS, Br- and L-PFOS showed significantly higher concentrations in males than females (all  $P < 0.05$ , Table 1). In all three species, there was no difference in the body condition between males and females (all  $t < 1.27$ , all  $P > 0.80$ ). Finally, TT3 levels were similar between sexes in all three species ( $t = 2.76$ ,  $P = 0.08$ ).

In great black backed gulls, TT3 was positively associated with PFUnDA, PFDoDA, PFTrDA, PFTeDA and Br-PFOS in females (all  $t > 2.10$ , all  $P \leq 0.04$ ; Fig. 2a–d, f, Table S3), while TT3 was negatively associated with PFHxS in males ( $t = -2.69$ ,  $P = 0.01$ ; Fig. 2e, Table S3). There was no association between TT3 and any PFAS in herring gulls and lesser black-backed gulls (all  $t \leq 0.79$ , all  $P \geq 0.43$ , Table S3).

In great black backed gull females, increasing levels of PFNA, PFDA, PFHxS, PFHpS, Br- and L-PFOS were associated with a reduced body condition (all  $t \leq -2.19$ , all  $P \leq 0.03$ ; Figs. S1, S2, Table S4), while increasing levels of PFNA and PFDA were associated with a reduced body condition (both  $t \leq -2.18$ , both  $P = 0.03$ ; Fig. S1, Table S4) in lesser black-backed gull males, but not females.

In great black backed gulls, there was a significant or marginally-significant increase in all PFAS from 2016 to 2018 (all  $t \geq 1.93$  and all  $P \leq 0.056$ ; Figs. 3 and 4, Table S5), while lesser black backed gulls showed an increase in PFTeDA and PFHxS, and a marginally significant decrease in PFUnDA levels from 2016 to 2018 (all  $t \geq 1.98$ , all  $P \leq 0.05$ ; Figs. 3, 4, Table S5). All PFAS showed similar concentrations among years in the herring gulls except for PFTrDA, which increased from 2016 to 2018 ( $t = 2.30$ ,  $P = 0.02$ ; Fig. 3, Table S5). Body condition did not change in any of the species from one year to another (all  $t < 1.14$ , all  $P > 0.26$ ). TT3 levels remained similar between 2016 and 2017 in all three species (all  $t < 1.88$ , all  $P > 0.42$ ).

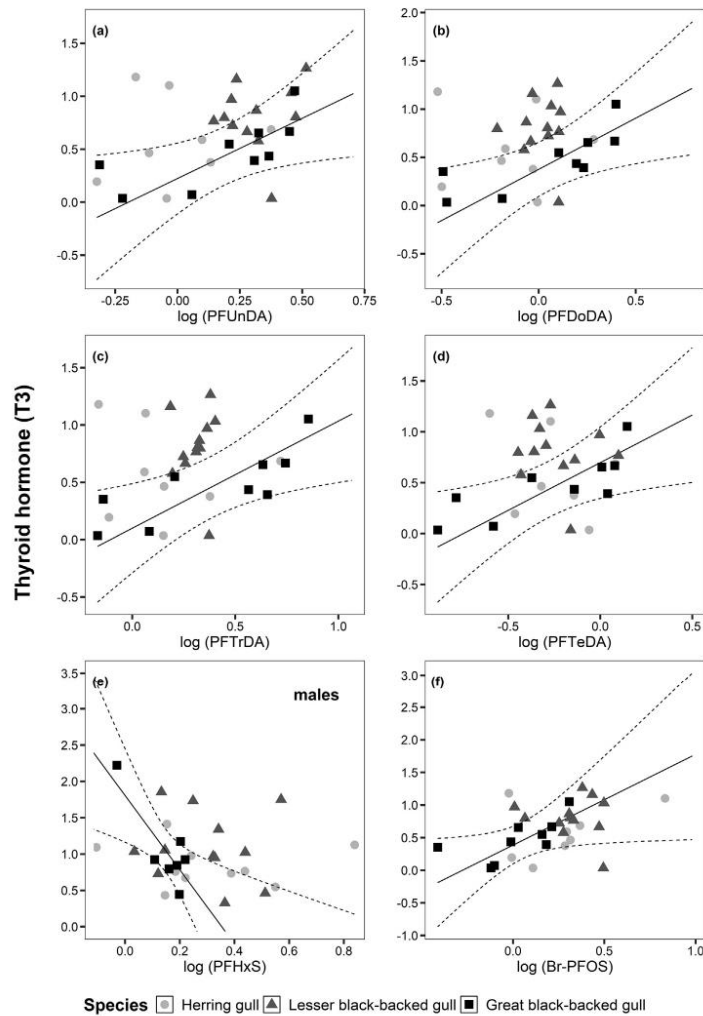
## 4. Discussion

Our study is the first to provide evidence that although not-known point sources of emission are present in the region, several PFAS were detected in seabird species from South Western France. Great and lesser black-backed gulls show that both plasma carboxylate and sulfonate concentrations are comparable to highly contaminated seabird species from Arctic regions, while herring gulls are exposed to relatively lower levels of PFAS. We found that PFAS are negatively associated with the body condition of the birds. Furthermore, TT3 levels were associated with several PFAS in a contrasted manner between sexes in the great black-backed gull, suggesting a potential disrupting mechanism of PFAS exposure. Finally, the great black-backed gulls documented an increasing trend of plasma PFAS concentration from 2016 to 2018.

Our results show that among carboxylates, PFUnDA and PFTrDA are the most abundant congener in all three species, a pattern that is commonly found in seabird species (Bustnes et al., 2008b; Melnes et al., 2017; Tartu et al., 2014). Because of the strong winds and oceanic currents that characterize the Atlantic Ocean, and considering that, to the best of our knowledge, there are no point sources of PFAS in the region, perfluorinated compounds should occur at a lower concentration than in the Mediterranean and the Arctic regions, considered as sinks for pollutants (Danovaro, 2003; Wong et al., 2018). However, the levels of carboxylates found in this study (ranging from a median of 0.6 ng/g of PFTeDA in Herring gulls to 5.8 ng/g of PFTrDA in great black-backed gulls) are similar to those reported for glaucous gulls (ranging from a median of 0.1 ng/g of PFOA to 3.8 ng/g of PFUnDA, Melnes et al., 2017) and lesser black-backed gulls (ranging from a median of 0.2 ng/g of PFDoDA to 5.9 ng/g of PFDA, Bustnes et al., 2008a) from Arctic regions, but lower than those found in other species (e.g. in black-legged kittiwakes from Svalbard; ranging from a mean of 1.0 ng/g of PFNA to 18.2 ng/g of PFTrDA, Tartu et al., 2014). One possible explanation may be related to the continental input of PFAS through the Gironde, Loire, and Charente estuaries (Munoz et al., 2019; Munoz et al., 2017a; Munschy et al., 2019), which may have contributed to the observed concentrations. Except for PFNA (which levels were higher in herring gulls and lesser black-backed gulls than great black-backed gulls), most carboxylates were higher in lesser and great black backed gulls than herring gulls. Although we cannot exclude that these three species differ in their ability to excrete PFAS from their body, our results suggest that the differences in exposure likely depend on the trophic niche occupied by the species. For instance, a recent study on PFAS in six seabird species from the Arctic regions showed that predatory birds (e.g. great skua *Stercorarius skua*, 44.8 ng/g of sumPFAS) showed the highest contaminant load compared with species from a lower trophic level (e.g. common eider *Somateria mollissima*, 1.3 ng/g of sumPFAS; Haarr et al.,



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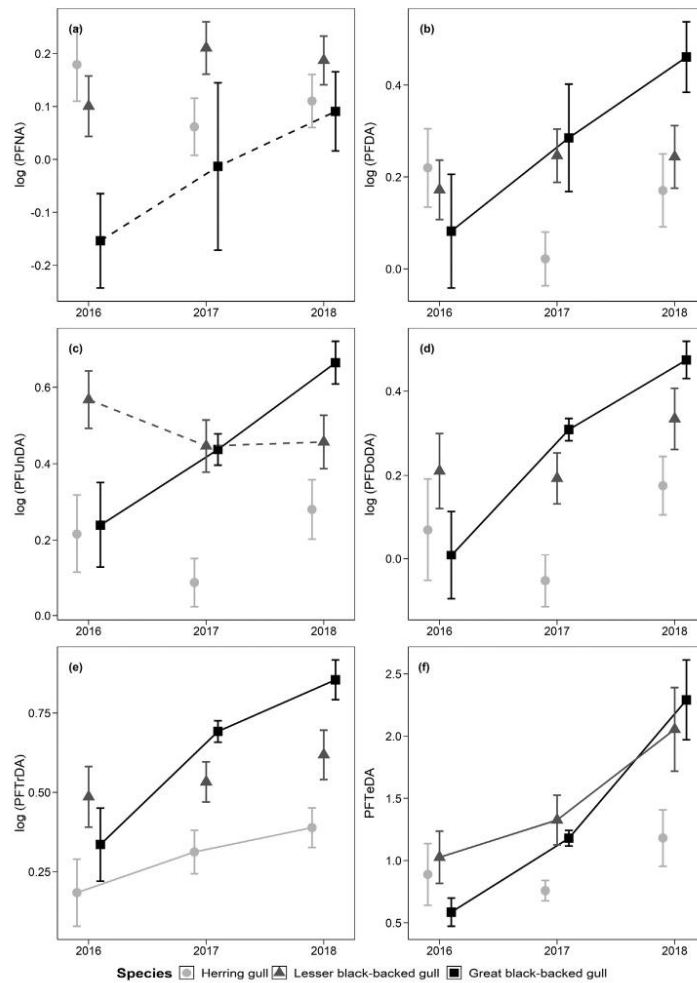
**Fig. 2.** Relationship between the concentration of the thyroid hormone TT3 (expressed as ng/mL) and log-transformed carboxylic (C-11 to C-14, panel a to d, respectively), and sulfonic PFAS (PFHxS and Br-PFOS, panel e and f, respectively) of the three seabird species from Ile de Re. The solid line represents the trend while curved dashed lines represent 95% confidence intervals. Shapes of data points and line colours are used to distinguish the three species as explained in the figure legend. Only significant trends are shown. Data refer to the period 2016–2017 for which both TT3 and PFAS were available (n = 58).

2018). Great black-backed gulls feed on higher trophic level preys and mainly forage along the shore (Maynard and Davoren, 2020), while lesser black-backed and herring gulls are known to have a generalist diet which also includes food items from both terrestrial and marine origin (Corman et al., 2016; Maynard and Davoren, 2020).

