Master's thesis

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Decline of a grey seal (*Halichoerus grypus*) population in Norway: can per- and polyfluoroalkyl substances be blamed?

Master's thesis in Environmental Toxicology
Supervisor: Bjørn Munro Jenssen
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Abstract

The grey seal (*Halichoerus grypus*) population of the Froan Archipelago has drastically decreased since 2001, and it is becoming a pressing challenge to understand the underlying causes for this decline. Particular attention has been paid to per-and polyfluoroalkyl substances (PFASs) in previous years because of their persistence, long-range transport, bioaccumulation, and toxicity, and some of them are now banned at the global scale. However, both legacy and emerging PFASs continue to be measured in the environment and biota. PFASs have previously been linked to endocrine disruption, immunotoxicity, and reproductive toxicity in marine mammals, and top predators such as grey seals are particularly vulnerable to high exposure. Grey seal pups are exposed to PFASs via placental transfer and lactation during their first weeks of life, a crucial time for their future survival. Previous studies have successfully used steroid hormones to assess the effect of persistent organic pollutants (POPs) exposure in marine mammals, suggesting that they might be an effective biomarker to assess the effect of PFASs in seal pups. In the present study, temporal trends of PFAS concentrations in the plasma of 164 seal pups sampled from 1992 to 2022 in the Froan archipelago were determined, in order to understand how PFAS concentrations varied in a declining population. Then, using steroid hormones as biomarkers, the effects of PFASs were assessed in 12 grey seal pups sampled in s2022. It was found that grey seal pups were exposed to the highest concentrations of PFAS in 2000. Moreover, while the concentration of PFASs significantly decreased from 1992 to 2022, the concentration of perfluoroalkyl carboxylic acids (PFCAs) significantly increased. Finally, emerging contaminants such as ADONA and Gen X were detected in more than half of the seal pups although long-chain PFASs were dominating. Negative correlations were found between steroid hormones, such as progesterone and cortisol, and several perfluoro sulfonic acids (PFSAs), suggesting that those compounds might disturb steroid homeostasis in grey seal pups and later affect their survival during the fasting period. However, because PFSAs accounted for only 23 % of the total variance in steroid levels, the disruption of the steroid homeostasis in the Froan pups is certainly multi-factorial, encompassing effects of other contaminants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). The Froan population should continue to be regularly monitored and steroid levels of grey seal pups from previous sampling years should be assessed, especially when PFAS concentrations were the highest. Ideally, sexually mature grey seals should be sampled to understand how PFASs affect sex steroid levels as they play a major role in reproductive success.

Sammendrag

Bestanden av gråsel (*Halichoerus grypus*) i Froan Archipelago har gått drastisk ned siden 2001, og det blir en presserende utfordring å forstå de underliggende årsakene til denne nedgangen. Spesiell oppmerksomhet har blitt gitt til per- og polyfluoralkylstoffer (PFAS) i tidligere år på grunn av deres utholdenhet, langtransport, bioakkumulering og toksisitet, og noen av dem er nå forbudt på global skala. Imidlertid måles både eldre og nye PFASer fortsatt i miljø og biota. PFASer har tidligere vært knyttet til hormonforstyrrelser, immunotoksisitet og reproduksjonstoksisitet hos sjøpattedyr, og toppredatorer som gråsel er spesielt utsatt for høy eksponering. Gråselunger eksponeres for PFASer via placentaoverføring og diegiving i løpet av de første ukene av livet, en kritisk tid for deres fremtidige overlevelse. Tidligere studier har med hell brukt steroidhormoner for å vurdere effekten av vedvarende organiske miljøgifter (POP) eksponering i marine pattedyr, noe som tyder på at de kan være en effektiv biomarkør for å vurdere effekten av PFASer i selunger. I denne studien ble tidsmessige trender for PFAS-konsentrasjoner i plasma fra 164 selunger samplet mellom 1992 og 2022 i Froan-øygruppen evaluert for å forstå hvordan PFAS-konsentrasjoner varierte i en synkende populasjon. Ved bruk av steroidhormoner som biomarkører ble effekten av PFAS vurdert hos 12 brune selunger som ble tatt prøver av i 2022. Det ble funnet at gråselungene ble utsatt for de høyeste konsentrasjonene av PFAS i 2000. Dessuten, mens de totale konsentrasjonene av PFAS gikk betydelig ned fra 1992 til 2022, har perfluoralkylkarboksylsyrer (PFCA) økt betydelig. Til slutt ble nye forurensninger som ADONA og Gen X påvist i mer enn halvparten av selungene, selv om langkjedede PFASer dominerte. Negative korrelasjoner ble funnet mellom steroidhormoner, som progesteron og kortisol, og flere perfluorsulfonsyrer (PFSA), noe som tyder på at disse forbindelsene kan forstyrre steroidhomeostase hos gråselunger som kan påvirke deres overlevelse i fasteperioden. Imidlertid utgjorde PFSA bare 23% av den totale variansen i steroidnivåer, forstyrrelsen av steroidhomeostasen hos Froan-valpene er absolutt multifaktoriell, og omfatter effekter av andre forurensninger som polyklorerte bifenyler (PCB) og polybromerte difenyletere (PBDE). Froan-populasjonen bør fortsatt overvåkes regelmessig, og steroidnivåene av gråselunger fra tidligere prøvetakingsår bør vurderes, spesielt når PFAS-konsentrasjonene er høyest. Ideelt sett bør seksuelt modne grå seler prøves for å forstå hvordan PFASer påvirker kjønnssteroidnivået, da de spiller en viktig rolle i reproduktiv suksess.

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Abbreviations

AR	Absolute recovery
CO2	Carbon dioxide
CCs	Calibration standards
CHLDs	Chlordanes
DDT	Dichlorodiphenyltrichloroethane
DR	Detection rate
EOF	Extractable organofluorine
ESI	Electrospray ionization
FPs	Fluoropolymers
HCOOH	Formic acid
HPA	Hypothalamus-pituitary-adrenal
HPG	Hypothalamus-pituitary-gonadal
HybridSPE	Hybrid Solid Phase Extraction
iLOD	Instrumental limit of detection
iLOQ	Instrumental limit of quantification
IS	Internal standard
IS-mix	Internal standard mix
LC-PFCAs	Long-chain perfluorocarboxylic acids
MEs	Matrix effects
MeOH	Methanol
mLOD	Method limit of detection
mLOQ	Method limit of quantification
MMs	Matrix-match samples
NH4CH3COO	Ammonium acetate
NH4COOH	Ammonium formate
NTNU	Norwegian University of Science and Technology
PASFs	Perfluoroalkane sulfonyl fluorides
PBDEs	Polybrominated diphenyl ethers
PC	Principal component
PCA	Principal component analysis
PCBs	Polychlorniated biphenyls
PFAAs	Perfluoroalkyl acids
PFASs	Per- and Polyfluoroalkyl Substances
PFCAs	Perfluoroalkyl carboxylic acids
PFECAs	Perfluoroalkyl ether carboxylic acids
PFESAs	Perfluoroalkyl ether sulfonic acids
PFOA	Perfluorooctanoic acid
PFPEs	Perfluoropolyethers
PFSAs	Perfluoroalkane sulfonic acids
POPs	Persistent organic pollutants
PP	Polypropylene
QCs	Quality control samples
RAs	Relative areas
RDA	Redundancy analysis
RR	Relative recovery
RSD	Relative standard deviation
SD	Standard deviation

SPs	Spiked samples
SPE	Solid Phase Extraction
TAs	Target analytes
UPC^2-MS/MS	Ultra-performance convergence chromatography tandem mass spectrometry
UPLC-MS/MS	Ultra performance liquid chromatography - tandem Mass spectrometer

10:2 FTS	1H,2H-Perfluorododecane sulfonate (10:2)
4:2 FTS	1H,2H-Perfluorohexane sulfonate (4:2)
6:2 FTS	1H,2H-Perfluorooctane sulfonate (6:2)
6:2 FTS 13 C2	1H,2H-Perfluorooctane sulfonate (6:2) 13C2
6:6 PFPi	Bis(tridecafluorohexyl)phosphinic acid
6:8 PFPi	Bis(heptadecafluorooctyl)phosphinic acid
7H-PFHpA	7H-Dodecafluoroheptanoic acid
8:2 FTS	1H,2H-Perfluorodecane sulfonate (8:2) (8:2 FTS)
8:8 PFPi	Bis(heptadecafluorooctyl)phosphinic acid
9CI-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate
ADONA	Dodecafluoro-3H-4,8-dioxanonanoate
Deca S	Sodium 1-decanesulfonate
DiSamPAP	Bis[2-(N-ethylperfluorooctane-1-sulfonamido)ethyl] phosphate
EtFOSA	Sulfluramid
EtFOSAA	N-ethylPerfluoro-1-octanesulfonamide acetic acid
EtFOSE	N-ethyl-N-(2-hydroxyethyl)-N-methylperfluorooctane sulfonamide
FOSAA	Perfluoro-1-octanesulfonamidoacetic acid
Gen X	2,3,3,3-tetrafluoro-2- (1,1,2,2,3,3,3-heptafluoropropoxy) propanoate
MeFOSA	N-methylPerfluoro-1-octanesulfonamide
MeFOSAA	2-(N-methylPerfluoro-1-octansulfonamido) acetic acid
MeFOSE	N-(2-hydroxyethyl)-N-methylperfluorooctane sulfonamide
P37DMOA	Perfluoro-3,7-dimethyloctanoic acid
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanoic acid sulfonate
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFDoDA	Perfluorododecanoic acid
PFDoDS	Perfluorododecane sulfonic acid
PFDS	Perfluorodecane sulfonic acid
PFECHS	Perfluoroethylcyclohexane sulfonic acid
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoro-1-heptanesulfonate
PFHxA	Perfluorohexanoic acid
PFHxDA	Perfluoro-n- hexadecanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFNS	Perfluorononane sulfonic acid
PFOA	Perfluorooctanoic acid
PFOA-13C8	Perfluorooctanoic acid 13C8
PFOS	Perfluorooctano sulfonic acid
PFOS-13C8	Perfluorooctanesulfonate 13C8 sodium salt
PFOSA	Perfluorooctane sulfonamide

PFPeA	Per-fluoropentanoic acid
PFPeS	Perfluoropentane sulfonic acid
PFTDA	Perfluorotetradecanoic acid
PFTriDA	Perfluorotri-decanoic acid
PFUnA	Perfluoroundecanoic acid
SamPAP	2-(N-ethylperfluorooctane-1-sulfonamido)ethyl phosphate
TriDeFHxSA	Tridecafluorohexane-1-sulfonic acid potassium salt

11-deoxyCOR	11-Deoxycortisol
11-ketoTS	11-ketostestosterone
17a-OHP	17α-Hydroxyprogesterone
17OH-P5	17-Hydroxypregnenolone
2,3,4-13C2-17αOHP	17α-hydroxyprogesterone-13C2
2,3,4-13C3-CORNE	Cortisone-13C3
2,3,4-13C-DHT	Dihydrotestosterone-13C
A5	Androstenediol
ALDO	Aldosterone
AN	Androstenedione
COR	Cortisol
CORNE	Cortisone
COS	Corticosterone
DHEA	Dehydroepiandrosterone
DHT	5α-Dihydrotestosterone
DOC	11-Deoxycorticosterone
E1	Estrone
P4	Progesterone
PREG	Pregnenolone
TS	Testosterone

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1. Introduction

1.1 PFASs

Per- and polyfluoroalkyl substances (PFASs) are a large group of more than 4000 compounds (OECD, 2018) and have been produced since the 1950s (Buck et al., 2011). They are defined as "fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it)" (OECD, 2021), and can be both polymeric and non-polymeric (Buck et al., 2011). An overview of PFAS classification as suggested by Wang et al., 2017 is shown in Table 1.1. Perfluoroalkyl acids (PFAAs) are an important group of non-polymeric PFASs which comprised, among others, perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs) (OECD, 2021). Groups containing both polymeric and non-polymeric PFASs are PFAA precursors, such as perfluoroalkane sulfonyl fluorides (PASFs) and fluorotelomer-based substances, as well as other PFASs such as fluoropolymers (FPs) and perfluoropolyethers (PFPEs) (OECD, 2021). PFASs can also be divided into long-chain and short-chain, with long-chain PFASs referring to PFCAs with ≥ 7 perfluoroalkyl carbons, PFSAs with ≥ 6 perfluoroalkyl carbons (e.g., PFOS), and precursors of PFCAs and PFSAs that can transform in long-chain PFASs once reaching the environment (OECD, 2015).

Table 1.1. Overview of PFASs classification based on Figure 1 of Wang et al., 2017.

Per- and polyfluoroalkyl substances (PFASs)	**Perfluoroalkyl acids (PFAAs)**	Perfluoroalkyl carboxylic acids (PFCAs)	*e.g., PFOA*
		Perfluoroalkyl sulfonic acids (PFSAs)	*e.g., PFOS*
		Perfluoroalkyl phosphonic acids (PFPAs)	*e.g., PFBPA*
		Perfluoroalkyl phosphinic acids (PFPiAs)	*e.g., C4/C4 PFPiA*
		Perfluoroalkyl Ether Carboxylic Acids (PFECAs) & Sulfonic Acids (PFESAs)	*e.g., ADONA*
	PFAAs precursors	Perfluoroalkane sulfonyl fluorine (PASF)-based substances	*e.g., MeFOSA*
		Fluorotelomer-based substances	*e.g., 4:2 FTOH*
	Others	Fluoropolymers	*e.g., PTFE*
		Perfluoropolyethers (PFPEs)	

Because PFASs share a number of unique physico-chemical properties such as low aqueous surface tension and a very high stability both chemically and thermally (Buck et al.; 2011), they are used in multiple applications such as in fire-fighting foams, textile, paper as surface protectors, the electronic industry, machinery and equipment, the production of plastic and rubber, as well as in coatings (Glüge et al., 2020). However, while those unique properties have made PFASs attractive to the industrial sector, they are also the cause of concerns surrounding these compounds. Indeed, the strength of the fluorine-carbon bond in PFASs renders them highly resistant to further degradation and they can persist for a very long time once reaching the environment (O'Hagan, 2008; Cousins et al., 2020). PFASs can be released in the environment throughout their whole life cycle, i.e., during their production, transport, use, and final disposal (Evich et al., 2022), where they can be long-range transported via oceanic and atmospheric transport (Stock et al., 2007; Muir et Miaz, 2021). They are therefore ubiquitous and found in remote areas such as in the Arctic (Wong et al., 2018; Ali et al., 2021). Moreover, they are mainly proteinophilic and bioaccumulate in protein-rich tissues in organisms (Kelly et al., 2009; Jones et al., 2003). In humans, legacy PFASs such as the well-studied perfluorooctanoic acid (PFOA) and perfluoro octane sulfonate (PFOS), have previously been linked to a plethora of toxic effects such as immunotoxicity, endocrine disruption, reproductive and developmental toxicity, and cancers (See Fenton et al., 2021 and references therein). Because of their persistence, long-range transport, bioaccumulation, and toxicity, some PFASs are now banned under the Stockholm Convention, the only treaty to protect human health and the environment from persistent organic pollutants (POPs) at the global level. Under the Stockholm Convention, PFOS has been restricted since 2009 while PFOA has been prohibited in 2019 followed by Perfluorohexanesulfonic acid (PFHxS) in 2022 (UNEP, 2009, 2019, 2022). During the next conference of the parties (COPs) of the Stockholm Convention in May 2023, the listing of long-chain perfluoro carboxylic acids (LC-PFCAs) and their salts under one of the three annexes (A, B and C) will be discussed. If listed under Annex A, LC-PFCAs will be prohibited.

Faced with growing concern about PFASs on a national and global scale, the industrial sector has started to replace the commonly used PFASs (or legacy PFASs) with so-called emerging PFASs, most of which are the short-chain equivalents of legacy PFASs (EPA, 2021). Other emerging compounds are fluoropolyethers such as perfluoroalkyl ether carboxylic acids (PFECAs) and perfluoroalkyl ether sulfonic acids (PFESAs) (Manojkumar et al., 2023). While emerging PFASs were first thought to be safe alternatives to legacy PFASs, they have been

detected in the environment (Wang et al.,2013; Gebbink et al., 2017) and biota (Jouanneau et al., 2021; Munoz et al., 2022) and recent studies on mammals suggest that they might exert toxic effects such as endocrine disruption (Zhang, S., et al., 2021), reproductive toxicity (Cui et al., 2020), and developmental toxicity (Gaballah et al., 2020).

The difficulty for PFASs management lies in the large number now being identified (4730, OECD, 2018), even if only 283 were judged to be commercially relevant (Buck et al., 2021). Moreover, their different physico-chemical properties make it challenging to predict their behavior once released in the environment. For example, PFSAs have been found to be more bioaccumulative than PFCAs (Martin et al., 2003), while longer-chain PFASs tend to sorb to soil particles in greater proportions than the shorter-chain PFASs (e.g., PFBA), the latter can therefore be considered more mobile (Nguyen et al., 2020). A lot of uncertainties remain about the fate of legacy and emerging PFASs, and their broad restriction is being discussed by policymakers (Cousins et al., 2020).

1.2. Grey seals and the Froan Archipelago population

Because PFASs can both bioaccumulate in the organisms and biomagnify throughout the food web (Kelly et al., 2009), top predators such as grey seals (*Halichoerus grypus*) are highly vulnerable to PFAS exposure and high concentrations have previously been reported in pinnipeds (Routti et al., 2015; Grønnestad et al., 2017). In marine mammals, PFASs have been suggested to exert adverse effects such as endocrine disruption (Bourgeon et al., 2017), immunotoxicity (Routti et al., 2019; Soloff et al., 2017) and reproductive toxicity (Sonne et al., 2009).

Grey seals are mainly distributed in cold temperate areas in the North Atlantic with the main breeding sites found off the coasts of North America (northeastern), Iceland, Denmark (the Faroe Islands), Norway, and the United Kingdom (Jefferson et al., 2015) (Figure 1.1). Females give birth to a single pup every year between September and March, but pupping season vary between locations, with seals off the coast of the United Kingdom being the first to breed and seals in Canada and in the Baltic Sea being the last (Jefferson et al., 2015). Females stay with their pup until it is weaned at 15-18 days old, and the pup will then remain fasting 2 to 4 weeks ashore before reaching the sea (Jefferson et al., 2015). In the Froan archipelago, a particular neonatal aquatic behavior was observed both in suckling and weaning pups (Jenssen et al., 2010). If suckling pups dispersing on long distances (>2000m) had significantly lower body mass (BM) than suckling pups staying on land or dispersing on short distances, no differences

in BM were observed in weaned pups with and without aquatic behavior, reflecting two strategies: one where weaned pups go to the sea and learn feeding and diving skills, and the other where they stay on land and increase their energy stores. (Jenssen et al., 2010).

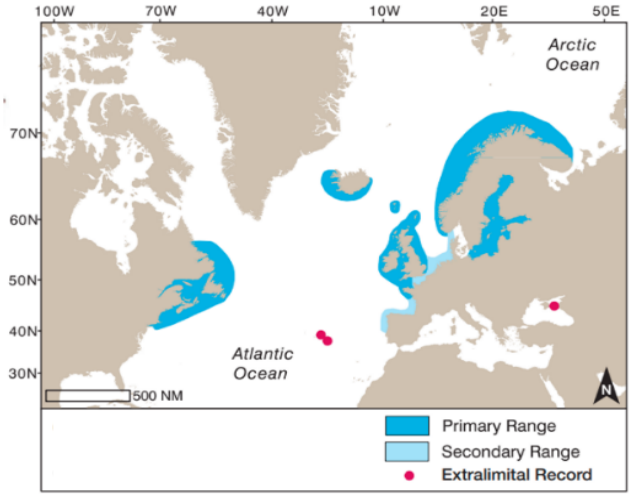

Figure 1.1. Global distribution of grey seals (*Halichoerus grypus*) as retrieved from Jefferson et al., 2015.

Globally, grey seal populations are thriving with an estimated world population of 450,000 grey seals (Jefferson et al., 2015; NAMMCO, 2016d). With this in mind, the decline of a grey seal population in the Froan Archipelago (Trøndelag region) off the coast of central Norway, is most alarming. Indeed, the Froan Archipelago was once the main breeding ground for grey seals in Norway with 200 to 283 pups being born each year from 1979 to 2002. However, the population is now rapidly declining with only 60 pups born in 2018 (Nilssen et al., 2020) (Figure 1.2).

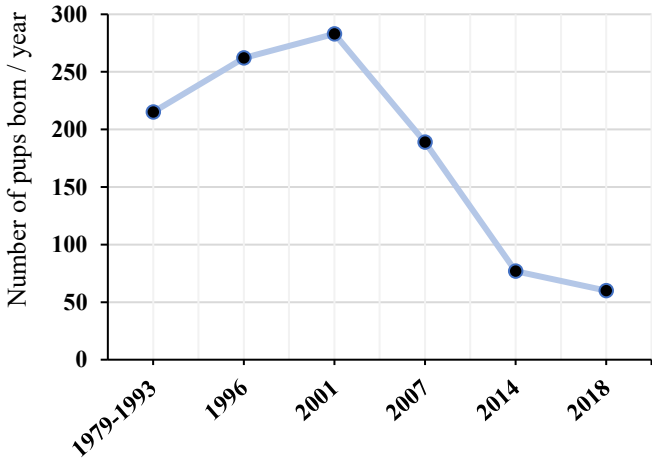

Figure 1.2. Number of pups being born each year from 1979 to 2018 in the Froan Archipelago with values found in Nilssen et al., 2020.

This declining trend seems to be specific to some areas, as the total grey seal pup production is increasing in Norway, whereas it is decreasing in both Trøndelag and Nordland region (NAMMCO, 2016d). Previous pinniped populations decline has been attributed to causes such as reductions in prey availability (Wilson et Hammond, 2019) as well as infections (Grachev et al., 1989). Exposure to contaminants has also been suggested to be another cause for seal population decline (Bergman, 1999). Indeed, because seals live in coastal waters, they are exposed to high concentrations of man-made chemicals (Jefferson et al., 2015).

During their first weeks of life, grey seal pups are highly vulnerable as they are still developing and undergo rapid morphological changes such as quadrupling their body mass and building up a thick blubber layer to further sustain the energy requirements of the fasting period (Jefferson et al., 2015; Nordoy et al., 1985; Schweigert, 1993). However, they may have already been exposed to several contaminants such as PFASs. Indeed, PFASs are mainly transferred to the pups via the placenta although they can also be transferred via maternal milk, and thus the PFAS load in seal pups represents that of the mother (Grønnestad et al., 2017).

1.3. Steroid hormones as a potential biomarker of PFAS effect in grey seal pups

In mammals, steroid hormones constitute an important group and are all derived from the cholesterol molecule. They are either synthesized via the hypothalamus-pituitary-adrenal (HPA) axis or via the hypothalamus-pituitary-gonadal (HPG) axis in the adrenal gland and gonads, but also in the placenta during gestation, and are essential for many physiological processes such as metabolism, immune function, salt-water balance, and reproductive function (Chakraborty et al., 2021). They can be further divided into 5 groups: glucocorticoids (e.g., cortisol), mineralocorticoids (e.g., corticosterone), androgens (e.g., testosterone), estrogens (e.g., estradiol) and progestogens (e.g., progesterone) (Chakraborty et al., 2021). Among steroids, glucocorticoids and in particular cortisol, have often been studied in seals (Ortiz et al., 2001; Oki et al., 2004; Atkinson et al., 2011; Kershaw et al., 2016). Glucocorticoids are often defined as stress hormones as they stimulate glucogenesis to provide sufficient energy in response to situations of high energy demand such as fasting (Nordoy, 1985; Whirledge et al., 2010). Mineralocorticoids are implicated in saltwater balance whereas sex steroid hormones (estrogens, androgens, and progestogens) are mainly implicated in the development and function of the reproductive system (Chakraborty et al., 2021).

PFAS effects and exposures have previously been assessed in biota using biomarkers, which were defined in 1994 by Peakall as “biological responses to a chemical or chemicals that give a measure of exposure and sometimes of toxic effect”. The biomarker can be measured at the molecular, cellular, or physiological level (WHO, 1993). In marine mammals, steroid levels have previously been successfully used as a biomarker of exposure and/or effect to persistent organic pollutants (POPs) (Ciesielski et al., 2023, 2017; Galligan et al., 2019; Gustavson et al., 2015) and only once to PFASs (Pedersen et al., 2016). Pedersen et al. (2016) determined that PFAS concentrations were in general positively correlated with steroid hormone levels in brain tissues of polar bears. However, uncertainties remain and there is a need for more studies assessing the effect of PFASs on steroid homeostasis in marine mammals.

A first objective of the present study was to understand how levels of legacy and emerging PFASs have fluctuated in a declining grey seal population over 30 years, by assessing the temporal trends of PFAS concentrations in plasma of 164 grey seal pups sampled from 1992 to 2022 in the Froan Archipelago. Then, it was determined whether PFAS burden could be related to the population decline via the disturbance of steroid homeostasis, by exploring the relationships between steroid levels and PFAS concentrations in plasma of 12 grey seal pups sampled in 2022. This study is the first to explore the relationships between PFAS burden and steroid levels in pinnipeds and the first to assess PFAS temporal trends in North Atlantic pinnipeds.

2. Materials and Methods

2.1. Study area and sampling

Grey seal pups (pre-weaning, n=12) were sampled from September 2022 to October 2022 in the Froan Nature Reserve (64° 10' N, 09° 20' E), an archipelago located in the Trøndelag region on the coast of central Norway. The sample locations are shown in Figure 2.1.

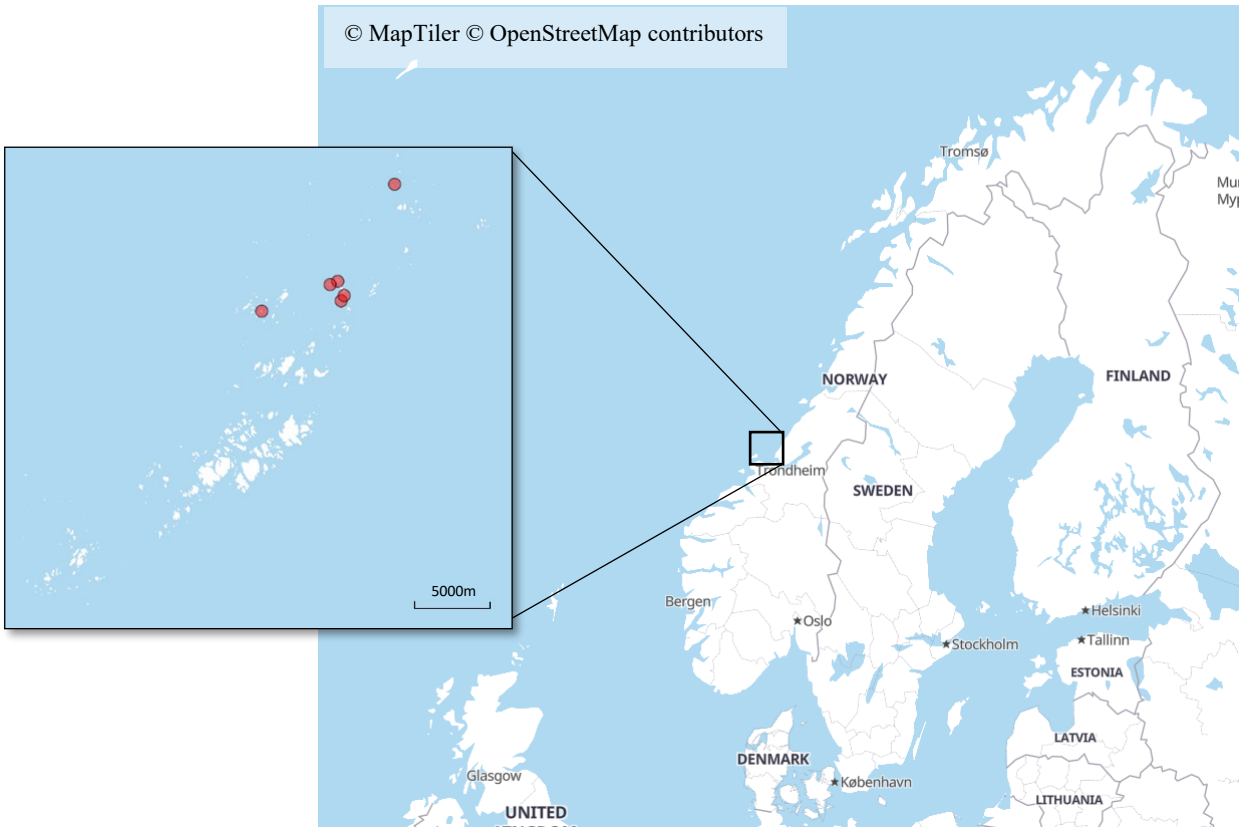

Figure. 2.1. Location map of the Froan Nature Reserve located on the coast of central Norway with the 6 sample sites represented as red circles.

Seal pups were hand captured, one-by-one, using a catching net that could be opened lengthwise. Pups were manually restrained during sampling which lasted for about 10 min. Date, time, and location of the sampling were noted. Blood samples were collected at the base of one of the hind flippers, using vacuum sodium-heparin blood collection tubes and 22-G needles, and kept in a cooling box at 0-5°C throughout the sampling. At the end of each field day, samples were centrifuged for 10min at 3000 rpm and plasma samples were transferred to polyethylene cryo vials and stored at -20°C until further analysis in the laboratory. The body mass (BM) was measured after sampling using a calibrated weight. Other morphometric

measurements were taken with a measuring tape. Length measurements (cm) were taken from the base of the hind flippers to the nose, and girth measurements (cm) were taken at the base of the fore flippers. Sex was determined based on morphological criteria. Further observations such as the presence or absence of infections, the location of the mother (at sea, ashore, not seen, attending) as well as the location of the male, were noted. Finally, pups were classified into one of four age categories based on morphological criteria according to previous studies (Kovacs et Lavigne, 1986; Jenssen et al., 2003) (Table 2.1). Permission to sample grey seal pups in the Froan Nature Reserve was granted by the County Governor of Trøndelag (2022/8005) and the Norwegian Food Safety Authority (FOTS ID 29830).

Table 2.1. Morphological criteria used to classify the seal pups in four age categories based on Table I in Kovacs et Lavigne, 1986

Age category	Morphological criteria	Mean age (days)
1	Yellow tint to pelage; lacking coordination; neck, hips, and ribs clearly visible; umbilicus present	2.4 ± 4.4
2	Pelage white; improved coordination, shoulder to hip region filled out; ribs covered by a layer of blubber; umbilicus not present	4.8 ± 3.1
3	Pelage white to light grey; fat sheath extends posteriorly from neck; body barrel shaped; lanugo intact except for slight loss in the facial region toward the end of stage	12.1 ± 2.9
4	Lanugo being shed, exposing the juvenile pelage	16.0 ± 3.0

Data from other sampling periods were used to assess the temporal trends of PFAS concentrations in seal pups. From 1992 to 2020, 153 pups (1992: n=85; 1993: n=29; 2000: n=6; 2005: n=19; 2006 = 1; 2020: n=13) were sampled in the Froan Archipelago with a blood sampling method identical to the one used in September and October 2022. However, length and girth were not measured for pups sampled before 2020 and the mother and male locations were not noted. Information regarding the seal pups sampled from 1992 to 2022 in the Froan Archipelago is shown in Table A.1.1.

2.2. Chemicals and materials

Analytical standards of 43 target analytes (TAs) for the PFAS analysis: Sodium 1-decanesulfonate (DecaS), 2,3,3,3-tetrafluoro-2- (1,1,2,2,3,3,3-heptafluoropropoxy) propanoate (Gen X), Per-fluoropentanoic acid (PFPeA), Perfluorohexanoic acid (PFHxA), 1H,2H-

Perfluorohexane sulfonate (4:2) (4:2 FTS), 7H-Dodecafluoroheptanoic acid (7H-PFHpA), Dodecafluoro-3H-4,8-dioxanonanoate (ADONA), tridecafluorohexane-1-sulfonic acid potassium salt (TriDeFHxSA), Perfluorooctanoic acid (PFOA), 1H,2H-Perfluorooctane sulfonate (6:2) (6:2 FTS), Perfluoro-1-heptanesulfonate (PFHpS), Perfluorononanoic acid (PFNA), Perfluoro-3,7-dimethyloctanoic acid (P37DMOA), Perfluorooctane sulfonamide (PFOSA), Perfluorooctano sulfonic acid (PFOS), N-methylPerfluoro-1-octanesulfonamide (MeFOSA), Perfluorodecanoic acid (PFDA), Sulfluramid (EtFOSA), N-ethylPerfluoro-1-octanesulfonamide acetic acid (EtFOSAA), 1H,2H-Perfluorodecane sulfonate (8:2) (8:2 FTS), 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate (9CI-PF3ONS), Perfluoro-1-octanesulfonamidoacetic acid (FOSAA), Perfluoroundecanoic acid (PFUnA), 2-(N-methylPerfluoro-1-octansulfonamido) acetic acid (MeFOSAA), N-ethyl-N-(2-hydroxyethyl)-N-methylperfluorooctane sulfonamide (EtFOSE), Perfluorododecanoic acid (PFDoDA), N-(2-hydroxyethyl)-N-methylperfluorooctane sulfonamide (MeFOSE), 1H,2H-Perfluorododecane sulfonate (10:2) (10:2 FTS), Perfluorotri-decanoic acid (PFTriDA), bis[2-(N-ethylperfluorooctane-1-sulfonamido)ethyl] phosphate (diSAmPAP), Perfluorotetradecanoic acid (PFTDA), Perfluoro-n-hexadecanoic acid (PFHxDA), Perfluorobutanoic acid (PFBA), Perfluorobutanoic acid sulfonate (PFBS), Perfluoropentane sulfonic acid (PFPeS), Perfluoroheptanoic acid (PFHpA), Perfluorohexane sulfonic acid (PFHxS), Perfluoroethylcyclohexane sulfonic acid (PFECHS), Perfluorononane sulfonic acid (PFNS), Perfluorodecane sulfonic acid (PFDS), 2-(N-ethylperfluorooctane-1-sulfonamido)ethyl phosphate (SamPAP), Perfluorododecane sulfonic acid (PFDoDS), Perfluorododecanoic acid (PFDoDA), Bis(tridecafluorohexyl)phosphinic acid (6:6 PFPi), Bis(heptadecafluorooctyl)phosphinic acid (6:8 PFPi), and Bis(heptadecafluorooctyl)phosphinic acid (8:8 PFPi) were all purchased from Chiron AS (Trondheim, Norway). Following the internal standard method, the 3 isotopically labeled internal standards (ISs) used for PFAS quantification, namely 1H,2H-Perfluorooctane sulfonate (6:2) 13C2 (6:2 FTS 13 C2), Perfluorooctanesulfonate 13C8 sodium salt (PFOS-13C8), and Perfluorooctanoic acid 13C8 (PFOA-13C8), were all purchased from Chiron AS (Trondheim, Norway).

