Polycyclic Aromatic Compounds in Canadian Arctic Seabirds: Spatiotemporal Trends and Bioaccumulation Patterns

By

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Abstract

Polycyclic aromatic compounds (PACs) are a complex class of organic contaminants associated with petroleum. Many PACs have been identified as a concern due to their persistence in the environment, their potential to bioaccumulate, and their toxicity to living organisms. In the Canadian Arctic, emissions from natural seeps and increasing industrial activities, particularly shipping and mining, pose significant threats to the region's vulnerable marine ecosystems. While numerous contaminants have been reported in this region, targeted monitoring of PACs remains limited. Seabirds serve as established bioindicators of marine ecosystem health and are commonly used for environmental monitoring. This thesis presents two complementary studies investigating PAC contamination in Arctic seabirds using gas chromatography-tandem mass spectrometry. The first study was a spatial and temporal analysis of PACs in seabird eggs collected from high Arctic and low Arctic colonies between 1993 and 2024. Higher PAC concentrations were detected in eggs from seabirds breeding in the high Arctic, with considerable interannual variation at both sites and many of the higher PAC concentrations occurring in the past decade. The second study focused on species- and tissue-specific PAC accumulation in three species of Arctic seabirds. Substantial variation in PAC concentrations and chemical profiles was observed between tissue types and among species. This variability reflects species-specific differences in PAC exposure, accumulation, and metabolism, driven by a combination of foraging ecology, breeding phenology, and physiological factors. As activity in the Arctic and interest in Canada's northern shipping routes continue to grow, the need for monitoring oil and gas related contaminants in the Arctic region is essential to detect emerging trends, identify potential sources and support informed decision-making to ensure the protection of Canada's Arctic wildlife.

Dedication

I would like to dedicate this thesis to all those who have supported, guided, and encouraged me along the way.

To my family-thank you for your constant love and support throughout this stage of my life.

To my supervisor, Dr. Gregg Tomy-thank you for everything. I will forever be thankful our paths crossed and for your unwavering support and belief in me. Thank you for creating such a special environment in the COGRAD lab that has truly felt like a second home.

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Chapter 1: Introduction

1.1 Polycyclic Aromatic Compounds

Polycyclic aromatic compounds (PACs) are a complex class of organic contaminants composed of two or more fused aromatic rings. These compounds are ubiquitous in the environment and have been detected in a wide range of environmental compartments including air, water, soil, sediment, and biota [1–4]. As PACs are a broad and structurally diverse group, they can exhibit considerable variation in their physicochemical properties, but in general possess high melting and boiling points and are hydrophobic in nature. Many of these compounds have been recognized as a concern because of their potential to bioaccumulate, their persistence in the environment, and their toxicity to humans and other organisms.

1.2 Classification of Polycyclic Aromatic Compounds

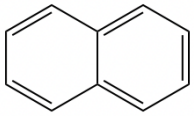
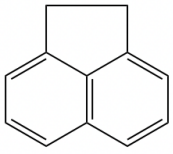
Structural properties, such as the presence of substituents or heteroatoms, can allow PACs to be further categorized into different sub-classes. The four groups of PACs that were included in this thesis are polycyclic aromatic hydrocarbons (PAHs), alkylated polycyclic aromatic hydrocarbons (APAHs), hetero-polycyclic aromatic compounds (HPACs), and halogenated hetero-polycyclic aromatic compounds (Halo-HPACs).

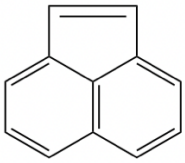
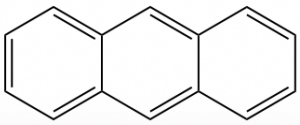
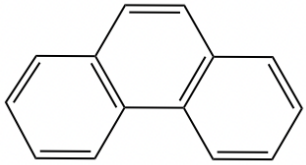
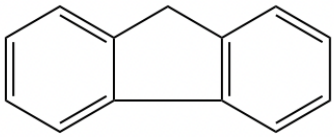
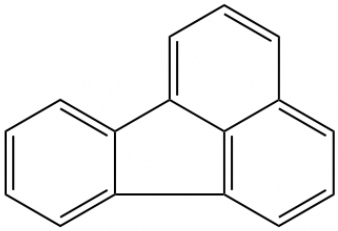
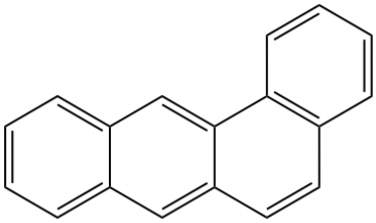
1.2.1 Polycyclic Aromatic Hydrocarbons

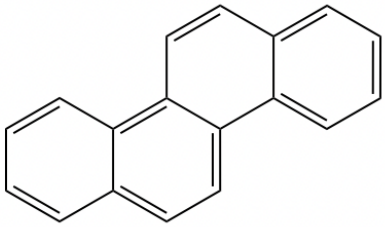
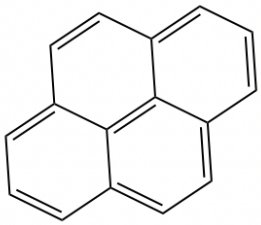
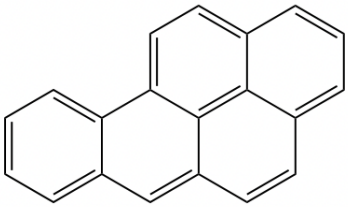
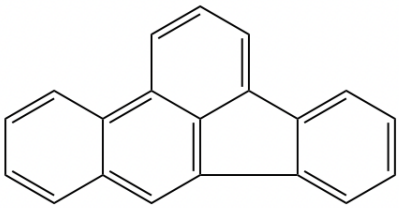
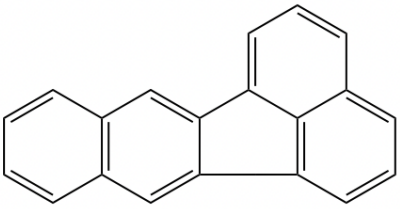
The most widely studied group of PACs are polycyclic aromatic hydrocarbons (PAHs). PAHs are composed exclusively of carbon and hydrogen atoms, with no substituents attached to the aromatic rings. PAHs are commonly classified according to the number of aromatic rings they contain. Low molecular weight (LMW) PAHs contain 2 or 3 rings, while high molecular weight (HMW) PAHs contain 4, 5, or 6 rings.

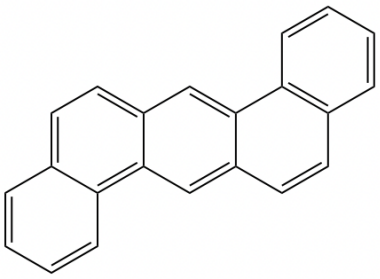
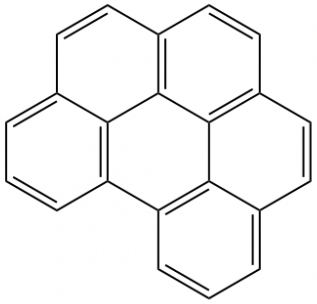
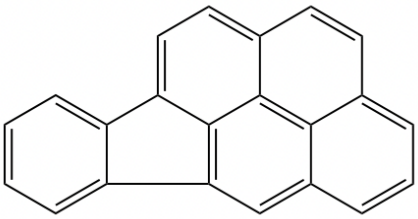
In the 1970s, the United States Environmental Protection Agency (US EPA) identified 16 PAHs (Table 1.1) as "high priority pollutants"[5]. The same 16 compounds were also added to the *Toxic Substance List* under the Canadian Environmental Protection Act (CEPA) in 1994[6]. Since their identification, the 16 priority PAHs have been widely used as representatives for the broader group of PACs and have consequently been the focus of the majority of research in this area.

Table 1.1 16 priority polycyclic aromatic hydrocarbons (PAHs) identified by the US EPA

Compound	Structure
Naphthalene	
Acenaphthene	

Acenaphthylene	
Anthracene	
Phenanthrene	
Fluorene	
Fluoranthene	
Benzo[a]anthracene	

Chrysene	
Pyrene	
Benzo[a]pyrene	
Benzo[b]fluoranthene	
Benzo[k]fluoranthene	

Dibenz[a,h]anthracene	
Benzo[g,h,i]perylene	
Indeno[1,2,3-c,d]pyrene	

While they may have been an appropriate selection at the time, the 16 priority PAHs might not be the best representatives of this diverse group of compounds nearly 50 years later. There are numerous PACs that are not currently included in the prioritization list and receive minimal attention, despite showing greater bioaccumulation potential, toxicity, and persistence than the 16 priority PAHs[7]. These compounds were originally selected primarily based on the analytical instrumentation available at the time and the availability of corresponding analytical standards[7]. Recent developments in analytical instrumentation as well as the improved

availability of analytical standards have allowed for the detection and quantitation of a more diverse range of compounds. More recently, efforts have been made to expand this list to include PACs that may be more representative of this diverse group of contaminants[8–10].

1.2.2 Alkylated Polycyclic Aromatic Hydrocarbons

Alkylated polycyclic aromatic hydrocarbons (APAHs) are derivatives of unsubstituted PAHs that contain one or more alkyl substituents. The position and extent of alkylation play a key role in the environmental fate of each APAH[11]. For example, increasing the extent of alkylation increases the compound's hydrophobicity and consequently increases its bioaccumulation potential. Similar to PAHs, APAHs are commonly classified as either alkylated low molecular weight (ALMW) or alkylated high molecular weight (AHMW) based on the number of aromatic rings they contain.

Although APAHs are typically more abundant in the environment than unsubstituted compounds, historically they have not received as much attention[11,12]. The main challenges associated with quantifying APAHs are the large number of isomers that exist for each compound as well as the lack of standards that are available for most isomers[13].

1.2.3. Hetero-Polycyclic Aromatic Compounds

Hetero-polycyclic aromatic compounds (HPACs) are an additional sub-class of PACs that contain one or more heteroatoms. Most commonly, a carbon atom in the polycyclic ring is replaced by oxygen, nitrogen, or sulfur, resulting in polycyclic aromatic oxygen heterocycles

(PAOH), polycyclic aromatic nitrogen heterocycles (PANH), and polycyclic aromatic sulfur heterocycles (PASH), respectively.

The addition of a heteroatom increases the polarity of these compounds relative to their homocyclic analogues and consequently leads to differences in their chemical properties and environmental behaviours[14]. Importantly, as a result of their greater polarity, HPACs are increasingly water soluble, making them more bioavailable compared to homocyclic PACs[15]. While PAHs and APAHs tend to adsorb predominantly to sediment or soil particles, HPACs are more readily available for uptake by living organisms.

Despite the occurrence of these compounds in the environment, they continue to be studied significantly less than homocyclic PACs. For example, quinoline, a PANH, was added to Schedule 1 under CEPA in 2011 due to its recognized risks to both environmental and human health [16]. However, reports of this compound in the environment and biota remain limited. This is largely due to analytical challenges associated with measuring HPACs, including the lack of commercially available standards, the presence of numerous structural isomers, and the lack of certified reference materials available[17]. Additionally, there are very few existing methods for the extraction and quantitation of HPACs. Due to their higher polarity, typical PAC extraction methods require modifications to incorporate more polar solvents to ensure efficient recovery of these compounds[17].

1.2.4. Halogenated Hetero-Polycyclic Aromatic Compounds

Halogenated hetero-polycyclic aromatic compounds (Halo-HPACs) contain one or more halogen substituents, most commonly bromine or chlorine, attached to a heterocyclic

backbone[17–19]. The addition of halogen atoms makes these compounds increasingly more polar than their non-halogenated analogues. However, Halo-HPACs still share many of the same analytical challenges as HPACs, resulting in limited detection and reporting in environmental samples. Consequently, compounds in this group remain largely unknown, highlighting a significant knowledge gap.

1.3 Sources of Polycyclic Aromatic Compounds

Both natural and anthropogenic sources release PACs into the environment. The most common natural sources include volcanic eruptions, forest fires, and natural oil and gas seeps [20], whereas residential wood combustion, transportation, and industrial emissions are among the greatest anthropogenic sources of these compounds[21].

Sources of PACs are also commonly classified as petrogenic or pyrogenic. Petrogenic PACs originate from unburned petroleum and its byproducts, including crude oil, gasoline and diesel fuel[22]. Petrogenic PACs are generally widespread due to the storage, transport, and leakage of these products[22]. In contrast, pyrogenic PACs are formed during the incomplete combustion of organic matter, such as fossil fuels, coal, and wood, at very high temperatures. Pyrogenic PACs are generally more abundant in urban areas due to the high occurrence of vehicle emissions and industrial processes, however many of these compounds are also capable of long-range atmospheric transport to more remote regions[23].

Information about the potential sources of PACs can be inferred based on the relative abundance of specific compounds present within a sample. Petrogenic sources typically produce

a high abundance of lower molecular weight PACs (2-3 rings) with many alkylated and heterocyclic compounds[24]. In contrast, pyrogenic PACs are predominantly unsubstituted compounds, as high temperatures tend to eliminate alkyl side chains[25]. High temperatures also promote the formation of larger compounds, resulting in a greater abundance of high molecular weight PACs (4, 5 or 6 rings) originating from pyrogenic sources[12,26].

1.4 Toxicity of Polycyclic Aromatic Compounds

The toxicity of numerous PACs has been studied in a wide range of organisms including invertebrates[27], fish[28], mammals[29], and humans[30]. Exposure occurs primarily through ingestion, inhalation, and dermal contact[30]. Due to their lipophilic nature, PACs can readily cross biological membranes and accumulate in fatty tissues, particularly in the liver, lungs, kidneys, and adipose tissue[31].

The liver plays a key role in the metabolism and detoxification of potentially harmful compounds like PACs. However, biotransformation can also occur in the liver, producing metabolites that are more toxic than the parent compound[30]. For example, benzo[a]pyrene, often identified as the most toxic PAH, is metabolized by cytochrome P450 to produce benzo[a]pyrene diol epoxide (BPDE), which is capable of forming DNA adducts that can lead to mutations and malignant transformations[32].

Exposure to PACs has been associated with a range of adverse health effects, including carcinogenic, mutagenic, teratogenic, and endocrine-disrupting effects[20]. The toxicity of each compound is largely dependent on factors such as molecular size, bioavailability, concentration,

and duration of exposure. Some of the most reported effects of PAC exposure include embryotoxicity, DNA damage, and hormone disruption[20]. However, despite concerns about these compounds, the full toxicology of most PACs remains poorly understood.

Among the various groups of PACs, the toxicity of the 16 priority PAHs is the most well-documented[22,29,33,34]. Although they remain relatively understudied, APAHs have been gaining increasing attention in recent years and are believed to be more toxic than their unsubstituted analogues[11,35,36]. Since APAHs typically account for more than 85% of PACs present in crude oil[1], these compounds are a significant concern especially in areas with greater risk of petrogenic PAC pollution. Both the position and extent of alkylation is believed to influence a compound's toxicity. However, due to the presence of numerous isomers, there remains much to be studied, especially for individual APAH compounds.

Similarly, the toxicity of most PACs containing heteroatoms also remains largely unknown. Although HPACs are generally detected at lower environmental concentrations compared to homocyclic PACs, many are believed to exhibit significantly greater toxicity[15,37]. Much of their toxicity is likely attributed to these compounds' greater bioavailability[15]. Research focused on Halo-HPACs is particularly limited, however, some halogenated PACs have demonstrated toxicities comparable to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), both of which are listed under Annex C of the Stockholm Convention[18,38].

1.5 The Canadian Arctic

The Canadian Arctic is a vast area of over four million square kilometers, representing more than 40% of Canada's land mass[39]. The Arctic was once considered a pristine environment due to its remote location and minimal industrialization and urbanization compared to the rest of the world. However, advances in environmental contaminant monitoring have revealed that the Arctic remains vulnerable to pollution despite its remoteness and general lack of human activity.

In 1991, the Northern Contaminants Program (NCP) was established in response to concerns about contaminant levels in the Arctic region[40]. Numerous contaminants have since been detected in the Arctic, including microplastics[41], heavy metals[42], and persistent organic pollutants (POPs)[42]. Importantly, monitoring also revealed that numerous contaminants are capable of long-range atmospheric transport, allowing them to travel from distant sources and deposit in remote Arctic environments[42]. Although local sources are limited, the presence of numerous contaminants in the Arctic highlights the vulnerability of this region to various forms of pollution.

In 2011, PACs were identified as emerging contaminants in the Arctic[43]. Currently, the primary source for PAC contamination in the Arctic is natural oil and gas seeps, specifically those located in the Baffin Bay-Davis Strait region[44,45]. The most well documented natural seep is in Scott Inlet, however additional seeps have also been identified in nearby areas such as Lancaster Sound[46–48]. Natural seeps are significant sources of petrogenic PACs, likely contributing to the high abundance of these compounds detected in Arctic. While natural seeps are currently believed to be the largest contributors to PAC levels in the Arctic, anthropogenic sources are also becoming a growing concern.

The Canadian Arctic is currently undergoing significant changes in its landscapes as climate change progresses. This region is particularly vulnerable, warming at a rate nearly four times faster than the rest of the world[49]. Warming temperatures have resulted in significant reductions in the extent and thickness of sea ice in this area. These environmental changes are expected to increase the risk of various forms of contamination as this region becomes more accessible.

Although current ship activity in the Arctic remains relatively low compared to major global shipping routes, increases have already been observed[50]. A variety of marine vessels operate in this region, including government vessels, icebreakers, cargo ships, and passenger vessels. Notably, pleasure craft (private yachts) are the fastest growing vessel type and are also the least regulated[51]. The rise in shipping activity consequently increases the risk of ship-related pollution into surrounding environments. As PACs are associated with oil and gas pollution, their release into the environment is a significant concern[21] and can occur through continuous ship emissions and through large, accidental oil spills.

In addition to shipping, industrial activity is also expected to increase in the Arctic region in the coming years, notably mining and offshore oil and gas exploration. Canada's Arctic is one of the world's richest reserves of high-grade iron ore[52] and also contains much of the world's undiscovered oil and gas reserves[21]. There is particular interest in the Canadian offshore waters in the Baffin Bay and Davis Strait region[53]. While there are currently restrictions in place preventing extensive industrial activity, the future remains uncertain. Should any of these restrictions be lifted in the future, the risk of PAC contamination poses a significant risk to Arctic ecosystems.

1.6 Seabirds for Environmental Monitoring

The Canadian Arctic is home to a large population of wildlife, including an estimated 10 million seabirds[54]. Seabirds are established bioindicators of marine ecosystems and have been used for environmental monitoring for many decades[55]. Because they typically rely on their local environment for acquiring nutrients, contaminant levels in seabirds are largely indicative of environmental contaminant levels.

