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Levels of Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs) in Feathers of Eurasian Eagle-Owls (*Bubo bubo*) in Norway

Marie Johanne Dahlberg Persson

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Supervisor: Veerle Jaspers, IBI
Co-supervisor: Mari Engvig Løseth, IBI
Torgeir Nygård, NINA

Norwegian University of Science and Technology
Department of Biology

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Abstract

In the current study per- and polyfluroalkyl substances (PFASs) were investigated in 72 feathers from Eurasian Eagle-Owls (*Bubo bubo*) collected in four geographically similar areas in Norway and in six different years. The objectives of the present study were to investigate accumulation of PFASs in feathers of Eagle-Owls, spatial and temporal trends of PFAS accumulation, and if PFAS exposure could be associated with the owls' trophic position (^{15}N) and feeding habits (^{13}C). No significant differences in PFAS levels were detected between sexes or sampling years. The highest concentration of ∑PFASs was 98.6 ng/g dw from a feather from Frøya, coastal Central Norway (2013), while the lowest was 4.0 ng/g from Lurøy, Northern Norway (2015). The median level of ∑PFAS detected in feathers from Southern Norway (41.59 ng/g dry weight (dw)), were more than twice as highs as in the feathers from the other areas (Coastal Central Norway: 21.37 ng/g dw; inland Central Norway: 10.77 ng/g dw; Northern Norway: 17.48 ng/g dw). The most abundant PFASs at all locations were linear PFOS (Perfluorooctane sulfonic acid)>PFTriDA (Perfluorotridecanoic acid)>PFUnDA(Perfluoroundecanoic acid)>PFDoDA (Perfluorododecanoic acid). The PFAS profile was dominated by PFSAs (perfluoroalkyl sulfonic acids) in feathers from Northern Norway (54.07 %) and coastal Central Norway (51.76 %). While for Southern Norway and inland Central Norway ∑PFCAs (perfluroalkyl carboxylic acids) was the main PFAS group (56.31 % and 59 % respectively). The pattern of median ∑PFASs contamination was Southern Norway> Coastal Central Norway> Northern Norway > Inland Central Norway. Stable isotopes (^{13}C and ^{15}N) were analyzed to elucidate dietary sources. A great variation in stable isotope data confirms the great diversity of the Eagle-Owls' diet ($-25.62 < \delta^{13}C < -16.65$ ‰; $4.84 < \delta^{15}N < 15.8$ ‰), and reflecting possible dietary shifts of Eagle-Owls in some areas. The ^{15}N data showed higher levels in feathers form Northern Norway (9.79 – 14.18 ‰) and coastal Central Norway (6.22 – 15.8 ‰), indicating that they feed on higher trophic levels. Accordingly, ^{13}C data from these areas revealed that some of these owls have marine food sources in their diet ($\delta^{13}C > -20$‰). The PFAS levels were only correlated with the stable isotope values in Northern Norway. The reason for this is unclear, but it may be explained by the lower sample sizes from the other areas which reduced the statistical power of the tests performed. Baseline values were unkown in the present study and more research is needed to investigate biomagnification of PFASs in Eagle-Owls. Further research of key prey species of Eagle-Owls are warranted to elucidate if the stable isotope values and PFAS levels represent spatial variation in PFAS exposure. Several factors can influence the contaminant accumulation, trophic behavior and feeding habits of the Eagle-Owls. Individuals differences in age and sex may be due to variation in physiology, diet, reproductive status or metabolic capacity. In conclusion, feathers seems to be useful as an alternative or complementary matrix to study PFAS exposure in Norwegian Eagle-Owls but more research is needed.

Sammendrag

I denne studien ble per- og polyfluorerte alkylforbindelser (PFASer) undersøkt i 72 fjær fra hubro (Bubo bubo). Fjærene ble samlet inn i 13 kommuner i Norge (fordelt på fire områder på bakgrunn av deres geografiske beliggenhet) under seks år. Målene med studiet var å undersøke opphopning av PFASer i fjær fra hubroer og om det fantes forskjeller i PFAS nivåer mellom kjønn. Videre ble det undersøkt om det fantes potensielle tidstrender eller geografiske forskjeller i PFAS nivåer, samt om eksponering for PFASer kunne assosieres med uglenes trofiske posisjon i næringskjeden (^{15}N) og diett (^{13}C). Ingen signifikante forskjeller i PFAS-nivåer ble funnet mellom kjønn eller innsamlingsår. Høyeste konsentrasjon (98.6 ng/g tørrvekt) ble målt i en fjær fra en hunn fra Frøya i Sør-Trøndelag (2013, Kyst Midt-Norge), mens laveste konsentrasjon (4.0 ng/g tørrvekt) ble funnet i fjær fra en hann fra Lurøy, Nordland (2015). I fjærene fra Sør-Norge var median-nivåene av ∑PFAS (41.59 ng/g tørrvekt), mer enn dobbelt så høye som nivåene i fjær fra de andre områdene (Kyst Midt-Norge: 21.37 ng/g tørrvekt; innland Trøndelag: 10.77 ng/g tørrvekt; Solværøyene (Nord-Norge):17.48 ng/g tørrvekt). De dominerende forbindelsene var lineær PFOS (Perfluorooctane sulfonic acid) > PFTriDA (Perfluorotridecanoic acid) > PFUnDA(Perfluoroundecanoic acid) > PFDoDA (Perfluorododecanoic acid). PFAS-profilen var dominert av PFSA(perfluorerte alkylsulfonater) i Nord-Norge og kyst Midt-Norge, mens ∑PFCA (perfluorerte alkyl karboksylsyrer) var dominerende i fjærene fra Sør-Norge og innland Trøndelag. Følgende trend ble funnet for median ∑PFAS konsentrasjoner i prøvene: Sør-Norge> Kyst Midt-Norge> Nord-Norge > Innland-Trøndelag. Stabile isotoper ble analysert for å studere uglenes diett. Det var stor variasjon i dataene, noe som reflekterer hubroens varierte diett og kan også indikere potensielle diettskift i noen områder. ^{15}N dataene avslørte at hubroene i Nord-Norge og kyst Midt-Norge spiser på høyere trofiske nivå, enn hubroer fra de to andre områdene. Samtidig viste ^{13}C verdiene at ugler fra disse områdene har innslag av marint føde i sin diett (δ^{13}C > - 20‰). PFAS nivåer var kun korrelert med de stabile isotopene i prøvene fra Nord-Norge. Årsaken til dette er ikke klar, men kan kanskje forklares av færre observasjoner/prøver fra de andre områdene. Dette kan ha redusert styrken i de statistiske testene som ble utført og resultatene bør tolkes med varsomhet. Basislinjer for de stabile isotopene var ukjente og mer forskning er nødvendig for å finne ut om PFASer oppkonsentreres via næringskjeden til norske hubroer. Undersøkelser av stabile isotoper og PFAS nivåer i viktige byttedyr kan være nyttig for å undersøke geografiske forskjeller i PFAS eksponering hos norske hubroer. Faktorer som påvirker opphopning av miljøgifter, kan være individuelle forskjeller i alder og kjønn, fysiologi, reproduktiv status og metabolsk kapasitet. På bakgrunn av resultatene som er presentert her kan det antas at fjær er et nyttig prøvemateriale for å studere PFAS-eksponering i norske hubroer, enten som et alternativ eller som supplement til andre prøvematriser. Mer forskning er nødvendig for å avgjøre om nivåer av PFASer i fjær kan relateres til nivåer i blod eller indre organer.

Abbreviations

%	Per cent
‰	Per mille
°C	Degrees Celsius
∑	Sum
α	Alpha, significance level
β	Bet
χ	Chi
δ	Delta
$\delta^{13}C$	Delta C-13 – ratio of stable carbon isotopes, ^{13}C: ^{12}C
$\delta^{15}N$	Delta N-15 – ratop of stable nitrogen isotopes, ^{15}N: ^{14}N
^{13}C	Stable isotope of carbon
^{15}N	Stable isotope of nitrogen
AFFF	Aqueous film forming foam
ANOVA	Analysis of variance
BEH	Ethylene Bridged Hybrid
C	Carbon
Cm	Centimetre
DNA	Deoxyribonucleic acid
dw	Dry weight
ECF	Electrochemical fluorination
ESI	Electrospray ionization
F	Fluorine
g	Gram
HPLC	High-performance liquid cromatography
HCl	Hydrochloride
ISTD	Internal standard solution
IUCN	International Union for Conservation of Nature
LOD	Limit of detection
LOQ	Limit of quantification
M	Molar (mol/litre)
MeOH	Methanol
Min	Minutes
mL	Millilitre
mm	Millimetre
mM	millimolar
MS	Mass spectrometry
n	Number of observations
N_2	Dinitrogen
NaOH	Sodiumhydroxide
ng	Nanogram (10^{-9} gram)
Ng/g	Nanograms per gram
NINA	Norwegian Institute for Nature Research
NSTD	Native standard solution
NTNU	Norwegian University of Science and Technology
OHC	Organohalogenated contaminant
p	Probability of rejecting the null hypothesis
PCB	Polychlorinated biphenyl
POPs	Persistent Organic pollutants
PP	Polypropylene
r_s	Spearman's correlation coefficient, rho
rpm	Rounds per minute
RSTD	Recovery standard solution

R_{sample}	Feather sample
$R_{standard}$	International reference standard
SD	Standard deviation
SE	Standard error
SRM	Selected reaction monitoring
t	t-value
Tukey's HSD	Tukey's Honest Significance Difference test
µL	Microliter
µm	Micrometre
UNEP	United Nations Environment Programme
UPLC	Ultraperformance liquid cromatography
USA	United States of America
ww	Wet weight

Compounds

FASA	Perfluoroalkane sulfonamides
FTOH	Fluorotelomer alcohols
FTS	Fluorotelomer sulfonate
PFAAs	Perfluoroalkyl acids
PFCAs	Perfluoroalkyl carboxylic acids
PFASs	Per – and polyfluoroalkyl substances
PFSAs	Perfluoroalkane sulfonic acids
PFPeA	Perfluoropentanoic acid
PFHxA	Perfluorohexanoic acid
PFHpA	Perfluoroheptanoic acid
PFOA	Perfluorooctanoic acid
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnDA	Perfluoroundecanoic acid
PFDoDA	Perfluorododecanoic acid
PFTriDA	Perfluorotridecanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFPeDA	Perfluoropentadecanoic acid
FPePA (5:3 FTA)	3-Perfluoropentyl propanoic acid (5:3)
FHpPA (7:3FTA)	3-Perfluoroheptyl propanoic acid (7:3)
PFBS	Perfluorobutane sulfonic acid
L-PFHxS	Linear Perfluorohexane sulfonic acid
Br-PFHxS	Branched Perfluorohexane sulfonic acid
L-PFOS	Linear Perfluorooctane sulfonic acid
Br-PFOS	Branched Perfluorooctane sulfonic acid
L-PFDS	Linear Perfluorodecane sulfonic acid
Br-PFDS	Branched Perfluorodecane sulfonic acid
L-FOSA	Linear Perfluorooctane sulfonamide
Br-FOSA	Branched Perfluoroctane sulfonamide
L-FOSAA	Linear Perfluorooctane sulfonamidoacetic acid
Br-FOSAA	Branched Perfluorooctane sulfonamidoacetic acid
L-MeFOSAA	Linear N-Methyl Perfluorooctane sulfonamidoacetic acid
Br-MeFOSAA	Branched N-Methyl Perfluorooctane sulfonamidoacetic acid
L-EtFOSAA	Linear N-Ethyl Perfluorooctane sulfonamidoacetic acid
Br-EtFOSAA	Branched N-Ethyl Perfluorooctane sulfonamidoacetic acid
4:2 FTS	Sodium 1H,1H,2H,2H-perfluorohexane sulfonate(4:2)
6:2 FTS	Sodium 1H,1H,2H,2H-perfluorooctane sulfonate(6:2)
8:2 FTS	Sodium 1H,1H,2H,2H-perfluorodecane sulfonate(8:2)

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1. Introduction

1.1 Birds As Biomonitoring Species

Birds feeding at high trophic levels are widely recognized as biomonitors of environmental pollution. As they are especially vulnerable to environmental changes, they are useful sentinel species for potential effects of contaminants on humans and the environment (Furness and Greenwood, 1993, Herzke et al., 2002, Jaspers et al., 2006, Ratcliffe, 1967, Newton, 1988). In Norway birds of prey have been used for biomonitoring of organic pollutants for several years (Gjershaug et al., 2008, Nygård and Polder, 2012b).

The Eurasian Eagle-Owl (*Bubo bubo,* Eagle-Owl) is the largest owl species in Norway. It is a nocturnal raptor and can be recognized by its large size and feather tufts ("ears") on the top of its head. The Eagle-Owl is a residential and territorial bird. However, it is highly adaptive and can be found in many different environments, ranging from deserts to forests and mountainous areas (Jacobsen and Røv, 2007). In Norway it is distributed along the coast from Agder in the south to Helgeland (Nordland) in the north, and also scattered in the inland (Hagen, 1952, Hagen, 1989, Jacobsen and Røv, 2007). The Eagle-Owl is a versatile hunter and preys on a wide range of vertebrate animals, such as small voles, rats, hares, frogs, seabirds and even fish. Its diet depends on the availability of prey where it is situated and might differ between habitats (Willgohs, 1974, Obuch and Bangjord, 2016, Fosså, 2013). Since the 19th century the Eagle-Owl populations in Norway have declined due to hunting and disturbances by humans. The Eagle-Owl was protected in 1971 and is classified as endangered on the Norwegian Red List for Species (Henriksen and Hilmo, 2015). Eagle-Owls in Norway are vulnerable to collisions with electrical wires and fences, and death by electrocution is the main threat for this species (Jacobsen and Røv, 2007).

Regarding the biomonitoring potential of the Eagle Owl, Nygård & Polder (2012b) reported that an Eagle-Owl egg from Hitra probably had the highest concentration of per- and polyfluorinated alkyl substances (PFASs) detected in any bird sample in Norway (1000 parts per billion (ppb)/1000 ng/g fresh weight of sum PFASs). These levels exceeded the concentrations detected in glaucous gulls (*Larus hyperboreus*) from Svalbard, which were the highest reported in any Arctic seabird species (Verreault et al., 2005b), as well as in eggs from European shag *(Phalacrocorax aristotelis*) and eider ducks (*Somateria mollissima)* sampled in

Sklinna, central Norway (Herzke et al., 2009). As a top predator Eagle-Owls are susceptible to bioaccumulation of environmental contaminants including PFASs.

1.2 Characteristics Of PFASs

Despite being produced for more than 50 years, PFASs are regarded as emerging pollutants and have received attention due to their presence in the environment, humans and wildlife (Giesy and Kannan, 2001, Ahrens, 2011, Giesy and Kannan, 2002, Houde et al., 2011, Kannan et al., 2004). PFASs are a large group of organic compounds which are used in numerous applications (Buck et al., 2011). These compounds are highly stable, which makes them resistant to biodegradation and susceptible to bioaccumulate and biomagnify through food webs (Tomy et al., 2004). The presence of PFASs in the environment is of great importance because of their bioaccumulative properties and potential to cause toxic effects in organisms (Stahl et al., 2011, DeWitt et al., 2012, Lau, 2012).

PFASs contain a hydrophobic alkyl chain and a hydrophilic functional group. Perfluoroalkyl substances are compounds where all the hydrogen atoms on the alkyl chain are replaced by fluorine atoms. Polyfluoroalkyl substances, however, are compounds where at least one of the hydrogens is replaced by a fluorine atom. Two of the most important classes of PFASs are perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs), also known as perfluoroalkyl acids (PFAAs) (Buck et al., 2011). Due to the high electronegativity of fluorine and its three nonbonding electron pairs, fluorine can form very strong covalent bonds with carbon and hydrogen. The C-F bond is one of the strongest known covalent bonds in organic chemistry (Smart and Fernandez, 1994). The strong bond between carbon and fluorine results in high thermal and chemical stability due to shielding of the carbon by the fluorine atoms, making the compounds resistant to biodegradation, metabolism, acids, oxidation, reduction and high temperatures (Kissa, 2001, Buck et al., 2011, Parsons et al., 2008). PFASs are amphipathic, both hydrophobic and lipophobic, meaning that they repel both water and lipids (Buck et al., 2011). These unique properties make PFASs highly useful in surfactants and polymers where the perfluoroalkyl moiety is incorporated (Buck et al., 2011, Kissa, 2001). Several PFASs are manufactured for commercial and industrial use, in paint, surface treatments and coatings in textiles and cookware, fire-fighting foams, ski waxes and emulsifiers (Key et al., 1997, Lau et al., 2007).

The most studied PFASs are perfluorooctanoic acid (PFOA) and PFOS (Perfluorooctane sulfonic acid), both ubiquitous in the environment and accumulating in top predators, such as birds of prey (Jaspers et al., 2013, Kannan et al., 2001). Despite low volatility and high water solubility, both PFOA and PFOS have been detected in remote regions, such as the Arctic (Butt et al., 2010, Letcher et al., 2010). Two hypothesis have been proposed to explain the fate and transport of PFOA and PFOS in the environment (Lau et al., 2007, Stock et al., 2007); either indirect atmospheric transport and subsequent depositions or direct release and long-range transport by ocean currents (Prevedouros et al., 2006, Yamashita et al., 2008). Volatile precursor chemicals, such as fluorotelomer alcohols (FTOHs) and fluorinated sulphonamides (FASAs), are transported in the atmosphere. Abiotic and biotic degradation, such as atmospheric oxidation of FTOHs and FSAs, lead to production of PFSAs and PFCAs and subsequent wet and dry depositions (Stock et al., 2007). Direct transport of PFSAs and PFCAs by ocean currents and sea spray aerosols (Armitage et al., 2006) involves oceanic transport of directly emitted PFCAs and PFSAs from manufacturing processes or intentional additives or residuals from consumer products (Butt et al., 2010). Prevedouros et al. (2006) estimated that 2-12 tons of PFOA are transported to the Arctic per year by oceanic transport.

PFASs can be absorbed by oral, dermal or respiratory routes, with oral uptake being the most important pathway. PFOS and PFOA bind primarily to serum albumin, but can also bind to β-lipoproteins and fatty acid binding proteins in the liver and can interfere with endogenous compounds (Stahl et al., 2011, Jones et al., 2003). The chain length and functional group of the PFASs influence the binding site and binding affinity (Chen and Guo, 2009). In contrast to legacy POPs that accumulate in lipid-rich tissues, PFASs mainly bind to serum proteins and accumulate in blood and protein rich tissues such as the kidneys and liver (Jones et al., 2003). Their potential to bioaccumulate is greatly governed by the carbon chain length of the compound and trophic position (Gomez-Ramirez et al., 2012, Van de Vijver et al., 2003, Martin et al., 2004). Long chain PFCAs ($>C_8$) and PFSAs ($>C_6$) are more bioaccumulative than short chain PFASs (Kannan et al., 2005, Tomy et al., 2004, Buck et al., 2011). In terrestrial and marine wildlife, PFOS is the most abundant PFAS and concentrations are often higher than those measured in humans (Houde et al., 2006). In chicken, PFOS and PFOA are primarily found in the liver and kidneys (Yoo et al., 2009). PFOS and PFOA are not metabolized, and excretion is the only way to eliminate these compounds, leading to bioaccumulation in the organism (Stahl et al., 2011). PFOA are more readily excreted than PFOS (Yoo et al., 2009). Laboratory studies have associated PFAS exposure with a range of adverse effects, such as

tumor induction, hepatotoxicity, developmental toxicity, immunotoxicity, endocrine disruption and neurotoxicity (Lau et al., 2007, Stahl et al., 2011).

The 3M Co., which was historically the main manufacturer of PFOS, phased out its production of PFOS, PFOA, and related compounds in 2002 (Lau et al., 2007). Despite this inititative, new and emerging compounds are still in production (Lau et al., 2007). Due to its great persistence in the environment and potential toxicity and accumulation, PFOS was added to the Stockholm Convention for Persistent organic pollutants in 2009 where it has been classified as an Annex B substance (UNEP, 2014). This has resulted in international restrictions for the use and import of this compound. Both PFOA and long chained PFCAs are regulated in the EU and Canada (Scheringer et al., 2014). Despite regulations on use and production of PFOS in Europe and North America, China and other Asian countries has continued to produce PFOS for use in industry (Chen et al., 2009, Xie et al., 2013, Wang et al., 2014). Brazil has an exemption from the Stockholm Convention to produce EtFOSA (N-Ethyl-Perflurooctansulfonamide), a PFOS-precursors for use in Sulfluramid ant baits (Löfstedt Gilljam et al., 2015). Increased production and release of PFOS in China and Brazil is likely to contribute to accumulation in the environment and in Arctic biota (Miller et al., 2015).

As a result of restrictions in the production and use of some PFASs, such as PFOS, decreasing trends have been documented in recent wildlife studies (Ahrens et al., 2009), yet levels of other substances, such as PFCAs, are still increasing in the environment (Ahrens et al., 2011, Lau et al., 2007). Due to their persistent nature and ability to accumulate within protein-rich biological tissues, some PFASs may biomagnify in top predators, such as birds of prey (Ahrens et al., 2011, Eriksson et al., 2016, Sletten et al., 2016, Jaspers et al., 2013, Holmström et al., 2010). Temporal trend studies in eggs of Swedish peregrine falcons (*Falco peregrinus*) (1974-2007) (Holmström et al., 2010) and tawny owl (*Strix aluco*) eggs (1986 – 2009) (Ahrens et al., 2011) from Central Norway, indicated increasing trends in ∑PFCAs concentrations, while PFOS concentrations were decreasing. Different spatial trends in PFCAs and PFOS have been found in eggs from guillemot (*Uria aalge*) collected in North-Western Europe (Löfstrand et al., 2008).

1.3 The Use Of Stable Isotopes

Diet is an important source for PFAS accumulation in birds (Stahl et al., 2011, Kannan et al., 2001). Sinclair et al. (2006) found that PFOS concentrations in livers of waterfowl were 2.5 fold greater in piscivorous birds than in non-piscivorous birds from New York State in the

United States of America (USA) (Sinclair et al., 2006). Analysis of stable isotopes is a useful tool to assess the link between diet and trophic position to contaminant levels in avian tissues (Haukås et al., 2007, Bourgeon et al., 2013). By studying stable nitrogen and carbon isotopes it is possible to study the effect of differences in diet between locations on the contaminant load in the study species. Trophic position of wildlife within a marine food chain and trophic transfer of contaminants through food webs can be determined (Hobson, 1992, Kelly, 2000). Stable nitrogen isotope ratios (δ^{15}N) reflect trophic position in the food chain, because ^{15}N enriches more with each step in the food chain, compared to the lighter ^{14}N isotope (Kelly, 2000, Hobson, 1992). Stable carbon isotopes ratios (δ^{13}C) reflect carbon sources in the diet and are used to distinguish between land and marine based energy sources (Kelly, 2000). Marine and terrestrial plants have distinct carbon isotope ratios, and carbon is incorporated differently in the photosynthetic steps in these plants. In marine photosynthesis heavier carbon isotopes are incorporated, thus higher values of δ^{13}C reflect a marine diet (Kelly, 2000).

Among the most frequently analyzed PFASs (i.e. C6-C14 PFCAs and C4, C6, C8, and C10 PFSAs) most will biomagnify(Martin et al., 2003b, Butt et al., 2010), and levels of PFASs in Eagle-Owls may vary depending on the trophic position of the prey. Owls feeding on herbivorous voles (*Arvicola amphibius*), will be expected to have lower levels of PFASs than owls feeding on insectivorous or piscivorous birds (Meyer et al., 2009).

1.4 The Use Of Feathers For Biomonitoring Of Pollutants In Birds Of Prey

During the breeding season, the Eagle-Owl is very sensitive to human disturbances and activity near the breeding area. If disturbed it can abandon its nest possibly leading to predation of eggs and chicks (Mikkola and Willis, 1983). By using shed feathers as sample matrices for detecting environmental pollutants, it is possible to take samples without stressing and disturbing the birds.

In general, PFASs in birds have been measured preferably in blood and blood-rich organs, such as liver and spleen (Jones et al., 2003). Since the Eagle-Owl is a protected species, lethal sampling is not possible due to legal and ethical concerns (Henriksen and Hilmo, 2015). Monitoring of protected raptors is thus limited to non-destructive sampling, including blood

samples, plucked feathers, preen oil, moulted feathers, regurgitated pellets and excrement and tissues from carcasses (Espín et al., 2016, Lind, 2012). Addled or deserted eggs and plasma/serum have been used as less invasive sampling methods to measure organohalogenated compounds and PFAS concentrations in Eagle-Owls and other birds (Gomez-Ramirez et al., 2012, Herzke et al., 2002, Nygård et al., 2006, Gjershaug et al., 2008, Nygård and Polder, 2012b, Espín et al., 2016).

Sampling keratinous tissues, such as feathers, for contaminant analysis and biomonitoring has several advantages (Burger, 1993). Due to ethical and practical interests feathers are a more suitable sample matrix when studying vulnerable and endangered bird species. Feathers are easy to collect and can be stored at room temperature (Jaspers et al., 2007). Vulnerable species can be sampled by collecting feathers at the nest. Sampling of feathers is not restricted to a limited time period. Compared with sampling of eggs, feathers might offer a better estimate of the overall contaminant loads in birds since both males and females can be sampled, including non-breeding individuals and juvenile birds (Espín et al., 2016, García-Fernández et al., 2013). By sampling feathers over time, it is also possible to analyze time-trends by sampling the same individual repeatedly without harming the bird, or by using museum collections (Burger and Gochfeld, 2000, Dietz et al., 2006).

Meyer et al. (2009) showed that feathers could also be a promising tool for biomonitoring of PFASs. Feathers from birds of prey are well suited and considered less invasive to sample, as they are relatively large and less feathers are required than when sampling small passerine birds (Dauwe et al., 2005). During development and growth, feathers are connected to the bloodstream and thus circulating compounds (both essential and non-essential) are incorporated into the growing feathers. This provides the possibility of detecting contaminants that are present in the bird during this period of growth. Feathers have been used for biomonitoring of heavy metals (Burger, 1993) and organic pollutants (Dauwe et al., 2005, Jaspers et al., 2006, Jaspers et al., 2007). Significant correlations between contaminant concentrations in feathers and blood or internal tissues have been reported for birds of prey (Jaspers et al., 2013, Jaspers et al., 2007, Jaspers et al., 2011, Eulaers et al., 2011). Furthermore, levels of PFASs in internal tissues from Belgian barn owls (*Tyto alba*) correlated with PFASs concentrations in feathers (Jaspers et al., 2013), yet another unpublished study could not find good correlations between PFASs in blood and feathers (pers.comm. Veerle Jaspers). More research is needed to investigate the relationship between PFASs in feathers and internal tissues.

1.5 Aims

This project is financed by the national "Management plan for Eagle-Owl in Norway". The Eagle-Owl is on the Norwegian Red List for Species (Henriksen and Hilmo, 2015) where it is classified as "Endangered". Electrocution by power-lines , food shortage and exposure to environmental contaminants have been identified as important factors for the decline of the Eagle-Owl population (Madslien et al., 2017, Bourgeon et al., 2012, Jacobsen and Røv, 2007, DN, 2009, Jacobsen and Gjershaug, 2014). About 450 feathers are stored at NINA and were collected in the time period 2009-2016, through work connected with research and management efforts under the national "Management plan for Eagle-Owl in Norway", mainly conducted by NINA, and administered by the County Governor in Nordland.

The aim of this study was to examine the concentrations of PFASs in Eagle-Owl feathers from six counties divided into four broader areas according to their geographical locations. It was expected that regional differences in pollutant levels existed, as a result of exposure and differences in prey availability. Furthermore the study aimed to investigate if PFAS concentrations differed between males and females, and between years. Females might have lower levels of pollutants than males, since they can transfer some of their contaminant burden to their eggs (Kannan et al., 2001). The potential for bioaccumulation of PFASs within the species was also investigated using stable isotopes to assess trophic level (δ^{15}N) and carbon source (δ^{13}C). These compounds are known to bioaccumulate, hence it is expected that concentrations of PFASs will correlate with δ^{15}N. It was expected that Eagle-Owls with a high content of seabirds in their diet, such as in coastal Central Norway, would have higher levels of PFASs than Eagle-Owls from areas with high densities of rodents as prey, for instance in Northern Norway. The δ^{13}C ratio was used to assess feeding habitat and energy sources (i.e. marine vs. terrestrial) of the sampled Eagle-Owls.