The analytical standards of the steroid analysis: Androstenedione (AN), Dehydroepiandrosterone (DHEA), Testosterone (TS), 5α-Dihydrotestosterone (DHT), Aldosterone (ALDO), Cortisol (COR), 11-Deoxycortisol (11-deoxyCOR), Corticosterone (COS), 11-Deixycorticosterone (DOC), Cortisone (CORNE), Pregnenolone (PREG),

17stHydroxypregnenolone (17OH-P5), Progesterone (P4), 17α-Hydroxyprogesterone (17α-OHP), and Estrone (E1) were purchased from Cerilliant (Texas, USA). The analytical standard of 11-ketostestosterone (11-ketoTS) was purchased from Sigma-Aldrich (Steinheim, Germany) and the analytical standard of Androstenediol (A5) was purchased from Toronto Research Chemicals, Inc (North York, ON, Canada). In total, 17 steroids were analyzed. Following the internal standard method, the 3 isotopically labeled ISs used for steroid quantification, namely Cortisone-13C3 (2,3,4-13C3-CORNE), Dihydrotestosterone-13C (2,3,4-13C-DHT), and 17α-hydroxyprogesterone-13C2 (2,3,4-13C2-17αOHP) were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA).

Methanol (MeOH), ammonium formate (NH4COOH), Polypropylene (PP) tubes (15 mL) and the amber glass LC vials (2 mL) were purchased from VWR International AS (Oslo, Norway). The Superclo Hybrid Solid Phase Extraction (HybridSPE)-phospholipid cartridges (30 mg, 1 mL) were purchased from Sigma-Aldrich (Darmstadt, Germany). A 12-port Visiprep DL (Disposable Liners) Solid Phase Extraction (SPE) vacuum manifold was purchased from Supelco, Inc (Bellefonte, PA, USA). The cryovials (1.8 mL) were purchased from Sartsedt AG & Co (Germany). Ammonium acetate (NH4CH3COO) and formic acid (HCOOH) were purchased from Merck (Darmstadt, Germany). Ultrapure carbon dioxide (CO2) was purchased from AGA industrial gases (Lindingö, Sweden). Finally, ultrapure water was prepared via a water purification system (Qoption, Elga Labwater, Veolia Water Systems LTD, U.K.).

2.2.1. Standard mix

The $\sum_{43}$PFAS were mixed together in MeOH to make up a 500 µg/L PFAS mix (PFAS-mix) in a 15 mL PP tube with a final volume of 1mL. The same was done with the $\sum_{17}$steroid to make up a 1000 µg/L steroid mix (steroid-mix) of 1mL. Finally, a 1000 µg/L internal standard mix (IS-mix) was made up in a 15 mL PP tube by mixing the PFAS ISs (PFOA-^{13}C8, PFOS-^{13}C8, 6:2 FTS ^{13}C2) and steroid ISs (CORNE-^{13}C, 17-OHP-^{13}C3, DHT-^{13}C3) in MeOH for a final volume of 1mL. The standard solutions were stored at -20°C in between uses.

2.2.2. Calibration standard

A 14-point calibration curve was made for each PFAS. Calibration standards (CCs) were made up in MeOH with 0.1% ammonium formate (w/v), spiked with 10 µL of IS-mix, and fortified

with concentrations of PFAS ranging from 0 to 100 ng/mL (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100 ng/mL). Calibration curves were also made for each steroid where CCs were made up in MeOH with 0.1% ammonium formate (w/v), spiked with 10 µL of IS-mix, and fortified with concentrations of steroid ranging from 0 to 50 ng/mL. Each CCs were made up in 15mL PP tube for a final volume of 500 µL.

2.3. Sample preparation

Sample preparation is an important step for separating the TAs from other matrix components in the sample as they can affect their detection and accurate quantification (Sargazi et al., 2021). These effects are known as matrix effects (ME) and are one of the main challenges in mass spectrometry-based methods (Huang et al., 2012). In this experiment, a HybridSPE extraction was performed according to previous work with minor modifications (Sait et al., 2023; Vike-Jonas et al., 2021). In the HybridSPE method, proteins are removed from the samples by centrifugation after the addition of a protein-precipitation solvent, here MeOH with 0.1% ammonium formate (w/v), and the phospholipids are removed by passing the supernatants through HybridSPE cartridges (Honda et al., 2018).

2.3.1. Plasma sample extraction

Prior to sample preparation, the 12 plasma samples were thawed at room temperature after having been stored at -20°C. Aliquots of 150 µL of each sample were transferred to 15 mL PP tubes where they were spiked with 10 µL of the IS-mix (500 ppb) and fortified with 450 µL of MeOH with 0.1% ammonium formate (w/v) for protein precipitation. The samples were vortexed for 30 seconds and centrifuged at room temperature for 10min at 4000 rpm. The HybridSPE cartridges were conditioned with 1 mL of MeOH with 0.1% ammonium formate (w/v) and the supernatants were then passed through the cartridges with the use of a SPE vacuum manifold. The eluents were collected into new 15mL PP tubes, transferred to 2 mL LC vials, and stored at -20 °C until further instrumental analysis. Finally, following a ratio of 1 method blank for 4 samples, background contamination was considered by making up 3 method blanks in MeOH with 0.1% ammonium formate (w/v) and spiked with 10 µL of IS-mix. The blanks were prepared at the same time as the samples following the HybridSPE protocol.

Plasma samples from 1992 to 2020 were prepared by PhD student Shannen Thora Lea Sait following the same method.

2.3.2. Quality control samples

A pooled sample was made up from 7 plasma samples of pups of different age and sex (4 males and 3 females between 2 and 16 days old) to be as representative as possible of the sample set. Specifically, 500 µL of each of the 7 samples were added to a PP tube (15 mL) for a final volume of 3500 µL. From the pooled sample, quality control samples (QCs) were made to assess the method performance for PFAS and steroid hormones analysis. Each QCs contained 150 µL of the pooled sample and 450 µL of MeOH with 0.1% ammonium formate (w/v). For PFASs, 21 QCs were made: 3 QCs spiked with 10 µL of the IS-mix prior to the extraction, 3 QCs in triplicates spiked with 10 µL IS-mix and fortified with the PFASs TA-mix at three fortification levels (5, 10, 20 ng/mL) prior to the extraction and labelled "spiked samples" (SPs), and 3 QCs in triplicates spiked with 10 µL IS-mix and fortified with the PFAS-mix at a three fortification levels (5, 10, 20 ng/mL) post-extraction and labelled "matrix-match samples" (MMs).

The method was the same to make the 21 QCs for steroids except that the SPs and MMs were fortified at three fortification levels (5, 10, 20 ng/mL) with the steroid-mix. For every 10 QCs, one method blank was made in MeOH with 0.1% with ammonium formate and spiked with 10 µL of the IS-mix to account for background contamination. QCs were vortexed for 30 seconds and centrifuged at room temperature for 10min at 4000 rpm. The supernatants were then passed through the pre-conditioned HybridSPE cartridges with the use of an SPE vacuum manifold. The eluents were collected into new 15mL PP tubes, transferred to 2 mL LC vials, and stored at -20 °C until further instrumental analysis.

2.4. Instrumental analysis

2.4.1. PFASs

The protocol used for PFAS analysis was based on Vike-Jonas et al. (2021) with minor modifications. In this experiment, ultra performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) was carried out using an Acquity UPLC I-Class system (Waters, Milford, USA) coupled to a triple quadrupole mass analyser (QqQ; Xevo TQ-S) with a ZSpray

electrospray ionization (ESI) ion source (Waters, Milford, USA). PFASs separation was achieved with a Kinetex C18 column (30 x 2.1 mm, 1.3 µm, 100 Å) serially connected to a phenomenex guard column in ESI negative mode (ESI -). The mobile phases consisted of water with 2mM ammonium acetate (A), and MeOH (co-solvent, B). The gradient elution program used was extrapolated from Vike-Jonas et al., (2021) and is shown in Table A.1.2. The flow rate was 250 µL min^{-1} and the injection volume was 4 µL. The wash solvent was MeOH:ultrapure water (50:50). The optimal source settings used for UPLC-MS/MS can be found in Table A.1.3.

PFASs analysis for plasma samples from 1992 to 2020 was performed by PhD student Shannen Thora Lea Sait, Department of Chemistry, NTNU following the same method.

2.4.2. Steroids

The protocol used for the analysis of steroid hormones was based on Sait et al. (2023) who, by extrapolating the method of de Kock et al., 2018, developed a method for the multiresidue determination of 19 steroid hormones in grey seal blood plasma using ultra-performance convergence chromatography tandem mass spectrometry (UPC^2-MS/MS). The main advantages of UPC^2-MS/MS are that it is a much faster method than UPLC and exhibits greater separation efficiencies (Gopaliya, 2014).

The separation of steroid hormones was performed using a Waters ACQUITY UPC^2 system (Milford, MA, USA) coupled to a triple quadrupole (QqQ; Xevo TQ/XS) mass spectrometer with a Zspray ESI ion source (Waters, Milford, U.S.), with a Viridis® Charged Surface Hybrid (CSH) Fluoro-Phenyl UPC^2 column (130 Å, 1.7 µm, 2.1 mm x 100 mm) equipped with a Viridis® CSH Fluoro-phenyl VanGuard™ pre-column (2.1 mm x 5 mm) in ESI positive mode (ESI+). The mobile phases (MPs) consisted of ultrapure CO2 (supercritical fluid, A), and MeOH containing 30mM ammonium acetate (co-solvent, B). The gradient elution program used was extrapolated from de Kock et al. (2018) and is shown in Table A.1.4. The flow rate was 200 µL min^{-1} and the injection volume was 1 µL. The weak wash solvent was heptane, and the strong wash solvent was MeOH. The optimal source settings used for UPC^2-MS/MS can be found in Table A.1.5.

2.5. Target analyte quantification

Both PFASs and steroids were quantified using the internal standard method. Indeed, by using ^{13}C-labeled ISs, the fluctuations between measurements due to instrumental conditions and MEs were compensated for (Christian et al., 2013; Cortese et al., 2020). A known amount of 500 ppb IS-mix (10 µL) was added to each sample, and relative areas (RAs) were obtained by calculating the ratio of the TA area to the IS area in every sample and for all TAs (Christian et al., 2013). The ^{13}C-labeled PFAS ISs were PFOA-^{13}C8, PFOS-^{13}C8, and 6:2 FTS 13C2. PFOA-^{13}C8 was the PFAS-IS for PFOA, 6:2 FTS ^{13}C2 was the PFAS-IS of 6:2 FTS, and PFOS-^{13}C8 was the PFAS-IS for all the other PFASs. For steroids, ^{13}C-labeled Steroid-ISs were CORNE-^{13}C, 17-OHP-^{13}C3, and DHT-^{13}C3. CORNE-^{13}C was the steroid-IS for COR, CORNE, ALDO, COS, 11-deoxyCOR and KetoTS, while 17-OHP-^{13}C3 was the steroid-IS for 17-OHP, DOC, 17aOHP5 and E1. Finally, DHT-^{13}C3 was the steroid-IS for all the other steroids (P4, KetoTS, PREG(P5), DHT, TS, AN, A5 and DHEA). ISs are usually chemically similar to the TAs (Christian et al., 2013). The RAs obtained were corrected for blank contamination by subtracting each RAs of the plasma samples to the average RAs of the three method blanks for all TAs. The blank corrected RAs were then divided by the slope of the RAs of the SPs for each TAs. Values below the instrumental limit of detection (iLOD) were replaced by "<iLOD". Finally, concentrations were corrected with the dilution factor (=0,5[mL]/0,15[mL] ≈ 3.3) and expressed in ng/mL on a wet weight (ww) basis.

2.6. Quality control

2.6.1. Matrix effects

MEs at the three TA fortification levels (5, 10, 20 ng/mL) were quantified by calculating the ratio of the slope of MMs to the slope of CCs made up in solvent (MeoH with 0.1% ammonium formate) for each TA as shown in Equation 1. The MMs were spiked with 10 µL of the 500 ppb IS-mix and fortified with TAs at three fortification levels (5, 10, 20 ng/mL) post-extraction. The CCs were fortified with TAs concentrations ranging from 0 to 50 ng/mL (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 ng/mL) and spiked with 10 µL of the 500 ppb IS-mix.

$$ME\%_{5,10,20\ ng/mL} = \frac{Slope_{MM}}{Slope_{CC}} \cdot 100 \quad (1)$$

2.6.2. Instrumental calibration

The calibrations of both UPC^2-MS/MS and UPLC-MS/MS were verified by injecting calibration standards solution prepared in MeOH containing 0.1% w/v ammonium formate and fortified with TA concentrations ranging from 0 to 100 ng/mL (0, 0.01, 0.02, 0.05, 0.1, 0.2; 0.5, 1, 2, 5, 10, 20, 50, 100 ng/mL). Regression coefficients (R^2) were calculated, and the calibration was judged satisfactory when $R^2 > 0.99$. Additionally, one procedural blank made of MeOH was analyzed with every batch of 5 samples to monitor background contamination and one calibration standard fortified with 20 ng/mL of TA was analyzed regularly to evaluate potential shifts in the signal.

2.6.3. Extraction efficiency

The extraction efficiency of the HybridSPE method for each TA can be determined by calculating the recoveries (Kollipara et al., 2011). In this experiment, absolute recovery (AR) and relative recovery (RR) were determined at three fortification levels (5, 10, 20 ng/mL) for each TA. AR was determined by the ratio of the average responses of SPs fortified prior extraction to the average response of MMs fortified post-extraction for all TAs as shown in Equation 2. RR was determined by the ratio of the average responses of SP to the average responses of SP in the ISs over the ratio of the average responses of MMs to the average responses of MMs in the ISs for all TAs as shown in Equation 3.

$$AR\%_{5,10,20 ng/mL} = \frac{\bar{X}_{SP}}{\bar{X}_{MM}} \cdot 100 \quad (2)$$

$$RR\%_{5,10,20\ ng/mL} = \frac{\bar{X}_{SP}\ /\bar{X}_{SP,IS}}{\bar{X}_{MM}/\bar{X}_{SP,IS}} \cdot 100 \quad (3)$$

2.6.4. Precision

Relative standard deviation (RSD) of the AR and RR were used as an indicator of precision, which is considered as the repeatability of the results obtained (Wells et Dantus, 2004). RSD was calculated as the ratio of the standard deviation (SD) to the mean as shown in Equation 4.

$$RSD\% = \frac{SD\left(\sum\left(SP_{5,10,20\frac{ng}{mL}}/\bar{X}_{MM\,5,10,20\,ng/mL}\right)\right)}{\bar{X}\left(\sum\left(SP_{5,10,20\frac{ng}{mL}}/\bar{X}_{MM\,5,10,20\,ng/mL}\right)\right)} \cdot 100 \quad (4)$$

In this experiment, both absolute and relative RSDs of the recoveries were determined, using absolute and relative areas respectively.

2.6.5. Sensitivity

In this experiment, the instrumental limit of quantification (iLOQ) was determined visually by evaluating for which concentration of the target analyte in the calibration standards the peak was well defined. As visual evaluation can be considered arbitrary, peer verification was applied when choices were more difficult to make. The instrumental limit of detection (iLOD) was determined by dividing iLOQ by 3.3 (EMA, 1995; Lister, 2005) as shown in Equation 5. The method limit of detection (mLOD) and the method limit of quantification (mLOQ) were determined from the iLOD and iLOQ by considering the dilution factor (=0,5[mL]/0,15[mL] ≈ 3.3) as shown in Equation 6 and 7.

$$iLOD = \frac{iLOQ}{3.3} \quad (5)$$

$$mLOD = iLOD \cdot 3.3 \quad (6)$$

$$mLOQ = iLOQ \cdot 3.3 \quad (7)$$

2.7. Data analysis and statistical treatment

UPLC-MS/MS and UPC^2-MS/MS data were acquired using MassLynx (Version 4.1) software while quantification processing was performed using TargetLynx (Waters, Milford, USA). Excel (Microsoft 365, version 2304) was used for simple calculations and for general descriptive statistics. PFASs and steroids concentrations were reported as ng/mL on a w/w basis. Only TAs for which values were above the mLOD in at least 70% of the samples were kept and further considered for statistical analysis. Values below the mLOD were handled by substituting them by half the mLOD value (Croghan et al., 2003). Because only one seal pup was sampled in 2006, it was not further considered in the analysis. Bivariate and multivariate statistical analysis were performed using R studio (Version 4.1.2). The level of significance was set at 0.05.

For each numerical variable, the Shapiro-Wilk test was used to assess the normal distribution of the data. Because normal distribution could not be verified for all variables even after log10-transformation (Shapiro-Wilk: $p<0.05$), both parametric and non-parametric tests were applied. To explore differences between sexes and age for each variable, student t-test (parametric) and Mann Whitney U test (non-parametric) were used. To explore the differences between sampling years (1992, 1993, 2000, 2005, 2020, 2022) for each variable, a one-way analysis of variance (ANOVA) with post-hoc Tukey HSD (parametric) and a Kruskal-Wallis test followed by a Dunn Test for pairwise comparison of groups (non-parametric) were used. To assess temporal trends of PFAS concentrations, linear regression models were used.

Multivariate analysis was carried out to explore the relationships between the steroid hormone concentrations and the explanatory variables (PFAS levels and biological variables) in plasma of grey seal pups sampled in 2022 (n = 12), and between PFAS levels and the explanatory variable body mass for pups sampled from 1992 to 2022 (n=164). A principal component analysis (PCA) based on a correlation matrix (centered and scaled) was first run and the correlations between the variables were further explored using Spearman's rank test. Redundancy analysis (RDA) with forward permutation tests was then applied using the R package "vegan" to assess if the explanatory variables had a significant effect on the total variance of the response variables (PFAS concentrations or steroid concentrations).

3. Results

3.1. Method validation

3.1.1. Sensitivity

The estimated mLODs for the majority of the PFASs ranged from 0.01 to 0.20 ng/mL (Table A.2.1). Higher mLODs were reported for PFOcDA (2.02 ng/mL), PFBA (5.05 ng/mL), TriDeFHxSA (20.20 ng/mL), SamPAP (50.50 ng/mL), Gen X (1.01 ng/mL), 6:6 PFPi (2.02 ng/mL), 6:8 PFPi (5.05 ng/mL) and 8:8 PFPi (5.05 ng/mL). The value obtained for mLODs were generally in accordance with those previously reported in the literature using a similar method, namely UPLC-MS/MS (Boisvert et al., 2019; Routti et al., 2015). The calibration curves demonstrated a satisfactory regression coefficient for all PFASs ($R^2 > 0.9$) except for PFBA ($R^2 = 0.28$) and TriDeFHxSA ($R^2 = 0.72$), which were considered not quantifiable and not further considered in the analysis.

The estimated mLODs for the majority of the steroid hormones ranged from 0.20 to 2.02 ng/mL (Table A.2.2). Higher mLODs (5.05 ng/mL) were reported for P4, P5, AN and A5. In general, the mLOD values were higher than those reported previously in the literature (Sait et al., 2023; Styrishave et al. 2017) using an identical analytical method. Moreover, the 14-point calibration curves for the analytes with concentrations ranging from 0 to 50 ng/mL (0 – 50 ng/mL) was made up using MeOH and demonstrated a satisfactory regression coefficient for all 17 steroids ($R^2 > 0.9$).

3.1.2. Matrix effects

The MEs ranged from -38% to 33% for PFASs with outlier values found for PFBA (-3345%) and TriDeFHxSA (-515%), and from -33% to 30% for steroids with the exception of ALDO (46%) and 11-KetoTS (77%) (Table A.2.3). Those outliers are justified by the poor linearity of the calibration curve for both PFBA and TriDeFHxSA (Table A.2.1). The negative MEs represent ion suppression while positive MEs represent ion enhancement (Cortese et al., 2020). The measured MEs were in accordance with the literature for both steroids (Sait et al., 2023)

and PFASs (Trimmel et al., 2021) using the same analytical methods. In the present study, MEs were compensated for by using isotopically labelled ISs.

3.1.3. Extraction efficiency

With the exceptions of PFBA (10,2%), TriDeFHxSA (164.8%), MeFOSAA (12,5%), SamPAP (0.66%) and DiSamPAP, (9.3%), the ARs of the PFASs ranged from 43.8% to 81.3% at the highest fortification level (20 ng/mL) (Table A.2.4). AR values were both similar (Roos et al., 2013) and lower (Taylor et al., 2021, Grønnestad et al., 2017) to those previously reported in the literature. This could be explained by recoveries being reported as relative rather than as absolute. The importance of the recoveries values lies more in their precision assessed by the RSD (%) than in their intrinsic value (ICH, 2019). RSD values for the ARs were lower or equal to 18% for all PFASs at high fortification level (20ng/mL) except for PFBA (88%), TriDeFHxSA (101%), MeFOSAA (51.8%), SAMPAP (158.6%) and DiSamPAP (73.7%). PFBA and TriDeFHxSA were already considered as not quantifiable because of the poor linearity of their calibration curves. MeFOSAA, SamPAP and DiSamPAP exhibited both low recovery and low precision even when looking at their RRs and were therefore considered not quantifiable.

With the exceptions of AN, ALDO, and 17a-OHP5, the ARs of the steroids ranged from 54.5% to 99.2% at the highest fortification level (20 ng/mL) (Table A.2.5). For the three fortification levels (5, 10, 20 ng/mL), AN exhibited the lowest ARs ranging from 0.6% to 1.1%, and ALDO had low ARs ranging from 19% to 22.2%. ARs were similar to those measured in previous studies using HybridSPE to extract steroids from plasma samples (Sait et al., 2023). RSD values for the ARs were lower or equal to 15% for all steroids at high fortification level (20ng/mL) except for 17a-OPH5 (62%) and AN (30%). Only AN demonstrated both a low absolute recovery and low precision. However, AN exhibited much higher relative recoveries (RRs) ranging from 86% to 161%, suggesting strong MEs. ALDO had poor recoveries and good precision even when looking at RRs and 17aOPH5 showed good recoveries but poor precision.

3.2. Biological variables

One objective of the present study was to assess the temporal trends of PFAS concentrations in grey seal pups sampled from 1992 to 2022 (n=165) in the Froan Archipelago. For the 165 grey seal pups, age was not considered in the statistical analysis as it was only available for pups sampled in 1993 (n=26) and 2022 (n=12), for a total of 38 pups. Similarly, length and girth were not considered in the temporal trends analysis as these variables were only available for 25 pups sampled in 2020 and 2022. Finally, data from the only pup sampled in 2006 were discarded because a single sample cannot be representative of trends in 2006. Therefore, BM was the only biological variable considered when assessing the temporal trends of PFAS concentrations.

The second objective of this experiment was to assess the potential relationships between steroid levels and PFAS concentrations in plasma samples of grey seal pups sampled in 2022. As sex, length, girth, BM, and age were available for all the pups sampled in 2022 (n=12), these biological variables were considered in the statistical analysis. The mean values ± standard deviation of the biological variables for the seal pups sampled in 2022 are shown in Table 3.1.

For all the pups, the sample location was not considered as a biological variable because they were all sampled in the Froan Archipelago, with no more than 20 kilometers between each sample (Figure 2.1).

Depending on normality of the data, both Student T-test and Mann Whitney U tests were carried out to explore the sex differences for the pups sampled from 1992 to 2022. Because sex data were not available for pups sampled in 2000 and 2005, they were not included in the analysis. No sex differences were found for each individual PFAS nor for ∑PFAS, ∑PFCA, ∑PFSA and body mass ($p > 0.05$) for pups sampled from 1992 to 2022. Moreover, sex and categorical age differences in biological variables (body mass, length, girth), PFAS concentrations, and steroid levels, were explored for pups sampled in 2022 and no significant differences were found. Therefore, sex and age were not further considered in the analysis. The lack of sex differences is in accordance with previous studies on pinnipeds (Taylor et al., 2021; Grønnestad et al., 2017) and is not surprising as grey seals become sexually mature between 3 and 5 years of age for females and at 6 years of age for males (Hall et Russell, 2018). It should also be mentioned that other studies focusing on PFAS concentrations in adult seals have not found any statistical sex differences either (Boisvert et al., 2019; Routti et al., 2016).

Table 3.1. Mean, standard deviation (sd), and range of the biological variables for the grey seal pups (n=12) sampled in the Froan Archipelago in 2022. Because no significant sex and age differences were found, grey seal pups were pooled together.

	Mean ± sd (min – max)
Weight	37.07 ± 14.57 (15 – 64.2)
Length	113.75 ± 14.63 (83 – 132)
Girth	90.29 ± 14.15 (70 -110)

3.3. Occurrence of PFASs in plasma of grey seal pups sampled from 1992 to 2022

42 PFASs were jointly analyzed in plasma samples of 164 (without pup sampled in 2006) grey seal pups captured from 1992 to 2022 in the Froan Archipelago, including 86 samples from 1992, 29 from 1993, 6 from 2000, 13 from 2020 and 12 from 2022 (Table 3.2). Out of these 42 PFASs, 16 were detected in more than 70% of the individuals (based on values >mLOD) and further considered for the statistical analysis. These included 7 PFCAs: PFPeA, PFOA, PFNA, PFUnA, PFDoDA, PFTriDA and PFTDA), 8 PFSAs: PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoDS and PFECHs, and 1 PFAA precursor: PFOSA. PFUnA, PFDoDA, PFTriDA, PFTDA, PFHxS, PFHpS, and PFOS were detected in all individuals. The detection rate (DR) and geometric mean for all 42 PFASs are shown in Table A.2.6.

Table 3.2. Concentrations of the 16 PFASs with a detection rate (DR) above 70% in plasma of grey seal pups sampled from 1992 to 2022 (n=164) in the Froan Archipelago. Concentrations are presented as the geometric mean (GM) ± standard deviation (sd) with median (m) in brackets and range in parentheses. All values are given in ng/mL.

		1992 (n=85)	**1993 (n=29)**	**2000 (n=6)**	**2005 (n=19)**	**2020 (n=13)**	**2022 (n=12)**
	PFASs (DR%)	GM ± sd [m] (range)	GM ± sd [m] (range)	GM ± sd [m] (range)	GM ± sd [m] (range)	GM ± sd [m] (range)	GM ± sd [m] (range)
PFAA precursor	PFOSA (73.3)	0.04 ± 0.09 [0.04] (<mLOD-0.53)	0.05 ± 0.03 [0.05] (<mLOD-0.15)	0.04 ± 0.04 [0.04] (0.02-0.12)	0.03 ± 0.03 [0.03] (<mLOD-0.10)	0.02 ± 0.02 [0.02] (<mLOD-0.06)	<mLOD
PFCAs	PFPeA (76.2)	1.15 ± 4.25 [1.09] *(<mLOD -30.4)*	0.84 ± 0.88 [0.82] *(<mLOD -4.46)*	0.73 ± 0.35 [0.61] (<mLOD -1.18)	0.71 ± 0.67 [0.55] (<mLOD -1.88)	1.08 ± 2.26 [0.82] (<mLOD -7.03)	0.40 ± 0.36 [0.31] (<mLOD -0.89)
	PFOA (90.2)	0.73 ± 0.70 [0.71] (<mLOD -4.57)	0.54 ± 0.80 [0.62] (<mLOD -4.04)	1.16 ± 1.41 [0.93] (0.55-4.20)	0.70 ± 0.64 [0.74] (0.19-2.59)	0.31 ± 0.22 [0.32] (<mLOD -0.80)	0.49 ± 0.28 [0.56] (0.13-1.18)
	PFNA (99.4)	0.55 ± 0.39 [0.53] (0.09-1.89)	0.58 ± 0.36 [0.58] (<mLOD -1.84)	1.25 ± 0.51 [1.19] (0.80-2.26)	2.27 ± 1.26 [2.17] (0.71-4.76)	1.64 ± 1.55 [1.59] (0.37-5.84)	3.33 ± 1.22 [3.80] (0.13-1.18)
	PFUnA (100)	2.27 ± 1.16 [2.23] (0.90-8.07)	2.50 ± 1.22 [2.47] (1.09-5.75)	5.63 ± 2.11 [5.71] (3.86-9.58)	8.62 ± 3.28 [9.12] (4.35-15.0)	5.31 ± 2.64 [5.61] (2.58-12.4)	7.53 ± 3.74 [6.84] (5.28-18.0)
	PFDoDA (100)	0.75 ± 0.32 [0.69] (0.38-1.81)	0.76 ± 0.37 [0.73] (0.21-1.70)	1.57 ± 0.57 [1.52] (1.00-2.40)	2.24 ± 0.87 [2.25] (1.08-4.27)	1.51 ± 0.87 [1.30] (0.58-3.31)	2.11 ± 0.97 [2.18] (1.32-4.29)
	PFTriDA (100)	2.77 ± 0.92 [2.73] (1.23-5.95)	2.83 ± 1.24 [2.98] (1.01-5.74)	5.24 ± 1.54 [4.83] (3.85-7.82)	7.27 ± 2.55 [6.91] (3.83-13.2)	4.02 ± 1.75 [3.98] (1.71-8.57)	4.59 ± 1.99 [4.28] (2.79-9.36)
	PFTDA (100)	0.28 ± 0.12 [0.29] (0.06-0.61)	0.25 ± 0.15 [0.32] (0.06-0.66)	0.48 ± 0.23 [0.50] (0.27-0.84)	0.62 ± 0.23 [0.58] (0.39-1.17)	0.47 ± 0.24 [0.51] (0.20-1.04)	1.09 ± 0.54 [0.98] (0.59-2.37)
	PFPeS (93.3)	0.19 ± 0.16 [0.23] (<mLOD-0.71)	0.22 ± 0.16 [0.24] (0.02-0.73)	0.37 [0.36] (0.15-0.88)	0.13 ± 0.30 [0.13] (0.02-1.30)	0.12 ± 0.46 [0.13] (0.01-1.66)	0.12 ± 0.05 [0.12] (<mLOD-0.2)

PFSAs	PFHxS (100)	8.48 ± 4.68 [8.99] (1.81-22.9)	9.88 ± 4.34 [10.01] (4.99-25.8)	15.54 [15.33] (9.60-26.0)	11.62 ± 10.14 [11.76] (5.28-49.3)	2.95 ± 1.81 [2.94] (1.07-8.09)	3.74 ± 2.54 [3.44] (1.83-10.5)
	PFHpS (100)	2.22 ± 1.43 [2.36] (0.47-7.39)	2.69 ± 1.70 [2.53] (0.62-9.20)	4.38 ± 1.06 [4.14] (3.56-6.47)	2.95 ± 1.51 [3.03] (1.46-7.79)	0.58 ± 0.43 [0.54] (0.25-1.48)	0.72 ± 0.48 [0.64] (0.32-1.85)
	PFOS (100)	103.6 ± 64.20 [107.81] (35.0-405.5)	122.89 ± 67.21 [122.05] (49.5-328.98)	208.85 ± 35.91 [209.95] (156.6-250.4)	137.26 ± 73.15 [138.33] (62.3-327.7)	43.80 ± 20.47 [43.66] (19.3-91.2)	45.0 ± 19.04 [41.64] (26.8-89.5)
	PFNS (94.5)	0.23 ± 0.22 [0.26] (<mLOD-1.24)	0.31 ± 0.23 [0.34] (<mLOD-1.07)	0.76 ± 0.21 [0.74] (0.59-1.11)	0.42 ± 0.24 [0.46] (0.14-1.03)	0.10 ± 0.08 [0.11] (<mLOD-0.25)	0.12 ± 0.09 [0.16] (0.02-0.32)
	PFDS (98.8)	0.23 ± 0.20 [0.24] (0.04-1.16)	0.27 ± 0.22 [0.31] (<mLOD-0.89)	0.98 ± 0.26 [1.00] (0.64-1.29)	0.92 ± 0.53 [0.95] (0.30-2.26)	0.29 ± 0.14 [0.33] (0.10-0.56)	0.29 ± 0.15 [0.34] (0.08-0.66)
	PFDoDS (72.1)	0.15 ± 0.11 [0.15] (<mLOD-0.62)	0.17 ± 0.08 [0.16] (<mLOD-0.41)	0.18 ± 0.07 [0.18] (0.13-0.29)	0.20 ± 0.16 [0.19] (<mLOD-0.63)	0.08 ± 0.02 [0.08] (<mLOD-0.11)	0.05 ± 0.11 [0.03] (0.01-0.21)
	PFECHS (98.2)	0.15 ± 0.14 [0.17] (<mLOD-0.75)	0.23 ± 0.17 [0.26] (<mLOD-0.79)	0.31 ± 0.06 [0.32] (0.23-0.38)	0.20 ± 0.13 [0.24] (0.08-0.52)	0.13 ± 0.08 [0.15] (0.03-0.32)	0.42 ± 0.44 [0.36] (0.25-1.88)
	$\sum_{16}$PFAS	125.83 ± 71.76 [132.00] (45.55-455.13)	146.29 ± 75.41 [144.34] (62.49-383.76)	248.57 ± 44.36 [255.32] (182.37-297.08)	178.07 ± 88.35 [178.27] (81.28-419.45)	64.19 ± 27.50 [61.00] (28.67-128.43)	71.14 ± 25.70 [63.82] (48.84-120.87)
	$\sum_{8}$PFSA	115.78 ± 69.62 [121.43] (37.74-434.99)	137.35 ± 72.57 [137.69] (56.48-367.12)	231.85 ± 42.94 [232.72] (171.91-285.48)	154.54 ± 83.03 [151.84] (69.65-389-74)	48.40 ± 21.91 [46.82] (20.89-96.11)	50.63 ± 21.65 [47.31] (30.09-100.47)
	$\sum_{7}$PFCA	9.08 ± 4.89 [8.47] (3.94-38.54)	8.48 ± 3.62 [8.68] (3.79-16.53)	16.01 ± 5.38 [16.73] (10.40-24.59)	22.56 ± 7.85 [23.87] (11.63-37.51)	15.41 ± 6.46 [14.46] (7.78-32.32)	19.71 ± 7.28 [18.56] (13.45-38.40)

<mLOD: below the limit of detection. n.d. not detected. Note: DR% calculated based on values > mLOD.

The distributions of each 16 PFASs were calculated by dividing each of their concentrations by the concentration of $\sum_{16}$PFAS for every sampling year as shown in Figure 3.1. From 1992 to 2022, PFSAs accounted for the majority of PFASs, with PFOS being the predominant compound (distribution > 60% for each sampling year). The second and third major PFASs were PFHxS and PFTriDA from 1992 to 2005 and PFUnA and PFTriDA from 2020 to 2022.