Exposure to contaminants occurs primarily through contact with contaminated air, water, and food sources[1]. Since they feed at relatively high trophic levels in Arctic marine ecosystems, seabirds are susceptible to bioaccumulating high levels of many contaminants[55]. Liver tissue is commonly used for monitoring organic contaminants in adult seabirds, although serum and muscle tissue has also been used. Contaminants are frequently detected in the liver because it is the organ primarily responsible for detoxification. The liver also has a relatively high lipid content and is prone to accumulating high amounts of lipophilic contaminants.

Seabirds have proven to be valuable bioindicators of Arctic ecosystem health and have been used for monitoring contaminants in the Canadian Arctic including organochlorines[56], heavy metals[57], and plastics[58]. However, limited data exists for PACs specifically, with the first report of these compounds in seabirds published only recently in 2020[44]. Monitoring PACs in seabirds is important because they are associated with numerous adverse health effects. Some of the negative effects of PAC exposure on seabirds can include physiological stress, reduced breeding success, and increased mortality[44].

Arctic seabirds are predominantly migratory, spending spring and summer months at their chosen breeding colonies and winters primarily at sea. Although different species of seabirds

may share similar overwintering sites and breeding colonies, they may differ in their foraging ecologies, including range, depth, and preferred prey[44]. For example, thick-billed murres (*Uria lomvia*) are pursuit divers, meaning they may dive several meters for prey, whereas northern fulmars (*Fulmarus glacialis)* tend to feed at or just below the water's surface[59,60]. These differences in foraging strategies are important because it can influence each species' exposure to environmental contaminants like PACs. In the following sections (1.6.1-1.6.3), three species of Arctic seabirds that were the focus of this thesis will be discussed in more detail.

Two of the major Arctic seabird monitoring sites in Canada are Prince Leopold Island (PLI) and Coats Island (CI). The PLI Field Station (74°02'N, 90°00'W) was established with the support of the Northern Contaminants Program (NCP) administered by Environment and Climate Change Canada (ECCC)[61]. PLI supports is a multi-species seabird colony over 150,000 breeding pairs and research at this site has produced one of the strongest data sets in the Arctic region[61]. PLI is home to approximately 6% of Canada's thick-billed murre population, 11% of Canada's northern fulmar population, and 16% of Canada's black-legged kittiwakes. The CI Field Station (62°51'N, 82°29'W) was established by later by the Canadian Wildlife Service of ECCC in 1984[62]. The most recent estimates indicate there are greater than 30,000 breeding pairs of seabirds inhabit CI[62].

1.6.1 Thick-Billed Murre (*Uria lomvia*)

Thick-billed murres (*Uria lomvia,* hereafter murres) are one of the dominant marine birds found in the Canadian Arctic, both in terms of population and biomass[63]. During breeding season, they typically occupy very large colonies along steep, rocky sea cliffs. Murres are among

the deepest diving species and can dive to depths of up to 200m to capture prey, including small fish, shrimp, and crustaceans[64]. In the Arctic, their foraging range can vary during breeding season but murres have a typical foraging range between 50-100 km from their colony. Murres tend to lay a single egg in late May to early June, with an incubation period of approximately 30 days[65].

1.6.2 Black-Legged Kittiwake (*Rissa tridactyla)*

Black-legged kittiwakes (*Rissa tridactyla*, hereafter kittiwakes) are marine gulls that breed in coastal colonies between June and September. In contrast to murres which dive to significant depths to catch their prey, kittiwakes only conduct shallow dives much closer to the surface to capture small fish[66]. During breeding season, kittiwakes typically forage between 10-50 km from their colony. Kittiwakes can lay between one and three eggs, with two being the most common clutch size. They lay their eggs early to mid-June and have a typical incubation period of 25 days[67].

1.6.3 Northern Fulmar (*Fulmarus glacialis)*

Northern fulmars (*Fulmarus* glacialis, hereafter fulmars) are another abundant species of Arctic seabird. Like kittiwakes, fulmars are surface feeders and only perform shallow dives. Unlike the other two species discussed previously, fulmars also have the tendency to follow fishing and whaling vessels to scavenge for fish and other discards[68,69]. They also have a much greater foraging range compared with murres and kittiwakes, with typical ranges > 100 km from

their colonies, with evidence of foraging up to 1000 km. Fulmars lay a single egg per breeding season, in late April to early June, and have an incubation period of approximately 50 days[70].

1.7 Seabird Eggs and Maternal Transfer

Given that seabirds rely on their local environment to supply nutrient reserves for their eggs, contaminant burdens in eggs can reflect Arctic pollution levels, similar to adult seabird tissue. During egg formation, female seabirds transfer nutrients that are required for embryo development such as proteins, lipids, minerals, and hormones[71]. However, in addition to essential nutrients, contaminants can also be transferred to eggs through a process known as maternal transfer[55]. This process is believed to be influenced by both physiological factors as well as the physico-chemical properties of the contaminants. Given that PACs are lipophilic, they are transferred along with lipids to eggs during their formation. Although PACs have been detected in eggs in numerous studies[55,72,73], there is relatively limited information available regarding details of the process.

Species-specific biological and physiological traits can contribute to differences in contaminant accumulation in eggs. Specifically, variations in egg size and lipid content among species can influence the extent of contamination in eggs, particularly for lipophilic contaminants like PACs. Clutch size may also result in differences in contaminant burdens among species. Species that lay a single egg are likely to offload a greater proportion of contaminants to that egg, while those laying multiple eggs may distribute the contaminants across their clutch. Even within a single individual, contaminant levels can vary between eggs. For example, one study found that poly- and perfluoroalkyl substances (PFAS) were 73% lower

in second-laid eggs compared to first-laid eggs[71]. Additionally, interspecies differences in maternal transfer may also reflect variation in each species' ability to metabolize, detoxify, and excrete contaminants. Species that can metabolize contaminants more efficiently may consequently transfer lower concentrations to their eggs.

There are several advantages to using seabird eggs for monitoring compared to liver tissue. One benefit is that sample preparation is generally much easier for eggs. Collection and dissection of adult birds to retrieve liver samples is a much more labour-intensive and time-consuming process whereas egg collection is much easier and less invasive. Because of this, eggs are also easier to collect in larger numbers compared to other tissues, which is important when performing meaningful statistical analyses. Additionally, while livers typically reflect more recent exposure, eggs are reflective of both recent exposure as well as contaminants accumulated over longer periods of time and from multiple locations. This allows eggs to be used to investigate longer-term accumulation of contaminants that may not be possible with other tissues.

While eggs are important indicators for monitoring environmental health, they also play a key role in assessing the health of seabird populations. Embryos have significantly lower xenobiotic metabolizing capabilities compared to adult birds[74], making them increasingly vulnerable to contaminants such as PACs. While adult birds may be able to metabolize or detoxify a substantial amount of these compounds, embryos lack these same mechanisms. There is evidence that exposure of seabird embryos to PACs can lead to reduced hatching success, developmental abnormalities, and altered biochemical responses[1]. Monitoring PAC levels in eggs is necessary to assess these risks, which pose significant threats to seabird reproductive success.

Environmental monitoring data from seabirds and their eggs is also essential to help inform policies and guide decision-making to ensure the health of Arctic ecosystems. For example, in 2020 environmental monitoring data from fulmars was used to provide evidence to inform policies involving plastic pollution[75]. Long-term monitoring can help assess how the environment is responding to policies and regulations that have been implemented to assess their effectiveness. Additionally, environmental monitoring ensures that concentrations of contaminants in food sources are within the level safe for human consumption, as many Indigenous communities in the Arctic rely on seabirds and their eggs as essential parts of their diets[54]. Monitoring contaminant levels in seabirds is therefore important for assessing risks to human health as well as risks to wildlife and their environment.

1.8 Thesis Hypotheses

The hypotheses tested in this thesis were as follows:

Chapter 3: PAC concentrations would differ between seabirds breeding in the high Arctic versus the low Arctic, and PAC concentrations would increase over time as activity in the Arctic has increased.

Chapter 4: Heterocyclic PACs would be detected in addition to homocyclic PACs in seabird egg and liver tissues, and species-specific differences in foraging ecology and the timing of egg-laying would influence variation in PAC concentrations and profiles among the three seabird species.

1.9 Thesis Structure

My thesis is divided into 5 chapters. *Chapter 1* introduces PACs, their sources and toxicity, the evolving environment in the Canadian Arctic, how seabirds and their eggs can be used for environmental monitoring, and my thesis hypotheses. *Chapter 2* describes the methodologies used for sample processing and analysis of the target analytes to test my hypotheses. *Chapter 3* presents a spatial and temporal analysis of PACs in Canadian Arctic seabirds. *Chapter 4* provides an interspecies and tissue-specific comparison of PACs in the livers and eggs of three species of seabirds from Prince Leopold Island. *Chapter 5* provides an overall conclusion and recommendations for future research in relation to this work.

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Chapter 2: Methodology

2.1 Sample Collection and Preparation

The first step in any analytical process is sample collection. All samples used in this thesis were provided by the Environment and Climate Change Canada (ECCC) National Wildlife Specimen Bank in Ottawa, Ontario. Sample collections were conducted in accordance with relevant guidelines and under the necessary permits. Adult birds were dissected, and livers were collected following standard protocols. Egg and liver samples were then homogenized, aliquoted, and stored at -40°C at the National Wildlife Research Centre (NWRC) in Ottawa, Ontario, Canada prior to preparation and analysis.

The next step, and arguably the most important step in any analysis, is sample preparation. Sample preparation refers to the process of isolating the analyte of interest from the sample matrix to ensure it is suitable for analysis[1]. This step is crucial because a sample matrix typically contains numerous components that may interfere with the analyte of interest. The removal of interferents is therefore necessary to ensure accurate detection in subsequent analyses. Additionally, sample preparation can convert the analyte into a more suitable form for analysis, as the original sample may not be compatible with all analytical instruments[2]. For example, if the original sample is in the solid state, the analyte often must be transferred to an appropriate solvent for analysis. Sample preparation can also increase the concentration of the analyte to improve the detectability.

The most common sample preparation technique is extraction. This technique involves transferring the target analyte from the sample matrix to an appropriate extracting solvent,

depending on the chemical properties of the analyte. For the extraction of PACs from biota samples, techniques such as traditional liquid-liquid extraction (LLE), Soxhlet extraction and QuEChERS (quick, easy, cheap, effective, rugged and safe) methods have been used. However, these techniques each have their own limitations. Conventional LLE tends to be time-consuming and labour intensive, Soxhlet extraction requires large amounts of solvent and in the QuEChERS method, it is challenging to select the appropriate solvent for the efficient extraction of a diverse range PACs[3]. Two extraction techniques were used for the work in this thesis: microbead beating extraction and accelerated solvent extraction (ASE) and are discussed in the following sections.

2.2 Microbead Beating Extraction

Microbead beating extraction is an effective technique used for both biological and environmental samples. This technique involves the agitation of a tube containing a sample and ceramic or stainless-steel microbeads in 3-dimensions to disrupt or lyse the sample to extract the target analyte. Historically, this technique has been used for biomedical purposes to extract DNA or other cell contents from biological samples. However, microbead beating has also been effective for the extraction of PACs from various matrices. A validated method for the extraction of PAHs and APAHs from avian eggs was previously developed in our laboratory[3] and was used for all extractions in Chapter 3 of this thesis. The general workflow for microbead beating extraction that was used is shown below in Figure 2.1.

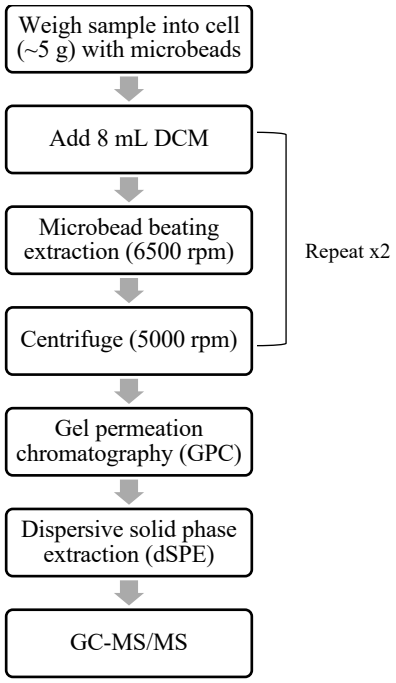

Figure 2.1 Workflow for microbead beating extraction of PACs from avian eggs

In this thesis, microbead beating extraction was used to extract PAHs and APAHs from seabird eggs. Approximately 5 g of seabird egg sample was weighed into a stainless-steel tube containing 0.65 g of ceramic microbeads. 8 mL of dichloromethane (DCM) was added to the tube and extracted using a Precellys Evolution Homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) at 6500 rpm with 3 cycles of 20 s with 120 s between cycles (total time of 5 min). The tubes were then centrifuged at 5000 rpm for 10 min at 10 °C. The extract (bottom layer) was then added to a glass tube and the tubes were rinsed twice with an additional 8 mL of DCM, vortexed, and centrifuged again for 10 min at 10 °C. The rinses were both combined with the original extract. Following extraction, the extracts were dried with sodium sulfate and filtered through a glass funnel containing glass wool using 3 x 5mL hexane rinses then reduced in volume to approximately 2 mL using ultra-high purity (UHP) nitrogen. Extracts were then

transferred to glass GPC tubes and brought up to 10 mL with hexane. 0.5 mL of each extract was transferred to a pre-weighed aluminum tray for lipid determination. Finally, extracts were reduced in volume to 2.5 mL using UHP nitrogen and made up to 5 mL with DCM.

2.3 Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) was first reported by Richter et al.[4] and involves the use of elevated temperatures and pressures with the appropriate organic solvent to extract target analytes from solid or semi-solid samples. During extraction, the cell is filled with solvent under high pressure (500 – 3000 psi) and is heated (50 – 200 °C). There are many advantages of ASE, most notably the significant reduction in extraction time and volume of solvent required[4]. The general workflow for ASE can be found below in Figure 2.2.

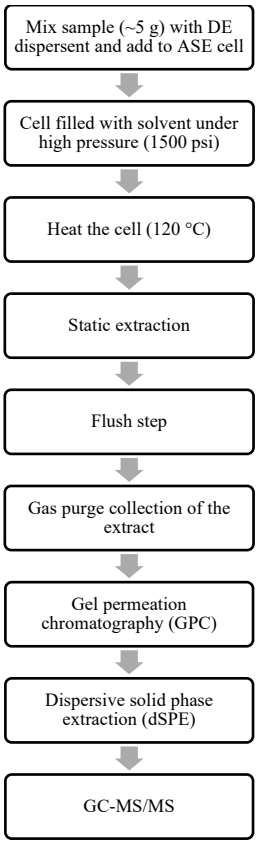

Figure 2.2. Workflow for accelerated solvent extraction of PACs in biota samples.

In Chapter 4 of this thesis, ASE was used to simultaneously extract homocyclic and heterocyclic PACs from seabird eggs and livers. A validated method for the extraction of PAHs and APAHs from biota by ASE was previously developed in our laboratory[5] and was used with slight modifications. 34 mL stainless-steel ASE cells were prepared with two glass fibre filters at the bottom. Approximately 5 g of seabird egg or liver sample was mixed with 3.5 g of diatomaceous earth (DE) dispersant before being added to the cell. The remaining volume of the cell was filled with Ottawa sand. Samples were extracted using a Dionex 350 Accelerated Solvent Extractor (ThermoFisher, Mississauga, ON). The solvent used for extraction was a 50:50 mixture of dichloromethane (DCM):ethyl acetate. The ASE parameters used were as follows:

1500 psi system pressure, 120°C oven temperature, 15 min static time with 2 static cycles, 60% flush volume and a nitrogen purge for 80 s at 150 psi.

Following extraction, extracts were dried with sodium sulfate and passed through a glass funnel containing glass wool into new 60 mL glass tubes and reduced in volume to 10 mL using a gentle stream of UHP nitrogen. 0.5 mL of each extract was then transferred to a pre-weighed aluminum tray for lipid determination. Finally, the extract volume was reduced to 2.5 mL using UHP nitrogen and brought up to 5 mL with DCM.

2.4 Gel Permeation Chromatography

Gel permeation chromatography (GPC), also known as size-exclusion chromatography (SEC), separates compounds based on their molecular size. GPC contains a stationary phase, composed of porous beads, and a mobile phase. The sample containing the target analyte is injected onto the head of the column where larger molecules are excluded from the small pores of the stationary phase and are eluted first. Smaller molecules are retained longer in the stationary phase and elute later.

A common use of GPC in sample preparation is to remove any interfering compounds that were simultaneously extracted along with the target analyte. For the analysis of PACs, GPC is used primarily to remove lipids and pigments from the target analyte(s).

The same protocol was used for extracts obtained through both the ASE and microbead extraction methods. Extracts of 5 mL were injected onto the head of a J_2-Scientific GPC column (AccuPrepMPS™, Columbia, Missouri, USA) containing 60 g of S-X3 Biobeads (29.5 mm x i.d.

400 mm) as the stationary phase. The following parameters were used: 5 mL injection volume, 8 psi pressure, 5 mL/min flow rate, and 50:50 DCM:hexane mobile phase. The first 140 mL was discarded, and the analytes of interest were eluted in the fraction from 140 to 360 mL. The fractions were then reduced in volume to approximately 2 mL using rotary evaporation.

2.5 Dispersive Solid Phase Extraction

Dispersive solid phase extraction (dSPE) is a sample preparation technique commonly used as a cleanup step to remove unwanted interferents from a sample extract. Anastassiades et al. first introduced dSPE[6] for the removal of polar matrix components such as organic acids, pigments, and sugars from food extracts for pesticide analysis. It is an effective alternative for more conventional approaches like solid-phase extraction (SPE), which involves passing the sample through a column containing the stationary phase. In contrast to SPE, the sorbent stationary phase is applied directly to the liquid sample extract with the goal of promoting the partitioning of interferents onto the stationary phase while the target analytes remain in the liquid phase. This technique therefore requires an appropriate solvent choice and must carefully consider the polarity of the target analytes.

There are many advantages of using dSPE over traditional SPE. It is a highly effective technique because of the extensive contact between the sorbent and solvent whereas in SPE, the solvent is passed over the stationary phase. By dispersing the solid stationary phase directly in the sample extract, dSPE simplifies the cleanup process and allows more samples to be processed simultaneously, faster processing times and requires less solvent compared to SPE[7].