The objectives of the present study were to:

1. Quantify the levels of selected PFASs in feathers of the Eurasian Eagle-Owl.
2. Investigate if levels of PFASs in feathers vary between the sexes of the birds.
3. Investigate if levels of PFASs in feathers vary between locations.
4. Investigate whether PFASs levels can be explained by differences in trophic level (δ^{15}N) and feeding habits (δ^{13}C) as indicated by stable isotope analysis.

2. Materials And Methods

2.1 Sampling Of Feathers

The feather material was provided by the Norwegian Institute for Nature Research (NINA) and were collected by staff and collaborators. Shed Eurasian Eagle-Owl (*Bubo bubo*) feathers were collected from 13 different municipalities in Norway, in the period 2009-2016 (Figure 1). To examine geographic trends in concentrations, individual Eagle-Owl populations were pooled into four broad regions according to geographical locations. A total number of 72 feathers from adult birds were selected for this study (Table 1, Appendix). The feather types varied between flight feathers and coverts, but feather type was not determined for the individual feathers.

Table 1 *Overview of sampling areas, year and sexes (F:females, M:males) of the Eagle-Owls.*

Year	**1979**		**1989**		**2013**		**2014**		**2015**		**2016**		**Total,**
Sex	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	***n* (F/M)**
Southern Norway					3	2	1		2	2	1	2	13 (7/6)
Central Norway, coastal			1		2	3	1	1	2	1	5	2	18 (11/7)
Central Norway, inland							1		1	1			3 (2/1)
Northern Norway		1			9	2	3	4	9	3	4	2	37 (25/12)

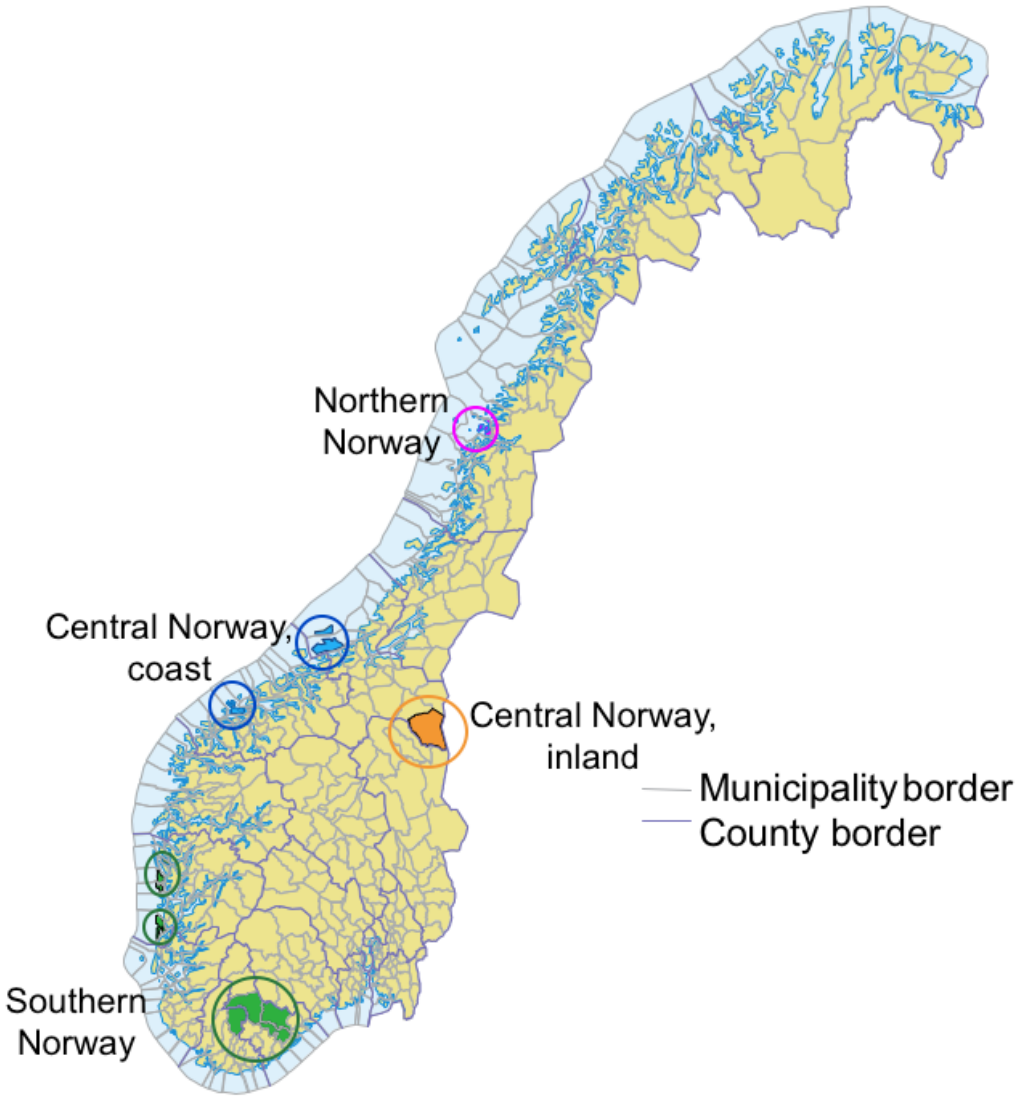

Figure 1 Map of sampling locations (municipalities); Southern Norway (green) , Central Norway coastal (blue), Central Norway inland (orange), and Northern Norway (red). The circles are provided for easier identification of the sampling locations on the map. Map basis: Kartverket (Creative Commons Attribution ShareAlike 3.0).

2.2 Molecular Sexing Of Feathers

Molecular sexing of the feathers was performed by Oddmund Kleven at NINA, according to the method described in Kleven et al. (2013). Briefly, genomic DNA was extracted from the feather calamus using a semi-automated system (Maxwell®16 Reasearch System Progema) and the Maxwell 16 tissue DNA purification kit following the manufacturer's protocol. DNA was amplified using the Z-002D primers (Dawson, 2007) or a combination of the primers M5 (Bantock et al., 2008), MP and NP (Gomez-Ramirez et al., 2012). Females amplified two fragments and males a single fragment.

2.3 Chemical Analysis Of PFASs

Extraction of PFASs in feathers was conducted at the Bird Ecotoxicology laboratory at the Norwegian University of Science and Technology (NTNU) in Trondheim, Norway. Further analysis of the extracts was performed at the Department of Environmental Science and Analytical Chemistry (ACES), Stockholm University, by Raed Awad and Jonathan Benskin. The PFASs that were included in this study are listed in Table 2.

Table 2 Overview of target analytes and their acronyms within the target classes of perfluoroalkyl carboxylates (PFCAs), perfluoroalkyl sulfonates (PFSAs), perfluoroalkane sulfonamides (FASA) and fluorotelomer sulfonate (FTS).

Target Class	Target Substance	Acronym
PFCAs	Perfluoropentanoic acid	PFPeA
	Perfluorohexanoic acid	PFHxA
	Perfluoroheptanoic acid	PFHpA
	Linear Perfluorooctanoic acid	PFOA
	Perfluorononanoic acid	PFNA
	Perfluorodecanoic acid	PFDA
	Perfluoroundecanoic acid	PFUnDA
	Perfluorododecanoic acid	PFDoDA
	Perfluorotridecanoic acid	PFTriDA
	Perfluorotetradecanoic acid	PFTeDA
	Perfluoropentadecanoic acid	PFPeDA
FTAs	3-Perfluoropentyl propanoic acid (5:3)	FPePA (5:3 FTA)
	3-Perfluoroheptyl propanoic acid (7:3)	FHpPA (7:3FTA)
PFSAs	Perfluorobutane sulfonic acid	PFBS
	Linear Perfluorohexane sulfonic acid	L-PFHxS
	Branched Perfluorohexane sulfonic acid	Br-PFHxS
	Linear Perfluorooctane sulfonic acid	L-PFOS
	Branched Perfluorooctane sulfonic acid	Br-PFOS
	Linear Perfluorodecane sulfonic acid	L-PFDS
	Branched Perfluorodecane sulfonic acid	Br-PFDS
FASAs	Linear Perfluorooctane sulfonamide	L-FOSA
	Branched Perfluoroctane sulfonamide	Br-FOSA
	Linear Perfluorooctane sulfonamidoacetic acid	L-FOSAA
	Branched Perfluorooctane sulfonamidoacetic acid	Br-FOSAA
	Linear N-Methyl Perfluorooctane sulfonamidoacetic acid	L-MeFOSAA
	Branched N-Methyl Perfluorooctane sulfonamidoacetic acid	Br-MeFOSAA
	Linear N-Ethyl Perfluorooctane sulfonamidoacetic acid	L-EtFOSAA
	Branched N-Ethyl Perfluorooctane sulfonamidoacetic acid	Br-EtFOSAA
FTSs	Sodium 1H,1H,2H,2H-perfluorohexane sulfonate(4:2)	4:2 FTS
	Sodium 1H,1H,2H,2H-perfluorooctane sulfonate(6:2)	6:2 FTS
	Sodium 1H,1H,2H,2H-perfluorodecane sulfonate(8:2)	8:2 FTS
	Recovery Standards	
	$^{13}C_8$ labeled Perfluorooctanoic acid	M8-PFOA
	$^{13}C_8$ labeled Perfluorooctane sulfonic acid	M8-PFOS

2.3.1 Extraction Of PFASs

The extraction procedure was performed according to previously described methods and was performed in two batches (Powley et al., 2005, Jaspers et al., 2013). The feathers were washed twice in MilliQ water. Before washing, the calamus was removed and the feather length was measured. All equipment used was washed with methanol before and between samples. Two tweezers were used to separate and wash between the barbs. Then the feathers were left to dry at room temperature covered by a paper tissue. The dry feathers were cut in small pieces (circa 1 mm) with pre-cleaned scissors in aluminum foil trays.

The feather homogenate was transferred to and weighed in 50 milliliter (mL) polypropylene (PP) tubes, followed by immersing the feather samples in 20 mL hexane, vortex mixing for 30 seconds and ultrasonicating for 10 minutes. The hexane was decanted using a glass Pasteur pipet and the feather samples were left to dry in the fume hood until the hexane was evaporated. The feathers were subsequently fortified with 50 µL of 20 pg/ml solution of individual stable isotope-labeled internal standards (ISTD; see Table A.3). Exactly 2 mL of 200mM NaOH in methanol was added and the samples were left to soak for 60 minutes. Then 10 mL of methanol was added followed by vortexing. The samples were extracted three times in an ultrasonic bath for 10 minutes with vortex in between, after which the samples were left to soak overnight in the methanol.

The next day 200 µL of 2M HCl in methanol was added to each sample to adjust the pH. The samples were then vortexed and ultrasonicated for 10 minutes, then centrifuged for 5 minutes at 2000 rpm for sedimentation. Finally, the extracts were transferred to 15 mL PP tubes. The tubes containing the feather samples were rinsed with 2 mL MeOH, centrifuged and the supernatant was transferred to the new PP tube. Samples were evaporated to approximately 1 mL under an N_2 stream on a heated plate (40 °C) on a moving table with swirling motion, 70 times/min.

The concentrated extracts were cleaned-up using approximately 25 mg ENVI-carb and 50 µL of glacial acetic acid. The samples were vortexed thoroughly and centrifuged (10 000 rpm, 10 min). Exactly 0.5 mL of the supernatant was transferred to glass vials for transportation to Stockholm University. Before capping, the vials were covered with aluminum foil that was cleaned with methanol. The samples were spiked with recovery standards prior to analysis by UPLC-MS/MS.

2.3.2 Method Verification

To verify the extraction performance, a spike/recovery experiment with a suite of native standards (NSTD) of the corresponding target analytes, (concentrations are listed in Appendix, table A.3 and A.4) was carried out. For the first extraction batch, a 6 g homogenized feather sample was separated into 7 samples (approximately 0.7 g per sample), of which 5 were fortified with NSTD and ITSD and 2 were only fortified with ISTD (Appendix, Table A.2). Due to limited feather material, only four control samples could be run for the second extraction batch, of which two samples were spiked with NSTD and ITSD and two were spiked only with ISTD. All samples were processed as previously described in section 2.3.1). The average recovery from this experiment is shown in Appendix Table A.8, which shows good accuracy (X-Y%) and precision (X-Y% RSD) for most targets. Lower accuracy (X-Y%) was observed for targets where an exactly matched, isotopically-labelled internal standard was unavailable (e.g. PFTriDA (Perfluorotridecanoic acid), PFTeDA (Perfluorotetradecanoic acid), FPePA (3-Perfluoropentyl propanoic acid), FhpPA (3-Perfluoroheptyl propanoic acid), PFDS (Perfluorodecane sulfonic acid), FOSAA (Perfluorooctane sulfonamidoacetic acid)).

2.3.3 Quantification And Quality Assurance

A list of PFASs analyzed in the present work is provided in Table 2. Instrumental analysis was performed using a Waters UPLC system coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer operated in negative ion electrospray ionization (ESI−) mode. Target analytes were chromatographed on a BEH C18 analytical column (2.1×50mm, 1.7 μm particle size, Waters) operated at a flow rate of 0.4ml/min. The mobile phase comprised of 90% water / 10% acetonitrile containing 2 mM ammonium acetate (solvent A) and 100% acetonitrile containing 2mM ammonium acetate (solvent B). The gradient profile is provided in Table A.7. Detection of target analytes was accomplished in selected reaction monitoring (SRM) mode with two precursor/product ion transitions/analyte; one for quantification and the other for qualification (Appendix, Table A.6).

Quantification of target compounds was accomplished using an isotope dilution/internal standard approach with a linear calibration curve with 1/X weighting. The concentration of branched isomers was estimated using the calibration curve for the linear isomer. The primary ions were used for quantification for all targets, except for PFHxA, where the secondary ion (313>119) was used because of an interference noticed in the chromatograms of the primary ion in most of the samples.

Limits of detection (LODs) were estimated as the concentration producing a signal-to-noise ratio of 3. In cases where a blank signal was observable, detection limits were defined as the mean of n=4 blanks (analyzed in 2 separate batches) + 3× the standard deviation of the blanks.

2.4 Analysis Of Stable Isotopes

The analysis for bulk stable carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) isotopes in feathers was performed at the Stable Isotope Lab of the University of Koblenz-Landau (Germany). A subsample of homogenized cleaned feather material (mean ± SD: 1.51 ± 0.26 mg) was wrapped into a tin combustion cup and was analysed for its elemental and isotopic composition using a Flash 2000 HT elemental analyzer coupled via a ConFlo IV interface to a Delta V Advantage isotope ratio mass spectrometer (all Thermo Fisher Scientific, Bremen, Germany). The reported stable carbon and nitrogen isotope values are expressed as δ (‰) relative to the international reference standards Vienna PeeDee Belemnite and atmospheric nitrogen, respectively. An internal reference material (i.e., casein) was measured in duplicate every tenth sample revealing an imprecision (±1 SD) of 0.06 and 0.03 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

The following equation was used to express SI ratios:

$$\delta X\ (‰) \left(= \frac{R_{sample}}{R_{standard}} - 1\right) \times 10^3 \qquad \text{(Equation 1)}$$

X represents ^{13}C or ^{15}N and R corresponds to the isotopic ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the feather sample or standard.

2.5 Data Handling And Statistical Analyses

Data treatment and statistical analyses was performed using the statistical software RStudio, version 1.0.136 (RStudioTeam, 2016) and Microsoft ® Excel for Mac (version 15.33).

The following packages were used in RStudio: corrplot (Wei, 2013), EnvStats (Millard, 2013), FSA (Ogle, 2017), ggplot2 (Wickham, 2009), ggpubr (Alboukadel, 2017), plyr (Wickham, 2011), psych (Revelle, 2017) and reshape2 (Wickham, 2007). The Solver Add-in was used in Microsoft ® Excel (Fylstra et al., 1998). All concentrations are expressed on a ng/g dry weight basis.

2.5.1 Data Below The Limit Of Detection

There are numerous methods for dealing with data which are below limits of detection. Common procedures include using randomized values between 0 and LOD, LOD*DF or LOD/$\sqrt{2}$. Each of these procedures has advantages and disadvantages. Owing to great variability in sample weights, the stated LOD (which was based on an average sample weight) was not accurate for all samples. Consequently, a data imputation approach, which does not take into account LODs, was used to generate missing data.

Concentrations below LOD were imputed using the Solver add-in for Microsoft Excel (Microsoft Excel) (John, 1998) via the following procedure (pers.comm. Jonathan Benskin). First, the measured data were $\log_e$-transformed to approximate a log-normal distribution of data. The data were then plotted as a cumulative normal distribution curve (total number of data versus ln(concentration)). An ideal cumulative normal distribution (the 'model') was also determined based on the mean and standard deviation of the measured data. The squared error was determined by squaring the difference between the measured y-values and the modelled y-values for each data point. These were then summed to get the sum of squared error. The Solver add-in for Microsoft ® Excel was used to fit the measured data to the model data, by minimizing the sum of squared error cell by changing the mean and standard deviation. To do this Solver uses the GRG (Generalized Reduced Gradient) algorithm, which is designed for solving non-linear problems (such as cumulative normal distributions). Once the measured data were fitted to the model, the missing data could be obtained by using the inverse cumulative normal distribution function (Norm.Inv), together with the (now minimized) mean and standard deviations as well as the rank of the missing data. In cases where there were more than one missing data point, the rank of the data below LOD is not known, so the imputed data was randomized before assigning it back to a particular sample.

2.5.2 Statistical Analysis

Graphs and plots were made in RStudio. Only samples with concentrations above the analyte-specific limit of detection (LOD) in ≥ 40 % of the samples were included in tables, plots and graphs.

From a total of 72 Eagle-Owl feathers collected, one individual from Southern Norway was sampled in two different years. To avoid increased imbalance in the number of samples from this area, the sample collected in the year with fewest samples was selected for the statistical

analysis. Furthermore, one sample was from an unknown sampling year (from inland Central Norway) and was excluded from statistical analysis since differences in PFAS levels between years were investigated. The influence of time (year) was also investigated independently of areas.

When testing for differences between sex, year and areas, data that passed Shapiro-Wilk's test for normality and Levene's test for homogeneity were tested using analysis of variance (ANOVA) and Tukey's Honest significance test (HSD). If the data failed one or both of the mentioned normality and homogeneity tests, data was analyzed using Kruskal-Wallis test and Dunn's Post hoc test (Zar, 1999, Ruxton and Beauchamp, 2008). Differences in contaminant levels between sexes were investigated using Welch's t-test. The stable isotope data were not normally distributed. Correlations were therefore performed using Spearman's rank correlation which is more robust when analyzing non-parametric data (Gibbons and Chakraborti, 2011). Based on the results from the correlation matrices, statistically significant correlations were examined using linear regression and boxplots.

3. Results

3.1 Influence Of Sampling Year And Sex On Pfass Levels In Eagle-Owl Feathers

Median concentrations and ranges of PFASs measured in feathers of Eagle-Owls are presented in Table 3 for each collection area (Southern Norway (2013-2016), coastal Central Norway (1989, 2013-1016), inland Central Norway (2014-2015) and Northern Norway (1979, 2013-2016)). The mean concentrations and individual concentrations can be found in Appendix (Table A.14). No significant differences were observed in PFAS levels between sampling years (ANOVA, F=1.69, *p*=0.15) nor between years within each area (ANOVA, F=0.67, *p*=0.7) (Figure 2Figure **2**. However, the earliest sampling year (1979) had among the lowest concentrations in all four regions.

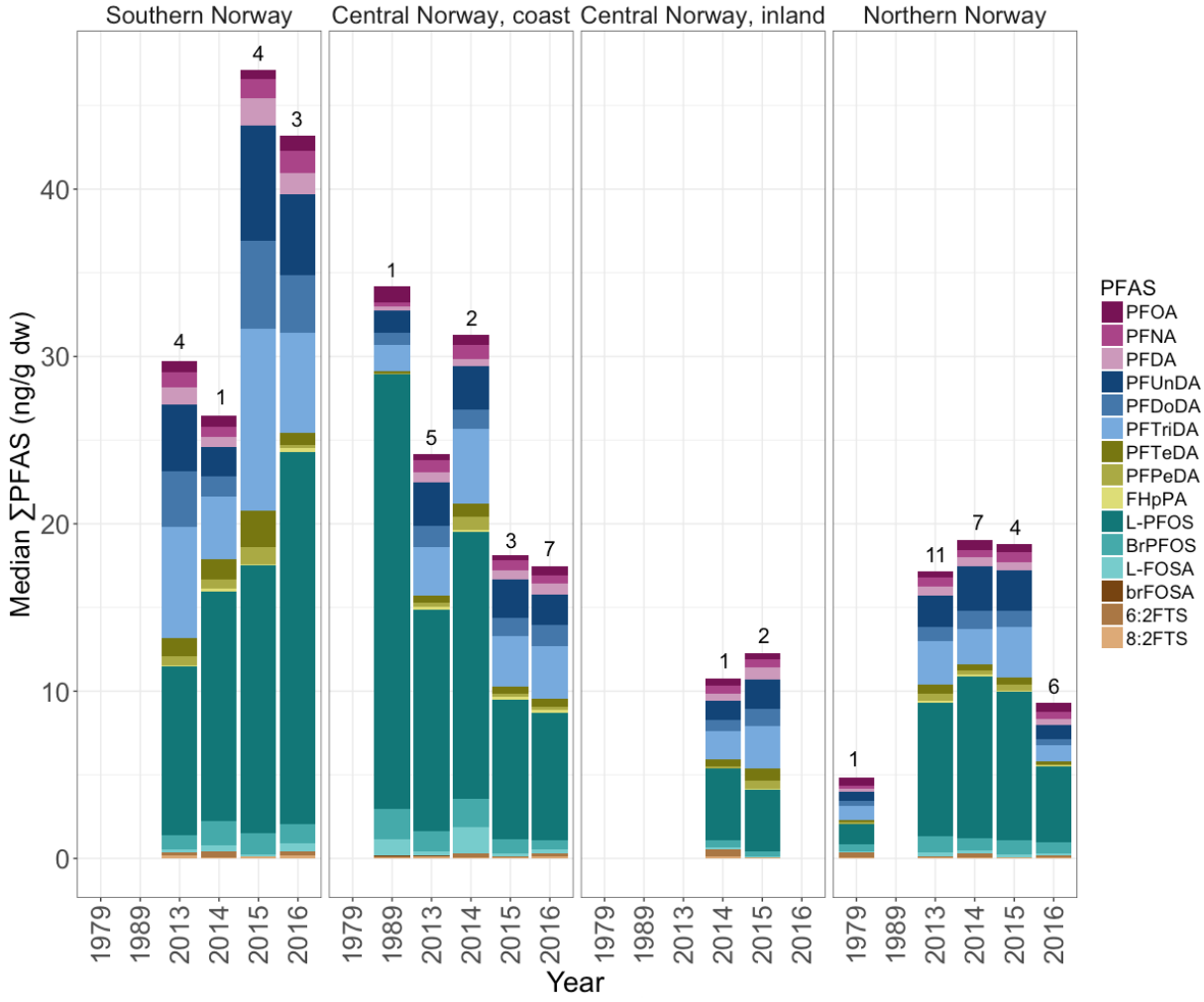

Figure 2 Composition profiles (ng/g dry weight) of selected PFASs in Eurasian Eagle-Owl feathers from Southern Norway (n= 12), Central Norway, coast (n = 18), Central Norway, inland (n = 3), and Northern Norway (n = 37). Y-axis represents mean ∑PFAS concentrations. Figure above each bar indicates number of samples per corresponding year.

Table 3 Concentrations (ng/g dw) of perfluoroalkyl substances (PFASs) in Eagle-Owl feathers collected in four different areas and 6 different year (1979, 1989, 2013-2016). Median. minimum (min) and maximum (max) concentrations (ng/g dw) of PFASs detected in >40 % of the samples, and stable isotope values (‰) in Eagle-Owl feathers from four areas in Norway.

	Southern Norway			**Central Norway. coastal**			**Central Norway. inland**			**Northern Norway**		
n	**12**			**18**			**3**			**37**		
	Median	**Min**	**Max**	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**
δ^{13}**C**	-22.32	-23.82	-18.84	-20.57	-23.79	-16.65	-22.46	-22.47	-17.69	-22.08	-25.62	-18.32
δ^{15}**N**	7.74	4.84	12.94	12.30	6.22	15.80	9.11	7.24	15.29	11.83	9.79	14.18
PFOA	0.65	0.44	1.10	0.39	0.22	1.02	0.37	0.36	0.45	0.51	<0.20	1.08
PFNA	1.02	0.41	2.13	0.60	0.27	1.36	0.44	0.38	0.61	0.51	<0.20	1.74
PFDA	1.15	0.27	2.67	0.59	0.22	1.95	0.52	0.42	0.94	0.48	0.11	1.85
PFUnDA	4.56	1.60	10.90	2.20	0.90	4.44	1.58	1.15	1.85	1.84	<0.10	9.33
PFDoDA	3.77	1.16	7.85	1.13	0.58	2.67	0.95	0.67	1.12	0.85	<0.10	3.25
PFTriDA	7.19	2.79	19.76	2.98	1.42	11.99	2.30	1.70	2.76	2.10	<0.20	9.37
PFTeDA	1.27	0.57	4.65	0.44	0.16	2.39	0.44	0.36	1.05	0.40	<0.10	1.89
PFPeDA	0.52	<0.10	2.83	0.17	0.01	1.55	0.21	0.12	0.80	0.25	<0.30	1.73
∑PFCAs	20.13	8.45	51.38	8.33	4.88	24.72	7.12	5.39	9.06	7.19	2.64	29.72
FHpPA (7:3 FTA)	0.08	0.03	0.75	0.17	0.02	0.84	0.03	0.01	0.06	0.07	0.01	0.23
L-PFOS	12.42	1.61	51.41	9.40	3.43	73.91	4.28	1.67	5.76	8.54	0.76	37.41
Br-PFOS	1.15	0.27	2.13	0.85	0.43	4.91	0.35	0.24	0.42	0.78	0.34	1.71
L-PFDS	0.11	0.01	0.96	0.11	0.01	0.41	0.03	0.01	0.06	0.04	0.006	0.26
∑PFSAs[2]	13.94	1.90	53.05	10.13	4.05	79.23	4.71	1.93	6.17	9.38	1.46	39.34
L-FOSA	0.18	<0.02	0.82	0.22	0.09	2.92	0.08	<0.02	0.10	0.19	<0.02	1.72
Br-FOSA	0.02	<0.02	0.07	0.02	0.005	0.08	0.01	<0.02	0.03	0.02	<0.02	0.07
6:2FTS	0.07	0.004	0.58	0.05	0.002	0.45	0.04	0.01	0.44	0.05	0.003	1.33
8:2FTS	0.17	0.03	0.54	0.11	0.02	0.52	0.06	0.04	0.09	0.07	0.01	0.39
∑PFAS	41.59	13.05	87.78	21.37	11.07	98.59	10.77	9.20	15.41	17.48	4.37	69.61

[1]∑PFCA: PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFPeDA. [2] ∑PFSA: L-PFOS, Br-PFOS.L-PFDS

Due to the potential of maternal transfer of contaminants to eggs, sex-dependent differences in sum PFAS (∑PFASs) concentrations were examined. No differences in feather concentrations of ∑PFASs were detected between males and females (Welch Two Sample t-test, t=-0.037, p=0.97) (Figure 3).