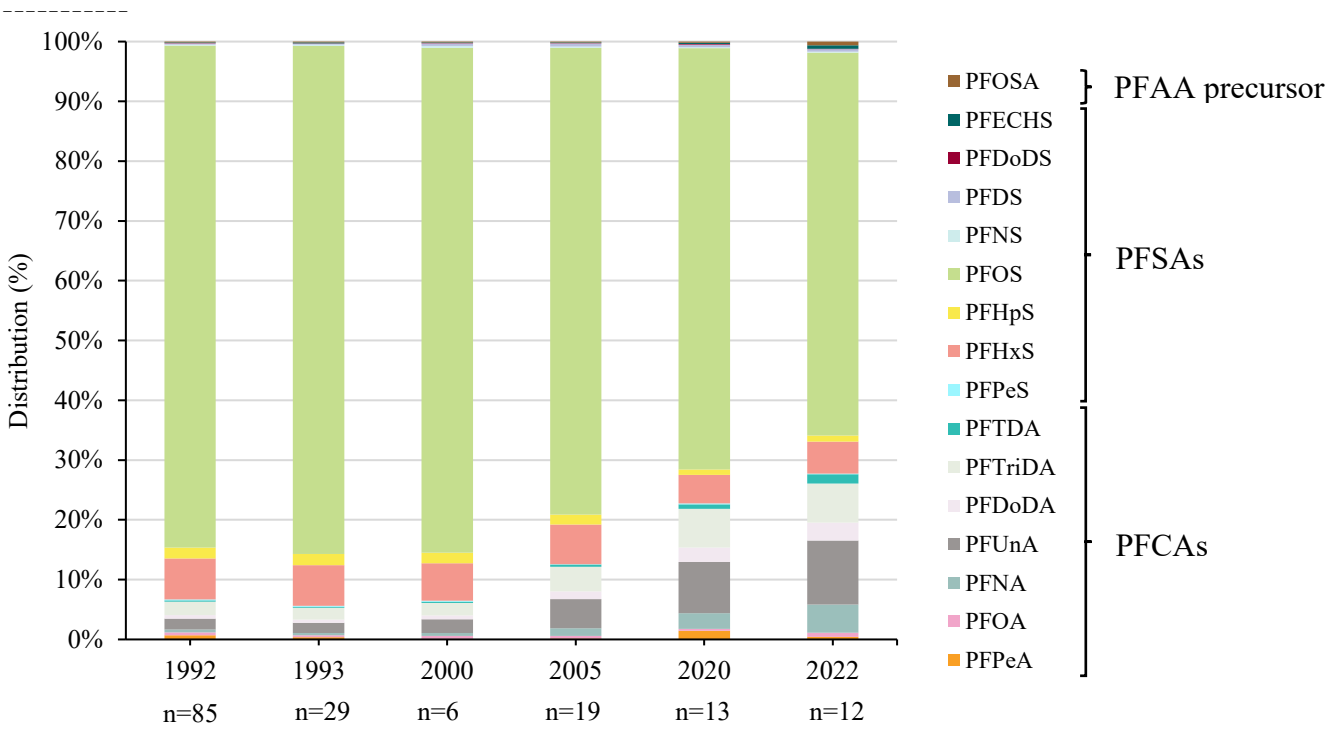

Figure 3.1. Distribution (%) per sampling year of each of the 16 PFASs with a detection rate (DR) above 70% in plasma of the grey seal pups (n=164) sampled from 1992 to 2020 in the Froan Archipelago.

3.4. Temporal trends of PFASs concentrations in seal pups sampled from 1992 to 2022

Median concentrations of $\sum_{16}$PFAS in plasma of grey seal pups (Figure 3.2) decreased significantly from 1992 to 2022 ($p < 0.05$). Precisely, $\sum_{16}$PFAS increased significantly from 1992 to 2000 ($p = 0.005$) with the highest concentrations measured in 2000, from ~126 ng/mL to ~ 249 ng/mL (Figure 3.2 and Table 3.2). No significant differences were found between 1992 and 1993, between 1993 and 2000, between 1993 and 2005, between 2000 and 2005 and between 2020 and 2022. Even if it seems that the concentrations of $\sum_{16}$PFAS started to decrease as early as 2005, they were still significantly higher in 2005 than in 1992 ($p = 0.03$). However, in 2020 and 2022, $\sum_{16}$PFAS concentrations were significantly lower than all previous sampling years ($p < 0.0001$). Indeed, in 2022, the concentrations of $\sum_{16}$PFAS dropped to a level even lower than those measured in 1992, from ~ 126 ng/mL to ~ 71 ng/mL.

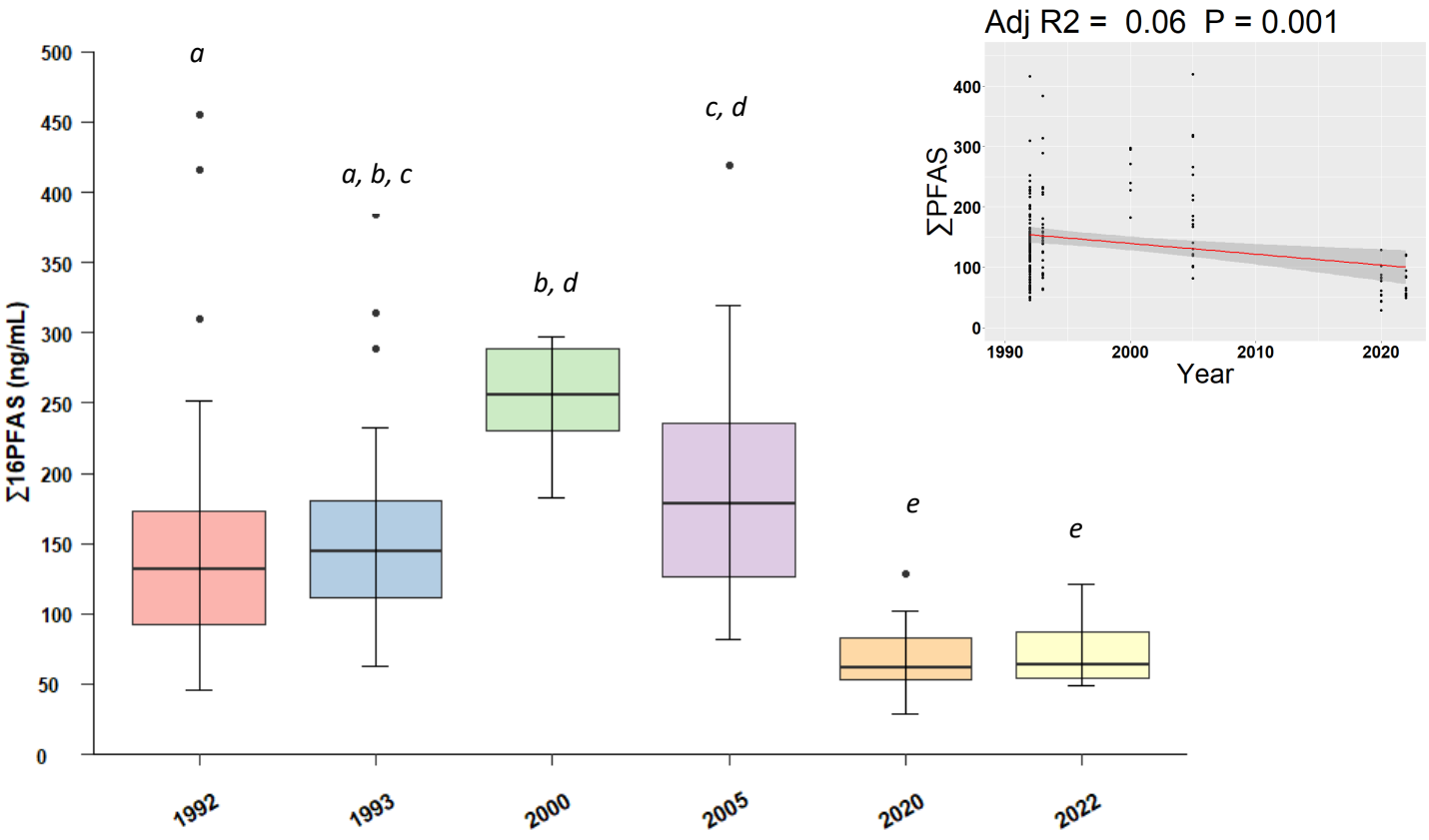

Figure 3.2. Concentrations per sampling year and temporal trend of $\sum_{16}$PFAS in plasma of 164 grey seal pups sampled from 1992 to 2022 in the Froan Archipelago. The absence of significative difference ($p > 0.05$) between two sampling years is highlighted with letters.

Median concentrations of PFOS in plasma of grey seal pups (Figure 3.3) followed the same trend and patterns as the $\sum_{16}$PFAS. Indeed, they decreased significantly from 1992 to 2022 ($p < 0.05$) and were the highest in 2000. In 2020, PFOS concentrations were significantly lower than all previous sampling years ($p < 0.0001$).

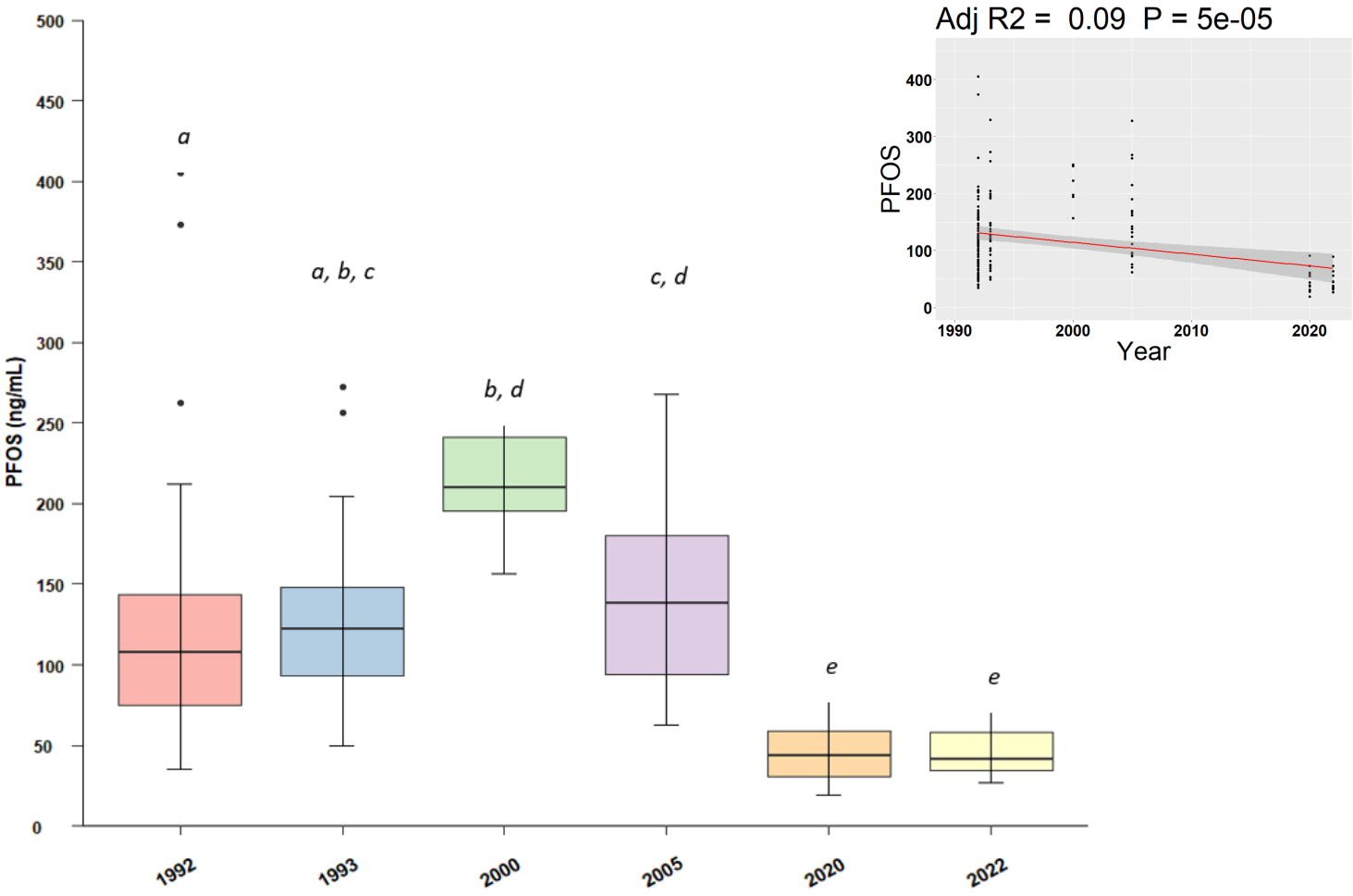

Figure 3.3. Concentrations per sampling year and temporal trend of PFOS in plasma of 164 grey seal pups sampled from 1992 to 2022 in the Froan Archipelago. The absence of significative difference ($p > 0.05$) between two sampling years is highlighted with letters.

Median concentrations of $\sum_{8}$PFSA in plasma of grey seal pups (Figure 3.4) seemed to follow the same pattern as the $\sum_{16}$PFAS. Indeed, they decreased significantly from 1992 to 2022 ($p < 0.05$). Precisely, they increased significantly from 1992 to 2000 ($p = 0.007$), from ~115 ng/mL to ~ 230 ng/mL, with the highest concentrations measured in 2000 and being twice as high as those measured in 1992 (Figure 3.3, Table 3.2). It seems that $\sum_{8}$PFSA concentrations started to decrease as early as 2005, but concentrations measured in 2005 were not significantly different

from any previous sampling years. Concentrations continued decreasing until reaching their lowest level in 2020 (~48 ng/mL) and remained low in 2022. $\sum_8$PFSA were significantly lower in 2020 and 2022 than in 1992 ($p < 0.000001$) with concentrations in 2022 (50 ng/mL) being less than half of those measured in 1992 (116 ng/mL). No significant differences were found between 1992 and 1993, between 1992 and 2005, between 1993 and 2000, between 1993 and 2005, between 2000 and 2005, and between 2020 and 2022.

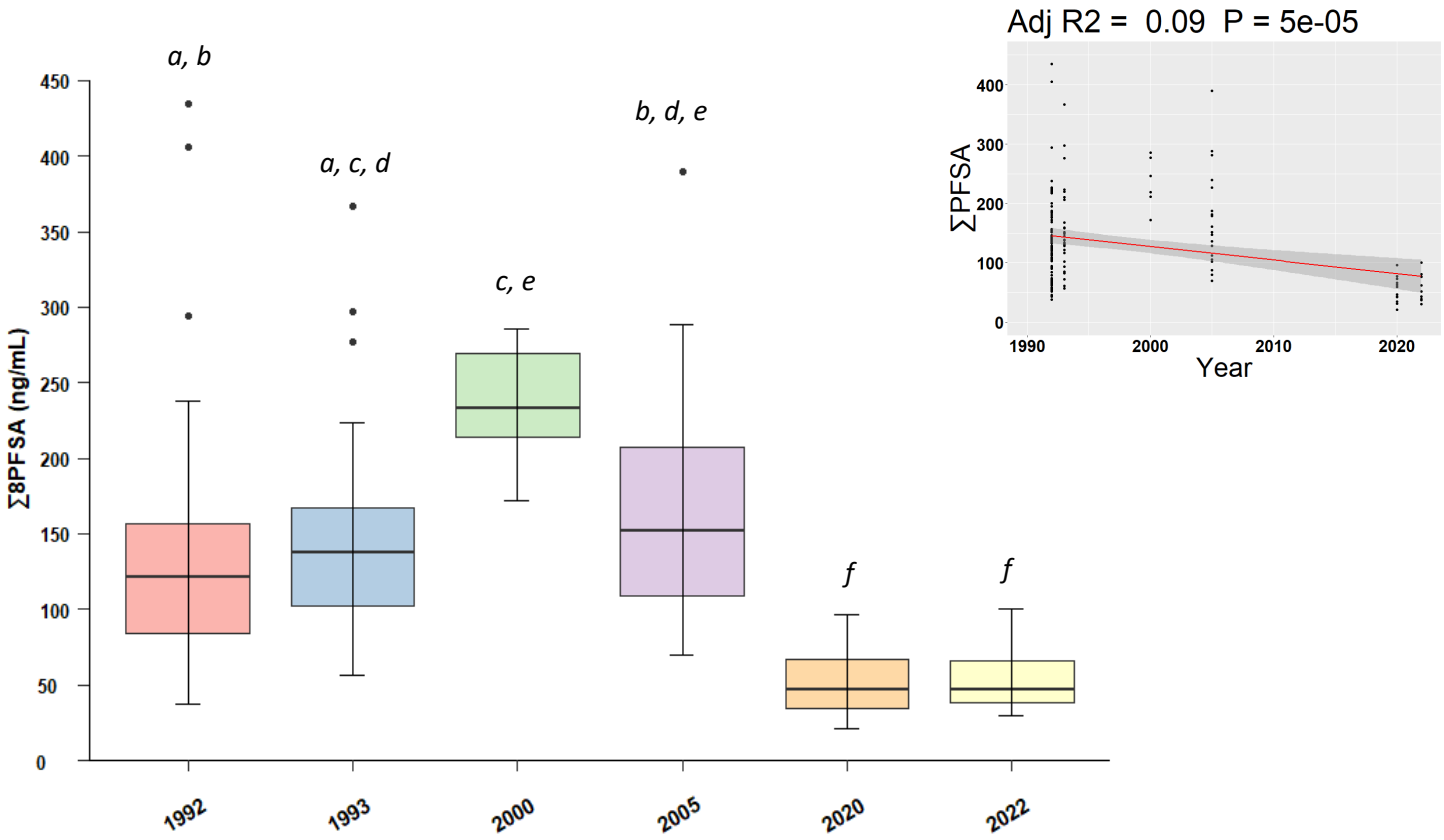

Figure 3.4. Concentrations per sampling year and temporal trend of $\sum_8$ PFSA in plasma of 164 grey seal pups sampled from 1992 to 2020 in the Froan Archipelago. The absence of significative difference ($p > 0.05$) between two sampling years is highlighted with letters.

Median concentrations of $\sum_7$PFCA (Figure 3.5) exhibited a different pattern than $\sum_{16}$PFAS and $\sum_8$PFSA. Indeed, concentrations of $\sum_7$PFCA increased significantly from 1992 to 2005 ($p < 0.000001$), from 9 to 23 ng/mL, and reached their highest concentrations in 2005 rather than in 2000 as did PFSAs (Figure 3.4). $\sum_7$PFCA levels measured in 1992 and in 1993 were significantly different than the $\sum_7$PFCA levels measured in all of the following sampling years ($p < 0.001$). Moreover, even if it seemed that concentrations were decreasing after 2005, they

did not drop back to levels measured in 1992 and even seemed to increase somewhat again in 2022 although not significantly. Indeed, $\sum_7$PFCA levels measured in 2022 were still significantly higher when compared with 1992 and 1993 ($p < 0.0001$). No significant differences were found in and between the sampling years 2000, 2005, 2020 and 2022.

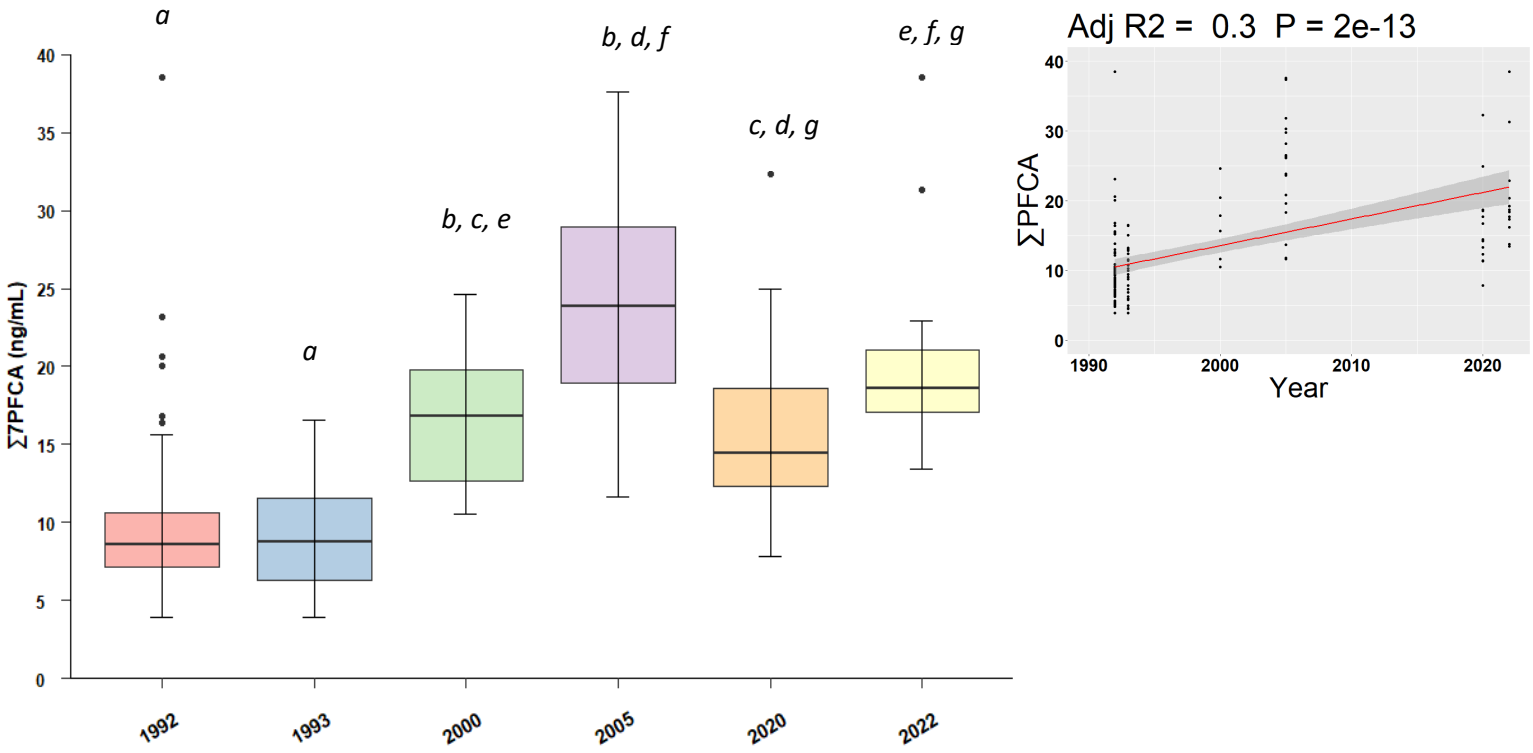

Figure 3.5. Concentrations per sampling year and temporal trend of $\sum_7$PFCA in plasma of 164 grey seal pups sampled from 1992 to 2020 in the Froan Archipelago. The absence of significative difference ($p > 0.05$) between two sampling years is highlighted with letters.

3.4.1. Relationships between PFASs levels and body mass

BM data were only available for 3 sampling years: 1992, 1993 and 2020. A PCA was used to explore the relationships among the 16 PFAS concentrations (response variables) and with the BM data (explanatory variable) (Figure 3.6). The first two components of the PCA explained 66.5% of the variance and displayed a structural grouping among PFASs with the majority of PFSAs and PFCAs correlating with each other. The variable BM was added as a passive variable on the PCA and showed a negative correlation with PFSAs although the length of the arrow from the origin suggested a small effect on the total variance. Correlations observed on the PCA were further explored using Spearman's correlation tests. Negative correlations were found between all PFSAs and BM (r: from -0.27 to -0.18, $p < 0.05$), except for PFPeS. (Figure A.2.1). However, no significant differences in BM were found between sampling years ($p > 0.05$). Moreover, even though BM contributed significantly to the variance of PFAS concentrations, it could only explain 2% of the total variance, which is negligible (RDA > 0.05, $R^2_{adj} = 0.02$).

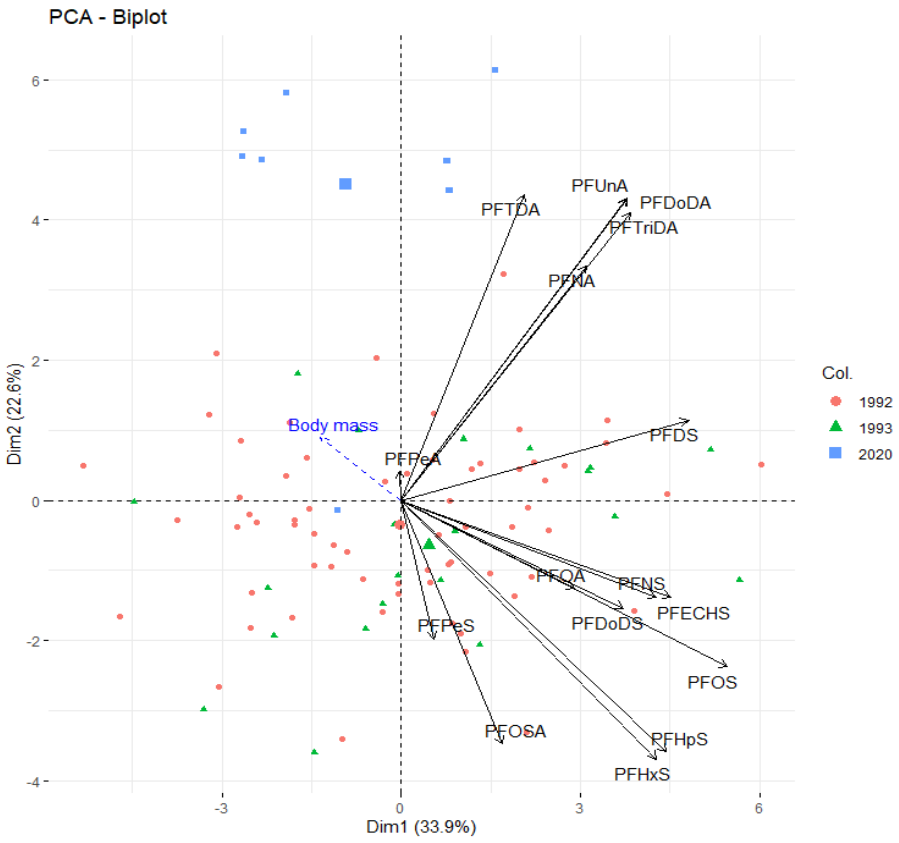

Figure 3.6. Biplot of 16 PFASs concentrations (ng/mL) in plasma of grey seal pups sampled from 1992 to 2022 (n=164) with the explanatory variable "body mass" added as a passive arrow (blue dash line). The larger shapes represent the mean values for each sampling year.

3.5. Occurrence of PFASs and steroids in pups sampled in 2022

Out of 46 PFASs analyzed in grey seal pups sampled in 2022, 13 PFASs were detected in more than 70% of the individuals (based on values > mLOD) and further considered for the statistical analysis (Table 3.3). These included 7 PFCAs: PFOA, PFNA, PFDA, PFUnA, PFDoDA, PFTriDA and PFTDA, and 6 PFSAs: PFHxS, PFHpS, PFOS, PFNS, PFDS, and PFECHs. The 13 PFASs were detected in plasma of all grey seal pups except PFNS (DR = 75%) and PFDS (DR = 91.7%). The DR and geometric means for the 46 analyzed PFASs are shown in Table A.2.7.

Table 3.3. Concentrations of the 13 PFASs with a detection rate (DR) above 70% in plasma of grey seal pups sampled in 2022 (n=12) in the Froan Archipelago. Concentrations are presented as the geometric mean (GM) ± standard deviation (sd) with median (m) in brackets and range in parentheses. All values are given in ng/mL.

PFAS category	Target analytes	DR %	GM ± sd [m] (range) ng/mL
PFCA	PFOA	100	0.49 ± 0.28 [0.56] (0.13-1.18)
	PFNA	100	3.32 ± 1.22 [3.80] (1.50 – 6.26)
	PFDA	100	2.49 ± 1.09 [2.54] (1.13 – 5.35)
	PFUnA	100	7.53 ± 3.74 [6.84] (5.28 – 17.97)
	PFDoDA	100	2.11 ± 0.97 [2.18] (1.32 – 4.29)
	PFTriDA	100	4.59 ± 1.99 [4.28] (2.79 – 9.36)
	PFTDA	100	1.09 ± 0.54 [0.98] (0.59 – 2.37)
PFAS	PFHxS	100	3.74 ± 2.54 [3.44] (1.83 – 10.48)
	PFHpS	100	0.72 ± 0.48 [0.64] (0.32 – 1.85)
	PFOS	100	44.99 ± 19.04 [41.64] (26.76 – 89.50)
	PFNS	75	0.12 ± 0.08 [0.16] (0.02 – 0.32)
	PFDS	91.7	0.29 ± 0.15 [0.34] (0.08 – 0.66)
	PFECHS	100	0.42 ± 0.44 [0.36] (0.25 – 1.88)
	$\sum_{13}$PFAS		72.74 ± 26.07 [65.25] (50.47-123.09)
	$\sum_{6}$PFSA		50.55 ± 21.62 [47.19] (29.90 – 100.26)
	$\sum_{7}$PFCA		14.56 ± 4.57 [14.25] (9.94 – 25.78)

Note: DR% calculated based on values > mLOD. PFCAs: Perfluoroalkyl carboxylic acids, PFSAs Perfluoro sulfonic acids

Out of 17 steroids analyzed in grey seal pups sampled in 2022, 5 steroids were detected in more than 70% of the individuals (based on values >mLOD) and further considered for the statistical analysis (Table 3.4). These included cortisol (COR), cortisone (CORNE), 11-Deoxycortisol (11deoxyCOR), 17α-Hydroxyprogesterone (17aOHP), and progesterone (P4). P4 is the precursor of all four other steroids while 17aOHP is the direct precursor of 11deoxyCOR

(Chakraborty et al., 2021). Moreover, 11deoxyCOR is the direct precursor of COR while COR is the direct precursor of CORNE (Chakraborty et al., 2021). An overview of the steroidogenesis in mammals as retrieved from Chakraborty et al., 2021 is shown on Figure A.3.1. The five steroids were detected in plasma of all of the 12 grey seal pups. The DR and geometric means for the 17 analyzed steroids are shown in Table A.2.8.

Table 3.4. Concentrations of the 5 steroids with a detection rate (DR) above 70% in plasma of grey seal pups sampled in 2022 (n=12). Concentrations are presented as the geometric (GM) ± standard deviation (sd) with median (m) in brackets and range in parentheses. All values are given in ng/mL.

Target analytes	DR (%)	GM ± sd [m] (range)
Cortisol (COR)	100	29.49 ± 14.14 [28.46] (11.87 – 62.59)
Cortisone (CORNE)	100	23.23 ± 6.52 [23.74] (15.21 – 33.18)
11-Deoxycortisol (11-deoxyCOR)	100	6.37 ± 3.04 [6.16] (3.99 – 15.60)
17α-Hydroxyprogesterone (17a-OHP)	100	4.03 ± 1.91 [4.68] (0.53 – 7.14)
Progesterone (P4)	100	0.40 ± 0.25 [0.42] (0.16 – 0.98)

Note: DR% calculated based on values > mLOD.

Distributions of the 13 PFASs and the 5 steroids were calculated by dividing each of their concentrations by the concentration of $\sum_{13}$PFAS and $\sum_{5}$steroid respectively as shown on Figure 3.7. PFSAs accounted for the majority of the $\sum_{13}$PFASs with a contribution of 70% (Figure 3.7a). PFOS was the main PFAS with a contribution > 60%. PFCAs accounted for 30% of the total distribution with the most important PFCAs being PFUnA followed by PFTriDA. The distributions of PFASs found in the plasma of grey seal pups sampled in 2022 are in accordance with those measured in the plasma of pups sampled from 1992 to 2022 (Figure 3.1).

COR and CORNE were the two major steroids measured in plasma of grey seal pups, with distributions of 47% and 35% respectively, followed by 11-deoxyCOR (10%), 17aOHP (7%) and P4 (1%) (Figure 3.7b)

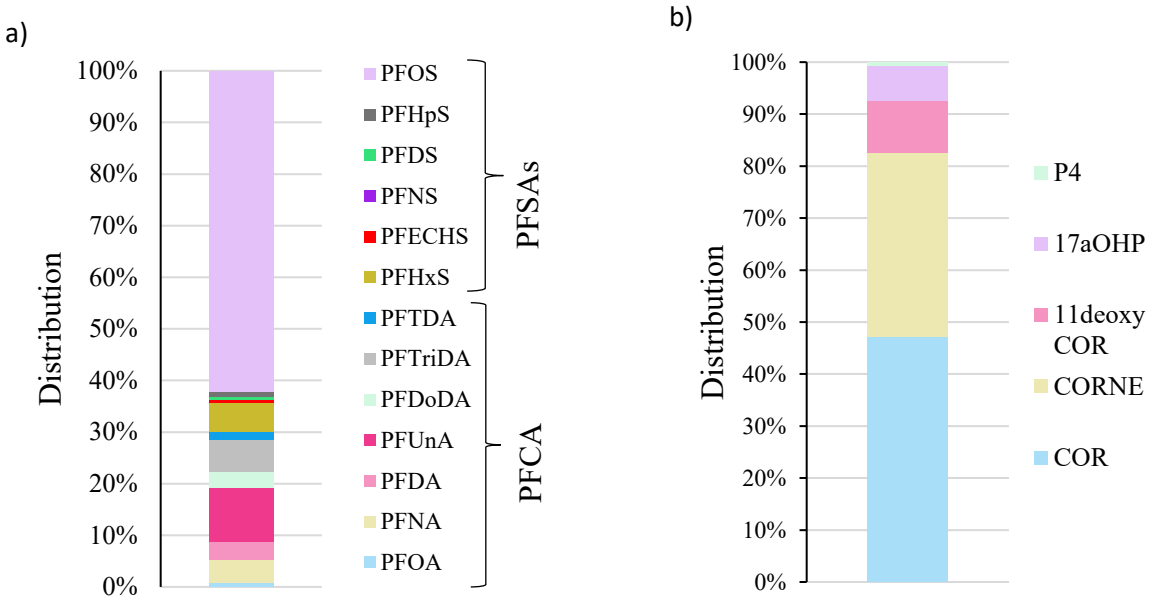

Figure 3.7. Distribution (%) of each of the 13 PFASs (a) and of the 5 steroids (b) detected in the plasma of more than 70% of the grey seal pups sampled in 2022.

3.6. Relationships between steroid levels, PFAS concentrations, and biological variables

A first PCA was run to assess the correlations among the 13 PFASs. The two first principal components (PCs) accounted for 83.5% of the variance (Figure 3.8a). PFECHS, PFOS, PFHpS, PFHxS and PFTDA, PFUnA, PFDoDA, PFTriDA formed two distinct groups and were consequently grouped as $\sum_4$PFSA and $\sum_4$PFCA for further analysis. PFNA, PFDA and PFNS were grouped together as $\sum_3$PFAS and PFDS and PFOA were grouped together as $\sum_2$PFAS (Fig. 3.8a). A summary of the 4 PFAS groups ($\sum_4$PFSA $\sum_4$PFCA, $\sum_3$PFAS and $\sum_2$PFAS) can be found in Table 3.5. A second PCA was run (Figure. 3.8b) to relate the structure in the PFASs variance to the explanatory variables (BM, girth, and length) with the two first PCs accounting for 82.7% of the variance. On the PCA, the biological variables appeared to be negatively correlated with $\sum_4$PFSA (Figure 3.8b). However, none of the biological variables significantly explained the overall variation in PFAS concentrations (RDA, $p > 0.05$). Moreover, no correlations between each of the biological variables and the PFAS concentrations were found, except for PFOA and PFNA which were positively correlated with the three biological variables (BM, length, and girth) (Table 3.6). Because of significatively high correlations between the variables girth and BM (Table 3.5; $r > 0.9$), only the variable BM was kept for further redundancy analysis to avoid multicollinearity.

