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Toxicology of the Svalbard Atlantic Puffin

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Arin Kendall Povelite Underwood

Cover: Atlantic Puffin. Photo: Arin K.P. Underwood

Abstract

The Arctic is a sink for pollutants that accumulate there mostly through mechanisms of long-range transport via atmospheric and ocean currents. The concentrations of persistent organic pollutants (POPs) then increase through trophic levels via biomagnification. The main objectives of this study were to identify the pollutants present in the Svalbard Atlantic puffin (*Fratercula arctica*) and to compare their contamination levels with other southern puffin colonies and other seabird species breeding in Svalbard with the purpose of conducting a risk assessment for the puffin. Samples were collected from 16 puffins in the Gåsøyane bird colony in Svalbard in summer, 2018, then analysed for contaminants, and compared to previously published data on other puffin colonies and Svalbard breeding seabird species. Game cameras were also used to assess food loads brought to chicks so prey species could be identified. The Svalbard puffins were found to be less contaminated than the puffin colonies in north east Norway, perhaps due to their distance from the Barents Sea as a pollution sink, as well as the low contamination level of the Svalbard puffin's main prey, polar cod. The Svalbard population POP levels were most similar to Røst puffins, with their exposure to Atlantic waters possibly a factor. Svalbard puffins also had POP levels comparable to those of Brünnich's guillemots and little auks, which was unexpected as little auks and Brünnich's guillemots exist at lower trophic levels. The black guillemots' and kittiwake's trophic level is similar to puffins, but the kittiwake had much higher levels. The glaucous gull also had significantly higher PCB levels as expected but low HCB, oxy-chlordane, and PFASs, which could stem from their diet. Based on these findings, the Svalbard puffin should be at low risk for biological effects from contamination though further study and monitoring is necessary as climate change is expected to exacerbate the influence and effects of POPs in the Arctic.

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1 Introduction

1.1 Contamination in the Arctic

The Arctic is often viewed as one of the last clean natural environments in the world and is characterized by extreme and barren conditions. In the 1970s however, scientific attention was drawn to the discovery of persistent organic pollutants (POPs) in Arctic biota (Jensen et al., 1972; Hung et al., 2016). POPs are long-lasting, far-reaching compounds that infiltrate food webs over time and cause insidious detrimental effects (Letcher et al., 2010). These POPs have been in mass production for decades and are used to make products flame-retardant and resistant to water and degradation. It is these characteristics that then ensure the perseverance of the contaminants in the biosphere once they are released into the atmosphere and ocean (Helgason et al., 2008). As low temperatures limit POP breakdown and local pollution sources are scarce, the Arctic acts as a sink for POPs and demonstrates the far and long-term reach of global pollution (Borgå et al., 2005).

Many POPs were first produced in the 1930s and 40s, reached their highest concentrations in the 1960s and early 70s, and prompted scientific concern in the 1970s and 90s (Gabrielsen & Sydnes, 2009; Adetona et al., 2013). Various POPs were regulated and partially banned during this time, but the first international agreement on POPs was the 2004 Stockholm Convention. Under direction from the United Nations, the convention officially marked 12 POPs for reduction and elimination of their production, use, and release into the environment. These “dirty dozen” were organochlorine pesticides and industrial chemicals and their by-products deemed to have adverse effects: DDT (Dichlorodiphenyltrichloroethane), HCB (Hexachlorobenzene), chlordane, PCBs (polychlorinated biphenyls), aldrin, dieldrin, endrin, heptachlor, mirex, toxaphene, PCDD (Polychlorinated dibenzo-p-dioxins) and PCDF (Polychlorinated dibenzofurans). In 2009 an additional 9 POPs were banned: HCH (Hexachlorocyclohexane: alpha, beta, and gamma), chlordecone, HBB (Hexabromobiphenyl), PeCB (Pentachlorobenzene), penta-BDE (pentabromodiphenyl ether), octa-BDE (octabromodiphenyl ether), PFOS (Perfluorooctane sulfonic acid), endosulfan, and HBCDD (Hexabromocyclododecane), and currently siloxane, PFOA (Perfluorooctanoic acid), and SCCP (Short-chain chlorinated paraffins) are under consideration (http://www.pops.int).

The Stockholm Convention recognized four criteria for banning POPS: the compound must be persistent, capable of long-range transport, accumulate in biota, and cause a detrimental

effect to biota (http://www.pops.int). By design, POPs are persistent and semi-volatile, allowing them to change phase easily and so remain mobile in the environment. When atmospheric POPs encounter cold environments, they can condense and shift away from their gas phase to enter the biome (Bard, 1999). This global distillation ensures that atmospheric contaminants have a net movement from low to high latitude and altitude and over time the Arctic acts as a global sink for contaminants (Wania & MacKay, 1993; Leat et al., 2019). Long-range transport occurs mainly through atmospheric and ocean currents, but also river runoff, and biotic pathways (migratory fish, marine mammals and birds) (Borgå, 2002).

POPs are usually categorized as legacy or emerging. The legacy POPs are no longer in production and are all generally lipophilic organochlorines (OCs) that served as industrial chemicals (PCB, HCB) and pesticides (DDT) (Rigét et al., 2019). while emerging POPS are largely unregulated lipophobic Perfluoroalkyl substances (PFASs) and brominated flame retardants (BFRs) (Letcher et al., 2010). Since restrictions in the 1970s and 80s as well as the Stockholm Convention, PCBs and OC pesticides have shown decreasing trends in Arctic biota, with OCs also decreasing in the atmosphere. Despite bans though, HCB has shown either increases or slow decreases in Arctic biota due to by-production in chemical processes (Rigét et al., 2019). Long-chain PFCAs (PFNA, PFDA and PFUnA) are also increasing in Arctic biota with the exception of PFOS, due to a reduction in its use (Rigét et al., 2019). Currently some contaminant levels in the Arctic are greater than those in industrialized cities and the continued aim of the Stockholm Convention is to induce transitions to safe alternatives to POPs, create new policies surrounding emerging POPs, and clean up remaining POP stockpiles (Tartu et al., 2014; AMAP, 2017).

1.2 Bioaccumulation and Biomagnification

Once brought to the Arctic via long-range transport, contaminants in seawater can be adsorbed by marine organisms and accumulate over time depending on the species and the chemical (Borgå et al., 2001). This bioaccumulation is the net contamination result of adsorbed POPs in an organism after all elimination processes (e.g. feces, biotransformation, respiration) resulting in concentrations exceeding those of their surrounding environment (Borgå et al., 2004; Hallanger, 2010). Due to the short productivity periods in the Arctic, lipid storages are very important for energy expensive events like breeding and molting (Borgå et al., 2001) and Arctic biota tend to have longer life-spans as a result (Borgå et al., 2004). The

lipid rich diets, longer lives, and efficient energy transfer of Arctic biota all contribute to their vulnerability to bioaccumulation (Borgå et al., 2004). Bioaccumulation also leads to biomagnification, where contaminant levels become more concentrated with each trophic level (Borgå et al., 2004). This allows POP concentrations to increase as they are biomagnified from invertebrates to fish, to seabirds, and then to top predators like polar bears and glaucous gulls (Borgå et al., 2001; Gabrielsen and Sydnes, 2009). While many species uptake hydrophobic OCs at similar rates, the rate of elimination of those OCs regulates how it biomagnifies (Borgå et al., 2004). The bioaccumulation of contaminants in the Arctic is also subject to seasonal variation, with contaminant concentrations and their ability to bioaccumulate decreasing in zooplankton across the breeding season from May to October, while lipid stores increase (Hallanger, 2010). Meanwhile, the biomagnification of contaminants between trophic levels increases over breeding season. The strength of biomagnification and the contamination of seawater is also reliant on seasons and ocean currents, with Arctic waters having different contamination than Atlantic water (Hallanger, 2010).

1.3 Biological Effects

The effects POPs can have on biota vary depending on the physiochemical properties of the POPs, and the exposure, metabolism, sex, age, and seasonality (breeding, non-breeding) of the organism (Borgå et al., 2004). Given the diversity of these aspects, there exists a knowledge shortfall concerning the biological and toxicological effects of POPs on Arctic species. Most available research has been conducted on a few marine top predators (e.g. polar bears, killer whales, glaucous gulls) with high contaminant loads, leaving less information on species in lower trophic levels (Gabrielsen, 2007). Many possible effects of contamination can also be difficult to interpret as other factors such as climate change, pathogens, invasive species, and ecological interactions can affect exposure or physiological responses (Letcher et al., 2010).

Contaminants infiltrate organisms mostly through diet and can be stored in adipose and liver tissue over time (Borgå et al., 2004). During high energy requirements however, such as breeding and molting in seabirds, the lipophilic POPs can be mobilized in the bloodstream as adipose tissue is burned. Once mobilized, POPs can affect the proper function of enzymes, vitamins, and the endocrine and immune system, eventually affecting reproduction (Letcher et al., 2010; Tartu et al., 2014), body condition, and even survival (Sagerup et al., 2009; Erikstad

et al., 2013). As the Arctic has a short season of productivity, altered endocrine regulation of events such as breeding, molting, and migration could have harmful results, decreasing populations over time (Fauchald et al., 2015; AMAP, 2018). A compromised immune system could also have secondary effects in the Arctic, leaving organisms vulnerable to parasites and unable to fight off pathogens as effectively, causing potential mortality in offspring (Sagerup et al., 2009; Letcher et al., 2010). Another factor contributing to contamination is sex. Females have historically had lower contaminant levels than males due to the expulsion of contaminants through egg laying (egg quantity is also a factor) (Verreault et al., 2006; Verreault et al., 2010) .

1.4 Study Species: Atlantic Puffin

Fig 1: Adult Atlantic Puffin (*Fratercula arctica*), Gåsøyane colony, Svalbard July 2018. Photo: Arin K.P. Underwood

The Atlantic puffin (*Fratercula Arctica*, hereafter referred to as puffin) (Fig 1) is a low-arctic alcid (Veit & Manne, 2015) found on both sides of the North Atlantic Ocean. Its range extends across the coasts of Greenland, eastern Canada, the Faroe Islands, Iceland, the north-western coast of Europe, northern Russia, and Svalbard. Puffins are the most common breeding seabird in Norway with an estimated two million breeding pairs in the country. Less than 1% of the global population breeds in the high-Arctic in Svalbard (Anker-Nilssen et al., 2000; Barrett, 2002).

The Svalbard population has historically been small and scattered, with only a few hundred seen gathered around Spitsbergen in the late 1940s (Løvenskiold, 1964), though as of 2000

they are estimated at fewer than 10,000 breeding pairs throughout 50 known colonies (including Bjørnøya), the northernmost puffin colonies in the world (Anker-Nilssen et al., 2000). There are three recognized subspecies of puffin, described via size differences. The Spitsbergen populations are *F. a. naumanni*, with a larger body size than the southern puffin populations (*F.a. arctica*) that breed along the coast of mainland Norway (Anker-Nilssen et al., 2000). The Svalbard puffins also build nests in rock crevices rather than burrows due to the lack of suitable soil in the environment. Despite puffins being the most numerous seabird in the coastal Norwegian Sea (Barrett et al., 2002), there is a knowledge shortfall on the ecology and toxicology of the Svalbard colonies as their populations are recent, small, spread out, and generally difficult to access.

1.4.1 Diet

Puffins are pelagic pursuit divers, often hunting out at sea and diving some 75 meters in the water column for prey (Durant et al., 2003). They typically feed on shoaling fish such as Atlantic herring (*Clupea harengus*) and capelin (*Mallotus villosus)* though have been recorded to also feed on crustaceans, squid, and polychaete worms (Anker-Nilssen et al., 2000; Barrett, 2002). No diet data exists for Spitsbergen puffins, though the Bjørnøya population has been shown to prey mostly on capelin, daubed shanny (*Leptoclinus maculatus*), and sand eels (*Ammodytes sp.)* with some Atlantic herring and gadids being delivered to chicks (Hornset, 2017). Northern Norway populations generally focus on four different prey species: capelin, sand eels, herring, and Atlantic cod (*Gadus morhua*) (Barrett, 2002). Puffins can also be considered income breeders, continuing to feed while incubating their eggs during the breeding season as opposed to capital breeders that rely on stores during incubation (Jonsson, 1997; Barrett et al., 2012). During winter, populations are dispersed throughout the North Atlantic, Western Mediterranean, Norwegian and Barents Sea, and along the coasts of Iceland and Greenland. (Fayet et al., 2017). The migration routes and wintering grounds of the Svalbard population is generally unknown to date while Norwegian puffin populations have been recorded wintering in the Barents Sea, Norwegian Sea, and around Iceland and the Faroe Islands (Fayet et al., 2017). Norwegian and Scottish populations found in the Faroe Islands in winter 2008-2011 were found to feed on mostly fish such as sand eels with some invertebrates like nereid worms also being important energy sources. These populations were concluded to be generalist hunters with more varied prey in the winter while consuming mostly fish during the breeding season (Harris et al., 2015).

1.4.2 Status

Globally, puffin populations are decreasing in Iceland, Norway, and the Faroes, and they are listed as vulnerable on the IUCN red list (BirdLife International, 2018). Since puffins mainly eat shoaling fish they are also vulnerable to their population fluctuations (Helgason et al., 2008). Thus, the puffin's decline in the north east Atlantic is thought to originate from food stress. Their main prey (Atlantic herring and capelin) have both experienced severe decreases in the last few decades, with spring-spawning herring populations collapsing in the 1960s (Anker-Nilssen et al., 2000; Helgason et al., 2008). Overfishing and climate change are also potential threats to their populations, though environmental pollution is a growing suspect and threat in the declines, in relation to both POPs and oil spills (Anker-Nilssen et al., 2000; Sagerup et al., 2014). While the Norwegian Sea puffin population has declined over the last few decades, the Barents Sea populations are either stable or increasing (Fauchald et al., 2015). And while scarce, available population data for Svalbard tentatively shows the populations increasing from several hundred observed in the 1940s to some 10,000 breeding pairs in 2000 (Løvenskiold, 1964; Anker-Nilssen et al., 2000).

1.5 Aim of Study

Puffins can act as indicator species for changes in pelagic food webs (Anker-Nilssen et al., 2000) and as climate change and other anthropogenic factors increase pressure on their populations, it is important to establish baselines for their toxicology and ecology. The main aim of this study was to assess the contamination levels for OCs and PFASs in the Svalbard puffin population and fill a knowledge shortfall on what pollutants are present as their population has not been studied before.

Additional aims of this study were to compare the Svalbard POP levels to other puffin colonies and Svalbard breeding seabird species. Contamination levels were compared for species feeding at different trophic levels starting with little auks (*Alle alle*) preying on copepods, then Brünnich's guillemots (*Uria lomvia*) with copepods and fish, black guillemots (*Cepphus grille*) and black-legged kittiwakes (*Rissa tridactyla*) (hereafter referred to as kittiwakes) feeding on fish, and glaucous gulls (*Larus hyperboreus*) at the highest level feeding on other seabirds, crabs, offal, and fish. These arctic species were chosen as representatives of different trophic levels in Svalbard where the environment and food web would be similar to the puffins. The diet of these seabird species is central to understanding

the differences in their contaminant loads as concentrations increase with trophic level through biomagnification. POPs are hypothesized to be present in the Svalbard puffins and at lower levels than Northern Norway puffin colonies, since those populations are closer to contamination sources. It is also hypothesized that the Svalbard puffins will have POP levels higher than the little auk and Brünnich's guillemot, but lower than the glaucous gull, resembling those species most similar to its trophic level, black guillemot and kittiwake.

The final aim of this study was to collect size measurements and sex of a sample of puffins to check for correlations with contamination and gather information on their diet for possible explanations to POP levels. Puffins have been found to display sexual dimorphism in regards to culmen length, head-bill length, and bill depth, with males exhibiting larger values (Friars & Diamond, 2011). This sexual dimorphism has been suggested to be common in pelagic seabirds due to trophic niche segregation (Navarro et al., 2009) and bill size can affect food options (Temeles et al., 1993). As female seabirds historically have lower contamination due to excretion via egg-laying and bill size may affect prey options and thus POP loads, it is predicted that sex and size measurements will have some correlation with contamination. The Svalbard puffin diet is also expected to reflect that of other Svalbard seabird species at the same trophic level, mainly black guillemots and kittiwakes feeding on polar and Atlantic cod (Hop et al., 2002)

2 Materials and Methods

2.1 Sampling Area

Adult puffin samples were collected from the Gåsøyane bird sanctuary puffin colony in Svalbard from July 6th - July 30th, 2018 during the incubation and chick-rearing period. The colony is located in Sassenfjord, 30 kilometers north-east of Longyearbyen, 78° 27' 22.8276" N, 16°12' 47.0664" E (Fig 2). This is likely the largest puffin colony on Svalbard with approx. 1000 puffin nests. Nests were built underneath and between rocks on the northeast shoreline. July of 2018 in Spitsbergen had an average temperature of 7.2˚C with a total of 4.3 mm precipitation and an average of 4.6 m/s wind with continuous daylight (Adventdalen observation station (99870)).