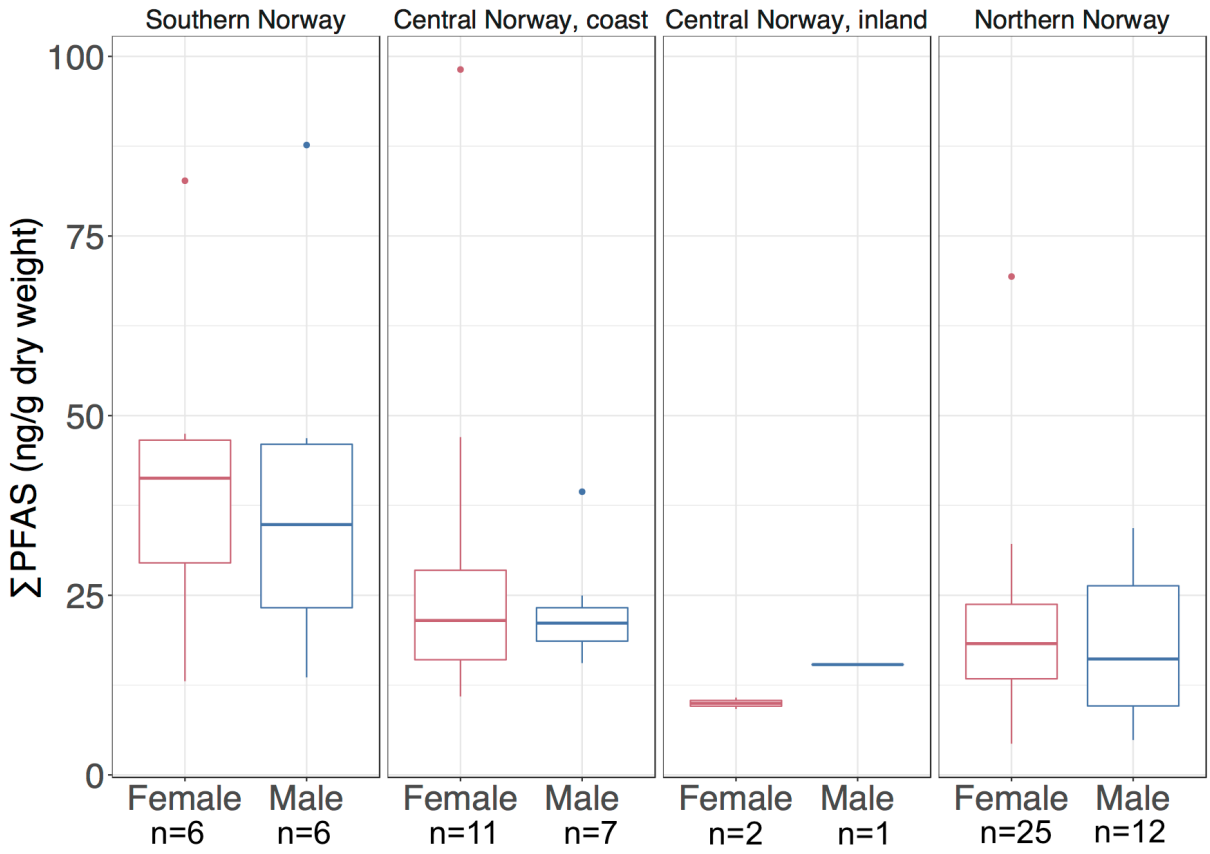

Figure 3 Box and whisker plots of concentrations of ∑PFAS (ng/g dw) within each sex from Southern Norway, Central Norway- coast, Central Norway - inland, and Northern Norway. The Y-axis shows the natural log-transformed ∑PFASs concentration (ng/g dw). The X-axis corresponds to the sex within each area. Horizontal lines represent the median values. Boxes correspond to the 25th – 75th percentiles. Whiskers represent 95 % confidence intervals. Outliers are presented as dots.

3.2 Differences In PFAS Levels Between Sampling Areas

The levels of PFASs were generally higher in Southern Norway and coastal Central Norway. Feathers collected in Southern Norway (median ∑PFASs=41.08 ng/g dw) displayed more than two times higher median concentration of ∑PFASs than that of feathers collected in Northern Norway (median ∑PFAS=17.46 ng/g dw, ANOVA, Tukey's HSD test, F= 6.35, p<0.01) and 3.5 times the median concentrations detected in feathers from inland Central Norway (median ∑PFAS=10.76 ng/g dw), p<0.05) (Table 3, Figure 2). Although not statistically significant (p=0.23) , it appeared that the levels of PFASs in coastal Central Norway (median

∑PFAS=21.30 ng/g dw) were higher than in Northern Norway. The lowest ∑PFAS concentration was found in feathers from inland Central Norway, however the sample size was very small from this area (n=3) (Figure 2). Overall, the pattern of mean ∑PFASs was Southern Norway (n = 12) > Central Norway (coastal) (n = 18) > Northern Norway (n = 37) > Central Norway (inland) (n =3). The highest concentration of ∑PFASs was measured in a feather from a female from Frøya, 2013 (97.14 ng/g dw), while the lowest concentration was measured in a feather collected from a male in Lurøy, 2015 (2.70 ng/g dw) (Tabell 3, Appendix Table A.13).

Overall, linear PFOS (L-PFOS) was the most prominent PFAS in the samples from Northern Norway (47.2 %) and coastal Central Norway (48.5%) (Southern Norway: 36.5 %, Central Norway (inland): 31.8 %). The highest median concentration of L-PFOS was detected in feathers from Southern Norway (12.24 ng/g [1.61 – 51.41 ng/g]), while the individual feather sample with the highest L-PFOS concentration was collected in coastal Central Norway (73.91 ng/g dw; Table 1). Linear and branched PFOS (br-PFOS) were the only PFSAs that were detected above LOD in all samples. The ratio between L-PFOS and Br-PFOS was about 90/10 for all samples. No significant differences in L-PFOS (ANOVA, p=0.056, F=2.6363) were detected between the four different areas, nor the sum of L-PFOS and branched PFOS (∑PFOS, p>0.05, Figure 4). The median levels of linear PFDS (median 0.06 ng/g dw) only contributed to 0.6% of the total PFSA (median, 9.89 ng/g dw) load. PFDS was detected in 42 % and 8% of the samples for the linear and branched isomer, respectively. Linear and branched PFHxS (Perfluorohexane sulfonic acid) were only detected in 4 % and 3 % of the samples respectively.

Other PFASs were detected at lower concentrations and less frequently than PFOS and the long chained PFCAs (Figure 2). The intermediate transformation product FHpPA (7:3FTA) was detected in 42 % of the samples. Highest median concentration of FHpPA (7:3FTA) was measured in feathers from coastal Central Norway (0.17 ng/g, Table 1). The other fluorotelomeric acid that was analyzed for in this study, FPePA (5:3 FTA), was only detected in 6 % of the feathers. The PFOS precursor linear FOSA (Perfluoroctane sulfonamide) was found in about 85 % of the samples, and branched FOSA was detected in 49 % of the samples (Appendix Table A. 9). While 4:2FTS (Sodium 1H,1H,2H,2H-perfluorohexane sulfonate (4:2))was not detected in any of the feather samples, 6:2 FTS (Sodium 1H,1H,2H,2H-perfluorooctane sulfonate(6:2)) (<0.05 - 1.33 ng/g dw) and 8:2FTS (Sodium 1H,1H,2H,2H-perfluorodecane sulfonate(8:2)) (<0.1 – 0.54 ng/g dw) were above LOD in 46 % and 39 % of the feather samples, respectively (Table A. 9, Table A. 12, Appendix).

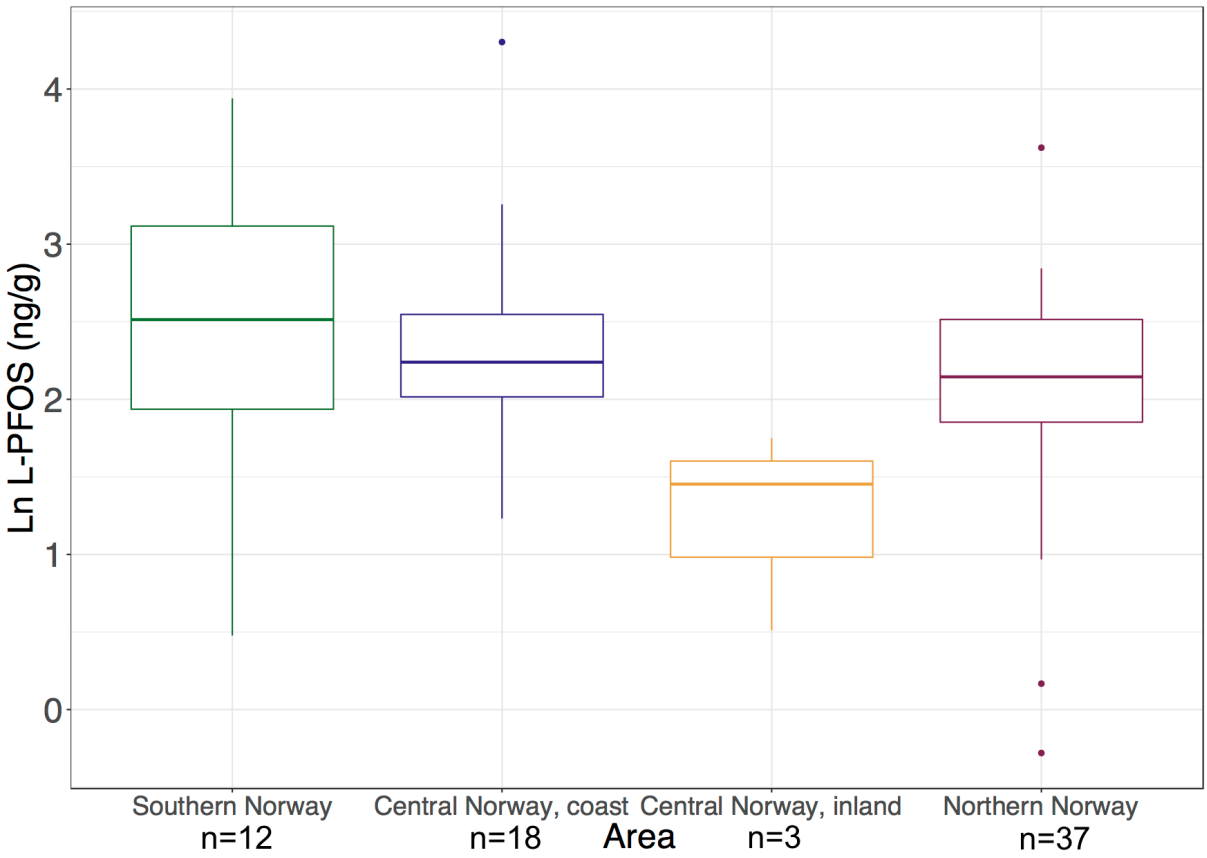

Figure 4 Box plot of $\log_e$-transformed concentration of ln L-PFOS (ng/g dw) in feathers from Southern Norway, Central Norway, coast, Central Norway, inland, and Northern Norway. Horizontal lines represent the median values. Boxes correspond to the 25th – 75th percentiles. Whiskers represent 95 % confidence intervals. Outliers are presented as dots.

The second most prevalent compounds were the longer chained PFCAs, PFTriDA. PFUnDA (Perfluoroundecanoic acid) and PFDoDA (Perfluorododecanoic acid) (Table 3, Figure 2).The sumPFCAs (∑PFCAs) comprised between 42.8 % (Northern Norway) and 59 % (Central, Norway, inland) of the total ∑PFASs. In the feathers from Southern Norway, ∑PFCAs were the most prominent PFASs, accounting for 56.3 % of the total PFAS load. The median ∑PFCAs concentration was also highest in Southern Norway (20.13 ng/g dw) (Figure 5). For all areas, PFTriDA was the dominating long-chained PFCA, with median levels of PFTriDA being 7.19 ng/g in Southern Norway, 2.98 ng/g in 2.30 ng/g in Inland Central Norway and 2.10 ng/g Northern Norway (Table 3). The levels of PFTriDA were significantly different between Southern Norway and the other areas (ANOVA Tukey's HSD, F (3,66)= 11.15, Coastal Central Norway: p<0.01; Inland Central Norway: p=0.03; Northern Norway, p<0.001). Levels of PFDoDA were also significantly different between Southern Norway and Northern Norway (ANOVA Tukey's HSD, F (3,66)= 16.25 , p<0.001), as well as between Southern Norway and

coastal Central Norway *(p*<0.05). In all areas there was an odd-even chain-length pattern for some of the PFCAs, where the concentrations of the even-numbered PFCAs were lower than the adjacent odd-numbered PFCAs. This was evident for PFUnDA>PFDA (Perfluorodecanoic acid), PFTriDA>PFDoDA, but not for PFPeDA (Perfluoropentadecanoic acid) > PFTeDA.

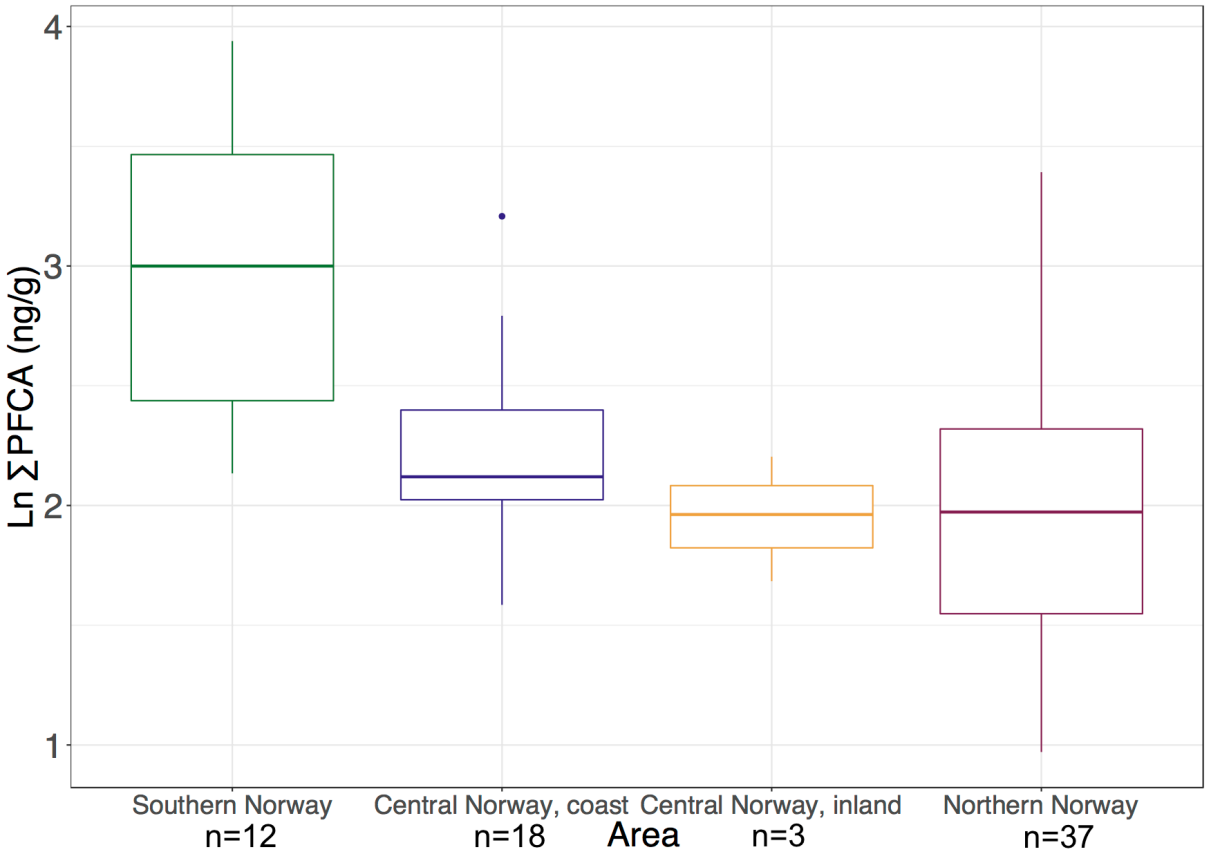

Figure 5 Box plot of $\log_e$-transformed concentrations of ∑PFCAs (ng/g dw) in feathers from Southern Norway, Central Norway, coast, Central Norway, inland, and Northern Norway. Horizontal lines represent the median values. Boxes corresponds to the 25th – 75th percentiles. Whiskers represent 95 % confidence intervals. Outliers are presented as dots.

PFOA was detected in 97 % of the samples and in all areas (Table A. 9, Appendix). Median concentrations of PFOA were 0.65 ng/g dw in Southern Norway, 0.39 ng/g dw in Coastal Central Norway, 0.37 ng/g dw in Inland Central Norway and 0.51 ng/g dw in Northern Norway. No significant differences in PFOA levels were observed between areas (ANOVA Tukey's HSD, F (3,66)=2.77, *p>0.05)*.

3.3 Isotopic Values

Trophic position and foraging habitats of the Eagle-Owl were assessed through determination of δ^{15}N and δ^{13}C in feathers (Table 3, Figure 6, Figure 7).

Figure 5 shows the isotopic values in the feathers from the different areas. Stable carbon isotope values of feathers ranged widely, varying between -23.82 and -18.84 ‰ in Southern Norway, -23.79 and - 16.65 ‰ in coastal Central Norway, -22.46 and – 17.69 ‰ in inland Central Norway, and - 25.62 and -18.32 ‰ in Northern Norway (Table 3). Stable nitrogen values were also variable within and among locations, ranging from 4.84 (Southern Norway) to 15.80 ‰ (coastal Central Norway) (Table 3).

Stable isotope values were compared between sampling areas to identify any diverging trends in trophic behavior between owls breeding in different areas in Norway. The stable nitrogen isotopes were significantly lower in the feathers from Southern Norway than in the feathers from coastal Central Norway (Kruskal-Wallis and Dunn's post hoc test (KW), $\chi^2(2) = 2.46$ $p>0.05$) and Northern Norway, respectively (KW, $\chi^2(2) = 2.68$, $p<0.05$). No significant differences in stable nitrogen signatures were detected between the other areas. Regarding the δ^{13}C values, significantly less negative δ^{13}C were detected in coastal central Norway as compared with Southern Norway (KW, $\chi^2(2)=2.05$, $p<0.05$) and Northern Norway (KW, $\chi^2(2) = 2.53$, $p<0.05$).

A significant difference in δ^{15}N was observed between sexes (Mann Whitney U-test (MWU), U = 742, $p<0.05$), with females having a slightly higher δ^{15}N ratio than males. No significant differences in δ^{13}C values were detected between female and male owls (MWU, U= 707, $p = 0.1$). However, it is important to note that the number of females and males was unbalanced for some areas (Table 1). No significant difference in either stable isotope values between sampling years was observed in the feathers (δ^{15}N :KW, $-0.047 < \chi^2(2) < 2.89$, $p\geq 0.5$ for all years ; δ^{13}C: $-0.98 < \chi^2(2) < 2.68$, $p>0.1$).

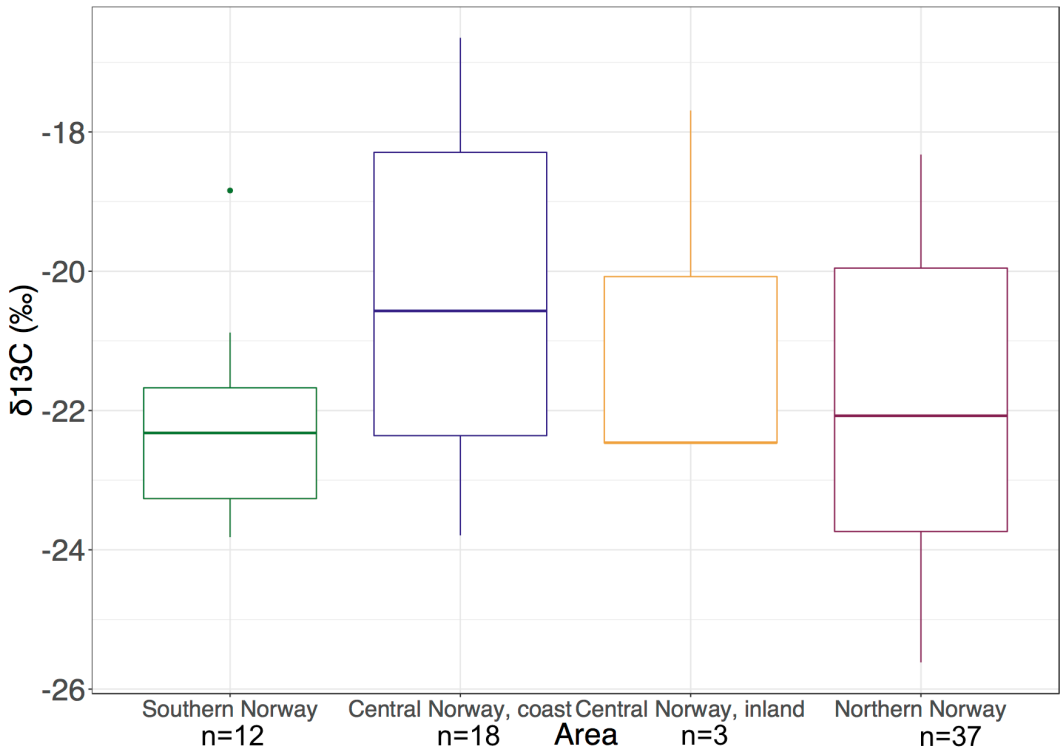

Figure 6 Box plot of $\delta^{13}C$ (‰) in feathers from Southern Norway, Central Norway, coast, Central Norway, inland, and Northern Norway. Horizontal lines represent the median values. Boxes corresponds to the 25th – 75th percentiles. Whiskers represent 95 % confidence intervals. Outliers are presented as dots.

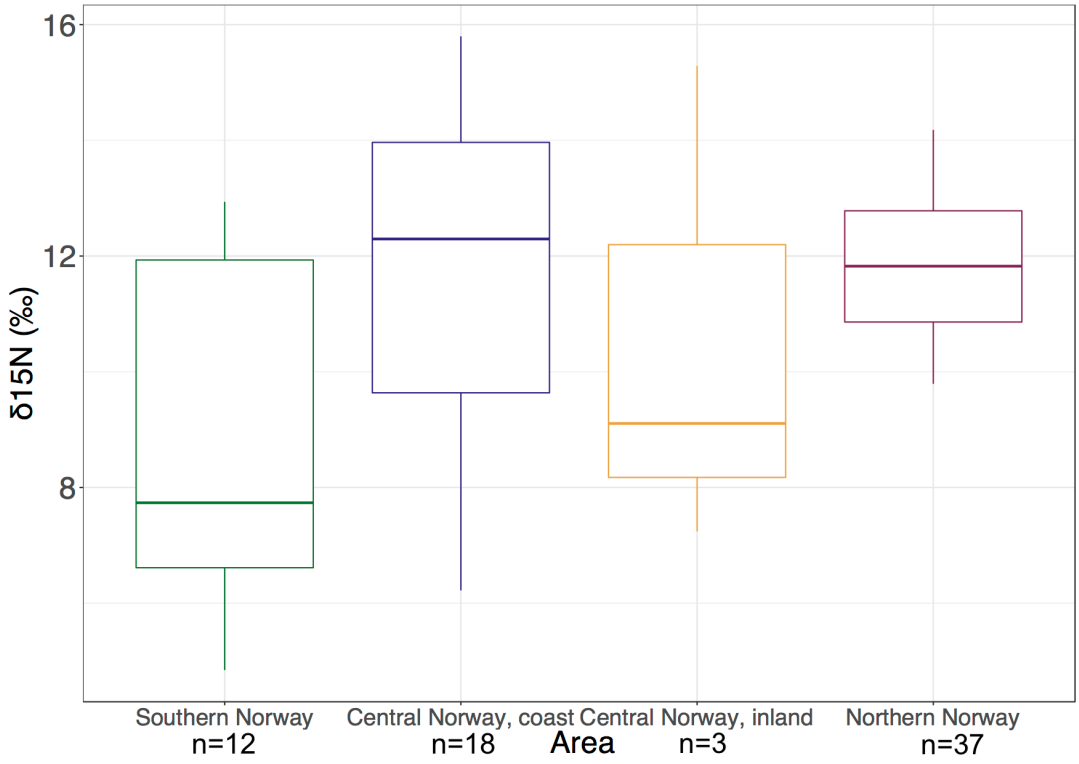

Figure 7 Box plot of $\delta^{13}C$ (‰) in feathers from Southern Norway, Central Norway, coast, Central Norway, inland, and Northern Norway. Horizontal lines represent the median values. Boxes corresponds to the 25th – 75th percentiles. Whiskers represent 95 % confidence intervals. Outliers are presented as dots.

3.4 Trophic Positions, Feeding Habits And Contaminant Bioaccumulation

A significant and positive correlation between $\delta^{13}C$ and $\delta^{15}N$ was observed in feathers from all areas (Spearman's rank correlation, $0.81 < r_s < 0.83$ and $p<0.01$, Table 4, Appendix Figure A1), except for feathers from Southern Norway ($r_s = 0.29$, $p=0.37$, Table 4). An overlap in isotopic values among sampling areas was also observed, and made it possible to divide the sampled feathers into three groups according to their stable isotope ratios (Figure 8). However, no significant differences were detected between levels of PFASs between these groups.

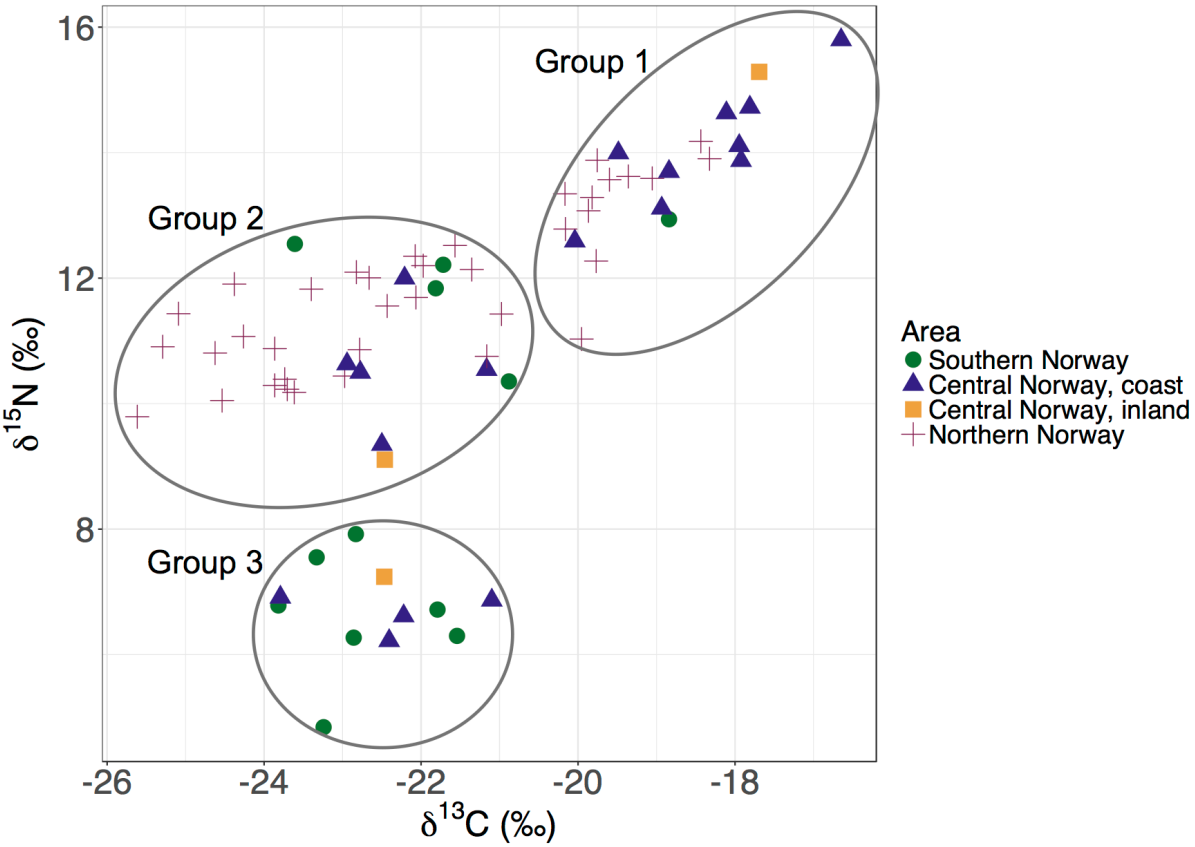

Figure 8: $\delta^{13}C$ and $\delta^{15}N$ (‰) in individual Eagle-Owl feathers collected in four different areas across Norway. The individual isotope values are represented by points in different shapes and colors according to the areas where the feather was collected. The circles represents individual feathers grouped according to their feeding habits ($\delta^{13}C$) and trophic position($\delta^{15}N$).

Table 4: Spearman's rank correlations between PFASs and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). Significance levels is set to α=0.05. Values in bold are significant.