Among sulfonates, L-PFOS was the most abundant, followed by Br-PFOS, PFHxS, and PFHpS and all occurred at very high concentrations. For instance, PFHxS ranged from a median value of 1.2 ng/g in great black-backed gulls to 2.2 ng/g in lesser black-backed gulls, while other studies from highly contaminated areas reported lower PFHxS plasma concentrations (a median below 1 ng/g in lesser black-backed gulls, Bustnes et al., 2008b; a median below 0.7 in glaucous gulls, Melnes et al., 2017; all samples below 0.2 ng/g in black-legged kittiwakes,

Tartu et al., 2014). Furthermore, L-PFOS ranged from a median of 11.6 ng/g in herring gull females to a median of 54.7 ng/g in lesser black-backed gull males. Thus, lesser black-backed gulls in our study showed very high sulfonate levels, even higher than Norwegian populations (a median of 40 ng/g of PFOS in males, Bustnes et al., 2008b), and in this species, sulfonate levels are significantly higher than those observed in great black-backed gulls. In addition, plasma concentrations of most PFAS showed significantly higher levels in males than in females, but this difference was not related to the body condition of the birds, with males showing a similar body condition than females. Because females transfer contaminants in the eggs, it is thus possible that females have lower levels of circulating PFAS in plasma. However, previous work pointed out contrasting results between PFAS in eggs

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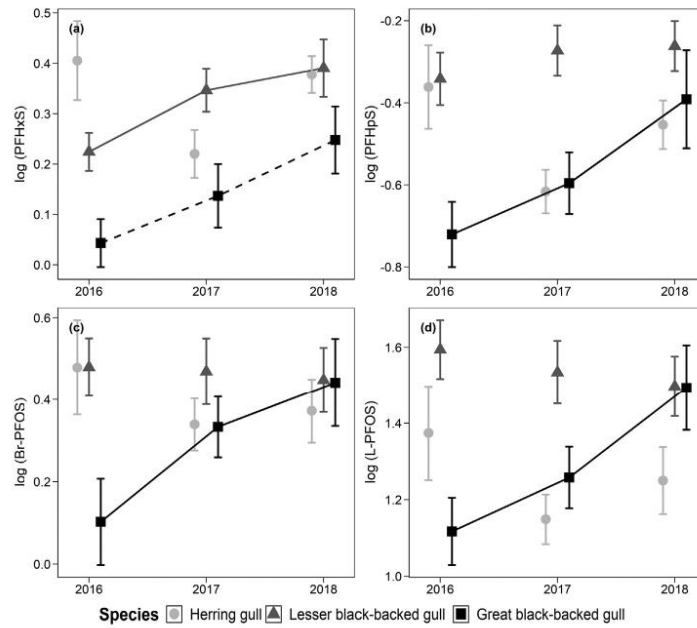
**Fig. 3.** Error bar plots mean  $\pm$  standard error of carboxylate concentrations in 2016, 2017, and 2018 in the seabird species from Ile de Re. The shapes and the three different grades of grey are used to distinguish the three species as explained in the figure legend. Only significant trends are shown. Dashed lines indicate a trend close to significance.

and plasma, suggesting that the extent of PFAS transfer to the eggs may significantly vary among the studied species (Bustnes et al., 2008a; Herzke et al., 2009; Verreault et al., 2005). Given that in this study we did not analyse PFAS levels in eggs, it is not possible to clarify whether the species differ in terms of PFAS excreted in eggs. Although Verreault et al. (2006) found that the contaminant content in glaucous gull eggs fluctuated irrespectively of the laying order, other work suggests that the majority of PFAS are found in the first or the first two eggs, while negligible concentrations of PFAS are found in the third egg, as previously shown in Audouin's gulls *Larus audouinii* (Vicente et al., 2015). Our results showed that females that laid three eggs had similar PFAS concentrations than females that laid two eggs, therefore our results should not be affected by the difference in PFAS deposition in eggs.

Because of the higher bioaccumulative properties and biomagnification of longer-chain PFAS (Boisvert et al., 2019; Simonnet-Laprade

et al., 2019), these compounds tend to occur at higher concentrations in wildlife tissues (Conder et al., 2008; Muir et al., 2019; Muir and de Wit, 2010). Therefore, individuals feeding at a higher trophic position are likely to be exposed to higher concentrations of long-chained carboxylates. Previous work also showed that longer chained PFAS are more likely to induce adverse health effects in seabirds compared to shorter chained PFAS. For instance, negative associations between PFAS and baseline corticosterone in black-legged kittiwakes were only found for PFTTrDA and PFTeDA (Tartu et al., 2014), while such association was not found for shorter-chain PFAS. Additional work on the same bird population found higher protein oxidative damage in those birds having higher concentrations of PFDoDA, PFTTrDA and PFTeDA (Costantini et al., 2019). Similarly, previous work found a positive association between PFTTrDA and metabolic rate in the same species (Blévin et al., 2017b). These results were further corroborated by experimental work on rat *Rattus* sp. cell cultures, showing that the cytotoxicity of PFAS increases

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**Fig. 4.** Error bar plots mean  $\pm$  standard error of sulfonate concentrations in 2016, 2017, and 2018 in the seabird species from Ile de Re. The shapes and the three different grades of grey are used to distinguish the three species as explained in the figure legend. Only significant trends are shown. Dashed lines indicate a trend close to significance.

with increasing carbon chain length (Berntsen et al., 2017), and that comparing molecules with a similar chain length, a sulfonate functional group may lead to greater toxicity than a carboxyl group (Berntsen et al., 2017).

Our results are thus of particular interest as they suggest that birds feeding at a higher trophic position and showing a more marine diet (i.e. great black-backed gulls) should also be exposed to greater toxicological risks from carboxylates, while birds with a more generalist diet (i.e. lesser black-backed gulls) can be exposed to higher levels of sulfonates. This is likely the reason why we found associations between TT3 and PFAS only in great black-backed gulls, while no significant associations have been found in herring gulls and lesser black-backed gulls. Interestingly, our results were dependent on the sex of the birds. Indeed, although we found that PFHxS was associated with TT3 in great black-backed gull males, all other associations were found in females only. Despite the lower concentration of circulating plasma PFAS levels, females can deposit PFAS in eggs therefore we cannot be certain that females were exposed to lower PFAS concentrations than males, and further work including other tissues (e.g. liver or muscle) would clarify whether PFAS intake differs between the sexes of sampled birds. Despite the absolute concentrations to which they are exposed, a possible explanation for the results in females may rely on the fact that incubation can be extremely costly for female birds (Hanssen et al., 2005), thus they may be more susceptible to PFAS exposure. Previous work in birds suggest a modulation of thyroid function induced by exposure to various environmental contaminants. Smits et al. (2002) reported decreased TT3 levels in American kestrels *Falco sparverius* experimentally exposed to PCBs, while Verreault et al. (2004) reported a decrease in T4:T3 ratio in the glaucous gull. Similarly, exposure to organochlorines was associated with reduced TT3 in kittiwakes (Blévin et al., 2017b), and with reduced T3 and T4 in glaucous gulls (Melnes et al., 2017; Verreault et al., 2004). However, specifically related to PFAS, further work on seabirds found a positive association with thyroid functioning (i.e. between PFOS and

TT3 in glaucous gulls, Melnes et al., 2017; between several PFAS and TT4 in black-legged kittiwakes and northern fulmars, Nøst et al., 2012). Although being conducted in fish, an experimental approach showed that exposure to PFOS in zebra fish (*Danio rerio*) led to increased thyroid hormones secretion (Liu et al., 2011). Thus, our results on great black backed gulls are in line with previous studies. In this study, increasing TT3 levels in this species were found with increasing concentrations of longer chain PFAS (PFUnDA, PFDoDA, PFTtDA, and PFTeDA) and Br-PFOS. This suggests that despite carboxylates and sulfonates are functionally different, their effect on TT3 is similar. However, this was not the case for PFHxS, which showed a decrease in TT3 levels with increasing concentrations. PFHxS is highly toxic and causes thyroid disruption by lowering thyroid hormone levels in rats (Ramhøj et al., 2020), but it remains unclear why this effect was only found in great black-backed gull males. Indeed, females showed similar levels than males, and the other species exhibited higher PFHxS concentrations than those found in great black backed gulls, thus this result would strongly benefit from experimental support. Because the avian thyroid gland secretes almost exclusively T4 (Darras et al., 2006), most T3 is derived from the deiodination of T4 (Darras et al., 2006). A possible explanation is that in great black-backed gulls, exposure to PFHxS may negatively impact either the transport of T4 (by reducing the activity of serum binding proteins) or deiodination processes. It is therefore strongly warranted to supplement *in vitro* experiments to verify the effect of PFHxS on T4 transformation. Our results do not provide evidence for a causal relationship PFAS exposure and circulating thyroid hormones. But the contrasting results found between sexes strongly call for further work to experimentally investigate the effect of PFAS exposure on thyroid functioning of birds.

Sex-related differences were also found while investigating the relationship between exposure to PFAS and the birds' body condition. In female great black-backed gulls, we found that increasing concentrations of PFNA, PFDA, and all four sulfonates were

negatively associated with body condition, while in lesser black-backed gulls, a similar negative relationship between PFNA, PFDA, and body condition was only found in males. Previous work in humans and various animal models found that exposure to certain PFAS is suspected to disrupt fatty acids metabolism and promote adipogenesis (Cheng et al., 2016; Wan et al., 2012; Xu et al., 2016; Yeung et al., 2007). More specifically, these changes in lipid content are related to the capacity of PFAS to alter the expression of genes involved in the metabolism of lipids and fatty acids (Jacobsen et al., 2018; Wan et al., 2012). To date, work on the effect of PFAS on lipid metabolism and body condition in birds remains extremely limited. The negative association we found is in contrast with a previous study on black-legged kittiwakes, showing that PFNA was positively associated with body condition in males (Tartu et al., 2014). One possible explanation for the negative relationship found in females may be related to the ability to deposit PFAS into the eggs. For instance, great black-backed gull females in a better body condition may be more efficient in eliminating PFAS through egg-deposition, although evidences to support this statement are lacking. However, this would not explain why in a closely related species (i.e. lesser black-backed gull) a similar association has been found in males. Our study results are novel but emphasize the need to experimentally investigate the potential association between exposure to PFAS and body condition in birds.

Finally, not only does our work provide evidence of high PFAS levels in seabirds from metropolitan France, but our results clearly suggest increasing blood concentration of most PFAS over a relatively short period of time (i.e. from 2016 to 2018) in great and lesser black-backed gulls. A previous study showed that birds caught later on over the breeding season had lower concentration of PFAS (Bustnes et al., 2008a). However, all birds included in this study were sampled during the same period of the year (difference of a few days from one year to another), and, more importantly, all birds were sampled while incubating eggs, thus during the same reproductive state, which should not affect the results. Additionally, we cannot exclude that we unintentionally captured older birds in more recent years (assuming that PFAS levels increase with age in these species). However, considering that all individuals included in this study were adults, and assuming that PFAS concentrations in birds reach a steady level relatively early in life as previously shown for organochlorines (Bustnes et al., 2003), these trends should not be affected by the age of the bird. Information on temporal trends of PFAS in birds' tissues in recent years are scarce and do not exhibit any overall trend (Jouanneau et al., 2020; Land et al., 2018; Muir et al., 2019; Sun et al., 2019). Thus, although only three years of data could be included in the present study, our results provide valuable information on PFAS trends in South western France. Further work including several years of study is strongly warranted to corroborate these findings.