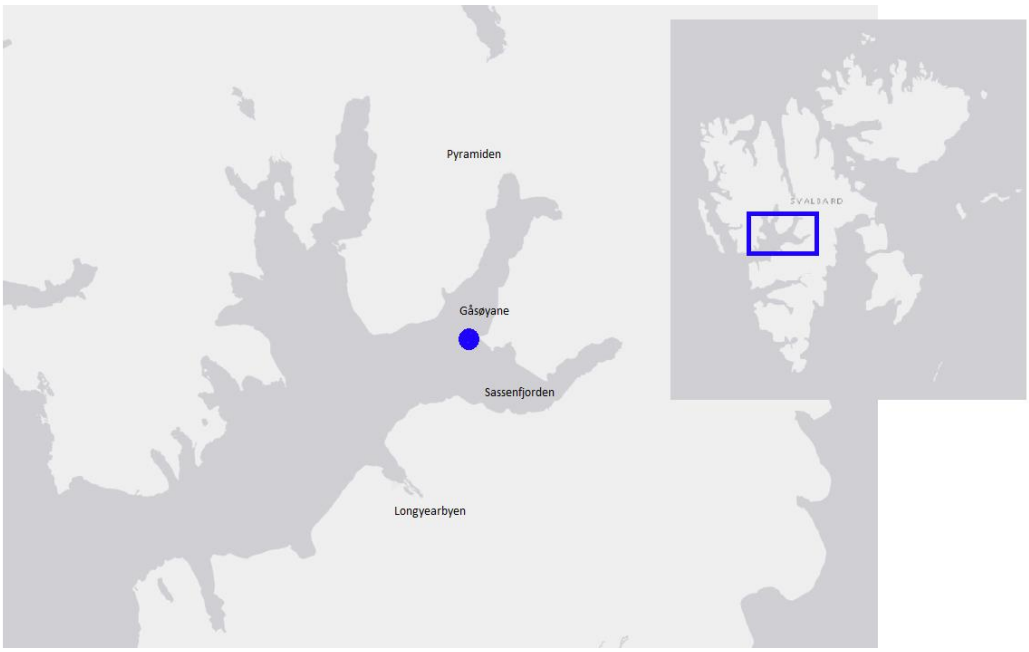

Fig 2: Gåsøyane bird sanctuary puffin colony location in Sassenfjord, Svalbard (QGiS)

2.2 Field Procedures

Puffins were captured using hand nets and a 2.4 x 12-meter mist net with two shelves (19 mm mesh size). 20 adults were captured for blood collection, with 2-3 ml being collected from the brachial vein. Of those captured, four were unsuitable for blood collection and were released to alleviate possible stress. 16 samples were frozen at -20˚C and sent to Tromsø for analysis at the end of the field season. Additionally, the adults were measured for head and bill length, bill depth and length, and mass. All adult birds were ringed for later identification and feather samples were collected for sex determination through DNA analysis. Five game cameras were also positioned at the entrances to five nests in the colony and were programmed to take pictures at the detection of movement in order to capture images of adults delivering food loads so prey species could be identified. The cameras were left at their prospective nest entrance from July 30th to August 18th. The SD cards were collected, downloaded, and individually examined for any evidence of food delivery into the nest. Identification of the prey species was conducted via the 113 photographs with visible food loads. Fig B2 shows examples of fish species used for identification from the Atlas of the Barents Sea Fishes by Wienerroither et al. (2011).

2.2.1 Ethical Considerations

Permission to conduct field work on wild seabirds was granted by the Norwegian Food safety Authority (permit #16061) and all guidelines were followed with regards to replacement, reduction, and refinement to ensure minimal stress to the study species. Presence in the colony during capture was kept to a maximum of one hour in each of two capture plots to minimize disturbance and the colony was visited three times.

2.3 Analyses of PFASs and OCs

The OCs and PFASs analyses of adult whole blood samples was conducted at the Norwegian Institute for Air Research (NILU) in Tromsø, Norway in early December 2018. The 16 samples were analyzed for the PFASs and OCs summarized in table 1.

Table 1*:* List of perfluoroalkyl substances (PFASs) and organochlorines (OCs) analyzed in whole blood samples of Atlantic Puffins (*fratercula arctica*) from Sassenfjord, Svalbard, July 2018. Compounds are divided into perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonates (PFSAs), DDTs, OCs, and PCBs.

Group	Abbreviation	Analyte
PFCAs	PFHxA	Perfluorohexanoate
	PFHpA	Perfluoroheptanoate
	PFOA	Perfluorooctanoate
	PFNA	Perfluorononanoate
	PFUnA	Perfluoroundecanoic acid
	PFDoA	Perfluorododecanoic acid
PFSAs	PFHxS	Perfluorohexane sulfonate
	brPFOS	Branched Perfluorooctane sulfonate
	linPFOS	Linear Perfluorooctane sulfonate
OCs	HCB	Hexachlorobenzene
	a-HCH	alpha-Hexachlorocyclohexane
	b-HCH	beta-Hexachlorocyclohexane
	g-HCH	gamma-Hexachlorocyclohexane
	oxy-chlordane	Oxy-chlordane
	t-chlordane	trans-chlordane
	c-chlordane	cis-chlordane
	t-Nonachlor	Trans-nonachlor
	c-Nonachlor	Cis-nonachlor
	Mirex	Dechlorane
DDTs	o,p'-DDT	1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl) ethyl] benzene
	p,p'-DDT	1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene
	o,p'-DDD	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene
	p,p'-DDD	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene
	o,p'-DDE	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene
	p,p'-DDE	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene
PCBs	PCB 28/31	Polychlorinated biphenyl 28/31
	PCB 52	Polychlorinated biphenyl 52
	PCB 99	Polychlorinated biphenyl 99
	PCB 101	Polychlorinated biphenyl 101

	PCB 105	Polychlorinated biphenyl 105
	PCB 118	Polychlorinated biphenyl 118
	PCB 138	Polychlorinated biphenyl 138
	PCB 153	Polychlorinated biphenyl 153
	PCB 180	Polychlorinated biphenyl 180
	PCB 183	Polychlorinated biphenyl 183
	PCB 187	Polychlorinated biphenyl 187
	PCB 194	Polychlorinated biphenyl 194

2.3.1 PFASs Extraction and Cleanup

The samples were prepared for analysis using the published method for blood on December 4th, 2018 (Hanssen et al., 2013). The 16 whole blood samples were thawed for 30 min and briefly vortexed to mix. The samples to be analyzed were 16 full blood samples, three blanks, and one standard reference: 200 µl AM-S-Y1804 human serum with known PFASs concentrations as a control. 400 µl of full blood was transferred to an Eppendorf-centrifuge tube with the pipette tip changed between each sample and the pipette being cleaned with iso-propanol after each transfer to prevent cross contamination of blood samples. 20 µl of internal standard (ISTD) (PFASs 0.5 ng/µl) was added to each sample, with PFASs: MPFHxS ($PFHxS^{18}O_2$), MPFOS ($^{13}C_4PFOS$), MFOSA ($^{13}C_8FOSA$), MPFBA ($^{13}C_4PFBA$), MPFHxA ($^{13}C_2PFHxA$), MPFOA ($^{13}C_4PFOA$), MPFNA ($^{13}C_5PFNA$), MPFDA ($^{13}C_2PFDA$), MPFUnDA ($^{13}C_2PFUnDA$) and MPFDoDA ($^{13}C_2PFDoDA$) (Hanssen et al., 2013). To extract the PFASs from the blood, 0.8 ml of methanol was added with the blanks and standard reference receiving 1 ml as there was no danger of overflowing. All tubes were then capped and vortexed for several seconds to mix. The tubes were also put in an ultrasonic bath for three 10-minute increments for more thorough mixing via ultrasonic waves with a few seconds of vortexing in between each session. To allow sedimentation, the tubes were centrifuged for 10 min at 10,000 rpm. During centrifugation, additional 1.7 ml Eppendorf centrifuge tubes were prepared with 25 mg ENVI-Carb and 50 µl glacial acetic acid for further clean up. The ENVI/Carb powder binds to and sinks unwanted components in the sample while the glacial acid prevents the PFASs from attaching to it. After centrifugation, 0.6 ml of the solvent from each tube was added to the new Eppendorf centrifuge tubes and then vortexed to mix. The tubes were centrifuged a second time for 10 minutes at 10,000 rpm then 0.5 ml of the supernatant was transferred to a vial. 20 µl Recovery Standard (RSTD) (0.1 ng/µl RSTD in methanol (3,7-diMeo-PFOA)) was added to each vial, which were then capped, vortexed, labeled, and stored in the fridge to await analysis.

2.3.2 OCs Extraction and Cleanup

Before lab work, all glassware was burned at 400˚C and washed in cyclohexane. To start the extraction process, 1 ml of whole blood from each sample was transferred to a 15 ml glass tube, which was weighed beforehand. There were 16 sample tubes, 3 blanks, and 0.5 ml of 1 standard reference (SRM 1958 05.09.2018). 20 µl of 4 separate internal standards was added to each tube, containing isotope labelled PCBs, pesticides, PBDEs and DDTs, followed by vortexing to mix. Next, 2 ml of deionized water saturated with ammonium sulfate and 2 ml of ethanol were added to each tube and then vortexed to denaturize proteins. After mixing, 6 ml of n-hexane was added and then the tubes were vortexed to drive lipophilic compounds into the hexane phase. Phase separation occurred by leaving the tubes in the fume hood for 15 minutes followed by an additional five minutes in the centrifuge at 2000 rpm due to slight emulsion. An empty 15 ml glass tube was weighed and then a glass pipette rinsed with hexane was used to transfer as much supernatant as available in the sample tubes to the 15 ml glass, which was weighed a second time. All samples were then run through the same steps a second time starting with adding the 6 ml of n-hexane to achieve a clearer solvent. Two extraction steps of the sample resulted in two doses with the compounds of interest extracted from the blood. The combined extracts were concentrated with the RapidVap to 0.2 ml and then gentle application of nitrogen was used to evaporate the samples until completely dry. The three blanks and SRM were excluded from this process as their final weights were not needed. All sample tubes were weighed so they could be compared to the original empty tube weight and the precipitate weight could be found. After the EOM (extractable organic matter) determination the samples were re-dissolved in 0.5 ml of hexane and vortexed to mix. Columns were then packed with 0.1 g Na_2SO_4 in the bottom and 0.9 g acidified silica on the top with one frit on the bottom of the column and one on the top. The acidified silica was previously prepared by burning silica at 450˚C for 8 hours and then shaking it with concentrated H_2SO_4 for four hours in a ratio of 5% acid and 95% silica. The samples were put into the w/sp robot for final clean up and left for the night with the procedure FLOKORT.SPE. When the silica cleanup was finished, isooctane was added to each tube and the RapidVap was used to evaporate all tubes to 0.2 ml. The extracts were then rinsed with isooctane before being transferred to GC vials with inserts. Nitrogen evaporation was conducted to evaporate samples further to 20 µl final volume. 10 µl of the RSTD (^{13}C-PCB 159 (~200pg/µl)) was added to each tube. All tubes were then labelled and stored in the fridge to await analysis.

2.4 Data Below the Limit of Detection and Limit of Quantification

For the data sets, any contaminant that was below the limit of detection (LOD) for more than 80% of the samples was omitted from data analysis. Given this criterion, for the PFASs: PFOSA, PFBS, PFPS, PFHpS, PFNS, PFDcS, PFBA, PFPA, PFHxA, PFHpA, 6:2FTS, and 8:2 FTS were not included in the analysis. For the OCs, a-HCH, b-HCH, g-HCH, o,p'-DDT, p,p'-DDT, o,p'-DDD, p,p'-DDD, o,p'-DDE, PCB 28/31, PCB 52, PCB 101, and PCB 194 were also excluded. In total, ten out of the original 22 PFASs and 16 out of the original 28 OCs tested were included in data analysis. In the remaining samples that had less than 20% below the limit of detection, the LOD was replaced with a value half of the LOD value provided in the POPs analysis. The limit of quantification (LOQ) was 3 times the LOD and the values for LOQ were included in the analysis.

2.5 Sex Determination

The sex of each puffin was determined via DNA analyses using DNA from feather samples. DNeasy 96 Blood and Tissue Kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from the feather samples in accordance with the manufacturer's protocol. The primers M5 (Bantock et al., 2008) and P8 (Griffiths et al., 1998) were used to determine sex as they amplify the sex-linked CHD-W and CHD-Z genes. These genes are different lengths and so result in one band for males and two bands for females. The M5 primer was 6FAM fluoro-labelled. Polymerase chain reaction (PCR) was run using and following the protocol of Qiagen's Multiplex PCR Kit, though with 8.4 µL reaction volume. The PCR products were mixed with GeneScan 500 LIZ (Applied Biosystems) size standard and Hi-Di formamide. Capillary electrophoresis was used to separate alleles on an ABI 3500*xl* Genetic Analyzer while sizes were assigned using GeneMapper software (Applied Biosystems). Analyses were performed at NINA/Trondheim.

2.6 Data for Pollution Levels of Other Puffin Colonies, Svalbard Seabird Species, and Diet

Contamination data for comparisons with other puffin populations was obtained from Sagerup et al (2014) and Helgason et al (2008). Helgason et al (2008) provided data from Hornøya, Hjelmsøy, and Røst colonies collected in 2003 while Sagerup et al (2014) had data from

Hornøya collected in 2013. The contamination data from these colonies at Hjelmsøy (71˚), Hornøya (70˚), and Røst (68˚) can then be compared to the Svalbard puffin data at 78˚ to visualize any differences (Fig 3).

For interspecies comparisons, little auk and Brünnich's guillemot contamination data for OCs was obtained from Borgå et al (2007). Kittiwake contamination was found in Blévin et al (2017). Zahn (2019) had data on black guillemots and Kjetil Sagerup provided raw data on glaucous gull contamination. Tables 2 and 3 summarize the origin and time of all sample collections. The contamination levels of these seabird species breeding in Svalbard and occupying different and similar trophic levels to the puffin can then be compared to the Svalbard puffin's contamination.

For contamination levels of Atlantic and polar cod, capelin, and Atlantic herring, raw data was obtained from Nicholas Warner and Katrine Borgå (Contaminants in Polar Regions (COPOL)). The contamination levels of the different prey options can then be paired with the diet data for the Svalbard puffins and compared to the other puffin colony's diet to speculate on any potential contamination differences between the colonies.

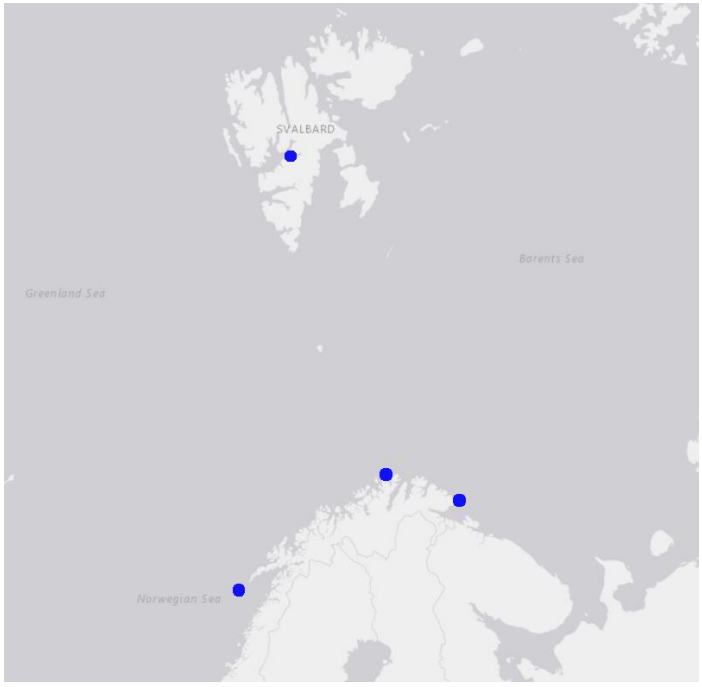

Figure 3: Atlantic puffin Colony locations for data used in intraspecies comparisons: Svalbard, Hjelmsøy, Hornøya, and Røst

Table 2: Data used in colony location comparisons for Atlantic Puffin: source, origin, sample type, and collection time.

Source	Location	Latitude	Sample type	Sample Collection
Primary study	Svalbard	78°	Whole blood	July 2018
Helgason 2008	Hjelmsøy	71°	Fresh eggs	2003
Helgason 2008 Sagerup 2014	Hornøya	70°	Fresh eggs Whole blood	2003 June 2004, 2005, 2006
Helgason 2008	Røst	67°	Fresh eggs	2003

Table 3: Other Svalbard breeding seabird species used in comparisons: sample origin, sample type, and collection time (Blévin et al., 2017; Borgå et al., 2007; Zahn et al., 2019).