Area[a]	**Southern Norway** n=12				**Central Norway, coastal** n=18				**Northern Norway** n=37			
	***δ*13C**		***δ*15N**		***δ*13C**		***δ*15N**		***δ*13C**		***δ*15N**	
PFAS	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*
PFOA	0.17	0.6	0.18	0.57	0.14	0.59	0.02	0.94	-0.03	0.85	-0.1	0.55
PFNA	0.1	0.75	-0.03	0.91	0.14	0.58	0.17	0.5	0.56	**<0.01**	0.45	**<0.01**
PFDA	0.09	0.78	0.01	0.97	-0.11	0.65	0.02	0.95	0.5	**<0.01**	0.43	**0.01**
PFUnDA	-0.1	0.76	-0.27	0.39	-0.05	0.84	0.13	0.6	0.43	**0.01**	0.4	**0.01**
PFDoDA	-0.01	0.98	-0.13	0.68	0.03	0.91	0.24	0.34	0.46	**<0.01**	0.48	**<0.01**
PFTriDA	-0.03	0.91	-0.22	0.48	-0.11	0.65	0.05	0.83	0.47	**<0.01**	0.44	**0.01**
PFTeDA	-0.07	0.83	-0.13	0.68	-0.11	0.66	-0.06	0.82	0.35	**0.04**	0.3	0.07
PFPeDA	0.14	0.66	-0.06	0.85	0.14	0.59	0.28	0.25	0.07	0.68	0.01	0.96
∑PFCA	-0.04	0.9	-0.19	0.56	-0.09	0.74	0.04	0.86	0.46	**<0.01**	0.43	**0.01**
FHpPA	0.55	0.07	0.14	0.66	0.04	0.88	-0.1	0.69	0.21	0.22	0.13	0.43
LPFOS	0.21	0.51	0.34	0.28	-0.18	0.48	-0.19	0.45	0.39	**0.02**	0.43	**0.01**
BrPFOS	0.4	0.2	0.62	**0.03**	0.2	0.43	0.06	0.82	0.29	0.08	0.14	0.42
L.PFDS	0.50	0.10	0.40	0.20	-0.09	0.72	-0.14	0.58	0.10	0.56	-0.03	0.85
∑PFSA	0.21	0.51	0.34	0.28	-0.18	0.48	-0.20	0.43	0.39	**0.02**	0.42	**0.01**
LFOSA	0.62	**0.03**	0.48	0.11	0.16	0.54	0.29	0.24	0.41	**0.01**	0.63	**<0.01**
brFOSA	0.19	0.56	-0.12	0.71	0.07	0.79	-0.05	0.84	-0.15	0.38	0.1	0.55
6.2FTS	0.37	0.24	0.2	0.53	0.11	0.67	0.08	0.75	0.06	0.71	0.14	0.4
8.2FTS	0.51	0.09	0.25	0.43	0.19	0.45	0.25	0.32	0.07	0.68	0.06	0.73
∑PFAS	0.17	0.6	0.07	0.83	-0.19	0.46	-0.11	0.66	0.44	**0.01**	0.43	**0.01**
*δ*13C	1	-	0.29	0.37	1	-	0.84	**<0.01**	1	-	0.81	**<0.01**
*δ*15N	0.29	0.37	1	-	0.84	<0.01	1	-	0.81	0	1	-

[a]Spearman's rank correlation was not possible for Central Norway, inland, due to limited sample size (n=3).

With all areas, years and sexes combined, ∑PFASs concentrations were not significantly correlated with $\delta^{15}N$ nor the $\delta^{13}C$ values in the feathers ($\delta^{13}C$: r_s= 0.22, p=0.06 ; $\delta^{15}N$: r_s=0.04, p=0.76). Both $\delta^{15}N$ and $\delta^{13}C$ showed a significant positive correlation between ∑PFASs in feathers from Northern Norway, but not for the other areas (Table 4, Figure 10, Figure 11). The stable isotopes were also significantly correlated with ∑PFOS (L-PFOS+Br-PFOS), and all PFCAs, except PFOA, PFTeDA and PFPeDA, in the samples from Northern Norway. The stable nitrogen values and L-FOSA concentrations were significantly correlated in Northern Norway (r_s=0.41, $p \leq 0.01$). A positive correlation was also observed between $\delta^{13}C$ and L-FOSA in Southern Norway (r_s=0.62 , p=0.03). Due to the small sample size (n=3) it was not possible to perform correlations test on the samples from inland Central Norway (Figure 10, Figure 11). Stable carbon isotope values and L-FOSA were significantly correlated in all areas ($0.41<r_s<0.62$, $0.01<p<0.03$) except in coastal Central Norway (r_s=0.16, $p>0.54$). Branched PFOS displayed a significant positive correlation with $\delta^{15}N$ (r_s=0.62, $p>0.03$) in the feathers from Southern Norway, but not with the carbon isotope.

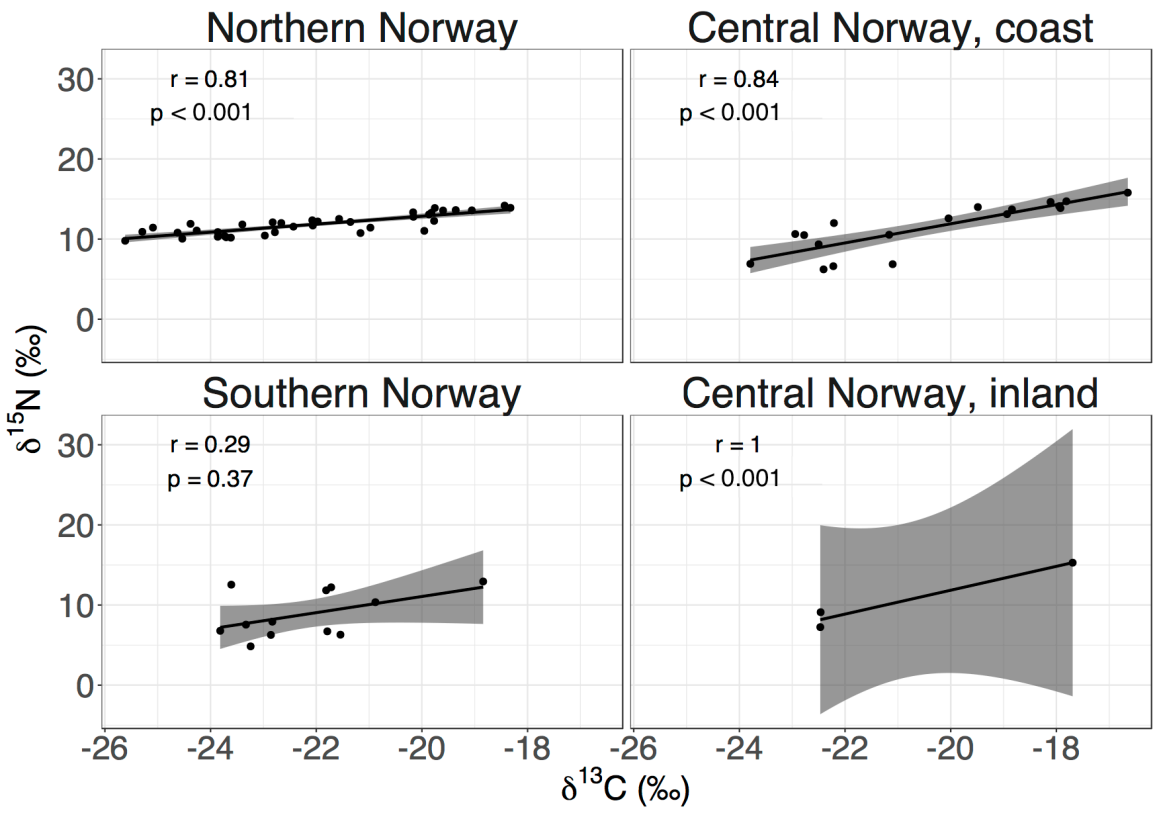

Figure 9 Relationships between stable carbon isotope ratios , $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰} in central Norway (coast), Central Norway (inland), Northern Norway and Southern Norway. The Spearman rank correlation was performed for all four areas. Grey areas represent 95 % confidence intervals. Dots represents individual samples. Significance level was set to $\alpha = 0.05$.

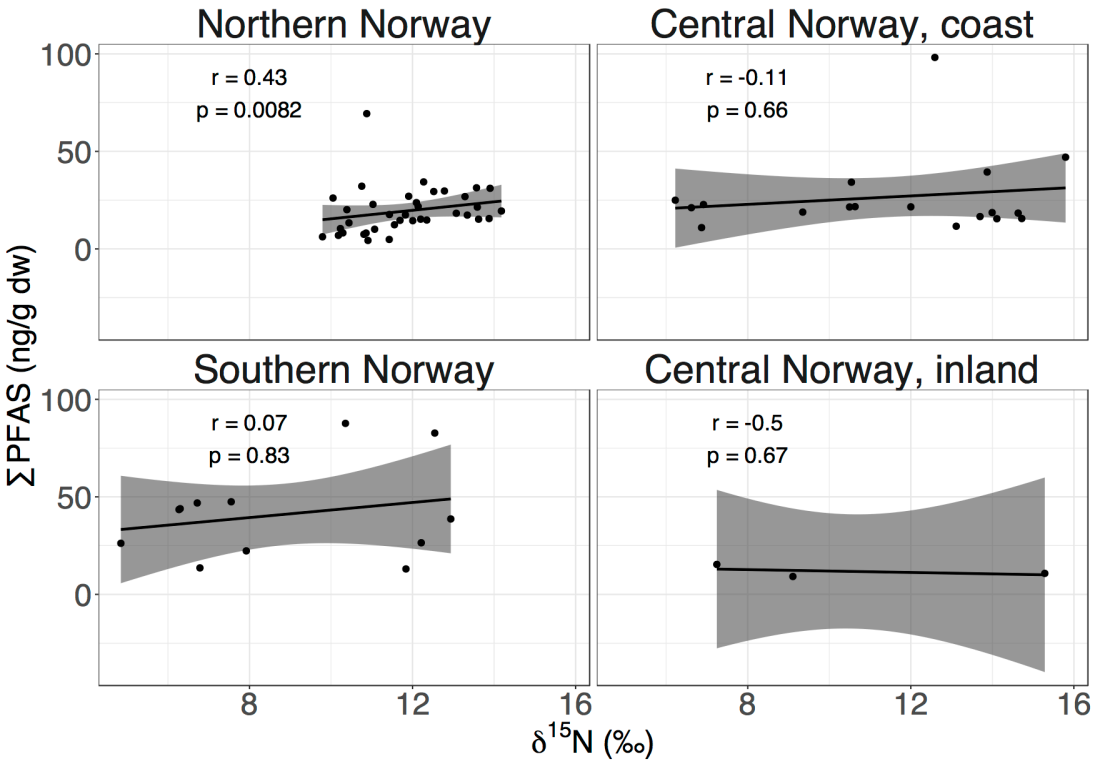

Figure 10 Relationship between ∑PFAS and $\delta^{15}N$ (‰}. The Spearman rank correlation was performed for all four areas. Grey areas represent 95 % confidence intervals. Dots represents individual samples. Significance level was set to $\alpha = 0.05$.

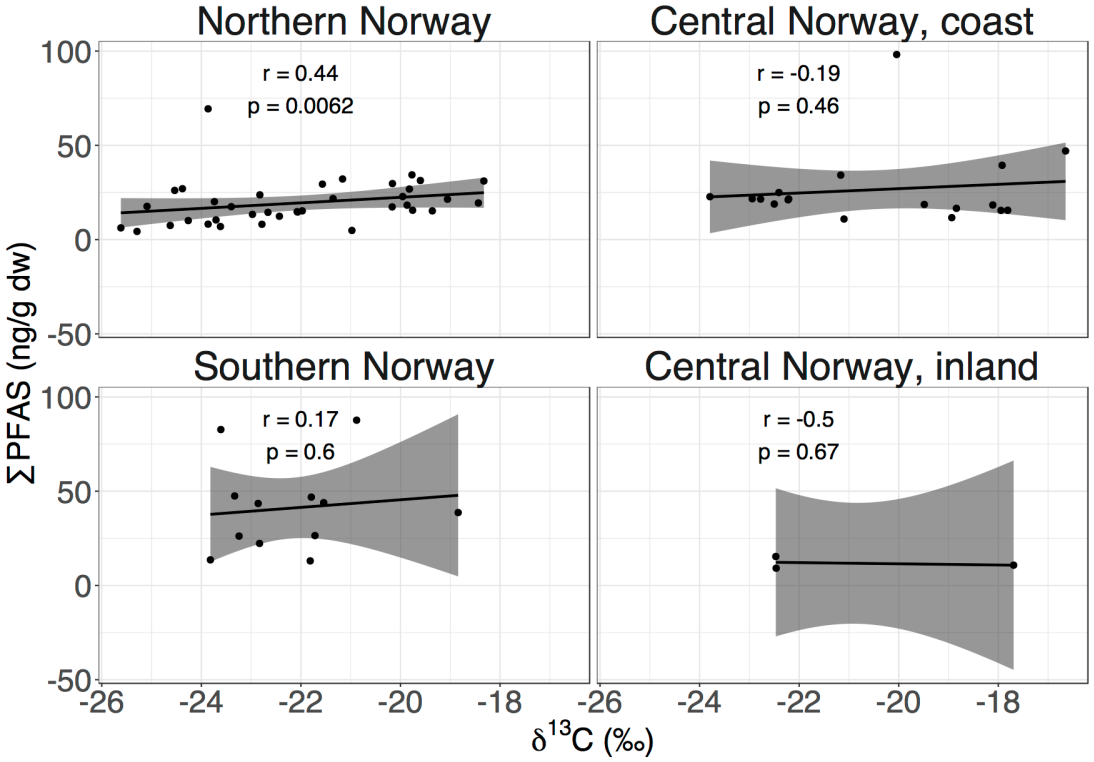

Figure 11 Relationship between ∑PFAS and δ13C (‰}. The Spearman rank correlation was performed for all four areas. Grey areas represent 95 % confidence intervals. Dots represents individual samples. Significance level was set to $\alpha = 0.05$.

4. Discussion

Contaminant bioaccumulation and the relationship with trophic habits in feathers from Eagle-Owls was investigated. Feathers provide the opportunity to examine the relationship between contaminants and trophic ecology of the Eagle-Owl, between years and sampling areas in Norway.

4.1 Levels Of PFASs

4.1.1 Influence Of Sampling Year And Sex On Pfass Levels In Eagle-Owl Feathers

No significant differences were found in PFAS levels between sexes of the Eagle-Owls. These findings are consistent with a general lack of sex differences for PFAS concentrations in wildlife (Leat et al., 2013, Lucia et al., 2017, Routti et al., 2016, Butt et al., 2007b, Verreault et al., 2005a). The reason for this is unclear. In the current study, the low sample size and unbalanced ratio between sexes may have failed to detect significant differences in contaminant concentrations. More research is needed to confirm potential sex differences for PFAS accumulation in birds.

The influence of time (sampling year) was investigated independently of sampling area and within each area, but no differences for PFASs were detected between years. The earliest sampling year (1979) had among the lowest concentrations in all 4 regions. This is consistent with what is known about the manufacturing of these substances. According to Paul et al. (2008) and Prevedouros et al. (2006) environmental monitoring showed a strong upward trend of PFASs in biota from the 1970s as a result of increased use and emissions. The present study only covered six years of sampling, and the sample size for each year was both small and unbalanced. The short sampling period might also affect the results. However, contaminant exposure can be affected by temporal variations of PFAS concentration within the Eagle-Owl population and may confound observed spatial trends in PFAS contamination.

4.1.2 Differences In PFAS Levels Between Sampling Areas

Overall, L-PFOS was the predominant PFAS detected in all feather samples. The overall predominance of L-PFOS in all samples is in accordance with the reported high occurrence of this compound in previous wildlife studies, including studies on birds of prey (Eriksson et al., 2016, Lind, 2012, Jaspers et al., 2013, Sletten et al., 2016). No significant differences for PFOS

levels, neither for the linear or branched isomer, were detected when comparing the different PFOS accumulation between areas. Lin-PFOS was found in higher concentrations than br-PFOS. High proportions of lin-PFOS have been observed in previous bird studies (Gebbink et al., 2011, Gebbink and Letcher, 2010). PFOS is typically manufactured in a 70%linear and 30% branched mix. The isomer ratio in the feathers from the present study was approximately 90% linear and 10 % branched for all samples. Higher levels of the linear isomer in biota and throughout the food web could be the result of a combination of the isomer profile as manufactured, preferential uptake of the linear isomer and preferential excretion of the branched isomers (Eriksson et al., 2016, Gebbink and Letcher, 2010, Houde et al., 2008, Benskin et al., 2010, De Silva et al., 2009, Benskin et al., 2009). Despite regulations and restrictions on use and production of PFOS in Europe and North America, China has continued to produce PFOS for use in industry (Chen et al., 2009, Xie et al., 2013, Wang et al., 2014). Increased production in China may depreciate the progress in reducing PFOS accumulation in the environment and biota accomplished through phase-outs and restrictions (Miller et al., 2015).

The concentrations in this study are comparable to the PFOS levels found in tail feathers of Belgian barn-owls (*Tyto alba*), but lower than in feathers from grey heron (*Ardea cinerea*), herring gull (*Larus argentatus)* and Eurasian sparrowhawk (*Accipiter nisus*) also sampled in Belgium (Meyer et al., 2009, Jaspers et al., 2013). PFOS levels in liver and feathers of the Belgian barn-owls were highly correlated (Jaspers et al., 2013), and the median concentration of PFOS in feathers from these owls were 15.8 ng/g ww which is similar to the levels detected in the feathers from Eagle-Owls. Median PFOS levels in the Eagle-Owl feathers were also in the same range as in studies from other terrestrial environments, such as in tawny owl eggs from Norway and Sweden (Eriksson et al., 2016, Ahrens et al., 2011), still the levels in the feathers of the current study were mostly lower than median PFOS levels in livers from Eagle-Owls (Lind, 2012). Levels of linear PFDS showed quantifiable levels at low concentrations in the feathers, which is similar to what was detected in plasma, red blood cells and muscle in Great Lakes herring gulls (Gebbink and Letcher, 2012). Gebbink et al. (2012) also found that PFDS was preferentially accumulated in the brain of these gulls, which might explain the low levels of this compound in the feathers. It is imporatn to note that PFAS profiles are tissue-specific with large variations between tissues, thus comparisons of PFAS levels in feathers and other tissues are not directly comparable (Nordén et al., 2013)

In Eagle-Owl liver FOSA concentrations were similar or higher than in feathers in the present study (Lind, 2012), while FOSA was not detected in feathers from barn-owls (Jaspers et al., 2013). Linear FOSA was detected in ~85 % of the samples, while br-FOSA was detected in ~49 % of the samples. Biotransformation of FOSA precursors (N-ethyl perfluorooctanesulfonamides) to FOSA (Letcher et al., 2014) and FOSA to PFOS have been demonstrated in livers of several vertebrate species, such as Sprague-Dawley rats (*Rattus norvegicus*) and polar bears (*Ursus maritimus*) (Ross et al., 2012, Greaves and Letcher, 2013). This suggests that some of the FOSA present in the blood during feather growth might not have been metabolized or degraded to PFOS in the Eagle-Owls, but rather excreted to the feathers. However, no information is available on concentrations at lower trophic levels, thus it is not possible to assess whether the levels of FOSA are attributable to metabolism of this PFAS or to low environmental exposure levels.

The 6:2FTS and 8:2 FTS were detected in 46 % and 39 % of the samples, respectively, but no significant differences in levels of these compounds were found between areas. The 6:2 FTS may be a precursor to PFHxA (Key et al., 1998) which was not detected in any of the feather samples. Eriksson et al. (2016) suggested that the occurrence of 6:2FTS could be a result of local aqueous film– forming foams (AFFFs) contamination and subsequent bioaccumulation in ospreys (*Pandion haliaetus*). The levels of 6:2 FTS in the feathers were lower than in eggs from both tawny owls and ospreys from Sweden (Eriksson et al., 2016). But the distribution pattern of 6-.2 FTS in feathers and eggs might not be comparable due to the difference in composition of these two matrices. The toxicological implications of 6:2 FTS accumulation are uncertain. Laboratory studies have reported that 6:2 FTS is not bioaccumulative in either fish or rats (Serex et al., 2008, Hoke et al., 2015), and is unlikely to undergo aquatic foodchain biomagnification in rainbow trout (*Onchorhynchus mykiss*) (Yeung and Mabury, 2013). Low levels of 6:2 FTS, 8:2 FTS and FHpPA (7:3 FTOH) might result in metabolism of these precursors to PFCAs (Gebbink and Letcher, 2012, Martin et al., 2005).

Median ∑PFCA levels in the feathers were significantly higher in Southern Norway than in the other areas. The predominating PFCA was PFTriDA for all areas. The dominance of PFTriDA and long-chained PFCAs has previously been observed in Swedish and Norwegian tawny owl

eggs (Eriksson et al., 2016, Ahrens et al., 2011) and Eagle-Owl livers (Lind, 2012). Sparrowhawk eggs from Agder in Southern Norway displayed high proportions of PFCAs (55% of the total ∑PFAS) (Herzke et al., 2015). This is in accordance with the present study. Higher proportions of PFCAs may be related to local pollution sources, such as firefighting practice grounds or ski wax which is known to contain high proportions of these compounds (Nilsson et al., 2010).

Detection of long-chained PFCAs may be explained by the fact that bioaccumulation of PFCAs increases with increasing chain length (Martin et al., 2003a, Martin et al., 2003b, Butt et al., 2007a). The dominance of PFTriDA within the ∑PFCAs in the terrestrial environment might reflect the input from precursor compounds originating from atmospheric transport, where fluorotelomer-based precursors degrade to odd- and even-numbered PFCAs in similar yield (Ahrens et al., 2011, Butt et al., 2007b, Armitage et al., 2009). The odd-even pattern may be changed during physiological and metabolic processes, yielding an increasing proportion of odd-numbered PFCAs through bioaccumulation (Martin et al., 2003a, Benskin et al., 2009). High concentrations of odd-chained PFCAs have been found in several bird species. The main source of this pattern in terrestrial ecosystems is suspected to be the degradation of FTOHs (Ellis et al., 2004). In marine environments, however, the PFCA pattern is often dominated by perfluorononanoic acid (PFNA) (Herzke et al., 2009, Dietz et al., 2008, Smithwick et al., 2006).

Despite being one of the most studied PFCAS, PFOA is typically not detected or found in low concetrations in biota (Butt et al., 2010). The low concentrations of PFOA in Eagle-Owl feathers is consistent with other reports of PFASs and is thought to be due to the low bioaccumulation potential of PFOA (Tomy et al., 2004, Martin et al., 2004, Yoo et al., 2009). The low levels of PFOA detected in this study are in concordance with a study by Meyer et al. (2009) who could not detect PFOA in feathers of birds from Belgium. Levels of PFOA in liver tissue of Eagle-Owls from Sweden were low or below the LOD (0.064 – 0937 ng/g ww) (Lind, 2012). In tawny owl eggs from Norway and Swedish peregrine falcon eggs, levels of PFOA were lower than those reported here (Ahrens et al., 2011, Holmström et al., 2010). However, levels of PFASs in eggs and feathers might not be comparable. In feathers from barn-owl PFOA was the predominant PFAS yet it was not discovered in other tissues, and it was suggested that this was due to external contamination from athmospheric depositions (Jaspers et al., 2013). The median level of PFOA (0.5 ng/g dw) in the present study was accordingly lower than in

the study by Jaspers et al. (2013) (37.1 ng/g ww). In this study the feathers were washed with distilled water and hexane prior to the extraction procedures in order to remove external contamination. The effect of these washing procedures on the contaminant levels are not well known and more studies are needed to investigate this. This suggests, however, that compared with long chained PFCAs and PFOS, PFOA is not a significant contaminant in Eagle-Owls.

PFASs found in the Eagle-Owls are likely due to local environmental occurrence arising from long-range atmospheric transport and/or sea current transport (Young et al., 2007, Shoeib et al., 2006), or local use of PFAS containing products (Stock et al., 2007, Herzke et al., 2015). Proximity to urbanized areas is associated with elevated PFAS levels (Gebbink et al., 2009). Ahrens et al. (2010) reported that the highest concentrations of ∑PFASs in aqueous samples from the North Sea were near the coast of Germany, while lower concentrations were detected near the Norwegian coast. This was considered to be due to lower human population and less industrial activity in this area. The same study also observed that the composition profile of PFASs was influenced by local sources caused by human activities (Ahrens et al., 2010). The Norwegian current and gulf stream is assumed to influence the spatial patterns of seawater concentrations for PFASs along the Norwegian coast (Theobald et al., 2007, Butt et al., 2010) . No obvious pattern was detected between PFOS concentrations in the feathers, while the PFCAs showed highest concentrations in the southern area. The sample size in this study was quite small for statistical analyses, but the data gives an indication of a trend of PFASs in Eagle-Owls in the Norwegian environment. Differences in pollutant levels between the different areas may be due to spatial differences in exposure levels and different sources of pollution.

Species-specific differences in toxicokinetics and accumulation for different PFASs, leading to different relative patterns between species, might also occur (Haukås et al., 2007, Eriksson et al., 2016, Galatius et al., 2013, Huber et al., 2015, Yeung et al., 2009). The differences in PFAS composition in diet and the local habitat where the animals reside may also influence differences in PFAS patterns between the areas. Differences in biotransformation capacity could affect the transformation of precursors, such as FOSA, into their final degradation products (Fisk et al., 2001). This may explain the differences in PFAS profiles found in birds.

No toxic reference value (TRV) are available for PFASs in feathers. Since PFAS accumulation in internal tissues was not assessed in the present study, it is difficult to discuss the potential

toxicological implications the observed PFAS levels may have for the Eagle-Owl population. The estimated TRV for PFOS in liver of avian top predators (600 ng/g ww) (Newsted et al., 2005) is several times higher than the concentrations measured in the most contaminated Eagle-Owl feather (98.59 ng/g dw). Because it is uncertain if PFAS concentrations in feathers and internal tissues are correlated, it is difficult to predict if the observed PFAS levels in this study present a health risk for the Eagle-Owls.

It has been reported that Norwegian Eagle-Owls are exposed to a wide range of environmental contaminants, both POPs, heavy metals and rodenticides (Madslien et al., 2017, Nygård and Polder, 2012a, Nygård et al., 2006). Accumulation of pollutants could have consequences for the Eagle-Owls' health and reproduction, especially during poor feeding conditions.

4.2 Relationship Between Trophic Position, Feeding Habitat And Contaminant Bioaccumulation

Ratios of $\delta^{15}N$ have been positively correlated with trophic level (Hobson, 1992), while depleted $\delta 13C$ ratios are associated with more marine and offshore food items (Hobson et al., 2002). Overall there was a great variation in both stable isotopes values in the feathers, which reflects the diverse diet of the Eagle-Owls in Norway (Willgohs, 1974, Obuch and Bangjord, 2016). The geographical differences in $\delta^{15}N$ should be interpreted with caution since the baseline values are unknown. A strong positive correlation between the stable isotopes were associated with the samples from Northern Norway and coastal central Norway . No patterns between $\delta^{15}N$ and $\delta^{13}C$ were observed for Southern Norway. The small sample size (n=3) from inland central Norway made it impossible to perform correlation tests for this area. Furthermore, Eagle-Owls from Northern Norway and coastal central Norway feed at a higher trophic level according to their $\delta^{15}N$ values, than Eagle-Owls from Southern Norway and inland central Norway. This was unexpected since Eagle-Owls from Northern Norway are known to have voles, a terrestrial rodent, as their main prey. As expected, the $\delta^{13}C$ values indicate that Eagle-Owls from coastal Central Norway feed on a more marine or offshore diet ($\delta^{13}C > -20$ ‰) than Eagle-Owls, however this was also true for the feathers collected in Northern Norway(Hobson, 1992). In years of low vole densities, Eagle-Owls in Northern Norway switch

to seabirds, waders and other bird groups, which are readily available at this site (pers. Comm. Torgeir Nygård) and may explain the observed isotopic vaues.

This divergence in isotopic values for Eagle Owls could reflect possible shifts in trophic behavior and diet during periods with low abundance of certain prey species. There was a difference in stable isotope ratios between feathers from females and males. Eagle Owls are sexual dimorphic with females being larger than males, and the difference in stable nitrogen isotopes might be a result of females capturing larger or higher trophic prey species than males (Mueller, 1986, Hagen, 1952). However, no differences in PFAS levels were detected between the sexes.

Consistent relationships between δ^{15}N and PFASs are not reported in the literature (Van de Vijver et al., 2003, Leat et al., 2013). Leat et al. (2013) suggested that the relationship between trophic level and bioaccumulation of PFASs might not be detectable within species, especially for species where diet does not vary. However the Eagle-Owl preys on a range of vertebrate species depending on their availability (Obuch and Bangjord, 2016, Willgohs, 1974) and this may explain the significant correlation found between δ^{15}N and PFASs in Northern Norway where this variability in prey items may be more pronounced.