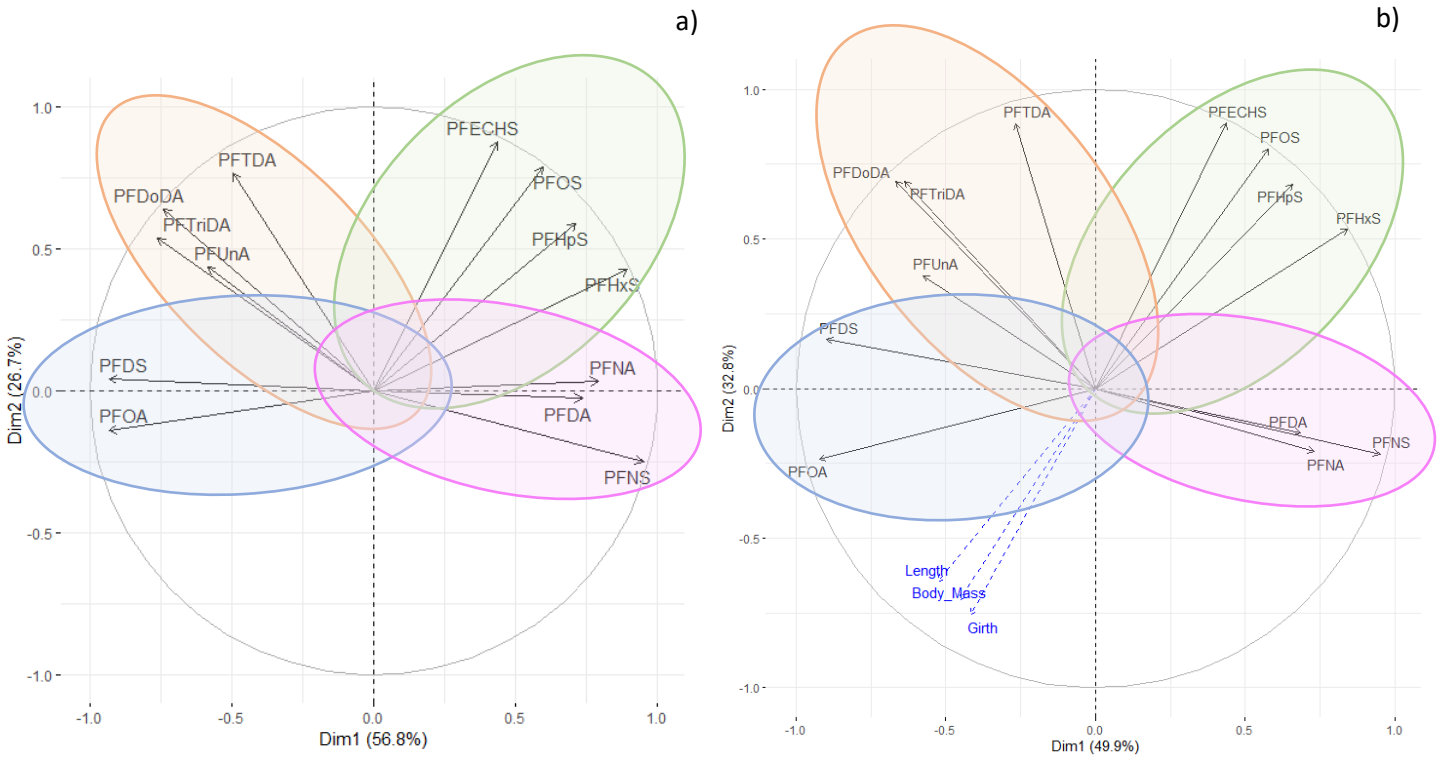

Figure 3.8. PCAs of 13 PFASs concentrations (ng/mL) in plasma of grey seal pups sampled in 2022 (n=12) (a) with the biological variables (body mass, girth, and length) added as passive arrows (blue dash lines) (b). The PCAs were based on log-transformed concentrations.

Table 3.5. Groupings of PFASs based on the correlations observed on the PCA (Figure 3.6a)

Group	PFASs
$\sum_4$PFSA	PFECHS, PFOS, PFHpS, PFHxS
$\sum_4$PFCA	PFTDA, PFUnA, PFDoDA, PFTriDA
$\sum_3$PFAS	PFNA, PFDA, PFNS
$\sum_2$PFAS	PFDS, PFOA

Table 3.6. Significant correlations ($p < 0.05$) between the biological variables (body mass, length, and girth) and the PFAS concentrations as well as among the biological variables in grey seal pups (n=12) using Spearman's Rank Correlation.

	PFOA		**PFNA**		**Body Mass**		**Length**		**Girth**	
Variables	***r***	**p**	***r***	**p**	***r***	**p**	***r***	**p**	***r***	**p**
Body Mass	0.63	0.03	0.65	0.02			0.81	0.001	0.93	1.3E-0.5
Length	0.65	0.02	0.62	0.03	0.81	0.001			0.75	0.005
Girth	0.68	0.02	0.58	0.04	0.93	0.00001	0.75	0.005		

To further explore the relationships between steroid concentrations and the explanatory variables ($\sum_4$PFSA, $\sum_4$PFCA, $\sum_2$PFAS, $\sum_3$PFAS, body mass, and length), a PCA was run with the explanatory variables entered as passive variables (Figure 3.9). The first two principal components accounted for 95.9% of the variance. The steroid hormones were highly correlated to each other, and $\sum_4$PFSA, $\sum_4$PFCA, and $\sum_3$PFAS seemed to be negatively correlated with the steroid levels while $\sum_2$PFASs seemed to be positively correlated with the steroid levels.

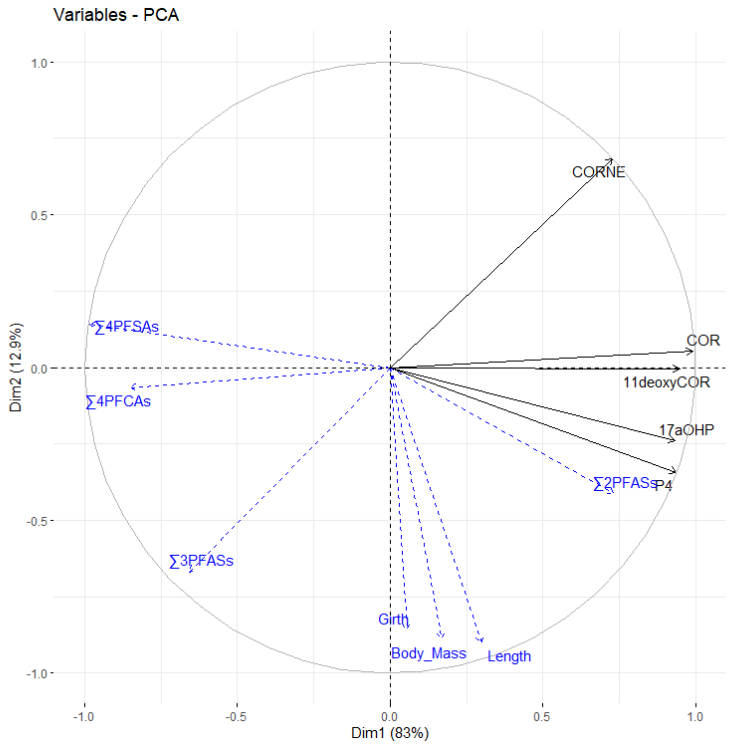

Figure 3.9. PCA of 5 steroids concentrations (ng/mL) in plasma of grey seal pups sampled in 2022 (n=12) with the explanatory variables (body mass, length, $\sum_4$PFSAs, $\sum_4$PFCAs, $\sum_3$PFASs and $\sum_3$PFASs) added as passive arrows (blue dash lines).

Associations between the explanatory variables (PFAS concentrations, length, and body mass) and the steroid levels were further explored using Spearman rank correlations (Figure 3.10, Table 3.7) and negative correlations were found between P4 and PFHpS, PFOS, PFHxS (r = -0.68 to -0.92, $p < 0.01$). PFHxS was also negatively correlated with 17aOHP while PFHpS was also negatively correlated with 11deoxyCOR. Furthermore, both PFHxS and PFHpS were negatively correlated to COR. Moreover, PFOA was positively correlated with levels of P4 and 17aOHP. No correlations were found between CORNE and any of the PFAS.

With respect to biological variables, positive associations were found between the P4 levels and the biological variables (BM and length). PFNA and PFOA were positively correlated to the three biological variables (BM, girth, and length), while PFDA was positively correlated to girth. Steroid hormones were highly correlated to each other: P4 and COR correlated to all of the other steroid hormones, while CORNE was only correlated to COR and 11deoxyCOR. 17aOHP were only correlated with P4 and COR. In general, structurally similar PFASs were highly correlated to each other.

According to the RDA, only the variable $\sum_4$PFSA (i.e., PFECHS, PFOS, PFHpS, PFHxS) contributed significantly to the total variance in steroids concentrations ($p < 0.05$, $R^2_{adj} = 0.23$). Because PFECHS was not correlated with any steroid hormone, it can be said that only PFOS, PFHPs, and PFHxS significantly contributed to the total variance of steroid hormones, up to 23%.

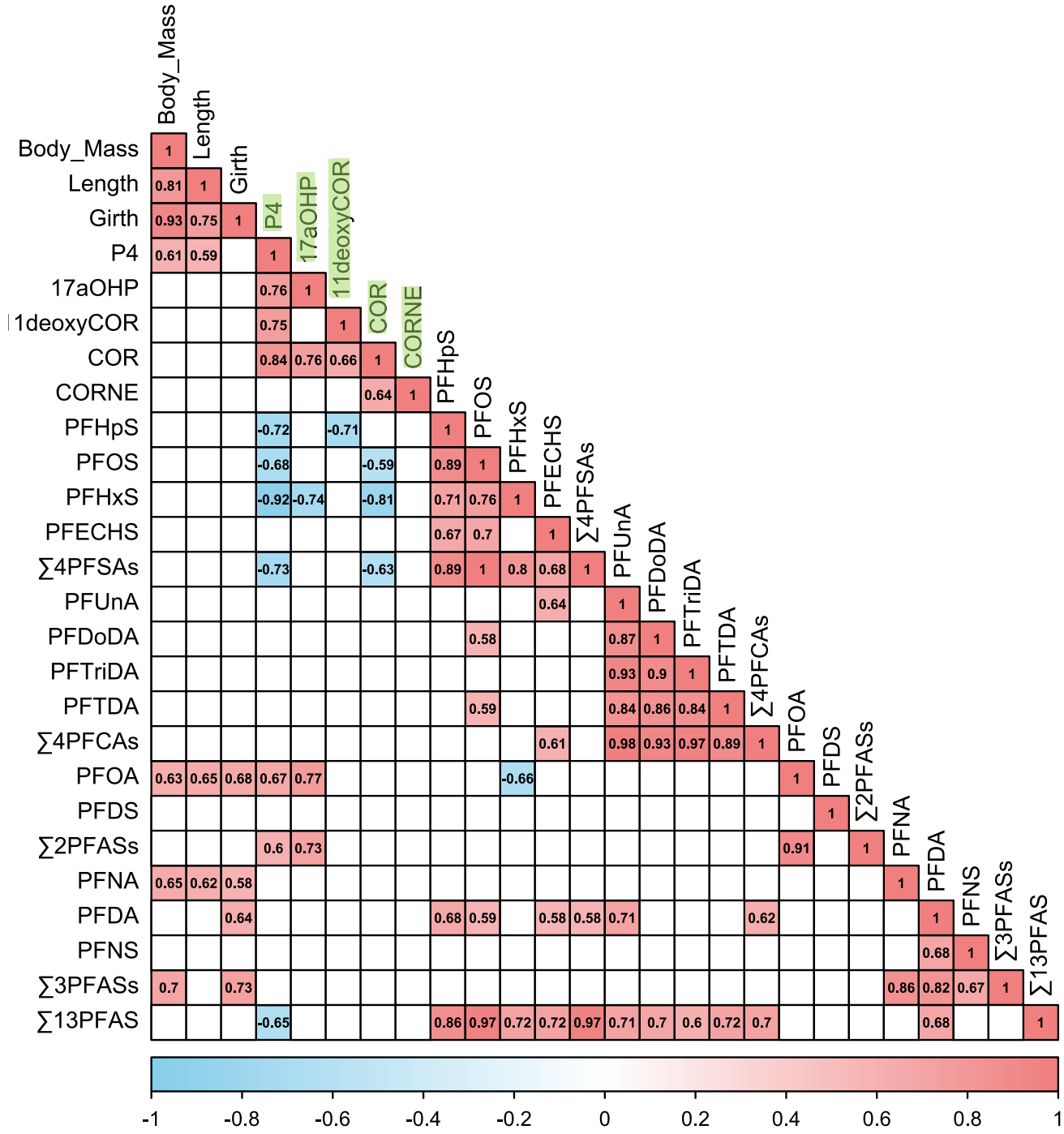

Figure 3.10. Spearman's correlation matrix among and between the PFAS concentrations, steroid levels, and biological variables (body mass, length, and girth) in plasma of grey seal pups sampled in 2022. Only the significant correlations are visible on the matrix ($p < 0.05$) with the respective correlation coefficients (r) displayed. Steroids are colored green on the x-axis. Significant correlations are reported in Table 3.7 for better visualization.

Table 3.7. Significant correlations ($p < 0.05$) between the explanatory variables (PFAS concentrations, BM, and length) and the steroid hormones levels in grey seal pups (n=12) using Spearman's Rank Correlation.

	P4		**17aOHP**		**11deoxyCOR**		**COR**		**CORNE**	
Variables	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Body Mass	0.61	0.04								
Length	0.59	0.04								
PFHpS	-0.72	0.008			-0.71	0.009				
PFOS	-0.68	0.01					-0.59	0.04		
PFHxS	-0.92	0.00002	-0.74	0.006			-0.81	0.002		
PFOA	0.67	0.02	0.77	0.004						

4. Discussion

4.1. Occurrence and patterns of PFASs in plasma of grey seal pups sampled from 1992 to 2022

In the present study, PFOS was the predominant PFAS measured in plasma of grey seal pups sampled from 1992 to 2022 in the Froan Archipelago and contributed to more than 60% of $\sum_{16}$PFAS for each sampling year. Considering that PFOS has been extensively produced globally and is known for its high persistence (Wang et al, 2009), this finding is not surprising and is in accordance with previous studies focusing on pinnipeds over the last three decades. Indeed, PFOS was found to be the predominant PFAS in liver tissues of ringed seals (*Pusa hispida*) sampled in Svalbard between 1990 and 2010 (Routti et al., 2016), as well as in plasma of mother-pup pairs of hooded seals sampled in 2008 in Greenland (Grønnestad et al., 2017). Moreover, it was the dominant compound in liver samples of Australian sea lions (*Neophoca cinerea*) and Australian fur seals (*Arctophalus pusillus doriferus*) pups sampled between 2017 and 2020 (Taylor et al., 2021). Finally, while not focusing on pinnipeds, one study has found that PFOS was also the main compound measured in liver tissues of Eurasian otters (*Lutra lutra*) sampled off the coast of central Norway (Nordland region) in 2017 and 2018 (Herzke et al., 2023), which is relatively close to the Froan Archipelago. Therefore, PFOS remains the main PFAS measured in marine mammals at the global scale despite its global restriction since 2009 under the Stockholm Convention (UNEP, 2009). In walruses sampled in Svalbard (Norway), levels of genes involved in immune function were positively associated with PFAS levels that were about ten times lower than those measured in the present study (Routti et al., 2019)

In the present study, the second and third main PFAS measured were PFHxS and PFTriDA from 1992 to 2005, and PFUnA and PFTriDA from 2020 to 2022. In liver tissues of ringed seal sampled in Greenland in 2012 and 2013, the second most predominant PFAS was PFUnA, behind PFOS, while the third was PFNA (Gebbink et al., 2016). In Australian fur seals and Australian sea lions' pups, the second and third most predominant PFAS were PFNA and PFOA respectively (Taylor et al., 2021). In the present study, PFOSA accounted for less than 1% of the $\sum_{16}$PFAS but as it is a PFAA precursor, it is further bio transformed to a stable PFAA in the environment and in the organisms (Zhang, W., et al., 2021).

It is worth mentioning that most studies measured PFAS concentrations in liver rather than in plasma and reported them as ng/g of ww (Routti et al., 2016; Herzke et al., 2023). It was previously found that while PFAS distribution is relatively similar between blood and liver, concentrations of ∑PFAS measured in liver samples of harbor seals were higher than those measured in blood samples (Ahrens et al., 2009). Indeed, because PFASs are proteinophilic, they mainly bind and accumulate in protein-rich tissues such as the liver (Jones et al., 2003). In 2022, the concentrations of PFOS measured in plasma of the Froan seal pups were 45 ng/mL, which were significantly higher than those measured in plasma samples of hooded seal pups from Greenland (mean : 30.4 ± 13 ng/g ww) (Grønnestad et al., 2017) and in liver samples of Australian fur seal pups (median : 27,4 ng/g ww) (Taylor et al., 2021). To the author's knowledge, there are no other studies assessing the levels of PFAS in the offspring of pinnipeds in the North Atlantic (excluding the Arctic).

The number and identity of PFAS compounds analyzed vary substantially between studies on marine mammals. Indeed, some decides to focus only on the most common PFASs, namely PFOS and PFOA (Law et al., 2008), while more recent studies have started to include emerging PFASs (Wang et al., 2021). In this study, 42 PFASs were jointly analyzed in plasma of grey seal pups sampled from 1992 to 2012 in the Froan Archipelago, which is rather high considering that, in general, an average of 20 compounds are analyzed in studies on PFASs (Spaan et al., 2020).

Ignoring emerging PFASs could lead to underestimating the PFAS burden in biota (Spaan et al., 2020; Herzke et al., 2023). Indeed, Herzke et al., 2023 has found that 41% of the total extractable organofluorine (EOF) remained unidentified in liver samples of Eurasian otters sampled in Norway. It should however be kept in mind that while the remaining organofluorine content could be explained by the presence of unidentified PFAS, it could also be related to the presence of other fluorinated compounds that are not PFASs, although a strong linearity was measured between the amount of fluorine in targeted PFAS and the EOF (Herkze et al., 2023). As for Spaan et al., 2020, they found that respectively 45% and 44% of the total EOF remained unidentified in liver tissues of ringed and harbour seals sampled in Sweden in 2015. However, the total EOF was fully identified by the targeted PFASs in liver tissues of grey seals sampled in Sweden in 2016 (Spaan et al., 2020). In view of the growing number of PFASs to be analyzed, EOF analysis is a promising tool to assess PFAS contamination in plasma samples and should be further refined (Aro et al., 2022).

Moreover, applying non-target and suspect screening could be beneficial such as when it was applied to liver samples of indo-pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena Palmer*) sampled in China, and allowed to identify 44 more PFASs than the target screening (Wang et al., 2021). Those new screening approaches allow to detect suspected and unknown compounds in a sample by assigning signals to compounds using reference libraries (Pourchet et al., 2020).

In the present study, two short-chain PFASs (i.e., PFPeS and PFPeA), were detected in more than 70% of the individuals. Moreover, while they were not considered in the statistical analysis, several other short chain compounds such as PFBS, 7H-PFHpA, and PFHxA (Table A.2.6), were detected in more than 50% of the individuals. This is quite alarming considering that these compounds are used as replacements for PFOS and PFOA (EPA, 2021). Finally, it is worth mentioning that Gen X was also detected in more than 50% of the individuals (Table A.2.6), which can be considered significant for this PFECA which has been used as a replacement for PFOA since its worldwide ban in 2019 (EPA, 2021; UNEP, 2019). Those results indicate that emerging PFASs can indeed bioaccumulate in biota and that more should be known about their potential risk.

4.2. Temporal trends of PFASs in grey seal pups from 1992 to 2022

One objective of the present study was to assess the temporal trends of PFAS concentrations in plasma samples of free-ranging grey seal pups sampled from 1992 to 2022 in order to understand how PFAS burden fluctuated in a rapidly decreasing seal population. When considering the pup production of the Froan grey seal population, it can be observed that the highest number of pups being born each year was reported in 2001 (Figure 1.2). Accordingly, the highest concentrations of PFASs were measured in plasma samples of grey seal pups sampled in 2000. The grey seal pups exposed to the highest PFAS concentrations in 2000 became sexually mature between 2003 and 2005 for the females and in 2006 for the males (Hall et Russell, 2018). Interestingly, in 2007, the number of pups being born each year had already drastically decreased. It could then be suggested that the 2000 pups are the 2007 sexually mature adults facing reproductive failure. Future studies could focus on tracking the age of the reproductive adults to assess if the breeding population is being renewed or not. The commonly used method to determine the age of adult seals is to count the cementum rings in the canine teeth (Hewer, 1960). However, in view of the rapid decline of the Froan population, this

destructive method cannot be considered. One solution could be to use skin or blood samples to obtain DNA methylation profiles as successfully developed by Robeck et al., 2023, who found high correlations between certain methylation profiles (epigenetic clocks) and age in several species of pinnipeds such as the harbor seals (*Phoca vitulina*).

The concentration of $\sum_{16}$PFAS has significantly decreased from 1992 to 2022 in plasma of the Froan pups. To be precise, the $\sum_{16}$PFAS increased significantly from 1992 to 2005, with the highest concentrations measured in 2000. The concentrations then started to decrease significantly and in 2020 and 2022, the $\sum_{16}$PFAS levels were the lowest ever measured in the plasma of the Froan grey seal pups. Similar tendencies were found for the $\sum_{8}$PFSA and PFOS concentrations in plasma of the Froan pups.

Decreasing trends for PFOS were also reported in plasma samples of white whales (*Delphinapterus leuca*) sampled in 1996 and 2014 in Svalbard (Villanger et al., 2020). Moreover, a similar pattern has been observed in juvenile female harbor porpoises sampled in the Baltic and North Sea where PFHxS and PFHpS levels in liver samples decreased significantly from 1991 to 2008 (Huber et al., 2012). PFOS and PFHxS have also decreased since 2004 in plasma of ringed seals sampled in Svalbard (Routti et al., 2016). Thus, although PFOS remains the dominant PFAS measured in marine mammals, negative temporal trends have been reported worldwide and its restriction on a global scale under the Stockholm Convention seems to be effective. However, marine mammal populations should continue to be monitored globally to understand whether this declining trend will continue or if $\sum_{16}$PFAS concentrations will start to level off given their high persistence (Wang et al., 2019).

On the contrary, the concentration of $\sum_{7}$PFCAs has significantly increased since 1992 in the Froan seal pups, with the highest concentrations measured in 2005. This interesting finding was also assessed in white whales sampled in Svalbard in 1996 and in 2014 where PFOS concentrations decreased by 44% from 1996 to 2014 whereas the total concentration of long-chain PFCAs increased by 43% (Villanger et al., 2020). Moreover, an increasing trend was observed for ∑PFCAs with a carbon chain length comprised between 9 and 13 (long-chain PFCAs) in liver sample of juvenile harbor seals sampled between 1991 and 2008 in the Baltic and North Sea (Huber et al., 2012). Interestingly, similar trends were found in plasma of mother-cub pairs of polar bears sampled in Svalbard in 1998 and 2008 (Bytingsvik et al., 2012). Indeed, concentrations of long-chain PFCAs were significantly higher in plasma of polar bear pairs sampled in 2008 when compared with pairs sampled in 1998 (Bytingsvik et al., 2012). In contrast, the concentrations of ∑PFCAs have significantly decreased since 2004 in plasma of

ringed seals sampled in Svalbard (Routti et al., 2016). In the present study, only one short chain PFCA, namely PFPeA, was detected in more than 70% of the sampled pups and no significant temporal trends were found (Figure A.2.6).

Even if the concentration of $\sum_{16}$PFAS has significantly decreased since 1992, structurally different PFASs showed different trends, demonstrating once again the difficulty of determining the common fate of this large and complex contaminant group. For future research, PFAS trends in the Froan population should continue to be monitored in order to understand how concentrations of legacy and emerging PFAS will vary in the coming years.

4.3. Occurrence and patterns of steroids hormones in pups sampled in 2022

4.2.1. Progestogens (PREG, P4, 17aOHP, 17aOHP5)

In pinnipeds, progestogens have only been measured once in plasma of suckling and recently weaned grey seal pups from the Baltic Sea (Sait et al., 2023). In the present study, only the following progestogens: Progesterone (P4) and 17-OH-Progesterone (17*a*OHP), were detected in more than 70% of the individuals. These two hormones are both implicated in the synthesis of mineralocorticoids (e.g., corticosterone, COS and aldosterone, ALDO), glucocorticoids (e.g., cortisol, COR), androgens (e.g., testosterone, TS) and estrogens (e.g., estrone, E1) (Chakraborty et al., 2021; Bremer et Miller, 2014). The levels of P4 and 17a-OHP were close to those measured in plasma of grey seal pups sampled in 2020 in the Baltic Sea (n=9) (Sait et al., 2023). It should be noted that in Europe, the highest concentrations of PFAS have been measured in the Baltic and North Sea (Muir et Miaz, 2021). With the exception of cortisone (CORNE), P4 was highly correlated with all of the others steroid hormones as it was expected since it is an important precursor of corticoids and other sex steroids (Chakraborty et al., 2021).

4.2.2. Mineralocorticoids (COS, ALDO, DOC)

P4 is further hydroxylated to 11-Deoxycorticosterone (DOC) from which mineralocorticoids (e.g., corticosterone, COS and aldosterone, ALDO) are synthesized, and whose main role of mineralocorticoids is to maintain the balance of salt and water (Chakraborty et al., 2021; Bremer et Miller, 2014). In the present study, DOC and COS were detected in 58% and 50% of the individuals respectively, and therefore not included in the analysis. However, they were detected in plasma of all 9 grey seal pups sampled in the Baltic Sea in 2020 and mean

concentrations measured were 3.73 ng/mL for COS and 0.38 ng/mL for DOC (Sait et al., 2023). ALDO is the main mineralocorticoid but was only detected in one sample in the present study, while it was detected in 67% of the grey seal pups sampled in the Baltic Sea though the mean concentration was below the LOD (Sait et al., 2023). It should be noted however, that in the present study, the extraction efficiency for ALDO using HybridSPE was quite poor (low recoveries) suggesting that the method might need further refinement for this compound. Using an identical method, Sait et al., (2023) found a poor precision for ALDO due to peak instability. Interestingly, ALDO levels were found to increase during the fasting period in 15 northern elephant (*Mirounga angustirostris*) pups sampled in California (USA) to conserve the salt-water balance, suggesting an important role for this hormone in the survival of seal pups (Ortiz et al., 2000). ALDO was detected in plasma of all 15 northern elephant seal pups and the mean concentration measured at the beginning of the fast was 0.2 ng/mL (Ortiz et al., 2000). It would then be interesting to analyze the plasma samples of the Froan pups again with a refined method for the detection and quantification of ALDO, as this hormone might play a crucial role in the survival of seal pups during the fasting period.

4.2.3. Glucocorticoids (COR, CORNE, 11deoxyCOR)

Glucorticoids play an important role in cell metabolism and immune cell-function with cortisol being the main glucocorticoid in mammals (Chakraborty et al., 2021). Cortisol levels measured in this study (29.49 ± 14.14 ng/mL) were significantly lower than the mean cortisol levels measured in plasma of grey seal pups sampled in Scotland in 2012 (Survilienė et al., 2022). In the study of Survilienė et al., (2022), grey seal pups were all sampled in 2012 but plasma samples were divided into two groups and analyzed in 2014 (n=32) and 2015 (n=29). The steroid levels were assessed using enzyme-linked immunosorbent assay (ELISA) kits and mean steroid levels measured in 2014 and 2015 were 107.62 ± 4.95 ng/mL and 49.4 ± 4.13 ng/mL respectively, a difference that could be attributed to plate variation due to inter-plate confounds (Survilienė et al., 2012). Finally, plasma of pre-weaned and post-weaned pups were pooled together for the analysis (Survilienė et al., 2022) even though it was found that corticosteroids levels significantly increased during the fasting period of pinniped pups (Ortiz et a., 2003).

The cortisol levels measured in the present study were also lower than levels measured in plasma of post-natal Australian fur seal pups sampled between 1997 and 1999 (59.4 ± 6.4 ng/mL) (Atkinson et al., 2011) and of Steller sea lion (*Umetopias jubatus*) pups sampled between 2005 and 2008 in Alaska (138.3 ± 39.8 ng/mL) (Keogh et al., 2013). However, it should be noted that the lactation period of Australian fur seals lasts 10 to 12 months, and even

longer in Steller sea lions, during which the mother will regularly leave the pup alone to go on foraging trips (Jefferson et al., 2015). Interestingly, cortisol levels in plasma of post-weaned grey seal pups sampled in the Baltic Sea (17.6 ng/mL) were close to levels measured in the present study and this was also the case for the cortisol precursor, 11-deoxycortisol (11-deoxyCOR) and the inactive metabolite of cortisol, cortisone (CORNE) (Sait et al., 2023).

4.2.4. Androgens (11-keto-TS, TS, DHT, DHEA, AN, A5) & estrogens (E1)

In the present study, 11-ketostestosterone (11-ketoTS), 5α-Dihydrotestosterone (DHT), Androstenediol (A5), and estrone (E1), were not detected while Dehydroepiandrosterone (DHEA) and Testosterone (TS) were detected in only one pup with concentrations below the LOD. In suckling and recently weaned pups from the Baltic Sea, DHEA, TS, and DHT were detected in more than 60% of the samples, but concentrations were below the LOD while A5 was not detected (Sait et al., 2023). Finally, precision and sensitivity of the method used in the present study were low for androstenedione (AN) which was also the case in the study of Sait et al., (2023) using an identical method (HybridSPE-UPC^2/MS-MS). It is not surprising to find no or low levels of sex steroid hormones as the sampled pups were sexually immature (Atkinson, 1997). It would then be relevant to sample sexually mature grey seals to assess the relationships between PFAS concentrations and sex steroids to study if steroid hormone disruption in adult seals could explain the reproductive failure observed in the Froan population. Indeed, sex steroids are crucial for the development and function of the reproductive system (Chakraborty et al., 2021).

4.4. Relationships between steroids, PFAS concentrations and biological variables

Several studies have assessed the effects of persistent organic pollutants (POPs) on steroid levels in marine mammals (Ciesielski et al., 2023; Galligan et al., 2019; Gustavson et al., 2015), but only one study has assessed the effects of PFAS burden on steroid levels in marine mammals (Pedersen et al, 2016) and to the author's knowledge, the present study is the first one to explore the relationships between PFAS concentrations and steroid levels in pinnipeds.

In the present study, P4 was negatively correlated with $\sum_{13}$PFAS, PFHxS, PFHpS and PFOS, while it was positively correlated with PFOA. It should however be noted that P4 and PFOA were both positively correlated with the biological variables (body mass, and length). Several sulfated steroid hormones, such as sulfated cholesterol, were measured in milk of Atlantic grey seal mothers (Watson et al., 2021) and this can indicate that as pups are gaining in body mass

and length, they are exposed to higher concentrations of cholesterol via lactation which can further be transformed in pregnenolone and progesterone. However, sulfated cholesterol concentrations in milk were found to decrease and reach very low concentrations 7 days after parturition (Watson et al., 2021). If both PFOA and PFNA were positively correlated to BM in grey seal pups sampled in 2022, most PFSAs were negatively correlated to BM in grey seal pups sampled in 1992, 1993 and 2020. However, no significant effect of BM on PFAS concentrations was found in pups sampled in 2022, and BM had a negligible effect on pups sampled in 1992, 1993, and 2020. This is in accordance with a previous study who did not find any significant effect of BM on the total PFAS variation in plasma of hooded seal pups (Gronnestad et al., 2017).

In brain tissue from East Greenland polar bears sampled from 2011 to 2012, significant positive correlations were found between several PFCAs (PFUnDA, PDoDA and PFTrDA) and P4, while no significant relationships were found between P4 with PFOS and PFOA (Pedersen et al., 2016). It should be mentioned that PFOS and PFOA concentrations measured in brain tissues of polar bears were respectively lower and higher than those measured in the present study (Pedersen et al., 2016).

In humans, no significant relationships were found between PFOS, PFOA and PFHxS with P4 in cord sera of newborns of a birth cohort from China between 2013 and 2014 although concentrations of PFOS and PFHxS were 10 times lower than those measured in the present study (Liu et al., 2020). Interestingly, a positive relationship between PFBS and P4 was found in newborns cord sera (Liu et al., 2020), but this compound was not considered here as its detection rate was below 70%.

In vitro, P4 production was inhibited in mouse Leydig tumor cells (mLTC-1) after exposure to individual PFAS including PFHxS, PFOS and PFOA, although the concentrations used were much higher than those measured in the present study (Zhao et al., 2017). Zhao et al., 2017 suggested that P4 depletion could be due to reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP) decreased by PFASs. Another explanation for P4 depletion by certain PFASs could be the inhibition of 3β-hydroxysteroid dehydrogenase activity. Indeed, P4 is synthesized from pregnenolone (PREG) by the 3β-hydroxysteroid dehydrogenase (3β-HSD) (Chakraborty et al., 2021) and it was found that PFOS and PFOA both inhibited 3β-HSD activity by 25% in human testis microsomes and by 40 and 98% in rat testis microsomes (Zhao et al., 2010). However, concentrations used were similar to

concentrations found in occupational workers (~114.1 mg/l), which were much higher than those measured in the grey seal pups.

In the present study, another progestogen synthesized from P4 or from 17a-hydroxy pregnenolone (17a-OH-P5), 17aOHP, was negatively related to PFHpS levels but positively related to PFOA levels. However, in one human study, no correlations were found between 17aOHP and PFAS levels in cord sera of newborns from China (Liu et al., 2020). In Pedersen et al., 2016, 17aOHP was not detected in brain tissues of polar bears from Greenland.

In the present study, cortisol levels were negatively correlated with PFOS and PFHxS. Moreover, a negative relationship was found between 11deoxyCOR, the precursor of cortisol, and PFHpS. In one human study, prenatal PFOS concentrations were found to be negatively correlated with cortisol levels measured in cord blood samples at lower concentrations than those measured in the present study (Goudarzi et al., 2017). However, in cord sera of newborns sampled between 2013 and 2014 in a birth cohort from China, positive relationships were found between several PFASs such as PFOS, PFOA and PFHxS, and 11deoxyCOR levels (Liu et al., 2020). In the previously mentioned study, PFOS and PFHxS levels were much lower than those measured in the Froan pups while PFOA levels were higher (Liu et al., 2020).

In the current study, cortisol levels measured in pre-weaning grey seals were lower than those measured in pups of several pinniped species (Survilienė et al 2022; Keogh et al 2013; Atkinson et al., 2011) and where negatively correlated with two PFSAs (PFOS and PFHxS). Moreover, levels of cortisol and other steroids were relatively similar to those measured in plasma of grey seal pups sampled in the Baltic Sea (Sait et al., 2023) in which the highest concentrations of PFASs in Europe were measured alongside the North Sea (Muir et Miaz, 2021). In northern elephant seal pups, it has previously been found that glucocorticoid concentrations increase from weaning to fasting (Ortiz et al., 2003). Indeed, to face the energy requirements of the fasting period, grey seal pups rely mainly on lipid catabolism from the blubber (Jefferson et al., 2015; Nordoy et al., 1985; Reilly, 1991; Schweigert, 1993) and glucocorticoids increase lipolysis for gluconeogenesis (Kuo et al., 2015). Therefore, it can be hypothesized that low cortisol levels will render the seal pups more vulnerable in the high energy demanding fasting period. For future research, grey seal pups could be followed, and blood samples taken during both the weaning and fasting period to see how those concentrations fluctuate and how they relate to PFAS exposure. If steroid hormones appear to be an effective biomarker of PFAS effect on steroid homeostasis in grey seal pups, the lack of reference studies render the comparison of the present results limited.