## 5. Conclusions

Our study provides the first evidence of the presence of high levels of PFAS in seabirds from South western France. Despite some PFAS showed similar levels of other seabird species, L-PFOS and some other PFAS showed either comparable or higher levels than highly contaminated seabird species, and may therefore pose a threat to long-lived seabirds. This hypothesis is further corroborated by our results showing an association between PFAS and the level of the thyroid hormone TT3. Similarly, we provide evidence that PFAS may interfere with lipid accumulation and body condition in birds, and we call for further work to experimentally test this hypothesis. Work on species-specific mechanisms of contaminant excretion and susceptibility to PFAS exposure would prove useful to understand the consequences of PFAS exposure in different species. Our results also document an increase in blood PFAS concentrations

over time, particularly in great black-backed gulls, suggesting that PFAS concentrations may also be increasing in the investigated species. Because PFAS have detrimental effects on birds, these and other seabird populations should be monitored as an increase of PFAS exposure may impact on population viability both in the short- and long-term.

## CRediT authorship contribution statement

**M. Sebastiano:** Writing – original draft, Resources, Investigation. **W. Jouanneau:** Writing – review & editing, Resources, Investigation. **P. Blévin:** Writing – review & editing, Resources, Investigation. **F. Angelier:** Writing – review & editing, Resources, Investigation. **C. Parenteau:** Resources, Formal analysis. **J. Gernigon:** Resources, Investigation. **J.C. Lemesle:** Resources, Investigation. **F. Robin:** Resources, Investigation. **P. Pardon:** Resources, Formal analysis. **H. Budzinski:** Writing – review & editing, Resources, Formal analysis. **P. Labadie:** Writing – review & editing, Resources, Formal analysis. **O. Chastel:** Writing – review & editing, Resources, Investigation, Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.144611>.

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## Appendix B

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# APPENDIX C



*Thick-billed murre (Uria lomvia) nesting on the rocks*



Long-term tracking of an Arctic-breeding seabird  
indicates high fidelity to pelagic wintering areas

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## Long-term tracking of an Arctic-breeding seabird indicates high fidelity to pelagic wintering areas

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**ABSTRACT:** Site fidelity is driven by predictable resource distributions in time and space. However, intrinsic factors related to an individual's physiology and life-history traits can contribute to consistent foraging behaviour and movement patterns. Using 11 yr of continuous geolocation tracking data (fall 2008 to spring 2019), we investigated spatiotemporal consistency in non-breeding movements in a pelagic seabird population of black-legged kittiwakes *Rissa tridactyla* breeding in the High Arctic (Svalbard). Our objective was to assess the relative importance of spatial versus temporal repeatability behind inter-annual movement consistency during winter. Most kittiwakes used pelagic regions of the western North Atlantic. Winter site fidelity was high both within and across individuals and at meso (100–1000 km) and macro scales (>1000 km). Spatial consistency in non-breeding movement was higher within than among individuals, suggesting that site fidelity might emerge from individuals' memory to return to locations with predictable resource availability. Consistency was also stronger in space than in time, suggesting that it was driven by consistent resource pulses that may vary in time more so than in space. Nonetheless, some individuals displayed more flexibility by adopting a strategy of itinerancy during winter, and the causes of this flexibility are unclear. Specialization for key wintering areas can indicate vulnerability to environmental perturbations, with winter survival and carry-over effects arising from winter conditions as potential drivers of population dynamics.

**KEY WORDS:** Spatial distribution · Individual consistency · Migration · Repeatability · Nearest neighbor distance · Biologging · Global Location Sensors · GLS

### 1. INTRODUCTION

Site fidelity, the propensity of organisms to return to previously occupied locations, is a common form of behavioural consistency across taxa (Switzer 1993, Börger et al. 2008, Piper 2011). Such spatial consistency is closely related to resource quality and pre-

dictability over space and time (Switzer 1993). Site fidelity for foraging patches may be favoured by individuals living in unpredictable environments, notably when resources are patchily distributed but spatially and temporally predictable (Switzer 1993). Returning to the same high-quality patch is reinforced by individuals' acquisition of local knowledge during previ-

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ous journeys, likely facilitating foraging efficiency (the 'always-stay' strategy; Switzer 1993, Irons 1998, Piper 2011). However, if changes in resource predictability and availability occur, fluctuations in behavioural consistency of individuals are expected to generate a decrease in site fidelity over time (the 'win-stay, lose-shift' strategy; Kamil 1983, Switzer 1993). The spatiotemporal predictability in resources is also a strong driver of migratory movements during which individuals follow consistent seasonal changes in environmental conditions (Mueller & Fagan 2008). Examples of individuals tracking predictable resources along a migratory pathway, sometimes over remarkable distances, include whales following planktonic blooms or geese following the 'green wave' of plant phenological development in spring (Alerstam & Hedenström 1998, Kölzsch et al. 2015, Abrahms et al. 2019). Resource tracking across space and time is likely driven by a trade-off between (1) memory of location and timing of profitable patches and (2) exploration of novel environments when memory is unable to locate profitable patches, with repeatability governed by both extrinsic and intrinsic factors that generate or constrain profitability (Fagan et al. 2013).

In marine systems, top predators, such as seabirds, are dependent on patchily distributed resources (Weimerskirch 2007, Fauchald 2009). Because predictability in pelagic resources is habitat- and scale-dependent, seabirds often rely on specific higher-quality foraging zones (e.g. shelf edges, frontal zones, upwellings) that are predictable at meso (100–1000 km) and macro scales (>1000 km; Weimerskirch 2007). Specialization in foraging behaviour would be optimal when resources are predictable, thus stimulating site fidelity in high-quality patches that show consistency in productivity over time (Barraquand & Benhamou 2008, Carroll et al. 2018). Extrinsic factors related to interactions with other species or the environment (e.g. competition, prey availability, heterogeneity in resources) can therefore have profound influence in determining individuals' movements across landscapes and distribution (Fayet et al. 2017). However, oceans are dynamic environments and currently undergoing major changes that can affect resource predictability (Cury et al. 2008, Polovina et al. 2008, Hoegh-Guldberg & Bruno 2010). Temporal and spatial changes in marine resource predictability and availability can lead to shifts in the distribution of seabirds (Hamer et al. 2001, 2007, Ceia et al. 2014, Orben et al. 2015). The resilience and adaptability of populations to such changes are closely related to phenotypic plasticity and variability in behavioural traits of individuals, with populations showing high

specialization in distribution, foraging behaviour, and diets being more sensitive to an alteration of their environment (Canale & Henry 2010, Patrick et al. 2015, de Grissac et al. 2016). Although seabirds often show within-individual wintering site fidelity (Ceia & Ramos 2015 and references therein), some species like the Cory's shearwater *Calonectris borealis* or the long-tailed skua *Stercorarius longicaudus* are highly flexible with some individuals shifting their winter distribution at the ocean scale (e.g. from the western to the eastern Atlantic Ocean; Dias et al. 2011, van Bemmelen et al. 2017). Flexibility in movement behaviour that is affected by extrinsic factors may only be measurable over large time scales, stressing the need for long-term movement tracking.

Intrinsic factors related to physiological or life-history traits, such as sex, age, nutrition state, or breeding status and investment, can also contribute to individual variation in foraging and movement patterns (Phillips et al. 2017). Failed breeders often depart on migration earlier than successful breeders, and this difference sometimes persists into the overwintering period with the previous breeding status affecting the winter distribution and the timing of the arrival at and departure from the wintering site (Phillips et al. 2005, Catry et al. 2013, Bogdanova et al. 2017). Personality can also influence movement patterns, with bold individuals typically displaying higher foraging site fidelity, while shy individuals are shifting sites and travelling farther (Patrick & Weimerskirch 2014, Krüger et al. 2019, Harris et al. 2020). Moulting patterns, flight capabilities, and levels of stress hormones can affect the migration timing and an individual's ability to engage in long migratory flights (Dawson et al. 2000, Guglielmo et al. 2001, Schultner et al. 2014, Cherel et al. 2016). Moreover, important life history stages that compose the annual life cycle such as migration or reproduction are controlled by physiological processes whose timing or duration is regulated internally by an individual's biological clock (the endogenous processes behind the biological rhythms) in response to temporally fixed cues (e.g. photoperiod; Kumar et al. 2010). Therefore, movement patterns driven by intrinsic factors with strong biological clock control (e.g. changes in physiology, body condition, moult) will tend to occur consistently between years over an individual's life (Kumar et al. 2010, Wascher et al. 2018). Overall, the mechanisms underlying inter-annual movement consistency involve complex interplay between extrinsic and intrinsic factors, and the relative contributions of those factors is likely to vary over time (Phillips et al. 2017).

Using a long-term tracking dataset of 11 yr of continuous non-breeding movement data (fall 2008 to spring 2019) of black-legged kittiwakes *Rissa tridactyla* breeding in Svalbard (High Arctic Norway), we investigated inter-annual variations in space utilization and movement phenology during the non-breeding period. The North Atlantic populations of kittiwakes largely congregate in winter at a shared staging area in the Western Atlantic Ocean (Frederiksen et al. 2012). Although the high degree of overlap in winter suggests site fidelity among and within populations, these results rely on 2 yr of data only (Frederiksen et al. 2012). Moreover, other studies showed that kittiwakes may alter their winter distribution according to intrinsic factors (e.g. stress hormone levels, breeding statuses, or reproductive investment; Schultner et al. 2014, Bogdanova et al. 2017, Whelan et al. 2020), which highlights the importance of multiple years of tracking data to assess the consistency of movement over time.

We estimated inter-annual consistency in movement among and within individuals (2 to 7 yr repeated per individual) and how this consistency varied over the course of the non-breeding period across a decade. Our main objective was, therefore, to assess the relative importance of spatial versus temporal repeatability underlying inter-annual movement consistency. Site fidelity can emerge from resource specialization where resources are predictable so that individuals would show high distribution repeatability for consistent high-quality foraging areas (Switzer 1993). Higher repeatability for space utilization would then suggest that individuals are specialized on certain resources and are more vulnerable to extrinsic factors (e.g. environmental conditions, resource availability). Timing of climatic events (e.g. sea ice breakup, peak in primary productivity) can show high interannual variations associated with fluctuations in strength of large-scale climatic and oceanographic systems like the North Atlantic Oscillation (NAO; Visbeck et al. 2001) or ocean gyres (Polovina et al. 2008). Individual specialization driven by individuals following consistent patches learned on previous winters might thus be expected to show consistency in space over time. In contrast, higher repeatability in time than space would suggest that individual specialization is driven by factors occurring consistently at the same time each year in relation to the individual biological clock. The temporal and spatial consistency in movement patterns can thus inform about the relative importance of extrinsic versus intrinsic parameters behind the site fidelity observed in a given population, but intrinsic and extrinsic fac-

tors often remain related to one another (i.e. hormonal clocks may change with foraging success driven by environmental variation), and the dominance of one factor over the other does not preclude the interaction or importance of both.