The differences in feeding behavior between the sampling areas were generally not reflected by the differences in PFAS concentrations. The samples from Southern Norway displayed higher PFAS concentrations than samples from the other areas. This could be related to several factors, including differential sample sizes, difference in locale pollution sources, local diet and remobilization of PFASs from internal tissues in association with metabolic or reproductive status (Lucia et al., 2017). Feeding at higher trophic level could be leading to increased PFAS levels in Eagle-Owls from coastal Central Norway, but no correlations were detected between PFAS levels and stable isotopes in the feathers from this area. During the last 5 years production of Eagle-Owl nestlings has been good in Northern Norway, yet poor in Southern Norway and Central Norway due to low abundance of prey species (Heggøy and Øien, 2016, Husdal, 2016, Pearson, 2014, Stenberg, 2014). Remobilization of PFASs from the liver during starvation or periods with limited food sources for Eagle-Owls in Southern Norway, may influence the levels of PFASs in the blood during feather growth. Southern Norway is also a more densly populated area and the sampling locations there were in closer vicinity to urban areas which could reflect the higher PFAS concentrations in these feathers.

The effect of diet on PFAS accumulation in Eagle-Owls needs further research. To study biomagnification of PFASs in the Eagle-Owls PFAS and stable isotope data for key prey species should be assessed.

4.3 Statistical Analyses And Handling Of Non-Detects

In this thesis distribution-based values were imputed for the non-detectable values. An advantage with this method of handling data below LOD (non-detects) is that any standard statistical method can be used for analyzing the data (Baccarelli et al., 2005). Several studies have found that simple substitution techniques only are reliable if a small percentage of the values are non-detects (Baccarelli et al., 2005, Helsel, 2006). Using a distribution based method produce estimates of the non-detectable values with smaller bias and error rates, than values generated by replacement techniques (Croghan and Egeghy, 2003). The sample weights varied considerably in this study as a result of the feather material varying greatly in size (both length and weight). Due to the varying sample weights of feathers in this thesis, the estimated LODs for each sample were not completely accurate. Thus, these values should be interpreted with caution in view of this uncertainty (pers. comm. Jonathan Benskin).

4.4 Feathers As Biomonitoring Tool

Feathers are coupled to the blood and its circulating compounds only during feather growth, and as the feather stops to grow, the blood vessels atrophy and are disconnected from the birds' circulatory system (Burger and Gochfeld, 1992). Therefore the levels detected in the feathers are reflecting the circulating levels of PFASs during the growth period of the feathers, but not at the time of sampling (Meyer et al., 2009). Factors that could affect the feather pollutant concentrations, but which remain unknown in the present study, are information on age and body condition, reproductive status, as well as time of moult of the sampled birds (Espín et al., 2016, García-Fernández et al., 2013, Jaspers et al., 2007)

Levels of OHCs have been shown to vary according to feather type. In West Greenland white-tailed eagles (*Haliaeetus albicilla*) higher levels of OHCs were detected in body feathers than in tail feathers and primary wing feathers (Jaspers et al., 2011). The "ideal" feather for biomonitoring studies of contaminants, however, depends on the molting pattern of the species (García-Fernández et al., 2013). Eagle-Owls have primary moult post breeding, which is

completed over 2-3 years or more (Bildstein and Bildstein, 2007, Hardey, 2006). Feather types were not assessed for each individual feather in the current study, and the material consisted of a mix of flight feathers and coverts. Scandinavian Eagle-Owls moult their flight and tail feathers at a slower pace than other large owls, usually replacing only 1-2 feathers each year (Solheim, 2011, Cramp, 1985). Moult strategy and preening may influence the inter-feather variability in PFAS levels due to differences in age and possible external contamination on the feathers (Espín et al., 2016, García-Fernández et al., 2013).

External contamination may confound the results presented here, and can originate from two main sources; atmospheric deposition or preen oil. In Belgian barn-owls the highest levels of PFOS were found in preen oil (up to 1208 ng/g ww), thus preening the feathers might influence the levels of PFOS detected in the feathers (Jaspers et al., 2013, Jaspers et al., 2008). Jaspers et al. (2013) further suggested that levels of PFOA in barn owl feathers were possibly due to external deposition, while PFOS levels reflected the internal concentrations via the preen oil. However, these processes are not well understood for PFAS contamination. The feathers were washed with both distilled water and hexane prior to the extraction procedures in order to remove external contamination. To what extent these washing procedures are reducing external contamination has not been investigated for PFASs and more research is needed to examine this further. More studies are warranted to earn a better understanding of how PFASs are incorporated into bird feathers.

In view of the analytical issues mentioned, PFAS determination in feathers may be influenced by the amount of feathers sampled. Since feathers are very light a fairly large amount of material is necessary in order to quantify PFASs in the samples. This may also be the reason why lower levels of PFASs in feathers are reported compared with internal tissues (Meyer et al., 2009, Jaspers et al., 2013, Herzke et al., 2011). However, how the levels of PFASs in feathers are related to the overall body burden of the sampled birds needs further research.

4.5 Further Remarks

In this study it was not possible to obtain equal sample sizes for all samples, due to a great variation in feather size and mass among the feathers and this may cause a bias to the presented results. It is important to keep sample weights approximately similar in order to get more accurate estimations of LODs and LOQs. It was not possible to investigate any time trends in PFAS accumulation of the Eagle-Owls. However, it would have been interesting to include more years in order to elucidate possible differences in PFAS accumulation pattern in light of restrictions and phase out of PFOS and PFOA related compounds this past decade.

Ideally this study should have been conducted with a larger sample size and an even distribution of samples between the different years, sexes and areas to achieve greater statistical power to the tests performed. However, this was not feasible for the current thesis.

Overall, statistically significant differences in ∑PFASs and ∑PFCAs concentrations were observed among the feathers from Southern Norway and the other areas. However, the samples allocated to this Southern area, were from both coastal and terrestrial locations. Thus, it might have been more reasonable to group the feathers from the coastal locations in Southern Norway together with the feathers from coastal Central Norway as these habitats may be more similar. This would have reduced the sample size for Southern Norway with n=5, consequently reducing the power of the statistical tests performed. By inspecting the data (Table, Appendix), it does not seem like this would have changed the significance of the observed results, as there was little difference in pollutant levels and stable isotopes ratios between the samples originally allocated to the Southern Norway group.

Considering the assumption that partitioning of PFASs into feather is a result of the PFASs binding to keratin proteins in the feathers (García-Fernández et al., 2013) . It would have been interesting to investigate if the accumulation of PFASs in feathers are correlated with protein content of the feathers. Thus, the mass and size of the feather and corresponding protein concentrations might affect the levels of PFASs due to differences in protein content in the feathers. However, I am not sure if it is possible to extract both PFASs and proteins from the same feather.

4.6 Conclusion

The present study detected a wide range of PFASs in feathers of Norwegian Eagle-Owls, confirming that they are exposed to PFASs in their environments.

No significant differences in ∑PFAS were detected between sexes or years. A feather from 1979 had significantly lower levels of PFASs than all other samples, which reflect current knowledge on manufacturing and emissions of PFASs. A significant higher concentration of ∑PFAS was found in feathers from Southern Norway than the other areas, reflecting spatial differences in PFAS exposure. It is uncertain if the observed concentrations of PFASs in Eagle-Owls poses a health risk for the studied individuals due to lack of TRV for PFASs in feathers. Issues that need further attention and may affect the observed contaminant concentrations are differences between feather types (size and mass) and external contamination of PFASs on the feathers. The matrix-specific differences in PFAS accumulation between feathers and internal tissues needs more research. However, feathers seems to be useful as an alternative or complementary matrix to study PFAS exposure in Norwegian Eagle-Owls.

δ^{13}C and δ^{15}N were positively correlated in Northern Norway and coastal central Norway. No clear evidence exist from stable isotopes that trophic position influences concentrations of PFASs in Eagle-Owls. However, sample sizes were small for most areas which might reduce the probability of detecting significant relationships due to lack of statistical power. It seems likely that the pollutant levels detected in the owls are influenced by pollution level in the area where the Eagle-Owls resides and that they are exposed through their diet. Baseline values were unkown in the present study and more research is needed to investigate biomagnification of PFASs in Eagle-Owls. Further research of key prey species of Eagle-Owls are warranted to elucidate if the stable isotope values and PFAS levels represent spatial variation in PFAS exposure, and if possible point sources of PFASs exist in the proximity of Eagle-Owl habitats.

Several factors can influence the contaminant accumulation, trophic behavior and feeding habits of the Eagle-Owls. Individuals differences in age and sex may be due to variation in physiology, diet, reproductive status or metabolic capacity. The present findings indicate that Eagle-Owls in Northern Norway could have access to higher quantity of preferred prey, both water voles and possibly seabirds. Eagle-Owls in Northern Norway might have better body condition and live in a habitat of higher quality than owls from the other areas. The prey availability for Eagle-Owls have been considered poor in the areas in Southern Norway and

coastal central Norway (Øien et al., Jacobsen and Gjershaug, 2014, Pearson, 2014), resulting in reduced productivity in these areas (Heggøy and Øien, 2016, Stenberg, 2014, Husby and Pearson, 2015a, Husby and Pearson, 2015b).

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5. Appendices

Appendix A. Sampling material

Overview of sampling material is given in Table A.1.

Table A. 1 Detailed overview of samples; ID-number (genelabnumber from NINA). sex. sampling year. municipality (municip.). county. area and length of each feater in millimeter (mm). The two feathers from the same individual are flagged with the corresponding ID.

ID	Sex	Year	Municip.	County	Area	Feather length, mm
454	Female	2013	Åmli	Aust-Agder	Southern Norway	138
456 (586)	Female	2013	Åmli	Aust-Agder	Southern Norway	262
613	Female	2013	Meland	Hordaland	Southern Norway	217
453	Male	2013	Åmli	Aust-Agder	Southern Norway	187
463	Male	2013	Fjell	Hordaland	Southern Norway	199
616	Female	2014	Bømlo	Hordaland	Southern Norway	213
589	Female	2015	Froland	Aust-Agder	Southern Norway	168
586 (456)	Female	2015	Åmli	Aust-Agder	Southern Norway	152
594	Male	2015	Bygland	Aust-Agder	Southern Norway	209
590	Male	2015	Åmli	Aust-Agder	Southern Norway	127
718	Female	2016	Åseral	Vest-Agder	Southern Norway	216
728	Male	2016	Bømlo	Hordaland	Southern Norway	166
726	Male	2016	Sund	Hordaland	Southern Norway	129
778	Female	1989	Frøya	Sør-Trøndelag	Central Norway. coastal	220
624	Female	2013	Frøya	Sør-Trøndelag	Central Norway. coastal	245
651	Female	2013	Frøya	Sør-Trøndelag	Central Norway. coastal	246
618	Male	2013	Frøya	Sør-Trøndelag	Central Norway. coastal	204
649	Male	2013	Frøya	Sør-Trøndelag	Central Norway. coastal	215
630	Male	2013	Hitra	Sør-Trøndelag	Central Norway. coastal	132
782	Female	2014	Haram	Møre & Romsdal	Central Norway. coastal	250
784	Male	2014	Haram	Møre & Romsdal	Central Norway. coastal	230
609	Female	2015	Frøya	Sør-Trøndelag	Central Norway. coastal	241
604	Female	2015	Hitra	Sør-Trøndelag	Central Norway. coastal	203
601	Male	2015	Hitra	Sør-Trøndelag	Central Norway. coastal	226
690	Female	2016	Frøya	Sør-Trøndelag	Central Norway. coastal	190
662	Female	2016	Hitra	Sør-Trøndelag	Central Norway. coastal	188
670	Female	2016	Hitra	Sør-Trøndelag	Central Norway. coastal	210
665	Female	2016	Hitra	Sør-Trøndelag	Central Norway. coastal	213
653	Female	2016	Hitra	Sør-Trøndelag	Central Norway. coastal	245
667	Male	2016	Frøya	Sør-Trøndelag	Central Norway. coastal	225
668	Male	2016	Hitra	Sør-Trøndelag	Central Norway. coastal	198

634	Female	2014	Røros	Sør-Trøndelag	Central Norway. inland	217
639	Female	2015	Røros	Sør-Trøndelag	Central Norway. inland	137
638	Male	2015	Røros	Sør-Trøndelag	Central Norway. inland	187
636	Female	?	Røros	Sør-Trøndelag	Central Norway. inland	265
785	Male	1979	Lurøy	Nordland	Northern Norway	224
251	Female	2013	Lurøy	Nordland	Northern Norway	111
188	Female	2013	Lurøy	Nordland	Northern Norway	198
230	Female	2013	Lurøy	Nordland	Northern Norway	205
205	Female	2013	Lurøy	Nordland	Northern Norway	215
215	Female	2013	Lurøy	Nordland	Northern Norway	215
201	Female	2013	Lurøy	Nordland	Northern Norway	221
238	Female	2013	Lurøy	Nordland	Northern Norway	224
190	Female	2013	Lurøy	Nordland	Northern Norway	233
209	Female	2013	Lurøy	Nordland	Northern Norway	243
261	Male	2013	Lurøy	Nordland	Northern Norway	116
234	Male	2013	Lurøy	Nordland	Northern Norway	223
459	Female	2014	Lurøy	Nordland	Northern Norway	207
458	Female	2014	Lurøy	Nordland	Northern Norway	227
361	Female	2014	Lurøy	Nordland	Northern Norway	240
342	Male	2014	Lurøy	Nordland	Northern Norway	182
391	Male	2014	Lurøy	Nordland	Northern Norway	191
358	Male	2014	Lurøy	Nordland	Northern Norway	213
325	Male	2014	Lurøy	Nordland	Northern Norway	294
578	Female	2015	Lurøy	Nordland	Northern Norway	135
550	Female	2015	Lurøy	Nordland	Northern Norway	171
538	Female	2015	Lurøy	Nordland	Northern Norway	174
529	Female	2015	Lurøy	Nordland	Northern Norway	184
540	Female	2015	Lurøy	Nordland	Northern Norway	186
576	Female	2015	Lurøy	Nordland	Northern Norway	214
524	Female	2015	Lurøy	Nordland	Northern Norway	218
532	Female	2015	Lurøy	Nordland	Northern Norway	219
565	Female	2015	Lurøy	Nordland	Northern Norway	235
531	Male	2015	Lurøy	Nordland	Northern Norway	168
574	Male	2015	Lurøy	Nordland	Northern Norway	196
526	Male	2015	Lurøy	Nordland	Northern Norway	201
832	Female	2016	Lurøy	Nordland	Northern Norway	200
806	Female	2016	Lurøy	Nordland	Northern Norway	217
812	Female	2016	Lurøy	Nordland	Northern Norway	237
802	Female	2016	Lurøy	Nordland	Northern Norway	245
809	Male	2016	Lurøy	Nordland	Northern Norway	222
813	Male	2016	Lurøy	Nordland	Northern Norway	255

Appendix B. Quantification of PFASs

Table A. 2 Internal standard

Compound	Conc. (pg/µL)
MPFAC-MXA	19.89
M5PFPeA	28.84
M4PFHpA	18.4
M8FOSA	18.4
M2 6:2FTS	20.38
M3HFPO-DA	19.74
d3-N-MeFOSAA	20.91
d5-N-EtFOSAA	19.56
Cl-PFHxPA	20.83

Table A. 3 Overview of native standard solution and conentrations (picogram/ µL).

Compound	**Acronym**	**Conc. (pg/µL)**
See the table below	PFAC-MXB	20.01
Perfluorooctanesulfonamide	FOSA-I	42.62
Perfluoro-1-octanesulfonamidoacetic acid	FOSAA	49.94
Sodium 1H,1H,2H,2H-perfluorohexane sulfonate(4:2)	4:2FTS	45.66
Sodium 1H,1H,2H,2H-perfluorooctane sulfonate(6:2)	6:2FTS	48.32
Sodium 1H,1H,2H,2H-perfluorodecane sulfonate(8:2)	8:2FTS	46.11
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	HFPO-DA	49.94
3-Perfluoropropyl propanoic acid (3:3)	FPrPA	48.29
3-Perfluoropentyl propanoic acid(5:3)	FPePA	50.33
3-Perfluoroheptyl propanoic acid(7:3)	FHpPA	49.44
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	53.3
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	84.68

Table A. 4 Overvies of the compounds in PFAC-MXB.in the native standard solution.

Abbreviation	PFAS
PFBA	perfluorobutanoate
PFPeA	Perfluoropentanoate
PFHxA	Perfluorohexanoate
PFHpA	Perfluoroheptanoate
PFOA	Perfluorooctanoate
PFNA	Perfluorononanoate
PFDA	Perfluorodecanoate
PFUnDA	Perfluorundecanoate
PFDoDA	Perfluorododecanoate
PFTriDA	Perfluorotridecanoate
PFTeDA	Perfluorotetradecanoate
PFPeDA	Perfluoropentadecanoate
PFHxDA	Perfluorohexadecanoate
PFODA	Perfluorooctadecanoate
PFBS	Perfluorobutane sulfonate
PFHxS	Perfluorohexane sulfonate
PFOS	Perfluorooctane sulfonate
PFDS	Perfluorodecane sulfonate

Table A. 5 Analytes of interest

Target Class	Target Compounds	Acronym[1]	Native		Surrogate	
			Standard	Supplier	Standard	Supplier
PFCAs	Perfluorobutanoic acid	L-PFBA	L-PFBA		$^{13}C_4$-PFBA	
PFCAs	Perfluoropentanoic acid	L-PFPeA	L-PFPeA		$^{13}C_5$-PFPeA	
PFCAs	Perfluorohexanoic acid	L-PFHxA	L-PFHxA		$^{13}C_2$-PFHxA	
PFCAs	Perfluoroheptanoic acid	L-PFHpA	L-PFHpA		$^{13}C_4$-PFHpA	
PFCAs	Linear Perfluorooctanoic acid	L-PFOA	L-PFOA		$^{13}C_4$-PFOA	
PFCAs	Branched Perfluorooctanoic acid	B-PFOA	L-PFOA	Well Labs	$^{13}C_4$-PFOA	Well Labs
PFCAs	Perfluorononanoic acid	L-PFNA	L-PFNA		$^{13}C_5$-PFNA	
PFCAs	Perfluorodecanoic acid	L-PFDA	L-PFDA		$^{13}C_2$-PFDA	
PFCAs	Perfluoroundecanoic acid	L-PFUnDA	L-PFUnDA		$^{13}C_2$-PFUnDA	
PFCAs	Perfluorododecanoic acid	L-PFDoDA	L-PFDoDA		$^{13}C_2$-PFDoDA	
PFCAs	Perfluorotridecanoic acid	L-PFTrDA	L-PFTrDA		$^{13}C_2$-PFDoDA	
PFCAs	Perfluorotetradecanoic acid	L-PFTeDA	L-PFTeDA		$^{13}C_2$-PFDoDA	
PFCAs	Perfluoropentadecanoic acid	L-PFPeDA	L-PFTeDA		$^{13}C_2$-PFDoDA	
PFSAs	Perfluorobutane sulfonic acid	PFBS	L-PFBS		$^{13}C_2$-PFHxA	
PFSAs	Linear Perfluorohexane sulfonic acid	L-PFHxS	L-PFHxS		$^{18}O_2$-PFHxS	
PFSAs	Branched Perfluorohexane sulfonic acid	B-PFHxS	L-PFHxS		$^{18}O_2$-PFHxS	
PFSAs	Linear Perfluorooctane sulfonic acid	L-PFOS	L-PFOS		$^{13}C_4$-PFOS	
PFSAs	Branched Perfluorooctane sulfonic acid	B-PFOS	L-PFOS		$^{13}C_4$-PFOS	
PFSAs	Linear Perfluorodecane sulfonic acid	L-PFDS	L-PFDS		$^{13}C_2$-PFUnDA	
PFSAs	Branched Perfluorodecane sulfonic acid	B-PFDS	L-PFDS		$^{13}C_2$-PFUnDA	
FASAs[1]	Linear Perfluorooctane sulfonamide	L-FOSA	L-FOSA		$^{13}C_8$-FOSA	
FASAs	Branched Perfluoroctane sulfonamide	B-FOSA	L-FOSA	Well Labs	$^{13}C_8$-FOSA	Well Labs
FASAs	Linear Perfluorooctane sulfonamidoacetic acid	L-FOSAA	L-FOSAA		d3-MeFOSAA	
FASAs	Branched Perfluorooctane sulfonamidoacetic acid	B-FOSAA	L-FOSAA		d3-MeFOSAA	
FASAs	Linear N-Methyl Perfluorooctane sulfonamidoacetic acid	L-MeFOSAA	L-MeFOSAA		d3-MeFOSAA	
FASAs	Branched N-Methyl Perfluorooctane sulfonamidoacetic acid	B-MeFOSAA	L-MeFOSAA		d3-MeFOSAA	
FASAs	Linear N-Ethyl Perfluorooctane sulfonamidoacetic acid	L-EtFOSAA	L-EtFOSAA		d5-EtFOSAA	

FASAs	Branched N-Ethyl Perfluorooctane sulfonamidoacetic acid	B-EtFOSAA	L-EtFOSAA		d5-EtFOSAA	
FTS[2]	4:2 Fluorotelomer sulfonate	4:2 FTS	4:2 FTS	Well Labs	$^{13}C_2$-6:2 FTS	Well Labs
FTS	6:2 Fluorotelomer sulfonate	6:2 FTS	6:2 FTS	Well Labs	$^{13}C_2$-6:2 FTS	Well Labs
FTS	8:2 Fluorotelomer sulfonate	8:2 FTS	8:2 FTS	Well Labs	$^{13}C_2$-6:2 FTS	Well Labs
Alt.	2-(6-chloro-dodecafluorohexyloxy)-tetrafluoroethane sulfonate	F-53B		R. Vestergren	$^{13}C_2$-PFDA	Well Labs
	Recovery Standards					
	$^{13}C_8$ labeled Perfluorooctanoic acid	M8-PFOA		Well Labs		
	$^{13}C_8$ labeled Perfluorooctane sulfonic acid	M8-PFOS		Well Labs		

Table A. 6 Retention times and monitored ions**.**

Target Analyte	Typical Retention Time (min)	Quant. Ion	Qual Ion	IS	IS Ion	Data quality
L-PFPeA	1.20	263/219	263/169	13C-PFPeA	266/222	Quantitative
L-PFHxA	2.10	313/269	313/119	13C-PFHxA	315/270	Quantitative
L-PFHpA	2.55	363/319	363/169	13C-PFHpA	367/322	Quantitative
L-PFOA	2.89	413/369	413/169	13C-PFOA	417/372	Quantitative
L-PFNA	3.19	463/419	463/219	13C-PFNA	468/423	Quantitative
L-PFDA	3.48	513/469	513/269	13C-PFDA	515/470	Quantitative
L-PFUnDA	3.75	563/519	563/269	13C-PFUnDA	565/520	Quantitative
L-PFDoDA	4.01	613/569	613/169	13C-PFDoA	615/570	Quantitative
L-PFTriDA	4.28	662.9/619	663/169	13C-PFDoA	615/570	Quantitative
L-PFTeDA	4.52	712.9/669	713/169	13C-PFDoA	615/570	Quantitative
L-PFPeDA	4.75	762.9/719	763/169	13C-PFDoA	615/570	Semi-quantitative
FPePA (5:3 FTA)		341/237	341/217			
FHpPA (7:3FTA)		441/337	441/148			
PFBS	2.02	298.9/80	298.9/99	18O-PFHxS	403/84	Quantitative
L-PFHxS	2.92	399/80	399/99 399/119	18O-PFHxS	403/84	Quantitative
B-PFHxS	2.85	399/80	399/99 399/119	18O-PFHxS	403/84	Semi-quantitative
L-PFOS	3.54	498.9/80	498.9/99	13C-PFOS	503/80	Quantitative
B-PFOS	~3.44	498.9/80	498.9/99	13C-PFOS	503/80	Semi-quantitative
L-PFDS	4.09	598.9/80	599/99	13C-PFOS	503/80	Quantitative
B-PFDS	~4.01	599/80	599/99	13C-PFOS	503/80	Semi-quantitative
L-FOSA	4.44	498/78	498/478 498/169	13C-FOSA	506/78	Quantitative
B-FOSA	4.35	498/78	498/478 498/169	13C-FOSA	506/78	Semi-quantitative
L-FOSAA	4.90	556/419	556/498	D3-MeFOSAA	573/419	Quantitative
B-FOSAA	4.78	556/419	556/498	D3-MeFOSAA	573/419	Semi-quantitative

L-MeFOSAA	5.43	570/419	570/483	D3-MeFOSAA	573/419	Quantitative
B-MeFOSAA	5.37	570/419	570/483	D3-MeFOSAA	573/419	Semi-quantitative
L-EtFOSAA	5.57	584/419	584/526	D5-EtFOSAA	589/419	Quantitative
B-EtFOSAA	5.32	584/419	584/526	D5-EtFOSAA	589/419	Semi-quantitative
4:2 FTS	3.63	327/307	327/80.6	13C-FTS	429/409	Qualitative
6:2 FTS	4.64	427/407	427/80.6	13C-FTS	429/409	Qualitative
8:2 FTS	5.3	527/507	527/80.6	13C-FTS	429/409	Qualitative
Recovery standards						
M8-PFOA	2.89	421/376				
M8-PFOS	3.54	506.9/80				

Table A. 7 Mobile phase gradient profile

LC Gradient Program			**LC Flow Rate**
Time (min)	Mobile phase A (%)[1]	Mobile Phase B (%)[2]	(mL/min)
0.0	90	10	0.40
0.3	90	10	0.40
4.5	20	80	0.40
4.6	0	100	0.40
7.5	0	100	0.55
9.5	90	10	0.40

[1] Mobile phase A: 90 % water and 10 % acetonitrile containing 2 mM ammonium acetate.
[2] Mobile phase B: 100 % acetonitrile containing 2 mM ammonium acetate.

Table A. 8 The recovery average of the target analytes.

Analytes	**PFPeA**	**PFHxA**	**PFHpA**	**PFOA**	**PFNA**	**PFDA**	**PFUnDA**	**PFDoDA**	**PFTriDA**	**PFTeDA**	**FPePA**	**FHpPA**
Recovery:	97	95	119	111	116	133	126	132	57	22	25	15
SEM	4.6	15.1	2.5	5.2	1.3	6.7	2.8	4.4	3.9	2.7	6.7	2.4
Analytes	PFBS	L-PFHxS	L-PFOS	L-PFDS	L-FOSA	4:2FTS	6:2FTS	8:2FTS	L-FOSAA	L-MeFOSAA	L-EtFOSAA	
Recovery:	79	102	124	66	144	113	116	107	67	100	124	
SEM	3.0	7.2	20.4	7.2	4.6	6.5	5.1	9.6	12.1	8.8	9.7	

Appendix C. Limit of detection and limit of quantification

Table A. 9 Overview of method limit of detection (LOD) and limit of quantification (LOQ). number of samples below and above the stated LOD. and detection frequency (DF).

Compound	LOD	LOQ	Below LOD (n)	Above LOD (n)	Detection frequency
PFPeA	0.60	2.00	72	0	0.00
PFHxA	0.90	3.00	72	0	0.00
PFHpA	0.20	0.67	65	7	0.10
PFOA	0.20	0.67	2	70	0.97
PFNA	0.10	0.33	1	71	0.99
PFDA	0.10	0.33	0	72	1.00
PFUnDA	0.20	0.67	1	71	0.99
PFDoDA	0.10	0.33	1	71	0.99
PFTriDA	0.30	1.00	3	69	0.96
PFTeDA	0.10	0.33	8	64	0.89
PFPeDA	0.10	0.33	15	57	0.79
FPePA	0.10	0.33	68	4	0.06
FHpPA	0.10	0.33	42	30	0.42
PFBS	0.30	1.00	66	6	0.08
L-PFHxS	0.50	1.67	69	3	0.04
br-PFHxS	0.50	1.67	70	2	0.03
L-PFOS_80	0.05	0.17	0	72	1.00
L-PFOS_99	0.05	0.17	0	72	1.00
brPFOS_80	0.05	0.17	0	72	1.00
brPFOS_99	0.05	0.17	0	72	1.00
L-PFDS	0.07	0.23	42	30	0.42
brPFDS	0.07	0.23	66	6	0.08
L-FOSA	0.02	0.07	11	61	0.85
brFOSA	0.02	0.07	37	35	0.49
4:2FTS	0.05	0.17	72	0	0.00
6:2FTS	0.05	0.17	39	33	0.46
8:2FTS	0.10	0.33	44	28	0.39
L-FOSAA	0.10	0.33	69	3	0.04
br-FOSAA	0.05	0.17	72	0	0.00
L-MeFOSAA	0.05	0.17	72	0	0.00
br-MeFOSAA	0.05	0.17	72	0	0.00
L-EtFOSAA	0.05	0.17	68	4	0.06
br_EtFOSAA	0.05	0.1665	68	4	0.06

Appendix D. Levels of perfluoroalkyl substances in feathers of Eagle-Owls

The levels of PFASs in the feathers of the individual Eagle-Owls sampled are presented in Table A.10, A11, A.12 and A.13.