For the projects presented in this thesis, dSPE was used as an additional, final cleanup step following GPC prior to injection for gas chromatography-tandem mass spectrometry analysis. This step is critical to ensure a clean sample is injected for high quality analysis with minimal interference. To complete dSPE, aliquots of 4 g of silica gel, 1 g of deactivated alumina, and 0.5 g of sodium sulfate were pre-rinsed with 3 x 10 mL of a 70:30 mixture of DCM:hexane and added to each extract. After 30 minutes, extracts were filtered through glass funnels containing glass wool into new glass tubes and reduced to a final volume of 200 µL with a gentle stream of UHP nitrogen. Samples were spiked with 20 µL of 1 ng/µL of IPIS and were stored in amber GC vials at 4 °C until analysis.

2.6 Extraction of Hetero-Polycyclic Aromatic Compounds

Traditional extraction techniques for PACs are typically targeted toward homocyclic PACs, namely PAHs and APAHs, and therefore employ the use of predominantly nonpolar solvents. However, the analysis in Chapter 4 of this thesis was expanded to include HPACs and Halo-HPACs, both of which are increasingly polar. As a result, slight modifications had to be made to the sample processing methods to ensure efficient extraction of both homocyclic and heterocyclic PACs.

For extraction by ASE, 100% DCM is typically used as the solvent to extract PAHs and APAHs. DCM was previously tested for the extraction of HPACs by Vitharana et al.[8] but showed extremely low recoveries. By adding 50:50 DCM:ethyl acetate, both homocyclic and heterocyclic PACs recoveries were improved dramatically.

Because silica is highly polar, the protocol for dSPE also had to be modified for the extraction of HPACs and Halo-HPACs to ensure these compounds were not retained on the stationary phase. First, the dispersant was pre-rinsed with 2x10 mL of ethyl acetate prior to adding it to the sample extract to remove any impurities. An additional rinse with 10 mL ethyl acetate was also done when transferring the extract through glass wool to ensure the target analytes were removed from the dispersant.

2.7 Gas Chromatography

Gas chromatography (GC) has become one of the most important and widely used analytical techniques since it was first reported by James and Martin in 1952[9]. GC allows for the separation of volatile analytes within a sample mixture. Like other chromatographic techniques, GC involves a stationary phase, typically a thin layer of liquid on inert solid support, and a mobile phase, an inert carrier gas such as helium, hydrogen, or nitrogen[10]. Compounds are separated primarily based on their boiling points, with more volatile compounds eluting first and less volatile eluting later. However, separation can also be influenced by other physio-chemical properties of the analytes such as polarity and vapour pressure, column temperature, carrier gas flow rate, column length, or polarity of the stationary phase[11].

In GC, samples are injected through an injection port where they are volatilized at high temperatures. The constant flow of carrier gas carries the volatilized sample through the column where analytes partition between the stationary phase and the mobile phase. Analytes exit the GC and enter the detector through a heated transfer line. Common GC detectors include flame

ionization detectors (FID), electron capture detectors (ECD), nitrogen phosphorous detectors (NPD) and mass spectrometers (MS)[10].

There are many advantages of GC including its high selectivity and resolution, good accuracy and precision, and high sensitivity[12]. GC coupled with MS has become the most widely used approach for the quantitation of PACs in environmental samples[12–14].

For all work in this thesis, an Agilent 7890 GC coupled with a triple quadrupole Agilent 7000C mass spectrometer fitted with an electron ionization (EI) source was used for all analyses. An Agilent J&W HP-5ms ultra inert column (30 m x 0.25 mm x 0.25 µm) was used with helium as the carrier gas at a constant flow rate of 1.2 mL/min. Splitless injections of 1 µL were made using a PAL RSI 85 auto sampler into the injection port at 250 °C. The GC oven temperature is held at 60 °C for 1 min then raised to 210°C at 35 °C/min, further ramped up to 260 °C at 2 °C/min, 300 °C at 10 °C/min and held for 5 min, and finally to 325 °C at 50 °C/min and held for 5.5 min. Both the transfer line and MS source temperature are set at 320 °C and UHP nitrogen was used as the collision gas at 60 psi.

2.8 Mass Spectrometry

Mass spectrometry (MS) is a technique used to identify and quantify molecules based on their mass-to-charge (m/z) ratio. J. J. Thompson was the first to measure the charge-to-mass ratio of electrons in 1897 and built what is recognized as the first mass spectrometer with Francis Aston[15].

A mass spectrometer is composed of an ion source, a mass analyzer and a detector. Chromatographic techniques such as GC or high-performance liquid chromatography (HPLC) are often coupled with MS. Both GC-MS and HPLC-MS are common techniques for the quantitative analysis of environmental contaminants. The chromatographic technique choice largely depends on the identity of the target analytes, with GC being the preferred choice for volatile analytes and HPLC for non-volatile compounds. Because all PACs that were investigated in this thesis were volatile or semi-volatile, gas chromatography-tandem mass spectrometry (GC-MS/MS) was used for all quantitative analyses.

2.8.1 Ionization Techniques

The first step in MS is the conversion of neutral analytes into ions by the ion source. There are numerous ionization techniques for MS, including electron ionization (EI), chemical ionization (CI), and electrospray ionization (ESI)[10]. Ionization techniques are commonly classified as either hard or soft based on their energy and the resulting fragmentation. Hard techniques like EI use more energy and result in extensive fragmentation, often destroying the molecular ion. Alternatively, softer techniques like CI use less energy, leading to minimal fragmentation with the molecular ion often being preserved[10].

Electron ionization (EI) is a common ionization technique for GC-MS and was used for all work in this thesis. In EI, high-energy electrons are emitted from a hot filament and accelerated within the ion source. As neutral analyte molecules pass through the ion source, they interact with the high-energy electrons to generate analyte ions, shown below in Equation 2.1.

$$M + e^- \rightarrow M^{+\cdot} + 2e^- \qquad \textbf{Equation 2.1}$$

Following ionization, the ions are accelerated by an electric field and separated according to the m/z ratio in the mass analyzer[16]. In this thesis, a triple quadrupole mass spectrometer (QqQMS) was used and is described in the next section. Additional mass analyzers include single quadrupoles, ion traps, Fourier transform ion cyclotron resonance (FTICR) and time-of-flight (TOF) mass analyzers[16].

2.8.2 Triple Quadrupole Mass Spectrometry

Triple quadrupole mass spectrometry (QqQMS), also referred to as tandem mass spectrometry (MS/MS), was introduced in 1978 by Christie Enke and Richard Yost in their publication titled "Selected Ion Fragmentation with a Tandem Quadrupole Mass Spectrometer"[17]. Although the name suggests three quadrupoles, QqQMS uses only two true quadrupoles (Q1, Q3) which act as mass filters, while q2 acts as a collision cell[16]. A schematic for a QqQMS can be found in Figure 2.3. The first quadrupole (Q1) selects a precursor ion from the ion source based on a specific m/z ratio, while all other ions are filtered out. The selected precursor ion then enters the collision cell (q2) where it collides with inert gas molecules like nitrogen or argon and subsequently undergoes fragmentation to produce product ions. Q3 then monitors for specific fragments produced within the collision cell, which go on to the detector.

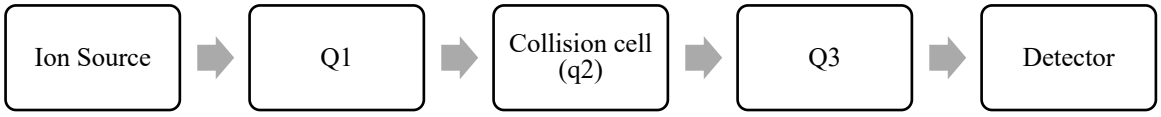

Figure 2.3. Triple quadrupole mass spectrometer schematic.

A triple quadrupole mass spectrometer can be operated in full scan mode, selected ion monitoring (SIM) mode or multiple reaction monitoring (MRM) mode[18]. When running a full scan, all ions are scanned across a wide *m/z* range to generate a complete spectrum. Full scans are much less selective and are typically used for qualitative analysis only[19]. In SIM mode, only the precursor ion is selected, while in MRM mode, both precursor and product ions are detected. Multiple compounds can have the same precursor ion whereas product ions can be more selective for a specific analyte. Therefore, MRM has greater selectivity and sensitivity compared to SIM, and is the most common for quantitative analysis[17,19].

2.9 Method Validation

Method validation is a critical step in the analytical process to ensure the selected method for sample preparation is suitable for its intended purpose. Several guidelines have been proposed for laboratories to follow when conducting method validation. *The Eurachem Guide: The Fitness for Purpose of Analytical Methods*[20] , first published in 1995, is a widely adopted set of guidelines used to help guide quality chemical analysis in laboratories.

Common method performance characteristics that are typically tested during method validation include accuracy, precision, ruggedness, limit of detection (LOD) and limit of quantitation (LOQ). These performance characteristics provide insight into the suitability of a method for the desired purpose and are discussed below in further detail. In this thesis, method validation was previously completed for the method used in Chapter 3. However, a full method validation for the simultaneous extraction of 49 homocyclic and 47 heterocyclic PACs was conducted for Chapter 4, carried out in strict accordance with the Eurachem Guidelines for Analytical Methods.

2.9.1 Accuracy

Accuracy, also commonly referred to as "trueness", expresses the closeness of a result to a reference value[20]. Accuracy is important in method validation, as it indicates how well the results align with the true value, ensuring the method is suitable for its intended purpose. In method validation, accuracy is commonly calculated as the percent of analyte recovered. The Eurachem Guide at multiple spiking levels. In the method validation in this thesis, the accuracy of our method was calculated using six replicates (n=6) at three concentration levels (10, 100, 500 pg/µL).

2.9.2 Precision

Precision includes repeatability, intermediate precision, and reproducibility. Repeatability ("within-run precision") is expected to show the smallest variation as it is a measure of variation

in the method on the same day. Intermediate precision, also called “interday precision”, measures the variability in the method performed on different days. As the name suggests, intermediate precision should show variation that is intermediate to repeatability and reproducibility. Reproducibility refers to variation between laboratories using the same method and is therefore expected to give the greatest variability[20].

In the method validation in Chapter 4, repeatability was determined using six replicates (n=6) on the same day for all three concentration levels (10, 100, 500 pg/µL) and calculating the relative standard deviation (RSD). Intermediate precision was determined over three consecutive days each with 4 replicates (n=4) using a single concentration level (100 pg/µL). A one-way ANOVA test (α =0.05) was performed to analyze the precision for each compound among the three days.

2.9.3 Ruggedness

Ruggedness of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate changes in method parameters[20]. To test ruggedness, deliberate changes are made to the method and the effect on performance is investigated. In the method validation conducted in this thesis, ruggedness was tested by making small changes to the extraction method. ASE temperature was increased from 120 °C to 140 °C in four replicates (n=4) and the mass of silica gel in the dispersant was increased from 4 g to 5 g in four replicates (n=4). A single concentration level (100 pg/µL) was used to determine ruggedness. Student t. tests (two-tailed, t=0.05) were performed to determine if either of the changes result in statistically significant differences.

2.9.4 Limit of Detection and Limit of Quantitation

Limit of Detection (LOD) and limit of quantitation (LOQ) are closely related but distinct performance characteristics. LOD refers to the lowest concentration of the analyte that can be detected by the method. LOQ is the lowest concentration of the analyte that can be quantitatively measured by the method. In this thesis, the method limits of detection (LOD) and limits of quantification (LOQ) were calculated using six replicates (n=6) of chicken egg samples spiked at the lowest concentration level (10 pg/µL). Blanks (n_b=6) were prepared using chicken eggs spiked only with RIS. An adjusted standard deviation, s_0', was calculated using the ratio given in the Eurachem guidelines: $s_0' = s_0\sqrt{\frac{1}{n} + \frac{1}{n_b}}$, where s_0=the standard deviation, n=number of samples, and n_b=number of blanks [20]. Analyte specific LODs and LOQs were calculated as 3 x s_0' and 10 x s_0', respectively

2.10 Statistical Analysis

For statistical analysis, PACs were first broken down into various groupings. Homocyclic PAC groupings included total PACs (Σ_{43}PAC), total priority PAHs (Σ_{16}PAH), the sum of parent lower molecular weight compounds (i.e. 2-3 ring compounds; Σ_6PLMW), the sum of alkylated lower molecular weight compounds (Σ_{17}ALMW), the sum of parent higher molecular weight compounds (i.e. 4, 5 or 6 ring compounds; Σ_{10}PHMW), and the sum of alkylated higher molecular weight compounds (Σ_{10}AHMW). Heterocyclic PACs groupings included total HPACs (Σ_{43}HPAC), the sum of polyaromatic nitrogen heterocycles (Σ_{23}PANHs), the sum of polyaromatic sulfur heterocycles (Σ_{16}PASHs) and the sum of polyaromatic oxygen heterocycles (Σ_4PAOHs).

Given that PACs are lipophilic, all concentrations were lipid-normalized (lipid weight; lw) for consistency across samples. All analytes were also blank corrected using the corresponding mean concentration present in the procedural blanks that accompanied each sample batch. Finally, all concentrations that were below our method detection limits were recorded as non-detects and assigned a value of 0 for statistical analyses. All statistical analyses were conducted using RStudio Software version 4.2.2 (R Development Core Team 2022).

The majority of common statistical analyses are based on parametric tests, which rely on specific assumptions about the data, particularly that the data are normally distributed and exhibit homogeneity of variance. Therefore, before performing statistical analyses, it is essential to test both the distribution and variance of the data. Many commonly used parametric tests, such as ANOVA and t-tests, rely on the assumption that the data are normally distributed. However, environmental data often deviate from a normal distribution. The Shapiro-Wilkes test is a popular method to test the normality of data[21] and was used for all statistical analyses in Chapters 3 and 4. Similarly, many statistical tests assume equal variance across groups. Violating this assumption can lead to unreliable results if parametric tests are used inappropriately[22]. The Levene test is commonly used to test if the data has equal variance[23] and was used in both Chapter 3 and Chapter 4.

2.10.1 Statistical Analysis in Chapter 3

For the environmental samples in Chapter 3, none of the PAC groupings showed a normal distribution or equal variances, so non-parametric tests were used to compare groups. An alpha value of 0.05 was used to evaluate statistical significance of models.

To assess differences in PAC levels between the two locations, Mann Whitney U tests were run for various PAC groupings. The Mann Whitney U test is a common alternative for a *t* test to compare differences between two independent groups when data is not normally distributed and the assumption of homogeneity of variance is violated[24]. This test was used to assess whether there is a statistically significant differences in PAC concentrations between PLI (high Arctic) and Appatuurjuaq (low Arctic).

To identify interannual variation in PAC concentrations, multiple statistical tests were conducted and PLI and Appatuurjuaq were analyzed individually. The Kruskal-Wallis rank sum test was first used to identify whether there were any differences in PAC concentrations among the years that were analyzed. This test is a common alternative for a one-way ANOVA test to compare three of more groups when the data is not normally distributed[25]. The p-values were adjusted using the Bonferroni method. The Bonferroni method is a conservative correction method to reduce the likelihood of false positives[26]. This p-value adjustment method ensures that the differences in PACs are statistically significant with a low likelihood of false positives. Finally, the Dunn's Post-Hoc test was done to get year comparisons. While the Kruskal-Wallis rank sum test was used to test if there are any differences exist among groups (yes or no), this test identifies which specific groups (years) are significantly different from each other[27]. This test identified which individual years had PAC concentrations that were significantly different from each other.

2.10.2 Statistical Analysis in Chapter 4

The environmental data in Chapter 4 was log transformed to meet assumptions of normality as assessed using the Shapiro-Wilk tests, and homogeneity of variance using Levene's Tests, allowing for the use of parametric analyses. Two-way ANOVA tests were used for pairwise comparisons of species within each tissue type to identify if differences in PAC concentrations existed among species. To further explore species-level differences within each tissue type for each of the PAC groupings, pairwise comparisons among species were conducted using the R package emmeans v.1.10.5[28]. Once again, p-values were adjusted using the Bonferroni method for multiple comparisons. An alpha value of 0.05 was used to evaluate statistical significance of models.

Linear Discriminant Analysis (LDA) was also used in Chapter 4 to compare PACs among species and between tissue types in the same diagram. LDA is a multivariate classification method that identifies linear combinations of predictor variables (i.e., PAC concentrations) that best separate predefined groups of interest, in our case tissue type and seabird species. LDAs maximize the ratio of between-group to within-group variance, projecting the data onto a reduced number of axes (linear discriminants) that best capture group separation. Two separate LDA for homocyclic as well as heterocyclic PACs were used in this study to determine which PACs best separate liver and egg tissue for each species. For each LDA, species was used as the predictor variable and was separated by tissue type (liver and egg). Only compounds that were detected in more than 70% of the samples were considered in each LDA to ensure that the data was not zero-inflated, as done in previous studies looking at PACs in seabirds[29]. All the

concentrations were log transformed before use in the LDA to limit skewedness of the data.

LDAs were conducted using the R packages tidyverse v. 2.0.0, and MASS v. 7.3-58.1[30,31].

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Chapter 3: Polycyclic Aromatic Compounds in Canadian Arctic Seabirds: Spatial and Temporal Trends

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3.1 Abstract

The Canadian Arctic is undergoing significant environmental changes. As climate change continues to drive the reduction in the extent and thickness of sea ice, this region is experiencing an increase in shipping and other industrial activity, notably mining. In addition to existing natural oil and gas seeps, greater industry has led to growing concerns about the release of oil and gas contamination into vulnerable Arctic ecosystems. Polycyclic aromatic compounds (PACs) are a broad class of organic contaminants that are associated with oil and gas, many of which are persistent, bioaccumulative and toxic. This study examines spatial and temporal PAC trends in murre eggs between 1993 and 2024 from Prince Leopold Island, Nunavut (high Arctic; 74°02'N, 90°00'W) and Appatuurjuaq (Coats Island), Nunavut (low Arctic; 62°51'N, 82°29'W). Forty-eight polycyclic aromatic hydrocarbons (PAHs) and alkylated polycyclic aromatic hydrocarbons (APAHs) were quantitated using gas chromatography-tandem mass spectrometry. Overall, concentrations of total PACs, alkylated high molecular weight (AHMW) PACs and high molecular weight (HMW) PACs were greater in eggs from birds breeding in the high versus low Arctic, suggesting potential spatial differences in PAC exposure. While concentrations of PACs did not change dramatically from 1993 to 2024 at either location, considerable variation among individual years was observed. As interest in Canada's northern shipping routes continues to grow, monitoring of PACs in seabird eggs will survey as a practical sentinel system to assess potential changes in contamination that may pose risks to Arctic wildlife and their environments.

3.2 Introduction

The Canadian Arctic is experiencing dramatic environmental shifts, with rising temperatures and reductions in sea ice extent and thickness, predominantly driven by climate change[1]. This region is particularly sensitive to warming, with the average temperature rising nearly four times faster than the rest of the world[2]. Due to its remote location and harsh conditions, Canada's Arctic has experienced less human activity that deleteriously influences habitats (e.g., industrialization, urbanization) than much of the rest of the world. However, as the reduction in the extent of sea ice continues to improve the accessibility of this region, the Arctic is becoming increasingly vulnerable to various forms of contamination.