Source	Species common name	Species Scientific name	Sample type	Location	Sample Collection
Borgå 2007	Little auk	*Alle alle*	liver tissue	North Barents Sea, 76˚	May 1999
Primary source	Atlantic Puffin	*Fratercula arctica*	whole blood	Sassenfjord, Svalbard	July 2018
Blévin 2017	Black-Legged Kittiwake	*Rissa tridactyla*	blood	Kongsfjorden, Svalbard	July 2012, June 2014
Zahn 2019	Black Guillemot	*Cepphus grylle*	plasma	Kongsfjorden, Svalbard	June-July 2015
Borgå 2007	Brünnich's guillemot	*Uria lomvia*	liver tissue	North Barents Sea, 76˚	May 1999
Sagerup data	Glaucous Gull	*Larus hyperboreus*	plasma	Kongsfjorden, Svalbard	June, 2015-17

2.7 Statistical Analysis

To allow for comparisons between tissues from different species, wet weight average concentration was converted to pg/ml lipid weight (lw) using the equation: $\frac{(wet\ weight\ x\ 100)}{Lipid\ \%}$ (Sagerup, 2009) and 95% confidence intervals were found by calculating the standard deviation (SD) of data and using the equation: $1.96\ x\ \frac{SD}{\sqrt{n}}$ (Altman & Bland, 2005). Species comparisons between PFASs was kept in wet weight (ww) as PFASs do not accumulate in lipids. The statistical significance threshold was set to $p < 0.05$ and all statistical analyses were run with R version 3.5.2 (R Development Core Team, 2016). To test for sex, mass, and size variation in contaminant levels, we performed a Principal component analyses (PCA) on both PFASs and POPs separately to obtain principal components. The different contaminant concentrations were generally correlated to each other and the principal component would thus give a simpler visualization of the contaminant levels. Moreover, using principal components instead of running these tests multiple times for each contaminant separately would also minimize the risk of type I error (i.e. finding a spurious relation). Linear model PCA was performed using the calcpc function. The assumptions of linearity were visually assessed using residual distributions and Q-Q plots. A linear model was then used to test for sex, mass, and body size (head bill length, bill depth, and bill length) effect on PFASs and OCs. Inspection of model residuals supported the assumption of residual normality and

homoscedasticity. The original puffin sampling occurred at three different dates from early to late July, but preliminary analysis showed that the sampling date had no effect on contaminant levels. Therefore, sampling date was not included in the model testing for sex, mass, and size effects.

3 Results

3.1 Fluorinated and Organochlorine Compounds Levels

The PFASs had higher contamination levels than the OCs and PCBs, with linPFOS, PFUnA, and PFTriA reaching averages of 6627 pg/ml ww, 5798 pg/ml ww, and 2932 pg/ml ww, respectively. Of the OCs, only p,p'-DDE at 3018 pg/ml ww came close to the PFASs contamination levels with PCB 153 the second highest at 1453 pg/ml ww (Fig 4). Though the confidence intervals overlapped for linPFOS and PFUnA, and PFTriA and p,p'-DDE quite a lot so this is a conservative outlook. The levels of the different contaminants were generally correlated within the individuals (Fig 5, 6) and some birds were more contaminated than others.

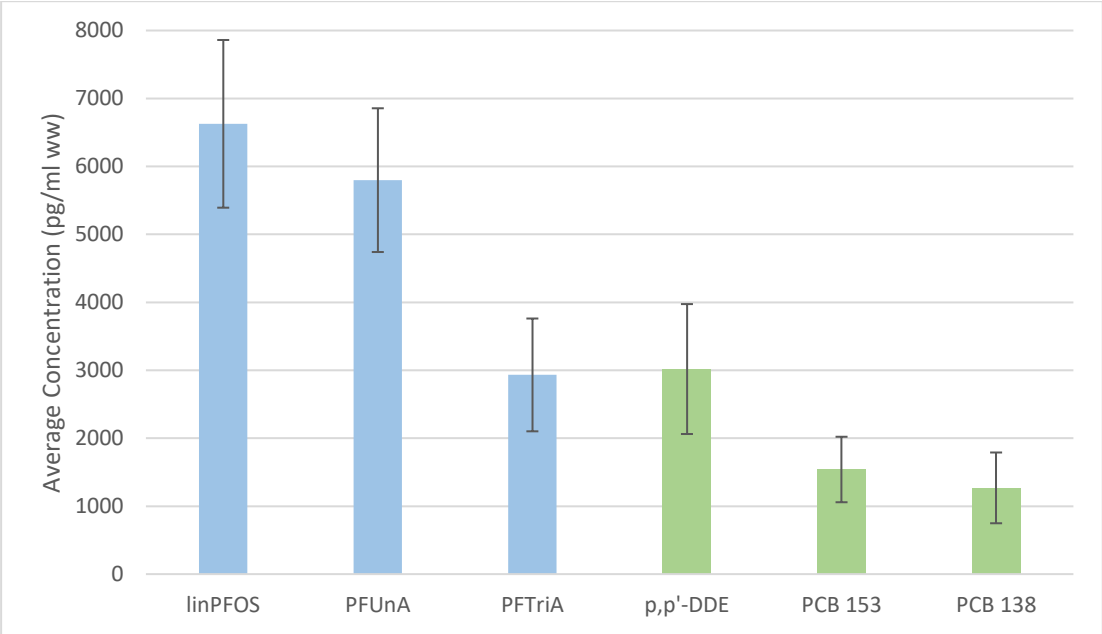

Fig 4: The 3 highest average concentrations for each category of POP in pg/ml ww with 95 % confidence intervals. Puffin samples collected in Sassenfjord, Svalbard, 2018. blue: PFASs, green: OCs

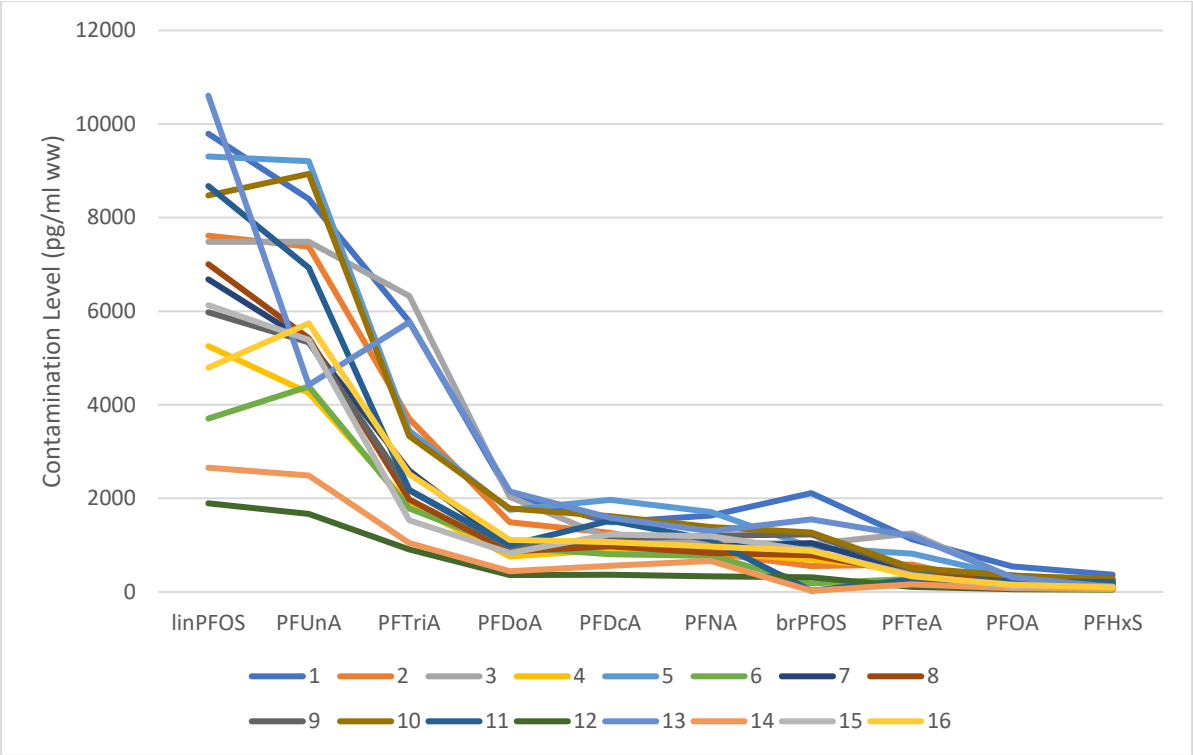

Fig 5: PFASs contamination levels by individual puffin 1-16 in pg/ml ww. Samples collected in Sassenfjord, Svalbard, 2018.

Of the OCs analyzed, p,p'-DDE (by-product of DDT) had the highest average concentration, then PCB 153, and PCB 138. The test for OCs showed oxychlordane, t-chlordane, t-Nonachlor, c-Nonachlor, and Mirex present in most puffin samples, with c-chlordane detected but below the limit of quantification. Of the 6 DDTs tested, only p,p'-DDE was found present. For PCBs, levels were found in PCB 99, PCB 105, PCB 118, PCB 138, PCB 153, PCB 180, PCB 187. Puffin 16 (female) was the most contaminated bird while puffin 12 (male) had low levels and the other 14 birds were relatively evenly matched (Fig 6).

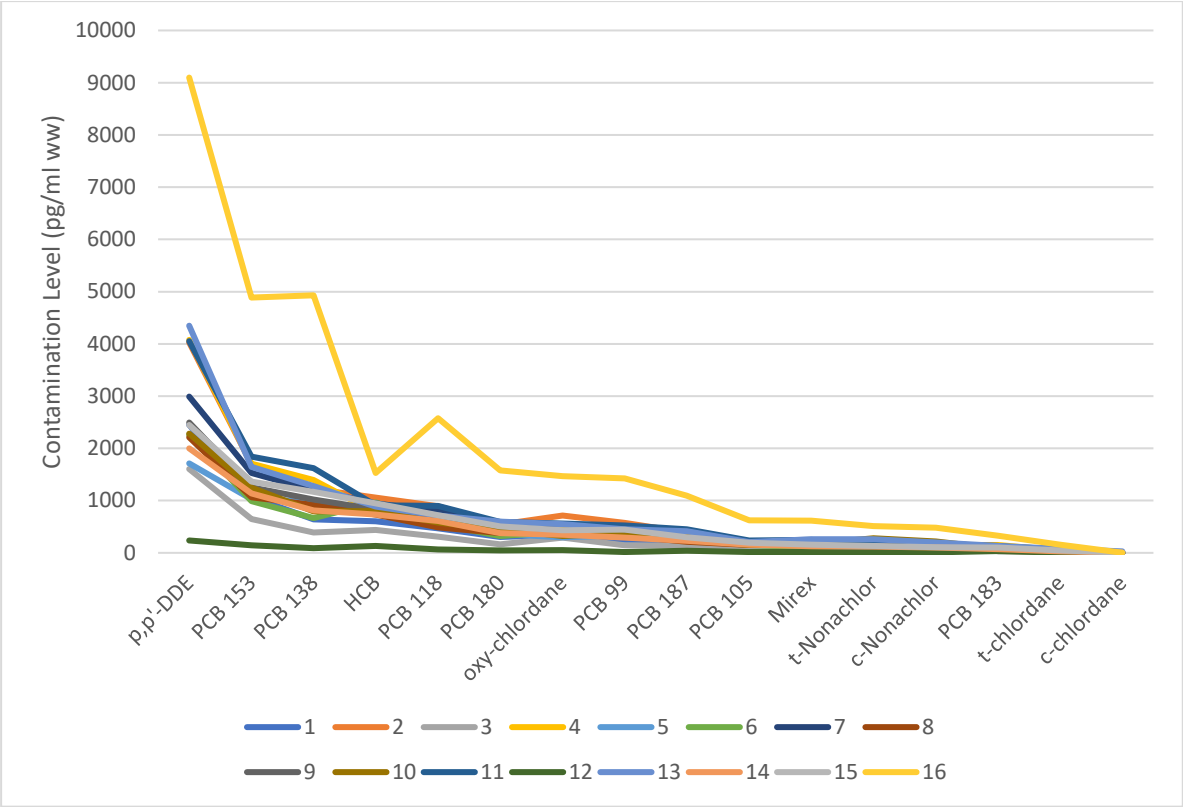

Fig 6: Organochlorine contamination levels by individual puffin 1-16 in pg/ml ww. Samples collected in Sassenfjord, Svalbard, 2018.

3.2 Comparisons of Contaminant Levels Between Puffin Populations

Using data from the literature (table 2), contamination levels were compared between puffins in Svalbard at 78˚, Hjelmsøy at 71˚, Hornøya at 70˚, and Røst at 68˚ (Fig 3). The colony at the highest latitude (Svalbard) had the lowest contamination, with some confidence interval overlap with Røst, which had the next lowest contamination, and the two Northern Norway colonies Hjelmsøy and Hornøya at the highest concentrations. For the PCBs, PCB 153 had the continuously highest concentration among all the colonies, followed by PCB 138, then PCB 188, with PCB 99 and 180 the lowest (Fig 7). For the other OCs, the DDT byproduct p,p′-DDE had the highest consistent concentration, followed by HCB, and oxychlordane (Fig 8).

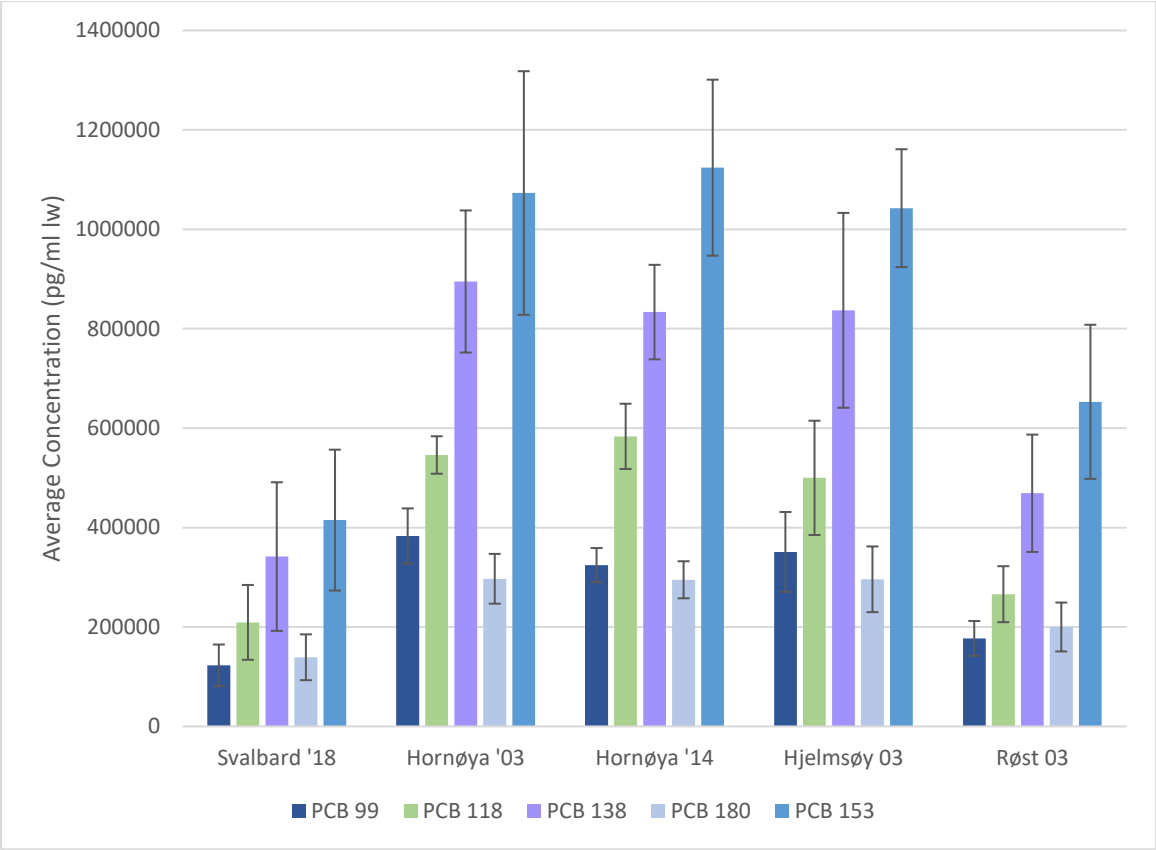

Fig 7: PCB average concentrations (pg/ml lw) by decreasing latitude in puffin colonies of Northern Norway with 95% confidence intervals (Helgason et al., 2008; Sagerup et al., 2014).