Table A. 10: Individual (ID) concentrtaions (ng/g dw) of perfluorocarboxylic acids (PFCAs) in feathers of Eagle-Owls.

ID	B[1]	weight. g	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTriDA	PFTeDA	PFPeDA	FPePA	FHpPA
188	1	0.1804	<0.6	<0.9	<0.2	0.73	0.91	0.74	2.48	1.10	3.29	0.58	0.25	<0.1	<0.1
190	1	0.3951	<0.6	<0.9	<0.2	0.42	0.53	0.59	3.05	1.28	3.96	0.75	0.38	<0.1	0.10
201	1	0.28	<0.6	<0.9	<0.2	0.36	0.35	0.56	1.68	0.76	2.10	0.51	0.41	<0.1	<0.1
205	1	0.3027	<0.6	<0.9	<0.2	0.40	0.76	0.65	1.88	0.85	2.61	0.71	0.63	<0.1	<0.1
209	1	0.5315	<0.6	<0.9	<0.2	0.54	0.52	0.62	1.76	0.92	1.92	0.22	<0.1	<0.1	0.13
215	1	0.2785	<0.6	<0.9	<0.2	<0.2	0.57	0.61	1.84	0.82	2.68	0.78	0.40	<0.1	<0.1
230	1	0.2969	<0.6	<0.9	<0.2	0.37	0.53	0.34	2.06	0.89	3.23	0.79	0.60	<0.1	<0.1
234	1	0.3646	<0.6	<0.9	<0.2	0.28	0.36	0.21	0.79	0.60	1.56	0.42	0.42	<0.1	0.16
238	1	0.3386	<0.6	<0.9	<0.2	0.27	0.35	0.48	1.42	0.85	2.08	0.50	0.21	<0.1	0.18
251	1	0.2252	<0.6	<0.9	<0.2	0.34	0.18	0.34	1.38	0.74	1.52	<0.1	0.04	<0.1	<0.1
261	1	0.1041	<0.6	<0.9	<0.2	0.36	0.67	0.84	4.54	1.45	5.19	1.13	0.60	<0.1	<0.1
453	1	0.1529	<0.6	<0.9	<0.2	0.51	0.73	0.98	4.85	4.11	8.51	1.34	0.60	<0.1	<0.1
454	1	0.1674	<0.6	<0.9	<0.2	0.67	1.11	1.22	4.64	4.64	12.53	4.26	2.55	<0.1	<0.1
456	1	0.6522	<0.6	<0.9	<0.2	0.33	1.07	0.82	2.71	2.39	6.08	1.63	1.00	<0.1	0.39
524	1	0.3315	<0.6	<0.9	<0.2	0.50	0.55	0.49	2.21	0.90	3.60	0.91	0.88	<0.1	0.10
526	1	0.2531	<0.6	<0.9	<0.2	0.33	0.22	0.20	0.84	0.35	0.75	<0.1	<0.1	<0.1	<0.1
529	1	0.1531	<0.6	<0.9	<0.2	0.52	0.68	0.42	2.73	0.94	3.28	0.88	0.72	<0.1	<0.1
531	1	0.1315	<0.6	<0.9	<0.2	0.35	0.21	0.32	1.66	0.61	0.95	<0.1	<0.1	<0.1	<0.1
532	1	0.326	<0.6	<0.9	<0.2	0.68	0.51	0.46	1.55	0.62	1.39	0.32	0.22	<0.1	<0.1
538	1	0.2288	<0.6	<0.9	<0.2	0.49	0.85	0.50	1.32	0.59	1.83	0.36	<0.1	<0.1	<0.1
540	1	0.2451	<0.6	<0.9	<0.2	1.02	1.30	1.00	4.24	1.16	3.30	0.55	0.65	<0.1	<0.1
550	1	0.1384	<0.6	<0.9	<0.2	0.48	0.73	0.95	3.67	1.92	5.06	1.03	0.47	0.11	<0.1

565	1	0.3637	<0.6	<0.9	<0.2	<0.2	0.13	0.11	0.72	0.38	0.83	<0.1	<0.1	<0.1	<0.1
574	1	0.2711	<0.6	<0.9	<0.2	0.56	0.49	0.79	3.43	1.16	3.13	0.67	0.73	<0.1	0.15
576	1	0.2929	<0.6	<0.9	0.23	0.62	1.74	1.85	9.33	3.25	9.37	1.89	1.66	<0.1	<0.1
578	1	0.1299	<0.6	<0.9	0.31	0.67	0.66	0.39	2.63	1.20	2.92	0.36	0.12	<0.1	<0.1
586	1	0.1715	<0.6	<0.9	0.23	0.60	2.13	2.67	10.90	7.85	19.76	4.65	2.83	<0.1	<0.1
589	1	0.1113	<0.6	<0.9	<0.2	0.46	1.03	1.49	4.49	4.42	8.41	1.68	0.28	<0.1	<0.1
590	1	0.0824	<0.6	<0.9	0.36	0.71	0.47	0.27	2.96	1.86	4.11	1.03	0.10	<0.1	<0.1
594	1	0.2971	<0.6	<0.9	<0.2	0.44	1.25	1.79	9.35	6.01	13.31	2.69	1.77	<0.1	0.28
601	1	0.3097	<0.6	<0.9	<0.2	0.32	0.59	0.58	2.27	1.08	3.05	0.41	0.11	<0.1	0.22
604	1	0.2127	<0.6	<0.9	<0.2	0.22	0.49	0.40	0.90	0.66	1.59	0.43	0.17	<0.1	<0.1
609	1	0.6118	<0.6	<0.9	<0.2	0.32	0.76	0.67	2.55	1.14	3.65	0.99	0.96	<0.1	0.17
618	1	0.2296	<0.6	<0.9	<0.2	0.39	0.75	0.56	2.23	0.95	2.57	0.41	0.21	<0.1	0.37
624	1	0.7028	<0.6	<0.9	<0.2	0.32	0.61	1.95	3.04	2.25	4.75	2.10	1.18	<0.1	0.84
630	1	0.1374	<0.6	<0.9	<0.2	0.39	0.69	0.62	4.44	2.67	11.99	2.39	1.55	<0.1	0.16
325	2	0.726	<0.6	<0.9	<0.2	0.56	0.24	0.41	1.56	0.69	1.15	0.14	<0.1	<0.1	0.16
342	2	0.1343	<0.6	<0.9	<0.2	0.57	0.46	0.62	2.68	1.38	4.77	0.78	0.53	<0.1	<0.1
358	2	0.3851	<0.6	<0.9	<0.2	0.55	0.40	0.37	2.85	1.19	2.16	0.24	<0.1	<0.1	<0.1
361	2	0.4541	<0.6	<0.9	<0.2	1.08	0.97	1.25	4.76	1.94	4.13	0.40	0.26	<0.1	<0.1
391	2	0.258	<0.6	<0.9	<0.2	0.81	0.34	0.37	1.31	0.48	1.48	0.33	0.52	<0.1	<0.1
458	2	0.3448	<0.6	<0.9	<0.2	0.59	0.43	0.51	2.79	1.07	3.67	0.52	0.32	<0.1	0.13
459	2	0.3318	<0.6	<0.9	<0.2	0.49	0.92	0.70	2.19	0.75	1.66	0.30	0.19	0.13	0.23
463	2	0.2353	<0.6	<0.9	<0.2	0.68	0.78	0.71	2.57	1.16	4.08	0.80	0.48	<0.1	<0.1
613	2	0.3445	<0.6	<0.9	<0.2	0.83	1.00	1.08	3.34	2.52	4.80	0.67	0.11	<0.1	0.29
616	2	0.3458	<0.6	<0.9	<0.2	0.64	0.64	0.59	1.76	1.20	3.74	1.19	0.56	<0.1	0.17
634	2	0.3294	<0.6	<0.9	<0.2	0.45	0.44	0.42	1.15	0.67	1.70	0.44	0.12	<0.1	<0.1
636	2	0.6116	<0.6	<0.9	<0.2	0.26	0.58	1.03	3.49	1.10	1.88	0.27	0.08	<0.1	<0.1
638	2	0.2909	<0.6	<0.9	<0.2	0.37	0.61	0.94	1.58	0.95	2.76	1.05	0.80	<0.1	<0.1
639	2	0.1226	<0.6	<0.9	<0.2	0.36	0.38	0.52	1.85	1.12	2.30	0.36	0.21	0.23	<0.1
649	2	0.2254	<0.6	<0.9	<0.2	0.85	1.36	0.68	2.16	0.97	2.92	0.44	0.22	<0.1	0.15

651	2	0.5607	<0.6	<0.9	<0.2	0.36	0.47	0.58	2.58	1.28	2.50	0.18	<0.1	<0.1	<0.1
653	2	0.5836	<0.6	<0.9	0.26	1.02	0.52	1.00	1.60	1.24	2.34	0.39	0.14	<0.1	0.23
662	2	0.1703	<0.6	<0.9	<0.2	0.81	0.52	0.39	1.07	0.58	1.42	0.49	<0.1	<0.1	0.64
665	2	0.2919	<0.6	<0.9	<0.2	0.55	0.30	0.60	2.34	1.72	4.25	0.80	0.38	<0.1	0.14
667	2	0.4672	<0.6	<0.9	<0.2	0.35	0.61	0.48	1.78	1.12	3.14	0.44	0.13	<0.1	0.46
668	2	0.2898	<0.6	<0.9	0.25	0.52	0.72	0.67	2.01	1.24	3.14	0.56	0.24	<0.1	0.18
670	2	0.2097	<0.6	<0.9	<0.2	0.58	0.59	0.88	3.06	1.58	3.73	0.57	0.17	<0.1	<0.1
690	2	0.1927	<0.6	<0.9	<0.2	0.39	0.39	0.72	1.81	0.91	2.68	0.36	0.17	0.39	<0.1
718	2	0.3006	<0.6	<0.9	<0.2	1.10	0.41	0.48	1.60	1.31	2.79	0.57	0.19	<0.1	<0.1
726	2	0.1465	<0.6	<0.9	<0.2	0.93	1.30	1.27	4.84	3.44	5.97	0.76	0.13	<0.1	0.23
728	2	0.1296	<0.6	<0.9	<0.2	0.64	1.31	2.61	5.04	6.14	12.71	3.68	0.96	<0.1	0.75
778	2	0.3488	<0.6	<0.9	<0.2	0.98	0.27	0.22	1.35	0.69	1.58	0.16	<0.1	<0.1	<0.1
782	2	0.5451	<0.6	<0.9	0.28	0.78	0.83	0.54	3.32	1.58	6.61	1.28	1.38	<0.1	0.21
784	2	0.3984	<0.6	<0.9	<0.2	0.40	0.83	0.37	1.83	0.76	2.27	0.34	0.16	<0.1	0.12
785	2	0.411	<0.6	<0.9	<0.2	0.51	<0.1	0.15	<0.2	<0.1	<0.3	<0.1	<0.1	<0.1	<0.1
802	2	0.397	<0.6	<0.9	<0.2	0.34	0.33	0.26	0.85	0.42	1.14	0.19	<0.1	<0.1	<0.1
806	2	0.3769	<0.6	<0.9	<0.2	0.55	0.55	0.46	0.84	0.26	0.57	<0.1	<0.1	<0.1	<0.1
809	2	0.3476	<0.6	<0.9	<0.2	0.55	0.40	0.41	1.42	0.73	1.81	0.39	0.19	<0.1	0.10
812	2	0.437	<0.6	<0.9	<0.2	0.57	0.46	0.31	0.61	0.20	<0.3	<0.1	<0.1	<0.1	<0.1
813	2	0.5785	<0.6	<0.9	<0.2	0.40	0.59	1.41	6.48	2.37	7.09	1.41	1.73	<0.1	0.13
832	2	0.2132	<0.6	<0.9	<0.2	0.79	0.20	0.31	0.50	0.20	<0.3	<0.1	<0.1	<0.1	<0.1

[1]B=batch number

Table A. 11 Individual PFSA concentrations

ID	B	weight. g	PFBS	L-PFHxS	br-PFHxS	L-PFOS_80	L-PFOS_99	brPFOS_80	brPFOS_99	L-PFDS	brPFDS
188	1	0.1804	<0.3	<0.5	<0.5	19.10	15.22	1.91	1.21	<0.07	<0.07
190	1	0.3951	<0.3	<0.5	<0.5	13.85	14.29	1.11	0.79	<0.07	<0.07
201	1	0.28	0.76	<0.5	<0.5	6.97	7.43	1.18	0.68	<0.07	<0.07
205	1	0.3027	<0.3	<0.5	<0.5	9.82	10.46	1.99	0.81	<0.07	<0.07
209	1	0.5315	<0.3	<0.5	<0.5	7.81	7.17	0.78	0.74	<0.07	<0.07
215	1	0.2785	<0.3	<0.5	<0.5	8.15	7.92	0.71	0.57	<0.07	<0.07
230	1	0.2969	0.35	<0.5	<0.5	11.76	11.44	1.67	0.47	0.08	<0.07
234	1	0.3646	<0.3	<0.5	<0.5	2.10	3.43	0.44	0.25	<0.07	<0.07
238	1	0.3386	<0.3	<0.5	<0.5	6.29	7.44	1.54	0.97	<0.07	<0.07
251	1	0.2252	<0.3	<0.5	<0.5	6.95	6.75	0.81	0.12	<0.07	<0.07
261	1	0.1041	0.64	<0.5	<0.5	14.19	16.45	1.21	0.12	<0.07	<0.07
453	1	0.1529	<0.3	<0.5	<0.5	3.18	4.61	0.73	0.11	<0.07	<0.07
454	1	0.1674	<0.3	<0.5	<0.5	11.71	10.48	0.56	1.05	0.74	<0.07
456	1	0.6522	<0.3	<0.5	<0.5	10.02	8.44	0.96	0.65	<0.07	<0.07
524	1	0.3315	<0.3	<0.5	<0.5	8.11	8.64	1.93	0.95	<0.07	<0.07
526	1	0.2531	<0.3	<0.5	<0.5	4.02	3.35	1.04	0.44	<0.07	<0.07
529	1	0.1531	<0.3	<0.5	<0.5	11.12	13.61	1.16	0.39	0.09	<0.07
531	1	0.1315	<0.3	<0.5	<0.5	5.00	4.71	1.15	0.18	<0.07	<0.07
532	1	0.326	<0.3	<0.5	<0.5	6.59	6.16	1.32	0.54	<0.07	<0.07
538	1	0.2288	<0.3	<0.5	<0.5	8.15	7.83	1.25	0.49	<0.07	<0.07
540	1	0.2451	<0.3	<0.5	<0.5	17.49	16.88	1.97	0.81	0.19	0.08
550	1	0.1384	0.35	<0.5	<0.5	12.21	12.84	1.54	0.25	0.23	<0.07
565	1	0.3637	<0.3	<0.5	<0.5	1.51	2.75	0.87	0.48	<0.07	<0.07
574	1	0.2711	<0.3	<0.5	<0.5	13.57	13.97	1.17	0.92	<0.07	<0.07
576	1	0.2929	<0.3	<0.5	<0.5	37.36	37.46	2.13	1.22	0.26	0.10
578	1	0.1299	<0.3	<0.5	<0.5	9.63	9.04	1.03	0.48	0.07	<0.07
586	1	0.1715	<0.3	<0.5	<0.5	27.78	30.18	2.60	0.92	0.26	<0.07

589	1	0.1113	<0.3	<0.5	<0.5	24.77	22.41	1.79	1.00	0.11	<0.07
590	1	0.0824	0.53	<0.5	<0.5	1.40	1.82	0.37	0.17	<0.07	<0.07
594	1	0.2971	<0.3	<0.5	<0.5	9.31	7.48	1.18	1.17	<0.07	<0.07
601	1	0.3097	<0.3	<0.5	<0.5	12.07	10.74	1.04	0.69	<0.07	<0.07
604	1	0.2127	0.62	<0.5	<0.5	5.34	5.84	1.11	0.56	<0.07	<0.07
609	1	0.6118	<0.3	<0.5	<0.5	8.53	8.17	0.92	0.65	0.11	<0.07
618	1	0.2296	<0.3	<0.5	<0.5	12.85	10.05	1.54	0.91	0.07	<0.07
624	1	0.7028	<0.3	<0.5	<0.5	74.62	73.20	6.50	3.32	0.41	0.10
630	1	0.1374	<0.3	<0.5	<0.5	13.74	12.75	0.96	0.83	0.14	0.04
325	2	0.726	<0.3	<0.5	<0.5	8.32	8.95	1.20	0.28	<0.07	<0.07
342	2	0.1343	<0.3	<0.5	<0.5	13.05	14.77	0.60	0.77	<0.07	<0.07
358	2	0.3851	<0.3	<0.5	<0.5	8.46	8.62	0.72	0.62	<0.07	<0.07
361	2	0.4541	<0.3	<0.5	<0.5	14.22	13.87	2.45	0.96	0.11	<0.07
391	2	0.258	<0.3	<0.5	<0.5	8.95	10.40	0.87	0.46	<0.07	<0.07
458	2	0.3448	<0.3	<0.5	<0.5	11.26	11.14	1.46	0.72	0.13	<0.07
459	2	0.3318	<0.3	<0.5	<0.5	8.60	9.30	1.69	0.60	<0.07	<0.07
463	2	0.2353	<0.3	<0.5	<0.5	7.74	10.45	0.87	0.89	0.33	<0.07
613	2	0.3445	<0.3	<0.5	<0.5	20.60	19.79	2.61	1.65	0.96	<0.07
616	2	0.3458	<0.3	<0.5	<0.5	14.78	12.70	1.77	1.04	0.11	<0.07
634	2	0.3294	<0.3	<0.5	<0.5	4.18	4.38	0.24	0.60	<0.07	<0.07
636	2	0.6116	<0.3	<0.5	<0.5	8.33	8.33	0.63	0.45	<0.07	<0.07
638	2	0.2909	<0.3	<0.5	<0.5	5.88	5.64	0.46	0.24	<0.07	<0.07
639	2	0.1226	<0.3	<0.5	<0.5	1.95	1.39	0.19	0.28	<0.07	<0.07
649	2	0.2254	<0.3	<0.5	<0.5	14.70	12.58	1.98	0.56	0.21	<0.07
651	2	0.5607	<0.3	<0.5	<0.5	9.15	8.36	0.79	0.30	0.11	<0.07
653	2	0.5836	<0.3	<0.5	<0.5	5.62	6.08	0.49	0.49	0.10	<0.07
662	2	0.1703	<0.3	0.54	<0.5	4.02	2.83	0.61	0.33	0.15	<0.07
665	2	0.2919	<0.3	<0.5	<0.5	9.49	10.07	0.67	0.19	0.11	<0.07
667	2	0.4672	<0.3	<0.5	<0.5	9.11	8.92	0.83	0.79	0.12	<0.07

668	2	0.2898	<0.3	<0.5	<0.5	7.73	7.19	0.28	0.78	<0.07	<0.07
670	2	0.2097	<0.3	<0.5	<0.5	10.93	9.40	0.86	0.47	0.13	<0.07
690	2	0.1927	<0.3	<0.5	<0.5	8.10	7.18	1.36	0.51	0.08	<0.07
718	2	0.3006	<0.3	<0.5	<0.5	3.82	3.53	0.34	0.45	<0.07	<0.07
726	2	0.1465	<0.3	0.55	0.35	21.94	22.54	1.46	0.78	0.07	<0.07
728	2	0.1296	<0.3	<0.5	<0.5	52.84	49.98	1.72	1.32	0.12	<0.07
778	2	0.3488	<0.3	0.64	0.25	25.59	26.33	2.45	1.16	<0.07	<0.07
782	2	0.5451	<0.3	<0.5	<0.5	24.69	24.65	3.54	1.57	0.32	0.08
784	2	0.3984	<0.3	<0.5	<0.5	7.06	7.35	1.16	0.55	<0.07	<0.07
785	2	0.411	<0.3	<0.5	<0.5	1.17	1.19	0.58	0.35	<0.07	<0.07
802	2	0.397	<0.3	<0.5	<0.5	6.01	5.53	0.68	0.88	<0.07	<0.07
806	2	0.3769	<0.3	<0.5	<0.5	2.62	4.01	1.03	0.97	<0.07	<0.07
809	2	0.3476	<0.3	<0.5	<0.5	7.24	7.80	0.62	0.25	<0.07	<0.07
812	2	0.437	<0.3	<0.5	<0.5	3.01	3.06	0.73	0.25	<0.07	<0.07
813	2	0.5785	<0.3	<0.5	<0.5	10.12	13.09	1.28	0.59	0.11	0.10
832	2	0.2132	<0.3	<0.5	<0.5	2.52	2.74	0.39	0.28	<0.07	<0.07

Table A. 12 Individual FOSA and FTS concentrations

ID	B	weight. g	L-FOSA	brFOSA	4:2FTS	6:2FTS	8:2FTS	L-FOSAA	br-FOSAA	L-MeFOSAA	br-MeFOSAA	L-EtFOSAA	br_EtFOSAA
188	1	0.1804	0.38	0.04	<0.05	<0.05	0.12	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
190	1	0.3951	0.29	<0.02	<0.05	<0.05	0.39	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
201	1	0.28	0.20	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
205	1	0.3027	1.21	0.03	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
209	1	0.5315	0.41	0.02	<0.05	<0.05	0.17	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
215	1	0.2785	0.56	0.03	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
230	1	0.2969	0.22	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
234	1	0.3646	<0.02	<0.02	<0.05	<0.05	0.11	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
238	1	0.3386	<0.02	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
251	1	0.2252	0.24	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
261	1	0.1041	0.21	0.05	<0.05	0.19	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
453	1	0.1529	<0.02	<0.02	<0.05	0.07	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
454	1	0.1674	0.17	0.03	<0.05	<0.05	0.12	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
456	1	0.6522	0.17	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
524	1	0.3315	0.13	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
526	1	0.2531	0.15	0.04	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
529	1	0.1531	0.20	0.02	<0.05	0.12	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
531	1	0.1315	0.12	0.04	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
532	1	0.326	0.12	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
538	1	0.2288	0.20	0.02	<0.05	<0.05	0.12	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
540	1	0.2451	0.07	0.04	<0.05	<0.05	0.18	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
550	1	0.1384	1.72	0.01	<0.05	<0.05	0.18	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
565	1	0.3637	0.08	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
574	1	0.2711	0.09	0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
576	1	0.2929	0.19	0.00	<0.05	0.07	0.25	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
578	1	0.1299	0.21	0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
586	1	0.1715	0.34	0.02	<0.05	<0.05	0.15	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05

589	1	0.1113	<0.02	<0.02	<0.05	0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
590	1	0.0824	<0.02	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
594	1	0.2971	0.16	0.02	<0.05	<0.05	0.20	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
601	1	0.3097	0.11	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
604	1	0.2127	0.20	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
609	1	0.6118	1.14	<0.02	<0.05	<0.05	0.15	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
618	1	0.2296	0.24	0.03	<0.05	<0.05	0.15	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
624	1	0.7028	2.12	0.05	<0.05	<0.05	0.14	0.12	<0.05	<0.05	<0.05	0.25	0.25
630	1	0.1374	0.22	0.05	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	0.11	0.11
325	2	0.726	0.31	0.02	<0.05	0.10	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
342	2	0.1343	0.20	0.07	<0.05	0.11	0.22	<0.1	<0.05	<0.05	<0.05	0.17	0.17
358	2	0.3851	0.16	0.04	<0.05	0.14	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
361	2	0.4541	<0.02	<0.02	<0.05	0.40	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
391	2	0.258	0.13	0.03	<0.05	1.33	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
458	2	0.3448	0.19	<0.02	<0.05	0.20	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
459	2	0.3318	0.24	<0.02	<0.05	0.38	0.13	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
463	2	0.2353	0.19	<0.02	<0.05	0.58	0.21	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
613	2	0.3445	0.82	0.04	<0.05	0.29	0.54	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
616	2	0.3458	0.36	<0.02	<0.05	0.36	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
634	2	0.3294	0.10	0.03	<0.05	0.44	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
636	2	0.6116	0.49	0.00	<0.05	0.17	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
638	2	0.2909	<0.02	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
639	2	0.1226	<0.02	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
649	2	0.2254	0.20	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
651	2	0.5607	1.04	<0.02	<0.05	<0.05	0.21	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
653	2	0.5836	0.19	<0.02	<0.05	<0.05	0.43	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
662	2	0.1703	0.22	0.08	<0.05	0.35	0.42	0.66	<0.05	<0.05	<0.05	<0.05	<0.05
665	2	0.2919	0.09	0.06	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
667	2	0.4672	0.35	<0.02	<0.05	0.16	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05

668	2	0.2898	0.37	<0.02	<0.05	0.20	0.52	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
670	2	0.2097	0.17	<0.02	<0.05	0.45	0.13	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
690	2	0.1927	0.21	0.03	<0.05	0.22	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
718	2	0.3006	<0.02	<0.02	<0.05	0.21	0.19	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
726	2	0.1465	0.50	0.07	<0.05	0.40	0.29	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
728	2	0.1296	0.61	<0.02	<0.05	0.07	0.20	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
778	2	0.3488	0.99	0.03	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
782	2	0.5451	2.92	0.08	<0.05	0.14	0.14	0.13	<0.05	<0.05	<0.05	<0.05	<0.05
784	2	0.3984	0.14	<0.02	<0.05	0.23	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
785	2	0.411	<0.02	<0.02	<0.05	0.26	<0.1	<0.1	<0.05	<0.05	<0.05	0.75	0.75
802	2	0.397	0.03	<0.02	<0.05	0.13	0.10	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
806	2	0.3769	0.15	0.03	<0.05	0.12	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
809	2	0.3476	0.29	0.02	<0.05	0.11	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
812	2	0.437	0.13	<0.02	<0.05	<0.05	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
813	2	0.5785	0.11	<0.02	<0.05	0.07	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05
832	2	0.2132	<0.02	<0.02	<0.05	0.19	<0.1	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05

Table A. 13 Individual concentrtaions of ∑PFCA, ∑PFOS and ∑PFAS

Name	Sex	Year	Area	Municipality	∑PFCA[1]	∑PFDS[1]	∑PFAS[3]
778	Female	1989	Central Norway. coast	Froeya	5.25	27.77	34.20
651	Female	2013	Central Norway. coast	Froeya	8.02	9.30	18.63
624	Female	2013	Central Norway. coast	Froeya	16.20	78.82	98.17
618	Male	2013	Central Norway. coast	Froeya	8.06	12.67	21.56
649	Male	2013	Central Norway. coast	Froeya	9.59	14.91	24.97
630	Male	2013	Central Norway. coast	Hitra	24.72	14.14	39.41
782	Female	2014	Central Norway. coast	Haram	16.32	27.22	47.01
784	Male	2014	Central Norway. coast	Haram	6.95	8.06	15.56
604	Female	2015	Central Norway. coast	Hitra	4.88	6.43	11.63
609	Female	2015	Central Norway. coast	Froeya	11.03	9.14	21.67
601	Male	2015	Central Norway. coast	Hitra	8.41	12.27	21.12
662	Female	2016	Central Norway. coast	Hitra	5.32	3.89	10.92
653	Female	2016	Central Norway. coast	Hitra	8.25	6.34	15.48
690	Female	2016	Central Norway. coast	Froeya	7.42	8.58	16.57
665	Female	2016	Central Norway. coast	Hitra	10.93	10.21	21.49
670	Female	2016	Central Norway. coast	Hitra	11.16	10.83	22.76
668	Male	2016	Central Norway. coast	Hitra	9.09	7.99	18.36
667	Male	2016	Central Norway. coast	Froeya	8.05	9.83	18.87
634	Female	2014	Central Norway. inland	Roeros	5.39	4.70	10.76
639	Female	2015	Central Norway. inland	Roeros	7.12	1.90	9.17
638	Male	2015	Central Norway. inland	Roeros	9.06	6.11	15.35
785	Male	1979	Northern Norway	Luroey	2.78	1.65	4.85
251	Female	2013	Northern Norway	Luroey	4.70	7.31	12.38
238	Female	2013	Northern Norway	Luroey	6.16	8.11	14.63
201	Female	2013	Northern Norway	Luroey	6.74	8.13	15.24
209	Female	2013	Northern Norway	Luroey	6.54	8.25	15.56
215	Female	2013	Northern Norway	Luroey	7.91	8.67	17.34
205	Female	2013	Northern Norway	Luroey	8.47	11.54	21.43
230	Female	2013	Northern Norway	Luroey	8.82	12.67	21.79
190	Female	2013	Northern Norway	Luroey	10.96	15.02	26.82
188	Female	2013	Northern Norway	Luroey	10.09	18.72	29.43
234	Male	2013	Northern Norway	Luroey	4.66	3.11	8.13
261	Male	2013	Northern Norway	Luroey	14.78	15.99	31.34
459	Female	2014	Northern Norway	Luroey	7.19	10.09	18.27
458	Female	2014	Northern Norway	Luroey	9.90	12.29	22.84
361	Female	2014	Northern Norway	Luroey	14.79	15.75	31.08
325	Male	2014	Northern Norway	Luroey	4.79	9.38	14.81
358	Male	2014	Northern Norway	Luroey	7.80	9.21	17.46
391	Male	2014	Northern Norway	Luroey	5.64	10.35	17.63
342	Male	2014	Northern Norway	Luroey	11.78	14.60	27.00
565	Female	2015	Northern Norway	Luroey	2.64	1.43	4.34

532	Female	2015	Northern Norway	Luroey	5.75	7.31	13.38
538	Female	2015	Northern Norway	Luroey	5.97	8.85	15.24
578	Female	2015	Northern Norway	Luroey	8.96	10.09	19.44
524	Female	2015	Northern Norway	Luroey	10.04	9.81	20.12
529	Female	2015	Northern Norway	Luroey	10.17	13.14	23.74
550	Female	2015	Northern Norway	Luroey	14.31	13.42	29.71
540	Female	2015	Northern Norway	Luroey	13.23	18.58	32.15
576	Female	2015	Northern Norway	Luroey	29.72	39.09	69.36
526	Male	2015	Northern Norway	Luroey	2.81	4.42	7.50
531	Male	2015	Northern Norway	Luroey	4.26	5.52	10.09
574	Male	2015	Northern Norway	Luroey	10.95	14.81	26.09
832	Female	2016	Northern Norway	Luroey	2.83	2.97	6.21
812	Female	2016	Northern Norway	Luroey	3.13	3.53	6.94
806	Female	2016	Northern Norway	Luroey	3.50	4.31	8.21
802	Female	2016	Northern Norway	Luroey	3.59	6.55	10.43
809	Male	2016	Northern Norway	Luroey	5.91	7.96	14.47
813	Male	2016	Northern Norway	Luroey	21.48	12.54	34.35
613	Female	2013	Southern Norway	Meland	14.36	22.33	38.67
454	Female	2013	Southern Norway	Aamli	31.63	11.90	43.93
463	Male	2013	Southern Norway	Fjell	11.26	9.98	22.29
453	Male	2013	Southern Norway	Aamli	21.64	4.32	26.19
616	Female	2014	Southern Norway	Boemlo	10.32	15.14	26.45
589	Female	2015	Southern Norway	Froland	22.26	24.99	47.48
586	Female	2015	Southern Norway	Aamli	51.38	30.74	82.69
590	Male	2015	Southern Norway	Aamli	11.51	1.89	13.57
594	Male	2015	Southern Norway	Bygland	36.62	9.57	46.86
718	Female	2016	Southern Norway	Aaseral	8.45	4.07	13.04
726	Male	2016	Southern Norway	Sund	18.63	23.36	43.49
728	Male	2016	Southern Norway	Boemlo	33.08	52.93	87.66

[1] ∑PFCA: PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFPeDA

[2] ∑PFSA: L-PFOS, Br-PFOS, L-PFDS

[3] ∑PFAS: PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFPeDA, FHpPA(7:3 FTA), L-PFOS, Br-PFOS, L-PFDS, L-FOSA, Br-FOSA, 6:2 FTS, 8:2 FTS

Table A. 14 Mean, standard deviation (SD), median, minimum, and maximum concentrations (ng/g dw) as well as standard error (SE) of the individual PFASs (> 40% of the samples) in Eagle-Owl feathers from Southern Norway (2013-2016), coastal Central Norway (1989, 2013-2016), inland Central Norway (2014-2015) and Northern Norway (1979, 2013-2016). For compounds with concentrtaions <LOD, a value, imputed using a cumulative normal distribution curve was applied in statistics. The „n“ presented in the table represents the numer of samples per year within each area.