4.5. Future recommendations

In the present study, 3 PFSAs (i.e., PFHxS, PFHpS, and PFOS) could explain the total steroid variation in grey seal pups, but only up to 23%. This finding suggests that while PFSAs might have a significant effect on steroid homeostasis in grey seal pups, they might not be the only factors involved. Indeed, exposure to other contaminants could be implicated. For example, in bottlenose dolphin (*Tursiops truncatus*) sampled in Florida (USA), cortisol levels were found to be negatively correlated with several contaminants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), chlordanes (CHLDs), mirex and dieldrin (Galligan et al., 2019). PCBs have also been found to affect steroid homeostasis in polar bears (Ciesielski et al., 2023; Gustavson et al., 2015) and in pilot whales (Hoydal et al., 2017). Several of those contaminants have previously been measured in plasma of grey seal pups from the Froan Archipelago (Jenssen et al., 1995, 1996, 2003).

Then, one main limitation of the present study was the small sample size for several sampling years. For example, only 6 pups were sampled in 2000 while 12 pups were sampled in 2022, which could lead to type I and type II errors when assessing PFAS temporal trends as well as the relationships between PFAS burden and steroid levels (Knudson et Lindsey, 2014). It would therefore be relevant to analyze previous sampling years to have a better appreciation of PFAS effects on steroid homeostasis in grey seal pups. In particular, it would be of interest to measure steroid levels in pups sampled in 2000 and exhibiting the highest PFAS burden. While it could be argued that the plasma samples are too old to be analyzed for steroid content, it was found that P4 concentrations were not affected by a storage of 22 years at -25°C in human serum samples (Holl et al., 2008).

Moreover, following the pups from their birth to the end of the fasting period could be relevant to understand how steroid hormones fluctuate during this high energy demanding period. Non-invasive methods such as hair and whiskers to measure steroid levels should be explored (Keogh et al., 2021; Otsuki et al., 2021) as they may allow more seal pups to be sampled in future years because of the greater ease of obtaining them than blood samples. Moreover, it would be interesting to assess the mean age of the reproductive mature grey seals to determine if the breeding population is being renewed by, for example, analyzing DNA methylation profiles in blood or skin samples (Robeck et al., 2023). Furthermore, suspect, and non-target screening (Wang et al., 2021) as well as measuring the total extractable organofluorine (EOF) (Herzke et al., 2023) should be considered to obtain a global picture of PFAS contamination in

grey seal pups. Finally, to understand if and how PFAS affect reproductive success in the Froan population, relationships between PFAS burden and sex steroid levels in sexually mature grey seals should be determined. However, this type of study is difficult to set up because of the challenge and cost of sampling adult free-ranging pinnipeds.

5. Conclusion

The present study is the first one to assess temporal trends of PFAS concentrations in pinnipeds from the North Atlantic and to explore the relationships between PFAS burden and steroid levels in pinnipeds. In the Froan population, grey seal pup production reached its highest peak in 2000 almost at the same time that the highest PFAS levels were measured in plasma of seal pups. While PFAS concentrations were lower in 2022 than in 1992, the concentrations of long-chain PFCAs were significantly higher in 2022 than in 1992. These results show on the one hand that the regulations put in place seem effective, but it also highlights the difficulty of predicting the environmental fate of the large and complex group that PFAS represents. Therefore, the Froan population should keep being monitored to assess how PFAS concentrations will evolve in the coming years. Levels of important steroid hormones, such as progesterone and cortisol, were generally lower than those measured in previous studies on pinnipeds, while they were close to levels measured in grey seal pups from the Baltic Sea, one of the two most polluted seas with PFASs in Europe. These low cortisol levels could affect the capacity of the grey seal pups to cope with high demanding energy events such as the fasting period. It could then be relevant to measure steroid levels in grey seal pups from previous years, especially in pups with the highest PFAS burden. Moreover, pups could be followed from their birth to the end of the fasting period with regular blood sampling to study how steroid hormone levels fluctuate during this period. Finally, mother-pup pairs could be sampled together to study/investigate how steroid and PFAS concentrations vary between them and to understand if PFAS affect sex steroids in sexually mature grey seals. In general, the present study found negative correlations between several PFSAs and steroid hormones suggesting that PFASs might affect steroid homeostasis in grey seal pups. It is, however, difficult to discuss and compare the results obtained due to the small sample size and the lack of reference studies on grey seals and on marine mammals in general. ∑PFSA (PFOS, PFHxS and PFHpS) was the only significant variable to explain the total steroid variance but could only account for 23% of the variance. Therefore, more factors such as exposure to other groups of contaminants might affect steroid homeostasis in grey seals, and future studies should consider including other compounds such as PCBs and PBDEs in their analysis.

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Appendices

1. Material & methods

Table A.1.1. Information regarding the grey seal pups sampled from 1992 to 2022 in the Froan Archipelago including the sampling location, age, sex, body mass, length, girth, and locations of mother and male when available. N/A : not applicable.

ID	**Year**	Location	**Age (categories)**	**Sex**	**Body mass (kg)**	**Length (cm)**	**Girth (cm)**	**Mother**	**Male**
D6505	1992	Hamnøyan	N/A	N/A	34	N/A	N/A	N/A	N/A
D6511	1992	Helvete M.	N/A	F	42	N/A	N/A	N/A	N/A
D6514	1992	Ormskj.	N/A	M	37.5	N/A	N/A	N/A	N/A
D6515	1992	Grunnlåta	N/A	F	34	N/A	N/A	N/A	N/A
D6516	1992	Båskj.	N/A	F	25.5	N/A	N/A	N/A	N/A
D6525	1992	Hamnøyan	N/A	M	26	N/A	N/A	N/A	N/A
D6542	1992	Sandskj.	N/A	M	52.5	N/A	N/A	N/A	N/A
D6545	1992	Sandskj.	N/A	F	26.5	N/A	N/A	N/A	N/A
D6546	1992	Inner Sandskj.	N/A	F	43.5	N/A	N/A	N/A	N/A
D6553	1992	Slettskj.	N/A	F	30	N/A	N/A	N/A	N/A
D6557	1992	By Tinnskj.	N/A	F	32	N/A	N/A	N/A	N/A
D6560	1992	Båskj. M.	N/A	M	50.5	N/A	N/A	N/A	N/A
D6561	1992	Tinnskj.	N/A	F	37	N/A	N/A	N/A	N/A

D6566	1992	Sandskj.	N/A	M	38.5	N/A	N/A	N/A	N/A
D6568	1992	Gronnskjara	N/A	M	32.5	N/A	N/A	N/A	N/A
D6569	1992	Helvete S.	N/A	F	41	N/A	N/A	N/A	N/A
D6570	1992	Helvete Ø.	N/A	M	17.5	N/A	N/A	N/A	N/A
D6572	1992	Helvete Ø.	N/A	F	16	N/A	N/A	N/A	N/A
D6577	1992	Helvete N.	N/A	F	N/A	N/A	N/A	N/A	N/A
D6579	1992	Sandskj.	N/A	F	44.5	N/A	N/A	N/A	N/A
D6582	1992	Hamnøyan	N/A	M	43.5	N/A	N/A	N/A	N/A
D6586	1992	Leiskj.	N/A	F	39.5	N/A	N/A	N/A	N/A
D6592	1992	Hamnøyan	N/A	M	54.5	N/A	N/A	N/A	N/A
D6599	1992	Lille ørnøyskj.	N/A	F	25.5	N/A	N/A	N/A	N/A
D6600	1992	Smeitskj.	N/A	F	31	N/A	N/A	N/A	N/A
D6605	1992	Oterglittan	N/A	M	31	N/A	N/A	N/A	N/A
D6606	1992	Oterglittan	N/A	F	36.5	N/A	N/A	N/A	N/A
D6615	1992	Leiskj.	N/A	M	22	N/A	N/A	N/A	N/A
D6616	1992	Masteskj.	N/A	F	32	N/A	N/A	N/A	N/A
D6617	1992	Leiskj.	N/A	F	30.5	N/A	N/A	N/A	N/A
D6618	1992	Leiskj.	N/A	M	30	N/A	N/A	N/A	N/A
D6623	1992	Grunnlåta	N/A	F	30.5	N/A	N/A	N/A	N/A
D6624	1992	Brattfl.	N/A	F	40	N/A	N/A	N/A	N/A
D6625	1992	Helvete S.	N/A	F	22.5	N/A	N/A	N/A	N/A

D6626	1992	Helvete N.	N/A	M	20.5	N/A	N/A	N/A	N/A
D6627	1992	Helvete M.	N/A	N/A	22	N/A	N/A	N/A	N/A
D6629	1992	Leiskj	N/A	F	31.5	N/A	N/A	N/A	N/A
D6671	1992	Helvete N.	N/A	F	19	N/A	N/A	N/A	N/A
D6673	1992	Leiskj	N/A	M	49	N/A	N/A	N/A	N/A
D6674	1992	Tvillingan S.	N/A	F	36	N/A	N/A	N/A	N/A
D6675	1992	Ormskj.	N/A	F	42.5	N/A	N/A	N/A	N/A
D6676	1992	Brattfl.	N/A	M	26	N/A	N/A	N/A	N/A
D6677	1992	Grunnlåta	N/A	M	20	N/A	N/A	N/A	N/A
E6546	1992	Stordagsjama	N/A	M	47.5	N/A	N/A	N/A	N/A
E6571	1992	Helvete Ø.	N/A	F	41.5	N/A	N/A	N/A	N/A
E6715	1992	Dagskjær	N/A	M	23	N/A	N/A	N/A	N/A
E6718	1992	Rundormskj.	N/A	F	52.5	N/A	N/A	N/A	N/A
E6724	1992	Dagskjær	N/A	M	52.5	N/A	N/A	N/A	N/A
E6727	1992	Hamnoyan	N/A	F	45.5	N/A	N/A	N/A	N/A
E6731	1992	Helvete N.	N/A	M	42	N/A	N/A	N/A	N/A
E6732	1992	Tinnskjæret	N/A	N/A	41	N/A	N/A	N/A	N/A
E6739	1992	Grønnskj. Ø	N/A	F	41	N/A	N/A	N/A	N/A
E6740	1992	Grønnskj. Ø.	N/A	M	46	N/A	N/A	N/A	N/A
E6741	1992	Grønnskjara	N/A	N/A	43	N/A	N/A	N/A	N/A
E6751	1992	Helvete Ø.	N/A	M	41.5	N/A	N/A	N/A	N/A

E6752	1992	Grunnlåta	N/A	M	43	N/A	N/A	N/A	N/A
E6764	1992	Båskj. M.	N/A	F	40	N/A	N/A	N/A	N/A
E6780	1992	Leiskj.	N/A	F	39.5	N/A	N/A	N/A	N/A
E6786	1992	Grunnlåta	N/A	M	37.5	N/A	N/A	N/A	N/A
E6787	1992	Skj. Ø Br.flæsa	N/A	M	31	N/A	N/A	N/A	N/A
E6788	1992	Brattfl	N/A	M	42.5	N/A	N/A	N/A	N/A
E6789	1992	Skj. Ø Br.flæsa	N/A	M	31	N/A	N/A	N/A	N/A
E6790	1992	Brattflæsa	N/A	F	36.5	N/A	N/A	N/A	N/A
E6792	1992	Helvete N.	N/A	M	40.5	N/A	N/A	N/A	N/A
E6793	1992	Brattfl.	N/A	M	38	N/A	N/A	N/A	N/A
E6795	1992	Vona	N/A	F	30.5	N/A	N/A	N/A	N/A
E6796	1992	Vona	N/A	F	26.5	N/A	N/A	N/A	N/A
E6797	1992	Rundormskj.	N/A	F	38	N/A	N/A	N/A	N/A
E9977	1992	Ormskjæra	N/A	F	40	N/A	N/A	N/A	N/A
E9981	1992	Ormskjæra	N/A	N/A	32	N/A	N/A	N/A	N/A
E9982	1992	Ormskj.	N/A	F	41	N/A	N/A	N/A	N/A
E9984	1992	Dagskjær	N/A	F	17.5	N/A	N/A	N/A	N/A
E9986	1992	Brattfl.	N/A	F	41.5	N/A	N/A	N/A	N/A
E9988	1992	Tinnskj.	N/A	M	22.5	N/A	N/A	N/A	N/A
E9989	1992	Bleikskj.	N/A	N/A	19	N/A	N/A	N/A	N/A
E9990	1992	Bleikskj.	N/A	M	20.5	N/A	N/A	N/A	N/A

E9991	1992	Taillarholmen	N/A	M	17	N/A	N/A	N/A	N/A
E9992	1992	Sandskj.	N/A	F	49	N/A	N/A	N/A	N/A
E9993	1992	Skattaskj.	N/A	M	29	N/A	N/A	N/A	N/A
E9994	1992	Oterglittan	N/A	M	16	N/A	N/A	N/A	N/A
E9995	1992	Skattaskj.	N/A	M	19.5	N/A	N/A	N/A	N/A
E9996	1992	Slettskj.	N/A	F	31.5	N/A	N/A	N/A	N/A
E9997	1992	Tinnskj.	N/A	F	16	N/A	N/A	N/A	N/A
E9998	1992	Helvete S.	N/A	F	20	N/A	N/A	N/A	N/A
E9999	1992	Helvete S.	N/A	F	36.5	N/A	N/A	N/A	N/A
N000	1993	Froan (N/S)	1	F	17.5	N/A	N/A	N/A	N/A
D6681	1993	Froan (N/S)	1	F	18.5	N/A	N/A	N/A	N/A
D6684	1993	Froan (N/S)	4	F	36	N/A	N/A	N/A	N/A
D6685	1993	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
D6686	1993	Froan (N/S)	1	N/A	N/A	N/A	N/A	N/A	N/A
DD687	1993	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
D6688	1993	Froan (N/S)	3	M	41	N/A	N/A	N/A	N/A
D6690	1993	Froan (N/S)	1	F	20.5	N/A	N/A	N/A	N/A
D6691	1993	Froan (N/S)	1	M	19.5	N/A	N/A	N/A	N/A
D6692	1993	Froan (N/S)	2	F	30	N/A	N/A	N/A	N/A
D6693	1993	Froan (N/S)	2	M	30.5	N/A	N/A	N/A	N/A
E3013	1993	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

E3621	1993	Froan (N/S)	3	M	50.5	N/A	N/A	N/A	N/A
E3624	1993	Froan (N/S)	4	M	48	N/A	N/A	N/A	N/A
E3644	1993	Froan (N/S)	3	M	55	N/A	N/A	N/A	N/A
E3645	1993	Froan (N/S)	4	F	50.5	N/A	N/A	N/A	N/A
E3660	1993	Froan (N/S)	3	F	42.5	N/A	N/A	N/A	N/A
E3673	1993	Froan (N/S)	2	M	39	N/A	N/A	N/A	N/A
E3676	1993	Froan (N/S)	4	F	40	N/A	N/A	N/A	N/A
E3690	1993	Froan (N/S)	2	M	30.5	N/A	N/A	N/A	N/A
E3709	1993	Froan (N/S)	2	F	30	N/A	N/A	N/A	N/A
E3710	1993	Froan (N/S)	4	M	47	N/A	N/A	N/A	N/A
E3711	1993	Froan (N/S)	4	M	53	N/A	N/A	N/A	N/A
E3716	1993	Froan (N/S)	2	F	38	N/A	N/A	N/A	N/A
E3722	1993	Froan (N/S)	3	M	44	N/A	N/A	N/A	N/A
E3723	1993	Froan (N/S)	3	N/A	45.5	N/A	N/A	N/A	N/A
E3742	1993	Froan (N/S)	2	F	30	N/A	N/A	N/A	N/A
E3791	1993	Froan (N/S)	3	N/A	23	N/A	N/A	N/A	N/A
E6390	1993	Froan (N/S)	2	N/A	N/A	N/A	N/A	N/A	N/A
2000-01	2000	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2000-02	2000	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2000-03	2000	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2000-04	2000	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

2000-06	2000	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2000-08	2000	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-01	2005	Finnvaeret	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-02	2005	Finnvaeret Ø	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-03	2005	Finnvaeret Ø	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-04	2005	Andstein	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-06	2005	Andstein	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-07	2005	Andstein	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-08	2005	Froan (N/S)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-09	2005	Selstein	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-10	2005	Andstein	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-11	2005	Kristianskj	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-12	2005	Kristianskj	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-13	2005	Kristianskj	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-14	2005	Kristianskj	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-15	2005	Kristianskj	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-16	2005	Selstein	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-17	2005	Selstein	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-19	2005	Leiskjaera	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-20	2005	Leiskjaera	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005-21	2005	Leisjaera	N/A	N/A	N/A	N/A	N/A	N/A	N/A

2006-01	2006	Froan (N/S)	N/A	N/A	54	110	103	N/A	N/A
2020-01	2020	Froan (N/S)	N/A	F	39.9	118	96	N/A	N/A
2020-02	2020	Froan (N/S)	N/A	F	39.5	108	100	N/A	N/A
2020-03	2020	Kråkoya	N/A	M	49	113	80	N/A	N/A
2020-04	2020	Ormskjæret	N/A	M	17	111	55	N/A	N/A
2020-05	2020	Tindskjæret	N/A	F	16.7	108	55	N/A	N/A
2020-06	2020	Kvalværet	N/A	F	38.2	105	87.5	N/A	N/A
2020-07	2020	Kniskfiskskjæret	N/A	M	25.1	106	80	N/A	N/A
2020-08	2020	Grunnlåta	N/A	F	23.6	98	92	N/A	N/A
2020-09	2020	Sandværet S.	N/A	M	38.8	117	66	N/A	N/A
2020-10	2020	Sandværet S.	N/A	M	20	102	92	N/A	N/A
2020-11	2020	N.E. Sandskjæret	N/A	F	38.6	104	95	N/A	N/A
2020-12	2020	N.E. Sandskjæret	N/A	M	43	112	53	N/A	N/A
2020-14	2020	Sandskjæret	N/A	M	26.1	101	75	N/A	N/A
F-22-01	2022	Tryggholmen	3	M	42	106	99	At sea	Not seen
F-22-02	2022	Skjelsmeitskjæret	3	M	44.5	128	99.5	Ashore	At see
F-22-03	2022	Henriksholmen Sør	2	M	27.5	116	83	At sea	Not seen
F-22-04	2022	Grunnlåta V	1.5	M	22.2	100	71	At sea	Not seen
F-22-05	2022	Grunnlåta V	4	F	51.2	119	110	Not seen	Not seen
F-22-06	2022	Grunnlåta V	3	M	49	120	100	By pup	Not seen
F-22-07	2022	Grunnlåta V	1	F	15	83	70	At sea	Not seen

F-22-08	2022	Fåfengøya S.V.	3	M	35.2	132	99	Ashore	At sea
F-22-09	2022	Gullholmen	1	M	19.7	98	71	At sea	At sea
F-22-10	2022	Gullholmen	2.5	F	31.2	120	81	At sea	At sea
F-22-11	2022	Gullholmen	3.5	M	64.2	130	100	At sea	At sea
F-22-12	2022	Skjelsmeitskjæret	3.5	F	43.2	113	100	Attending	At sea

Table. A.1.2. Gradient elution program for the UPLC-MS/MS analysis. The composition is given at the percentage of water with 2 mM ammonium acetate (A) and MeOH (B). The flow was kept constant at 250 $\mu L min^{-1}$.

Time [min]	**%A**	**%B**	**Step**
Init	80	20	Init
0.1	80	20	6
0.2	50	50	6
0.8	30	70	6
1.5	20	80	6
2.8	15	85	5
4.5	0	100	6
5.5	0	100	6
5.6	80	20	6
6	80	20	6

Table A.1.3. UPLC-MS/MS optimal source settings

ESI -	
Source temperature	150°C
Capillary voltage	2 kV
Cone voltage	25 V
Source offset	40 V
Desolvation temperature	450°C
Desolvation gas flow rate	650 L/hr
Cone gas flow rate	150 L//hr
Nebulizer gas pressure	6.0 bar

Table. A.1.4. Gradient elution program for the UPC^2-MS/M analysis. The composition is given at the percentage of CO2 (A) and MeOH with ammonium acetate (B). The flow was kept constant at 200 µLmin^{-1}.

Time [min]	**%A**	**%B**	**Step**
Init	98	2	Init
0.5	98	2	6
3.0 (4.0)	83	17	6
3.5 (4.0)	83	17	6
4.0 (5.0)	98	2	6
5.0 (6.0)	98	2	6

Table A.1.5. UPC^2-MS/MS optimal source settings

ESI +	
Source temperature	150°C
Capillary voltage	2.8 kV
Cone voltage	20 V
Source offset	80 V
Desolvation temperature	500°C
Desolvation gas flow rate	1000 L/hr
Cone gas flow rate	150 L//hr
Nebulizer gas pressure	6.0 bar

2. Results

Table A.2.1. Linearity (R^2), method limit of detection (mLOD), and method limit of quantification (mLOQ) of the 46 PFASs analyzed in plasma of grey seal pups sampled in 2022 (n=12)

PFASs	Linearity (R^2)	mLOD (ng/mL)	mLOQ (ng/mL)
Deca S	0,997	0.05	0.17
PFBA	0,282	5.05	16.67
PFPeA	0,999	0.20	0.67
PFHxA	0,999	0.10	0.33
PFHpA	0,999	0.05	0.17
PFOA	0,999	0.10	0.33
PFNA	0,998	0.05	0.17
PFDA	0,998	0.05	0.17
PFUnDA	0,998	0.02	0.07
PFDoDA	0.998	0.05	0.17
PFTriDA	0.998	0.20	0.67
PFTDA	0.999	0.20	0.67
PFHxDA	0.998	0.02	0.07
PFOcDA	0.998	2.02	6.67
P37DMOA	0.999	0.10	0.33
7H-PFHpA	0.999	0.05	0.17
TriDeFHxSA	0.718	20.20	66.67
PFBS	0.999	0.05	0.17
PFPeS	0.999	0.05	0.17
PFHxS	0.999	0.01	0.03
PFHpS	0.999	0.05	0.17
PFOS	0.999	0.10	0.33
PFNS	0.999	0.01	0.03
PFDS	0.999	0.01	0.03
PFDoDS	0.999	0.01	0.03
PFECHS	0.999	0.01	0.03
PFOSA	0.998	0.02	0.07
MeFOSA	0.998	0.02	0.07
EtFOSA	0.997	0.03	0.01
FOSAA	0.999	0.05	0.17
MeFOSAA	0.999	0.05	0.17
EtFOSAA	0.999	0.03	0.01
MeFOSE	0.995	0.05	0.17
EtFOSE	0.999	0.20	0.67
4 :2 FTS	0.999	0.10	0.33
6 :2 FTS	0.999	0.01	0.03
8 :2 FTS	0.998	0.02	0.07
10 :2 FTS	0.999	0.01	0.03
Gen X	0.995	1.01	3.33
ADONA	0.999	0.01	0.03
9Cl-PF3ONS	0.999	0.05	0.17
SAMPAP	0.992	50.50	166.67
DiSAMPAP	0.997	0.05	0.17
6 :6 PFPi	0.996	2.02	6.67
6 :8 PFPi	0.991	5.05	16.67
8 :8 PFPi	0.994	5.05	16.67

Table A.2.2. Linearity (R^2), method limit of detection (mLOD), and method limit of quantification (mLOQ) of the 17 steroids analyzed in plasma of grey seal pups sampled in 2022 (n=12)

Steroids	Linearity (R^2)	mLOD (ng/mL)	mLOQ (ng/mL)
COR	0,996	0.20	0.67
CORNE	0,999	0.51	1.67
ALDO	0,999	0.51	1.67
COS	0,997	2.02	6.67
11-deoxyCOR	0,999	2.02	6.67
17a-OHP	0,998	0.20	0.67
DOC	0,999	0.05	0.17
17aOH-P5	0,997	0.10	0.33
11-KetoTS	0,999	0.20	0.67
P4	0,996	5.05	16.67
P5	0,976	5.05	16.67
DHT	0,995	1.01	3.33
TS	0,998	0.10	0.33
AN	0,983	5.05	16.67
E1	0,999	2.02	6.67
A5	0,968	5.05	16.67
DHEA	0,998	2.02	6.67

Table A.2.3 Matrix effects (%) of the 46 PFASs and 17 steroids analyzed in plasma of grey seal pups sampled in 2022 (n=12).

Steroids	Matrix effects (%)	PFASs	Matrix effects (%)	PFASs	Matrix effects
COR	14	Deca S	-38	PFDS	-7
CORNE	-4	PFBA*	-3445	PFDoDS	2
ALDO	46	PFPeA	-23	PFECHS	-12
COS	6	PFHxA	-41	PFOSA	-2
11-deoxyCOR	-12	PFHpA	-21	MeFOSA	-8
17a-OHP	6	PFOA	-21	EtFOSA	-6
DOC	30	PFNA	-18	FOSAA	-11
17aOH-P5	11	PFDA	-36	MeFOSAA	7
11-KetoTS	77	PFUnA	-7	EtFOSAA	-5
P4	6	PFDoDA	-10	MeFOSE	-21
P5	-1	PFTriDA	0	EtFOSE	-10
DHT	-11	PFTDA	6	4 :2 FTS	-28
TS	20	PFHxDA	-4	6 :2 FTS	-8
AN	-21	PFOcDA	4	8 :2 FTS	10
E1	27	P37DMOA	-2	10 :2 FTS	-9
A5	16	7H-PFHpA	-23	Gen X	2
DHEA	-33	TriDeFHxSA*	-515	ADONA	-10
		PFBS	-8	9Cl-PF3ONS	-3
		PFPeS	-14	SAMPAP	33
		PFHxS	-11	DiSAMPAP	6
		PFHpS	-10	6 :6 PFPi	7
		PFOS	-33	6 :8 PFPi	22
		PFNS	-6	8 :8 PFPi	6

**Those compounds were considered as not quantifiable because of poor linearity of the calibration curve*

Table A.2.4. Absolute (a) and relative (b) recoveries of the 46 PFASs at three different fortification levels (5, 10, 20 ng/mL). The 46 PFASs were analyzed in plasma of the grey seal pups sampled in 2022 (n=12)

	Absolute recovery (%) ± RSD (%)			Relative recovery (%) ± RSD (%)		
	5 ng/mL	10 ng/mL	20 ng/mL	5 ng/mL	10 ng/mL	20 ng/mL
Deca S	50,3 (±6.0)	55,8 (±7.0)	58,5 (±5.0)	75,6 (±7.7)	99,4 (±11.3)	119,2 (±8.5)
PFBA	16,7 (±89.0)	10 (±86.8)	10,2 (±88.0)	25 (±88.7)	17,8 (±86.7)	20,8 (±87.2)
PFPeA	49,9 (±20.1)	52,9 (±9.8)	57,4 (±3.4)	75 (±19.2)	94,4 (±8.4)	117 (±7.1)
PFHxA	52,6 (±12.2)	56,8 (±7.6)	61,4 (±6.4)	79,1 (±12.4)	101,3 (±8.8)	125,2 (±8.1)
PFHpA	46,4 (±25.2)	53,3 (±4.5)	64,4 (±2.5)	69,7 (±25.0)	95 (±12.2)	131,4 (±5.9)
PFOA	48,4 (±9.9)	55,8 (±0.2)	62,7 (±1.6)	58,7 (±14.3)	86 (±10.5)	109,4 (±6.8)
PFNA	56,8 (±9.2)	59,5 (±3.7)	67,1 (±5.0)	85,3 (±8.6)	106 (±7.3)	136,8 (±7.9)
PFDA	55,6 (±5.6)	62,9 (±5.9)	66,2 (±1.6)	83,5 (±6.8)	112 (±13.0)	135 (±5.3)
PFUnA	60,2 (±10.3)	61,4 (±10.0)	64,7 (±2.1)	90,5 (±10.4)	109,4 (±8.6)	132 (±1.8)
PFDoDA	54,5 (±11.2)	57 (±3.3)	58 (±6.3)	81,8 (±10.7)	101,6 (±8.1)	121,9 (±10.0)
PFTriDA	59,1 (±3.5)	59,9 (±9.5)	61,4 (±3.4)	88,7 (±1.9)	106,7 (±8.5)	125,3 (±4.3)
PFTDA	48,5 (±11.8)	55,5 (±2.8)	57,8 (±3.3)	72,9 (±11.5)	98,9 (±6.1)	118 (±7.1)
PFHxDA	34,1 (±19.7)	40 (±8.8)	53,5 (±3.1)	51,2 (±20.5)	71,3 (±11.1)	109,2 (±5.1)
PFOcDA	26,4 (±27.1)	31,3 (±2.5)	43,8 (±12.6)	39,7 (±26.5)	55,8 (±10.8)	89,2 (±13.9)
P37DMOA	48,7 (±12.2)	54,9 (±7.5)	61,9 (±3.5)	73,2 (±11.8)	97,9 (±5.7)	126,1 (±6.8)
7H-PFHpA	45,3 (±18.4)	57,9 (±7.6)	62,7 (±0.7)	68 (±18.8)	103,3 (±9.9)	127,8 (±3.4)
TriDeFHxSA	86,7 (±16.2)	144,8 (±173.2)	164,8 (±101.0)	130,3 (±15.1)	258,1 (±173.2)	336,2 (±103.6)
PFBS	46,4 (±9.1)	53,6 (±4.2)	62,4 (±3.2)	69,7 (±9.8)	95,6 (±6.1)	127,2 (±5.6)
PFPeS	0,47 (±7.9)	0,57 (±7.3)	0,64 (±2.7)	70,1 (±8.4)	100,9 (±8.4)	131,5 (±4.2)
PFHxS	52,2 (±8.2)	59,6 (±4.5)	63,6 (±2.7)	78,4 (±7.3)	106,2 (±6.5)	129,7 (±3.1)
PFHpS	49,8 (±15.2)	56,7 (±5.4)	62,2 (±2.4)	74,9 (±14.6)	101 (±5.8)	127 (±5.6)
PFOS	89,4 (±4.4)	81,5 (±7.3)	81,3 (±3.7)	134,3 (±4.5)	145,2 (±2.9)	165,7 (±7.4)
PFNS	46,3 (±13.1)	52,2 (±8.3)	61,8 (±6.1)	69,6 (±13.1)	93 (±3.9)	126 (±9.9)
PFDS	47,6 (±10.7)	53,5 (±2.8)	61 (±3.5)	71,6 (±10.8)	95,4 (±9.4)	124,4 (±7.0)
PFDoDS	46,5 (±13)	54,8 (±13)	58,9 (±8.6)	69,9 (±12.7)	97,7 (±4.4)	120,2 (±9.7)
PFECHS	46,9 (±13)	54,3 (±13)	61,5 (±4.3)	70,5 (±12.6)	96,7 (±6.8)	125,4 (±7.3)
PFOSA	43 (±12.2)	52,8 (±5.8)	62,9 (±4.9)	64,7 (±12.5)	94 (±5.8)	128,3 (±7.9)
MeFOSA	45,8 (±8.3)	53,3 (±7.1)	59,6 (±4.8)	68,9 (±7.9)	95 (±5.5)	121,6 (±8.3)
EtFOSA	48 (±12.2)	51,2 (±3.2)	59,4 (±3.2)	72,1 (±11.8)	91,3 (±4.3)	121,1 (±7.0)
FOSAA	30,2 (±3.9)	15,2 (±9.0)	9,7 (±5.5)	45,4 (±2.4)	27,1 (±5.3)	19,9 (±9.0)
MeFOSAA	5,1 (±38.5)	4,7 (±35.7)	12,5 (±51.8)	7,61 (±38.1)	8,38 (±38.5)	25,6 (±51.8)
EtFOSAA	47,9 (±10.1)	52,5 (±5.2)	57,5 (±5.1)	72 (±9.3)	93,5 (±4.0)	117,3 (±8.9)
MeFOSE	44,5 (±27.2)	48,9 (±5.7)	60,7 (±5.7)	66,9 (±27.7)	87,1 (±12.3)	123,9 (±9.4)
EtFOSE	44,2 (±10.7)	49,3 (±9.2)	59,2 (±4.2)	66,4 (±10.3)	87,8 (±4.3)	120,7 (±4.7)
4:2 FTS	47,9 (±13.7)	57,3 (±7.6)	60,1 (±2.9)	72 (±13.7)	102,1 (±9.1)	122,5 (±6.6)
6:2 FTS	46,4 (±19.9)	56,1 (±3.2)	71,3 (±2.7)	63,8 (±19.1)	90,8 (±13.5)	130,4 (±2.2)
8:2 FTS	42,3 (±19.8)	49,1 (±8.6)	55,5 (±6.0)	63,6 (±20.0)	87,5 (±6.7)	113,2 (±3.8)
10:2 FTS	48,3 (±22.8)	54,2 (±10.8)	57,3 (±7.2)	72,5 (±21.8)	96,4 (±9.8)	116,8 (±11.0)
Gen X	32,9 (±54.7)	55,6 (±3.9)	60,8 (±13.1)	49,5 (±54.3)	99 (±9.7)	124 (±16.1)
ADONA	48,2 (±12.7)	54,8 (±6.2)	62,9 (±2.5)	72,4 (±12.7)	97,6 (±9.1)	128,2 (±3.4)
9Cl-PF3ONS	46 (±8.1)	54,7 (±5.9)	61,6 (±4.1)	69,1 (±7.9)	97,6 (±7.4)	125,7 (±7.3)
SAMPAP	6,2 (±173.2)	0,85 (±92.2)	0,66 (±158.6)	9,3 (±173.2)	1,5 (±96.9)	1,3 (±158.1)
diSAMPAP	0,54 (±56.)	0,94 (±40.0)	9,37 (±73.7)	0,81 (±56.2)	1,67 (±44.4)	19,1 (±73.6)

6:6 PFPi	48,7 (±49.9)	43,6 (±40.7)	57,3 (±3.6)	73,2 (±51.1)	77,7 (±38.4)	116,8 (±3.9)
6:8 PFPi	44,5 (±183.9)	25 (±27.5)	46,1 (±5.6)	66,9 (±156.6)	44,6 (±34.2)	93,9 (±6.5)
8:8 PFPi	24,1 (±43.4)	30,9 (±27.1)	45,1 (±17.7)	36,2 (±42.5)	55 (±18.2)	92 (±16.2)

Table A.2.5. Absolute and relative recoveries (%) of the 17 steroids at three fortification levels (5, 10, 20 ng/mL) ± relative standard deviation (RSD %) using the HybridSPE method of extraction. The 17 steroids were analyzed in plasma of grey seal pups sampled in 2022 (n=12)