While the increase in industrial development and traffic in the region are a concern for Arctic communities, oil and gas contamination in particular is a growing concern that has been identified as a research priority in several existing reports led by Indigenous residents of Arctic Canada (Inuit)[3,4]. While natural oil and gas seeps in this region contribute to contamination, growth in anthropogenic activity is expected to introduce additional sources of oil and gas pollutants. The extent and potential impacts of these additional sources on the environment are still largely unknown.

The Canadian Arctic has relatively low levels of ship traffic compared to other regions globally, however, vessel traffic in this region has been steadily increasing in recent years[5]. A wide range of marine vessels operate in this area including fishing vessels, general cargo ships, government vessels and icebreakers. Ships associated with tourism, including passenger ships and pleasure craft, are currently the fastest growing vessel types in this Arctic[5]. As accessibility and interest in this region continues to grow, marine ecosystems near major shipping routes are

expected to encounter increasing levels of oil and gas related contaminants in the coming decades[6].

In addition to shipping, ongoing mining and other industrial activities in the Arctic further contribute to the risk of oil and gas contamination. There are increasing concerns about the potential impacts of these activities on the region's fragile marine ecosystems.[7] Although there are currently heavy restrictions in place to protect Canada's northern environments, the future of offshore oil and gas exploration remains uncertain[8]. Should these restrictions be lifted in the future, offshore oil and gas activity could become a significant concern as an additional source of pollution.

Polycyclic aromatic compounds (PACs) are a broad class of organic contaminants that are associated with oil and gas[9]. PACs can be further classified into subgroups depending on their structure, with the most widely studied being polycyclic aromatic hydrocarbons (PAHs). The U.S. Environmental Protection Agency (EPA) designated 16 PAHs as "High Priority Pollutants" because of their toxicity to humans and other organisms, and their prevalence and persistence in the environment[10]. The 16 priority PAHs have also been identified under the Toxic Substance List by the Canadian Environmental Protection Act (CEPA) since 1994[11]. Alkylated polycyclic aromatic hydrocarbons (APAHs) have received less attention relative to unsubstituted PAHs, however, there are numerous reports of many APAHs being more persistent, bioaccumulative and toxic than their parent compounds[12–14].

Seabirds are useful for monitoring contaminants like PACs because they are established bioindicators of marine ecosystems[15]. It is estimated that 10 million seabirds inhabit Canada's Arctic marine environments[16]. They feed at relatively high trophic levels in the Arctic marine

food webs and are therefore susceptible to bioaccumulating high levels of many compounds[17]. Female seabirds are also known to transfer contaminants such as hydrocarbons to their eggs, making them an effective way to monitor PACs[17]. Eggs are relatively easy to sample in large quantities and have been used for monitoring environmental contaminants in the Canadian Arctic since the 1970s[17]. Furthermore, seabirds and their eggs are important food sources for many Inuit communities[18]. Therefore, monitoring contaminant levels in seabird eggs is important for assessing risks to human health in addition to wildlife.

The need for longitudinal studies on PACs in the Arctic region has been emphasized in a recent study by Provencher et al[15]. Temporal studies have been conducted for many contaminants in Arctic seabirds including organochlorines[19], heavy metals[20] and plastics[21], however, there are currently limited data available regarding PACs specifically. Long-term monitoring is essential for understanding how environmental contaminants impact ecosystems over time to help assess risks to marine species. Long-term monitoring also provides reliable data to assess the effectiveness of existing regulations and helps guide decision-making for the implementation of new policies to help maintain and promote healthy marine ecosystems[17].

This paper presents a 31-year temporal and spatial analysis of 48 PACs using archived thick-billed murre (*Uria lomvia*) eggs from Prince Leopold Island, Nunavut (74°02'N, 90°00'W; PLI) and Appatuurjuaq (Coats Island), Nunavut (62°51'N, 82°29'W; CI). We predicted that PAC concentrations would differ among these locations, separated by 1300 km and different water and land masses, and we expected that PAC concentrations would increase over time as activity in the Arctic has increased. Our study aimed to enhance the understanding of current PAC levels

and how concentrations have changed over time in the Canadian Arctic to assess the evolving risks to seabirds and marine ecosystems.

3.3 Materials and Methods

3.3.1 Chemicals

All high purity (Optima grade) organic solvents, silica gel (923 grade, 100-200 mesh), alumina (60-325 mesh), and anhydrous sodium sulfate were purchased from Fisher Chemicals (Ottawa, Ontario, Canada).

Labelled PAH standards were purchased from Cambridge Isotope Laboratories, Inc (Andover, Massachusetts, USA). Napthalane-d_8, benz[a]anthracene-d_{12}, phenanthrene-d_{10}, fluoranthene-d_{10}, benzo[b]fluoranthene-d_{12}, benzo[g,h,i]perylene-d_{12}, indeno[1,2,3-cd]pyrene-d_{12}, dibenz[a,h]anthracene-d_{14}, accaphthylene-d_8, acenaphthene-d_{10}, fluorene-d_{10}, pyrene-d_{10}, benzo[k]fluoranthene-d_{12}, and chrysene-d_{12} were used as the recovery internal standard (RIS). Labelled anthracene (anthracene-d_{10}) was purchased from AccuStandard (New Haven, Connecticut, USA) and was used as an instrument performance internal standard (IPIS). Native PAH mix containing 16 PAHs was purchased from AccuStandard (New Haven, Connecticut, USA) and APAH mixes containing 37 compounds were purchased from Chiron (Trondheim, Norway).

3.3.2 Reference Egg Samples

As a standard reference material for avian eggs does not currently exist, in-house egg reference samples were created. Chicken eggs were purchased from a local retail store (Winnipeg, Manitoba, Canada). Eggs were cracked and homogenized using a Polytron extraction unit (Kinematica, Switzerland) and aliquoted into 5 g samples. With each sample batch (n=32), one aliquot of reference material was spiked with 20 µL of 1 ng/µL of native PAHs and APAHs and a second aliquot was used as a matrix blank. A total of nine spiked reference materials and nine matrix blanks were used in our analysis across nine sample batches.

3.3.3 Environmental Samples

Archived thick-billed murre (*Uria lomvia*) eggs stored at -40 °C were provided by the Environment and Climate Change Canada National Wildlife Specimen Bank in Ottawa, Ontario, Canada (Mallory and Braune, 2012). Ninety eggs from 18 sampling years were collected from Prince Leopold Island and 100 eggs from 20 sampling years were collected from Appatuurjuaq between 1993 and 2024 based on archived material availability. Eggs were homogenized and stored at -80 °C prior to extraction.

3.3.4 Sample Extraction and Processing

The analysis of all PACs was conducted in an ISO 17025/2018 accredited laboratory. PAHs and APAHs were extracted following a previously developed method by Xia et al. for the extraction of PACs specifically in avian eggs[22]. Each sample batch consisted of 29 environmental

samples, 1 procedural blank, 1 matrix blank, and 1 spiked reference sample. Approximately 5 g of seabird egg homogenate was weighed into a stainless-steel tube contained 0.65 g of ceramic microbeads. Samples were spiked with 20 µL of 1 ng/µL RIS and 8 mL of dichloromethane (DCM) was added to the tube. Samples were extracted using a Precellys Evolution Homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) at 6500 rpm with 3 cycles for 20 s with 120 s between cycles. Samples were then centrifuged at 5000 rpm at 10 °C for 10 min and the extract (bottom layer) was transferred to a 60 mL glass tube. Stainless steel tubes were rinsed with 2 x 8 mL of DCM, vortexed, and centrifuged again. Both rinses were combined with the original extract.

Following extraction, samples were dried with sodium sulfate and transferred to new 60 mL glass tubes through glass funnels containing glass wool with 3x5 mL hexane rinses. Extracts were reduced in volume to approximately 10 mL using a gentle stream of ultra-high purity (UHP) nitrogen. We transferred 0.5 mL of each extract to a pre-weighed aluminum tray for lipid determination, and then reduced extracts in volume to 2.5 mL using UHP nitrogen and made up to 5 mL with DCM.

Samples were then injected onto a gas-permeation chromatography (GPC) column (J_2-scientific AccuPrep, Columbia, Missouri, USA) with 60 g of S-X3 Biobeads with the following parameters: 5 mL injection volume, 8 psi pressure, 5 mL/min flow rate, 50:50 hexane:DCM mobile phase. The first 140 mL fraction was discarded and the fraction containing our analytes of interest was collected from 140 to 360 mL. Extracts were reduced in volume to approximately 2 mL using a rotary evaporator. A final cleanup step was done by pre-rinsing 4 g of silica gel, 1 g of deactivated alumina, and 0.5 g of sodium sulfate with 3 x 10 mL of a 70:30 mixture of

DCM:hexane and adding it to the extracts. After 30 min, extracts were filtered through glass funnels containing glass wool into new 60 mL glass tubes and reduced to a final volume of 200 µL with a gentle stream of UHP nitrogen. Extracts were spiked with 20 µL of 1 ng/µL of IPIS and were stored in amber GC vials at 4 °C until analysis.

3.3.5 Gas Chromatography-Tandem Mass Spectrometry

An Agilent 7890 GC coupled with a triple quadrupole Agilent 7000C mass spectrometer fitted with an electron ionization (EI) source was used for GC-MS/MS analysis. An Agilent J&W HP-5ms ultra inert column (30 m x 0.25 mm x 0.25 µm) was used with helium as the carrier gas at a constant flow rate of 1.2 mL/min. Splitless injections of 1 µL were made using a PAL RSI 85 auto sampler into the injection port at 250 °C. Both transfer line and source temperatures were set to 300 °C. Details for the oven temperature ramp and the multiple reaction monitoring (MRM) ion transitions for PAHs and APAHs are reported in Idowu et al.[14].

3.3.6 Quality Control/Quality Assurance

Each sample batch included a procedural blank, a matrix blank, and a spike reference sample. Reference samples were spiked with a known amount of each target analyte to assess the accuracy of our extraction and quantitation method. The overall accuracy for all 48 analytes across nine sample batches was 96 ± 23%. All samples were also spiked with a known amount of RIS to account for any losses during sample processing. A solvent blank (hexane) was run between every 20 GC-MS/MS injections.

3.3.7 Statistical Analysis

We compared concentrations of different groupings of PACs, including total PACs (Σ_{48}PAC), total priority PAHs (Σ_{16}PAH), the sum of parent lower molecular weight compounds (i.e. 2-3 ring compounds; Σ_{6}PLMW), the sum of alkylated lower molecular weight compounds (Σ_{17}ALMW), the sum of parent higher molecular weight compounds (i.e. 4, 5 or 6 ring compounds; Σ_{10}PHMW), and the sum of alkylated higher molecular weight compounds (Σ_{10}AHMW). We also included the sum of total high molecular weight compounds (Σ_{20}HMW) and the sum of total low molecular weight compounds (Σ_{23}LMW). A single parent heterocyclic compound (dibenzothiophene; ΣPHET) and its alkylated congeners (Σ_{4}AHET) were also included in our analysis. Given that PACs are lipophilic, all concentrations were lipid-normalized (lw). All analytes were also blank corrected using their corresponding mean concentration present in the procedural blanks. All PAC concentrations that were below our method detection limits were recorded as non-detects.

The distribution of the data for each PAC grouping was tested using a Shapiro-Wilks test, and variances tested using a Levene Test using the R package car v.3.1-3 (Fox and Weisberg 2019). None of the groupings showed a normal distribution or equal variances, thus non-parametric tests were used to compare groups where at least 50% of the samples had PAC concentrations above the detection limits. An alpha value of 0.05 was used to evaluate statistical significance of models. To investigate differences in PAC levels between PLI and Appatuurjuaq, Mann Whitney U tests were run. Additionally, differences in PACs among years within each site were examined using Kruskal-Wallis multiple comparison test, with *p*-values adjusted using the Bonferroni method. A Dunn's Post-Hoc test was used to compare between years using the

package FSA v.0.9.6 (Ogle 2023). All statistical analyses were conducted using RStudio Software v. 4.2.2 (R Development Core Team 2022). Additionally, ggplot2 v.3.5.1 (Wickham 2016) was used for all data visualization.

3.4 Results and Discussion

A total of 48 PACs were analyzed in 190 thick-billed murre eggs collected from Appatuurjuaq (n=100) and Prince Leopold Island (n=90) at various collection dates between 1993 and 2024. The concentrations (ng/g, lipid weight) can be found in Table S4. Comparisons were made between locations and among years to identify any trends.

3.4.1 Spatial Comparison

To compare PAC concentrations between Appatuurjuaq and Prince Leopold Island (PLI), the mean concentrations of various groups of PACs were evaluated. Summarized results are displayed in Figure 3.1.

The mean concentration of total PACs (Σ_{48}PAC) detected in Appatuurjuaq eggs (n=100) was 38.04 ng/g (range: 1.50-224.69 ng/g) and in PLI eggs (n=90) was 43.87 ng/g (range: 2.04-437.82 ng/g). In general, both sites showed relatively similar PAC distribution patterns. When comparing individual groups, alkylated high molecular weight ((Σ_{10}AHMW) compounds made the greatest contribution to the overall PAC concentrations in both locations, comprising 44% of total PACs in Appatuurjuaq eggs and 49% of total PACs in PLI eggs. At both sites, C2 chrysene

was the single PAC found in the highest relative abundance. Mean C2 chrysene values were 7.90 ng/g at Appatuurjuaq and 10.44 ng/g at PLI, accounting for 21% and 24% of total PACs at Appatuurjuaq and PLI, respectively. A single parent heterocyclic compound, dibenzothiophene (PHET), and its alkylated derivatives ((Σ_4AHET) were included in our analysis. However, dibenzothiophene was only detected in a single egg sample (0.92 ng/g, PLI in 2003) and thus was not included in Figure 3.1. Besides dibenzothiophene, its alkylated derivatives contribute the smallest proportion to total PAC concentrations, representing only 4% of total PACs in Appatuurjuaq eggs and 5% of total PACs in PLI eggs.

Our analysis revealed that three groups of PACs differed significantly between the two locations. Concentrations of Σ_{48}PAC, Σ_{10}AHMW and Σ_{20}HMW were all higher in PLI eggs compared to Appatuurjuaq eggs. Figure 3.2 summarizes changes in the concentrations (ng/g, lipid weight) of these three groups of PACs in each location over time.

Although PAC concentrations differed between Appatuurjuaq and PLI, their PAC profiles in Figures 3.3 and 3.4 were largely similar. These figures display the relative abundance of various PACs at each location and reveal many similarities between sites. For example, Figure 3.3 shows a relative abundance of approximately 0.4 for C2 compounds at both Appatuurjuaq and PLI. Although there were slight differences, the general distribution of compounds was very similar. While murres breeding at PLI generally appeared to be exposed to greater levels of PACs, this indicated that the sources of these compounds were likely similar at both locations.

To further investigate possible origin of PACs in our study, we compared the relative abundances of parent and alkylated PAHs at each site. Overall, alkylated compounds were detected in much higher concentrations compared to parent PAHs at both locations, accounting

for 77% and 71% of total PACs at Appatuurjuaq and PLI, respectively. Figure 3.3 shows the relative abundance of unsubstituted (C0) and alkylated (C1-C4) compounds in each location. Typically, a bell-shaped distribution is characteristic of petrogenic sources, as there is a greater abundance of C1-C4 compounds, which are indicative of petrogenic origins[23,24]. Conversely, a sloped distribution (C0>C1>C2>C3>C4) with primarily unsubstituted compounds would suggest predominantly pyrogenic origins because high temperatures tend to eliminate alkyl substituents[23,25]. We found a bell-shaped distribution with the highest relative abundance of C2 compounds at both PLI and Appatuurjuaq, suggesting petrogenic sources as major contributors for PACs at both locations. Although C2 compounds were present in the greatest relative abundance, the notable contribution of unsubstituted (C0) compounds indicated the presence of pyrogenic sources too.

Figure 3.4 presents a more detailed distribution of parent (C0) and C1-C4 alkylated compounds at Appatuurjuaq and PLI. Both locations showed many similarities in the relative abundances of compounds. With the exception of naphthalene, all compounds showed a greater abundance of alkylated derivatives compared to the parent compound. This again suggested the strong contribution of petrogenic sources, as they tend to produce a high abundance of alkylated compounds. Although naphthalene showed a higher relative abundance compared to its alkylated derivatives, naphthalene is ubiquitous in the environment and is typically less indicative of potential sources compared to other compounds[26].

Although murres in our study were likely exposed to a combination of both petrogenic and pyrogenic PACs from a variety of sources, pyrogenic sources were unlikely to be the dominant contributors to PACs currently at these two sites. Parent high molecular weight

(PHMW) compounds tend to be strongly indicative of pyrogenic sources; however, this group was among the smallest contributor to overall PAC concentrations in our study. In fact, only two years at Appatuurjuaq (2014, 2023) and one year at PLI (2023) showed Σ_{10}PHMW concentrations above detection limits. Taking this into consideration, along with our other results, we believe petrogenic sources were the predominant source of PACs at both Appatuurjuaq and PLI while pyrogenic sources contributed to a lesser extent. This finding is consistent with previous studies that have also revealed the high occurrence of petrogenic PACs in the Arctic region[27–29].

In our analysis, C2 chrysene was the most abundant single compound in eggs at both sites. There is limited data available regarding the potential sources of this particular compound, although the extent of alkylation on C2 chrysene suggests petrogenic origins are likely. Very little is currently known about the potential effects and toxicity of C2 chrysene. Given its high abundance in both locations, a better understanding of the potential impact of this particular compound on seabirds and other organisms is necessary.

A possible source for the high abundance of petrogenic PACs detected in our study could be natural oil and gas seeps in the region. Natural seeps have been identified in the Baffin Bay-Davis Strait region and are significant sources of petrogenic PACs[29]. Similarities may be explained by high and low Arctic breeding murres' relatively similar overwintering or partial overlapping spring staging in the Hudson Strait, very close to these natural seeps[30]. As murres are a migratory species, they spend time in this region prior to their arrival at their respective breeding colonies[31]. PACs accumulated during overwintering can contribute to the contaminant burden that is transferred to eggs[32], potentially explaining many of the similarities we observed.