PFAS	Area	Year	n	Mean	SD	Median	Min	Max	SE
PFOA	Southern Norway	2013	4	0.673	0.132	0.675	0.510	0.833	0.066
		2014	1	0.639	NA	0.639	0.639	0.639	NA
		2015	4	0.555	0.125	0.533	0.445	0.710	0.062
		2016	3	0.887	0.231	0.926	0.639	1.097	0.133
	Northern Norway	1979	1	0.509	NA	0.509	0.509	0.509	NA
		2013	11	0.387	0.145	0.359	0.196	0.734	0.044
		2014	7	0.665	0.211	0.571	0.490	1.085	0.080
		2015	12	0.536	0.206	0.505	0.220	1.020	0.059
		2016	6	0.533	0.157	0.551	0.338	0.788	0.064
	Central Norway. inland	2014	1	0.455	NA	0.455	0.455	0.455	NA
		2015	2	0.366	0.005	0.366	0.363	0.369	0.003
	Central Norway. coast	1989	1	0.977	NA	0.977	0.977	0.977	NA
		2013	5	0.463	0.220	0.387	0.320	0.853	0.098
		2014	2	0.587	0.268	0.587	0.397	0.777	0.190
		2015	3	0.288	0.055	0.318	0.224	0.321	0.032
		2016	7	0.602	0.237	0.547	0.352	1.022	0.090
PFNA	Southern Norway	2013	4	0.906	0.183	0.890	0.730	1.115	0.091
		2014	1	0.639	NA	0.639	0.639	0.639	NA
		2015	4	1.221	0.688	1.140	0.475	2.130	0.344
		2016	3	1.006	0.517	1.303	0.410	1.306	0.298
	Northern Norway	1979	1	0.189	NA	0.189	0.189	0.189	NA
		2013	11	0.522	0.209	0.529	0.179	0.914	0.063
		2014	7	0.537	0.287	0.433	0.240	0.971	0.109
		2015	12	0.671	0.463	0.601	0.133	1.739	0.134
		2016	6	0.423	0.143	0.431	0.203	0.592	0.059
	Central Norway. inland	2014	1	0.441	NA	0.441	0.441	0.441	NA
		2015	2	0.496	0.163	0.496	0.381	0.612	0.115
	Central Norway. coast	1989	1	0.265	NA	0.265	0.265	0.265	NA
		2013	5	0.776	0.341	0.687	0.470	1.356	0.152
		2014	2	0.827	0.001	0.827	0.827	0.828	0.000
		2015	3	0.613	0.136	0.588	0.490	0.760	0.079
		2016	7	0.523	0.141	0.525	0.298	0.722	0.053
PFDA	Southern Norway	2013	4	0.996	0.215	1.029	0.709	1.219	0.108
		2014	1	0.586	NA	0.586	0.586	0.586	NA
		2015	4	1.555	0.989	1.640	0.274	2.666	0.495
		2016	3	1.453	1.075	1.268	0.482	2.608	0.621
	Northern Norway	1979	1	0.148	NA	0.148	0.148	0.148	NA
		2013	11	0.544	0.185	0.589	0.211	0.835	0.056
		2014	7	0.606	0.312	0.510	0.369	1.252	0.118
		2015	12	0.624	0.473	0.479	0.114	1.847	0.136
		2016	6	0.528	0.441	0.363	0.257	1.414	0.180
	Central Norway. inland	2014	1	0.417	NA	0.417	0.417	0.417	NA

		2015	2	0.730	0.300	0.730	0.517	0.942	0.212
	Central Norway. coast	1989	1	0.217	NA	0.217	0.217	0.217	NA
		2013	5	0.876	0.603	0.617	0.559	1.952	0.270
		2014	2	0.458	0.118	0.458	0.374	0.541	0.083
		2015	3	0.549	0.134	0.576	0.404	0.667	0.077
		2016	7	0.676	0.215	0.673	0.389	0.999	0.081
	Southern Norway	2013	4	3.853	1.083	3.994	2.574	4.852	0.541
		2014	1	1.760	NA	1.760	1.760	1.760	NA
		2015	4	6.923	3.802	6.920	2.956	10.896	1.901
		2016	3	3.826	1.927	4.837	1.604	5.037	1.112
	Northern Norway	1979	1	0.577	NA	0.577	0.577	0.577	NA
		2013	11	2.081	1.006	1.840	0.791	4.544	0.303
		2014	7	2.591	1.129	2.677	1.314	4.757	0.427
PFUnDA		2015	12	2.861	2.324	2.424	0.724	9.331	0.671
		2016	6	1.783	2.321	0.846	0.500	6.476	0.947
	Central Norway. inland	2014	1	1.154	NA	1.154	1.154	1.154	NA
		2015	2	1.717	0.193	1.717	1.580	1.853	0.136
	Central Norway. coast	1989	1	1.346	NA	1.346	1.346	1.346	NA
		2013	5	2.891	0.933	2.583	2.165	4.439	0.417
		2014	2	2.572	1.055	2.572	1.826	3.318	0.746
		2015	3	1.907	0.879	2.269	0.905	2.547	0.508
		2016	7	1.952	0.625	1.808	1.067	3.062	0.236
	Southern Norway	2013	4	3.107	1.580	3.315	1.159	4.637	0.790
		2014	1	1.196	NA	1.196	1.196	1.196	NA
		2015	4	5.036	2.539	5.217	1.861	7.851	1.270
		2016	3	3.627	2.423	3.435	1.306	6.141	1.399
	Northern Norway	1979	1	0.278	NA	0.278	0.278	0.278	NA
		2013	11	0.932	0.248	0.846	0.601	1.448	0.075
		2014	7	1.070	0.493	1.071	0.477	1.936	0.186
PFDoDA		2015	12	1.090	0.810	0.920	0.349	3.254	0.234
		2016	6	0.698	0.845	0.338	0.203	2.373	0.345
	Central Norway. inland	2014	1	0.669	NA	0.669	0.669	0.669	NA
		2015	2	1.032	0.123	1.032	0.945	1.119	0.087
	Central Norway, coast	1989	1	0.694	NA	0.694	0.694	0.694	NA
		2013	5	1.623	0.789	1.284	0.945	2.666	0.353
		2014	2	1.171	0.579	1.171	0.762	1.580	0.409
		2015	3	0.963	0.262	1.084	0.663	1.143	0.151
		2016	7	1.196	0.384	1.235	0.577	1.717	0.145
	Southern Norway	2013	4	7.482	3.887	6.658	4.079	12.534	1.944
		2014	1	3.744	NA	3.744	3.744	3.744	NA
		2015	4	11.397	6.727	10.860	4.106	19.764	3.364
		2016	3	7.158	5.065	5.972	2.791	12.710	2.924
	Northern Norway	1979	1	0.843	NA	0.843	0.843	0.843	NA
PFTriDA		2013	11	2.741	1.115	2.610	1.523	5.190	0.336
		2014	7	2.716	1.445	2.161	1.148	4.769	0.546
		2015	12	3.034	2.399	3.022	0.747	9.369	0.692
		2016	6	1.995	2.538	0.944	0.575	7.089	1.036
	Central Norway. inland	2014	1	1.695	NA	1.695	1.695	1.695	NA
		2015	2	2.531	0.322	2.531	2.304	2.759	0.228
	Central Norway. coast	1989	1	1.576	NA	1.576	1.576	1.576	NA

		2013	5	4.944	4.041	2.917	2.501	11.985	1.807
		2014	2	4.440	3.063	4.440	2.274	6.606	2.166
		2015	3	2.760	1.059	3.046	1.588	3.646	0.611
		2016	7	2.958	0.927	3.141	1.419	4.249	0.350
	Southern Norway	2013	4	1.766	1.685	1.068	0.672	4.256	0.843
		2014	1	1.194	NA	1.194	1.194	1.194	NA
		2015	4	2.511	1.580	2.184	1.028	4.648	0.790
		2016	3	1.670	1.746	0.757	0.570	3.684	1.008
	Northern Norway	1979	1	0.160	NA	0.160	0.160	0.160	NA
		2013	11	0.592	0.284	0.576	0.118	1.128	0.086
		2014	7	0.388	0.210	0.332	0.138	0.778	0.080
PFTeDA		2015	12	0.609	0.519	0.459	0.079	1.894	0.150
		2016	6	0.413	0.497	0.185	0.133	1.410	0.203
	Central Norway. inland	2014	1	0.437	NA	0.437	0.437	0.437	NA
		2015	2	0.707	0.484	0.707	0.364	1.049	0.342
	Central Norway. coast	1989	1	0.157	NA	0.157	0.157	0.157	NA
		2013	5	1.103	1.052	0.437	0.183	2.392	0.470
		2014	2	0.810	0.670	0.810	0.336	1.284	0.474
		2015	3	0.613	0.329	0.432	0.415	0.992	0.190
		2016	7	0.514	0.150	0.492	0.356	0.802	0.057
	Southern Norway	2013	4	0.937	1.097	0.543	0.111	2.553	0.549
		2014	1	0.564	NA	0.564	0.564	0.564	NA
		2015	4	1.244	1.293	1.025	0.101	2.825	0.647
		2016	3	0.426	0.463	0.189	0.129	0.960	0.267
	Northern Norway	1979	1	0.078	NA	0.078	0.078	0.078	NA
		2013	11	0.366	0.204	0.404	0.025	0.626	0.062
		2014	7	0.270	0.203	0.258	0.035	0.526	0.077
PFPeDA		2015	12	0.475	0.487	0.342	0.030	1.663	0.141
		2016	6	0.367	0.670	0.081	0.054	1.732	0.274
	Central Norway. inland	2014	1	0.117	NA	0.117	0.117	0.117	NA
		2015	2	0.507	0.415	0.507	0.214	0.800	0.293
	Central Norway. coast	1989	1	0.014	NA	0.014	0.014	0.014	NA
		2013	5	0.642	0.672	0.216	0.058	1.546	0.301
		2014	2	0.770	0.866	0.770	0.158	1.383	0.612
		2015	3	0.415	0.470	0.175	0.115	0.957	0.271
		2016	7	0.183	0.106	0.168	0.044	0.385	0.040
	Southern Norway	2013	4	0.114	0.118	0.062	0.040	0.290	0.059
		2014	1	0.173	NA	0.173	0.173	0.173	NA
		2015	4	0.115	0.112	0.074	0.033	0.278	0.056
		2016	3	0.340	0.373	0.235	0.031	0.755	0.216
	Northern Norway	1979	1	0.022	NA	0.022	0.022	0.022	NA
		2013	11	0.087	0.054	0.082	0.013	0.181	0.016
FHpPA		2014	7	0.104	0.074	0.085	0.018	0.230	0.028
		2015	12	0.074	0.033	0.070	0.034	0.148	0.009
		2016	6	0.070	0.041	0.066	0.020	0.132	0.017
	Central Norway. inland	2014	1	0.010	NA	0.010	0.010	0.010	NA
		2015	2	0.043	0.025	0.043	0.026	0.061	0.018
	Central Norway. coast	1989	1	0.047	NA	0.047	0.047	0.047	NA
		2013	5	0.310	0.322	0.164	0.027	0.843	0.144
		2014	2	0.164	0.060	0.164	0.121	0.206	0.042

		2015	3	0.140	0.100	0.168	0.029	0.223	0.058
		2016	7	0.249	0.222	0.182	0.016	0.637	0.084
L-PFOS	Southern Norway	2013	4	11.071	6.796	10.096	3.898	20.194	3.398
		2014	1	13.743	NA	13.743	13.743	13.743	NA
		2015	4	15.644	12.785	15.993	1.612	28.978	6.392
		2016	3	25.774	24.063	22.237	3.674	51.410	13.893
	Northern Norway	1979	1	1.182	NA	1.182	1.182	1.182	NA
		2013	11	9.771	4.339	8.031	2.765	17.158	1.308
		2014	7	10.708	2.408	9.676	8.540	14.045	0.910
		2015	12	11.219	9.455	8.855	0.756	37.410	2.730
		2016	6	5.646	3.475	4.541	2.632	11.605	1.419
	Central Norway. inland	2014	1	4.277	NA	4.277	4.277	4.277	NA
		2015	2	3.713	2.893	3.713	1.667	5.759	2.046
	Central Norway. coast	1989	1	25.958	NA	25.958	25.958	25.958	NA
		2013	5	24.201	27.856	13.245	8.758	73.911	12.457
		2014	2	15.934	12.349	15.934	7.202	24.666	8.732
		2015	3	8.449	2.908	8.352	5.590	11.404	1.679
		2016	7	7.619	2.374	7.641	3.426	10.166	0.897
Br-PFOS	Southern Norway	2013	4	1.060	0.743	0.843	0.420	2.133	0.372
		2014	1	1.402	NA	1.402	1.402	1.402	NA
		2015	4	1.151	0.633	1.285	0.274	1.760	0.317
		2016	3	1.012	0.572	1.121	0.394	1.521	0.330
	Northern Norway	1979	1	0.464	NA	0.464	0.464	0.464	NA
		2013	11	0.913	0.386	0.929	0.343	1.561	0.116
		2014	7	0.957	0.387	0.740	0.666	1.706	0.146
		2015	12	0.988	0.335	0.879	0.664	1.676	0.097
		2016	6	0.662	0.279	0.633	0.335	1.001	0.114
	Central Norway. inland	2014	1	0.423	NA	0.423	0.423	0.423	NA
		2015	2	0.292	0.077	0.292	0.238	0.347	0.055
	Central Norway. coast	1989	1	1.807	NA	1.807	1.807	1.807	NA
		2013	5	1.769	1.779	1.222	0.546	4.909	0.796
		2014	2	1.705	1.202	1.705	0.855	2.555	0.850
		2015	3	0.829	0.040	0.838	0.786	0.864	0.023
		2016	7	0.620	0.193	0.532	0.430	0.936	0.073
L-PFDS	Southern Norway	2013	4	0.490	0.454	0.537	< 0.07	0.959	0.227
		2014	1	0.112	NA	0.112	0.112	0.112	NA
		2015	4	0.058	0.161	0.020	< 0.07	0.263	0.080
		2016	3	0.041	0.099	0.074	< 0.07	0.120	0.057
	Northern Norway	1979	1	< 0.07	NA	< 0.07	< 0.07	< 0.07	NA
		2013	11	< 0.07	0.044	< 0.07	< 0.07	0.076	0.013
		2014	7	< 0.07	0.092	< 0.07	< 0.07	0.131	0.035
		2015	12	0.029	0.132	< 0.07	< 0.07	0.257	0.038
		2016	6	< 0.07	0.072	< 0.07	< 0.07	0.107	0.030
	Central Norway. inland	2014	1	< 0.07	NA	< 0.07	< 0.07	< 0.07	NA
		2015	2	< 0.07	0.000	< 0.07	< 0.07	< 0.07	0.000
	Central Norway. coast	1989	1	< 0.07	NA	< 0.07	< 0.07	< 0.07	NA
		2013	5	0.189	0.135	0.144	0.073	0.414	0.061
		2014	2	0.124	0.274	0.124	< 0.07	0.318	0.194
		2015	3	< 0.07	0.103	< 0.07	< 0.07	0.108	0.059
		2016	7	0.088	0.073	0.111	< 0.07	0.154	0.028

	Southern Norway	2013	4	0.310	0.346	0.183	0.054	0.820	0.173
		2014	1	0.364	NA	0.364	0.364	0.364	NA
		2015	4	0.149	0.137	0.110	0.037	0.340	0.069
		2016	3	0.396	0.284	0.501	0.075	0.613	0.164
	Northern Norway	1979	1	0.049	NA	0.049	0.049	0.049	NA
		2013	11	0.349	0.320	0.240	0.058	1.207	0.096
		2014	7	0.183	0.085	0.195	0.044	0.312	0.032
L-FOSA		2015	12	0.275	0.458	0.141	0.071	1.720	0.132
		2016	6	0.130	0.092	0.121	0.028	0.294	0.037
	Central Norway. inland	2014	1	0.099	NA	0.099	0.099	0.099	NA
		2015	2	0.054	0.034	0.054	0.030	0.078	0.024
	Central Norway. coast	1989	1	0.992	NA	0.992	0.992	0.992	NA
		2013	5	0.764	0.836	0.241	0.202	2.118	0.374
		2014	2	1.528	1.963	1.528	0.139	2.916	1.388
		2015	3	0.485	0.572	0.199	0.113	1.144	0.330
		2016	7	0.227	0.100	0.213	0.087	0.368	0.038
	Southern Norway	2013	4	0.024	0.018	0.025	0.003	0.042	0.009
		2014	1	0.012	NA	0.012	0.012	0.012	NA
		2015	4	0.019	0.005	0.020	0.012	0.024	0.003
		2016	3	0.032	0.030	0.016	0.013	0.067	0.017
	Northern Norway	1979	1	0.017	NA	0.017	0.017	0.017	NA
		2013	11	0.021	0.015	0.018	0.006	0.052	0.005
		2014	7	0.027	0.022	0.020	0.009	0.071	0.008
Br-FOSA		2015	12	0.022	0.011	0.021	0.007	0.040	0.003
		2016	6	0.016	0.010	0.017	0.004	0.031	0.004
	Central Norway. inland	2014	1	0.035	NA	0.035	0.035	0.035	NA
		2015	2	0.006	0.003	0.006	0.004	0.008	0.002
	Central Norway. coast	1989	1	0.029	NA	0.029	0.029	0.029	NA
		2013	5	0.031	0.018	0.026	0.014	0.053	0.008
		2014	2	0.045	0.050	0.045	0.010	0.081	0.035
		2015	3	0.014	0.007	0.017	0.005	0.019	0.004
		2016	7	0.029	0.031	0.008	0.005	0.080	0.012
	Southern Norway	2013	4	0.240	0.253	0.178	0.027	0.578	0.126
		2014	1	0.358	NA	0.358	0.358	0.358	NA
		2015	4	0.020	0.022	0.012	0.004	0.053	0.011
		2016	3	0.227	0.170	0.209	0.067	0.404	0.098
	Northern Norway	1979	1	0.259	NA	0.259	0.259	0.259	NA
		2013	11	0.047	0.050	0.032	0.016	0.191	0.015
		2014	7	0.382	0.436	0.203	0.103	1.331	0.165
6:2FTS		2015	12	0.031	0.035	0.016	0.003	0.119	0.010
		2016	6	0.104	0.063	0.113	0.006	0.193	0.026
	Central Norway. inland	2014	1	0.438	NA	0.438	0.438	0.438	NA
		2015	2	0.022	0.020	0.022	0.008	0.036	0.014
	Central Norway. coast	1989	1	0.056	NA	0.056	0.056	0.056	NA
		2013	5	0.020	0.019	0.011	0.002	0.045	0.008
		2014	2	0.185	0.064	0.185	0.140	0.231	0.045
		2015	3	0.037	0.022	0.026	0.023	0.062	0.013
		2016	7	0.205	0.152	0.198	0.024	0.445	0.057
8:2FTS	Southern Norway	2013	4	0.228	0.217	0.166	0.044	0.537	0.108
		2014	1	0.081	NA	0.081	0.081	0.081	NA

	2015	4	0.110	0.080	0.103	0.030	0.205	0.040
	2016	3	0.227	0.057	0.195	0.193	0.294	0.033
Northern Norway	1979	1	0.079	NA	0.079	0.079	0.079	NA
	2013	11	0.110	0.102	0.084	0.039	0.393	0.031
	2014	7	0.096	0.063	0.072	0.032	0.217	0.024
	2015	12	0.087	0.078	0.053	0.012	0.254	0.023
	2016	6	0.066	0.034	0.070	0.016	0.101	0.014
Central Norway. inland	2014	1	0.094	NA	0.094	0.094	0.094	NA
	2015	2	0.046	0.015	0.046	0.035	0.056	0.010
Central Norway. coast	1989	1	0.070	NA	0.070	0.070	0.070	NA
	2013	5	0.136	0.054	0.141	0.074	0.215	0.024
	2014	2	0.091	0.064	0.091	0.046	0.136	0.045
	2015	3	0.081	0.060	0.068	0.029	0.146	0.035
	2016	7	0.225	0.224	0.128	0.021	0.524	0.085

Appendix E. Stable isotope analysis

Table A. 15: Stable isotope analysis. Mass indicated the weight of individual feathers in milligram (mg).

ID	mass	C:N	C	N	d13C	d15N
188	1.04	3.20	43.77	13.66	-21.57	12.52
190	1.41	3.12	41.78	13.41	-19.82	13.29
201	1.33	3.17	43.41	13.69	-21.97	12.20
205	1.24	3.18	45.34	14.26	-19.06	13.59
209	2.02	3.12	43.39	13.91	-19.76	13.88
215	1.47	3.18	44.33	13.92	-20.17	13.34
230	1.39	3.12	43.48	13.95	-21.35	12.14
234	1.72	3.20	43.49	13.60	-22.78	10.86
238	1.60	3.29	43.99	13.37	-22.07	11.69
251	1.59	3.10	42.74	13.78	-22.43	11.56
261	1.21	3.13	42.59	13.59	-19.60	13.57
325	1.54	3.25	42.35	13.04	-22.08	12.35
342	1.42	3.29	42.54	12.94	-24.38	11.91
358	1.88	3.14	43.90	13.97	-23.40	11.83
361	1.78	3.23	44.38	13.74	-18.32	13.90
391	1.38	3.12	43.91	14.06	-25.09	11.43
453	1.78	3.26	44.58	13.66	-23.24	4.84
454	1.58	3.13	41.49	13.27	-21.54	6.30
458	1.72	3.13	42.32	13.51	-18.36	15.37
459	1.41	3.11	44.19	14.19	-19.96	11.03
463	2.00	3.09	41.95	13.59	-19.87	13.07
456	1.47	3.18	44.21	13.90	-22.83	7.92
524	1.64	3.12	42.93	13.78	-23.74	10.39
526	0.86	3.17	41.70	13.15	-24.62	10.81
529	1.19	3.21	45.26	14.08	-22.82	12.10
531	1.12	3.26	45.45	13.94	-24.26	11.07
532	1.54	3.18	43.57	13.72	-22.97	10.44
538	1.44	3.13	44.08	14.08	-19.36	13.62
540	1.27	3.17	43.86	13.83	-21.16	10.75
550	0.99	3.30	44.95	13.63	-20.16	12.78
562	1.40	3.27	43.01	13.14	-25.29	10.91
565	1.83	3.11	43.47	13.96	-24.54	10.05
574	1.33	3.17	44.01	13.88	-23.86	10.88
576	1.47	3.20	44.96	14.05	-18.44	14.18
578	1.46	3.11	40.37	12.96	-23.61	12.55
586	1.38	3.25	42.45	13.05	-23.33	7.55
589	1.23	3.25	42.90	13.22	-23.82	6.78
590	1.30	3.26	42.85	13.15	-21.79	6.72
594	1.30	3.23	40.86	12.66	-22.22	6.62
601	1.99	3.15	42.90	13.62	-18.93	13.11
604	1.70	3.24	43.04	13.30	-22.94	10.63
609	1.31	3.15	42.40	13.44	-18.84	12.94

613	1.44	3.12	41.46	13.27	-21.72	12.21
616	1.59	3.13	42.57	13.59	-22.21	12.00
618	1.72	3.09	42.29	13.68	-20.04	12.59
624	1.66	3.10	43.60	14.07	-17.92	13.87
630	1.74	3.13	40.55	12.95	-17.69	15.29
634	1.22	3.26	43.84	13.45	-21.78	7.75
636	1.51	3.19	43.40	13.60	-22.47	7.24
638	1.19	3.13	42.18	13.47	-22.46	9.11
639	1.71	3.29	43.60	13.26	-22.41	6.22
644	1.94	3.14	43.30	13.77	-19.49	14.00
651	1.66	3.11	43.15	13.89	-17.95	14.11
653	1.76	3.25	44.09	13.57	-21.10	6.87
662	1.66	3.31	43.80	13.24	-22.77	10.50
665	1.70	3.20	43.75	13.69	-22.50	9.35
667	1.11	3.25	45.09	13.89	-18.11	14.63
668	1.26	3.27	40.97	12.52	-23.79	6.91
670	1.18	3.24	41.88	12.91	-18.84	13.70
690	1.84	3.44	43.64	12.71	-21.81	11.84
718	1.76	3.23	42.97	13.31	-22.86	6.27
726	1.38	3.25	43.84	13.47	-20.88	10.35
728	1.41	3.47	45.08	12.98	-21.16	10.54
778	1.41	3.21	43.37	13.51	-16.65	15.80
782	1.32	3.22	43.99	13.66	-17.81	14.72
784	1.69	3.27	44.12	13.48	-20.98	11.43
785	1.42	3.20	44.59	13.93	-23.71	10.23
806	1.64	3.19	44.75	14.04	-23.86	10.29
809	1.99	3.14	44.19	14.08	-22.66	12.01
812	1.79	3.18	41.42	13.04	-23.62	10.18
813	1.57	3.15	43.47	13.81	-19.77	12.27
832	1.61	3.27	43.82	13.39	-25.62	9.79

Appendix F. Correlation matrix and plots

A correlation matrix of compounds detected in more than 60% of the samples was calculated using the Spearman correlation coefficient, rho (r_s) (**Feil! Fant ikke referansekilden.**). Of all the PFASs analyzed, only linear FOSA was found to correlate significantly with both of the stable isotopes. All PFCAs were highly correlated, except for PFOA which onlys displayed a weak correlation with PFNA. L-PFOS and br-PFOS were also highly correlated, and a positive correlation between L-FOSA and both PFOS isomers was also detected.