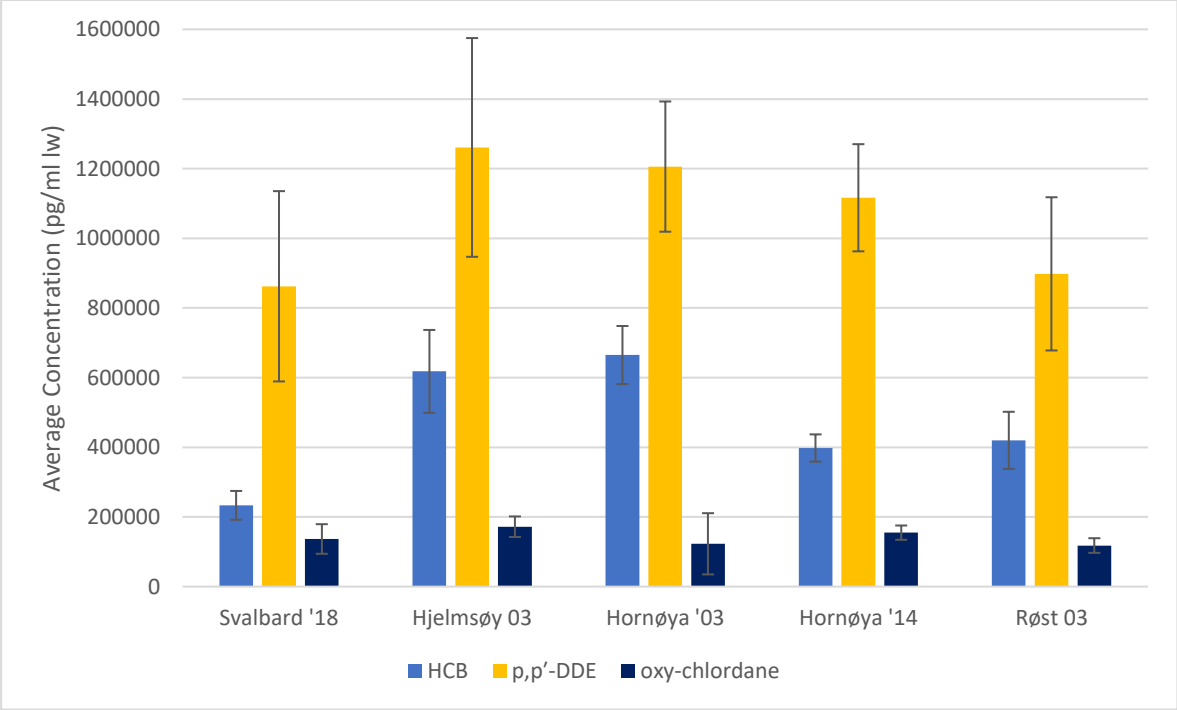

Fig 8: Pesticide average concentrations (pg/ml lw) by decreasing latitude in puffin colonies of Northern Norway with 95% confidence intervals (Helgason et al., 2008; Sagerup et al., 2014).

3.3 Comparisons of Contaminant Levels Between Svalbard Seabird Species

Using published data on other seabird species breeding on Svalbard (table 3), we found that the glaucous gull and kittiwake displayed the highest contamination levels for OCs with the gull showing significantly higher p,p'-DDE than other species and the highest oxy-chlordane level. The puffin showed the most similar contamination to the little auk and guillemot species (Fig 9, 10). The glaucous gull also had the highest PCB contamination, despite large confidence intervals, followed by the kittiwake, black guillemot, and puffin (Fig 10).

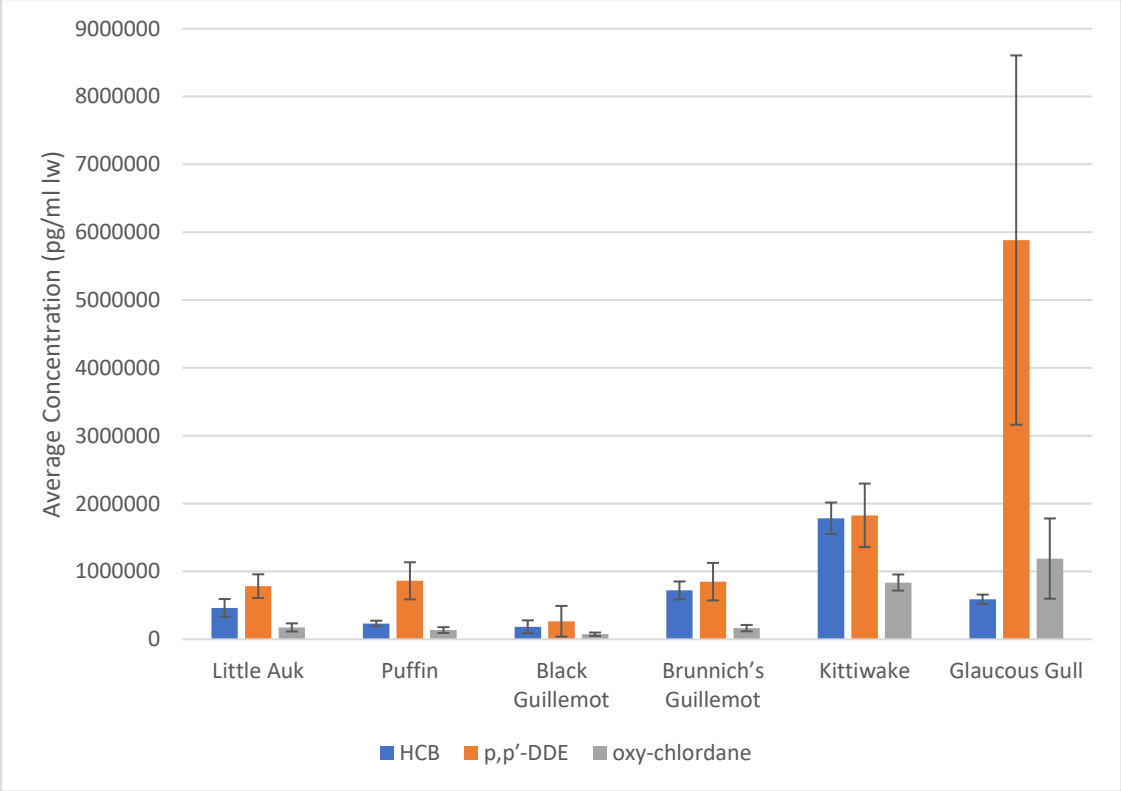

Fig 9: OC average concentrations (pg/ml lw) by species of increasing trophic level with 95% confidence intervals (Borgå et al., 2007; Sagerup et al., 2014; Zahn et al., 2019, Sagerup raw data).

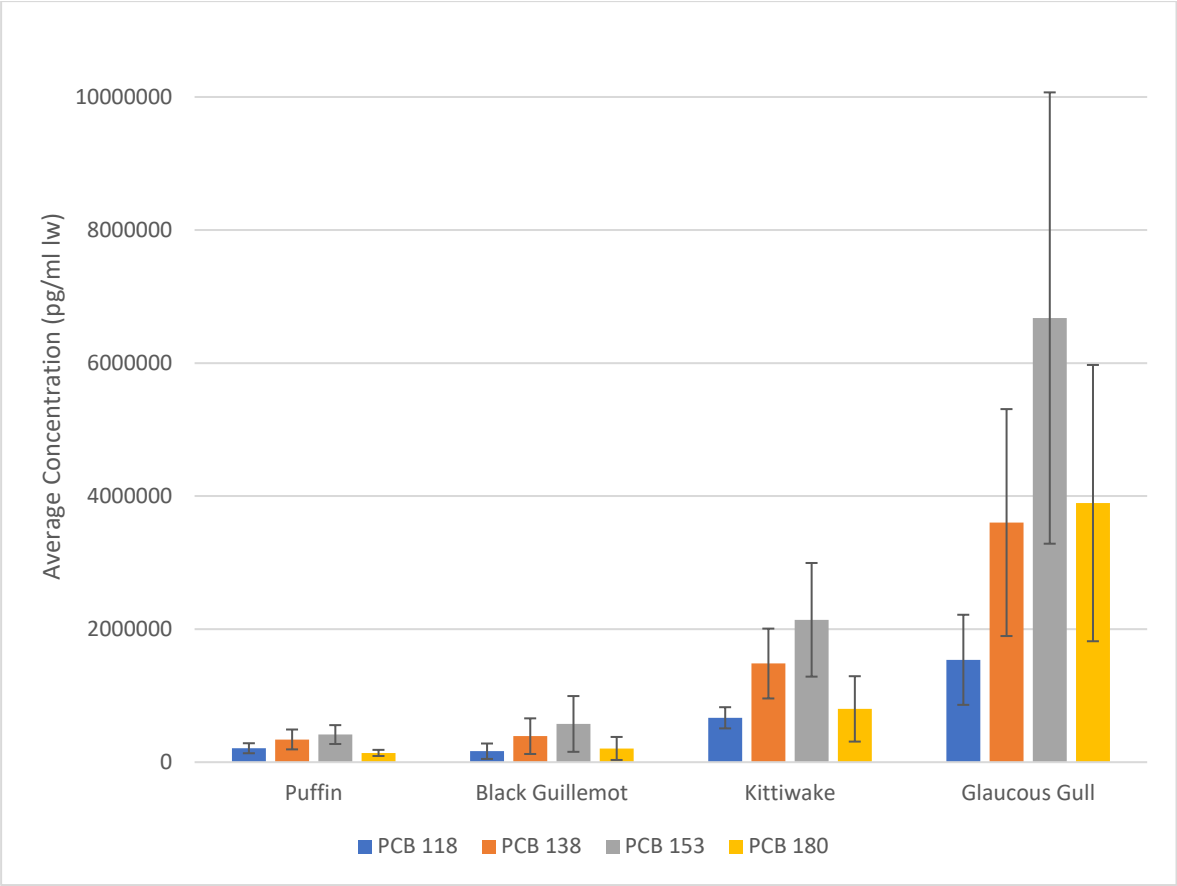

Fig 10: PCB average concentrations (pg/ml lw) by species of increasing trophic level and 95% confidence intervals (Borgå et al., 2007; Sagerup et al., 2014; Zahn et al., 2019, Sagerup raw data).

The PFASs only had contamination level data available for the kittiwake and glaucous gull. This comparison showed the kittiwakes to generally have much higher levels of PFUnA than the gull and puffin with the gull displaying the lowest contamination for all but linPFOS, though the confidence intervals overlapped with the kittiwake (Fig 11).

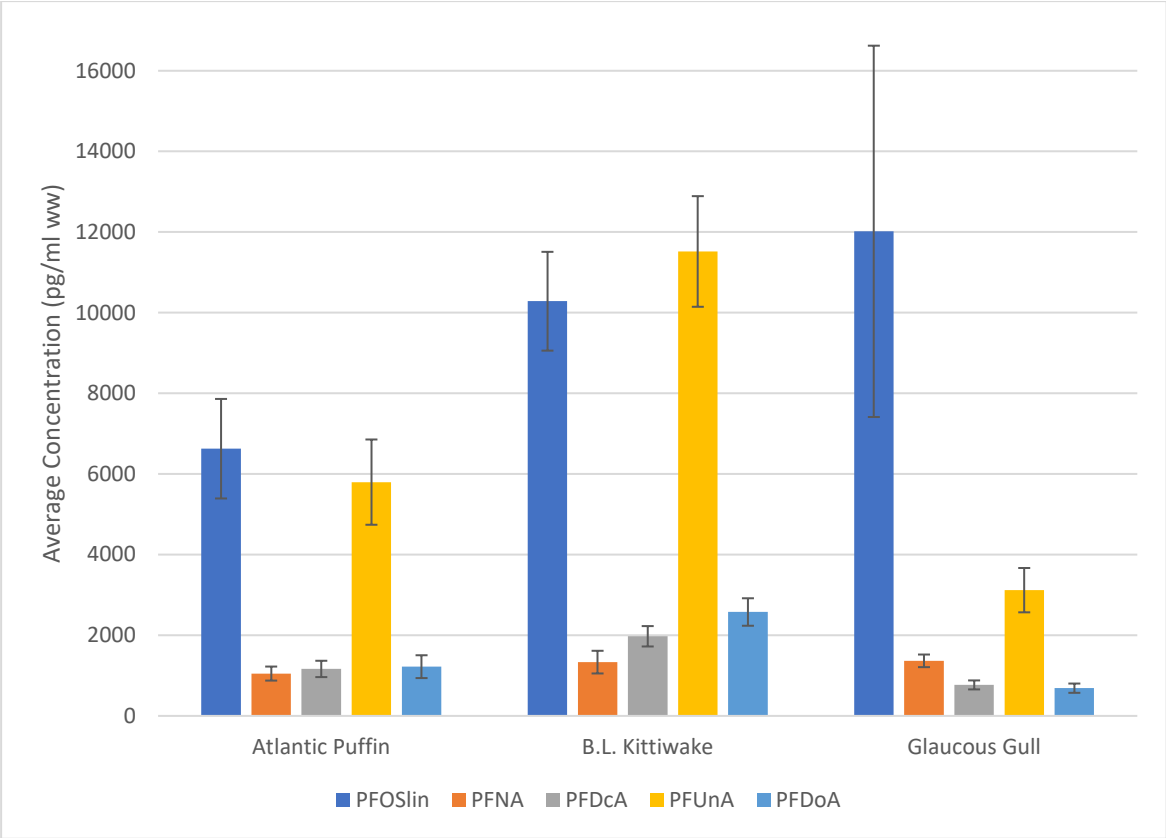

Fig 11: PFASs average concentrations (pg/ml ww) by species with 95% confidence intervals (Blévin et al., 2017, Sagerup raw data).

3.4 Sex, Mass, and Size Effects Determination and Contaminant Level

For the OCs and PFASs, the PC1 axis explained 88% and 73% of the variation respectively. Thus, only the PC1 was used in further analysis to test predictions (Fig C1). These PC1 values had a straightforward interpretation because they were negatively correlated with all the PFASs and positively correlated with all the OCs (table C1).

The male puffins had slightly higher contaminant concentrations on average than females for the PFASs (Fig 12) while the OCs had the opposite result with females exhibiting higher contaminant loads than the males (Fig 13). However, these differences were not statistically significant (Table 4). Mass and body size did not affect the PFAs levels but had a significant effect on OCs (Table 4). However, these effects were driven by a single outlier (puffin 16) (Fig C3). When this bird was removed, neither the effect of mass or body size on OCs was significant (Fig C4) ($p=0.287$ and $p=0.625$ respectively, for the mass and size effect (table C3)).

Table 4: Coefficient table results for PCA testing OCs and PFASs PC1 values against sex, mass, head bill length, bill depth, and bill length. All 16 puffins, samples from Sassenfjord, Svalbard 2018. Size measurement means available in appendix table A2.

* significant values become not significant when outlier puffin 16 removed

factor	Estimate	Standard Error	t value	p value
OCs				
Sex	-2.86	1.83	-1.56	0.15
Mass	-0.07	0.03	-2.38	0.04*
Head bill	0.18	0.36	0.52	0.62
Bill depth	0.79	0.29	2.76	0.02*
Bill length	0.26	0.33	0.78	0.46
PFASs				
Sex	-1.12	2.08	-0.54	0.61
Mass	0.01	0.03	0.21	0.84
Head bill	-0.01	0.40	-0.03	0.98
Bill depth	-0.12	0.32	-0.36	0.73
Bill length	-0.13	0.37	-0.34	0.75

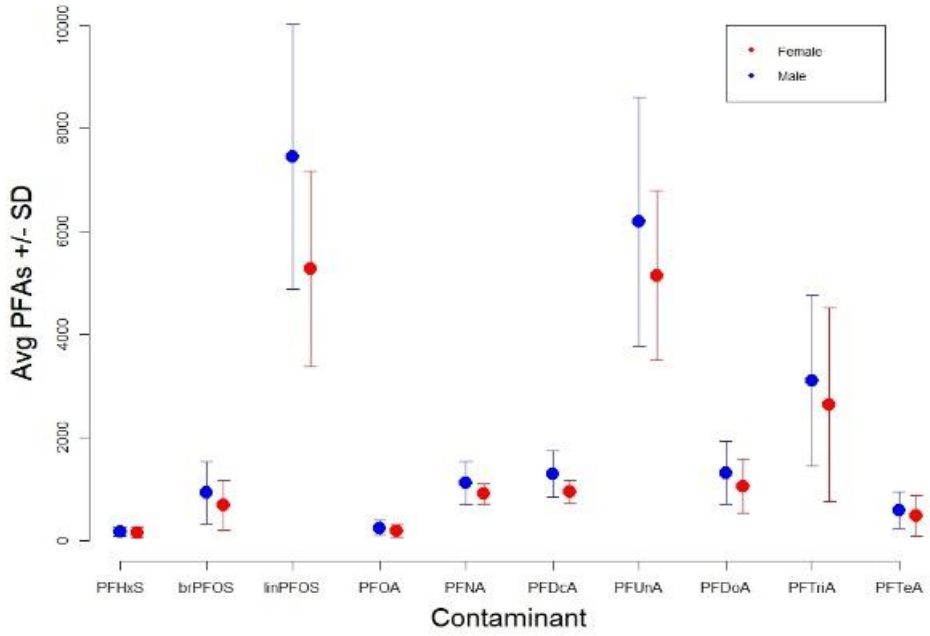

Fig 12: Average PFASs concentration (pg/ml ww) level by sex (Female, Male) with SD. Puffin samples from Sassenfjord, Svalbard 2018.