## 2. MATERIALS AND METHODS

### 2.1. Logger deployment and geolocation processing

From 2008 to 2018, we deployed 276 geolocators (light-loggers or Global Location Sensors, GLS) on black-legged kittiwakes *Rissa tridactyla* to track their non-breeding movements (Table S1 in the Supplement at [www.int-res.com/articles/suppl/m676p205\\_supp.pdf](http://www.int-res.com/articles/suppl/m676p205_supp.pdf)). Adults were captured using a nylon loop attached to a fishing rod at the colony site in Kongsfjorden, Svalbard (High Arctic Norway; 78° 54' N, 12° 12' E) between May (pre-breeding) and July (chick-rearing) and equipped with geolocators. We used mk18 and mk13 (British Antarctic Survey), mk4083 and mk4093 (Biotrack) and Intigeo F100 and C65 (MigrateTechnology) mounted on a Darvic leg band. Devices measured light intensity every minute and recorded the maximum light intensity every 5 or 10 min. They also measured conductivity (as proxy for bird immediate environment, i.e. immersion) every 3 or 30 s and stored the number of wet measurements for every 10 min period. We recaptured 83% of the individuals at their return to the colony and recovered the geocator (Table S1). Only complete annual tracks were used in the analyses after filtering out partial tracks caused by device failure or battery discharge. Overall, we acquired 200 complete tracks from 130 different individuals (see Fig. 1), covering 11 non-breeding seasons, continuously (fall 2008 to spring 2019). We tracked 33 individuals across multiple years (from 2 to 7 years) providing a total of 104 repeated tracks that were used to investigate within-individual consistency in movement. This repeated tracking was not always continuous, with gaps of 1 to 5 yr for 14 out of 33 individuals.

To infer geographic positions, geocator data were processed according to the procedure developed for the SEATRACK project (Bråthen et al. 2021) and based on the threshold method calculating positions from twilight events ('coord' function from GeoLight package; Hill & Braun 2001, Lisovski & Hahn 2012, Lisovski et al. 2020). The procedure automatically identifies twilight events from raw light data ('twilightCalc' function from GeoLight package; Lisovski

& Hahn 2012) and applies a set of filters to twilight events (removing or moving events from false day/night detections or noise) and positions (speed, distribution limits, angle filter). Thus, all the geolocator data were processed automatically and consistently for all years of the study. Because light sensors from different geolocator models may differ, each track was calibrated individually. As such, the calibration method avoided systematic bias in latitude related to potential differences in light sensors among geolocator models or years of production. Based on the approach by Hanssen et al. (2016) and van Bemmelen et al. (2019), the calibration method used a set of criteria that allowed calibration of tracks from kittiwakes breeding in the Arctic (79° N), where constant daylight prevents calibration at the time of deployment and recapture. By plotting the latitude against time for a range of sun elevation angles and for each track (Fig. S1 in the Supplement), the sun elevation angle that was manually selected (1) minimized the amplification of the latitudinal error close to the equinoxes, (2) resulted in matching latitudes at both sides of the equinox, (3) resulted in positions that fitted the latitude of the colony at the beginning and the end of the track and (4) fitted the shape and position of the oceans and continents when plotting the positions on a map (Fig. S2). The resulting sun elevation angle varied from  $-4.5$  to  $-2.5^\circ$  (mean angle  $-3.3^\circ$ ). The method also included rooftop calibration of geolocator models, with the purpose to select model specific thresholds that would result in approximately the same sun elevation angles among geolocator models. The mk-series geolocators from the British Antarctic Survey and Biotrack were assigned a threshold of 1 unit, while Intigeo geolocators from Migrate Technology were assigned a threshold of 11 units.

Although longitudes can still be determined reliably around the equinoxes, estimation of latitudes is inherently imprecise during this period, because day length is similar around the globe (Lisovski et al. 2012). Therefore, locations around equinoxes were excluded (8 Sep–20 Oct, 20 Feb–3 Apr; Bråthen et al. 2021). Additionally, continuous daylight during the polar summer (or continuous night during polar winter) does not allow geolocation-based tracking using light-level sensors. To fill these gaps and reduce biases along the trajectories, missing locations were re-estimated by interpolation between known locations using an algorithm that was specifically developed for SEATRACK (Fauchald et al. 2019), based on a method originally proposed by Technitis et al. (2015). In short, this algorithm is based on the determination of so-called space-time prisms, which are 3-

dimensional volumes defined by the coordinates ( $x, y$ ) and time ( $z$ ). The space-time prism delineates all the potential paths that can be followed by an individual moving from point A to point B, given 3 parameters: the distance from A to B, the time budget available, and the maximum rate of movement (Miller 1991). When projected onto a 2-dimensional plane, the space-time prism becomes the potential point area (hereafter *Ppa*; Technitis et al. 2015). Although the 3-dimensional representation of the space-time prism is useful to understand its concept (Neutens et al. 2007), it is naturally more convenient to work with only 2 dimensions when dealing with discrete time steps, as is the case in tracking studies, where locations are obtained at specific time intervals. Computing the *Ppa* in this context is straightforward (Technitis et al. 2015), given that the 3 above-mentioned parameters are known. Let us consider a startpoint (A) and start time ( $t_{i-1}$ ), and an endpoint (B) and end time ( $t_{i+1}$ ). Knowing the maximum rate of movement and the time  $t_i$  at which a new location ( $N_i$ ) is to be created, one can determine the circle defining the maximum range ( $rg_{i-1}$ ) from point A to the new location and the circle defining the maximum range ( $rg_{i+1}$ ) to point B, centered on B. The *Ppa* corresponds to the area of overlap between those 2 circles of maximum range, i.e. the area delimiting all locations that are reachable from both A and B, given the time budget and maximum movement rate. This process can be repeated any number of times, depending on the number of new locations that need to be generated. The new locations are generated in a random order (i.e. not chronological), thus creating a sort of correlated random walk respecting the constraints set by the relative position of A and B, the time budget, and the maximum movement rate. Here, we used a dynamic value for the maximum movement rate parameter, based on the distribution of observed movement rates as a function of time elapsed between 2 locations from the dataset. To do so we calculated, based on each individual track, the movement rates for random combinations of known locations separated by varying time-intervals. We used the 75<sup>th</sup> percentile from that distribution as the maximum movement rate (Fig. S3). The 75<sup>th</sup> percentile was computed by quantile regression, using the function 'rq' from package *quantreg* (Koenker 2020). Finally, the algorithm uses additional information to constrain the new positions obtained: (1) immersion data to determine attendance at the colony and force a new location to remain close to the colony during the breeding season, (2) land masks (land filters) to constrain positions over the ocean, (3) longitudes (obtained from the geolocator

data, as longitude can still be estimated during the equinoxes), and (4) light levels to determine whether the new position was north of the latitudinal limit of the polar day in summer or night in winter (i.e. continuous day/night recorded by the loggers).

In all further analyses, an annual track refers to the non-breeding period extending from the colony departure in fall to the return to the colony area the following spring. Departure from the colony and return to the colony were identified using Lavielle partitioning algorithm ('ts.LaviellePart' function from R package *adehabitatLT* v.0.3.25; Calenge 2006, Barraquand & Benhamou 2008) over a 5 d running maximum of the saltwater immersion data indicating a transition between land use (mostly dry) and continuous pelagic behaviour (mostly wet). Departure and arrival dates were adjusted according to visual inspection of the individual's locations right after the behavioural transition from land use to pelagic in fall, and right before the transition from pelagic to land use in spring. In spring, foraging trips after the first visit to the colony area were excluded, as individuals start to display a central place foraging behaviour, including long pre-laying trips as far as Iceland (Bogdanova et al. 2011).

## 2.2. Consistency in intertrack distances

To estimate the consistency in non-breeding movement over the entire annual tracks either among or within individuals, we used an approach based on the nearest neighbour analysis (for similar methods see Guilford et al. 2011, Dias et al. 2013, van Bemmelen et al. 2017). For each location of a focal track, we calculated the orthodromic distance to the nearest location on (1) a randomly selected track from another individual to estimate among-individual consistency and (2) a track from the same individual but from another year to estimate within-individual consistency. Pre-breeding movements were excluded, and positions were considered to be fixed at the colony after the first visit in the colony area in spring. This nearest neighbour distance was calculated over a large time window (60 d) to assess spatial consistency in movement. The 60 d time window was selected after running a sensitivity analysis using different time windows varying from 1 to 120 d (with 10 d intervals) to assess when apparent variation related to timing differences fades (Fig. S4). The time window we selected allows spatial comparison without overlaps between fall and spring migrations that would be created if using a larger time window (i.e. >100 d). We repeated the

method described above with a 1 d time window (i.e. daily comparison) to compare dissimilarities among tracks generated by both spatial and timing differences. We bootstrapped the resulting distances 10 000 times to calculate the mean intertrack distance per day among and within individuals. The results were log-transformed before further analysis to meet the assumptions of homoscedasticity and normality of residuals. We fitted linear mixed-effects models (LMER, R package *lme4* v.1.1-23; Bates et al. 2020) to determine if among- and within- individual distances differ, using the mean intertrack distance for each annual track, with individual and year as random factors. Sex had no effects on individuals with known sex (LMER; using the 60 d time window:  $\beta = 0.05$ , SE = 0.05, df = 107.1,  $p = 0.318$ ; using the 1 d time-window:  $\beta = 0.04$ , SE = 0.03, df = 116.7,  $p = 0.191$ ) and was therefore discarded from the final models on all individuals. Following the method used by van Bemmelen et al. (2017), the mean intertrack distance calculated over the large time window (60 d) was mapped to illustrate both among- and within-individual spatial consistency in movement during the non-breeding period.

## 2.3. Variability in migration schedule

To illustrate the variability in migration schedule, we extracted parameters associated to important migration phenology events: (1) the departure from the colony, (2) the start of the southward migration movement, (3) the crossing of the Arctic Circle in fall corresponding to the end of the post-breeding staging in the northern regions (Barents and Greenland Seas), (4) the start of the northward migration movement and (5) the arrival at the colony defined as the first visit to the colony area in spring. We estimated the variability in the timing of each phenological parameter at the population level. To do so, we calculated the difference between the latest date in a given year and the dates from all other individuals in the same year and then averaged these differences over the 11 yr of the study. We also estimated the within-individual variability in migration timing for each phenological parameter for individuals tracked multiple years ( $n = 33$  individuals, 104 annual tracks). This was done by calculating the difference between the latest date for a given individual and the dates from all the other years this individual was tracked and then averaged over all individuals. Mean dates are reported with their standard deviation. Finally, we estimated the individual repeatability  $r$  (intra-class correlation) for

the timing of each phenological parameter. This was done using the function 'rpt' of the R package rptR v.0.9.22 (Stoffel et al. 2017) and using only individuals with multiple years of tracking (see Table S3). We used 'year' as random effect only for the 'colony departure' to account for important interannual variation in this parameter. The repeatability estimate  $r$  is not a measure of absolute consistency but the proportion of total variance accounted for by differences between groups (i.e. in our case among individuals; Nakagawa & Schielzeth 2010). All analyses were carried out in R version 4.0.2 (R Core Team 2020).