	Absolute recovery (%) (± RSD %)			Relative recovery (%) ± RSD (%)		
	5 ng/mL	10 ng/mL	20 ng/mL	5 ng/mL	10 ng/mL	20 ng/mL
COR	67 (±7.3)	59,4 (±16.8)	67,4 (±11.9)	0 (±7.3)	126,4 (±11.2)	125,4 (±13.5)
CORNE	65,2 (±7.4)	69,6 (±9.4)	70,7 (±0.2)	120,2 (±8.7)	148 (±5.2)	131,6 (±16.4)
ALDO	19 (±13.4)	16,5 (±10.1)	22,2 (±14.3)	35 (±12.4)	35 (±9.0)	41,3 (±24.8)
COS	53,3 (±8.1)	49,2 (±8.5)	54,5 (±9.2)	98,2 (±11.0)	104,5 (±8.4)	101,4 (±17.4)
11-deoxyCOR	56,9 (±13.0)	58,7 (±16.4)	64,7 (±4.8)	104,9 (±2.8)	124,8 (±10.0)	120,4 (±12.1)
17a-OHP	78,5 (±32.4)	92,7 (±13.2)	92,4 (±5.1)	121 (±21.6)	142,2 (±6.9)	139,1 (±4.6)
DOC	40,3 (±6.0)	44,1 (±25.1)	55,5 (±5.1)	62,1 (±12.5)	67,6 (±23.1)	83,5 (±6.4)
P4	68,5 (±16.7)	65,5 (±13.7)	79,7 (±4.1)	96,1 (±6.0)	97,2 (±15.3)	98,5 (±7.5)
KetoTS	52,7 (±22.4)	56 (±7.1)	63 (±4.3)	97,1 (±8.7)	119 (±3.6)	117,2 (±18.2)
P5	52,3 (±17.9)	73 (±14.6)	79,3 (±2.6)	73,5 (±10.1)	108,4 (±7.1)	98 (±6.7)
17a-OHP5	112 (±30.4)	109,6 (±9.8)	99,2 (±61.8)	172,6 (±23.7)	168,2 (±15.7)	149,3 (±62.0)
DHT	60,3 (±24.8)	63,9 (±10.9)	80,2 (±2.6)	84,6 (±16.4)	94,9 (±5.5)	99,1 (±6.3)
TS	58,8 (±15.9)	69,3 (±7.4)	74,6 (±6.4)	82,6 (±6.5)	102,9 (±15.0)	92,2 (±6.5)
AN	0,6 (±45.2)	1,1 (±5.5)	1,1 (±29.5)	86,2 (±34.7)	161 (±20.5)	135,2 (±24.5)
E1	56,6 (±25.7)	56,1 (±9.2)	66,5 (±0.1)	87,1 (±17.4)	86 (±1.6)	100,1 (±1.9)
A5	67,4 (±1.6)	62,9 (±10.7)	78,3 (±5.3)	94,6 (±8.9)	93,4 (±6.3)	96,7 (±3.2)
DHEA	55,8 (±16.4)	88,5 (±8.0)	91,1 (±11.2)	78,4 (±19.6)	131,3 (±12.5)	112,6 (±4.5)

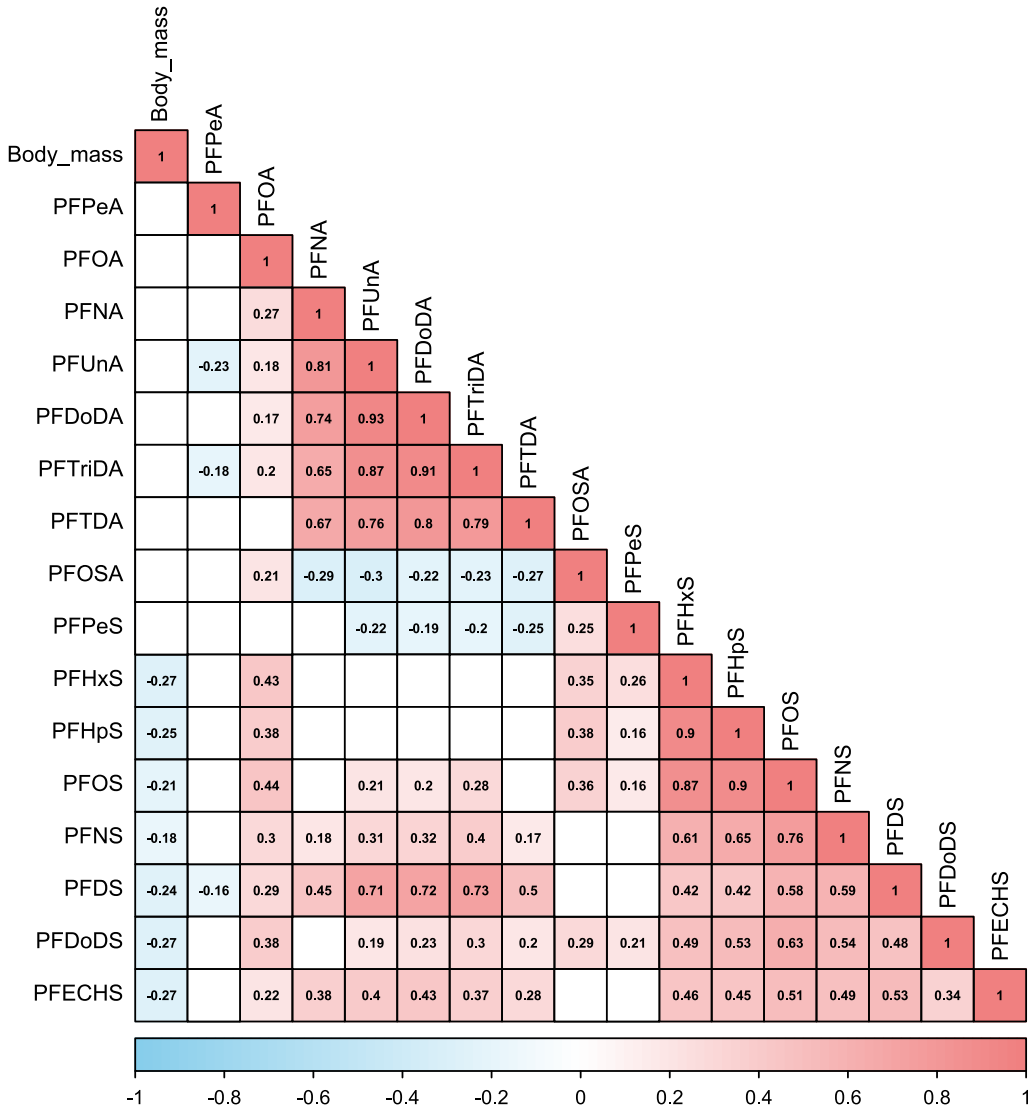


Figure A.2.1 Spearman's correlation matrix among the 16 PFASs and between the PFASs and the biological variable "body mass" in plasma of grey seal pups sampled from 1992 to 2022 (n=164) in the Froan Archipelago.

Table A.2.6. Concentrations and detection rates (DR%) of the 42 PFASs jointly analyzed in plasma of grey seal pups sampled from 1992 to 2022 (n=164) in the Froan Archipelago. Concentrations are presented as the geometric mean (ng/mL) ± standard deviation (sd) with range in parentheses.

		1992	1993	2000	2005	2020	2022
Target analytes	DR (%)	GM ± sd (range)	GM ± sd (range)	GM ± sd (range)	GM ± sd (range)	GM ± sd (range)	GM ± sd (range)
Deca S	50.9	0.26 ± 0.15 (<mLOD-0.72)	0.24 ± 0.13 (<mLOD-0.60)	0.28 ± 0.15 (<mLOD. -0.49)	0.24 ± 0.16 (<mLOD-0.42)	0.17 ± 0.04 (<mLOD-0.24)	0.14 ± 0.17 (<mLOD-0.42)
PFBA	6.7	2.69 ± 0.51 (<mLOD -3.29)	<mLOD	<mLOD	14.8 (<mLOD -14.8)	5.45 ± 3.00 (<mLOD -8.3)	22.82 ± 28.00 (7.88.-71.1)
PFPeA	76.4	1.15 ± 4.25 (<mLOD -30.4)	0.84 ± 0.88 (<mLOD -4.46)	0.73 ± 0.35 (<mLOD -1.18)	0.71 ± 0.67 (<mLOD -1.88)	1.08 ± 2.26 (<mLOD -7.03)	0.40 ± 0.36 (<mLOD -0.89)
PFHxA	57.0	0.43 ± 0.37 (<mLOD -1.99)	0.52 ± 0.51 (<mLOD -1.63)	0.46 ± 0.19 (<mLOD -0.71)	0.40 ± 0.31 (<mLOD -1.04)	0.56 ± 0.62 (<mLOD -1.63)	0.55 ± 0.22 (<mLOD -0.82)
PFHpA	33.3	0.24 ± 0.12 (<mLOD – 0.53)	0.27 ± 0.15 (<mLOD – 0.46)	0.13 (<mLOD -0.13)	0.28 ± 0.21 (<mLOD-0.79)	0.21 ± 0.07 (<mLOD -0.32)	0.82 (<mLOD – 0.82)
PFOA	90.3	0.73 ± 0.70 (<mLOD -4.57)	0.54 ±0.80 (<mLOD -4.04)	1.16 ±1.41 (0.55-4.20)	0.70 ± 0.64 (0.19-2.59)	0.31 ± 0.22 (<mLOD -0.80)	0.49 ± 0.28 (0.13-1.18)
PFNA	99.4	0.55 ± 0.39 (0.09-1.89)	0.58 ± 0.36 (<mLOD -1.84)	1.25 ± 0.51 (0.80-2.26)	2.26 ± 1.26 (0.71-4.76)	1.64 ± 1.55 (0.37-5.84)	3.33 ± 1.22 (0.13-1.18)
PFDA	23.0	1.46 ± 0.36 (<mLOD – 2.10)	<mLOD	1.65 ± 0.45 (<mLOD – 2.00)	2.32 ± 1.18 (<mLOD – 4.92)	2.50 ± 1.47 (<mLOD – 4.95)	2.49 ± 1.09 (1.13 – 5.35)
PFUnA	100.0	2.27 ± 1.16 (0.90-8.07)	2.50 ± 1.22 (1.09-5.75)	5.63 ± 2.11 (3.86-9.58)	8.62 ± 3.28 (4.35-15.0)	5.31 ± 2.64 (2.58-12.4)	7.53 ± 3.74 (5.28-18.0)
PFDoDA	100.0	0.75 ± 0.32 (0.38-1.81)	0.76 ± 0.67 (0.21-1.70)	1.57 ± 0.57 (1.00-2.40)	2.24 ± 0.87 (1.08-4.27)	1.51 ± 0.87 (0.58-3.31)	2.11 ± 0.97 (1.32-4.29)
PFTriDA	100.0	2.77 ± 0.92	2.83 ± 1.24	5.24 ± 1.54	7.27 ± 2.55	4.02 ± 1.75	4.59 ± 1.99

		(1.23-5.95)	(1.01-5.74)	(3.85-7.82)	(3.83-13.2)	(1.71-8.57)	(2.79-9.36)
PFTDA	100.0	0.28 ± 0.12 (0.06-0.61)	0.25 ± 0.15 (0.06-0.66)	0.48 ± 0.23 (0.27-0.84)	0.62 ± 0.23 (0.39-1.17)	0.47 ± 0.24 (0.20-1.04)	1.09 ± 0.54 (0.59-2.37)
PFHxDA	0.6	0.13 (<mLOD – 0.13)	<mLOD	<mLOD	<mLOD	<mLOD	<mLOD
PFOcDA	13.3	0.10 ± 0.07 (<mLOD – 0.30)	0.11 ± 0.05 (<mLOD – 0.22)	<mLOD	0.07 (<mLOD – 0.07)	<mLOD	<mLOD
P37DMOA	52.7	0.04 ± 0.04 (<mLOD-0.16)	0.03 ± 0.02 (<mLOD-0.08)	0.05 ± 0.03 (0.03-0.08)	0.11 ± 0.06 (0.04-0.23)	0.06 ± 0.04 (0.03-0.14)	n.d.
7H-PFHpA	56.4	0.20 ± 0.13 (<mLOD-0.57)	0.11 ± 0.04 (<mLOD-0.18)	0.14 ± 0.19 (<mLOD-0.33)	0.16 ± 0.30 (<mLOD-0.60)	0.24 ± 1.00 (<mLOD-2.14)	<mLOD
FOSAA	0	<mLOD	<mLOD	<mLOD	<mLOD	<mLOD	<mLOD
MeFOSAA	15.2	0.39 ± 0.57 (<mLOD – 2.44)	0.27 ± 0.37 (<mLOD – 0.83)	n.d.	0.47 (<mLOD – 0.47)	0.42 ± 0.46 (<mLOD – 0.85)	0.60 (<mLOD – 0.60)
EtFOSAA	17.0	0.04 ± 0.09 (<mLOD – 0.44)	0.03 ± 0.01 (<mLOD – 0.04)	<mLOD	0.03 (<mLOD – 0.03)	0.05 (<mLOD – 0.05)	<mLOD
PFOSA	73.3	0.04 ± 0.09 (<mLOD-0.53)	0.05 ± 0.03 (<mLOD-0.15)	0.04 ± 0.04 (0.02-0.12)	0.03 ± 0.03 (<mLOD-0.10)	0.02 ± 0.02 (<mLOD-0.06)	<mLOD
MeFOSA	0	<mLOD	<mLOD	<mLOD	<mLOD	<mLOD	<mLOD
EtFOSA	35.8	0.03 ± 0.04 (<mLOD – 0.3)	0.02 ± 0.01 (<mLOD – 0.03)	0.04 (<mLOD – 0.04)	0.02 (<mLOD – 0.02)	0.02 ± 0.01 (<mLOD – 0.03)	<mLOD
PFBS	58.2	0.04 ± 0.04 (<mLOD-0.19)	0.03 ± 0.02 (<mLOD-0.06)	0.05 ± 0.02 (<mLOD-0.07)	0.05 ± 0.02 (<mLOD-0.14)	0.06 ± 0.04 (<mLOD-0.15)	0.07 ± 0.02 (<mLOD-0.09)
PFPeS	91.5	0.19 ± 0.16 (<mLOD-0.71)	0.22 ± 0.16 (0.02-0.73)	0.37 ± 0.24 (0.15-0.88)	0.13 ± 0.30 (0.02-1.30)	0.12 ± 0.46 (0.01-1.66)	0.12 ± 0.05 (<mLOD-0.2)

PFHxS	100.0	8.48 ± 4.68 (1.81-22.9)	9.88 ± 4.34 (4.99-25.8)	15.5 ± 6.39 (9.60-26.0)	11.6 ± 10.14 (5.28-49.3)	2.95 ± 1.81 (1.07-8.09)	3.74 ± 2.54 (1.83-10.5)
PFHpS	100.0	2.22 ± 1.43 (0.47-7.39)	2.69 ± 1.70 (0.62-9.20)	4.38 ± 1.06 (3.56-6.47)	2.95 ± 1.51 (1.46-7.79)	0.58 ± 0.42 (0.25-1.48)	0.72 ± 0.48 (0.32-1.85)
PFOS	100.0	103.59 ± 64.20 (35.0-405.5)	122.89 ± 67.21 (49.5-328.98)	208.85 ± 35.91 (156.6-250.4)	137.26 ± 73.15 (62.3-327.7)	43.80 ± 20.47 (19.3-91.2)	44.99 ± 19.04 (26.8-89.5)
PFNS	94.5	0.23 ± 0.22 (<mLOD-1.24)	0.31 ± 0.23 (<mLOD-1.07)	0.76 ± 0.21 (0.59-1.11)	0.42 ± 0.24 (0.14-1.03)	0.10 ± 0.08 (<mLOD-0.25)	0.12 ± 0.09 (0.02-0.32)
PFDS	98.8	0.23 ± 0.20 (0.04-1.16)	0.27 ± 0.22 (<mLOD-0.89)	0.98 ± 0.26 (0.64-1.29)	0.92 ± 0.53 (0.30-2.26)	0.29 ± 0.14 (0.10-0.56)	0.29 ± 0.15 (0.08-0.66)
PFDoDS	72.1	0.15 ± 0.11 (<mLOD-0.62)	0.17 ± 0.08 (<mLOD-0.41)	0.18 ± 0.07 (0.13-0.29)	0.20 ± 0.16 (<mLOD-0.63)	0.08 ± 0.02 (<mLOD-0.11)	0.05 ± 0.11 (0.01-0.21)
PFECHS	98.2	0.15 ± 0.14 (<mLOD-0.75)	0.23 ± 0.17 (<mLOD-0.79)	0.31 ± 0.06 (0.23-0.38)	0.20 ± 0.13 (0.08-0.52)	0.13 ± 0.08 (0.03-0.32)	0.42 ± 0.44 (0.25-1.88)
4:2 FTS	8.5	0.04 ± 0.02 (<mLOD – 0.08)	0.03 ± 0.01 (<mLOD – 0.03)	0.03 (<mLOD – 0.03)	<mLOD	0.04 ± 0.03 (<mLOD – 0.06)	1.14 (<mLOD – 1.14)
6:2 FTS	40.0	0.84 ± 9.74 (<mLOD – 45.2)	1.10 ± 6.20 (<mLOD – 17.1)	0.30 ± 0.35 (<mLOD – 0.88)	0.24 ± 0.16 (<mLOD – 0.63)	0.61 ± 1.35 (<mLOD – 2.87)	0.78 ± 65.44 (<mLOD – 173.5)
8:2 FTS	0.6	0.09 ± 0.05 (n.d. – 0.13)	<mLOD	<mLOD	<mLOD	<mLOD	<mLOD
10:2 FTS	58.2	0.05 ± 0.02 (<mLOD-0.12)	0.05 ± 0.04 (<mLOD-0.16)	0.05 ± 0.03 (<mLOD-0.09)	0.07 ± 0.13 (<mLOD-0.45)	0.08 ± 0.06 (<mLOD-0.20)	0.04 ± 0.03 (0.02-0.08)
MeFOSE	0	<mLOD	n.d.	n.d.	<mLOD	n.d.	n.d.
EtFOSE	0.6	<mLOD	<mLOD	<mLOD	<mLOD	0.53 (<mLOD – 0.53)	n.d.
Gen X	51.5	1.43 ± 1.90	1.99 ± 1.76	1.07 ± 1.35	1.55 ± 1.12	1.20 ± 0.41	2.75 ± 0.42

		(0.23-7.94)	(0.39-7.63)	(0.50-2.99)	(0.60-4.44)	(0.90-1.83)	(2.47-3.06)
ADONA	8.5	0.02 ± 0.01 (<mLOD – 0.03)	0.02 ± 0.00 (<mLOD – 0.02)	<mLOD	<mLOD	<mLOD	0.03 ± 0.03 (<mLOD – 0.05)
9Cl-PF3ONS	3.0	<mLOD	<mLOD	0.03 ± 0.00 (<mLOD – 0.03)	0.03 ± 0.03 (<mLOD – 0.03)	0.03 (<mLOD – 0.03)	0.15 (<mLOD – 0.15)
SamPAP	13.9	2189.01 ± 3408.38 (<mLOD – 11448.14)	543.36 ± 54.56 (<mLOD – 583.31)	n.d.	217.65 (<mLOD – 217.65)	n.d.	n.d.
DiSamPAP	10.3	2.03 ± 2.50 (<mLOD – 6.67)	4.41 ± 5.10 (<mLOD – 11.56)	n.d.	2.39 (<mLOD – 2.39)	2.52 ± 0.15 (<mLOD – 2.62)	n.d.

<mLOD: below the limit of detection. n.d. not detected. Note: DR% calculated based on values > mLOD.

Table A.2.7. Concentrations and detection rates (DR%) of the 46 PFASs analyzed in plasma of grey seal pups sampled in 2022 (n=12) in the Froan Archipelago. Concentrations are presented as the geometric mean (ng/mL) ± standard deviation (sd) with range in parentheses.

Target analytes	DR %	GM ± sd (range)
Deca S	33.3	0.14 ± 0.17 (<mLOD – 0.42)
PFBA	0	n.d.
PFPeA	25	0.40 ± 0.36 (<mLOD – 3.06)
PFHxA	41.7	0.55 ± 0.22 (0.30 – 0.82)
PFHpA	8.3	0.82 (<mLOD – 0.82)
PFOA	100	0.49 ± 0.28 (0.13-1.18)
PFNA	100	3.32 ± 1.22 (1.50 – 6.26)
PFDA	100	2.49 ± 1.09 (1.13 – 5.35)
PFUnA	100	7.53 ± 3.74 (5.28 – 17.97)
PFDoDA	100	2.11 ± 0.97 (1.32 – 4.29)
PFTriDA	100	4.59 ± 1.99 (2.79 – 9.36)
PFTDA	100	1.09 ± 0.54 (0.59 – 2.37)
PFHxDA	8.3	<mLOD
PFOcDA	33.3	<mLOD
P37DMOA	0	n.d.
7H-PFHpA	0	<mLOD
TriDeFHxSA	0	n.d.
PFBS	16.7	0.07 ± 0.02 (<mLOD – 0.09)
PFPeS	50	0.12 ± 0.05 (<mLOD – 0.19)
PFHxS	100	3.74 ± 2.54 (1.83 – 10.48)
PFHpS	100	0.72 ± 0.48 (0.32 – 1.85)
PFOS	100	44.99 ± 19.04 (26.76 – 89.50)
PFNS	75	0.12 ± 0.09 (0.02 – 0.32)
PFDS	91.7	0.29 ± 0.15 (0.08 – 0.66)
PFDoDS	25	0.05 ± 0.11 (0.01 – 0.21)
PFECHS	100	0.42 ± 0.44 (0.25 – 1.88)
PFOSA	58.3	<mLOD
MeFOSA	0	n.d.
EtFOSA	16.7	<mLOD
FOSAA	0	n.d.
MeFOSAA	0	n.d.
EtFOSAA	33.3	0.03 ± 0.02 (<mLOD – 0.05)
MeFOSE	0	n.d.
EtFOSE	0	n.d.
4:2 FTS	8.3	1.14 (<mLOD – 1.14)
6:2 FTS	58.3	0.78 ± 65.44 (0.17 – 173.50)
8:2 FTS	0	<mLOD
10:2 FTS	41.7	0.04 ± 0.03 (0.02 – 0.08)
Gen X	16.7	2.75 ± 0.42 (<mLOD – 3.06)
ADONA	16.7	0.03 ± 0.03 (<mLOD – 0.05)
9Cl-PF3ONS	8.3	0.15 (<mLOD – 0.15)
SAMPAP	0	n.d.
diSAMPAP	0	n.d.
6:6 PFPi	8.3	<mLOD
6:8 PFPi	0	n.d.
8:8 PFPi	0	n.d.

<mLOD: below the limit of detection. n.d. not detected. Note: DR% calculated based on values > mLOD.

Table A.2.8. Concentrations and detection rates (DR%) of the 17 steroids analyzed in plasma of grey seal pups sampled in 2022 (n=12). Concentrations are presented as the geometric mean (ng/mL) ± standard deviation (sd) with range in parentheses.

Target analytes	DR (%)	GM ± sd (range)
COR	100	29.49 ± 14.14 (11.87 – 62.59)
CORNE	100	23.23 ± 6.52 (15.21 – 33.18)
ALDO	0	n.d.
COS	50	4.79 ± 4.11 (<mLOD – 13.12)
11-deoxyCOR	100	6.37 ± 3.04 (3.99 – 15.60)
17a-OHP	100	4.03 ± 1.91 (0.53 – 7.14)
DOC	58.3	0.07 ± 0.03 (<mLOD – 0.14)
P4	100	0.40 ± 0.25 (0.16 – 0.98)
KetoTS	0	n.d.
PREG (P5)	8.3	6.02 (<mLOD – 6.02)
17aOPH5	41.2	14.99 ± 5.49 (<mLOD – 23.65)
DHT	0	n.d.
TS	8.3	0.15 (<mLOD – 0.15)
AN	50	12.08 ± 34.45 (<mLOD – 99.76)
E1	0	n.d.
A5	0	n.d.
DHEA	8.3	14.99 (<mLOD – 14.99)

3. Discussion

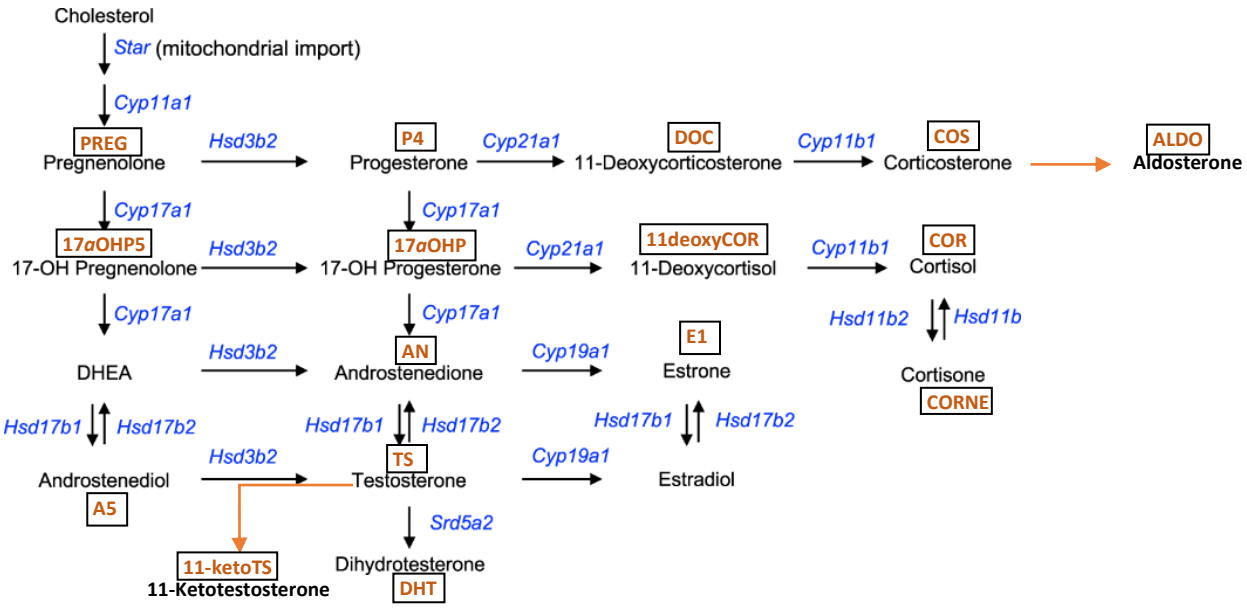


Figure A.3.1. Overview of steroidogenesis in mammals as retrieved from Chakraborty et al., 2021 with minor modifications from Turcu et al., 2020 and Bremer et Miller, 2014

Supplementary information

Table S.1. Concentrations (ng/mL) of the 46 analyzed PFASs in the plasma of each individual grey seal pups sampled from 1992 to 2022 (n=164)

Seal ID	DecaS	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTriDA	PFTDA	PFHxDA	PFOcDA	P37DMOA	7H-PFHpA	PFBS	PFPeS	PFHxS	PFHpS	PFOS
D6505	0,16	< LOD	1,26	< LOD	< LOD	0,93	0,64	< LOD	2,10	0,91	3,05	0,43	< LOD	n.d.	0,03	0,38	0,04	0,16	7,05	1,40	119,00
D6511	< LOD	n.d.	1,58	n.d.	0,25	2,40	1,89	< LOD	3,86	1,09	4,34	0,24	< LOD	0,07	n.d.	0,34	0,10	0,05	17,89	6,42	201,79
D6514	n.d.	n.d.	< LOD	n.d.	< LOD	0,34	1,07	2,09	6,60	1,73	5,27	0,49	< LOD	< LOD	0,07	0,12	0,07	0,14	6,39	2,48	189,63
D6515	0,20	n.d.	1,49	n.d.	0,49	0,58	0,93	< LOD	4,35	1,62	5,95	0,57	< LOD	< LOD	0,03	0,46	0,03	0,36	11,87	3,65	163,60
D6516	n.d.	n.d.	0,79	n.d.	< LOD	0,45	0,79	< LOD	2,52	1,08	4,26	0,45	n.d.	0,11	n.d.	0,12	0,04	0,09	11,58	3,41	123,88
D6525	< LOD	n.d.	1,49	n.d.	< LOD	1,32	0,91	< LOD	2,42	1,07	3,02	0,36	n.d.	< LOD	n.d.	0,35	< LOD	0,17	11,58	4,38	158,68
D6542	< LOD	n.d.	1,26	0,86	0,41	0,61	0,35	n.d.	2,29	0,85	3,02	0,41	< LOD	n.d.	0,03	0,26	n.d.	0,10	4,85	1,12	59,84
D6545	0,29	n.d.	1,87	0,27	0,36	0,43	0,35	< LOD	0,90	0,47	1,41	0,15	< LOD	0,07	0,03	0,23	0,02	0,39	2,48	1,31	38,80
D6546	< LOD	n.d.	1,53	< LOD	0,17	0,71	0,51	< LOD	1,72	0,68	2,53	0,13	< LOD	n.d.	0,03	0,40	0,06	0,09	9,79	1,91	100,31
D6553	0,13	n.d.	0,33	0,13	< LOD	0,41	0,59	< LOD	2,35	0,55	2,77	0,26	< LOD	n.d.	n.d.	0,08	< LOD	0,68	7,10	1,19	98,39
D6557	0,18	n.d.	0,50	0,59	0,26	0,54	0,32	< LOD	1,52	0,79	2,82	0,16	n.d.	n.d.	n.d.	0,57	0,01	0,29	3,30	1,14	46,30
D6560	n.d.	n.d.	0,86	n.d.	< LOD	0,95	0,39	< LOD	2,41	0,66	1,92	0,06	n.d.	n.d.	< LOD	0,40	0,06	0,29	6,77	1,34	94,14
D6561	0,14	< LOD	0,62	0,71	0,52	< LOD	0,34	< LOD	1,38	0,61	2,46	0,20	< LOD	n.d.	0,04	0,21	0,02	0,24	5,15	1,35	66,76
D6566	0,34	n.d.	1,50	0,88	0,32	1,61	0,67	< LOD	2,50	0,67	2,48	0,33	n.d.	n.d.	0,03	0,30	< LOD	0,29	14,89	2,70	127,98
D6568	0,13	n.d.	1,39	n.d.	n.d.	0,37	0,47	< LOD	1,02	0,47	1,23	0,08	n.d.	n.d.	0,04	0,17	0,06	0,45	8,99	1,14	81,47
D6569	0,37	n.d.	1,23	0,56	0,13	< LOD	0,67	< LOD	2,63	0,63	2,33	0,32	n.d.	n.d.	n.d.	0,17	0,07	0,07	5,70	2,06	107,81
D6570	0,21	n.d.	< LOD	n.d.	n.d.	0,54	0,32	< LOD	1,10	0,38	2,17	0,22	< LOD	n.d.	n.d.	0,31	0,03	0,19	8,68	2,11	80,30
D6572	0,31	n.d.	< LOD	0,35	0,19	0,79	0,29	< LOD	2,02	0,77	2,99	0,29	n.d.	n.d.	0,03	0,35	0,02	0,13	11,75	4,67	177,46

D6577	n.d.	2,33	3,07	n.d.	< LOD	1,68	0,98	< LOD	2,54	0,67	2,93	0,34	< LOD	< LOD	0,04	0,42	0,07	0,02	7,76	2,69	129,11
D6579	n.d.	n.d.	0,83	0,20	n.d.	0,30	0,45	< LOD	2,37	0,95	2,53	0,46	< LOD	n.d.	0,04	0,21	< LOD	0,27	8,89	1,98	92,80
D6582	< LOD	n.d.	1,69	n.d.	< LOD	0,66	0,53	n.d.	1,67	0,56	2,74	0,27	n.d.	n.d.	0,01	0,26	< LOD	0,28	5,67	1,97	75,26
D6586	< LOD	n.d.	0,43	n.d.	< LOD	0,91	0,53	< LOD	1,92	0,74	2,92	0,33	< LOD	n.d.	< LOD	0,11	0,04	0,08	4,63	1,01	61,41
D6592	n.d.	n.d.	1,38	n.d.	0,22	0,12	0,32	< LOD	1,66	0,52	2,43	0,09	n.d.	n.d.	0,04	0,07	0,04	0,15	4,22	0,88	51,57
D6599	0,24	n.d.	< LOD	0,39	0,21	0,29	0,19	< LOD	2,20	1,28	4,70	0,49	n.d.	n.d.	0,14	0,40	0,03	0,09	9,92	2,36	110,30
D6600	0,16	< LOD	0,32	< LOD	0,14	0,83	1,86	< LOD	4,13	0,87	2,35	0,19	n.d.	n.d.	0,11	0,15	0,05	0,47	21,74	7,39	373,30
D6605	0,47	n.d.	5,58	1,99	0,24	0,21	0,53	< LOD	2,32	0,48	3,09	0,42	n.d.	n.d.	n.d.	0,43	0,04	0,53	4,76	1,02	83,79
D6606	n.d.	n.d.	0,54	0,46	< LOD	1,66	0,42	< LOD	1,74	0,52	2,40	0,31	n.d.	0,07	n.d.	0,42	0,05	0,49	14,93	2,48	102,71
D6615	0,40	n.d.	1,56	1,15	0,17	0,88	0,62	< LOD	2,18	0,59	2,01	0,12	< LOD	0,10	n.d.	0,31	n.d.	0,34	9,95	3,07	98,64
D6616	0,17	n.d.	0,50	0,30	< LOD	1,52	0,51	< LOD	2,13	0,74	3,06	0,25	n.d.	n.d.	n.d.	0,28	0,03	0,03	10,10	2,16	109,86
D6617	n.d.	n.d.	0,50	0,22	n.d.	0,23	0,32	< LOD	1,70	0,62	2,06	0,23	n.d.	n.d.	0,01	0,28	< LOD	0,10	5,78	2,21	65,07
D6618	< LOD	< LOD	< LOD	0,80	n.d.	1,10	0,54	< LOD	1,47	0,53	2,31	0,32	< LOD	< LOD	0,03	0,29	< LOD	0,33	5,39	1,27	50,65
D6623	0,36	n.d.	1,46	0,46	n.d.	n.d.	0,14	< LOD	3,43	0,96	3,42	0,29	< LOD	n.d.	0,12	0,49	0,03	< LOD	8,84	4,42	202,79
D6624	< LOD	< LOD	0,32	n.d.	< LOD	0,78	0,17	< LOD	1,86	0,57	2,58	0,22	< LOD	n.d.	0,02	0,23	0,09	0,15	4,53	1,01	67,88
D6625	< LOD	n.d.	0,54	0,12	0,37	3,04	1,39	n.d.	3,68	1,40	4,70	0,44	n.d.	n.d.	n.d.	0,23	0,04	0,22	12,98	3,30	154,05
D6626	< LOD	n.d.	2,04	n.d.	n.d.	1,75	0,47	< LOD	1,79	0,57	1,95	0,21	n.d.	n.d.	n.d.	0,07	0,12	0,23	9,46	1,88	112,87
D6627	n.d.	n.d.	3,81	n.d.	< LOD	0,48	0,38	< LOD	1,40	0,65	2,12	0,18	< LOD	n.d.	0,02	0,56	n.d.	0,46	11,74	4,17	206,20
D6629	< LOD	n.d.	1,09	0,42	0,11	0,52	0,47	< LOD	1,56	0,46	2,72	0,30	< LOD	n.d.	0,04	0,24	0,06	0,23	3,89	1,30	57,23
D6671	n.d.	n.d.	1,04	< LOD	< LOD	0,75	0,44	< LOD	3,42	1,03	3,15	0,31	n.d.	n.d.	n.d.	< LOD	0,03	0,38	11,59	2,88	170,48
D6673	0,26	n.d.	1,60	0,37	< LOD	0,45	0,41	< LOD	1,65	0,62	2,25	0,22	n.d.	n.d.	0,06	0,15	0,08	0,30	5,24	1,33	47,61
D6674	0,48	2,54	1,63	0,55	< LOD	0,68	0,17	< LOD	1,77	0,53	3,11	0,44	n.d.	n.d.	n.d.	0,22	n.d.	0,38	13,13	3,73	107,56
D6675	0,12	n.d.	3,41	0,52	0,13	0,47	0,48	< LOD	2,52	0,74	2,21	0,14	< LOD	n.d.	0,07	0,19	< LOD	0,32	4,25	1,31	77,54