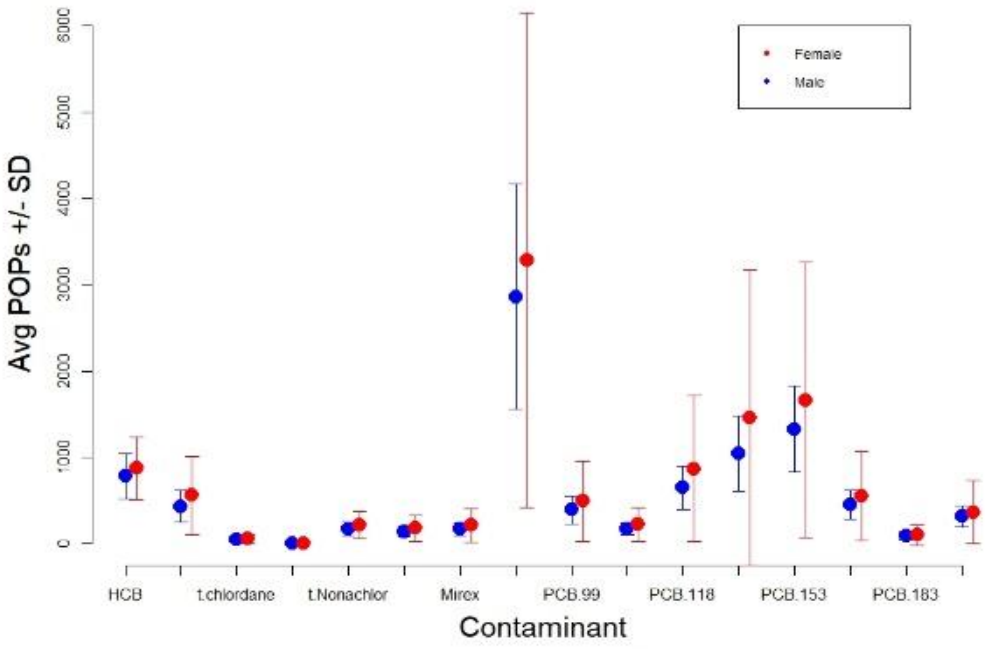

Fig 13: Average OCs concentration (pg/ml ww) level by sex (Female, Male). Contaminants: HCH, oxy-chlordane, t-chlordane, c-chlordane, t-Nonachlor c-Nonachlor, Mirex, p,p'-DDE, PCB 99, PCB 105, PCB 118, PCB 138, PCB 153, PCB 180, PCB 183, PCB 187. Puffin samples from Sassenfjord, Svalbard 2018.

3.5 Svalbard Puffin Diet

The most common food load contents evidenced in the game camera photographs were juvenile polar cod and members of the stichaeidae family along with Atlantic herring, and capelin (Fig 14). However, the quality of the photographs was poor, and identification was sometimes subjective (Fig B1). Results of prey species contamination loads for Atlantic Herring and cod, polar cod, and capelin are presented in appendix Fig B3.

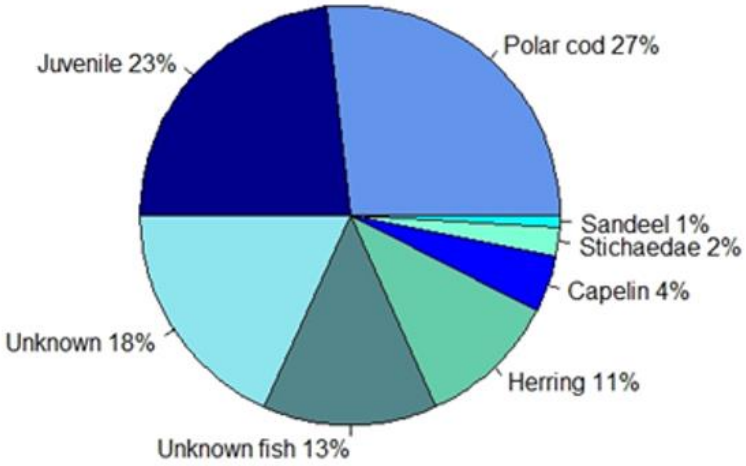

Fig 14: Food delivery visual identification. Unknown: not identifiable, Unknown fish: Herring, capelin, or sand eel, Juvenile: juvenile stichaeidae or polar cod.

4 Discussion

In this study, the Svalbard puffin population had more PFASs contamination than OCs with linear PFOS displaying the highest concentration. The Svalbard puffins also displayed lower contamination levels than those of northern Norway puffin populations and had generally low levels for OCs and PFASs when compared to other species breeding on Svalbard of different and similar trophic levels. These differences can be potentially explained through diet influences and origins as these are the main source of contamination for seabirds. This study would have benefited from a higher sample size for more firm results and control samples for the diet to improve identification accuracy.

4.1 Svalbard Puffin Contaminant Levels and Comparisons Between Other Puffin Colonies

Many OCs and PFASs were present in the Svalbard puffin population, with the highest concentrations being linPFOS, PFUnA, and p,p'-DDE. The PFASs exhibited higher levels than the OCs perhaps due to the lack of restrictions on the emerging POPs (Letcher et al., 2010). Of the four puffin colonies analyzed for POP contamination, the colonies at Svalbard and Røst (the highest and lowest latitudes respectively) had low contaminant levels while the two colonies at mid-latitudes (Hjelmsøy and Hornøya) were more contaminated. Svalbard was expected to have low POP levels because Arctic waters are traditionally less

contaminated at lower trophic levels (Gabrielsen & Henriksen, 2001). It was unexpected though that Røst would have similar levels to Svalbard as it is at the lowest latitude and was expected to be comparable to Hjelmsøy and Hornøya due to proximity to human activities and pollution sources.

Helgason et al (2008) compared the contamination of different species and colonies (puffin, common guillemot, kittiwake, and herring gull) of seabirds across northern Norway, but found no significant difference in the POP and mercury contamination levels between colonies, though the sample size was low and species-specific differences were not corrected for (Helgason et al., 2008). But geographic gradients have been found in other studies where different colony locations displayed dissimilar contamination based on their environment. Shearwaters, for example, were found to display higher contamination with higher latitude in the north Atlantic (Roscales et al., 2010). The study compared the Atlantic populations to the Mediterranean and found lower shearwater OC levels in the Atlantic, thought to be due to diet differences in colonies and the contamination of the different environments. Historically, seas subject to contaminated output are more polluted than open oceans with more isolated colonies (Roscales et al., 2010). Another study compared populations of common guillemots (*Uria aalge*) and Brünnich's guillemots in Alaska, and searched for geographic, species, and temporal differences in contamination. The Gulf of Alaska was found to have higher POP levels than the Bering Sea, potentially due to differences in winter and summer foraging areas, diet, and contamination of regional food webs (Vander Pol et al., 2004).

4.1.1 Migration and Diet Influences

For seabirds, diet is the most relevant route of contamination (Borgå et al., 2004). But seabirds can have contaminants in their systems from recent feeding (most studies are done during breeding season) and from mobilizing adipose stores made during the winter, usually in a different environment if migratory. Previous studies show that given stable conditions, blood samples can be used to reflects OC levels for both short and long-term periods (Roscales et al., 2010). Contaminants in blood samples also have a shorter retention time than levels in adipose tissue (Yamashita et al., 2007). Previous studies on great skuas (*Stercorarius skua*) showed that wintering areas of migratory birds affected their pollutant concentration and pattern, so resident and migratory birds inhabiting the same area would display different patterns based on winter diet (Leat et al., 2013). The influence of wintering area was attributed to the plasma samples from the skuas, thought to be due either to the long half-lives

of POPs in plasma or to adipose stores being mobilized for the high energy needs of breeding season. Different migration strategies between species and colonies can thus be expected to affect contamination levels (Leat et al., 2013).

Previous studies on puffin winter distribution found that colony size, productivity, winter resource availability, and latitude are the main drivers in puffin migratory patterns (Fayet et al., 2017). During breeding season, puffin colonies tend to forage close by and are relatively segregated, whilst populations mix some during the winter. In addition, colonies with less water productivity in the winter tend to migrate farther as do those experiencing intraspecific competition. The main wintering hotspots of puffins have been found to be in south Ireland, southwest of Iceland, the entrance to the Labrador Sea and the mid-north Atlantic (Fayet et al., 2017). Hjelmsøy puffins have been recorded mainly in the Barents Sea while Hornøya puffins are found mostly in the Barents Sea and occasionally in the Norwegian Sea. Røst puffins are recorded wintering along the west coast of Norway and the Norwegian Sea to Iceland and Greenland, with few in the North Barents Sea (http://seatrack.seapop.no). Thus, the most contaminated puffin colonies share a winter range while the less contaminated Røst colony winters farther west. There is no current data on where the Svalbard populations winter. Regarding summer foraging areas, the Hjelmsøy and Hornøya colonies are both exposed to the Barents Sea while Svalbard and Røst colonies face the Norwegian and Greenland Sea, areas they are also exposed to in winter. The West Spitsbergen Current (WSC) also supplies Atlantic waters from mainland Norway to Svalbard's west coast, transporting prey species there in a minimum of 32-38 days, and exposing the Røst and Svalbard puffins to the same waters (Vihtakari et al., 2018). In addition, the Barents Sea is historically more contaminated as it is a sink for POPs from Russian pollution sources such as metallurgical smelters and shipyards (AMAP, 2002; Savinov et al., 2003). These different environments could offer the best explanation for the contamination differences between colonies if it is assumed that Svalbard populations winter more in the Norwegian Sea than Barents Sea.

Different foraging areas also often offer different prey availability. Northern Norwegian colonies were documented to feed mainly on capelin, sand eel, herring, and Atlantic cod (Barrett et al., 2002), but the Svalbard population seemed to feed almost half the time on juvenile polar cod (Fig 14). This was expected as other Svalbard seabirds feeding at similar trophic levels also preyed on polar cod (e.g. Black guillemots and kittiwakes). It was difficult though to distinguish between the larval stages of many observed puffin prey and previous

studies collected control samples to account for this, which may improve the accuracy of future studies (Barrett, 2002). While juveniles were labeled as either polar cod or stichaeidae, they are more likely to be polar cod as there is evidence of stichaeidae employing defensive chemical measures to dissuade seabirds from preying upon them (Geir Wing Gabrielsen, personal communication). Therefore, any diet-based contamination in the Svalbard puffin is most likely driven by polar cod rather than other prey species. Fig B3 also demonstrates that polar cod was consistently the least contaminated prey species in samples taken from two fjords in northern Svalbard. Meanwhile, Atlantic cod in Liefdefjorden, the prey item of southern puffin colonies, was the most contaminated. The differences between fjords could be attributed to the different ocean current that supplies them. Liefdefjorden is influenced by Arctic water masses while Kongsfjorden is influenced by Atlantic water masses. Though no differences in POP levels were found in seawater of the two fjords, for zooplankton, Kongsfjorden generally displayed higher levels of some POPs (Hallanger et al., 2011). Isfjorden, the entrance to the Gåsøyane colony, is seasonally supplied by Atlantic water (Nilsen et al., 2008). The most abundant prey of the Svalbard colony (polar cod) being the least contaminated prey species could have an influence on why the Svalbard population is less contaminated.

Polar cod are gadoid fish that have a high abundance and ecological importance in the arctic (Nahrgang et al., 2010). They typically feed on euphausiids and amphipods, with 1-2 year cod preying on copepods, euphausiids, amphipods and other polar cod (Borgå et al., 2001). PBDE concentrations in polar cod liver have also been found to be much lower than levels in herring and Atlantic cod as Arctic waters are generally less contaminated than southern latitudes (Wolker et al., 2004). Polar cod are an important prey item to Arctic seabird species like little auk and the puffin as they have richer lipid stores than amphipods and are a more valuable energy source. Seabirds consuming a kg of fat fish achieve 2-3 times more energy than the same amount of protein rich crustaceans, there is more energy density and the fat can also serve as insulation (Gabrielsen, 2009). During winter many seabirds increase mass for both energy stores and insulation, with puffins being found to increase their mass by 20-30% from the end of breeding to winter (Anker-Nilssen et al., 2018). Between the Norwegian and Barents Sea, auks dominate the seabird populations with mostly Brünnich’s guillemots and little auks breeding in the Barents Sea and predominantly puffins in the Norwegian sea. Birds in the Barents sea also predominantly feed on fatty fish, while the Norwegian sea diet is mostly lean fish, with puffins consuming most of the fatty fish (Barrett et al., 2002). On

Spitsbergen, Franz Josef Land, and Novaya Zemlya, polar cod and crustaceans dominate seabird diets (Barrett et al., 2002).

For the different puffin colonies, a combination of this different diet and feeding location on the west coast of Svalbard as well as Røst being on the west coast and less exposed to the eastern runoff could explain both colony's lower contamination levels. It is noteworthy as well that for the two data sets available for Hornøya, the contaminant levels were similar 10 years apart, there was not too much temporal fluctuation. Therefore, the narrow sampling window has support for being used as a representation of the colony.

4.2 Comparisons of Contaminant Levels Between Svalbard Seabird Species

In comparing the contamination levels of different seabird species in Svalbard, it was predicted that little auks would display the lowest contamination, followed by the Brünnich's guillemot, then the black guillemots and kittiwakes, and finally the glaucous gull. This hierarchy was expected based on trophic position and the effects of biomagnification. Little auks feed mainly on copepods while Brünnich's guillemot feed on crustaceans and fish (polar cod) (Wolkers et al., 2005). Black guillemots and kittiwakes feed mostly on fish like polar cod, though black guillemots have been evidenced to feed opportunistically and both species can also prey upon amphipods and krill (Hop et al., 2002). Glaucous gulls are predators and scavengers that have a generalist diet consisting of spider crabs (a trophic level below polar cod), fish (polar cod), seabirds, and offal in Svalbard (Borgå et al., 2001; Hop et al., 2002). The Svalbard puffins were also evidenced to mostly feed on fish, especially polar cod (Hornset, 2017). Biomagnification also ensures that as species go up trophic levels, their contamination level increases, leaving little auks with the lowest expected levels and glaucous gulls with the highest (Borgå et al., 2001).

Despite the expectations based on trophic level, the little auks had similar contamination levels to puffins for oxy-chlordane and p,p'-DDE, with slightly lower HCB levels. The black guillemot had the lowest levels for these POPs while the Brünnich's guillemot had similar levels to the puffin and little auk with slightly higher HCB. The kittiwake had the highest HCB level while the gull had the highest p,p'-DDE as expected though overlapping confidence intervals took some significance from the higher oxy-chlordane level of the gull.

Speculating on the direct causes of contamination difference can be less than objective as species differences are numerous and often unaccounted for. Previous studies investigating PCB contamination between different seabird species found that the levels depended mostly on diet and biotransformation. These factors have high species-specificity though and levels can depend on seasonality, physiology, age, sex, unaccounted for environmental conditions, and the contaminant's properties (Borgå et al., 2004; Wolkers et al., 2005). Another study on two different guillemot species in Alaska found significant contamination differences between the species, attributed to different foraging depths, prey, and winter and summer foraging areas. POP contributions from wintering areas were also concluded to be small, with egg contamination reflecting local sources (Vander Pol et al., 2004).

Given these aspects, the different contamination levels in this study can still be speculated upon given the available background information on the samples. During breeding season for example, little auks in Svalbard eat mostly crustaceans like copepods though they also prey on pelagic amphipods, euphasiids, and decapod larvae. In some areas though, little auks have been found to prey on polar cod and there is evidence that they hunt mostly fish in the winter months (Mehlum & Gabrielsen, 1993; Anker-Nilssen et al., 2000). As the puffin diet was found to consist almost half of polar cod in a rough estimate (Fig 14) and given that the winter diet of little auks is evidenced to consist of more fish than copepods and the samples were collected in May, it is possible the collected samples were reflecting the similar winter diet of little auks and summer diet of puffins (Mehlum & Gabrielsen, 1993; Anker-Nilssen et al., 2000). This similar diet could account for their unexpectedly comparable contamination levels, though their breeding season trophic levels are more separate. The results for the glaucous gull was expected (given its higher trophic level) and the black guillemots were similar to puffins as expected but the kittiwake had consistently higher contamination than the puffin, perhaps because they were feeding opportunistically on a higher trophic level. The black guillemot and puffin had similar PCB levels while the kittiwake was higher but had levels less than the glaucous gull. This was expected as a previous study showed that PCB blood levels in both puffins and kittiwakes from Hornøya were 10-20 times lower than levels correlated with sub-lethal effects found in glaucous gulls (Sagerup et al., 2009). For PFASs though, kittiwakes had higher levels than both the puffin and the glaucous gull for all POPs but linPFOS. The PFASs comparison for the puffin and the glaucous gull was unexpected as the gull is an apex predator to seabirds and should have been more contaminated. The gull did have the highest linPFOS levels though, which follows with how historically, studies of

PFASs contamination have shown PFOS to be in the highest concentrations (Verreault et al., 2010; AMAP, 2018), though the confidence interval for the gull linPFOS is large and overlapped with the kittiwake results.

Glaucous gulls having unexpectedly low HCB, oxy-chlordane, and PFASs could also be attributed to them being generalists and feeding on a wide variety of prey. While those hunting other seabirds will theoretically be more contaminated, they may just as well feed upon prey items at lower trophic levels. If this is the case, their contamination would reflect that of a seabird that traditionally feeds on lower trophic levels, like puffins, or even lower if they feed on spider crabs as Svalbard populations of glaucous gull have been reported doing. The highest POP levels in the gulls were also p,p'-DDE and PCB 153, which is logical as p,p'-DDE has a very high food web magnification factor (Hop et al., 2002).