Based on the locations of Appatuurjuaq and PLI, petrogenic PACs could also be associated with vessel-related activity. Both sites are located near major northern shipping routes in Canada, although those shipping routes are much less used than what would be recognised as a main shipping route in southern Canada. PLI is located within Lancaster Sound, at the eastern entrance to the Northwest Passage, while Appatuurjuaq is situated in northern Hudson Bay, near the Arctic Bridge shipping route[5]. While petrogenic PACs in the Arctic region are typically associated with natural oil and gas seeps[33], they can also be found in ship-related fuel and oil prior to their combustion. PACs can be released into the environment through large oil or fuel spills, as well as through continuous, low-level emissions from ships[34].

Long-range atmospheric transport of PACs to the Arctic is also possible and can contribute to overall concentrations. However, lower molecular weight PACs are more likely to undergo this type of transport due to their greater volatility compared to higher molecular weight compounds[33]. Therefore, long-range transport is unlikely to account for the high abundance of AHMW PACs we detected as they are less prone to atmospheric transport. For this reason, we believe long-range atmospheric transport to be a relatively minor source for overall PAC levels detected in murres eggs at Appatuurjuaq and PLI.

The difference in PAC concentrations between Appatuurjuaq and PLI suggested spatial differences in exposure. Natural oil and gas seeps could once again explain this observation. While these natural seeps may not be directly adjacent to Appatuurjuaq and PLI, murres have a typical foraging range between 12 to 175 km km[30], which could bring them closer in proximity to these sources. The natural seeps in the Baffin Bay-Davis Strait region are much closer to PLI compared to Appatuurjuaq and could explain the greater PAC levels in PLI eggs. Alternatively,

murres breeding in the high Arctic (PLI) may spend more time in the Hudson Strait as they migrate towards their breeding colony, potentially increasing their exposure to natural seeps compared to murres breeding at Appatuurjuaq.

The proximity of each site to major shipping routes could also provide an explanation for our observed spatial variation. Although both PLI and Appatuurjuaq are located along popular shipping routes, PLI typically experiences greater traffic, particularly from tourism vessels[35]. Tourism vessels typically refer to passenger ships (cruise ships) and pleasure craft vessels (yachts) and are among the fastest growing ship type in the Arctic region. In a recent report on Canadian Arctic shipping, Dawson et al. (2021) reported that the average distance travelled by pleasure crafts in this region increased by nearly 4000% in 2010-2018 compared to 1990-1999[35]. Additionally, they reported that the greatest increase in distance travelled occurred through the Northwest Passage, near PLI, while a slight decrease in traffic was observed through Hudson Bay, near Appatuurjuaq[35].

The Northwest Passage, connecting the Arctic and Pacific oceans through Canada's Arctic Archipelago, is gaining significant interest as a future international shipping route. As climate change continues to drive reductions in Arctic sea ice, current research estimates the Northwest Passage will be ice-free during the summer months by 2030[36]. The Northwest Passage would offer a shorter alternative for current shipping routes through the Panama or Suez canals, potentially bringing substantial vessel traffic into this region[37]. While there are several routes that are referred to in general as the "Northwest Passage", they all include PLI as they move through the Lancaster Sound zone[38] . Although currently this route remains covered with sea ice for much of the year, tourism in this region continues to grow at a rapid pace. The substantial increase in

pleasure craft vessels, including both commercial and private yachts, is particularly concerning because they are among the least regulated vessel type[35]. Continued monitoring at these sites, along with comparisons to local vessel data, are necessary to understand the potential impacts of ship activity on PAC concentrations as well as risks to the Arctic environment and its large seabird populations.

3.4.2 Interannual Comparison

Our analysis revealed interannual variation in PAC concentrations at both locations. To investigate interannual trends more closely in each location, Appatuurjuaq and PLI were analyzed individually.

3.4.2.1 Appatuurjuaq

The interannual variation in Σ_{48}PAC concentrations at Appatuurjuaq is displayed in Figure 3.5. Statistical analysis for additional PAC groupings were also completed, however, there were no statistically significant differences detected among any years in other groupings, so these results are not included.

We statistically compared year differences in Σ_{48}PAC concentrations from Appatuurjuaq eggs (Kruskal-Wallis χ_{19}=46.288, $p < 0.001$). The highest Σ_{48}PAC concentrations were detected in 2018 (mean value: 111.14 ng/g) and the lowest in 2014 (mean value: 10.03 ng/g). These two years were the only two to show a significant difference in Σ_{48}PAC concentrations (Table S6), with 2018 being higher. Although interannual differences in PAC concentrations between other

years were not statistically significant, there was still considerable variation between individual years.

The majority of years in our analysis showed greater concentrations of AHMW compared to ALMW compounds in Appatuurjuaq murre eggs. Surprisingly, the mean Σ_{48P}AC concentration in 2018 eggs was comprised of predominantly ALMW compounds (50.69 ng/g; 46% of total PACs), and 2017 was similar (60.44 ng/g; 63% of total PACs). These findings suggest possible changes in the dominant sources of PACs in 2017 and 2018 compared to other years in our analysis. The high ALMW concentrations observed in 2017 and 2018 were particularly significant because these also represented the two years with the greatest Σ_{48}PAC concentrations among all 20 collection years. Interestingly, the high ALMW concentrations in eggs from 2017 and 2018 were predominantly driven by alkylated naphthalene compounds, indicating elevated environmental levels of these compounds.

We observed a surprising spike in the concentrations of Σ_{10}PHMW compounds at Appatuurjuaq in 2023 (29.66 ng/g, 37% of total PACs). This was notable because all other years were below detection limits for this group of compounds. PHMW compounds are strongly associated with pyrogenic sources, suggesting Appatuurjuaq murres may have been exposed to greater concentrations of pyrogenic PACs in 2023 compared to other years. Wildfires have been identified as the dominant source of pyrogenic PACs in western and northern Canada and predominantly produce PHMW compounds[33]. Interestingly, 2023 was Canada's worst wildfire season on record with an estimated 15 million hectares of land burned[39]. This could potentially explain the substantial rise in PHMW compounds seen in eggs from this year. More frequent and severe wildfires are expected with ongoing climate change and could have severe implications

on the Arctic environment[33]. PHMW compounds can also be derived from fossil fuel combustion, however, we did not find any indication of enhanced or unusual activity that would explain the substantial spike in 2023 compared to other years.

3.4.2.2 Prince Leopold Island

The annual variation for Σ_{48}PAC concentrations at PLI was also analyzed and is presented in Figure 3.6. To identify statistically significant differences, we compared Σ_{48}PAC concentrations among individual years (Kruskal-Wallis χ_{17}=45.104, $p < 0.001$). The greatest Σ_{48}PAC concentrations were detected in 2023 eggs (mean value: 142.47 ng/g) and the lowest in 2010 (mean value: 11.25 ng/g). The greater concentrations seen in 2023 compared to 2010 was the only statistically significant difference among years (Table S7). No statistical difference was detected between any other years but variation between individual years was still apparent.

PLI eggs from 2023 had the greatest overall Σ_{48}PAC compared to all other years, with PHMW compounds contributing the greatest amount (80.06 ng/g, 56% of total PACs). Interestingly, 2023 was also the only year we observed PHMW concentrations above detection limits, similar to our findings at Appatuurjuaq. This spike in PHMW concentrations further suggests murres in 2023 may have been exposed to elevated levels of PACs originating from pyrogenic sources. As previously discussed, the increase in PHMW PACs could potentially be linked to the record-breaking Canadian wildfires seen in 2023, as this spike coincides with extensive fires across the country[39]. The fact that similar observations were made at both sites suggests PHMW compounds are likely derived from similar sources.

Alternatively, the spike in PHMW compounds in 2023 may reflect elevated levels of these compounds at a shared overwintering site. The presence of this increase in eggs from both breeding colonies suggests that murres may have been exposed to similar sources during late winter, prior to migration. Because PHMW compounds are strongly indicative of pyrogenic sources, these findings suggest that unusual activity, such as increased combustion of organic material or fossil fuels[25], may have occurred at an overwintering site in 2023, leading to greater accumulation of these compounds in murre eggs.

Similar to Appatuurjuaq, PAC concentrations in PLI eggs were generally influenced by AHMW compounds in most years. ALMW compounds typically contributed less to overall PAC concentrations with the exception of 2024 eggs, which showed a significant spike in Σ_{17}ALMW contributions (43.76 ng/g; 55% of total PACs). The concentrations of ALMW compounds in 2024 were largely driven by alkylated naphthalene compounds, indicating greater exposure of murres to sources containing these compounds.

Interannual comparisons were also done for additional PAC groupings in murre eggs from PLI. The only other group that had statistically significant differences among any of the collection years at PLI was Σ_{10}AHMW (Table S8). Interannual variation of Σ_{10}AHMW PACs is shown in Figure 3.7. We found Σ_{10}AHMW concentrations differed significantly between 1993 and 2018, and also between 2010 and 2018, with 2018 being higher in both cases. The high concentrations of this group of compounds in 2018 were largely dominated by C2 chrysene, with a mean concentration of 26.72 ng/g. This finding further emphasizes the need for additional research into likely sources of C2 chrysene and its potential implications on marine wildlife.

3.5 Conclusions

The availability of archived egg samples from sites in Arctic Canada allowed us to undertake this initial, long-term temporal analysis of PACs in Canadian Arctic seabirds. Previously, Provencher et al.[15] reported decadal differences in Arctic seabird PAC concentrations but emphasized the need for further research in this area. Overall, we evaluated both spatial and interannual variation of PACs in thick-billed murre eggs collected from Prince Leopold Island and Appatuurjuaq. Our results revealed greater PAC concentrations in high Arctic (PLI) eggs compared to low Arctic (Appatuurjuaq), and although overall PAC concentrations did not change dramatically from 1993 to 2024, considerable variation among years is apparent. Many of the higher concentrations of various PACs have occurred in murre eggs collected in the last decade, especially in the high Arctic, and we speculate that this may be related to increased levels of shipping occurring in this region or wildfire activity over the same period. This study helps fill existing knowledge gaps and provides a benchmark for historic and current PAC levels to help track any future changes. As activity in the Arctic and the interest in Canada's northern shipping routes continue to grow, the need for annual monitoring of oil and gas related contaminants in the Arctic region is essential to detect emerging trends, identify potential sources and support informed decision-making to ensure the protection of Canada's Arctic wildlife. Continued collection and analysis of chemicals in seabird eggs, building on existing contaminant monitoring programs[14], provides a practical, reliable and robust monitoring system to address these needs.

3.6 Acknowledgements

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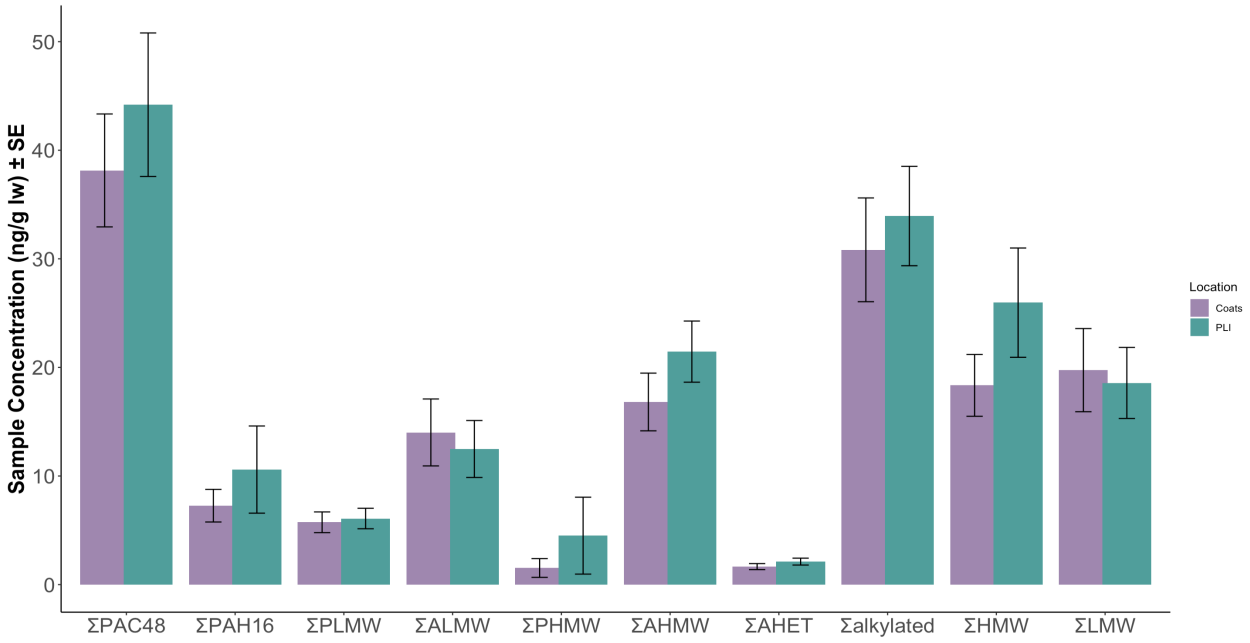

Figure 3.1 Concentrations (ng/g, lipid weight) of various groups of PACs in thick-billed murre (Uria lomvia) eggs collected from Appatuurjuaq (Coats Island) (n=100) and Prince Leopold Island (n=90) between 1993 and 2024. Mean values of total eggs are given with error bars representing standard error. The sum (Σ) of all 48 PACs, the US EPA's 16 priority PAHs, parent low molecular weight (PLMW) and their alkylated congeners (ALMW), parent high molecular weight (PHMW) and their alkylated congeners (AHMW) and alkylated heterocyclic compounds (AHET) are shown. The sum of total alkylated compounds, total high molecular weight (HMW) compounds, and total low molecular weight (LMW) compounds are also presented.

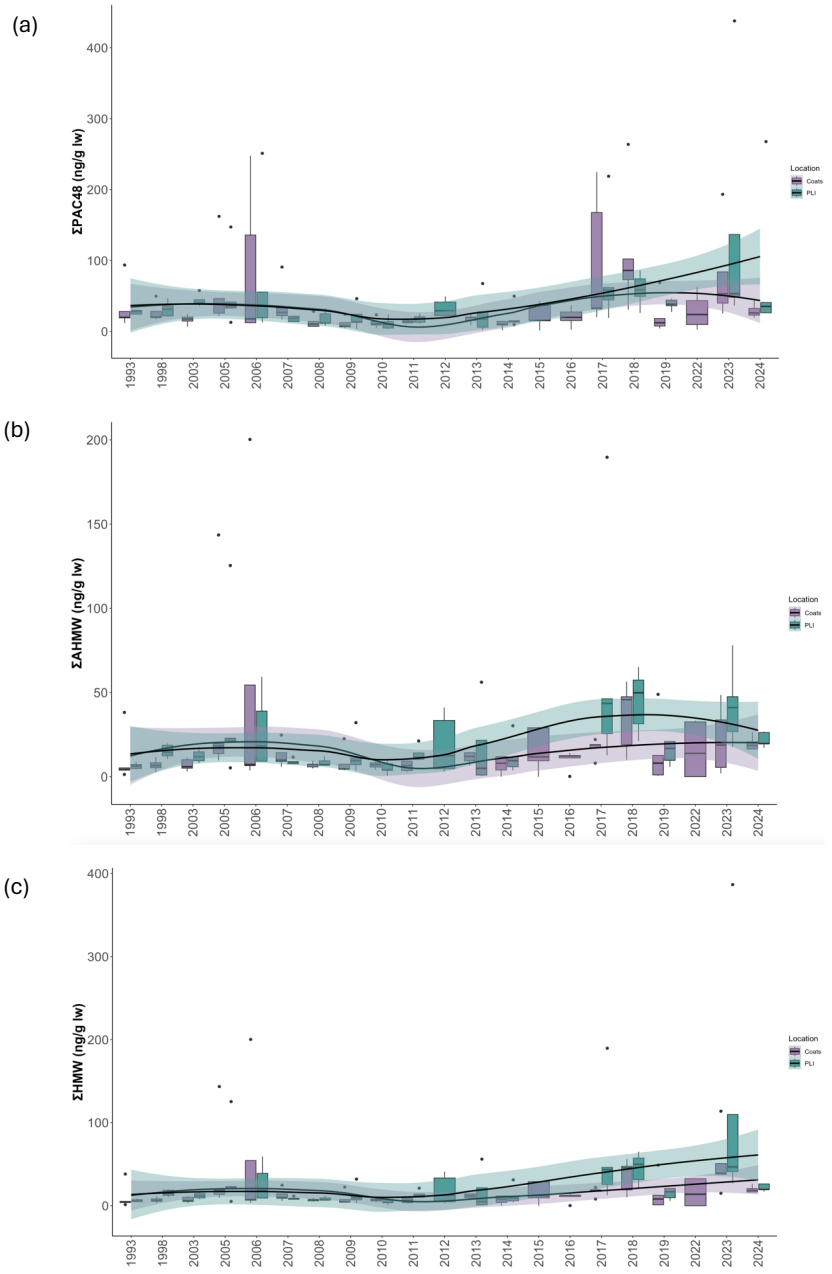

Figure 3.2 Box plots showing concentrations (ng/g, lipid weight) of (a) the sum of total PACs (Σ_{48}PAC), (b) the sum of alkylated high molecular weight compounds (Σ_{10}AHMW) and (c) the sum of total high molecular weight compounds (Σ_{20}HMW) in thick-billed murre (*Uria lomvia*) eggs collected from Appatuurjuaq (Coats Island) and Prince Leopold Island between 1993 and 2024.

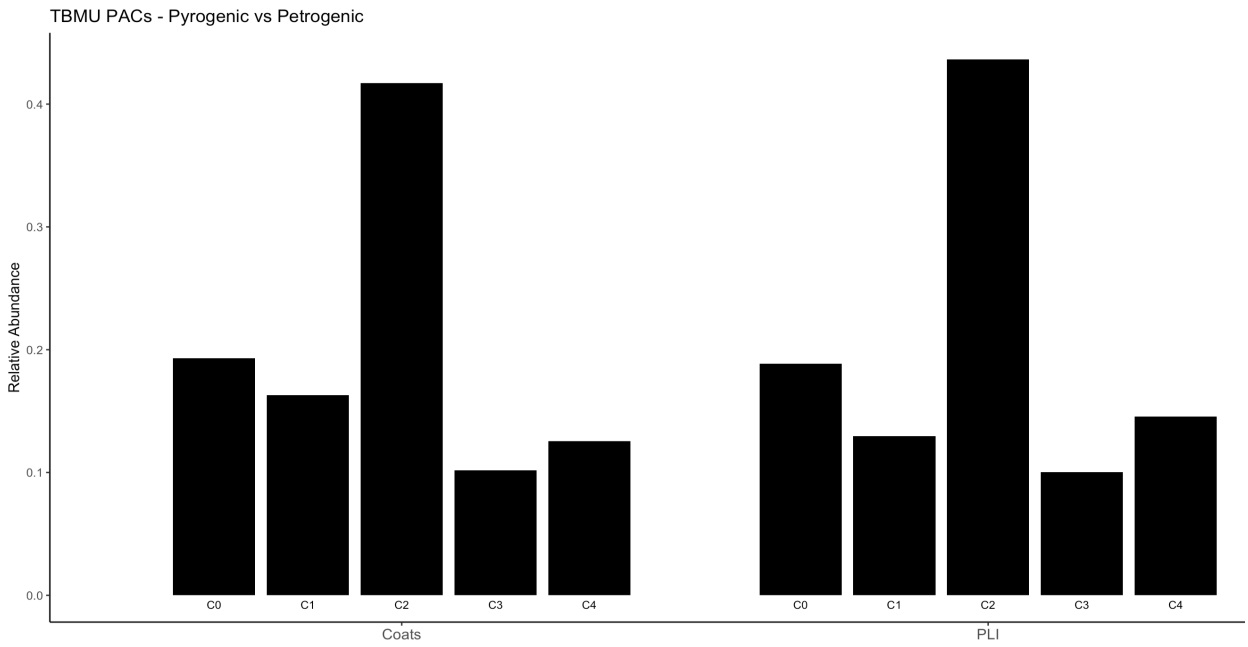

Figure 3.3 Relative abundance of parent PAHs (C0) and alkylated analogues (C1-C4) in thick-billed murre (*Uria lomvia*) eggs from Appatuurjuaq (Coats Island) and Prince Leopold Island between 1993 and 2024.