#### 2.4. Non-breeding staging areas

We used Hidden Markov Models (HMMs; Zucchini et al. 2017) to examine at-sea behaviour of individuals and identify the sequence of discrete behavioural states that best fitted the non-breeding tracks of individuals. HMMs were fitted to all individuals at once using R package moveHMM v.1.7 (Michélot et al. 2016) with gamma and von Mises distributions to describe the frequency of step length and turning angle distributions, respectively. Different initial parameter values were tested to ensure numerical maximization of the likelihood through the iteration process. A 4-state model was selected after examination of the pseudo-residuals (Michélot et al. 2016) and because it better fitted the geolocation data than simpler 2- or 3-state models based on AICs and initial inspection of distribution of movement parameters (see Table S4 and Fig. S5). States 1 and 2 were defined by short steps (<85 km) with either frequent shifts in direction (angle concentration of 0; State 1) or moderately directional movement (angle concentration of 1; State 2) and attributed to periods of staging in more intensively utilized areas (see Table S4). States 3 and 4 were defined by long steps (>150 km) with moderate shifts in direction (angle concentration of 0.5; State 3) or highly directional movement (angle concentration of 7; State 4) that characterized transient and commuting behaviours during travelling periods (see Table S4). In further analyses, the first 2 states were combined into a broader 'staging state' and the last 2 states into a 'travelling state' to determine stationary and travelling positions, respectively. We were only interested in the stationary positions (staging state), which we mapped separately for the fall migration, winter, and spring migration periods to illustrate the main staging areas during each period (see Fig. 1). Therefore, the 'staging areas' refer to more intensively utilized areas throughout the non-

breeding period. Finally, we calculated the 80 and 50% utilization distribution kernels (UDs) over the stationary positions projected using a Lambert Azimuthal Equal Area coordinate system and the R package adehabitatHR v.0.4.18 (Calenge 2006) with a smoothing factor ( $h$ ) of 200 km and grid cells of 50 × 50 km. We used the kernels to identify important staging areas for our study population and to illustrate the correspondence between these staging areas and the spatial consistency in the intertrack distance of individuals.

### 3. RESULTS

#### 3.1. Individual tracking and migratory routes

The mean annual departure date from the colony varied from 27 August to 25 September (5 September ± 9 d [SD]). Individuals staged within the Arctic in the Barents and Greenland Seas (mean annual staging from 38 to 64 d, 52 ± 7 d) before migrating southwest along a corridor between East Greenland and Iceland (Fig. 1). The mean annual date of onset of fall migration ranged from 10 to 29 October (19 October ± 5 d). All individuals spent the winter in the North Atlantic Ocean, with the main wintering area extending from the Grand Banks of Newfoundland to the mid-Atlantic ridge. The winter distribution of the population was therefore largely pelagic, but alternative staging areas were also used along the continental shelves of Northeast America and Western Europe (Fig. 1 and see UD of Fig. 3). The northeast movement of spring migration (mean annual starting date from 31 March to 9 April, 4 April ± 3 d) was spread over a larger front than in fall, with routes passing both north and south of Iceland (Fig. 1). The mean annual date of arrival in the colony area varied from 10 to 20 April (15 April ± 5 d).

#### 3.2. Non-breeding movement consistency in space

The nearest neighbour analysis conducted over a large time window (60 d) indicated high spatial consistency during the non-breeding period in the studied population, both within and among individuals (Fig. 2A, see also examples of repeated tracks from several individuals in Fig. S6). The mean within-individual intertrack distance was 261 km (95% CI: 180–376 km), significantly lower than the mean intertrack distance of 545 km found among individuals (95% CI: 377–

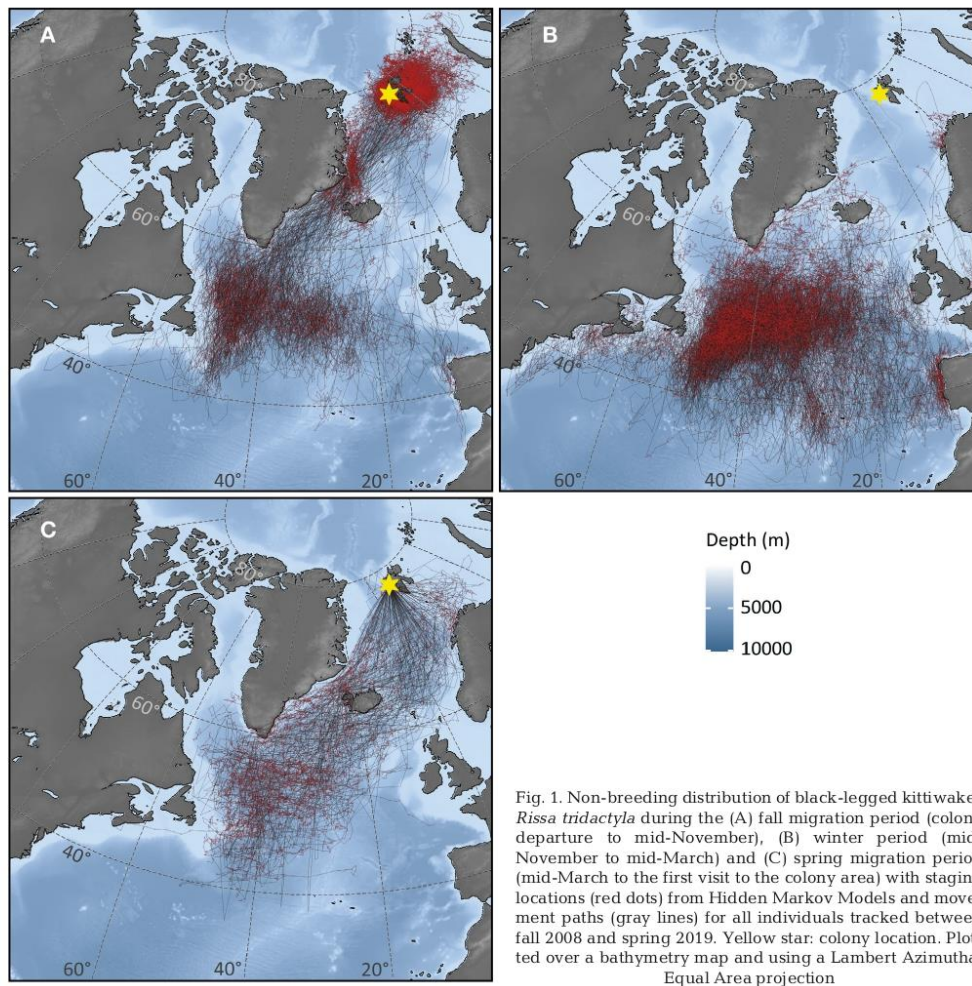


Fig. 1. Non-breeding distribution of black-legged kittiwakes *Rissa tridactyla* during the (A) fall migration period (colony departure to mid-November), (B) winter period (mid-November to mid-March) and (C) spring migration period (mid-March to the first visit to the colony area) with staging locations (red dots) from Hidden Markov Models and movement paths (gray lines) for all individuals tracked between fall 2008 and spring 2019. Yellow star: colony location. Plotted over a bathymetry map and using a Lambert Azimuthal Equal Area projection

793 km; LMER,  $\beta = 0.71$ , SE = 0.04, 95% CI: 0.62; 0.80, df = 218.9,  $p < 0.001$ ). The mean within-individual intertrack distance was consistent regardless of the number of years individuals were tracked (LM,  $\beta = 12.2$ , SE = 23.7,  $t_{31} = 0.5$ ,  $p > 0.609$ ), indicating that site fidelity persists over longer tracking periods (range 2 to 7 yr). Mapping intertrack distances (Fig. 3) showed areas of high spatial consistency among individuals in the western part of the North Atlantic Ocean as well as in the Greenland Sea (east coast of Greenland and Svalbard) and in the Barents Sea (between Svalbard

and Novaya Zemlya, Fig. 3A). These sectors of high spatial consistency correspond to the main staging areas identified with the Hidden Markov Models (Fig. 1) and illustrated with 80 and 50% utilization distribution kernels (Fig. 3A). Similarly, areas associated with high consistency within individuals were also associated with staging areas in the Northwest Atlantic Ocean and the Greenland and Barents Seas but also with areas along the coasts of Northeast America and, to a lesser extent, Western Europe (Iberian Peninsula and British Isles, Fig. 3B).



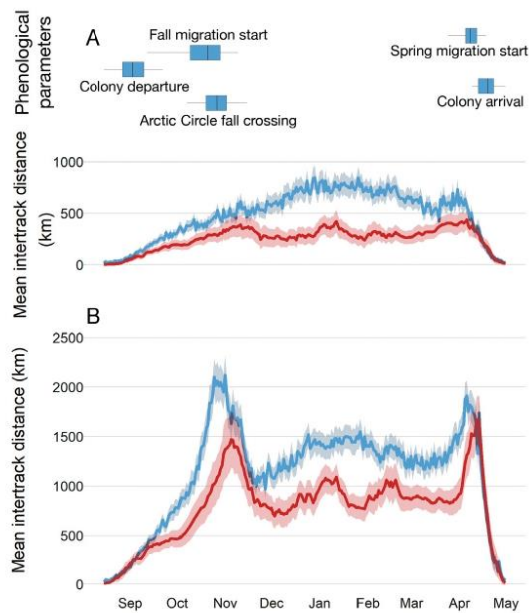


Fig. 2. Consistency in non-breeding movements of black-legged kittiwakes *Rissa tridactyla* from bootstrapped mean intertrack distance estimated among (blue) and within (red) individuals. (A) Consistency in space with distances estimated over a large time window (60 d), reducing timing effects in movement dissimilarities. (B) Consistency in space and time with distances estimated per day (1 d time window). Shaded areas: 95% confidence intervals of the bootstrapped mean intertrack distance. Boxplots show among-individual variation in the timing of phenological events associated with the migration periods (boxes: 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> percentiles; whiskers: 5<sup>th</sup> and 95<sup>th</sup> percentiles)

Across the non-breeding season, the kittiwake distribution was mostly pelagic with 73% of the locations (32 327 out of 44 071 locations in total) in areas deeper than 500 m. High spatial consistency within individuals was also found in coastal areas and shallow waters of the Barents Sea (see Fig. 3), but the use of these areas was limited. The utilization of deeper waters was even more evident during the winter period (mid-November to mid-March), with only 12% of the locations found in areas of 0–500 m depth (2816 out of 24 034 winter locations). Overall, spatial consistency within individuals remained high over deeper waters (Fig. S7), indicating that site fidelity was also common in pelagic areas.

### 3.3. Non-breeding movement consistency in time

The nearest neighbour distance analysis conducted over a short time window (daily comparison to include variations associated with timing effects) also showed higher consistency in mean intertrack distances (Fig. 2B; LMER,  $\beta = 0.39$ , SE = 0.02, 95% CI: 0.35; 0.44, df = 204.9,  $p < 0.001$ ) within (847 km, 95% CI: 694–1026) than among individuals (1257 km, 95% CI: 1034–1546). Using a short time window in the analysis revealed peaks in timing variability during fall (October to mid-November) and spring (mid-March to mid-April) associated with migratory stages (Fig. 2B). Individual repeatability estimation of phenological parameters during migration stages (Fig. 4B) showed low individual repeatability in timing of post-breeding colony departure ( $r = 0.20$ , 95% CI: 0.02; 0.39,  $p < 0.001$ ) driven by a relatively high intra-individual variability (see Fig. 4A and Table S4), high repeatability in the onset of the fall migration movement ( $r = 0.80$ , 95% CI: 0.65; 0.88,  $p < 0.001$ ), and moderate repeatability in the timing of the crossing of the Arctic Circle after the post-breeding staging in the Barents and Greenland Seas ( $r = 0.54$ , 95% CI: 0.33; 0.69,  $p < 0.001$ ). In spring, the very low repeatability estimates in the timing of the onset of the migration movement ( $r = 0.36$ , 95% CI: 0.12; 0.55,  $p = 0.002$ ) and the arrival in the colony area ( $r = 0.28$ , 95% CI: 0.04; 0.49,  $p = 0.016$ ) were driven by high consistency in the timing of these events among individuals (see Fig. 4B and Table S3). Overall, the timing of phenological events, both within and among individuals, showed higher variability during the fall period than during the spring period (Fig. 4A and Table S2), indicating the spring migration occurred during a shorter time interval.