As diet is the main contamination source in seabirds, factors that influence diet, like migration patterns, can also serve an impact. From Svalbard colonies, kittiwakes winter mainly in the North Atlantic while Brünnich's guillemots have been reported around Bjørnøya and Iceland. Glaucous gulls are seen between Bjørnøya and northern Norway and down to Iceland while little auk colonies are reported by Iceland and south of Greenland (http://seatrack.seapop.no). Typically seabirds from the European Arctic are more contaminated than those in the Canadian Arctic (Borgå et al., 2005) and between the Canadian Arctic and the Barents Sea, the POP levels have been found to be similar in copepods, euphausiids, amphipods, and polar cod (Borgå et al., 2005). At higher trophic levels though, POPs are higher in the Barents Sea than the Canadian Arctic (Borgå et al., 2005). Fish and invertebrates from the Barents Sea also historically have less PCB contamination than those in sub-Arctic and temperate regions. When compared to the Canadian Arctic they had relatively similar contamination levels. It is at higher trophic levels that POP concentrations are seen much higher in the Barents Sea when compared to ecosystems in the North American Arctic (Gabrielsen & Sydnes, 2009).

Other factors that can affect the contamination of prey species and thus seabirds are spatial scale outside of migration. Even within Svalbard, differences have been found in zooplankton species between fjords. This could be due to the different characteristics such as seasonal variation, and exposure to ocean currents. Some fjords are supplied by Atlantic water rather than Arctic which can affect contaminant level (Hallanger, 2010). The influence of Atlantic waters and thus prey species is also increasing in the arctic with food webs shifting as a result of climate change (Vihtakari et al., 2018). Contaminant level changes between fjords was not evidenced in species at a higher trophic level than zooplankton though, as bioaccumulation

and biomagnification require more time to become evident (Hallanger, 2010). So, at seabird level the different sampling locations around Svalbard may not have as large an impact on contamination between species. Though the original samples were collected from different locations, the puffins from Sassenfjord, the gulls from Kongsfjord, and over a wide time gradient. Differences due to spatial and temporal variation and small sample size should not be discounted. In general, other studies have found that trophic level, dietary carbon source, and regional breeding location (historical POP contamination) are the most relevant factors to explain interspecific contamination differences (Eulaers et al., 2013).

4.3 Risk Assessment

The effects of POP exposure in biota can be difficult to assess as correlation is complicated by unpredictable factors like differences in prey, physiology, or the environment (Letcher et al., 2010). To best assess the risk of POP exposure to puffins though, the known effects in other species can be evaluated, especially since a lot of research is available on the glaucous gull due to its position as a top avian predator. Glaucous gulls have historically been found with adverse health effects in Bjørnøya, the southernmost island of Svalbard, displaying high levels of PCB and p,p'-DDE in their livers. In fact, from 1986 to 2006, the Bjørnøya population was seen to drop by almost 65% with POP exposure, climate change, habitat loss, pathogens, and other factors as possible culprits, though no causal link was established (Verreault et al., 2010). Glaucous gulls are at the most potent risk for biological effects from POPs, due to their high trophic level, particularly those populations feeding in the eastern Barents Sea, close to contaminated river runoff from Russia (Gabrielsen & Sydnes, 2009). POP contamination in gulls has been evidenced to affect endocrine and immune responses, reproduction success, thermoregulation, basal metabolism, feather growth, and behavior (Verreault et al., 2010). In Antarctic snow petrels, legacy POPs were shown to affect endocrine function even at low levels, making the birds more susceptible to environmental stressors (Tartu et al., 2015). PFASs have also been evidenced to affect the thyroid system, body condition, and body mass of both Svalbard kittiwakes and Arctic skuas with high levels of linPFOS and PFUnDA in their blood (Ask, 2015). PFASs in general can have adverse effects on the immune and endocrine system, liver function, and development of biota (Lau et al., 2007).

When assigning POP level thresholds for adverse physiological and biochemical responses in biota taking into account everything that can cause adverse effects can cause difficulties in

assigning correlation. Reactions to POP exposure can vary depending on seasonal exposure, the physiology of separate species, age, body condition, and adaptive responses along with temporal and geographic trends of POPs as well as their possible additive effects and reactions to biotransformation (Letcher et al., 2010). Previous studies on seabird contamination have also concluded that risk assessments based on single life stages cannot be applied to other species or life stages (Guzzo et al., 2014). With all this in mind, the threshold levels available for when adverse effects may be expected in the eggs of some fish-eating and predatory birds, listed the PCB reproduction success (hatching, egg mortality, deformities, parental attentiveness) no-observed-effects-level (NOEL) as 1.3-11 μg/g ww. The lowest-observed-effects-level (LOEL) was 3.5-22 μg/g ww. In adult birds, mortality was seen with a combined PCB concentration higher than 300 μg/g ww in the brain. For herring gulls specifically, egg mortality and deformities were associated with 5.0 μg/g ww, while hatching success was affected at 2.3 μg/g ww for forster's tern and 7.6 μg/g ww for common terns (de Wit et al., 2004). The Bjørnøya gulls experiencing adverse effects had PCB levels at 0.701 μg/g ww from blood plasma (Verreault et al., 2010). Svalbard puffin's highest PCB level, PCB 153 was at 0.001453 μg/g ww. The Hornøya puffin population was also recorded as having a PCB concentration of 0.6 μg/g ww, 20% of the lowest levels for observed negative effects from PCBs (Sagerup et al., 2009).

Given past studies and these levels far below the threshold for effects, puffins can be expected to not experience adverse effects since they exist at a traditionally lower trophic level than the glaucous gull and have consistently lower contamination levels. Lack of obvious effects does not mean that they are completely unaffected however. The numerous and complicated factors that can influence contamination and biological effects may be obscuring small negative effects in species presently considered at low risk and only further research will reveal such theories. Most research is also concerning legacy POPs, but as POPs like PFASs are emerging, not as much research exists regarding their fate and effects in the Arctic (Hallanger, 2010). Thus, PFASs could already be having effects on seabirds like puffins, and it simply has not been studied or correlated yet. As PCBs and p,p'-DDE had the highest levels in the puffin colonies though, any potential effects in the future would most likely arise from these contaminants. This includes endocrine disruption (PCBs) and negative reproduction effects (p,p'-DDE) (Letcher et al., 2010).

Many factors can also affect how POPs accumulate in Arctic biota. Migration patterns and feeding habits can affect exposure while climate change affects sea-ice coverage and POP

storage (Rigét et al., 2019). The average Arctic temperature is increasing at almost twice the global rate (Bernstein et al., 2007) with melting sea ice and permafrost that had previously acted as sinks for greenhouse gases and pollutants releasing stores and worsening Arctic contamination (AMAP, 2018). The Svalbard archipelago is evidenced to experience less sea ice and warmer waters over time, leading to an Atlantification process that can change community composition with sub-Arctic and Atlantic marine species replacing high Arctic ones (Vihtakari et al., 2018). Puffins and black guillemots are traditionally low-Arctic auks while little auks and Brünnich's guillemots are high Arctic (Veit & Manne, 2015). The presence of large puffin colonies in Svalbard at all is likely a result of Atlantification. Climate induced changes in the atmospheric and ocean currents that act as Arctic transport for POPs can also potentially increase or decrease concentrations while forced food web changes can alter organism's exposure (Bustnes et al., 2012). Climate change can affect the phenology of seabird species as well, leading puffins to mistime breeding based on temperatures and then miss prey migrations and starve (Durant et al., 2003). Given that melting permafrost and ice release POP stores and warming temperatures make POPs more volatile and more likely to be adsorbed by biota, as well as increasing pathogen risk weakening immune systems of individuals, the effects of pollutants can be expected to worsen with climate change (Verreault et al., 2010; Ma et al., 2011).

5 Conclusions

Puffins are an indicator species for changes in pelagic food webs in the Norwegian and Barents Sea, thus current data on their population trends, diet choices, and success are important for future considerations of Arctic activity and regulations (Anker-Nilssen et al., 2000). POPs are present in the Svalbard populations, but the birds are at low risk of adverse effects compared to other puffin populations and seabird species. Future studies would benefit from obtaining more objective diet identification through collection and DNA analysis while higher samples sizes over temporal gradients and breeding status of birds would offer more significant conclusions and general trends. The recent colony growth and general presence of the low-Arctic puffin species in Svalbard makes these colonies unique and worthy of further study. They may be a symptom of climate change and as conditions in the Arctic are subject to Atlantification, establishing baselines and recognizing trends will become even more important for future protection and monitoring.

6 Works Cited

Adetona, O., Horton, K., Sjodin, A., Jones, R., Hall, D. B., Aguillar-Villalobos, M., … Naeher, L. P. (2013). Concentrations of select persistent organic pollutants across pregnancy trimesters in maternal and in cord serum in Trujillo, Peru. *Chemosphere*, *91*(10), 1426–1433. https://doi.org/10.1016/j.neuron.2015.05.044.HTR7

Altman, D. G., & Bland, J. M. (2005). Standard deviations and standard errors. *BMJ (Clinical Research Ed.)*, *331*(7521), 903. https://doi.org/10.1136/bmj.331.7521.903

AMAP. (2017). *AMAP Assessment 2016: Chemicals of Emerging Arctic Concern*. *AMAP*. Retrieved from file:///Users/Kirstine/Downloads/aar2016-ceac.pdf

AMAP. (2018). *Biological Effects of Contaminants on Arctic Wildlife and Fish*. Tromsø. https://doi.org/10.13140/RG.2.1.4083.2488

Anker-Nilssen, T., Bakken, V., Strøm, H., Golovkin, A. N., Bianki, V. V., & Tatarinkova, I. P. (2000). *The Status of Marine Birds Breeding in the Barents Sea Region*. Tromsø: Norsk Polarinstitutt.

Anker-Nilssen, T., Jensen, J.-K., & Harris, M. P. (2018). Bird Study Fit is fat: winter body mass of Atlantic Puffins Fratercula arctica. *Bird Study*. https://doi.org/10.1080/00063657.2018.1524452

Ask, A. V. (2015). Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs) Affect the Thyroid Hormone System , Body Condition , and Body Mass in Two Arctic Seabird Species, (May).

Bantock, T. M., Prys-Jones, R. P., & Lee, P. L. M. (2008). New and improved molecular sexing methods for museum bird specimens. *Molecular Ecology Resources*, *8*, 519–528. https://doi.org/10.1111/j.1471-8286.2007.01999.x

Bard, S. M. (1999). Global Transport of Anthropogenic Contaminants and the Consequences for the Arctic Marine Ecosystem. *Marine Pollution Bulletin*, *38*(5), 356–379.

Barrett, R. T. (2002). Atlantic puffin Fratercula arctica and common guillemot Uria aalge chick diet and growth as indicators of fish stocks in the Barents Sea. *Marine Ecology Progress Series*, *230*, 275–287. https://doi.org/10.3354/meps230275

Barrett, R. T., Anker-Nilssen, T., Gabrielsen, G. W., & Chapdelaine, G. (2002). Food consumption by seabirds in Norwegian waters. *ICES Journal of Marine Science*, *59*(1), 43–57. https://doi.org/10.1006/jmsc.2001.1145

Barrett, R. T., Nilsen, E. B., & Anker-Nilssen, T. (2012). Long-term decline in egg size of Atlantic puffins Fratercula arctica is related to changes in forage fish stocks and climate conditions. *Marine Ecology Progress Series*, *457*, 1–10. https://doi.org/10.3354/meps09813

Bernstein, L., Bosch, P., Canziani, O., Chen, Z., Christ, R., Davidson, O., … Madan, P. (2007). *Climate Change 2007 Synthesis Report*. Geneva. Retrieved from https://www.ipcc.ch/site/assets/uploads/2018/02/ar4_syr_full_report.pdf

BirdLife International. (2018). *Fratercula arctica , Atlantic Puffin*. *The IUCN Red List of Threatened Species*.

Blévin, P., Angelier, F., Tartu, S., Bustamante, P., Herzke, D., Moe, B., … Chastel, O.

(2017). Perfluorinated substances and telomeres in an Arctic seabird: Cross-sectional and longitudinal approaches. *Environmental Pollution*, *230*, 360–367. https://doi.org/10.1016/j.envpol.2017.06.060

Borgå, K. (2002). *Organochlorine contaminants in Arctic marine food webs: distribution in pelagic and sympagic fauna*. University of Tromsø.

Borgå, K., Fisk, A. T., Hoekstra, P. F., & Muir, D. C. G. (2004). Biological And Chemical Factors Of Importance In The Bioaccumulation And Trophic Transfer Of Persistent Organochlorine Contaminants In Arctic Marine Food Webs. *Environmental Toxicology and Chemistry*, *23*(10), 2367–2385.

Borgå, K., Gabrielsen, G. W., & Skaare, J. U. (2001). Biomagnification of organochlorines along a Barents Sea food chain. *Environmental Pollution*, *113*(2), 187–198. https://doi.org/10.1016/S0269-7491(00)00171-8

Borgå, K., Gabrielsen, G. W., Skaare, J. U., Kleivane, L., Norstrom, R. J., & Fisk, A. T. (2005). Why Do Organochlorine Differences between Arctic Regions Vary among Trophic Levels? *Environmental Science and Technology*, *39*(12), 4343–4352. https://doi.org/10.1021/es0481124

Borgå, K., Hop, H., Skaare, J. U., Wolkers, H., & Gabrielsen, G. W. (2007). Selective bioaccumulation of chlorinated pesticides and metabolites in Arctic seabirds. *Environmental Pollution*, *145*(2), 545–553. https://doi.org/10.1016/j.envpol.2006.04.021

Bustnes, J. O., Moe, B., Hanssen, S. A., Herzke, D., Fenstad, A. A., Nordstad, T., … Gabrielsen, G. W. (2012). Temporal dynamics of circulating persistent organic pollutants in a fasting seabird under different environmental conditions. *Environmental Science and Technology*, *46*(18), 10287–10294. https://doi.org/10.1021/es301746j

de Wit, C. A., Fisk, A. T., Hobbs, K. E., & Muir, D. C. G. (2004). *AMAP Assessment 2002: Persistent Organic Pollutants in the Arctic*. Oslo.

Durant, J. M., Anker-Nilssen, T., & Stenseth, N. C. (2003). Trophic interactions under climate fluctuations: The Atlantic puffin as an example. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1523), 1461–1466. https://doi.org/10.1098/rspb.2003.2397

Erikstad, K. E., Sandvik, H., Reiertsen, T. K., Bustnes, J. O., & Strøm, H. (2013). Persistent organic pollution in a high-Arctic top predator: Sex-dependent thresholds in adult survival. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1769). https://doi.org/10.1098/rspb.2013.1483

Eulaers, I., Jaspers, V. L. B., Bustnes, J. O., Covaci, A., Johnsen, T. V., Halley, D. J., … Eens, M. (2013). Ecological and spatial factors drive intra- and interspecific variation in exposure of subarctic predatory bird nestlings to persistent organic pollutants. *Environment International*, *57–58*, 25–33. https://doi.org/10.1016/J.ENVINT.2013.03.009

Fauchald, P., Anker-Nilssen, T., Barrett, R. T., Bustnes, J. O., Bårdsen, B., Christensen-Dalsgaard, S., … Systad, G. H. (2015). *The status and trends of seabirds breeding in Norway and Svalbard*. *Norwegian Institute for Nature Research* (Vol. 1151). Tromsø.

Fayet, A. L., Freeman, R., Anker-Nilssen, T., Shoji, A., Wanless, S., & Guilford Correspondence, T. (2017). Ocean-wide Drivers of Migration Strategies and Their

Influence on Population Breeding Performance in a Declining Seabird. *Current Biology*, *27*, 3871–3878. https://doi.org/10.1016/j.cub.2017.11.009

Friars, K. A., & Diamond, A. W. (2011). Predicting the Sex of Atlantic Puffins, Fratercula arctica, by Discriminant Analysis. *The International Journal of Waterbird Biology*, *34*(3), 304–311. Retrieved from https://www.jstor.org/stable/23018160?seq=1&cid=pdf-reference#references_tab_contents

Gabrielsen, G. W. (2007). *Levels and effects of persistent organic pollutants in arctic animals*.

Gabrielsen, G. W. (2009). 17. Seabirds in the Barents Sea. *Ecosystem Barents Sea*, 415–452.

Gabrielsen, G. W., & Henriksen, E. O. (2001). Persistent organic pollutants in Arctic animals in the Barents Sea area and at Svalbard: Levels and effects. *National Insitute of Polar Research*, (54), 349–364.

Gabrielsen, G. W., & Sydnes, L. K. (2009). Pollution in the Barents Sea. *Ecosystem Barents Sea.*, 415–452.

Griffiths, R., Double, M. C., Orr, K., & Dawson, R. J. G. (1998). A DNA test to sex most birds. *Molecular Ecology*, *7*, 1071–1075.

Hallanger, I. G. (2010). *Influence of climate on accumulation of contaminants in Arctic marine pelagic food webs*. University of Tromsø.