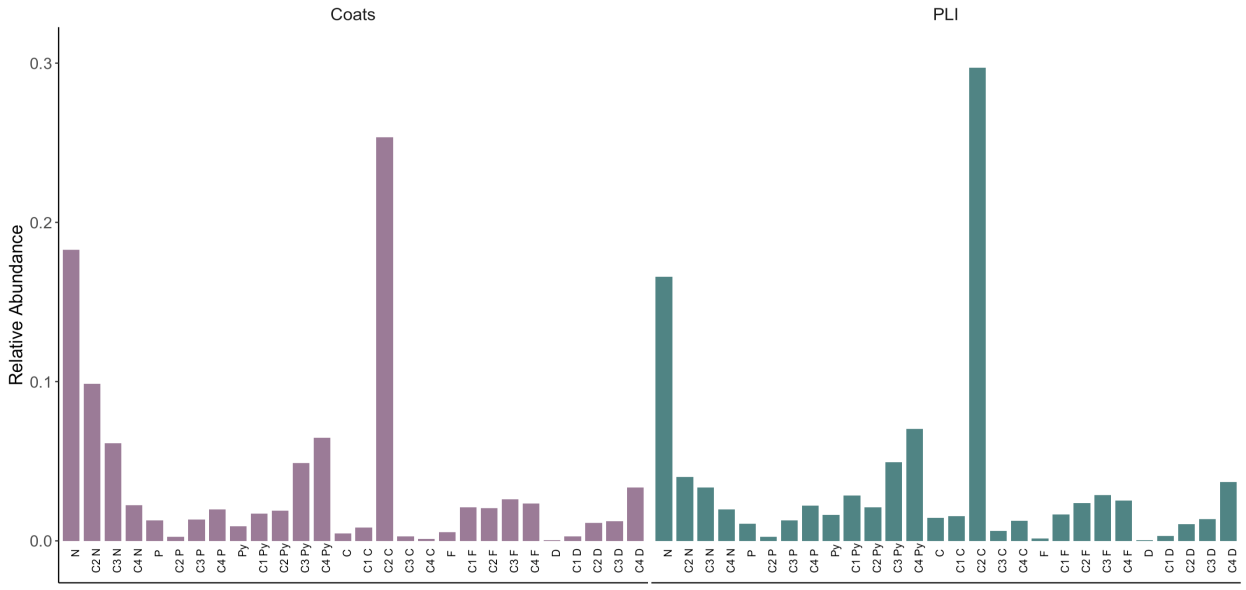

Figure 3.4 Relative abundance of unsubstituted and alkylated PACs in thick-billed murre (*Uria lomvia*) eggs from Appatuurjuaq (Coats Island) and Prince Leopold Island between 1993 and 2024. Relative abundance is presented for naphthalene (N), phenanthrene (P), pyrene (Py), chrysene (C), fluorene (F) and dibenzothiophene (D).

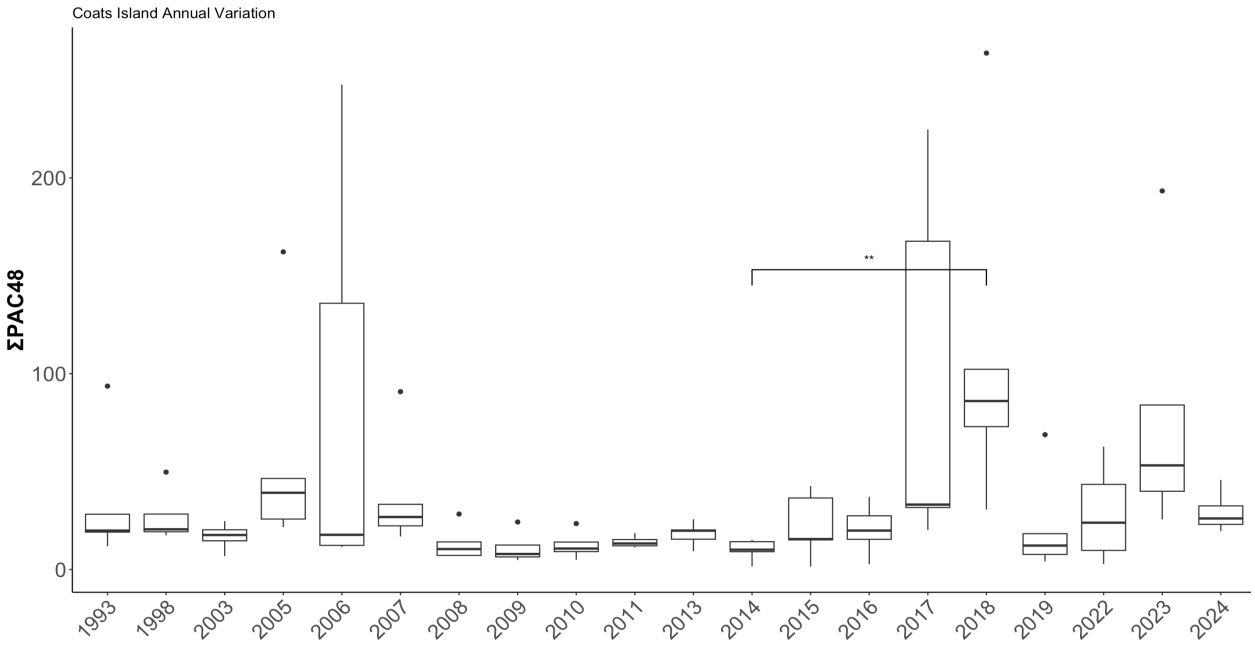

Figure 3.5 Box plot showing annual variation in Σ_{48}PAC concentrations (ng/g, lipid weight) in thick-billed murre (*Uria lomvia*) eggs collected from Appatuurjuaq (Coats Island) between 1993 and 2024.

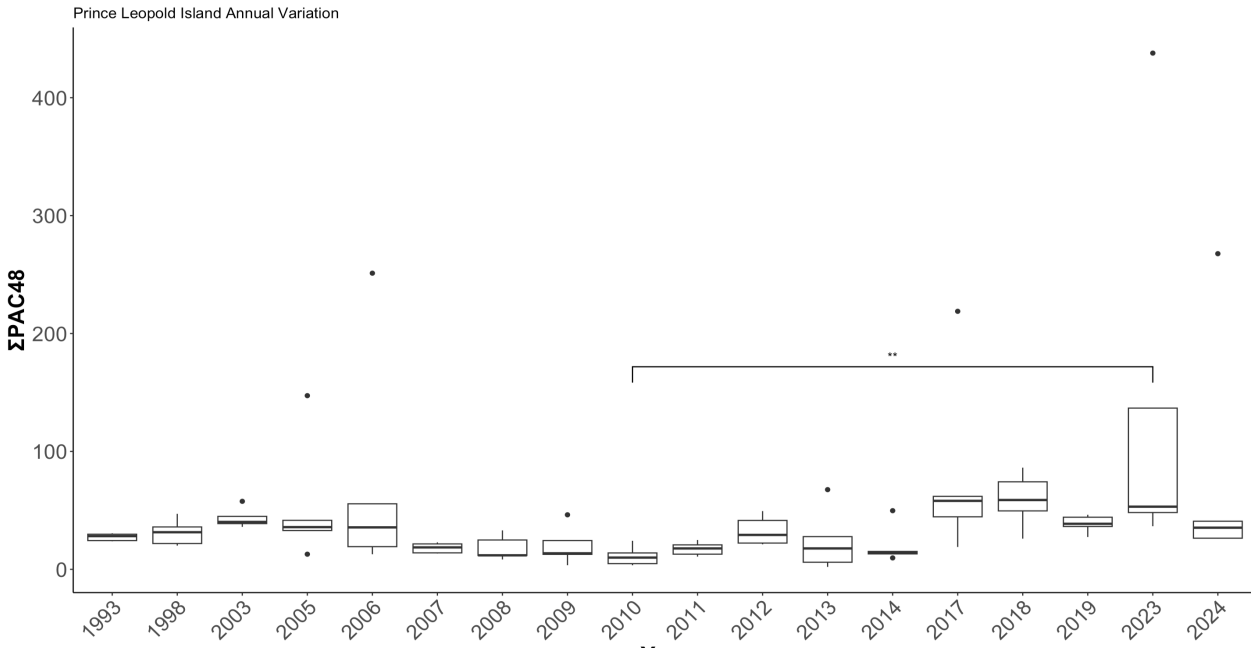

Figure 3.6 Box plot showing annual variation in Σ_{48}PAC concentrations (ng/g, lipid weight) in thick-billed murre (*Uria lomvia*) eggs collected from Prince Leopold Island between 1993 and 2024.

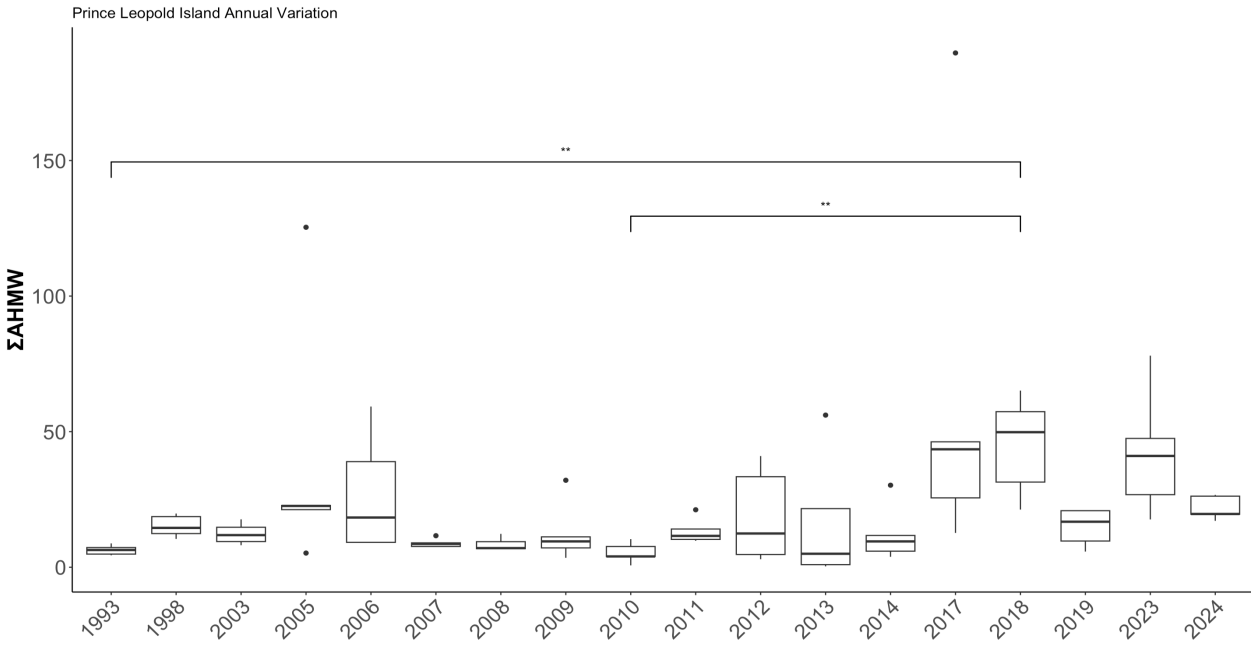

Figure 3.7 Box plot showing annual variation in Σ_{10}AHMW concentrations (ng/g, lipid weight) in thick-billed murre (*Uria lomvia*) eggs collected from Prince Leopold Island between 1993 and 2024.

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Chapter 4: Species- and tissue-specific bioaccumulation of polycyclic aromatic compounds in three Arctic seabirds

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4.1 Abstract

Polycyclic aromatic compounds (PACs) are a complex class of toxic organic contaminants associated with petroleum. Emissions of PACs from natural seeps, along with PAC emissions from increasing industrial activity in the Arctic, pose potential risks to marine wildlife, including Canada's seabird populations. In this study, we examined homocyclic and heterocyclic PACs in liver and egg tissues from three species of breeding Arctic seabirds; murres (*Uria lomvia*), fulmars (*Fulmarus glacialis*), and kittiwakes (*Rissa tridactyla*). Eggs and adult birds were collected from Prince Leopold Island, Nunavut (74°02'N, 90°00'W) in 2023. We extracted and analyzed 86 PACs, including many heterocyclic compounds that have not previously been analyzed in Arctic biota, by gas chromatography-tandem mass spectrometry. To ensure the method captured the full suite of compounds, a full method validation was also completed. Both homocyclic and heterocyclic PACs were detected in livers and eggs, and were significantly higher in liver, across all species. Northern fulmars had the highest PAC concentrations in liver tissue, whereas black-legged kittiwakes had the highest concentrations in eggs. Considerable differences in PAC profiles were also observed both among species and between tissue types. This variability likely reflects species-specific differences in PAC exposure, accumulation, and metabolism, driven by a combination of foraging ecology, breeding phenology, and physiological factors.

4.2 Introduction

Polycyclic aromatic compounds (PACs) are a complex class of organic contaminants with both natural and anthropogenic origins. They are primarily classified as either petrogenic, originating from unburned petroleum sources, or pyrogenic, resulting from the incomplete combustion of fossil fuels and organic matter[1–3]. PACs have been detected in a wide range of environmental compartments including water, soil, sediment, air, and biota[4,5]. Many of these compounds have been recognized as a concern due to their bioaccumulation potential, persistence in the environment, and toxicity[6].

Polycyclic aromatic hydrocarbons (PAHs) are the most widely studied group of PACs. In the 1970s, 16 PAHs were designated as "high priority pollutants" by the United States Environmental Protection Agency (US EPA) and are also listed on the Toxic Substances List under the Canadian Environmental Protection Act (CEPA)[7]. Since their identification, much of the focus has been on these 16 PAHs and their alkylated polycyclic aromatic hydrocarbon (APAH) analogues. However, more recent advancements in detection and quantitation have prompted efforts to expand this list, especially due to the potential for comparatively greater, but under-researched toxicological impacts on biota[8,9]. Additional groups of PACs have gained significant interest in recent years including hetero-polycyclic aromatic compounds (HPACs), in which one or more carbon atoms are replaced with a heteroatom (most commonly nitrogen, sulfur, or oxygen) and halogenated hetero-polycyclic aromatic compounds (Halo-HPACs)[10]. These compounds have been studied much less than their homocyclic analogues but have similar origins, have also been detected in a wide range of environmental compartments, and are estimated to have comparable or even greater toxicity[11–13].

Seabirds are established bioindicators of marine ecosystems and have been used for decades to monitor environmental contaminants[14]. Because they feed at relatively high trophic levels within food webs, seabirds are susceptible to bioaccumulating high levels of certain contaminants through their diet[14]. Additionally, many contaminants are known to undergo maternal transfer from female seabirds to their eggs[15], a process influenced by both physiological factors as well as the physico-chemical properties of the contaminants[16]. Given their lipophilic nature, PACs are transferred along with lipids to developing eggs and can be reflective of the contaminant burden in the female at the time of laying[17,18]. Oil can also be passed from the incubating parent's feathers into the developing embryo through the eggshell and membrane[19]. Consequently, both adult seabirds and eggs can serve as effective bioindicators for PACs in marine ecosystems.

Despite maternal transfer being a critical exposure pathway for developing eggs[20], our understanding of this process in seabirds, especially for PACs, remains limited. The xenobiotic metabolizing capabilities of embryos are typically very low[21], making them particularly vulnerable to PACs passed through maternal transfer. Evidence of PAC embryotoxicity in avian eggs includes developmental abnormalities, reduced hatching success, and biochemical disruptions [22–25], all of which pose significant risks to the reproductive success of seabirds.

Prince Leopold Island, Nunavut (74°02'N, 90°00'W; PLI) has been among the most important seabird monitoring sites in the Canadian Arctic since 1975 and has produced one of the strongest long-term contaminants datasets in Canada spanning 50 years[26]. Thick-billed murres (*Uria lomvia*, hereafter murres*),* northern fulmars (*Fulmarus glacialis*, hereafter fulmars), and black-legged kittiwakes (*Rissa tridactyla*, hereafter kittiwakes) are three of the prominent

seabird species that breed at PLI and were the focus of this study. While these species share a common breeding site and have overlapping overwintering areas[27], their foraging ecology and timing of egg-laying differ[27–29], which may influence their exposure to environmental contaminants[30]. Murres are capable of diving as deep as 200 m for over 3 min to forage on pelagic and benthic fish, particularly Arctic cod (*Boreogadus saida*) at the PLI colony[31,32]. In the Arctic, murres typically forage 50-100 km away from the colony. Conversely, fulmars often follow marine vessel traffic and primarily scavenge prey including fish and invertebrates (mostly copepods) on the surface of the water and are known to only conduct shallow dives of approximately 2-3 m, and > 100 km away from the colony[33–35]. Similarly, kittiwakes, like other gull species, forage at the water surface and opportunistically consume similar fish and invertebrate prey with a typical foraging range 10-50 km[36]. Overall, seabirds from PLI have been used to investigate a variety of contaminants including heavy metals[37], organochlorine compounds[38], polychlorinated dibenzo-p-dioxins (PCDDs)[39], and UV stabilizers including UV-328, a plastic additive recently listed on the Stockholm Convention[40]. However, there are currently limited data available regarding PACs at this breeding colony, and generally in the Canadian Arctic.