## 4. DISCUSSION

Kittiwakes showed high spatial consistency in non-breeding movements at both macro (>1000 km) and meso scales (100–1000 km), suggesting that individuals benefit from predictability in resources at these coarse scales. The significantly higher consistency within individuals than among individuals indicated specialization in space-use strategy at the individual level. With a deviation in routes among years of less than 300 km on average, the inter-annual spatial consistency of individuals indicated important site fidelity at the individual level, especially when considering the coarse resolution of geolocator measurements and the wide ocean-scale distribution and

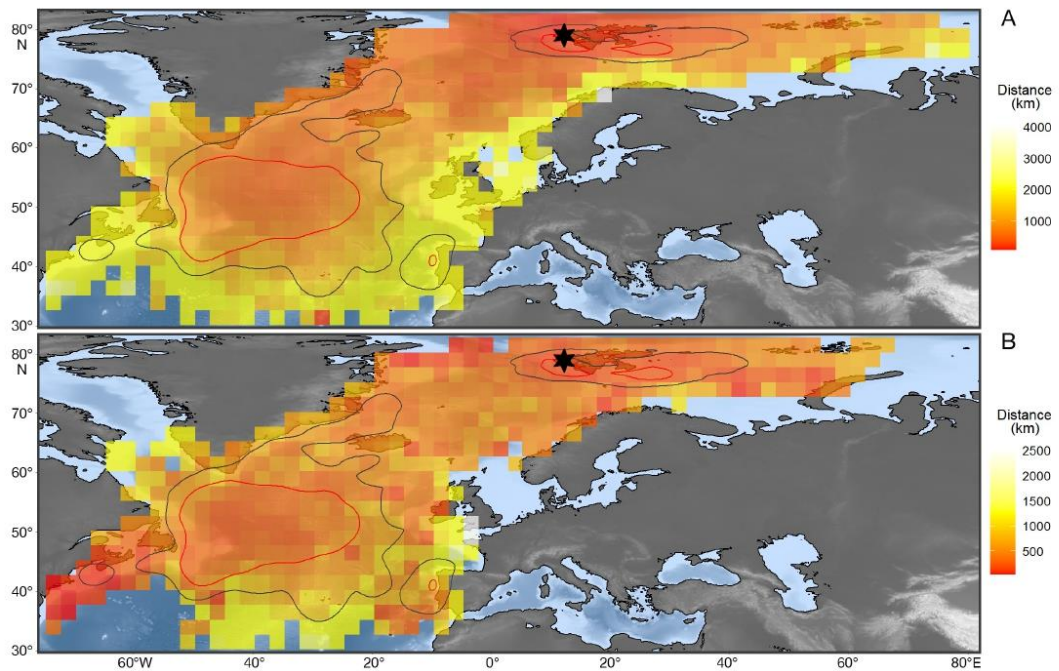
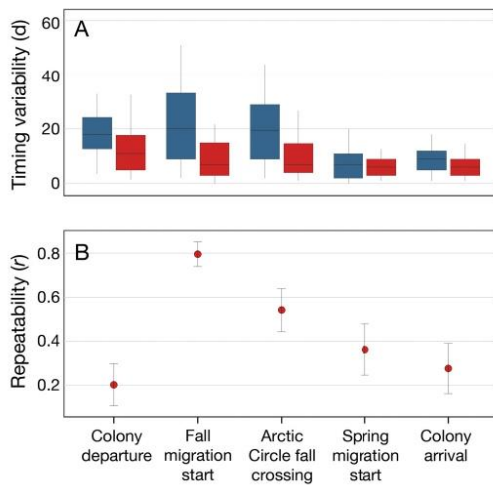


Fig. 3. Heat maps of mean intertrack distances showing (A) spatial consistency among individuals and (B) within individuals during the non-breeding period. Distances are estimated with the nearest neighbour distance analysis using a large time window (60 d) to exclude most of the variability associated with timing effect and illustrate consistency in space only. Darker grid cells (2.5° latitude × 2.5° longitude) indicate areas of higher spatial consistency in movement. Polygons: utilization distribution (80 % kernel in gray and 50 % kernel in red) calculated over the stationary locations only. Black star: colony location



long migrations of the studied population. In contrast, we found low repeatability in the timing of important phenological events in general, which suggests that those intrinsic timing mechanisms known to be repeatable across years, such as the individual's biological clock, may not have been a strong driver of the movement consistency of individuals over the years of the study (but see Section 4.3). Thus, repeatability is likely governed by memory of the location of high-quality patches relatively unconstrained by intrinsic factors. This individual spatial consistency was stable over the entire non-breeding sea-

Fig. 4. (A) Observed variation in timing of phenological parameters estimated at the population level (blue boxes) and individual level (red boxes). Boxes: 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> percentiles; whiskers: 5<sup>th</sup> and 95<sup>th</sup> percentiles. (B) Individual repeatability in timing of phenological parameters (inter-class correlation coefficient,  $r$ ) with standard errors, where 0 = low repeatability and 1 = high repeatability

son, with site fidelity to the wintering staging areas and also to the migratory staging sites used during fall in the Barents and Greenland Seas.

#### 4.1. Fidelity to deep-water areas

High fidelity to staging areas outside the breeding season is not uncommon in seabirds and is often associated with more predictable areas along continental shelves and less with oceanic habitats (Weimerskirch 2007). For instance, highly productive upwelling habitats along shelf edges in the Canary and Benguela current systems were associated with site fidelity during the non-breeding period in black-browed albatrosses *Thalassarche melanophrys*, Cory's shearwaters *Calonectris borealis* and long-tailed skuas *Stercorarius longicaudus* (Phillips et al. 2005, Dias et al. 2011, van Bemmelen et al. 2017). Similarly, kittiwakes tracked in this study used the shelf edge of the Grand Banks, an area known for its high biological productivity (Heywood et al. 1994, Maillet et al. 2005, Frederiksen et al. 2012). However, many individuals also wintered in deep waters, from the edge of the Grand Banks plateau to the mid-Atlantic Ridge. Despite this highly pelagic distribution, individuals showed area fidelity, suggesting this deep-water area can provide habitats with enough coarse-scale predictability in resources to stimulate area fidelity. In comparison, black-legged kittiwakes tracked in the North Pacific also showed some degree of individual fidelity to pelagic areas (~25% of locations within 400 km grid squares; Orben et al. 2015), but to a lesser extent than what we found in the North Atlantic (~75% of locations within 400 km of mean nearest neighbour distance). However, a similar degree of spatial consistency (median nearest neighbour distance 250 to 400 km) to what we found with kittiwakes was described in long-tailed skuas for the same oceanic area west of the mid-Atlantic ridge, which they used as a staging area during migration (van Bemmelen et al. 2017).

This oceanic region is crossed by the subpolar front extending from the Newfoundland Rise to the mid-Atlantic Ridge and characterized by a strong horizontal temperature gradient, eddies, and nutrient mixing and retention inducing biological productivity enhancement (Heywood et al. 1994, Scales et al. 2014, Hátún et al. 2016). There is evidence that this mid-ocean frontal zone is an important staging site and a diversity hotspot for multiple seabirds (e.g. Guilford et al. 2009, Egevang et al. 2010, Hedd et al. 2012, Montevecchi et al. 2012, Weimerskirch et al.

2015), as well as other marine predators such as sharks (Queiroz et al. 2016), tunas (Walli et al. 2009), chelonoid turtles (Eckert 2006), and cetaceans (Doksaeter et al. 2008, Skov et al. 2008). This large overlap in distribution has stressed the potential vulnerability of marine vertebrate populations relying on this area to large-scale changes in environmental conditions affecting resource predictability and availability (Frederiksen et al. 2012), such as the weakening of the subpolar gyre and warming of the North Atlantic (Häkkinen & Rhines 2004, Descamps et al. 2013, 2017, Fluhr et al. 2017, Hátún et al. 2017). Populations showing important site fidelity are expected to be more sensitive to extrinsic factors affecting resource predictability and might thus be particularly impacted by such carry-over effects (Phillips et al. 2017).

#### 4.2. Movement strategy in space

To a certain extent, marine predators are expected to adjust their space-use strategy in response to environmental changes affecting resource predictability of foraging patches over time (Davoren et al. 2003, Wakefield et al. 2015). This ability to respond to environmental variability by using a 'win-stay, lose-shift' strategy directly depends on the flexibility and plasticity in behavioural traits intrinsic to the population and individuals (Canale & Henry 2010). Area shifting was uncommon in our study, and we found an overall high spatial consistency, even for individuals tracked up to 7 years. This long-term area fidelity could be expected to arise if individuals benefited from consistency in resource availability in known staging areas over the course of the study, thus preventing the need for important shifts in distribution at meso or macro scale. Alternatively, long-term site fidelity can be reinforced by site familiarity and the benefits of acquiring information specific to an area, leading to individuals favouring an 'always-stay' strategy (Irons 1998, Wakefield et al. 2015). Advantages of returning to a known area include increased foraging efficiency through knowledge about food location and availability, movement efficiency by using prevailing wind corridors, dominance during competitive interactions as well as avoiding potential risks of visiting unfamiliar places, such as higher risks of getting stranded (Piper 2011). In contrast, some seabird populations show high flexibility in their non-breeding distribution with individuals possibly having several preferred migratory strategies (Dias et al. 2011, van Bemmelen et al. 2017). For example, some long-

tailed skuas shifted their winter distribution between years at the ocean scale, between the Benguela current and the Falkland current (van Bemmelen et al. 2017). They followed a specific route for each alternative wintering site and kept the same route over the years, indicating this shifting behaviour was not accidental but based on past experience. Similarly, Cory's shearwaters used wintering areas independently of prevailing wind currents encountered on route, suggesting the choice of using one or another alternative site was deliberate and predetermined (Dell'Ariccia et al. 2018). We also found that some kittiwakes showed flexibility in their migratory decisions by shifting their staging areas or by displaying a more exploratory behaviour with different degrees of itinerancy, although this occurred at a more modest scale than in long-tailed skuas and Cory's shearwaters. If a decrease in resource availability can stimulate area-shifting, the cause of the flexibility observed in some individuals can emerge from a diversity of factors both extrinsic or intrinsic, and thus remains unclear. For instance, the breeding status is likely to vary across years and is known to introduce inter-annual variability in individuals' space-use strategies (Phillips et al. 2017). In some species, failed breeders engaged in longer (Bogdanova et al. 2011) or shorter migration (Phillips et al. 2005) compared to successful breeders, indicating that the choice of wintering sites can be condition-dependent to the breeding investment.

#### 4.3. Plasticity in timing of movement

The dynamics of large-scale climatic and oceanographic systems, such as the subpolar gyre, generate strong interannual variations (up to several weeks) in the timing of biological productivity (i.e. phytoplankton bloom) in the North Atlantic (Gaard et al. 1998, Henson et al. 2009), with cascading effects on higher trophic levels (Henson et al. 2009, Eliassen et al. 2011). In response to these interannual fluctuations, marine predators following foraging patches learned on past travels are expected to show higher movement consistency in space than in time. This pattern is what we observed with individuals showing high spatial consistency, suggesting that individual specialization is driven more by space utilization in relation to extrinsic factors with higher predictability in space than in time.