Hallanger, I. G., Ruus, A., Warner, N. A., Herzke, D., Evenset, A., Schøyen, M., … Borgå, K. (2011). Differences between Arctic and Atlantic fjord systems on bioaccumulation of persistent organic pollutants in zooplankton from Svalbard. *Science of The Total Environment*, *409*(14), 2783–2795. https://doi.org/10.1016/j.scitotenv.2011.03.015

Hanssen, L., Dudarev, A. A., Huber, S., Øyvind, J., Nieboer, E., & Sandanger, T. M. (2013). Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma , assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. *Science of the Total Environment*, *447*, 430–437. https://doi.org/10.1016/j.scitotenv.2013.01.029

Harris, M. P., Leopold, M. F., Jensen, J. K., Meesters, E. H., & Wanless, S. (2015). The winter diet of the Atlantic Puffin Fratercula arctica around the Faroe Islands. *International Journal of Avian Science*, *157*(3), 468–479. https://doi.org/10.1111/ibi.12272

Helgason, L. B., Barrett, R., Lie, E., Polder, A., Skaare, J. U., & Gabrielsen, G. W. (2008). Levels and temporal trends (1983-2003) of persistent organic pollutants (POPs) and mercury (Hg) in seabird eggs from Northern Norway. *Environmental Pollution*, *155*(1), 190–198. https://doi.org/10.1016/j.envpol.2007.10.022

Hop, H., Borgå, K., Gabrielsen, G. W., Kleivane, L., & Skaare, J. U. (2002). Food Web Magnification of Persistent Organic Pollutants in Poikilotherms and Homeotherms from the Barents Sea. *Environmental Science and Technology*, *36*(12). https://doi.org/10.1021/es010231l

Hornset, K. (2017). *Monitoring Daily Activity and Parental Food Provisioning in the Atlantic Puffin (Fratercula arctica) by the Use of Camera Traps*. Norwegian University of Life Sciences.

Hung, H., Katsoyiannis, A. A., & Guardans, R. (2016). Ten years of global monitoring under the Stockholm Convention on Persistent Organic Pollutants (POPs): Trends, sources and transport modelling. *Environmental Pollution*, *217*, 1–3. https://doi.org/10.1016/j.envpol.2016.05.035

Jensen, S., Johnels, A. G., Olsson, M., & Otterlind, G. (1972). *DDT and PCB in herring and cod from the Baltic , the Kattegat and the Skagerrak*.

Jonsson, K. I. (1997). Capital and Income Breeding as Alternative Tactics of Resource Use in Reproduction. *Nordic Society Oikos*, *78*(1), 57–66.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., & Seed, J. (2007). Perfluoroalkyl Acids: A Review of Monitoring and Toxicological Findings. *Toxicological Sciences*, *99*(2), 366–394. https://doi.org/10.1093/toxsci/kfm128

Leat, E. H. K., Bourgeon, S., Hanssen, S. A., Petersen, A., Strøm, H., Bjørn, T. H., … Borgå, K. (2019). The effect of long-range transport, trophic position and diet specialization on legacy contaminant occurrence in great skuas, Stercorarius skua, breeding across the Northeast Atlantic. *Environmental Pollution*, *244*, 55–65. https://doi.org/10.1016/j.envpol.2018.10.005

Leat, E. H. K., Bourgeon, S., Magnusdottir, E., Gabrielsen, G. W., Grecian, W. J., Hanssen, S. A., … Borgå, K. (2013). Influence of wintering area on persistent organic pollutants in a breeding migratory seabird. *Marine Ecology*, *491*, 277–293. https://doi.org/10.3354/meps10455

Letcher, R. J., Bustnes, J. O., Dietz, R., Jenssen, B. M., Jørgensen, E. H., Sonne, C., … Gabrielsen, G. W. (2010). Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Science of the Total Environment*, *408*(15), 2995–3043. https://doi.org/10.1016/j.scitotenv.2009.10.038

Løvenskiold, H. L. (1964). *Avifauna Svalbardensis: with a discussion on the geographical distribution of the birds of Spitsbergen and adjacent islands* (129th ed.). Oslo: Norsk Polarinstitutt.

Ma, J., Hung, H., Tian, C., & Kallenborn, R. (2011). Revolatilization of persistent organic pollutants in the Arctic induced by climate change. *Nature Climate Change*, *1*(8), 255–260. https://doi.org/10.1038/nclimate1167

Mehlum, F., & Gabrielsen, G. W. (1993). The diet of high-arctic seabirds in coastal and ice-covered, pelagic areas near the Svalabrd archipelago. *Polar Research*, *12*(1), 1–20.

Melnes, M., Gabrielsen, G. W., Herzke, D., Sagerup, K., & Jenssen, B. M. (2017). Dissimilar effects of organohalogenated compounds on thyroid hormones in glaucous gulls. *Environmental Research*, *158*(7491), 350–357. https://doi.org/10.1016/j.envres.2017.06.007

Nahrgang, J., Camus, L., Broms, F., Christiansen, J. S., & Hop, H. (2010). Seasonal baseline levels of physiological and biochemical parameters in polar cod (Boreogadus saida): Implications for environmental monitoring. *Marine Pollution Bulletin*, *60*(8), 1336–1345. https://doi.org/10.1016/J.MARPOLBUL.2010.03.004

Nilsen, F., Cottier, F., Skogseth, R., & Mattsson, S. (2008). Fjord–shelf exchanges controlled by ice and brine production: The interannual variation of Atlantic Water in Isfjorden, Svalbard. *Continental Shelf Research*, *28*(14), 1838–1853.

https://doi.org/10.1016/J.CSR.2008.04.015

R Development Core Team. (2016). R: A Language and Environment for Statistical Computing. Retrieved from https://www.r-project.org

Rigét, F., Bignert, A., Braune, B., Dam, M., Dietz, R., Evans, M., … Wilson, S. (2019). Temporal trends of persistent organic pollutants in Arctic marine and freshwater biota. *Science of the Total Environment*, *649*(August 2018), 99–110. https://doi.org/10.1016/j.scitotenv.2018.08.268

Roscales, J. L., Muñoz-Arnanz, J., González-Solís, J., & Jiménez, B. (2010). Geographical PCB and DDT Patterns in Shearwaters (Calonectris sp.) Breeding Across the NE Atlantic and the Mediterranean Archipelagos. *Environmental Science & Technology*, *44*(7), 2328–2334. https://doi.org/10.1021/es902994y

Sagerup, K. (2009). *POP-cocktails: Hangover threats for seabirds? The response of three seabird species to exposure to persistent organic pollutants in the Barents Sea*. University of Tromsø.

Sagerup, K., Åsbakk, K., Polder, A., Skaare, J. U., Gabrielsen, G. W., & Barrett, R. T. (2009). *The effect of persistent organic pollutants (POPs) on the immune system of black-legged kittiwakes and Atlantic puffins in the Barents Sea*. University of Tromsø.

Sagerup, K., Åsbakk, K., Polder, A., Skaåre, J. U., Gabrielsen, G. W., & Barrett, R. T. (2014). Relationships between persistent organic pollutants and circulating immunoglobulin-y in black-legged kittiwakes and atlantic puffins. *Journal of Toxicology and Environmental Health - Part A: Current Issues*, *77*(9–11), 481–494. https://doi.org/10.1080/15287394.2014.886543

Sagerup, K., Helgason, L. B., Polder, A., Strøm, H., Josefsen, T. D., Skåre, J. U., & Gabrielsen, G. W. (2009). Persistent organic pollutants and mercury in dead and dying glaucous gulls (Larus hyperboreus) at Bjørnøya (Svalbard). *Science of the Total Environment*, *407*(23), 6009–6016. https://doi.org/10.1016/j.scitotenv.2009.08.020

Sagerup, K., Savinov, V., Savinova, T., Kuklin, V., & Muir, D. C. G. (2009). Persistent organic pollutants, heavy metals and parasites in the glaucous gull (Larus hyperboreus) on Spitsbergen. *Environmental Pollution*, *157*, 2282–2290.

Savinov, V. M., Savinova, T. N., Matishov, G. G., Dahle, S., & Næs, K. (2003). Polycyclic aromatic hydrocarbons (PAHs) and organochlorines (OCs) in bottom sediments of the Guba Pechenga, Barents Sea, Russia. *Science of The Total Environment*, *306*(1–3), 39–56. https://doi.org/10.1016/S0048-9697(02)00483-7

SEAPOP. (2018). SEATRACK: seabird tracking. Retrieved from http://seatrack.seapop.no

Tartu, S., Angelier, F., Herzke, D., Moe, B., Bech, C., Gabrielsen, G. W., … Chastel, O. (2014). The stress of being contaminatedα Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. *Science of the Total Environment*, *476–477*, 553–560. https://doi.org/10.1016/j.scitotenv.2014.01.060

Tartu, S., Angelier, F., Wingfield, J. C., Bustamante, P., Labadie, P., Budzinski, H., … Chastel, O. (2015). Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. *Science of The Total Environment*, *505*, 180–188. https://doi.org/10.1016/J.SCITOTENV.2014.10.008

Vander Pol, S. S., Becker, P. R., Kucklick, J. R., Pugh, Rebecca, S., Roseneau, D. G., &

Simac, K. S. (2004). Persistent Organic Pollutants in Alaskan Murre (Uria spp.) Eggs: Geographical, Species, and Temporal Comparisons. *Environmental Science and Technology*, *38*, 1305–1312. https://doi.org/10.1021/es034987r

Veit, R. R., & Manne, L. L. (2015). Climate and changing winter distribution of alcids in the Northwest Atlantic. *Frontiers in Ecology and Evolution*, *3*, 38. https://doi.org/10.3389/fevo.2015.00038

Verreault, J., Gabrielsen, G. W., & Bustnes, J. O. (2010). The Svalbard Glaucous Gull as Bioindicator Species in the European Arctic: Insight from 35 Years of Contaminants Research. *Reviews of Environmental Contamination and Toxicology*, *205*, 77–112. https://doi.org/10.1007/978-1-4419-5623-1

Verreault, J., Villa, R. A., Gabrielsen, G. W., Skaare, J. U., & Letcher, R. J. (2006). Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. *Environmental Pollution*, *144*, 1053–1060.

Vihtakari, M., Welcker, J., Moe, B., Chastel, O., Tartu, S., Hop, H., … Gabrielsen, G. W. (2018). Black-legged kittiwakes as messengers of Atlantification in the Arctic, (9037), 1–11. https://doi.org/10.1038/s41598-017-19118-8

Wania, F., & MacKay, D. (1993). Tracking the Distribution of Persistent Organic Pollutants. *Environmental Science and Technology*, *30*(9), 390–396.

Wienerroither, R., Johannesen, E., Wenneck, T. D. L., Bjelland, O., Dolgov, A., Prozorkevich, D., … Museum, B. (2011). *Atlas of the Barents Sea Fishes*.

Wolker, H., Bavel, B. Van, Derocher, A. E., Wiig, Ø., Kovacs, K. M., Lydersen, C., & Lindstrom, G. (2004). Congener-Specific Accumulation and Food Chain Transfer of Polybrominated Diphenyl Ethers in Two Arctic Food Chains. *Environmental Science and Technology*, *38*, 1667–1674. https://doi.org/10.1021/es030448a

Wolkers, H., Skaare, J. U., Borga, K., Hop, H., Muir, D. C. G., & Gabrielsen, G. W. (2005). Bioaccumulation of PCBs in Arctic seabirds : influence of dietary exposure and congener biotransformation. *Environmental Pollution*, *134*, 397–409. https://doi.org/10.1016/j.envpol.2004.09.016

Yamashita, R., Takada, H., Murakami, M., Fukuwaka, M., & Watanuki, Y. (2007). Evaluation of Noninvasive Approach for Monitoring PCB Pollution of Seabirds Using Preen Gland Oil. *Environmental Science and Technology*, *41*(14), 4901–4906. https://doi.org/10.1021/ES0701863

Zahn, S., Herzke, D., Borgå, K., Haarr, A., Le Bohec, C., Eckbo, N., … Planas-Bielsa, V. (2019). Individual variability in contaminants and physiological status in a resident Arctic seabird species. *Environmental Pollution*. https://doi.org/10.1016/j.envpol.2019.01.025

Appendix A: Data

Table A1: Raw data of contamination in individual puffins 1-16 for organochlorine and fluorinated substances

		Individual Puffin ID							
		1	2	3	4	5	6	7	8
	Sex	M	M	F	M	M	F	M	F
	Compound								
	HCB	601.1	1060.5	440	770	782.1	967.6	945.3	733.9
	oxy-chlordane	345	715	292	491	299	409	550	412
	t-chlordane	43	65	31	46	32	45	34	27
	c-chlordane	2	2	2	5	2	2	5	5
	t-Nonachlor	173	226	107	194	81	267	160	125
	c-Nonachlor	137	189	90	152	83.3	185	165	86
	Mirex	95.0	207.0	95.8	202	147	107	185	163
DDT	p,p'-DDE	2450	4026	1605	4076	1712	2260	2991	2212
PCBs	PCB 99	248	574	143	465	337	370	474	331
	PCB 105	117	221	82	192	188	152	220	148
	PCB 118	471	892	309	832	549	546	792	487
	PCB 138	640	1222	392	1395	986	667	1199	915
	PCB 153	1071	1694	648	1710	1025	988	1528	1062
	PCB 180	304	541	165	582	360	323	524	377
	PCB 183	33.55	118	33.55	149	33.55	33.55	133	33.55
	PCB 187	242	392	138	406	245	246	337	212
PFASs	PFHxS	368.01	111.14	230.68	176.90	149.48	42.96	176.55	286.28
	brPFOS	2109.65	553.24	1024.61	690.69	958.39	182.78	1045.11	786.06
	linPFOS	9791.52	7613.81	7478.25	5253.90	9306.33	3702.54	6680.35	7004.70
	PFOA	546.90	120.14	294.23	166.86	334.90	57.24	276.08	198.10
	PFNA	1632.57	830.76	1061.49	841.21	1709.42	770.89	962.01	832.91
	PFDcA	1491.89	1263.67	1142.59	899.02	1970.51	803.92	997.76	975.50
	PFUnA	8399.45	7378.00	7482.99	4254.60	9207.04	4389.84	5335.91	5423.07
	PFDoA	2075.04	1490.64	2030.63	757.72	1754.37	958.12	956.98	865.30
	PFTriA	5779.83	3696.82	6326.66	1867.00	3442.92	1783.75	2592.25	1977.63
	PFTriA	1125.08	584.39	1250.98	507.53	817.87	278.69	452.56	403.06

		Individual Puffin ID							
		9	10	11	12	13	14	15	16
	Sex	F	M	M	M	M	F	M	F
	Compound								
	HCB	831	799.2	908.7	131.1	886.3	734.3	948.2	1527.6
	oxy-chlordane	411	324	562	50	549	349	432	1471
	t-chlordane	40	60.7	75.4	3.2	75.7	27.2	48.3	159
	c-chlordane	2	8	2.375	2.375	28	2.375	2.375	2.375
	t-Nonachlor	141	278	163	10.7	261	120	132	511
	c-Nonachlor	128	216	159	11.6	199	101	107	479
	Mirex	152	166	247	17.8	260	129	157	614

DDT	p,p'-DDE	2495	2282	4052	237	4348	2000	2448	9098
PCBs	PCB 99	392	382	527	16.65	452	294	442	1424
	PCB 105	172	149	237	18.3	205	157	192	621
	PCB 118	680	578	899	65	716	609	720	2577
	PCB 138	1020	794	1621	92	1277	814	1169	4932
	PCB 153	1252	1247	1839	143	1648	1135	1365	4887
	PCB 180	467	448	590	48.3	597	379	506	1579
	PCB 183	95	86	111	33.55	135	72	102	329
	PCB 187	277	309	453	39	416	233	296	1094
PFASs	PFHxS	218.46	274.57	216.24	61.87	107.97	59.62	114.30	93.78
	brPFOS	1230.19	1268.09	25.00	313.58	1548.39	25.00	883.48	879.47
	linPFOS	5975.84	8471.44	8674.57	1893.70	10607.36	2655.38	6128.55	4789.92
	PFOA	356.33	349.34	215.89	59.52	309.50	80.29	119.34	154.85
	PFNA	1199.42	1387.77	1112.05	335.06	1299.72	659.36	1186.51	972.56
	PFDcA	1142.85	1621.30	1524.60	367.91	1585.48	561.09	1228.26	1062.48
	PFUnA	5331.71	8933.45	6930.18	1667.11	4424.84	2485.93	5385.35	5739.55
	PFDoA	959.42	1781.32	1003.43	362.31	2148.89	445.44	831.25	1114.81
	PFTriA	2174.64	3325.92	2183.17	908.80	5761.68	1047.14	1528.11	2513.97
	PFTriA	480.31	514.96	262.20	110.14	1196.06	161.50	363.03	330.91

Table A2: Size measurement averages by male and female Atlantic Puffin with Confidence intervals

	Males n = 10	Females n = 6
	Mean ± CI	Mean ± CI
Mass (g)	591.5 ± 22.25	573.33 ±25.64
Head Bill (mm)	86.77 ± 1.87	85 ±1.94
Bill Depth (mm)	44.15 ± 1.88	41.55 ±2.09
Bill Length (mm)	51.04 ± 2.12	51.08 ±1.37
Wing (mm)	187.5 ± 2.36	182.17 ±3.01

Table A3: The mean ± 95% CI of OCs and PFASs (pg/ml lipid weight) levels in Atlantic Puffin whole blood samples collected in Sassenfjord, Svalbard, 2018. Lipid weight calculated from equation: Lipid weight= $\frac{(wet\ weight\ x\ 100)}{Lipid\ \%}$. Mean Lipid % for puffin samples was 0.35.