As shipping and industrial activity continue to increase in the Canadian Arctic, along with the potential for future oil and gas development, there is a growing risk for the emission of PACs into surrounding ecosystems[41,42]. While some Arctic studies have measured PACs in seabirds or their eggs, research in this area is limited and has focused on PAHs and APAHs only[43,44]. Expanding research to include heterocyclic compounds is essential to assess their potential impacts on Arctic marine wildlife. Additionally, although PACs have been studied in seabird livers and eggs separately[1,42,45], there are currently no studies that have compared the two tissues

within a similar time frame to investigate how PACs may differ from female seabirds to their eggs.

In this study, we analyzed 86 PACs in the livers and eggs of three species of seabirds breeding at PLI in 2023. The main objectives of this study were to evaluate both interspecies and tissue-specific differences in PAC concentrations and profiles. Because heterocyclic PACs have similar origins to homocyclic compounds, we expected that we would be able to detect these compounds in addition to homocyclic compounds in both tissues. We also expected that species-specific differences in foraging ecology and the timing of egg-laying would influence variation in PAC concentrations and profiles among the three seabird species. To capture the full suite of compounds, we conducted a full method validation for the extraction and quantitation of all compounds in our study. Method validation was done in strict accordance with the Eurachem Guidelines for Analytical Methods[46].

4.3 Materials and Methods

4.3.1 Chemicals

All high purity (Optima grade) organic solvents, silica gel (923 grade, 100-200 mesh), alumina (60-325 mesh), Ottawa sand, diatomaceous earth (DE) dispersant, and anhydrous sodium sulfate were purchased from Fisher Chemicals (Ottawa, Ontario, Canada).

Labelled PAH standards were purchased from Cambridge Isotope Laboratories (Andover, Massachusetts, USA). Napthalane-d_8, benz[a]anthracene-d_{12}, phenanthrene-d_{10}, fluoranthene-d_{10}, benzo[b]fluoranthene-d_{12}, benzo[g,h,i]perylene-d_{12}, indeno[1,2,3-cd]pyrene-d_{12},

dibenz[a,h]anthracene-d_{14}, accaphthylene-d_8, acenaphthene-d_{10}, fluorene-d_{10}, pyrene-d_{10}, benzo[k]fluoranthene-d_{12}, and chrysene-d_{12} were used as the recovery internal standard (RIS). Labelled anthracene (anthracene-d_{10}) was purchased from AccuStandard (New Haven, Connecticut, USA) and was used as an instrument performance internal standard (IPIS).

Native PAH mix containing sixteen PAHs was purchased from AccuStandard (New Haven, Connecticut, USA). APAH and HPAC mixes, including both halogenated and non-halogenated compounds, were purchased from Chiron (Trondheim, Norway). Complete details can be found in the Supplementary Information (SI).

4.3.2 Method Validation Samples

Chicken eggs and chicken livers were purchased from a local retail store (Winnipeg, Manitoba, Canada). Eggs and livers were homogenized and aliquoted into 5 g samples. Samples were then purposely spiked with native PAH, APAH, HPAC, and halo-HPACs at three concentration levels (10, 100, and 500 pg/μL).

4.3.3 Environmental Samples

Murre, kittiwake, and fulmar egg and liver samples were collected from Prince Leopold Island, Nunavut, Canada (74°02'N, 90°00'W) in 2023. Collection dates can be found in the SI (Table S6). One egg per nest was collected from each nest, and these were placed in plastic bags and kept intact and at 4°C through transit. We collected adult birds of each species using noose poles and cervical dislocation and froze those within 12 h, as previously reported on[47]. In fall of

2023, adult birds of all three species were measured, sexed, dissected and livers collected following standard protocols during the Wildlife Contaminants Workshop, a week-long training opportunity for students attending the Nunavut Arctic College in Iqaluit, Nunavut[48]. Egg and liver samples were homogenized using chemically clean instruments, aliquoted and stored at -40°C prior to extraction at the National Wildlife Research Centre (NWRC) in Ottawa, Ontario.

4.3.4 Sample Extraction and Cleanup

The analysis of all PACs was completed in an ISO 17025/2018 accredited laboratory. Sample extraction and cleanup were completed using a modified method by Vitharana et al.[13] for extracting HPACs from sediments. 34 mL stainless steel ASE cells were prepared by placing two glass fibre filters at the bottom. We mixed 5 g of sample homogenate with 3.5 g of DE dispersant and added to each cell. We then spiked each sample with 20 µL of a 1 ng/µL mixture of d-PAH recovery internal standard (RIS) and filled each remaining cell volume with Ottawa sand (baked at 600°C for 6 h). Samples were extracted using a Dionex 350 Accelerated Solvent Extractor (ThermoFisher, Mississauga, ON). Extraction was completed using a 50:50 mixture of dichloromethane (DCM):ethyl acetate. The ASE parameters used were as follows: 1500 psi system pressure, 120°C oven temperature, 15 min static time with 2 static cycles, 60% flush volume and a nitrogen purge for 80 s at 150 psi.

Following extraction, we dried each extract with sodium sulfate and passed it through a glass funnel containing glass wool into a new 60 mL glass tube and reduced in volume to 10 mL using a gentle stream of ultra-high purity (UHP) nitrogen. We transferred 0.5 mL of each extract to a pre-weighed aluminum tray for lipid determination and the extract volume was reduced to

2.5 mL using UHP nitrogen and made them up to 5 mL with DCM. Extracts were then injected onto a gel-permeation chromatography (GPC) column (J_2-Scientific AccuPrepMPS^{TM}, Columbia, Missouri, USA) containing 60 g of S-X3 Biobeads (29.5 mm x i.d. 400 mm). The following parameters were used for GPC: 5 mL injection volume, 8 psi pressure, 5 mL/min flow rate, and 50:50 DCM:hexane mobile phase. The first 140 mL was discarded, and the analytes of interest were eluted in the fraction from 140 to 360 mL. We then reduced the volume of each extract to approximately 2 mL using rotary evaporation. A final cleanup step was done using dispersive solid-phase extraction (dSPE) with 4 g of silica gel (baked at 150°C for 16 h), 1 g of 5% deactivated alumina (baked at 250°C for 3h), and 0.5 g of sodium sulfate (baked at 600°C for 6 h) pre-rinsed with 1x20 mL Hex:DCM (30:70) and 2x10 mL ethyl acetate. After 30 min, extracts were transferred to new glass tubes through glass funnels containing glass wool using 2x5 mL Hex:DCM (30:70) and 1x10 mL ethyl acetate rinses. Volumes were then reduced to approximately 100 µL, brought up to 200 µL with hexane, and spiked with 20 µL of 1 ng/µL IPIS. Samples were transferred to amber GC vials and stored at 4°C prior to GC-MS/MS analysis.

4.3.5 Gas Chromatography Tandem Mass Spectrometry

An Agilent 7890 GC coupled with a triple quadrupole Agilent 7000C mass spectrometer with an electron ionization (EI) source was used for GC-MS/MS analysis. An Agilent J&W HP-5ms ultra inert column (30 m x 0.25 mm x 0.25 µm) was used with helium as the carrier gas at a constant flow rate of 1.2 mL/min. Splitless injections of 1 µL were made using a PAL RSI 85 auto sampler into the injection port at 250 °C. Both transfer line and source temperatures were

set to 300 °C. Details for the oven temperature ramp and the quantification and confirmation ions and the multiple reaction monitoring (MRM) ion transitions for PAHs and APAHs are reported in Idowu et al[49]. Instrument parameters and details of the MRM used for HPACs and Halo-HPACs are described by Vitharana et al.[13].

4.3.6 Method Validation

The performance characteristics of our method were calculated as described in the Eurachem Guide-The Fitness for Purpose of Analytical Methods[46].

The method limits of detection (LOD) and limits of quantification (LOQ) were calculated using six replicates (n=6) of chicken egg samples spiked at the lowest concentration level (10 pg/µL). Blanks (n_b=6) were prepared using chicken eggs spiked only with RIS. An adjusted standard deviation, s_0', was calculated using the ratio given in the Eurachem guidelines: $s_0' = s_0\sqrt{\frac{1}{n} + \frac{1}{n_b}}$, where s_0=the standard deviation, n=number of samples, and n_b=number of blanks [46]. Analyte specific LODs and LOQs were calculated as $3 \times s_0$' and $10 \times s_0$', respectively.

Accuracies of our method were calculated using six replicates (n=6) at all three concentration levels (10, 100, 500 pg/µL). Repeatability of our method was determined using six replicates (n=6) on the same day for all three concentration levels (10, 100, 500 pg/µL) and calculating the relative standard deviation (RSD). Intermediate precision was determined over three consecutive days each with four replicates (n=4) using a single concentration level (100 pg/µL). A one-way ANOVA test (α =0.05) was performed to analyze the precision for each compound among the three days.

The ruggedness of our method was tested by purposely making small changes in our method to determine if the performance characteristics remain unaffected. ASE temperature was increased from 120 °C to 140 °C in four replicates (n=4) and the mass of silica gel in the dispersant was increased from 4 g to 5 g in four replicates (n=4). A single concentration level (100 pg/µL) was used to determine ruggedness. Student *t*-tests (two-tailed, α =0.05) were performed to determine if either of the changes resulted in statistically significant differences.

4.3.7 Environmental Samples Statistical Analysis

In our statistical analysis for the environmental samples, we analyzed 86 PACs which were separated into 43 homocyclic and 43 heterocyclic compounds. PACs were further broken down into various groupings. Homocyclic PAC groupings include total PACs (Σ_{43}PAC), total priority PAHs (Σ_{16}PAH), the sum of parent lower molecular weight compounds (i.e., 2-3 ring compounds; Σ_{6}PLMW), the sum of alkylated lower molecular weight compounds (Σ_{17}ALMW), the sum of parent higher molecular weight compounds (i.e., 4, 5 or 6 ring compounds; Σ_{10}PHMW), and the sum of alkylated higher molecular weight compounds (Σ_{10}AHMW). Heterocyclic PACs groupings include total HPACs (Σ_{43}HPAC), the sum of polyaromatic nitrogen heterocycles (Σ_{23}PANH), the sum of polyaromatic sulfur heterocycles (Σ_{16}PASH) and the sum of polyaromatic oxygen heterocycles (Σ_{4}PAOH).

Given that PACs are lipophilic, all concentrations were lipid-normalized (lipid weight; lw). All analytes were also blank corrected using the corresponding mean concentration present in the procedural blanks. All concentrations that were below our method detection limits were recorded as non-detects and assigned a value of 0 for statistical analyses.

All PAC data were log transformed to meet assumptions of normality as assessed using Shapiro-Wilk tests, and homogeneity of variance using Levene's Tests in the R package car v.3.1-3[50]. First, to explore species-level differences within each tissue type for each of the PAC groupings, pairwise comparisons among species were conducted using the R package *emmeans* v.1.10.5[51], with p-values adjusted using the Bonferroni method for multiple comparisons. Results of the pairwise comparisons were visualized using the R package *ggplot2* v.3.5.1[52], significance brackets added using the R package *ggpubr* v. 0.6.0[53], and overall global model results were added to each visualization using the R package *rstatix*[54]. An α of 0.05 was used to evaluate statistical significance of models. To account for sequential Bonferroni corrections applied to the *p*-values, the significance level was adjusted to 0.001.

We used two separate linear discriminant analyses (LDA) for homocyclic as well as heterocyclic PACs in this study to determine which PACs best separate liver and egg tissue for each species. For each LDA, species was used as the predictor variable and was separated by tissue type (liver and egg). Only compounds that were detected in more than 70% of the samples were considered in each LDA to ensure that the data were not zero-inflated, as done in previous studies looking at PACs in seabirds[55]. All the concentrations were log transformed before use in the LDA to limit skew in the data. LDAs were conducted using the R packages tidyverse v. 2.0.0, and MASS v. 7.3-58.1[56,57]. All statistics were run using RStudio Software v. 4.2.2 (R Development Core Team 2022).

4.3.8 Quality Control/Quality Assurance

Each sample batch (n=32) included a procedural blank and a matrix blank. Reference samples, included with each sample batch and spiked with a known amount of each target analyte, were used to assess the accuracy of our method. The overall accuracy for all 86 PACs across all four environmental sample batches was 104 ± 37 %. All samples were spiked with a known amount of RIS to account for any losses during sample processing and with IPIS to account for any fluctuations between injections. A solvent blank (hexane) was run after approximately every 20 GC-MS/MS injections.

4.4 Results and Discussion

The results of our method validation are presented first, followed by those from our analysis of the environmental samples.

4.4.1 Method Validation

A summary of our method validation performance characteristics-including accuracy, repeatability, intermediate precision, limits of detection (LOD), and limits of quantitation (LOQ)-is presented in Table 1. Full validation data for individual compounds are available in the supplementary information (Table S4). In addition to these performance characteristics listed above, ruggedness was tested for the ninety-seven compounds. Seventy-four compounds showed no statistical difference in recoveries when the mass of DE dispersant was varied. Sixty-six compounds showed no significant change in recoveries with changes to the ASE temperature.

While some compounds were affected by these changes, both parameters are tightly controlled in our laboratory and are therefore unlikely to influence overall recoveries.

4.4.2 Environmental Samples

A total of 30 liver samples (10 fulmars, 10 kittiwakes, 10 murres) and 21 eggs (11 fulmars, 10 kittiwakes) were collected and analyzed for 86 PACs, including 43 homocyclic PACs and 43 heterocyclic PACs. For comparison purposes, we also include five murre eggs in our study had been previously extracted in our laboratory using a different method and were therefore analyzed for the 43 homocyclic compounds only. The concentrations of PACs in our samples (ng/g, lw) are summarized in Table 2.

The mean concentration of the sum of homocyclic PACs in all liver samples (n=30) was 2248.96 ng/g (range: 409.28-4454.58), and in eggs (n=26) was 97.95 ng/g (range: 18.87-436.67). For the sum of heterocyclic PACs, the mean concentration in livers (n=30) was 2721.11 ng/g (range: 702.97-16877.46) and in eggs (n=21) was 448.69 ng/g (range: 0.12-1589.97).

4.4.2.1 Interspecies Comparison

Our results revealed considerable interspecies variation in both PAC concentrations and profiles in the livers and eggs of the three species of seabirds sampled from PLI. To facilitate interspecies comparisons, livers and eggs were considered separately.

Livers

Comparisons of homocyclic and heterocyclic PAC concentrations among kittiwakes, fulmars, and murres are summarized in Figures 4.1 and 4.2 (right side). The highest mean concentrations of both homocyclic and heterocyclic PACs in livers were observed in fulmars (3306.19 ng/g and 4337.15 ng/g, respectively), and the lowest in murres (1346.44 ng/g and 1587.88 ng/g, respectively. Based on the Bonferroni-adjusted threshold (i.e. p=0.001), homocyclic PAC concentrations in fulmar livers were significantly greater compared to murres ($p < 0.001$), whereas all other pairwise differences among species had $p > 0.001$ (Table S7 and S8). For heterocyclic PACs, although fulmars had the highest mean concentrations, followed by kittiwakes and murres, all pairwise comparisons were not significant after considering Bonferroni corrections on significance (i.e., all $p > 0.001$).

The concentrations we observed in livers aligned with the foraging ecologies of each species. Murres are pursuit-diving seabirds that can forage at depths up to 200 m[36], whereas both fulmars and kittiwakes are surface feeders[55,58]. Given that PACs are associated with oil and gas, which tend to accumulate at the water's surface[42,55], surface-feeding species are likely exposed to greater concentrations through diet compared to those that forage at depth. Additionally, fulmars are known to follow ships, particularly fishing vessels, to scavenge for fish and discards[35,59,60]. Because ships are known sources of both petrogenic and pyrogenic PACs[2], this behaviour increases fulmar risk of PAC exposure and may help explain their elevated hepatic concentrations. Foraging range may also contribute to interspecies variation in PAC exposure. Although all three species are capable of foraging away from their colonies throughout the breeding season, fulmars typically forage farthest offshore and over broader ranges[27–29,36,47,55]. Their greater foraging range could potentially bring fulmars into contact with more distant sources of PACs that may be beyond the typical range of the other species.

We also assessed interspecies differences in liver PAC profiles. Across all species, ALMW compounds made up the largest proportion of homocyclic PAC concentrations in livers, accounting for 82% of total homocyclic PACs in fulmars, 79% in kittiwakes and 70% in murres. Similarly, heterocyclic PAC profiles were also consistent across species, with polyaromatic nitrogen heterocycles (PANH) comprising 95% of total HPACs in fulmars and 92% in kittiwakes.

To further investigate PAC profiles among species, we compared the relative abundance of unsubstituted (C0) and alkylated (C1-C4) compounds in the livers of fulmars, kittiwakes, and murres (Fig. 3). C3 compounds were the most abundant in all species, however, their relative abundance in murres was noticeably lower than in fulmars and kittiwakes. C2 compounds were also more prominent in murres than in the other species. A detailed breakdown of individual PACs (Fig. 4) confirmed these trends. C3 fluorene was the most abundant homocyclic compound in all species but its relative abundance in murres was approximately half that of kittiwakes and fulmars. Murres also showed higher relative levels of C3 naphthalene, further highlighting their distinct homocyclic PAC profiles. Heterocyclic compounds also varied among species. Besides indole, which was highly abundant in all samples, the dominant HPAC differed. The most abundant compound was quinoline in fulmars, C4 dibenzothiophene in kittiwakes, and C2 dibenzothiophene in murres.

The similarity in dominant PAC groups (ALMW and PANH) likely reflects shared exposure at PLI. Because most PACs are metabolized relatively quickly, liver concentrations generally represent more recent PAC exposure[61–64]. Given that all livers in our study were collected in early July 2023, they likely reflect PAC accumulation from foraging near their

shared breeding site. The high abundance of alkylated PACs across species suggests that PAC contamination in livers from our study was predominantly petrogenic in origin[65,66], a finding consistent with previous studies on Arctic seabirds[42,67]. The most likely source for these compounds is nearby natural oil and gas seeps, particularly in the Baffin Bay-Davis Strait region[42]. While the most well-known natural seep is located in Scott Inlet, additional seeps have also been reported in Lancaster Sound, which is even closer to PLI[68–70]. Seabirds may encounter PACs from these seeps while foraging, as their foraging ranges and the ability of oil and gas to disperse through the marine environment[70,71] can bring them into contact with these compounds.

While primary sources of PACs appear consistent across species, differences in the distributions of individual compounds indicates some degree of species-specific variation in exposure. As with concentration differences, ecological factors likely also influence the types of PACs encountered by seabirds. Surface-feeding fulmars and kittiwakes exhibited highly similar PAC profiles, potentially reflecting their similar, surface feeding foraging ecology, whereas deep-diving murres showed a slightly different suite of compounds.