D6676	0,13	3,29	10,58	0,30	< LOD	< LOD	0,15	< LOD	2,04	0,68	2,70	0,21	0,13	n.d.	0,06	0,37	< LOD	0,20	8,86	2,79	143,61
D6677	0,17	n.d.	3,54	n.d.	n.d.	0,39	1,09	< LOD	3,95	1,43	4,41	0,56	< LOD	n.d.	< LOD	0,44	n.d.	0,41	22,88	6,73	262,25
E6546	0,32	n.d.	1,85	< LOD	0,40	0,56	0,70	< LOD	1,45	0,47	2,41	0,27	n.d.	n.d.	< LOD	0,20	n.d.	0,02	8,12	3,01	80,38
E6571	n.d.	n.d.	0,34	n.d.	0,35	0,51	0,21	n.d.	1,32	0,45	2,24	0,28	< LOD	n.d.	n.d.	0,44	n.d.	0,07	7,64	2,48	67,86
E6715	0,19	n.d.	30,39	0,22	< LOD	0,32	1,46	< LOD	2,54	0,86	2,70	0,26	n.d.	< LOD	n.d.	0,07	n.d.	0,05	15,13	4,87	162,63
E6718	< LOD	n.d.	17,01	n.d.	0,31	0,61	0,60	< LOD	1,68	0,74	2,21	0,28	< LOD	n.d.	n.d.	0,20	n.d.	0,40	5,83	1,24	60,89
E6724	< LOD	n.d.	10,45	< LOD	n.d.	0,42	1,12	< LOD	3,14	1,22	3,85	0,42	n.d.	n.d.	0,10	n.d.	n.d.	0,13	9,67	2,36	102,20
E6727	< LOD	n.d.	6,67	0,25	0,12	0,68	0,80	n.d.	2,93	0,93	4,21	0,61	n.d.	n.d.	0,03	0,28	n.d.	0,12	7,07	1,66	74,54
E6731	< LOD	n.d.	0,93	0,41	0,23	1,18	1,15	1,13	2,70	0,84	3,70	0,40	< LOD	n.d.	0,03	0,19	0,10	n.d.	11,14	2,36	99,94
E6732	< LOD	n.d.	0,87	< LOD	< LOD	0,94	0,43	< LOD	2,29	0,62	2,75	0,39	n.d.	n.d.	0,09	0,25	n.d.	0,14	4,28	1,21	65,44
E6739	0,33	n.d.	0,44	0,38	0,20	1,06	1,02	< LOD	2,91	0,85	2,99	0,20	n.d.	n.d.	0,06	< LOD	n.d.	< LOD	11,70	3,81	159,58
E6740	n.d.	n.d.	0,46	0,30	< LOD	< LOD	0,09	< LOD	2,09	0,45	1,86	0,23	< LOD	n.d.	0,04	0,22	n.d.	0,14	4,10	1,31	46,83
E6741	0,33	n.d.	0,59	0,51	0,27	0,57	0,49	< LOD	2,51	0,67	2,03	0,21	n.d.	n.d.	0,04	0,07	n.d.	0,05	6,90	2,53	110,44
E6751	< LOD	n.d.	0,54	< LOD	< LOD	1,35	0,44	< LOD	2,21	0,60	2,42	0,14	n.d.	n.d.	n.d.	0,13	0,08	0,07	7,27	1,83	84,59
E6752	0,12	n.d.	0,99	n.d.	< LOD	n.d.	0,53	< LOD	2,52	0,83	3,67	0,33	n.d.	n.d.	n.d.	0,10	n.d.	0,02	4,99	1,54	71,89
E6764	0,29	n.d.	1,48	0,84	0,29	1,33	0,54	< LOD	4,48	1,50	4,05	0,44	n.d.	n.d.	n.d.	0,07	0,04	0,37	19,72	4,15	212,05
E6780	0,22	n.d.	0,40	0,35	n.d.	0,59	0,48	1,41	2,13	0,55	1,85	0,45	n.d.	n.d.	< LOD	< LOD	< LOD	< LOD	5,41	1,17	48,06
E6786	n.d.	n.d.	0,44	0,21	0,42	0,96	1,42	< LOD	2,28	0,61	2,39	0,31	n.d.	n.d.	0,02	0,14	n.d.	0,38	14,36	3,44	134,44
E6787	0,16	n.d.	0,28	0,65	< LOD	0,26	0,97	< LOD	1,65	0,66	2,29	0,32	n.d.	0,13	0,01	0,10	0,19	0,23	18,75	3,47	118,95
E6788	n.d.	n.d.	0,75	0,39	0,53	2,34	1,61	1,38	8,07	1,81	5,14	0,34	< LOD	< LOD	0,14	0,34	0,04	0,21	20,20	6,27	405,53
E6789	0,32	n.d.	< LOD	0,29	< LOD	0,70	1,17	< LOD	4,61	1,36	5,39	0,50	< LOD	< LOD	n.d.	0,07	0,14	< LOD	8,00	1,89	114,92
E6790	0,24	< LOD	3,63	0,16	< LOD	0,64	0,53	< LOD	3,49	1,04	2,95	0,28	n.d.	n.d.	n.d.	0,29	0,07	0,33	10,24	3,14	205,04
E6792	< LOD	n.d.	1,08	0,38	0,14	0,83	0,87	< LOD	3,98	1,36	3,74	0,32	n.d.	n.d.	0,03	0,08	0,01	0,14	10,09	3,14	157,33

E6793	n.d.	n.d.	< LOD	0,70	0,36	1,08	0,43	1,44	1,88	0,47	2,68	0,25	n.d.	0,06	n.d.	< LOD	0,03	0,04	6,14	1,42	53,95
E6795	0,31	n.d.	0,27	n.d.	< LOD	0,81	0,46	n.d.	3,49	1,18	3,88	0,47	< LOD	n.d.	n.d.	0,23	< LOD	0,28	9,83	2,81	119,91
E6796	0,35	n.d.	0,46	0,27	n.d.	1,20	0,85	< LOD	3,41	0,75	2,86	0,29	n.d.	< LOD	< LOD	0,11	0,02	0,43	8,70	1,80	129,12
E6797	< LOD	n.d.	< LOD	1,83	< LOD	0,67	0,20	< LOD	1,49	0,68	2,12	0,21	n.d.	0,07	0,06	0,11	0,02	0,71	3,07	0,88	40,73
E9977	< LOD	n.d.	0,43	n.d.	< LOD	4,57	1,33	< LOD	3,86	1,37	3,41	0,31	< LOD	0,30	0,02	< LOD	0,04	0,42	14,57	2,47	167,30
E9981	< LOD	n.d.	0,28	n.d.	n.d.	0,32	0,61	n.d.	1,91	0,69	2,07	0,37	n.d.	< LOD	< LOD	< LOD	0,02	0,25	7,18	2,69	126,44
E9982	0,20	n.d.	0,65	0,57	< LOD	1,89	1,28	< LOD	4,37	0,96	3,40	0,50	< LOD	< LOD	0,08	0,13	0,01	0,39	9,49	2,02	139,36
E9984	0,42	n.d.	0,25	0,92	< LOD	0,77	0,56	< LOD	2,74	0,81	3,20	0,37	n.d.	0,09	0,16	0,10	0,05	0,12	8,85	2,47	120,84
E9986	0,13	n.d.	1,11	0,17	0,31	0,78	0,30	< LOD	1,99	0,80	3,02	0,31	n.d.	n.d.	< LOD	0,34	< LOD	0,42	9,49	2,09	77,24
E9988	< LOD	n.d.	3,27	0,62	< LOD	0,97	0,44	n.d.	2,22	0,73	2,16	0,26	n.d.	< LOD	n.d.	0,28	0,05	0,26	12,10	3,51	127,73
E9989	0,54	n.d.	1,00	n.d.	n.d.	< LOD	0,59	n.d.	1,99	0,62	2,64	0,24	< LOD	0,08	0,04	< LOD	0,04	0,17	11,42	3,04	123,70
E9990	0,59	n.d.	0,96	n.d.	0,11	0,38	0,72	< LOD	2,37	0,51	2,48	0,33	n.d.	0,11	< LOD	0,07	n.d.	0,39	9,06	3,30	137,94
E9991	< LOD	n.d.	2,76	0,51	n.d.	n.d.	0,33	< LOD	1,26	0,63	2,31	0,25	< LOD	n.d.	n.d.	0,18	< LOD	0,29	3,95	0,76	53,44
E9992	0,19	n.d.	1,79	0,77	n.d.	n.d.	0,50	n.d.	1,70	0,75	2,81	0,23	n.d.	n.d.	n.d.	0,13	0,07	0,32	1,81	0,47	34,95
E9993	0,35	n.d.	< LOD	0,77	< LOD	1,97	1,13	< LOD	2,34	0,83	3,17	0,37	n.d.	n.d.	0,06	0,18	n.d.	0,23	16,60	2,25	89,12
E9994	0,72	< LOD	0,40	n.d.	< LOD	0,35	0,34	< LOD	0,99	0,47	1,25	0,13	n.d.	n.d.	0,01	< LOD	n.d.	0,23	13,12	3,31	116,21
E9995	0,71	n.d.	< LOD	n.d.	0,16	0,53	0,75	< LOD	2,10	0,50	2,75	0,08	< LOD	< LOD	n.d.	0,23	0,07	0,42	18,59	5,21	194,83
E9996	0,19	n.d.	< LOD	0,39	< LOD	1,51	1,08	< LOD	2,23	0,62	2,64	0,37	n.d.	n.d.	0,02	< LOD	0,01	0,04	15,77	4,90	155,44
E9997	0,28	n.d.	1,29	n.d.	n.d.	0,96	0,54	< LOD	2,67	0,95	3,19	0,25	n.d.	0,20	0,04	0,07	n.d.	0,38	16,81	3,18	147,38
E9998	0,27	< LOD	5,54	0,23	< LOD	1,07	0,44	< LOD	1,14	0,81	3,14	0,37	< LOD	n.d.	0,01	< LOD	n.d.	0,48	11,72	2,92	89,59
E9999	0,50	n.d.	0,57	0,39	n.d.	0,33	0,60	< LOD	2,23	0,63	1,70	0,25	< LOD	< LOD	n.d.	< LOD	n.d.	0,43	10,00	1,20	94,64
N000	< LOD	n.d.	0,52	1,53	< LOD	< LOD	0,19	< LOD	1,16	0,45	2,05	0,12	n.d.	0,10	n.d.	0,11	0,03	0,20	16,49	4,93	99,17
D6681	0,13	n.d.	1,13	n.d.	< LOD	0,35	0,26	< LOD	2,10	0,88	4,06	0,33	n.d.	0,08	n.d.	< LOD	n.d.	0,24	10,24	2,41	117,26

D6684	0,22	n.d.	0,78	n.d.	< LOD	0,30	0,47	n.d.	1,31	0,59	1,38	0,13	< LOD	< LOD	n.d.	< LOD	n.d.	0,38	7,55	2,35	72,86
D6685	0,12	< LOD	1,61	0,14	< LOD	n.d.	0,47	< LOD	1,44	0,48	1,81	0,13	< LOD	0,22	n.d.	0,09	n.d.	0,18	8,08	3,03	120,60
D6686	0,35	n.d.	1,39	0,37	< LOD	< LOD	0,36	n.d.	2,39	1,21	4,79	0,55	n.d.	0,12	< LOD	< LOD	n.d.	0,36	11,00	2,42	118,75
D6687	0,41	n.d.	4,46	1,48	0,15	0,68	0,83	< LOD	2,58	1,04	4,82	0,66	< LOD	0,17	n.d.	n.d.	0,04	0,12	9,53	3,26	191,40
D6688	0,22	n.d.	0,74	0,12	n.d.	0,14	0,29	< LOD	1,86	0,64	2,46	0,17	n.d.	< LOD	0,05	0,18	n.d.	0,31	10,87	3,50	122,05
D6690	0,60	n.d.	1,44	0,54	< LOD	0,88	0,78	< LOD	3,94	1,25	3,80	0,35	n.d.	n.d.	< LOD	0,09	n.d.	0,15	13,59	4,51	256,45
D6691	0,31	n.d.	1,13	0,27	< LOD	4,04	1,31	< LOD	4,37	1,41	3,93	0,33	< LOD	n.d.	0,06	0,15	n.d.	0,26	25,76	9,20	328,98
D6692	0,33	n.d.	0,23	0,27	0,38	1,41	0,76	< LOD	4,11	1,27	4,88	0,45	< LOD	n.d.	0,02	0,08	n.d.	0,16	14,06	4,49	199,74
D6693	0,48	n.d.	0,33	n.d.	< LOD	0,14	< LOD	n.d.	1,30	0,55	2,13	0,09	< LOD	0,10	n.d.	0,17	n.d.	0,28	10,44	2,64	68,49
E3013	0,14	n.d.	0,61	1,39	< LOD	1,88	1,84	< LOD	4,07	0,95	3,49	0,39	n.d.	< LOD	0,08	< LOD	n.d.	0,45	13,79	5,03	147,32
E3621	0,26	n.d.	0,54	< LOD	< LOD	0,29	0,44	n.d.	2,47	0,82	2,94	0,34	n.d.	n.d.	0,03	< LOD	n.d.	0,20	14,29	2,22	127,51
E3624	< LOD	n.d.	1,79	0,32	< LOD	0,48	0,95	< LOD	2,35	0,72	3,63	0,40	n.d.	0,08	n.d.	< LOD	n.d.	0,13	4,99	1,61	64,71
E3644	0,12	n.d.	0,56	0,62	0,12	0,84	0,38	< LOD	1,72	0,47	1,69	0,32	n.d.	< LOD	n.d.	0,12	n.d.	0,02	6,08	0,62	49,51
E3645	0,14	n.d.	1,46	1,63	< LOD	0,23	0,63	< LOD	1,46	0,42	1,95	0,11	n.d.	n.d.	0,02	< LOD	0,02	0,61	8,80	1,44	81,98
E3660	0,18	n.d.	< LOD	0,25	n.d.	1,51	1,07	< LOD	5,75	1,70	5,74	0,51	n.d.	n.d.	0,05	< LOD	0,05	0,22	16,46	5,69	272,67
E3673	n.d.	n.d.	1,72	1,45	0,41	0,11	0,57	< LOD	2,99	0,73	3,06	0,29	< LOD	n.d.	0,04	< LOD	0,02	0,47	5,41	1,93	93,09
E3676	< LOD	n.d.	1,14	n.d.	n.d.	0,86	0,85	< LOD	4,10	1,19	2,98	0,31	< LOD	n.d.	0,08	< LOD	< LOD	0,24	8,24	2,45	116,52
E3690	< LOD	n.d.	1,07	0,50	< LOD	0,57	0,49	< LOD	3,64	1,24	4,16	0,38	n.d.	n.d.	0,02	< LOD	0,03	0,23	7,44	1,99	147,88
E3709	< LOD	n.d.	0,51	n.d.	< LOD	0,57	0,37	< LOD	2,29	0,68	2,22	0,24	n.d.	n.d.	0,04	0,09	0,04	0,73	13,17	3,70	204,31
E3710	< LOD	n.d.	0,47	< LOD	< LOD	0,88	0,95	< LOD	4,85	1,35	4,04	0,32	n.d.	n.d.	0,01	n.d.	< LOD	0,16	10,02	3,58	195,46
E3711	< LOD	n.d.	< LOD	0,72	n.d.	0,72	1,04	< LOD	3,73	0,54	1,65	0,06	n.d.	n.d.	0,01	n.d.	0,03	0,03	13,17	3,75	133,60
E3716	0,23	n.d.	< LOD	0,51	< LOD	0,76	0,41	n.d.	1,96	0,34	2,07	0,19	n.d.	n.d.	n.d.	< LOD		0,35	7,21	1,44	75,20
E3722	0,32	n.d.	0,24	0,87	< LOD	0,17	0,59	< LOD	2,94	0,67	2,54	0,17	n.d.	n.d.	0,03	< LOD	0,03	0,30	10,43	2,18	143,99

E3723	0,21	n.d.	< LOD	0,18	< LOD	0,66	0,68	< LOD	1,09	0,21	1,01	0,14	< LOD	0,12	n.d.	n.d.	0,06	0,36	5,86	1,13	53,58
E3742	0,39	< LOD	0,82	0,39	0,46	0,48	0,82	< LOD	2,26	0,70	2,10	0,17	n.d.	n.d.	n.d.	n.d.	0,01	0,28	9,28	2,93	103,42
E3791	< LOD	n.d.	< LOD	0,96	< LOD	0,38	0,37	< LOD	3,04	1,02	4,59	0,48	n.d.	n.d.	0,03	< LOD	0,06	0,10	9,04	2,53	126,39
E6390	< LOD	< LOD	< LOD	< LOD	n.d.	0,83	0,34	n.d.	2,80	0,97	3,32	0,41	n.d.	n.d.	0,05	0,09	< LOD	0,32	6,32	2,22	138,07
2000_01	0,49	< LOD	1,18	0,44	0,13	0,62	1,28	1,37	5,48	1,59	5,07	0,44	n.d.	n.d.	0,04	0,33	0,03	0,15	10,89	3,87	194,41
2000_02	0,19	n.d.	< LOD	0,71	< LOD	0,76	1,10	< LOD	3,86	1,19	4,36	0,30	n.d.	n.d.	0,08	0,06	0,07	0,37	26,04	6,47	250,42
2000_03	0,36	n.d.	0,61	n.d.	n.d.	1,11	2,26	2,00	9,58	2,39	7,82	0,84	< LOD	< LOD	n.d.	< LOD	0,07	0,33	15,70	4,72	222,38
2000_04	0,17	n.d.	0,54	0,26	< LOD	2,02	1,43	< LOD	6,70	2,28	6,76	0,72	n.d.	n.d.	n.d.	n.d.	0,05	0,47	14,97	4,40	197,52
2000_06	n.d.	n.d.	< LOD	0,58	n.d.	4,20	1,06	< LOD	5,94	1,45	4,58	0,56	n.d.	n.d.	0,07	n.d.	0,03	0,88	22,05	3,83	247,76
2000_08	< LOD	n.d.	< LOD	n.d.	n.d.	0,55	0,80	< LOD	3,93	1,00	3,85	0,27	n.d.	< LOD	0,03	n.d.	< LOD	0,35	9,60	3,56	156,63
2005_01	< LOD	n.d.	< LOD	n.d.	< LOD	0,74	1,04	1,61	4,66	1,08	3,83	0,39	n.d.	n.d.	0,06	< LOD	0,14	0,15	12,29	3,39	111,62
2005_02	n.d.	n.d.	0,32	0,30	< LOD	0,59	1,29	< LOD	4,85	1,27	4,93	0,43	< LOD	n.d.	0,13	n.d.	0,05	0,08	8,00	2,96	75,84
2005_03	< LOD	n.d.	1,88	n.d.	0,41	1,09	2,72	< LOD	8,53	2,12	6,81	0,47	n.d.	n.d.	n.d.	< LOD	0,04	0,20	12,03	3,28	169,92
2005_04	< LOD	n.d.	0,53	n.d.	0,22	1,98	4,50	4,92	11,84	2,48	6,35	0,51	n.d.	n.d.	0,23	n.d.	n.d.	0,08	12,91	4,63	267,53
2005_06	n.d.	14,81	1,38	n.d.	0,38	0,32	2,17	1,34	11,80	3,15	10,65	0,85	< LOD	n.d.	n.d.	< LOD	0,03	0,23	7,83	2,38	124,57
2005_07	< LOD	< LOD	0,55	0,26	0,79	0,61	3,37	1,83	9,84	2,33	6,56	0,61	n.d.	0,07	0,04	0,09	n.d.	0,07	10,82	3,03	132,14
2005_08	< LOD	< LOD	< LOD	n.d.	< LOD	0,91	2,04	< LOD	5,82	2,15	6,77	0,51	n.d.	n.d.	0,10	< LOD	0,08	0,51	12,47	1,86	95,81
2005_09	0,11	n.d.	1,73	< LOD	< LOD	2,59	3,87	2,86	9,12	2,25	6,39	0,57	< LOD	n.d.	n.d.	< LOD	0,01	1,30	29,87	3,27	189,84
2005_10	n.d.	< LOD	< LOD	0,91	0,52	0,92	4,76	2,31	14,94	4,15	11,57	0,95	< LOD	n.d.	n.d.	< LOD	< LOD	0,16	12,94	3,17	261,64
2005_11	n.d.	n.d.	< LOD	0,65	0,13	1,41	2,76	4,27	15,03	4,27	13,16	0,88	n.d.	n.d.	n.d.	0,60	0,05	0,02	9,67	2,65	166,41
2005_12	< LOD	n.d.	< LOD	0,15	< LOD	0,30	1,75	< LOD	5,16	1,55	4,43	0,41	< LOD	n.d.	0,09	< LOD	0,02	0,34	11,76	2,30	90,45
2005_13	0,42	n.d.	< LOD	0,23	0,26	1,16	3,73	1,78	8,81	1,84	7,43	0,61	n.d.	n.d.	0,10	< LOD	0,05	0,06	13,09	4,08	142,16
2005_14	< LOD	n.d.	< LOD	1,04	< LOD	1,23	3,87	3,80	11,01	3,15	9,25	1,17	n.d.	n.d.	0,20	< LOD	0,10	0,57	49,33	7,79	327,67

2005_15	0,29	n.d.	0,36	0,30	< LOD	0,19	0,71	< LOD	4,35	1,20	4,38	0,44	n.d.	n.d.	n.d.	< LOD	0,02	0,08	5,28	1,46	62,26
2005_16	< LOD	n.d.	1,72	n.d.	0,19	1,54	4,02	2,39	11,52	2,54	9,61	0,88	n.d.	n.d.	0,16	< LOD	0,04	0,19	11,25	3,60	161,49
2005_17	n.d.	n.d.	< LOD	< LOD	0,18	0,55	1,71	3,12	11,81	2,79	8,67	0,51	n.d.	n.d.	0,12	< LOD	0,07	0,07	17,11	5,40	214,85
2005_19	< LOD	n.d.	0,55	0,67	< LOD	0,30	1,36	< LOD	7,78	2,12	8,19	0,58	< LOD	n.d.	0,07	0,08	0,06	0,06	6,83	1,46	70,20
2005_20	n.d.	n.d.	0,23	n.d.	0,17	0,43	1,37	1,57	7,75	2,19	6,91	0,71	n.d.	n.d.	0,15	< LOD	< LOD	0,13	6,15	1,82	92,60
2005_21	< LOD	n.d.	n.d.	0,28	< LOD	0,22	2,00	1,28	10,59	2,74	9,89	0,96	n.d.	n.d.	0,11	n.d.	0,07	0,04	8,88	2,69	138,33
2020_01	< LOD	8,31	0,31	n.d.	n.d.	0,12	1,43	< LOD	5,84	1,22	3,92	0,51	< LOD	n.d.	n.d.	n.d.	0,05	0,13	1,74	0,25	28,37
2020_02	0,24	n.d.	1,79	< LOD	< LOD	0,34	4,64	4,95	8,12	2,95	6,18	0,94	n.d.	n.d.	n.d.	< LOD	0,13	0,21	2,29	0,71	73,16
2020_03	n.d.	n.d.	0,63	n.d.	0,18	0,42	2,13	1,44	7,54	1,95	4,60	0,43	< LOD	n.d.	0,04	0,08	0,04	0,04	3,23	0,59	54,83
2020_04	0,14	n.d.	0,48	0,29	< LOD	< LOD	1,03	< LOD	5,61	1,83	4,65	0,57	< LOD	< LOD	0,04	< LOD	0,09	0,02	2,16	0,43	43,66
2020_05	< LOD	7,23	7,03	1,63	< LOD	0,59	0,80	< LOD	2,58	0,83	2,44	0,20	n.d.	n.d.	n.d.	< LOD	< LOD	1,66	8,09	1,48	61,15
2020_06	0,19	2,70	0,41	0,58	n.d.	< LOD	2,71	1,61	4,36	0,80	2,64	0,34	n.d.	n.d.	0,03	< LOD	0,10	0,80	3,32	0,65	37,10
2020_07	< LOD	< LOD	< LOD	n.d.	0,15	< LOD	1,00	< LOD	4,52	1,28	3,92	0,51	n.d.	n.d.	0,09	2,14	0,01	0,01	2,94	0,54	39,11
2020_08	0,19	n.d.	1,56	n.d.	< LOD	0,18	1,50	< LOD	3,58	1,30	3,75	0,43	< LOD	n.d.	n.d.	< LOD	0,08	0,13	2,48	0,41	27,69
2020_09	< LOD	n.d.	2,13	0,37	< LOD	< LOD	0,37	2,39	2,75	0,58	1,71	0,25	n.d.	n.d.	0,05	< LOD	0,06	0,08	1,07	0,27	19,30
2020_10	< LOD	n.d.	0,81	n.d.	n.d.	0,17	2,07	< LOD	7,32	2,45	5,21	0,53	n.d.	< LOD	0,14	n.d.	0,15	0,17	4,83	1,42	55,78
2020_11	0,13	n.d.	5,99	n.d.	< LOD	0,30	1,92	< LOD	4,80	1,29	3,98	0,43	< LOD	n.d.	n.d.	0,07	0,04	0,27	2,67	0,34	30,92
2020_12	0,15	n.d.	0,37	n.d.	0,32	0,80	5,84	3,55	12,39	3,31	8,57	1,04	< LOD	n.d.	n.d.	< LOD	0,03	0,02	3,28	0,54	91,18
2020_14	< LOD	n.d.	0,83	n.d.	0,19	0,33	1,59	< LOD	6,15	2,51	4,81	0,54	< LOD	n.d.	0,09	0,30	0,08	0,21	5,11	1,26	59,02
F_22_01	<LOD	n.d.	n.d.	n.d.	n.d.	0,62	2,64	3,78	9,79	2,57	6,32	0,99	n.d.	<LOD	n.d.	n.d.	<LOD	< LOD	3,44	1,17	56,37
F_22_02	0,42	n.d.	n.d.	n.d.	n.d.	1,18	4,47	2,28	6,42	1,36	4,35	0,96	n.d.	<LOD	n.d.	<LOD	<LOD	0,19	2,12	0,45	26,76
F_22_03	<LOD	n.d.	n.d.	n.d.	<LOD	0,55	3,89	2,88	7,44	2,55	4,25	1,73	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	7,86	1,85	89,50
F_22_04	0,05	17,13	n.d.	0,41	n.d.	0,13	3,77	2,59	7,15	2,14	4,32	0,87	n.d.	<LOD	n.d.	n.d.	n.d.	0,18	10,48	0,98	63,75

F_22_05	0,25	71,14	< LOD	0,82	0,82	0,58	3,83	5,35	17,97	4,29	9,36	2,37	n.d.	n.d.	n.d.	<LOD	<LOD	< LOD	3,98	1,59	73,16
F_22_06	0,06	n.d.	n.d.	n.d.	<LOD	0,33	3,10	2,94	5,55	1,42	2,79	0,59	n.d.	n.d.	n.d.	n.d.	n.d.	0,07	3,12	0,65	34,41
F_22_07	<LOD	28,25	n.d.	0,30	n.d.	0,29	2,45	1,81	5,32	1,32	3,20	0,86	n.d.	n.d.	n.d.	<LOD	0,09	0,07	4,99	0,88	45,22
F_22_08	<LOD	7,88	n.d.	0,72	<LOD	0,55	3,87	2,49	7,88	1,79	4,13	1,06	<LOD	n.d.	n.d.	n.d.	n.d.	0,10	3,37	0,41	38,06
F_22_09	<LOD	n.d.	0,89	n.d.	n.d.	0,30	1,50	1,62	5,61	2,21	4,64	1,01	n.d.	n.d.	n.d.	n.d.	0,06	< LOD	3,43	0,63	32,49
F_22_10	<LOD	n.d.	0,23	n.d.	n.d.	0,68	2,45	1,13	6,52	2,41	4,09	0,97	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,83	0,32	34,40
F_22_11	n.d.	n.d.	n.d.	n.d.	<LOD	0,74	6,26	2,39	5,28	1,36	3,32	0,76	n.d.	<LOD	n.d.	n.d.	n.d.	n.d.	2,06	0,47	36,14
F_22_12	n.d.	n.d.	0,31	0,67	n.d.	0,76	4,06	2,84	12,37	3,90	7,94	1,99	n.d.	n.d.	n.d.	n.d.	<LOD	0,15	4,68	0,62	45,66

<mLOD: below the limit of detection. n.d. not detected.

Table S.1. (Suite)

Seal ID	PFNS	PFDS	PFDoDS	PFECHS	4:2 FTS	6:2 FTS	8:2 FTS	10:2 FTS	FOSAA	MeFOSAA	EtFOSAA	PFOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	GenX	ADONA	9Cl-PF3ONS	SAMPAP	diSAMPAP
D6505	0,29	0,57	0,29	0,11	n.d.	< LOD	n.d.	0,07	< LOD	0,95	n.d.	0,05	< LOD	< LOD	n.d.	< LOD	4,13	n.d.	n.d.	10769,40	6,00
D6511	0,09	0,41	0,10	0,37	n.d.	n.d.	< LOD	n.d.	< LOD	0,60	0,08	0,02	n.d.	0,05	n.d.	< LOD	n.d.	n.d.	< LOD	11448,14	3,73
D6514	0,60	0,77	0,29	0,16	n.d.	n.d.	n.d.	0,11	< LOD	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.	7,29	n.d.	n.d.	9644,06	0,12
D6515	0,30	0,50	0,32	0,32	n.d.	n.d.	n.d.	0,05	< LOD	0,32	< LOD	0,11	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.	3535,53	n.d.
D6516	0,44	0,38	0,28	0,26	< LOD	< LOD	n.d.	0,04	< LOD	n.d.	n.d.	0,04	n.d.	n.d.	n.d.	< LOD	0,24	n.d.	n.d.	3198,74	n.d.
D6525	0,54	0,46	0,15	0,30	< LOD	< LOD	n.d.	0,04	< LOD	0,05	n.d.	0,02	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.	3350,00	n.d.
D6542	0,09	0,16	< LOD	0,01	< LOD	n.d.	n.d.	0,05	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	0,84	< LOD	< LOD	1987,40	n.d.
D6545	0,04	0,11	0,06	0,06	< LOD	0,11	n.d.	< LOD	< LOD	n.d.	< LOD	0,27	n.d.	n.d.	n.d.	n.d.	2,19	< LOD	n.d.	1855,11	n.d.
D6546	0,23	0,18	0,11	0,08	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	< LOD	6369,90	n.d.
D6553	0,16	0,11	0,08	0,07	< LOD	< LOD	n.d.	< LOD	< LOD	n.d.	0,04	< LOD	n.d.	< LOD	n.d.	< LOD	1,69	< LOD	< LOD	3523,55	n.d.

D6557	0,18	0,10	0,08	0,17	n.d.	< LOD	n.d.	< LOD	< LOD	0,10	0,06	0,02	n.d.	< LOD	n.d.	< LOD	2,81	< LOD	n.d.	2378,01	n.d.
D6560	0,09	0,15	< LOD	0,08	n.d.	n.d.	n.d.	n.d.	< LOD	0,25	n.d.	0,03	n.d.	0,05	n.d.	n.d.	1,04	n.d.	n.d.	1690,74	n.d.
D6561	0,11	0,04	0,24	0,07	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	< LOD	n.d.	< LOD	0,01	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
D6566	0,19	0,30	0,10	0,29	< LOD	< LOD	n.d.	< LOD	< LOD	< LOD	< LOD	0,04	n.d.	n.d.	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	n.d.
D6568	0,16	0,11	0,09	0,13	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	0,03	0,03	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	2618,85	n.d.
D6569	0,31	0,10	< LOD	0,29	0,05	45,16	0,13	0,03	< LOD	n.d.	n.d.	0,01	n.d.	< LOD	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	n.d.
D6570	0,07	0,13	< LOD	0,26	n.d.	n.d.	n.d.	0,07	< LOD	n.d.	n.d.	0,05	n.d.	0,01	n.d.	n.d.	4,54	< LOD	n.d.	n.d.	n.d.
D6572	0,16	0,20	0,18	0,29	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.	0,53	n.d.	0,02	n.d.	< LOD	0,70	0,01	n.d.	n.d.	n.d.
D6577	0,43	0,33	0,17	0,23	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	< LOD	0,08	n.d.	0,02	n.d.	n.d.	0,87	n.d.	n.d.	n.d.	n.d.
D6579	0,27	0,29	< LOD	0,28	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	n.d.	0,05	n.d.	n.d.	n.d.	n.d.	2,47	< LOD	n.d.	n.d.	n.d.
D6582	0,23	0,13	0,06	0,17	n.d.	n.d.	n.d.	0,07	< LOD	0,77	n.d.	0,04	n.d.	< LOD	n.d.	n.d.	0,98	n.d.	< LOD	207,41	4,92
D6586	0,08	0,06	0,09	0,05	< LOD	n.d.	n.d.	< LOD	< LOD	n.d.	< LOD	0,03	n.d.	0,03	n.d.	< LOD	0,52	< LOD	n.d.	n.d.	n.d.
D6592	0,13	0,11	< LOD	0,09	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.	1,68	n.d.	n.d.	n.d.	n.d.
D6599	0,61	0,43	0,14	0,22	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	< LOD	0,05	n.d.	< LOD	n.d.	n.d.	1,28	n.d.	n.d.	947,18	n.d.
D6600	1,24	0,64	0,13	0,54	< LOD	< LOD	n.d.	0,07	< LOD	n.d.	n.d.	0,02	< LOD	0,02	n.d.	n.d.	1,38	n.d.	n.d.	472,18	n.d.
D6605	0,19	0,17	0,16	0,07	< LOD	< LOD	n.d.	0,08	< LOD	n.d.	0,02	< LOD	n.d.	0,04	n.d.	< LOD	5,78	0,03	n.d.	n.d.	n.d.
D6606	0,15	0,28	0,16	0,22	< LOD	n.d.	n.d.	0,07	< LOD	n.d.	< LOD	0,01	< LOD	n.d.	n.d.	n.d.	2,34	n.d.	< LOD	n.d.	n.d.
D6615	0,04	0,19	0,08	0,15	n.d.	n.d.	< LOD	0,06	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
D6616	0,29	0,30	0,34	0,18	< LOD	< LOD	< LOD	0,04	< LOD	n.d.	< LOD	0,04	n.d.	0,02	n.d.	< LOD	1,02	< LOD	n.d.	n.d.	n.d.
D6617	0,18	0,10	< LOD	0,12	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	0,02	0,02	n.d.	0,03	n.d.	< LOD	0,40	< LOD	n.d.	n.d.	n.d.
D6618	0,12	0,16	0,10	0,05	< LOD	n.d.	n.d.	< LOD	< LOD	0,59	0,03	0,05	< LOD	0,04	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	2,60
D6623	0,47	0,45	0,15	0,26	0,03	0,38	n.d.	n.d.	< LOD	n.d.	0,05	0,03	< LOD	0,02	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
D6624	0,16	0,25	0,10	n.d.	< LOD	n.d.	n.d.	0,05	< LOD	0,11	< LOD	0,02	n.d.	0,03	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.
D6625	0,48	0,49	0,62	0,20	n.d.	2,05	n.d.	0,03	< LOD	n.d.	0,03	0,03	n.d.	0,02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D6626	0,28	0,28	0,28	0,09	< LOD	1,38	n.d.	0,03	< LOD	n.d.	< LOD	0,05	n.d.	< LOD	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
D6627	0,57	0,35	0,12	0,17	< LOD	n.d.	n.d.	0,12	< LOD	n.d.	0,06	0,08	n.d.	0,08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D6629	0,32	0,23	0,16	0,09	< LOD	0,81	n.d.	0,02	< LOD	n.d.	n.d.	0,06	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.
D6671	0,50	1,00	0,42	0,21	n.d.	1,59	n.d.	0,06	< LOD	n.d.	< LOD	0,05	n.d.	0,02	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
D6673	< LOD	0,11	< LOD	0,09	0,03	1,52	< LOD	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	0,04	n.d.	< LOD	0,57	< LOD	n.d.	n.d.	n.d.
D6674	0,30	0,37	0,26	0,51	< LOD	0,11	n.d.	0,06	< LOD	n.d.	< LOD	0,10	n.d.	0,03	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.