	Males n = 10		Females n = 6	
Compound	LOD	Mean ± CI	LOD	Mean ± CI
HCB	120	223786 ± 46314.51	120	249257.14 ± 83393.49
oxychlordane	4650	123342.86 ± 32915.22	4650	159238.10 ± 102910.54
t-chlordane	1960	13800 ± 3938.75	1960	15657.14 ± 11782.62
c-chlordane	4750	1710 ± 1409.06	4750	784.52 ± 207.67
t-Nonachlor	2260	47968.57 ± 14274.38	2260	60523.81 ± 36079.07
c-Nonachlor	750	40540 ± 10812.45	750	50861.91 ± 34739.42
Mirex	14200	48108.57 ± 12677.48	14200	60038.10 ± 45609.42
DDT's				
p,p'-DDE	54000	817771 ± 231348.42	54000	936666.67 ± 655386.49
PCBs				
PCB 99	33300	111932.86 ± 28569.76	33300	140666.67 ± 106273.84
PCB 105	36600	49694.29 ± 11539.48	36600	63428.57 ± 45252.94
PCB 118	33600	186114.29 ± 44673.06	33600	248000 ± 193569.79
PCB 138	58200	297000 ± 77347.03	58200	416190.48 ± 392428.18
PCB 153	51500	379142.86 ± 88610.73	51500	474857.14 ± 364199.91
PCB 180	96600	128580 ± 30462.08	96600	156666.67 ± 117672.75
PCB 183	67100	26704.29 ± 7959.04	67100	28411.90 ± 26364.63
PCB 187	78000	89571.43 ± 21342.01	78000	104761.91 ± 82156.11
PFASs				
PFHxS	NA	50201.2 ± 16119.99	NA	44370.22 ± 23401.04
brPFOS	50	268446.3 ± 107261.46	50	196577.4 ± 109564.08
linPFOS	NA	2126330 ± 455331.42	NA	1505078 ± 432656.26
PFOA	NA	71384.95 ± 25576.56	NA	54335.33 ± 26952.04
PFNA	NA	322773.9 ± 72780.10	NA	261744.5 ± 45554.12
PFDcA	NA	370011.3 ± 80260.71	NA	270876.9 ± 52148.51
PFUnA	NA	1769027 ± 426339.68	NA	1469195 ± 376670.42
PFDoA	NA	376056 ± 108713.13	NA	303510.1 ± 120158.40
PFTriA	NA	888185.6 ± 293257.03	NA	753513.9 ± 428109.17
PFTeA	NA	169538.1 ± 62638.05	NA	138354.9 ± 89390.61

Appendix B: Diet

Fig B1: Game cam photograph prey identification as puffin returns to nest with food load, late July/ early August 2018, Sassenfjord: Juvenile stichaeidae, juvenile polar cod, herring, capelin

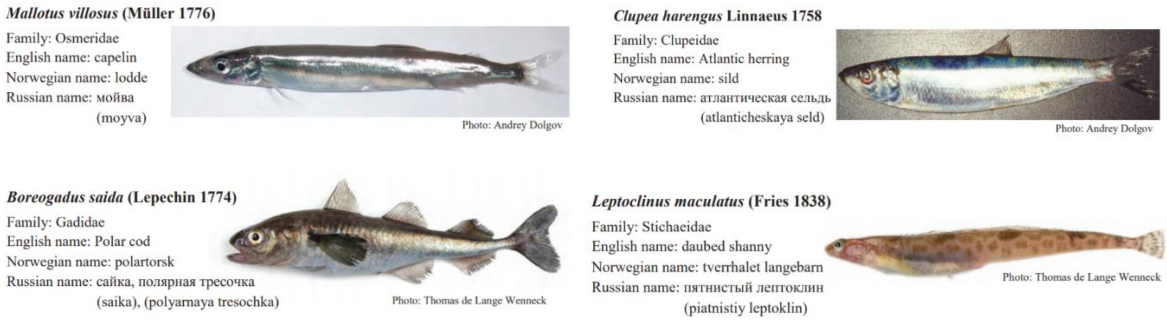

Fig B2: Atlas examples of adult prey species identified (Wienerroither et al., 2011)

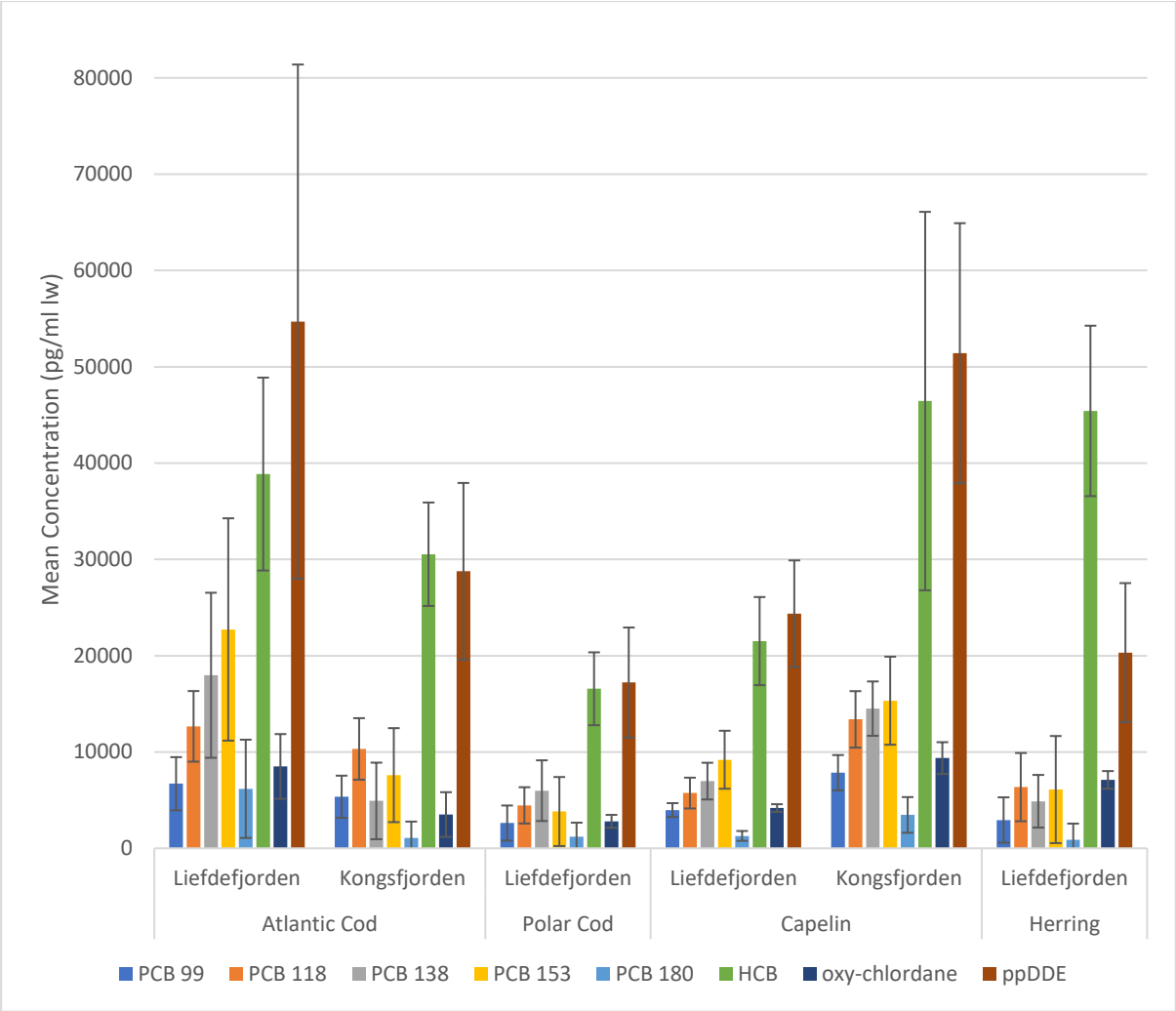

Fig B3: POP contamination of 4 different prey species muscle and whole fish samples: Atlantic cod, polar cod, capelin, and Atlantic Herring in pg/ml lipid weight with 95% confidence intervals. Data available for two fjords in northern Svalbard: Liefdefjorden and Kongsfjorden, June 2008 (COPOL)

Appendix C: Principal Component Analysis

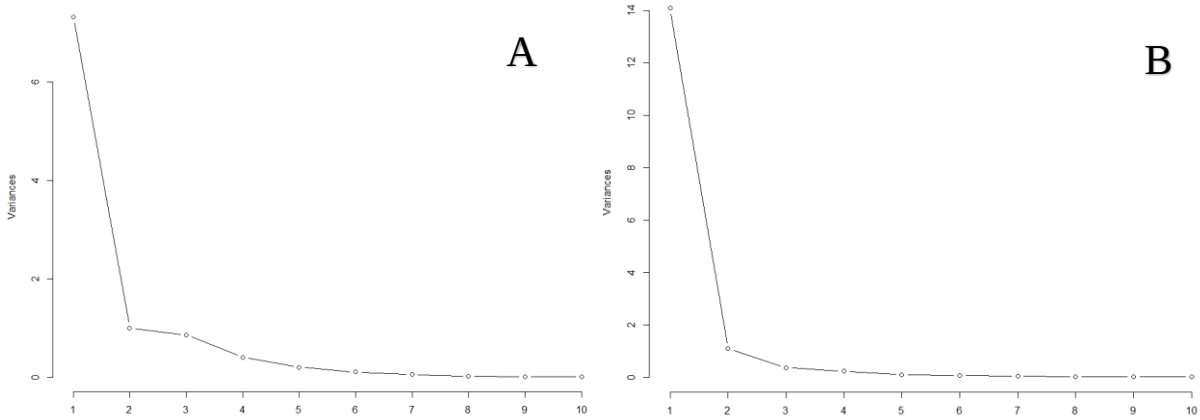

Fig C1: A: Scree plot of PFASs PC values B: Scree plot of OC PC values

Table C1: Loading values of each PFAS and OC contaminants, PFASs: negative correlation with final PC1 value for each puffin, OCs: positive correlation

PFHxS	brPFOS	linPFOS	PFOA	PFNA	PFDcA	PFUnA	PFDoA	PFTriA	PFTeA
-0.255	-0.31	-0.34	-0.33	-0.33	-0.32	-0.304	-0.34	-0.32	-0.32
HCB	oxy.chlordane	t.chlordane	c.chlordane	t.Nonachlor	c.Nonachlor	Mirex	p.p.DDE	PCB 99	PCB 105
0.236	0.26	0.26	0.03	0.24	0.26	0.26	0.26	0.27	0.26
PCB 118	PCB 138	PCB 153	PCB 180	PCB 183	PCB 187				
0.263	0.26	0.27	0.27	0.25	0.27				

Table C2: PFASs and OCs PC1 values for each puffin 1-16

Puffin ID	1	2	3	4	5	6	7	8
PC1 PFASs	-5.15	0.02	-2.51	1.52	-2.99	2.86	0.32	0.65
PC1 OCs	-1.82	1.37	-3.10	0.66	-1.74	-0.92	0.32	-1.66
Puffin ID	9	10	11	12	13	14	15	16
PC1 PFASs	-0.28	-2.55	0.09	4.76	-2.96	4.09	1.00	1.11
PC1 OCs	-0.71	-0.30	1.29	-5.28	1.42	-1.59	-0.36	12.3

Table C3: Coefficient table results for PCA testing OCs and PFASs PC1 values against sex, mass, head bill length, bill depth, and bill length. Excluding puffin 16 as an outlier for the OCs, PFASs retain puffin 16 as residual plots did not present it as an outlier for PFASs. Samples from Sassenfjord, Svalbard 2018.

factor	Estimate	Standard Error	t value	p value
OCs				
Sex	1.13	2.15	0.53	0.61
Mass	-0.04	0.03	-1.14	0.29
Head bill	-0.24	0.33	-0.72	0.49
Bill depth	0.18	0.36	0.51	0.63
Bill length	-0.13	0.33	-0.39	0.71
PFASs				
Sex	-1.12	2.08	-0.54	0.61
Mass	0.007	0.03	0.21	0.84
Head bill	-0.01	0.40	-0.03	0.98
Bill depth	-0.12	0.32	-0.36	0.73
Bill length	-0.13	0.37	-0.34	0.75

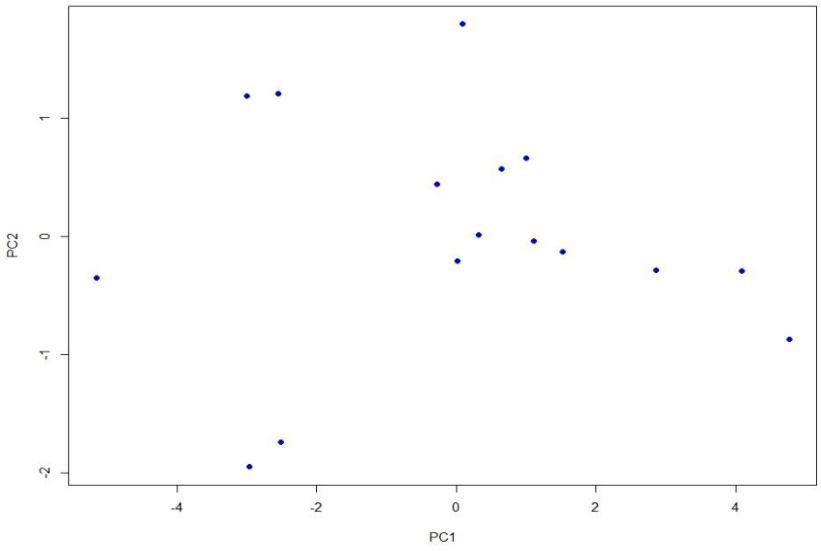

Fig C2: PC1 values by PC2 values for PFASs

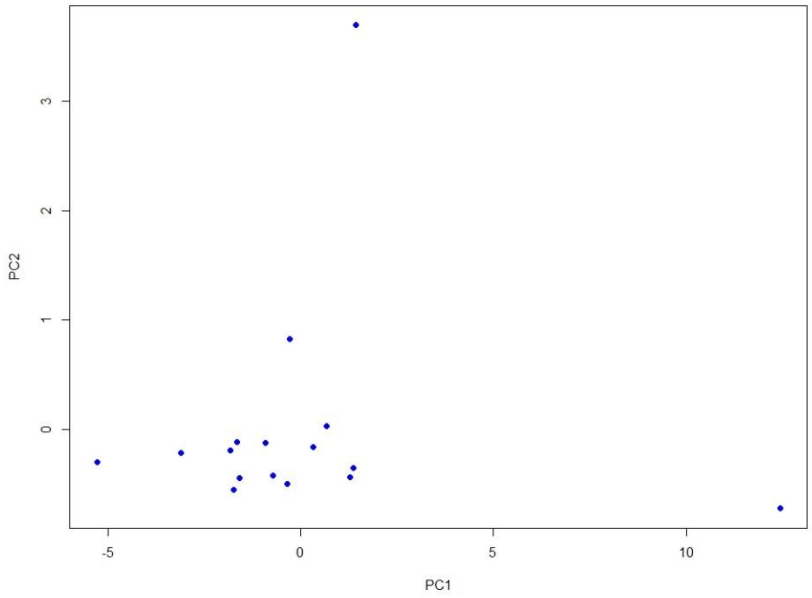

Fig C3: PC1 values by PC2 values for OCs, for PC1 values, puffin 16 is an outlier

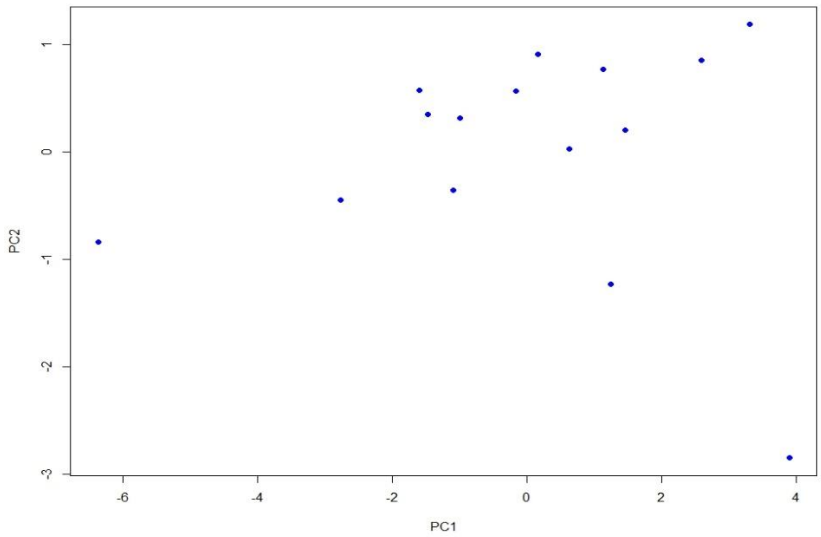

Fig C4: PC1 values by PC2 values for OCs, for PC1 values, puffin 16 removed as an outlier