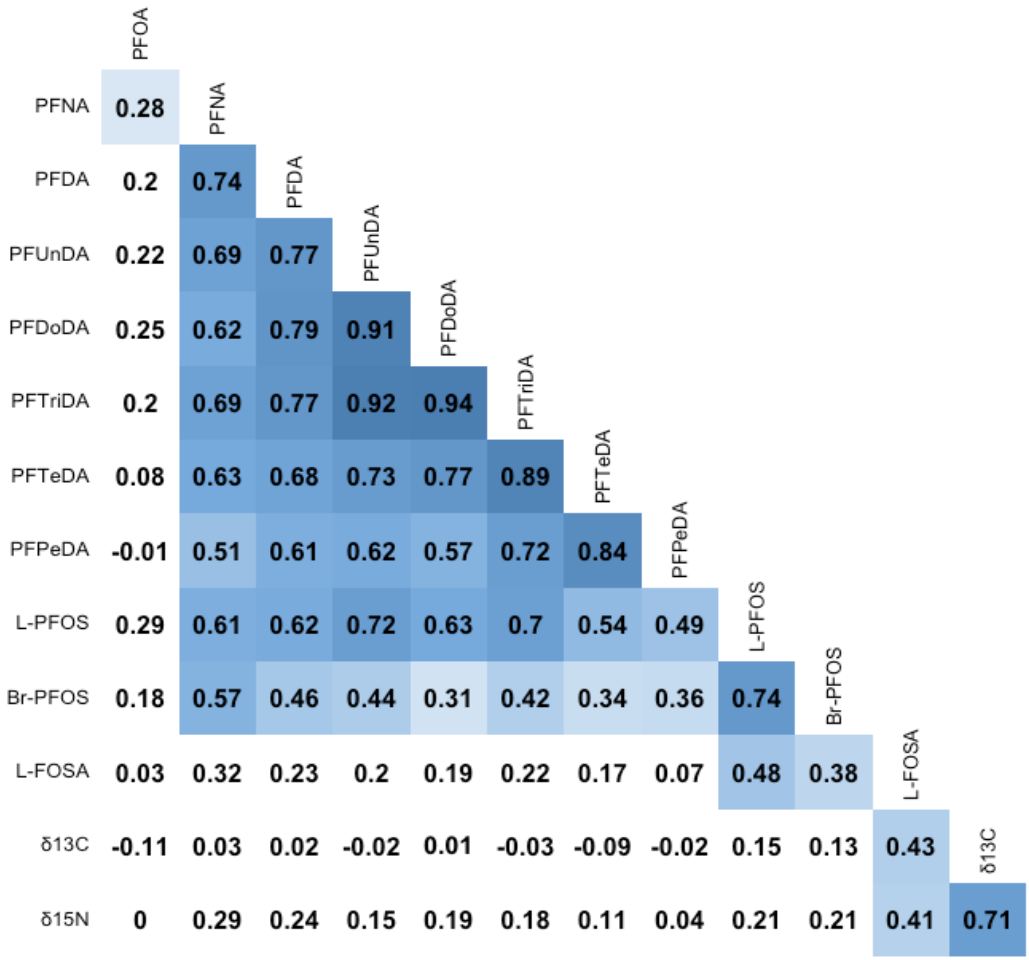

Figure A. 1 Correlation matrix illustrating Spearman rank correlation coefficients (r_s) between selected PFASs and stable isotopes. Blue indicates significant positive correlation. Blank indicates non-significant correlations (significance level set to $\alpha = 0.05$). The color intensity of the shaded boxes indicates the strength of correlation.

Correlation plots of PFASs and stable isotopes

The relationship between of selected PFASs (ng/g dw) and stable isotope values in feathers as presented in figure A.2-A.7.

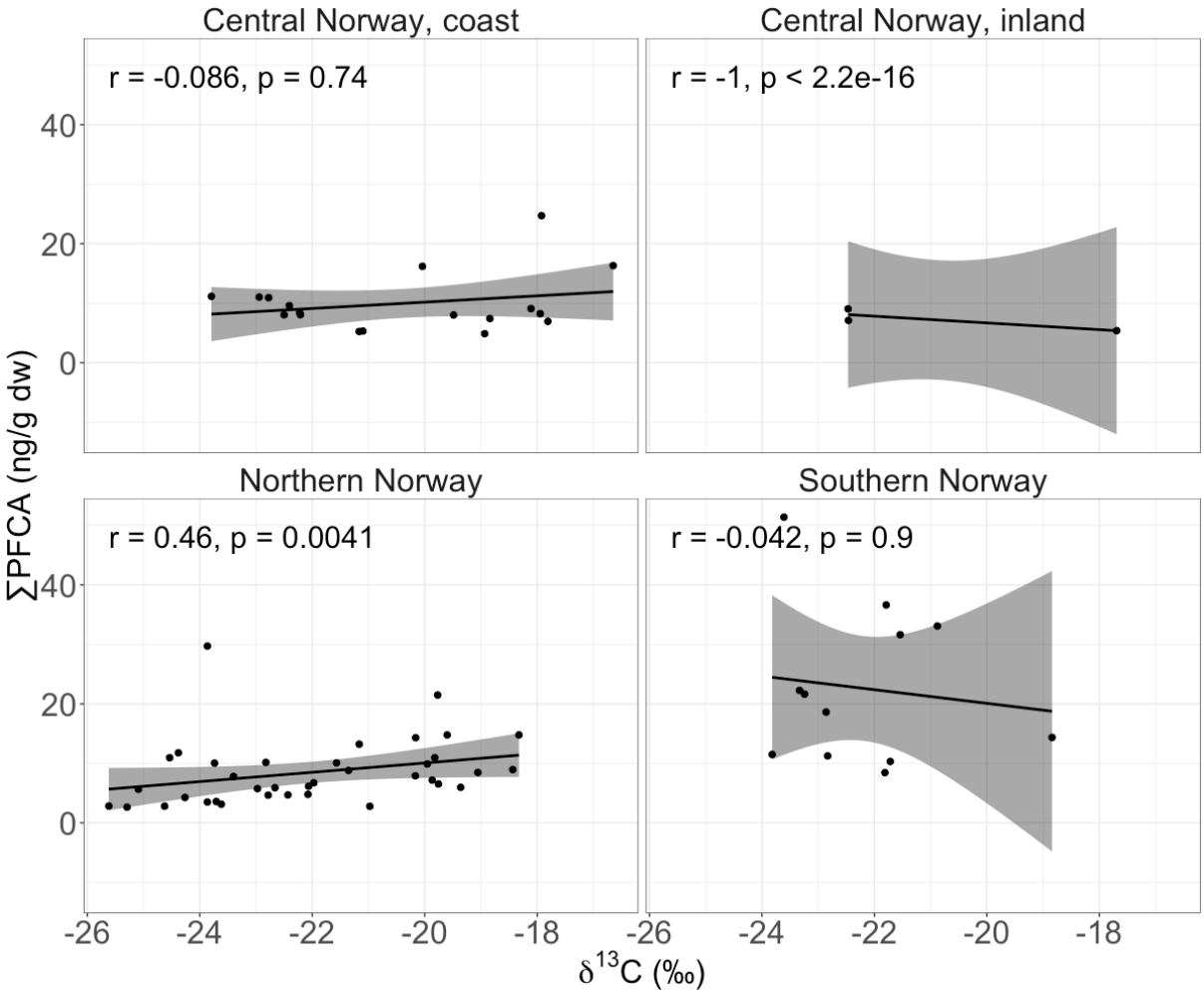

Figure A. 2 Relationship between ∑PFCA (ng/g dw) and $\delta^{13}C$ (‰). The Spearman rank correlation was performed for all four areas. Grey areas represent 95 % confidence intervals. Dots represents individual samples. Significance level was set to $\alpha = 0.05$.

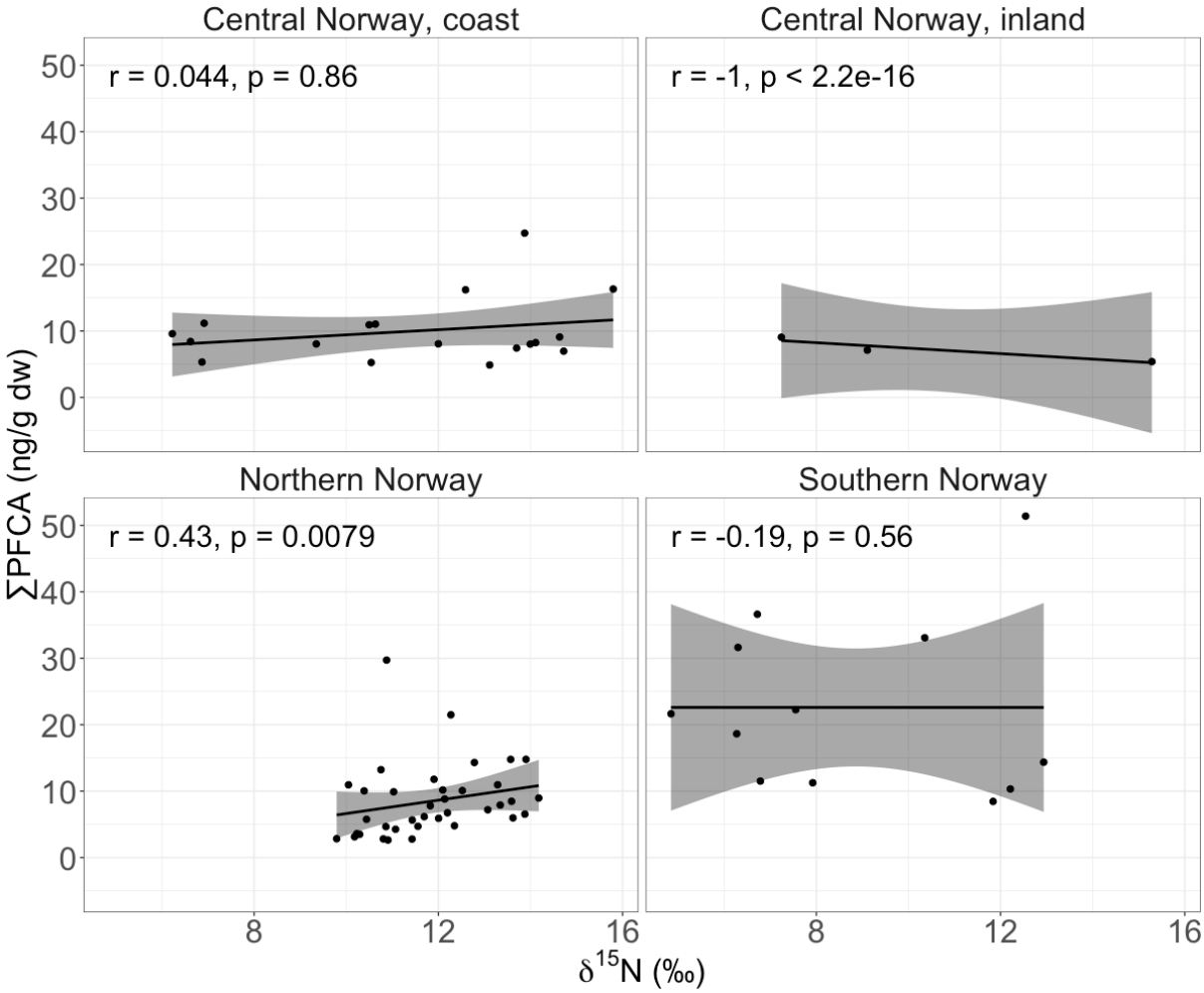

Figure A. 3 Relationship between ∑PFCA (ng/g dw) and $\delta^{15}N$ (‰). The Spearman rank correlation was performed for all four areas. Grey areas represent 95 % confidence intervals. Dots represents individual samples. Significance level was set to $\alpha = 0.05$.

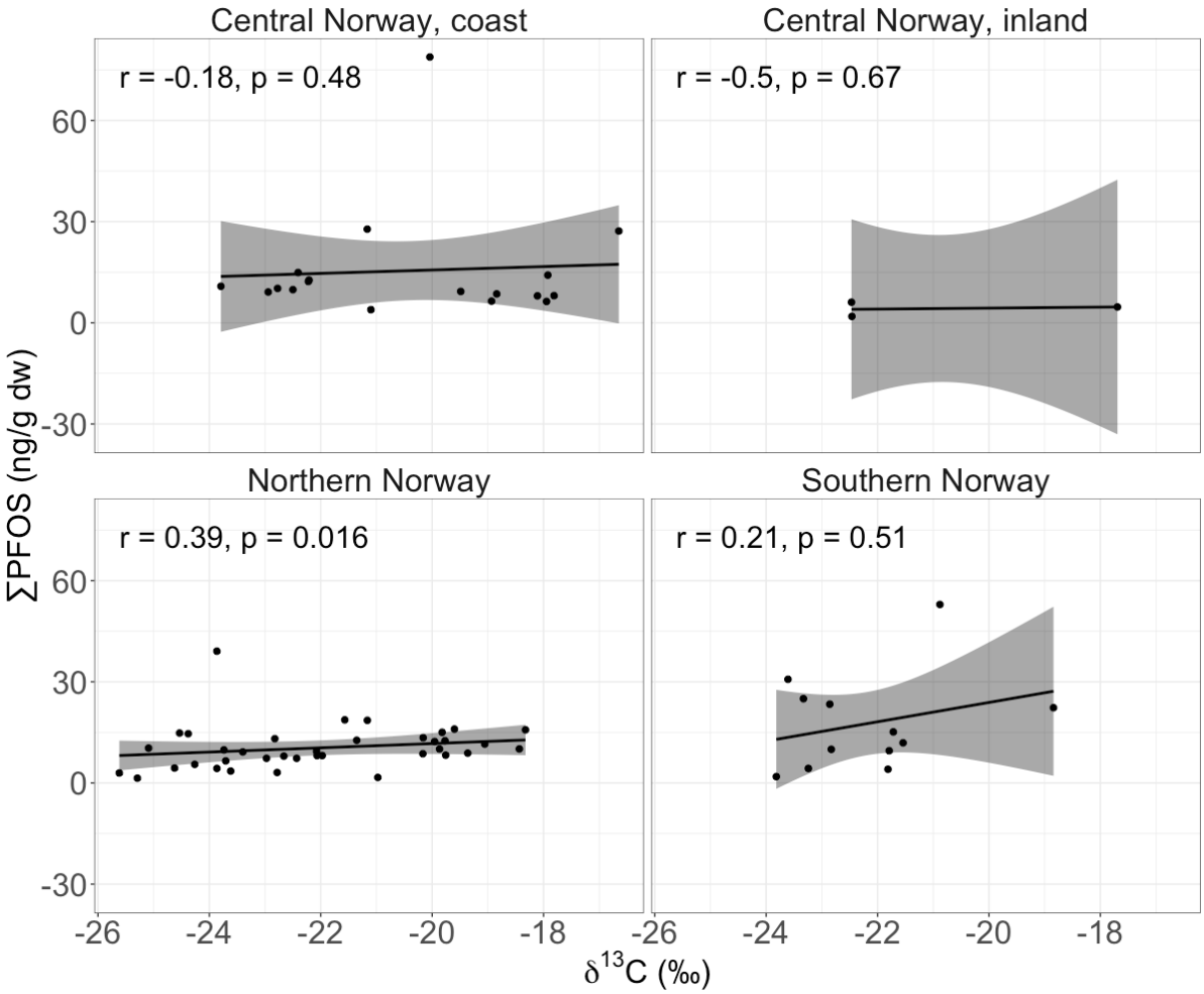

Figure A. 4 Relationship between ∑PFOS (ng/g dw) and $\delta^{13}C$ (‰). The Spearman rank correlation was performed for all four areas. Grey areas represent 95 % confidence intervals. Dots represents individual samples. Significance level was set to $\alpha = 0.05$.

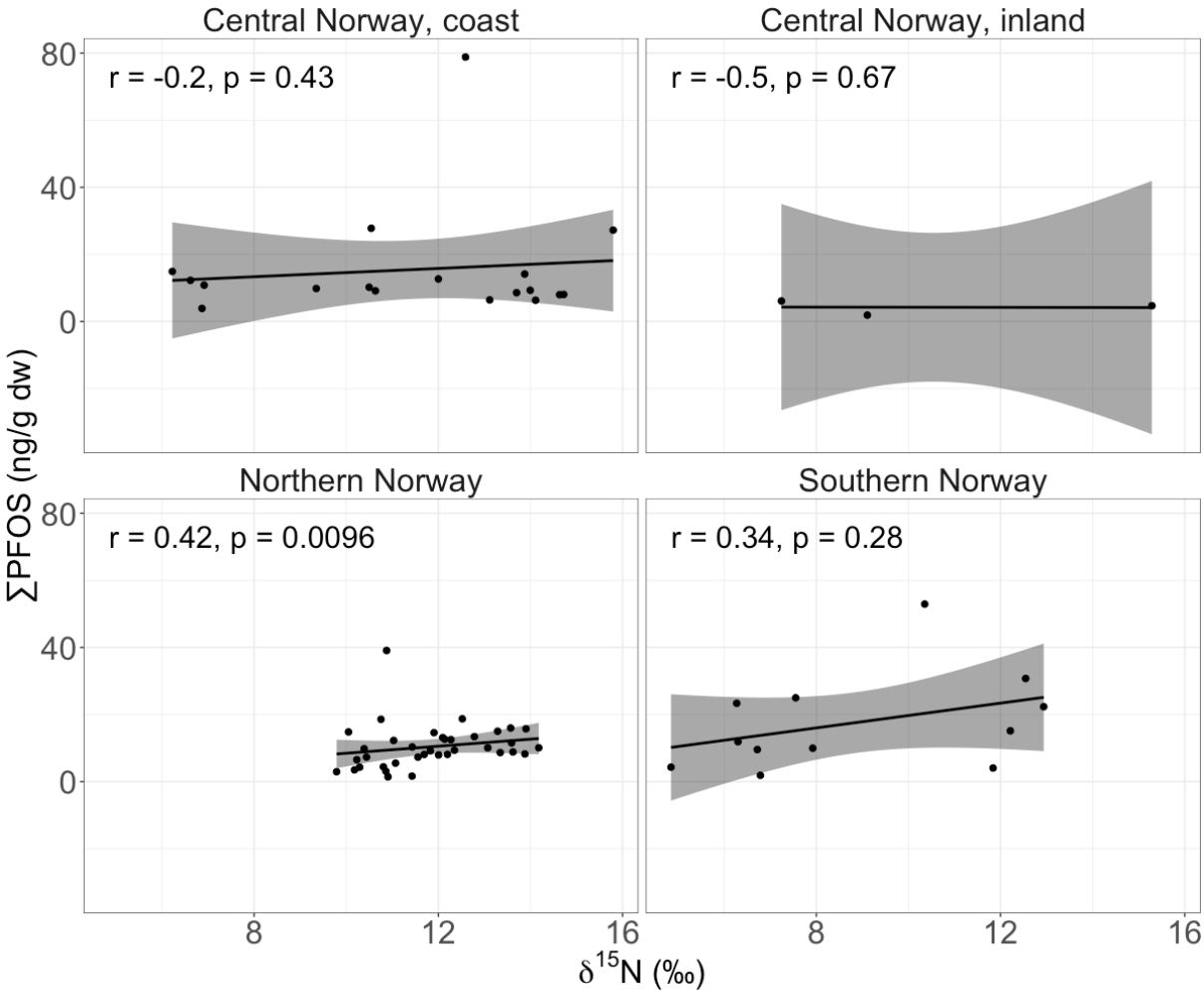

Figure A. 5 Relationship between ∑PFOS (ng/g dw) and $\delta^{15}N$ (‰). The Spearman rank correlation was performed for all four areas. Grey areas represent 95 % confidence intervals. Dots represents individual samples. Significance level was set to $\alpha = 0.05$.

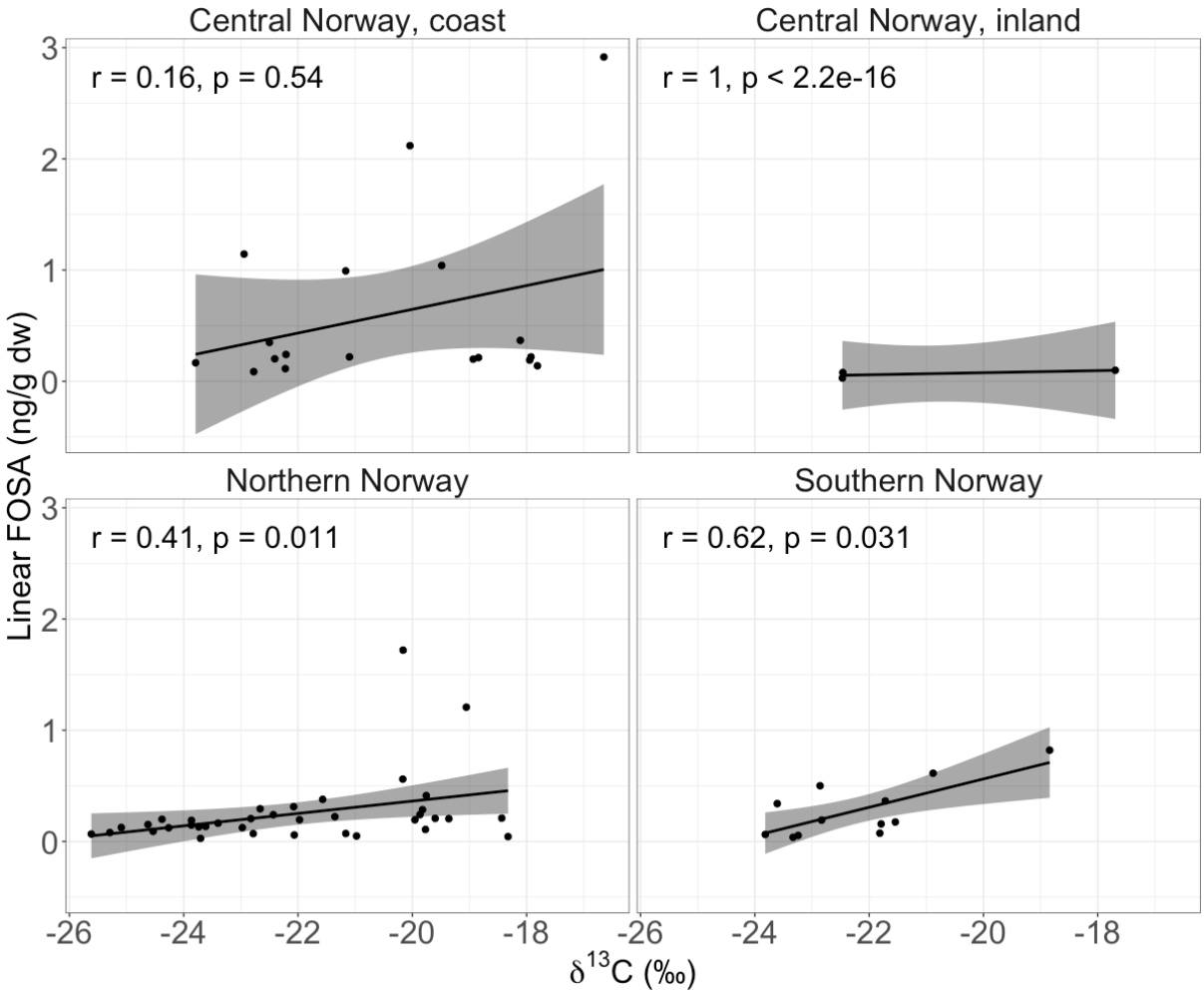

Figure A. 6 Relationship between linear FOSA (ng/g dw) and $\delta^{13}C$ (‰). The Spearman rank correlation was performed for all four areas. Grey areas represent 95 % confidence intervals. Dots represents individual samples. Significance level was set to $\alpha = 0.05$.

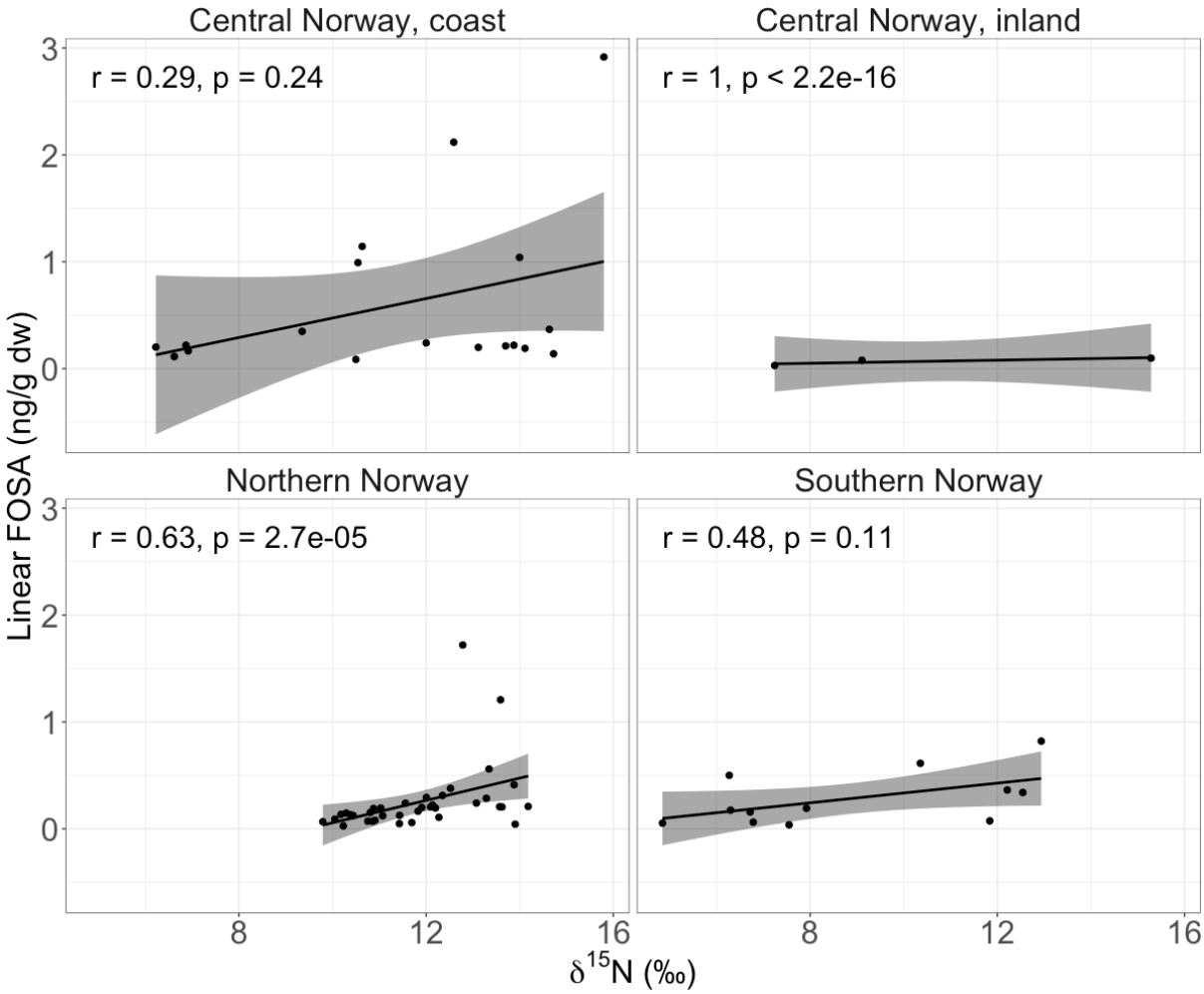

Figure A. 7 Relationship between linear FOSA (ng/g dw) and $\delta^{15}N$ (‰). The Spearman rank correlation was performed for all four areas. Grey areas represent 95 % confidence intervals. Dots represents individual samples. Significance level was set to $\alpha = 0.05$.