D6675	0,16	0,24	0,15	0,12	n.d.	n.d.	n.d.	0,03	< LOD	0,62	0,06	0,03	n.d.	n.d.	n.d.	< LOD	n.d.	< LOD	< LOD	881,61	3,73
D6676	0,47	0,57	0,16	0,21	0,08	n.d.	< LOD	0,05	< LOD	n.d.	0,44	0,04	n.d.	0,29	n.d.	< LOD	n.d.	0,03	< LOD	n.d.	0,14
D6677	0,64	0,73	0,21	0,55	n.d.	n.d.	n.d.	0,06	< LOD	0,24	0,02	0,06	< LOD	0,04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
E6546	0,19	0,13	< LOD	0,15	< LOD	1,46	n.d.	0,03	< LOD	n.d.	n.d.	0,02	n.d.	< LOD	n.d.	< LOD	0,33	n.d.	< LOD	n.d.	n.d.
E6571	0,30	0,16	0,14	0,06	n.d.	n.d.	n.d.	0,04	< LOD	n.d.	< LOD	0,11	n.d.	n.d.	n.d.	< LOD	0,94	n.d.	< LOD	n.d.	n.d.
E6715	0,17	0,24	< LOD	0,55	n.d.	1,61	< LOD	0,08	< LOD	n.d.	< LOD	0,08	< LOD	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	n.d.	n.d.
E6718	0,10	0,11	0,09	0,02	0,03	< LOD	n.d.	< LOD	< LOD	n.d.	n.d.	0,17	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.
E6724	0,34	0,10	0,06	0,23	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	< LOD	0,03	n.d.	n.d.	n.d.	n.d.	1,38	n.d.	n.d.	n.d.	n.d.
E6727	0,08	0,20	0,08	0,11	0,05	n.d.	n.d.	< LOD	< LOD	n.d.	< LOD	0,02	< LOD	n.d.	n.d.	n.d.	4,72	n.d.	< LOD	n.d.	n.d.
E6731	0,20	0,18	< LOD	0,26	< LOD	n.d.	n.d.	0,05	< LOD	n.d.	< LOD	0,02	n.d.	0,01	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
E6732	0,06	0,15	0,07	0,06	n.d.	< LOD	< LOD	< LOD	< LOD	0,80	< LOD	0,03	< LOD	< LOD	n.d.	< LOD	n.d.	0,01	n.d.	n.d.	n.d.
E6739	0,49	0,31	0,27	0,35	n.d.	< LOD	< LOD	< LOD	< LOD	n.d.	0,04	0,04	n.d.	< LOD	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
E6740	0,13	0,17	< LOD	< LOD	< LOD	< LOD	n.d.	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	0,04	n.d.	n.d.	0,36	n.d.	< LOD	n.d.	n.d.
E6741	0,06	0,14	< LOD	0,23	< LOD	1,16	< LOD	0,06	< LOD	n.d.	n.d.	0,05	n.d.	n.d.	n.d.	n.d.	6,73	< LOD	n.d.	n.d.	n.d.
E6751	0,27	0,16	0,11	0,10	< LOD	3,49	n.d.	0,03	< LOD	n.d.	< LOD	0,04	n.d.	< LOD	n.d.	n.d.	2,39	< LOD	n.d.	n.d.	n.d.
E6752	0,28	0,18	0,13	0,14	< LOD	0,22	n.d.	0,06	< LOD	n.d.	n.d.	n.d.	n.d.	< LOD	n.d.	< LOD	0,24	n.d.	n.d.	n.d.	n.d.
E6764	1,00	0,44	0,21	0,23	< LOD	3,93	n.d.	< LOD	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	1,89	n.d.	< LOD	n.d.	n.d.
E6780	< LOD	0,24	0,16	0,04	n.d.	8,58	< LOD	< LOD	< LOD	n.d.	< LOD	0,04	n.d.	0,03	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
E6786	0,48	0,31	0,32	0,27	n.d.	19,51	0,06	0,09	< LOD	n.d.	n.d.	0,02	n.d.	0,02	n.d.	< LOD	n.d.	0,02	n.d.	n.d.	n.d.
E6787	0,14	0,31	0,14	0,38	0,06	23,17	n.d.	0,03	< LOD	n.d.	n.d.	0,02	n.d.	0,02	n.d.	n.d.	3,08	0,02	n.d.	n.d.	n.d.
E6788	0,72	1,16	0,23	0,66	n.d.	< LOD	n.d.	0,12	< LOD	2,44	< LOD	0,07	n.d.	< LOD	n.d.	n.d.	3,02	n.d.	< LOD	n.d.	6,11
E6789	0,33	0,49	0,11	0,23	n.d.	< LOD	n.d.	0,03	< LOD	n.d.	< LOD	< LOD	< LOD	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	0,55
E6790	0,20	0,45	0,40	0,27	< LOD	0,16	n.d.	0,07	< LOD	n.d.	< LOD	0,47	n.d.	0,03	n.d.	< LOD	7,94	< LOD	n.d.	n.d.	n.d.
E6792	0,31	0,43	0,30	0,15	n.d.	4,13	< LOD	0,06	< LOD	n.d.	< LOD	0,06	n.d.	n.d.	n.d.	< LOD	2,76	n.d.	n.d.	n.d.	n.d.
E6793	0,10	0,12	< LOD	0,02	n.d.	0,19	n.d.	< LOD	< LOD	n.d.	0,04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
E6795	0,38	0,34	< LOD	0,32	< LOD	0,18	n.d.	< LOD	< LOD	n.d.	n.d.	0,09	n.d.	0,02	n.d.	n.d.	1,04	n.d.	n.d.	n.d.	n.d.
E6796	0,29	0,35	0,20	0,12	n.d.	< LOD	n.d.	0,03	< LOD	0,17	n.d.	0,04	n.d.	< LOD	n.d.	n.d.	0,90	< LOD	n.d.	n.d.	n.d.
E6797	0,10	0,11	0,09	0,05	n.d.	0,23	n.d.	< LOD	< LOD	n.d.	< LOD	0,04	n.d.	n.d.	n.d.	< LOD	3,20	< LOD	< LOD	n.d.	n.d.
E9977	0,45	0,42	0,09	0,12	n.d.	0,15	n.d.	0,07	< LOD	n.d.	< LOD	0,06	n.d.	n.d.	n.d.	< LOD	0,49	< LOD	n.d.	n.d.	n.d.
E9981	0,09	0,26	0,06	0,14	< LOD	0,13	n.d.	< LOD	< LOD	n.d.	n.d.	0,07	n.d.	0,02	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.

E9982	0,42	0,43	0,15	0,19	n.d.	0,13	n.d.	0,06	< LOD	0,80	< LOD	0,03	n.d.	0,04	n.d.	< LOD	2,56	0,01	n.d.	568,68	5,86
E9984	0,25	0,27	0,23	0,04	< LOD	< LOD	n.d.	< LOD	< LOD	n.d.	n.d.	0,02	n.d.	0,02	n.d.	< LOD	0,79	n.d.	n.d.	n.d.	n.d.
E9986	< LOD	0,20	0,15	0,18	< LOD	n.d.	n.d.	< LOD	< LOD	n.d.	< LOD	0,18	< LOD	n.d.	n.d.	n.d.	0,23	< LOD	n.d.	n.d.	n.d.
E9988	0,40	0,25	0,24	0,23	< LOD	0,11	n.d.	n.d.	< LOD	n.d.	n.d.	0,09	n.d.	0,03	n.d.	< LOD	0,73	< LOD	< LOD	n.d.	n.d.
E9989	0,23	0,23	0,08	0,19	< LOD	n.d.	n.d.	0,06	< LOD	n.d.	< LOD	0,05	n.d.	0,03	n.d.	< LOD	< LOD	< LOD	n.d.	n.d.	n.d.
E9990	0,26	0,20	0,27	0,30	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	< LOD	0,05	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
E9991	0,19	0,11	0,07	0,12	0,04	0,13	n.d.	0,02	< LOD	n.d.	n.d.	0,03	< LOD	0,04	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
E9992	0,08	0,08	< LOD	0,02	< LOD	< LOD	n.d.	< LOD	< LOD	n.d.	0,03	0,01	n.d.	0,02	n.d.	< LOD	2,73	< LOD	< LOD	n.d.	n.d.
E9993	0,16	0,31	0,09	0,16	n.d.	< LOD	n.d.	0,09	< LOD	n.d.	n.d.	< LOD	n.d.	0,02	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
E9994	0,55	0,46	< LOD	0,28	n.d.	n.d.	< LOD	< LOD	< LOD	n.d.	< LOD	0,08	n.d.	0,02	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
E9995	0,80	0,39	0,24	0,75	n.d.	n.d.	n.d.	n.d.	< LOD	0,61	0,11	0,08	n.d.	0,06	n.d.	< LOD	3,72	n.d.	< LOD	3550,71	6,67
E9996	0,32	0,16	< LOD	0,26	n.d.	n.d.	n.d.	0,08	< LOD	n.d.	0,03	0,06	n.d.	0,09	< LOD	< LOD	1,14	n.d.	n.d.	984,68	n.d.
E9997	0,49	0,21	0,33	0,32	n.d.	< LOD	< LOD	0,04	< LOD	n.d.	0,03	0,08	< LOD	0,01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
E9998	0,26	0,06	0,22	0,11	n.d.	< LOD	< LOD	0,06	< LOD	n.d.	0,05	0,03	< LOD	< LOD	n.d.	n.d.	n.d.	0,02	n.d.	n.d.	n.d.
E9999	0,33	0,30	0,08	0,15	< LOD	< LOD	< LOD	< LOD	< LOD	n.d.	0,03	0,03	n.d.	0,03	n.d.	n.d.	1,43	< LOD	n.d.	n.d.	n.d.
N000	0,17	0,08	0,15	0,44	< LOD	0,55	n.d.	n.d.	< LOD	n.d.	< LOD	0,11	n.d.	< LOD	n.d.	< LOD	0,86	< LOD	n.d.	n.d.	n.d.
D6681	0,43	0,29	0,21	0,33	< LOD	2,05	n.d.	0,05	< LOD	n.d.	< LOD	0,04	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.
D6684	0,13	0,13	n.d.	0,21	< LOD	16,63	< LOD	0,03	< LOD	n.d.	n.d.	0,07	n.d.	< LOD	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
D6685	0,45	0,24	0,14	0,31	n.d.	17,05	< LOD	0,10	< LOD	n.d.	n.d.	0,01	< LOD	0,01	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
D6686	0,25	0,42	0,22	0,14	n.d.	2,79	n.d.	0,04	< LOD	n.d.	0,02	0,08	< LOD	0,02	n.d.	< LOD	0,39	n.d.	n.d.	n.d.	n.d.
D6687	0,52	0,42	0,41	0,29	n.d.	< LOD	n.d.	0,16	< LOD	0,83	0,04	0,15	n.d.	0,03	n.d.	< LOD	1,42	< LOD	n.d.	506,14	11,56
D6688	0,21	0,34	0,11	0,30	< LOD	0,16	n.d.	< LOD	< LOD	0,04	< LOD	0,02	n.d.	< LOD	n.d.	< LOD	1,19	0,02	n.d.	n.d.	n.d.
D6690	0,57	0,54	0,25	0,52	< LOD	1,12	n.d.	0,06	< LOD	n.d.	< LOD	0,07	n.d.	< LOD	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
D6691	1,07	0,75	0,29	0,79	n.d.	0,12	n.d.	n.d.	< LOD	n.d.	< LOD	0,12	< LOD	0,02	n.d.	< LOD	1,15	< LOD	n.d.	n.d.	n.d.
D6692	0,53	0,35	0,13	0,30	n.d.	1,86	< LOD	0,15	< LOD	n.d.	n.d.	0,05	n.d.	< LOD	n.d.	n.d.	0,95	< LOD	n.d.	n.d.	n.d.
D6693	0,09	0,16	< LOD	0,15	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	< LOD	0,06	n.d.	0,02	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
E3013	0,30	0,31	0,19	0,22	< LOD	< LOD	< LOD	< LOD	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	2,92	< LOD	n.d.	n.d.	n.d.
E3621	0,17	0,24	< LOD	0,21	< LOD	13,40	< LOD	n.d.	< LOD	n.d.	n.d.	0,08	n.d.	0,03	n.d.	n.d.	7,63	n.d.	n.d.	n.d.	n.d.
E3624	0,29	0,09	0,06	0,05	n.d.	2,04	< LOD	0,05	< LOD	n.d.	< LOD	< LOD	< LOD	< LOD	n.d.	< LOD	2,73	< LOD	n.d.	n.d.	n.d.
E3644	0,11	0,01	< LOD	0,12	n.d.	< LOD	n.d.	0,05	< LOD	n.d.	n.d.	0,03	n.d.	< LOD	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.

E3645	0,19	0,10	0,14	0,19	< LOD	0,18	n.d.	< LOD	< LOD	0,67	< LOD	0,03	< LOD	0,03	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	2,73
E3660	0,59	0,89	0,27	0,63	< LOD	0,15	< LOD	< LOD	< LOD	0,09	n.d.	0,07	n.d.	< LOD	n.d.	< LOD	3,80	n.d.	n.d.	n.d.	n.d.
E3673	0,42	0,26	0,13	0,10	0,02	n.d.	n.d.	0,05	< LOD	n.d.	0,03	< LOD	n.d.	0,02	n.d.	< LOD	n.d.	0,02	n.d.	n.d.	n.d.
E3676	0,28	0,34	0,11	0,17	< LOD	< LOD	< LOD	0,03	< LOD	n.d.	0,02	0,02	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.
E3690	0,57	0,69	0,27	0,25	< LOD	n.d.	n.d.	0,03	< LOD	n.d.	0,04	0,06	n.d.	< LOD	n.d.	< LOD	1,92	n.d.	n.d.	n.d.	n.d.
E3709	0,49	0,30	0,22	0,38	0,03	n.d.	n.d.	0,02	< LOD	n.d.	n.d.	0,04	n.d.	< LOD	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
E3710	0,54	0,55	0,29	0,28	n.d.	0,36	n.d.	0,06	< LOD	n.d.	< LOD	0,08	n.d.	n.d.	n.d.	< LOD	2,38	< LOD	n.d.	n.d.	n.d.
E3711	0,21	0,13	< LOD	0,27	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	< LOD	0,04	n.d.	< LOD	n.d.	n.d.	4,70	< LOD	n.d.	n.d.	n.d.
E3716	0,08	0,18	0,15	0,08	< LOD	n.d.	n.d.	< LOD	< LOD	n.d.	n.d.	0,03	n.d.	0,02	n.d.	< LOD	< LOD	< LOD	< LOD	n.d.	n.d.
E3722	0,45	0,40	0,11	0,36	< LOD	< LOD	n.d.	< LOD	< LOD	n.d.	< LOD	0,08	n.d.	n.d.	n.d.	< LOD	3,72	0,02	n.d.	n.d.	n.d.
E3723	< LOD	< LOD	< LOD	< LOD	n.d.	0,34	n.d.	0,02	< LOD	0,69	n.d.	0,06	< LOD	< LOD	n.d.	n.d.	2,28	0,01	n.d.	583,31	2,71
E3742	0,31	0,31	0,08	0,19	< LOD	< LOD	n.d.	n.d.	< LOD	n.d.	< LOD	0,05	n.d.	< LOD	n.d.	< LOD	2,38	< LOD	< LOD	n.d.	n.d.
E3791	0,37	0,49	0,17	0,13	< LOD	< LOD	n.d.	0,06	< LOD	n.d.	n.d.	0,03	n.d.	0,02	n.d.	< LOD	1,96	< LOD	n.d.	n.d.	n.d.
E6390	0,77	0,61	0,24	0,26	< LOD	n.d.	n.d.	0,07	< LOD	n.d.	n.d.	0,05	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
2000_01	0,94	0,85	0,23	0,23	0,03	0,15	n.d.	< LOD	< LOD	n.d.	n.d.	0,02	n.d.	0,04	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
2000_02	0,59	1,07	0,14	0,38	n.d.	0,48	n.d.	0,07	< LOD	n.d.	n.d.	0,04	n.d.	n.d.	n.d.	n.d.	2,99	< LOD	n.d.	n.d.	n.d.
2000_03	1,11	1,29	0,13	0,36	n.d.	n.d.	n.d.	0,09	< LOD	n.d.	n.d.	0,12	n.d.	n.d.	n.d.	n.d.	0,50	< LOD	0,03	n.d.	n.d.
2000_04	0,59	0,92	0,29	0,25	n.d.	0,13	< LOD	0,04	< LOD	n.d.	n.d.	0,04	n.d.	n.d.	n.d.	n.d.	0,82	< LOD	0,03	n.d.	n.d.
2000_06	0,78	1,28	0,24	0,34	n.d.	0,88	< LOD	0,04	n.d.	n.d.	n.d.	0,04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2000_08	0,70	0,64	0,13	0,30	n.d.	n.d.	n.d.	0,02	< LOD	n.d.	< LOD	0,07	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.
2005_01	0,44	0,45	< LOD	0,26	n.d.	0,20	n.d.	0,06	< LOD	n.d.	< LOD	0,02	n.d.	n.d.	n.d.	n.d.	2,97	n.d.	n.d.	n.d.	n.d.
2005_02	0,14	0,54	0,18	0,24	n.d.	0,63	n.d.	0,03	< LOD	n.d.	n.d.	0,05	n.d.	n.d.	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
2005_03	0,57	1,14	0,15	0,26	n.d.	0,21	n.d.	< LOD	< LOD	n.d.	< LOD	0,03	n.d.	n.d.	n.d.	n.d.	1,64	n.d.	< LOD	n.d.	n.d.
2005_04	0,71	1,43	0,24	0,52	< LOD	0,31	n.d.	< LOD	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4,44	< LOD	n.d.	n.d.	n.d.
2005_06	0,36	0,92	0,26	0,13	n.d.	0,26	n.d.	0,07	< LOD	n.d.	n.d.	0,01	n.d.	< LOD	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
2005_07	0,36	0,53	0,13	0,16	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	< LOD	0,01	n.d.	n.d.	n.d.	n.d.	0,65	n.d.	< LOD	n.d.	n.d.
2005_08	0,46	0,80	0,07	0,10	n.d.	< LOD	< LOD	< LOD	< LOD	n.d.	n.d.	0,10	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	n.d.
2005_09	0,55	1,24	0,19	0,28	n.d.	< LOD	n.d.	0,09	< LOD	n.d.	0,03	0,08	n.d.	n.d.	n.d.	n.d.	1,15	n.d.	n.d.	n.d.	n.d.
2005_10	0,85	1,93	0,57	0,33	n.d.	n.d.	n.d.	0,36	< LOD	n.d.	n.d.	0,02	n.d.	n.d.	n.d.	n.d.	2,42	n.d.	n.d.	n.d.	n.d.
2005_11	0,44	1,62	0,36	0,25	n.d.	n.d.	< LOD	0,12	< LOD	n.d.	n.d.	0,03	n.d.	< LOD	n.d.	n.d.	0,98	n.d.	0,03	n.d.	n.d.

2005_12	0,32	0,44	< LOD	0,13	< LOD	0,12	< LOD	0,06	< LOD	0,47	< LOD	0,04	n.d.	< LOD	n.d.	n.d.	2,00	n.d.	n.d.	217,65	2,39
2005_13	0,52	0,84	0,11	0,23	< LOD	n.d.	n.d.	0,03	< LOD	n.d.	n.d.	0,03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2005_14	1,03	2,26	0,63	0,47	n.d.	0,14	< LOD	0,45	n.d.	n.d.	< LOD	0,04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	n.d.
2005_15	0,16	0,30	< LOD	0,11	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	< LOD	< LOD	n.d.	0,02	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	n.d.
2005_16	0,58	1,44	0,19	0,39	n.d.	0,29	< LOD	0,14	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	2,50	< LOD	< LOD	n.d.	n.d.
2005_17	0,73	1,22	0,26	0,27	n.d.	n.d.	< LOD	0,05	< LOD	n.d.	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	0,95	n.d.	< LOD	n.d.	n.d.
2005_19	0,15	0,71	< LOD	0,09	n.d.	n.d.	< LOD	0,02	< LOD	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.
2005_20	0,23	0,95	0,15	0,08	n.d.	n.d.	n.d.	0,06	< LOD	n.d.	n.d.	0,02	n.d.	n.d.	n.d.	n.d.	1,73	< LOD	< LOD	n.d.	n.d.
2005_21	0,53	1,10	0,11	0,16	n.d.	n.d.	< LOD	0,03	< LOD	n.d.	n.d.	0,02	n.d.	< LOD	n.d.	< LOD	0,60	n.d.	n.d.	n.d.	n.d.
2020_01	0,11	0,25	< LOD	0,11	n.d.	0,22	< LOD	< LOD	< LOD	0,85	< LOD	0,01	< LOD	n.d.	n.d.	< LOD	1,06	< LOD	< LOD	n.d.	2,41
2020_02	0,25	0,56	0,11	0,13	< LOD	2,87	< LOD	0,08	< LOD	n.d.	n.d.	0,02	n.d.	< LOD	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
2020_03	0,11	0,44	0,06	0,16	< LOD	n.d.	< LOD	0,05	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2020_04	0,08	0,33	< LOD	0,15	n.d.	n.d.	n.d.	0,03	< LOD	n.d.	n.d.	< LOD	n.d.	0,03	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
2020_05	0,06	0,23	0,08	0,21	0,06	0,11	n.d.	0,08	< LOD	n.d.	0,05	0,06	n.d.	< LOD	n.d.	0,53	n.d.	< LOD	n.d.	n.d.	n.d.
2020_06	0,06	0,14	n.d.	0,05	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	< LOD	n.d.	n.d.	0,03	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	n.d.
2020_07	0,08	0,28	< LOD	0,14	n.d.	n.d.	n.d.	0,12	< LOD	n.d.	< LOD	< LOD	n.d.	n.d.	n.d.	< LOD	0,90	< LOD	< LOD	n.d.	n.d.
2020_08	< LOD	0,21	< LOD	0,19	n.d.	n.d.	n.d.	0,04	< LOD	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.
2020_09	0,04	0,10	< LOD	0,03	0,02	< LOD	n.d.	n.d.	< LOD	n.d.	< LOD	n.d.	< LOD	0,01	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.
2020_10	0,23	0,36	< LOD	0,28	< LOD	1,96	n.d.	n.d.	< LOD	n.d.	n.d.	< LOD	n.d.	< LOD	n.d.	< LOD	1,20	< LOD	n.d.	n.d.	n.d.
2020_11	< LOD	0,36	< LOD	0,09	< LOD	< LOD	< LOD	< LOD	< LOD	0,20	< LOD	0,02	n.d.	n.d.	n.d.	< LOD	1,83	n.d.	< LOD	n.d.	2,62
2020_12	0,25	0,53	< LOD	0,32	< LOD	n.d.	< LOD	0,20	< LOD	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0,03	n.d.	n.d.
2020_14	0,11	0,44	0,09	0,21	n.d.	< LOD	n.d.	0,15	< LOD	n.d.	< LOD	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	n.d.
F_22_01	0,07	0,66	0,01	0,43	n.d.	0,21	n.d.	n.d.	100,00	n.d.	0,05	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F_22_02	n.d.	0,31	n.d.	0,25	n.d.	0,17	n.d.	n.d.	447,91	n.d.	n.d.	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F_22_03	0,10	0,41	0,21	0,54	n.d.	0,38	n.d.	n.d.	537,17	n.d.	n.d.	< LOD	n.d.	<LOD	n.d.	n.d.	n.d.	0,01	<LOD	n.d.	n.d.
F_22_04	0,16	0,15	0,03	0,48	n.d.	n.d.	n.d.	0,08	329,32	n.d.	<LOD	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	<LOD	n.d.	n.d.	n.d.
F_22_05	0,32	n.d.	n.d.	1,88	1,14	173,50	<LOD	0,03	23,35	0,60	<LOD	< LOD	n.d.	n.d.	n.d.	n.d.	3,06	n.d.	0,15	n.d.	n.d.
F_22_06	0,16	0,24	n.d.	0,28	n.d.	0,62	n.d.	n.d.	167,84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F_22_07	0,17	0,31	n.d.	0,43	n.d.	n.d.	n.d.	n.d.	186,13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2,47	n.d.	<LOD	n.d.	n.d.
F_22_08	0,20	0,34	n.d.	0,37	n.d.	n.d.	n.d.	n.d.	306,45	n.d.	<LOD	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOD	n.d.	n.d.

F_22_09	0,08	0,36	n.d.	0,35	n.d.	0,19	n.d.	0,02	297,59	n.d.	0,03	< LOD	n.d.	n.d.	n.d.	n.d.	n.d.	<LOD	<LOD	n.d.	n.d.
F_22_10	0,02	0,34	n.d.	0,35	n.d.	n.d.	n.d.	0,02	253,54	n.d.	0,01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F_22_11	n.d.	0,08	n.d.	0,35	<LOD	n.d.	n.d.	n.d.	264,13	n.d.	n.d.	n.d.	n.d.	<LOD	n.d.	n.d.	n.d.	0,05	<LOD	n.d.	n.d.
F_22_12	n.d.	0,42	n.d.	0,26	n.d.	0,66	n.d.	0,04	223,54	n.d.	0,04	n.d.	n.d.	n.d.	n.d.	n.d.	<LOD	<LOD	<LOD	n.d.	n.d.

<mLOD: below the limit of detection. n.d. not detected.

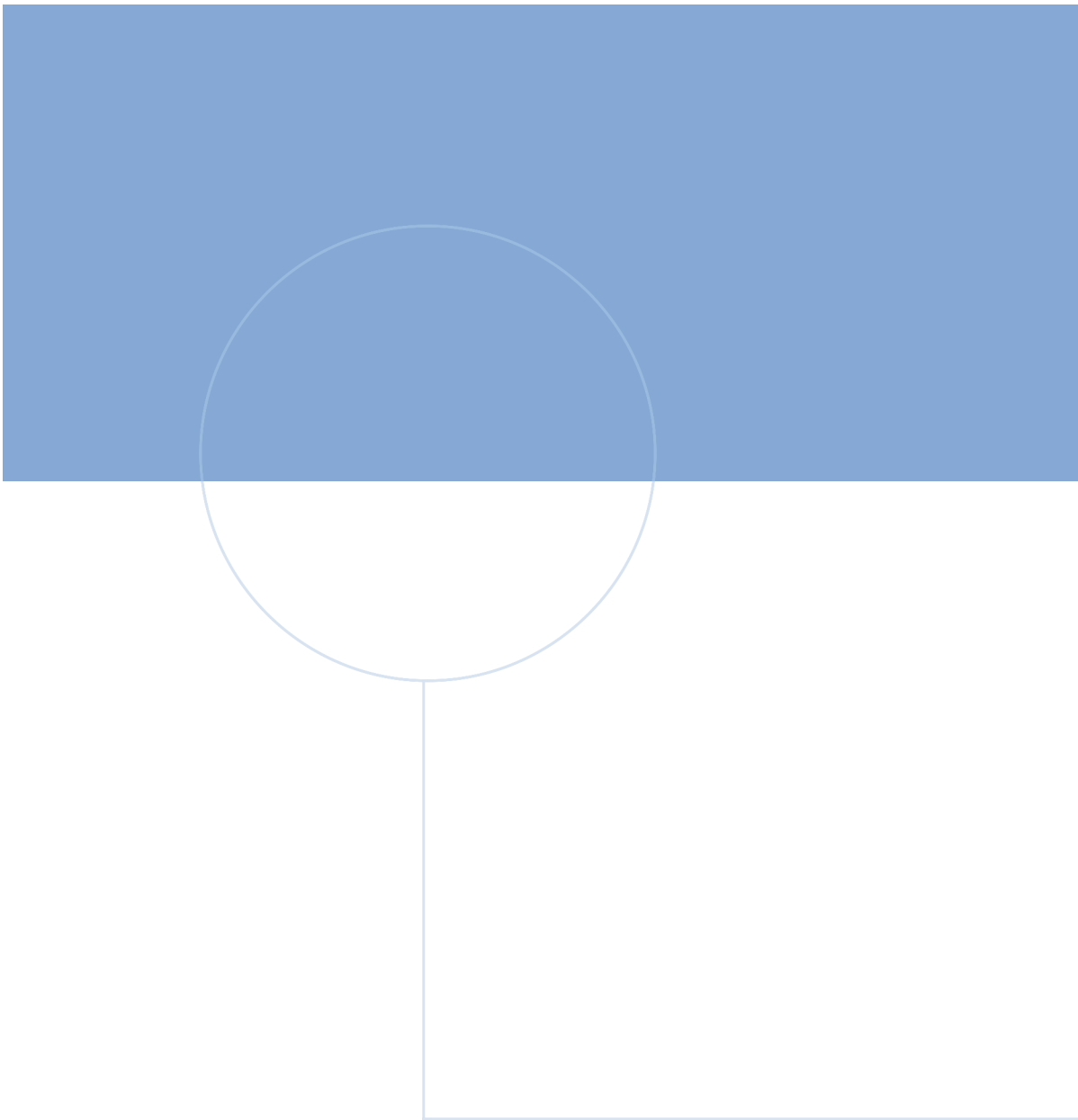
Table S.1. (suite et fin)*

	6:6 PFPi	6:8 PFPi	8:8 PFPi	TriDeFHxSA
F_22_01	n.d.	n.d.	n.d.	n.d.
F_22_02	n.d.	n.d.	n.d.	n.d.
F_22_03	n.d.	n.d.	n.d.	n.d.
F_22_04	n.d.	n.d.	n.d.	< LOD
F_22_05	n.d.	n.d.	n.d.	31,71
F_22_06	n.d.	n.d.	n.d.	< LOD
F_22_07	n.d.	n.d.	n.d.	28,6
F_22_08	n.d.	n.d.	n.d.	< LOD
F_22_09	< LOD	n.d.	n.d.	< LOD
F_22_10	n.d.	n.d.	n.d.	< LOD
F_22_11	n.d.	n.d.	n.d.	22,41
F_22_12	n.d.	n.d.	n.d.	< LOD

*TriDeFHxSA, 6:6 PFPi, 6:8 PFPi and 8:8 PFPi were only analyzed in the pups sampled in 2022

Table S.2. Concentrations (ng/mL) of the 17 analyzed steroids in the plasma of each individual grey seal pups sampled in 2022 (n=12)

ID Seal	COR	CORNE	ALDO	COS	11-deoxyCOR	17a-OHP	DOC	P4	KetoTS	PREG (P5)	17aOPH5	DHT	TS	AN	E1	A5	DHEA
F_22_01	36,13	22,92	<LOD	3,12	5,34	4,81	0,06	0,51	n.d.	n.d.	<LOD	n.d.	0,15	6,42	<LOD	n.d.	<LOD
F_22_02	27,95	15,21	n.d.	3,13	7,62	5,72	0,10	0,68	n.d.	n.d.	<LOD	n.d.	n.d.	5,89	<LOD	n.d.	<LOD
F_22_03	15,92	19,51	0,82	n.d.	4,90	4,31	<LOD	0,20	n.d.	6,02	<LOD	n.d.	n.d.	<LOD	<LOD	n.d.	<LOD
F_22_04	11,87	15,95	<LOD	n.d.	5,15	0,53	0,05	0,16	n.d.	n.d.	11,83	n.d.	<LOD	<LOD	<LOD	n.d.	<LOD
F_22_05	26,75	18,76	n.d.	n.d.	4,62	3,48	0,05	0,28	n.d.	n.d.	19,09	n.d.	n.d.	17,74	<LOD	<LOD	<LOD
F_22_06	53,13	32,01	<LOD	3,95	8,04	6,92	<LOD	0,60	n.d.	n.d.	13,27	n.d.	n.d.	5,42	<LOD	n.d.	n.d.
F_22_07	28,72	30,75	n.d.	<LOD	5,99	4,57	<LOD	0,26	n.d.	n.d.	n.d.	n.d.	n.d.	11,16	n.d.	n.d.	<LOD
F_22_08	25,24	25,80	n.d.	8,08	7,24	4,81	0,07	0,45	n.d.	n.d.	23,65	n.d.	<LOD	<LOD	<LOD	<LOD	<LOD
F_22_09	28,19	24,56	n.d.	<LOD	3,99	2,52	<LOD	0,26	n.d.	n.d.	<LOD	n.d.	<LOD	99,76	<LOD	n.d.	14,99
F_22_10	62,59	31,23	n.d.	13,12	15,60	6,76	0,14	0,98	n.d.	<LOD	<LOD	n.d.	<LOD	n.d.	<LOD	n.d.	n.d.
F_22_11	36,03	18,78	0,66	2,98	6,33	7,14	<LOD	0,76	n.d.	n.d.	n.d.	n.d.	<LOD	9,25	n.d.	n.d.	n.d.
F_22_12	34,54	33,18	<LOD	<LOD	6,93	4,50	0,05	0,39	n.d.	n.d.	10,68	n.d.	n.d.	<LOD	n.d.	n.d.	n.d.



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