Similarly, when investigating phenological parameters associated with migratory movement, we observed overall low repeatability in timing across

years. Notably, we found low repeatability in the timing of the colony departure, a phenological parameter often related to breeding status and reproductive investment (Bogdanova et al. 2011, Whelan et al. 2020). In another study, food supplementation enabled fed kittiwakes to initiate departure from the colony earlier than unfed kittiwakes, indicating that individuals experiencing lower breeding costs are able to transition into the non-breeding season in better condition and initiate migration earlier (Whelan et al. 2020). Interestingly, high repeatability only occurred for the timing of the onset of the fall migration. Considering the large range of departure dates in the population, this high within-individual repeatability suggests that intrinsic timing mechanisms played an important role in the phenology of the fall migration movement. This has been demonstrated with blue whales *Balaenoptera musculus*, where movement phenology was driven not by proximate environmental factors, but rather by the average timing of these factors across years at specific staging sites (Abrahms et al. 2019). This highlights the importance of memory and individual biological clock as intrinsic factors regulating the timing of individual movement in this long-lived species.

Additionally, the low individual repeatability in phenology of the spring migration was driven by low variation among individuals. This synchronicity in phenology in spring, coupled with high variation in timing of phenological parameters among individuals in fall, indicated that spring migration might be more time constrained. This is often the case for polar and subpolar migrants, as the optimal arrival date at the breeding site is a compromise between costs and benefits of an early arrival with individuals benefiting from e.g. a better nesting site or higher mating success while confronted with the risk of being exposed to harsh environmental conditions in early spring (Kokko 1999). These constraints are usually relaxed or absent in fall, leading to more variability in the timing of the fall migration (Nilsson et al. 2013), but carry-over effects arising from the breeding status and investment can also contribute to among-individual variations in post-breeding migration patterns (Harrison et al. 2011).

In conclusion, black-legged kittiwakes were remarkably consistent in their overwinter locations among years despite wintering primarily in pelagic regions of the North Atlantic. The consistency was stronger within than among individuals, implying that individuals were using memory to return to profitable locations year upon year. Consistency was also stronger in space than in time, suggesting that it was

driven by consistent resource pulses that vary in time more so than space, and that intrinsic drivers (photo-period, hormones, condition) that are known to be repeatable within individuals were somewhat less important. Of course, intrinsic and extrinsic factors often interact with one another (i.e. foraging success can influence body condition which can influence hormones), and in reality, both types of factors will be integrated by the individual to decide when to stay or go. Consistent use of key foraging locations is likely associated with foraging success, risk of starvation, and a strong driver of overwintering survival, linking variation in timing of resources to individual fitness and, ultimately, population trends.

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# APPENDIX D



*Glaucous gull (Larus hyperboreus) in flight*



## Appendix D

In the table below I aimed at gathering all published data on PFAS concentrations in seabird eggs (nonetheless, some publications may be missing). I limited these publications to those focusing on large and small gull species (Laridae family). Only PFAS found above the limit of detection are included, those not detected or not investigated are represented similarly (nd). Moreover, only PFAS examined in this thesis are provided. Values are in ng g<sup>-1</sup> wet weight.

### Black-legged kittiwake (*Rissa tridactyla*)

Tissue		Yolk	Whole eggs	Whole eggs
Sampling year		2019 – 2020	2017	2008
Location		Svalbard	Northern Norway	Canada
Reference		Paper A and C	Herzke <i>et al.</i> 2022	Braune <i>et al.</i> 2013
		Mean ± SD	Mean	Mean ± SD
Compounds	Carbon number			
PFHxS	C <sub>6</sub>	0.25 ± 0.15	0.03	nd
PFOS	C <sub>8</sub>	19.0 ± 8.65	12	9.58 ± 0.96
PFOA	C <sub>8</sub>	0.16 ± 0.10	0.11	nd
PFNA	C <sub>9</sub>	2.01 ± 1.20	0.64	nd
PFDCa	C <sub>10</sub>	2.98 ± 1.48	1.4	nd
PFUnA	C <sub>11</sub>	12.9 ± 6.19	6.5	nd
PFDoA	C <sub>12</sub>	3.32 ± 1.55	1.64	nd
PFTriA	C <sub>13</sub>	17.1 ± 8.23	8.69	nd
PFTeA	C <sub>14</sub>	3.85 ± 2.48	1.21	nd
PFHxDA	C <sub>16</sub>	0.23 ± 0.13	nd	nd
∑ <sub>PFASs</sub>	-	19.2 ± 8.63	-	-
∑ <sub>PFCA</sub> s	-	42.5 ± 19.9	-	9.71 ± 1.93
∑ <sub>PFAS</sub>	-	61.6 ± 27.2	32.2	

## Appendix D

**Yellow-legged gull (*Larus michahellis*)**

Tissue	Yolk	Yolk	Whole eggs	Whole eggs	Whole eggs	Whole eggs	
Sampling year	2021	2016	2009 - 2018	2010	2009	2009	
Location	Southern France	Italy	Spain	Spain	Spain	Spain	
Reference	<b>Paper D</b>	Parolini <i>et al.</i> 2020	Colomer-Vidal <i>et al.</i> 2022	Morales <i>et al.</i> 2012	Bertolero <i>et al.</i> 2015	Vicente <i>et al.</i> 2012	
Compounds	Carbon number	Mean ± SD	Mean ± SD	Mean ± SD (different colonies)	Mean	Mean ± SD	Range (min to max)
PFHxS	C <sub>6</sub>	1.76 ± 1.40	0.80 ± 0.50	nd	0.50	nd	nd
PFOS	C <sub>8</sub>	55.4 ± 27.4	46.2 ± 15.0	10.5 ± 4.2 to 101 ± 6	75.0	5.1 ± 31	10.1 to 54.0
PFOA	C <sub>8</sub>	0.93 ± 0.38	3.60 ± 1.50	nd	nd	nd	nd
PFNA	C <sub>9</sub>	0.98 ± 0.38	2.00 ± 0.70	nd	1.70	nd	nd
PFDcA	C <sub>10</sub>	1.46 ± 0.54	3.00 ± 1.00	nd	nd	nd	nd
PFUnA	C <sub>11</sub>	1.66 ± 0.81	3.10 ± 1.30	nd	nd	nd	nd
PFDcA	C <sub>12</sub>	2.00 ± 0.78	3.20 ± 1.40	nd	nd	nd	nd
PFTriA	C <sub>13</sub>	2.78 ± 1.18	nd	nd	nd	nd	nd
PFTeA	C <sub>14</sub>	2.62 ± 1.34	nd	nd	nd	nd	nd
PFHxDA	C <sub>16</sub>	0.66 ± 0.22	nd	nd	nd	nd	nd
∑PFSAs	-	57.1 ± 28.0	-	-	-	-	-
∑PFCAs	-	13.1 ± 4.66	-	-	-	-	-
∑PFAS	-	70.2 ± 31.1	62.5 ± 18.7	-	77.2 ± 0.03	-	-

## Appendix D

**European herring gull (*Larus argentatus*)**

Tissue		Whole eggs	Whole eggs		Whole eggs	Yolk	Whole eggs	Whole eggs
Sampling year		2017	2017		2015	2007 – 2009	1988 – 2008	1983 – 2003
Location		Southern Norway	Southern Norway (Inner fjord)	Southern Norway (Outer fjord)	Germany	Sweden	Baltic and North Seas	Northern Norway
Reference		Thorstensen <i>et al.</i> 2021	Knudtzon <i>et al.</i> 2021		Kotthoff <i>et al.</i> 2020	Nordén <i>et al.</i> 2013	Rüdel <i>et al.</i> 2010	Verreault <i>et al.</i> 2007
		Mean ± SD	Mean ± SD		Mean ± SD	Median	Range (min to max)	Maximum value
Compounds	Carbon number							
PFHxS	C <sub>6</sub>	nd	nd	nd	nd	1.16	nd	nd
PFOS	C <sub>8</sub>	nd	25.6 ± 42.3	38.5 ± 32.2		423	60 to 80	42
PFOA	C <sub>8</sub>	nd	nd	nd	nd	0.82	1 to 10	nd
PFNA	C <sub>9</sub>	nd	nd	nd	nd	7.6	nd	nd
PFDCa	C <sub>10</sub>	nd	nd	nd	nd	34.6	nd	nd
PFUnA	C <sub>11</sub>	nd	nd	nd	nd	45.1	nd	4.2
PFDoA	C <sub>12</sub>	nd	nd	nd	nd	19.3	nd	nd
PFTriA	C <sub>13</sub>	nd	nd	nd	nd	23.1	nd	2.8
PFTeA	C <sub>14</sub>	nd	nd	nd	nd	5.41	nd	nd
PFHxDA	C <sub>16</sub>	nd	nd	nd	nd	nd	nd	nd
∑PFSAs	-	-	-	-	37.0 ± 1.48	-	-	-
∑PFCAs	-	-	3.49 ± 2.78	5.11 ± 4.67	8.03 ± 2.04	-	-	-
∑PFAS	-	-	-	-	45.5 ± 3.16	-	-	-

## Appendix D

**American herring gull (*Larus smithsonianus*)**

Tissue	Whole eggs	Whole eggs	Yolk	Whole eggs	2007	
Sampling year	2012 – 2014	2012– 2013	2010	2008	Whole eggs	
Location	USA & Canada	USA & Canada	Canada	Canada	USA & Canada	
Reference	Su <i>et al.</i> 2017	Letcher <i>et al.</i> 2015	Gebbink <i>et al.</i> 2012	Gebbink <i>et al.</i> 2011	Gebbink <i>et al.</i> 2009	
		Mean	Mean approximation	Mean	Mean of colonies means	Mean of 15 colonies
Compounds	Carbon number					
PFHxS	C <sub>6</sub>	0.55	0.496	nd	nd	nd
PFOS	C <sub>8</sub>	142	201	nd	nd	nd
PFOA	C <sub>8</sub>	0.27	nd	nd	nd	nd
PFNA	C <sub>9</sub>	3.09	nd	nd	nd	nd
PFDCa	C <sub>10</sub>	5.03	nd	nd	nd	nd
PFUnA	C <sub>11</sub>	6.99	nd	nd	nd	nd
PFDoA	C <sub>12</sub>	3.10	nd	nd	nd	nd
PFTriA	C <sub>13</sub>	7.64	nd	nd	nd	nd
PFTeA	C <sub>14</sub>	2.78	nd	nd	nd	nd
PFHxDA	C <sub>16</sub>	nd	nd	nd	nd	nd
∑PFSAs	-	145		258 ± 39	141	289
∑PFCAs	-	29.0	-	88 ± 9	44.1	61.9
∑PFAS	-	-	207	-	-	-

## Appendix D

**Audouin's gull (*Larus audouinii*)**

Tissue	Whole eggs	Whole eggs	Whole eggs	Yolk
Sampling year	2009 - 2018	2010	2009	2009
Location	Spain	Spain	Spain	Spain
Reference	Colomer-Vidal <i>et al.</i> 2022	Morales <i>et al.</i> 2012	Bertolero <i>et al.</i> 2015	Vicente <i>et al.</i> 2015
		Colonies mean range (min to max)	Mean	Mean ± SD
Compounds	Carbon number			
PFHxS	C <sub>6</sub>	nd	0.57	nd
PFOS	C <sub>8</sub>	48 ± 10 to 101 ± 15	77.5	87.9 ± 23
PFOA	C <sub>8</sub>	nd	nd	nd
PFNA	C <sub>9</sub>	nd	1.28	nd
PFDCa	C <sub>10</sub>	nd	nd	nd
PFUnA	C <sub>11</sub>	nd	nd	nd
PFDoA	C <sub>12</sub>	nd	nd	nd
PFTriA	C <sub>13</sub>	nd	nd	nd
PFTeA	C <sub>14</sub>	nd	nd	nd
PFHxDA	C <sub>16</sub>	nd	nd	nd
∑PFASs	-	-	-	-
∑PFCAs	-	-	-	-
∑PFAS	-	-	79.4 ± 0.02	-

## Appendix D

**Glaucous gull (*Larus hyperboreus*)**

Tissue		Whole eggs	Whole eggs	Whole eggs
Sampling year		2017 - 2018	2008	2004
Location		Svalbard	Canada	Svalbard & Bear Island (Norwegian Arctic)
Reference		Herzke <i>et al.</i> 2022	Braune <i>et al.</i> 2013	Verreault <i>et al.</i> 2005
		Mean	Mean ± SD	Mean ± SD
Compounds	Carbon number			
PFHxS	C <sub>6</sub>	0.18	0.39 ± 0.04	nd
PFOS	C <sub>8</sub>	5.47	20.0 ± 1.13	104 ± 13.2
PFOA	C <sub>8</sub>	0.52	nd	nd
PFNA	C <sub>9</sub>	1.07	nd	nd
PFDCa	C <sub>10</sub>	0.46	nd	2.08 ± 0.46
PFUnA	C <sub>11</sub>	1.39	nd	21.4 ± 2.82
PFDoA	C <sub>12</sub>	0.41	nd	3.35 ± 0.62
PFTriA	C <sub>13</sub>	1.57	nd	15.1 ± 3.61
PFTeA	C <sub>14</sub>	0.27	nd	nd
PFHxDA	C <sub>16</sub>	nd	nd	nd
∑PFSA <sub>s</sub>	-	-	-	-
∑PFCA <sub>s</sub>	-	-	24.2 ± 0.71	41.8 ± 5.27
∑PFAS	-	11.3	-	-

## Appendix D

Ivory gull (*Pagophila eburnea*)

Tissue		Whole eggs					
Sampling year		2010	2007	2006			2004
Location		Greenland	Svalbard	Nagurskoe (Russian Arctic)	Kluyv Cape (Russian Arctic)	Domashny (Russian Arctic)	Canadian Arctic
Reference		Miljeteig <i>et al.</i> 2009; Lucia <i>et al.</i> 2015					
		Median	Median	Median	Median	Median	Median
Compounds	Carbon number						
PFHxS	C <sub>6</sub>	nd	0.37	0.77	0.69	0.79	nd
PFOS	C <sub>8</sub>	25.8	79.2	59.1	66.1	57.7	nd
PFOA	C <sub>8</sub>	1.33	nd	0.3	0.23	0.22	nd
PFNA	C <sub>9</sub>	4.89	1.04	1.34	0.99	1.49	nd
PFDCa	C <sub>10</sub>	nd	2.41	3.11	3.36	3.5	nd
PFUnA	C <sub>11</sub>	nd	12.6	12.9	11.7	10.7	nd
PFDoA	C <sub>12</sub>	nd	3.44	2.29	2.12	1.51	nd
PFTriA	C <sub>13</sub>	nd	10.7	8.21	7.86	5.67	nd
PFTeA	C <sub>14</sub>	nd	0.78	1.07	0.97	0.77	nd
PFHxDA	C <sub>16</sub>	nd	nd	nd	nd	nd	nd
$\sum$ PFSA	-	-	-	-	-	-	-
$\sum$ PFCA	-	-	-	-	-	-	-
$\sum$ PFAS	-	32.6	116	91.3	96.9	86.9	-

Appendix D

## Several other Laridea species

Species		<i>Larus californicus</i>	<i>Larus crassirostris</i>	<i>Larus crassirostris</i>	<i>Larus delawarensis</i>	<i>Larus glaucescens</i>	<i>Larus melanocephalus</i>
Common name		California gull	Black-tailed gull	Black-tailed gull	Ring-billed gull	glaucous-winged gull	Mediterranean gull
Tissue		Whole eggs	Whole eggs	Whole eggs	Whole eggs	Whole eggs	Whole eggs
Sampling year		2008	2015 - 2019	2012 – 2018	2008	2008	2006
Location		Canada	South Korea	South Korea	Canada	Canada	Belgium
Reference		Gebbink <i>et al.</i> 2011	Jang <i>et al.</i> 2022	Wang <i>et al.</i> 2021	Gebbink <i>et al.</i> 2011	Gebbink <i>et al.</i> 2011	Lopez-Antia <i>et al.</i> 2017
		Mean of colonies means	Median range (min to max)	Mean ± SD (two colonies)	Mean of colonies means	Mean of colonies means	Range (min to max)
Compounds	Carbon number						
PFHxS	C <sub>6</sub>	nd	0.21 to 4.48	nd	nd	nd	nd
PFOS	C <sub>8</sub>	nd	9.38 to 43.9	7.22 ± 6.24 & 6.66 ± 3.04	nd	nd	150 to 916
PFOA	C <sub>8</sub>	nd	0.39 to 5.73	0.35 ± 0.70 & 1.00 ± 1.18	nd	nd	nd
PFNA	C <sub>9</sub>	nd	0.97 to 3.81	nd	nd	nd	nd
PFDCa	C <sub>10</sub>	nd	2.07 to 4.59	nd	nd	nd	nd
PFUnA	C <sub>11</sub>	nd	6.46 to 10.3	nd	nd	nd	nd
PFDoA	C <sub>12</sub>	nd	1.69 to 2.30	nd	nd	nd	nd
PFTriA	C <sub>13</sub>	nd	4.77 to 9.63	nd	nd	nd	nd
PFTeA	C <sub>14</sub>	nd	1.23 to 1.53	nd	nd	nd	nd
PFHxDA	C <sub>16</sub>	nd	nd	nd	nd	nd	nd
∑PFSA <sub>s</sub>	-	74	-	14.8 ± 7.15 & 26.5 ± 9.32	49	50.5	-
∑PFCAs	-	18	-	7.66 ± 6.38 & 7.20 ± 3.14	16	2.65	-
∑PFAS	-	-	33.0 - 79.2	-	-	-	-



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## Transfert maternel et conséquences physiologiques des substances per- et polyfluoroalkylées dans les œufs d'oiseaux marins

### Résumé :

Le développement embryonnaire est une phase très sensible puisqu'elle voit la synthèse et la maturation de tous les organes et fonctions du futur organisme. Par conséquent, toute perturbation subie lors de cette phase peut avoir des conséquences ultérieures importantes. Dans un contexte de fort impact des activités humaines sur la faune sauvage, les oiseaux de mer sont particulièrement menacés car ils sont exposés à de nombreuses menaces, notamment les interactions avec les pêcheries, la destruction de l'habitat ou la pollution environnementale. Parmi celles-ci, cette dernière est peut-être la plus insidieuse, puisqu'elle peut également être transmise à la progéniture par transfert maternel dans les œufs, et provoquer des effets néfastes dès le développement. Le 20<sup>ème</sup> siècle a vu l'émergence de nombreuses substances synthétiques. Parmi elles, les substances per- et polyfluoroalkyles (PFAS) sont retrouvées dans les œufs d'oiseaux marins, mais leurs effets sont encore peu connus. Dans cette thèse, j'ai cherché à étudier le transfert maternel des PFAS chez un oiseau marin de l'Arctique, la mouette tridactyle (*Rissa tridactyla*). J'ai également examiné les conséquences éventuelles de l'exposition aux PFAS historiques et émergents pour l'embryon chez cette espèce et chez le goéland leucophée (*Larus michahellis*). J'ai trouvé des concentrations relativement élevées de PFAS historiques dans les œufs ainsi que certains composés émergents, notamment le 7:3 FTCA ou le PFEchS. Les caractéristiques physico-chimiques des PFAS affectent leur efficacité de transfert. Mes résultats suggèrent également que les PFAS circulants des femelles pourraient affecter le transfert d'hormones maternelles dans les œufs, ce qui pourrait affecter la progéniture à court et à long terme. Enfin, je n'ai trouvé aucune indication que les PFAS déposés dans les œufs puissent affecter l'embryon en développement sur certains biomarqueurs du vieillissement (longueur des télomères) ou du métabolisme. Je suggère que les deux populations étudiées ne sont pas impactées, au moins aux concentrations de PFAS mesurées dans leurs œufs. Néanmoins, des études supplémentaires seraient nécessaires pour évaluer comment les PFAS peuvent affecter le transfert endocrinien maternel et ses conséquences pour l'embryon.

Mots clés : Arctique, Mouette tridactyle, Goéland leucophée, Polluants organiques, Ecotoxicologie, Effets maternels, Développement

## Maternal transfer and physiological consequences of per- and polyfluoroalkyl substances in seabird eggs

### Summary:

The developmental period is a very sensitive phase since it sees the synthesis and maturation of all organs and functions of the future organism. Therefore, any disruption experienced early in life may have substantial subsequent consequences. In the context of the considerable impact of Human activities on wildlife, seabirds are particularly at risk since they are exposed to numerous threats, including fisheries interactions, habitat destruction, or environmental pollution. Among them, the later is maybe the most insidious, since it can also be transferred to the progeny via maternal transfer in eggs, and cause adverse effects as early as during the development. The 20<sup>th</sup> century saw the emergence of numerous synthetic substances. Among them, the per- and polyfluoroalkyl substances (PFAS) are found in seabird eggs, but little is known about their effects. In this thesis, I aimed at investigating the maternal transfer of PFAS in an Arctic seabird, the black-legged kittiwake (*Rissa tridactyla*). I also examined the eventual consequences of legacy and emerging PFAS exposure for the embryo in this species and in the yellow-legged gull (*Larus michahellis*). I found relatively high concentrations of legacy PFAS in eggs as well as some emerging compounds including 7:3 FTCA or PFEchS. PFAS physicochemical characteristics influenced their transfer efficiency. My results also suggested that females PFAS might affect their transfer of maternal hormones in eggs, which may ultimately affect offspring at short and long term. Finally, I found no indications that PFAS deposited in eggs may affect the developing embryo on biomarkers of ageing (telomere length) or metabolism. I therefore suggested that both studied population should be relatively safe at least at the PFAS concentrations measured in their eggs. Nonetheless, additional studies would be needed to assess how PFAS may affect the endocrine maternal transfer and its consequences for the embryo.

Keywords: Arctic, Black-legged kittiwake, Yellow-legged gull, Organic pollutants, Ecotoxicology, Maternal effects, Development



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