Two separate LDAs were used to assess differences in PAC profiles among species. In both the homocyclic and heterocyclic PAC models (Figs. 5, 6), livers from fulmars, kittiwakes, and murres showed extensive overlap with minimal separation between groups, also indicating that these three species have highly similar hepatic PAC profiles (i.e., similar compounds contribute in similar concentrations in the liver tissue). Although some variation in the relative abundance of individual compounds was observed, the overall similarity suggests common sources of exposure, likely associated with the species' common breeding site at PLI.

Eggs

Summarized results comparing homocyclic and heterocyclic PAC concentrations are presented in the left panels of Figures 4.1 and 4.2, respectively. We observed the highest mean concentrations of both homocyclic and heterocyclic PACs in kittiwakes (147.17 ng/g and 661.25 ng/g, respectively), whereas fulmars had the lowest (33.36 ng/g and 459.10 ng/g, respectively). Considering the adjusted significance level after Bonferroni corrections (i.e. p=0.001), pairwise comparisons revealed homocyclic PAC concentrations in kittiwake eggs were significantly higher than those in fulmars ($p < 0.001$), and murres also had significantly higher concentrations than fulmars ($p < 0.001$). Although mean concentrations were higher in kittiwakes compared to murres, the *p*-value after Bonferroni adjustment was greater than 0.001. When considering heterocyclic compounds, all pairwise comparisons were not significant after considering Bonferroni corrections (i.e. *p*-values > 0.001; Tables S7 and S8).

The trends we observed in eggs were somewhat unexpected, as PAC concentrations were not consistent with the species' foraging ecologies. Most notably, fulmars had the lowest egg concentrations despite their surface-feeding and close association with ships, both of which would increase their risk of PAC exposure (as we did observe in the liver results outlined above). While ecological factors such as foraging strategy and range may influence female seabirds' exposure to PACs prior to egg laying, other factors appear to play a more significant role in determining contaminant levels in eggs.

Temporal factors likely contributed to much of the interspecies variation we observed in PAC concentrations. Although all three species are income-breeders that primarily rely on local food sources near their breeding colonies to obtain nutrients for egg production[72,73], eggs can

reflect both recent exposure as well as contaminants accumulated over longer periods of time and from multiple locations[18,63,64,74,75]. Prior to arriving at their breeding colony, seabirds migrating to PLI often occupy areas with known natural oil and gas seeps, particularly during spring staging in the Baffin Bay – Davis Strait and Hudson Strait regions[76–78]. The timing of egg-laying may therefore influence their contaminant burdens, as fulmars typically lay their eggs earliest (late April to early May)[79], followed by murres (late June to early July)[80], with kittiwakes laying last (early to mid-July)[81]. Fulmars arrive and forage for nutrients to form eggs earlier than the other species, and may be constrained to forage in eastern Baffin Bay near Greenland where there is open water, as areas near PLI are often still ice-covered in May and early June[82]. Consequently, fulmars may spend less time in regions with natural seeps during migration, potentially reducing their exposure prior to egg laying. In contrast, since kittiwakes lay their eggs later, they may spend more time in the western Baffin Bay-Davis Strait, where currents would move PACs from seeps south along Lancaster Sound[55], potentially increasing their exposure to PACs from natural seeps before arriving at PLI.

Since local PAC sources are unlikely to remain constant, seasonal variations from natural seeps or anthropogenic inputs may also contribute to interspecific differences in egg contamination. Although fluctuations in natural seep activity in this region are not well understood, vessel traffic, including shipping and tourism, generally starts in June [83,84] in the Low Arctic; few ships enter Lancaster Sound before early August[84,85]. Fulmars are already at PLI by early May, murres soon after, and then kittiwakes arrive in numbers in June. We speculate that the seasonal rise in marine shipping activity may result in greater environmental PAC emissions, potentially contributing to the higher PAC concentrations observed in kittiwakes recently

migrating through these more active regions, as this species migrates north and lays later in the season.

Species-specific differences in egg size, lipid content, and composition may have influenced the extent of PAC contaminant burdens in eggs[20]. Interspecies differences in PAC concentrations may also reflect variation in each species' ability to metabolize, detoxify, and excrete contaminants[45]. For example, fulmars may metabolize and excrete PACs more efficiently than kittiwakes and murres, potentially explaining their unexpectedly low PAC concentrations in eggs. However, these relationships have not been examined to date.

In addition to PAC concentrations, we also analyzed PAC profiles in eggs across species. For homocyclic PACs, AHMW compounds contributed most to total concentrations in fulmar and kittiwake eggs. Mean concentrations of Σ_{10}AHMW were 23.26 ng/g in fulmars and 93.98 ng/g in kittiwakes, accounting for 70% and 64% of the total PAC concentrations in each species, respectively. In contrast, PHMW was the dominant group in murre eggs with a mean Σ_{10}PHMW concentration of 80.05 ng/g, representing 57% of total homocyclic PACs. Nitrogen-containing HPACs were the dominant group of HPACs for both fulmars and kittiwake eggs, comprising 94% in fulmars and 92% in kittiwakes.

To examine PAC profiles more closely, we compared the relative abundances of unsubstituted (C0) and alkylated (C1-C4) PACs (Fig. 3). Greater similarity was still observed in the PAC profiles of eggs from kittiwakes and fulmars, both dominated by C2 compounds. Although murre eggs still contained a considerable amount of C2 compounds, their relative abundance was approximately half that of the other two species, with murres instead showing higher abundances of C0 and C1 compounds. A more detailed distribution of individual

compounds (Fig. 4) further confirms these patterns. C2 chrysene was the most abundant compound across all species, however, its relative abundance in murres was approximately half the relative abundance compared to fulmars and kittiwakes. Additionally, murre eggs also contained unsubstituted PAHs – specifically phenanthrene, pyrene, and chrysene – which were absent in the other two species. Heterocyclic compounds also showed variation in eggs among species. Aside from indole and its derivatives, 3,6-dichlorocarbazole was most abundant in fulmars, while 2,8-dibromodibenzothiophene dominated in kittiwake eggs.

Murres had a markedly different PAC profile than the surface-feeding species: compounds like phenanthrene, pyrene, and chrysene may have been more concentrated below the surface, which could explain their higher proportional contribution in murres. However, temporal variation must also be considered, as the staggered timing of egg-laying may expose each species to different PACs due to fluctuations in regional activity. For instance, since PHMW compounds are typically indicative of pyrogenic sources[66], pyrogenic activity in the area may have been greater while murres were forming their eggs, potentially explaining their higher abundance of these compounds in their profiles. Physiological factors, such as metabolic capabilities and maternal transfer efficiency, may also influence species-specific egg PAC profiles. For example, C2 chrysene may be readily transferred to eggs in kittiwakes and fulmars, while murres may either metabolize this compound more effectively or transfer it less efficiently to their eggs, resulting in its lower relative abundance. However, this highlights an area that requires additional research.

The interspecific differences of PACs in eggs were visualized in the LDA plots in Figures 4.5 and 4.6. While our previous observations suggested that fulmars and kittiwakes were fairly

similar, the homocyclic PAC model (Fig. 5) showed clear separation among all species, with C3 phenanthrene as the main driver of this variation, particularly separating fulmar eggs. The heterocyclic PAC model (Fig. 6) also revealed significant differences between fulmars and kittiwakes, indicated by the substantial distance between the groups. In this model, C1 and C3 dibenzothiophene were the primary contributors to the separation of fulmar egg PAC profiles from the other two species, while C2 dibenzothiophene, carbazole and isoquinoline were most influential for separating kittiwake eggs from the other species.

4.4.2.2 Liver and Egg Tissue Comparison

Concentrations of both homocyclic and heterocyclic PACs were significantly higher in liver tissue than in eggs across species. This is consistent with the liver's role as the primary site for contaminant metabolism[85]. Adult seabirds are exposed to PACs through various pathways, including through ingestion, inhalation, and dermal absorption[64,86]. Due to its relatively high lipid content, the liver is especially prone to accumulating high levels of lipophilic compounds such as PACs. In contrast, PAC concentrations in eggs were much lower. Maternal transfer represents a major route of contaminant exposure to eggs[20], although it is likely that only a small portion of the female's contaminant burden is transferred. However, the extent and efficiency of maternal transfer, particularly for PACs in seabirds, is currently poorly understood.

Interestingly, species-specific trends in PAC concentrations differed between tissues across all species. We initially expected that the species with the highest liver concentrations would also have the highest concentrations in eggs, but this was not what we observed. The most notable example was fulmars, which had the highest PAC concentrations in liver tissue but the

lowest in eggs. When collection timing was consistent across species, as with liver samples, foraging ecology appeared to be the primary driver of PAC exposure. In contrast, for eggs, which were laid at different times, the timing of exposure likely played a more significant role, as PAC concentrations were higher with later egg-laying. Additional, species-specific differences in metabolic processing or maternal transfer efficiency may also contribute to the observed discrepancies, however, these physiological processes remain poorly understood in seabirds and require further research.

Differences were also evident in the specific compounds that predominated in each tissue. In livers from all species, homocyclic PACs were primarily AHMW. In contrast, eggs from fulmars and kittiwakes were dominated by ALMW compounds and from murres by PHMW compounds. The most abundant single homocyclic compound also varied between tissues. In all liver samples, C3 fluorene was the most abundant, whereas in eggs, C2 chrysene is the most abundant in kittiwakes and fulmars and benzo[a]pyrene was most abundant in murres.

Although PANH compounds consistently dominated both livers and eggs, heterocyclic PAC profiles also showed variation between tissues. Indole was the most abundant single heterocyclic compound across all samples, however, since indole is produced by biological processes and is ubiquitous in the environment, it may not be as indicative of tissue-specific accumulation as other compounds[87,88]. Interestingly, there were some HPACs that were detected consistently across all egg samples but were not detected in livers. For example, 3,6-dichlorocarbzole was present in all fulmar eggs (mean value: 79.97 ng/g) but was not detected in any fulmar livers. Similarly, 2,8-dibromodibenzothiophene was consistently detected in kittiwake eggs (mean value: 38.11 ng/g) but was detected in only 5 of the 11 livers, in much lower

concentrations (mean value: 3.33 ng/g). This suggests that the birds may be metabolising these chemicals in the livers, and liver tissues are not ideal for monitoring such compounds, whereas eggs provide a snapshot of the mother's exposure when she was forming the egg, and thus may be better proxy for these types of compounds.

We suspect that temporal and spatial drivers contributed to the observed variation in PAC profiles between tissues. For example, eggs were laid at different times for each species (up to a month earlier by fulmars compared to kittiwakes) which potentially represents different dietary items, foraging locations and thus PAC exposure. In contrast, liver samples were collected simultaneously across species, and interestingly we observed greater similarity in their PAC profiles compared to eggs. As mentioned earlier, eggs can reflect both recent maternal exposure as well as some longer-term accumulation, whereas livers generally reflect only recent exposure[61–64,75]. Therefore, livers are likely primarily indicative of local exposure near the PLI breeding colony, whereas eggs may reflect both local and overwinter exposure. Some compounds may only have been present in overwintering areas rather than at PLI, which could explain why some compounds were detected in eggs but were absent or present in much lower concentrations in livers.

The tissue-specific differences in PAC profiles may also reflect potential selectivity in the maternal transfer of individual compounds, likely influenced by their physicochemical properties. For example, C2 chrysene, a four-ring AHMW compound, was found in high concentrations in eggs but was much less abundant in livers. Generally, LMW PACs are more easily metabolized, while HMW PACs are more resistant to degradation[89]. Because of its larger molecular weight, a compound like C2 chrysene is more difficult to metabolize and may be more

likely offloaded into eggs[90]. Compound lipophilicity may also influence transfer efficiency, as female seabirds are believed to transfer lipophilic contaminants more easily to their eggs[91]. Given the higher lipid content of eggs (8.9 ± 1.7 %) compared to livers (4.0 ± 1.2 %), lipophilic PACs that are not readily metabolized may preferentially accumulate in eggs over liver tissue; this would mirror the pattern observed in persistent organic pollutants in Arctic seabird livers and eggs[18].

The observed tissue-specific variation was further supported by our LDA plots, as eggs and livers were clearly separated along the discriminant axes. Additionally, while livers show more consistency, there is more variation in eggs both between and within species, as indicated by the size and overlap of the ellipses. Both the homocyclic and heterocyclic models (Figs. 5,6) show extensive overlap among livers, indicating largely similar profiles, while egg samples show greater separation and larger ellipses, reflecting more inter- and intra-species variation.

Female northern fulmar matching liver and egg

Although our analysis included eggs and livers from the same species and breeding site, the majority of these were not from matching birds. We had one matching pair of a female and her egg to examine. Overall, the results from this matched liver and egg pair largely aligned with many of our findings from the broader tissue comparison. Concentrations of homocyclic and heterocyclic PACs were substantially higher in the liver (4211.20 ng/g and 16877.46 ng/g, respectively) compared to the egg (18.87 ng/g and 283.59 ng/g, respectively). Additionally, the liver was dominated by ALMW PACs (84% of homocyclic PACs), while the egg had a higher proportion of AHMW compounds (92%). C3 fluorene (2820.75 ng/g) and C2 chrysene (13.47

ng/g) were the most abundant homocyclic compounds in the liver and egg, respectively. Both tissues also had PANH as the dominant HPAC group, consistent with previous observations.

This matching pair also revealed some notable compound-specific differences between PACs in tissues (Fig. S1). With the exception of benzo[b]fluoranthene (1.60 ng/g), all homocyclic compounds were present in higher concentrations in this individual's liver sample compared to its egg. Interestingly, benzo[b]fluoranthene was also identified as the most efficiently transferred PAC in a previous study investigating maternal transfer of PACs in guitarfish[92], suggesting there may be specific properties of this compound that facilitate its efficient transfer to eggs. Several heterocyclic compounds were detected in relatively high concentrations in the egg, including 3,6-dichlorocarbzole (33.00 ng/g) and 2,8-dibromodibenzothiophene (31.66 ng/g), but were non-detectable in the liver.

4.5 Conclusions

Overall, our study revealed considerable interspecies and tissue-specific differences in PAC concentrations and profiles among northern fulmars, black-legged kittiwakes, and thick-billed murres, highlighting the complexity of contaminant exposure in Arctic seabirds. While broad similarities in PAC profiles suggested shared exposure to many compounds during the breeding season or overwintering, potentially influenced by natural seeps in these areas, closer examination revealed distinct species-specific differences, particularly between murres and fulmars/kittiwakes. Specifically, when comparing livers among species, foraging behaviour appears to explain much of the variation we observed in both PAC concentrations and profiles. However, for eggs, the staggered laying times of each species appeared to greatly influence

differences in both the extent of PAC exposure and the specific compounds accumulated. This study contributes to the limited understanding of maternal transfer of PACs in seabirds, a process that represents an important exposure route for developing eggs. With industrial activity and vessel traffic increasing in the Arctic region, the risk of PAC contamination in both seabirds and their eggs is expected to rise. The detection of heterocyclic compounds at concentrations comparable to those of homocyclic PACs highlights the importance of expanding monitoring efforts to include a wider range of PACs and better assess their potential impacts on Arctic wildlife.

4.6 Acknowledgements

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Table 4.1 Summarized performance characteristics for the extraction and quantitation of polycyclic aromatic compounds (PACs) in biota using accelerated solvent extraction and gas chromatography-tandem mass spectrometry.

Group of Compounds	**Accuracy (%)**			**Precision (%RSD)**			**Intermediate Precision (%RSD)**	**LOD (ng/g)**	**LOQ (ng/g)**
	10 pg/ µL	100 pg/ µL	500 pg/ µL	10 pg/ µL	100 pg/ µL	500 pg/ µL			
PAHs	75-120[a]	93-112	94-119	<17.3[b]	<10.5	<9.7	<31.0[c]	0.03-0.24	0.11-0.81
APAHs	70-144	76-140	79-146	<22.2[d]	<18.3	<17.2	<38.0[e]	0.03-0.23	0.09-0.75
HPACs	53-119	50-114	57-120	<38.0	<29.1	<30.3	<47.6[f]	0.02-0.17	0.07-0.57
Halo-HPACs	66-125	74-120[g]	62-120[h]	<14.9	<15.9	<8.9	<45.6	0.02-0.08	0.06-0.26

[a] exceptions for PAH accuracy at low level: pyrene (153), fluoranthene (130)

[b] exception for PAH low level precision: naphthalene ((35.6)

[c] exception for PAH intermediate precision: naphthalene (45.6)

[d] exception for APAH low level precision: C2-fluorene (46.7)

[e] APAH intermediate precision for 27 of the 33 compounds

[f] exception for HPAC intermediate precision: indole (104.4)

[g] exception for Halo-HPAC medium level precision: 3-bromoisoquinoline (136)

[h] exception for Halo-HPAC high level precision: 3-bromoisoquinoline (139)

Table 4.2 Mean concentrations (ng/g, lipid weight) of homocyclic and heterocyclic PACs detected in three seabird species-black-legged kittiwakes (*Rissa tridactyla*, BLKI), northern fulmars (*Fulmarus glacialis*, NOFU), and thick-billed murres (*Uria lomvia*, TBMU)-from Prince Leopold Island in 2023.

Homocyclic PACs							
Species	Sample Type	Σ_{43}PACs	Σ_{16}PAHs	Σ_{6}PLMW	Σ_{17}ALMW	Σ_{10}PHMW	Σ_{10}AHMW
Northern fulmar	Liver (n=10)	3306.19	188.72	5.34	2702.24	183.38	415.22
	Egg (n=11)	33.36	1.73	0.00	8.37	1.73	23.26
Black-legged kittiwake	Liver (n=10)	2120.04	63.33	8.60	1682.35	54.73	374.36
	Egg (n=10)	147.17	3.03	2.93	50.15	54.73	93.98
Thick-billed murre	Liver (n=10)	1346.44	102.42	67.39	945.31	35.03	298.72
	Egg (n=5)	141.64	91.21	11.15	8.24	80.05	42.19
Heterocyclic PACs							
Species	Sample Type	Σ_{43}HPACs	Σ_{42}HPACs (no indole)	Σ_{23}PANHs	Σ_{22}PANHs (no indole)	Σ_{16}PASHs	Σ_{4}PAOHs
Northern fulmar	Liver (n=10)	4337.15	1091.67	4125.64	880.16	211.20	0.32
	Egg (n=11)	459.10	140.37	431.27	112.54	27.29	0.54
Black-legged kittiwake	Liver (n=10)	2318.76	794.99	2125.24	601.48	193.43	0.09
	Egg (n=10)	661.25	130.90	606.32	75.97	54.92	0.00
Thick-billed murre	Liver (n=10)	1587.88	794.14	1312.29	518.55	274.87	0.72

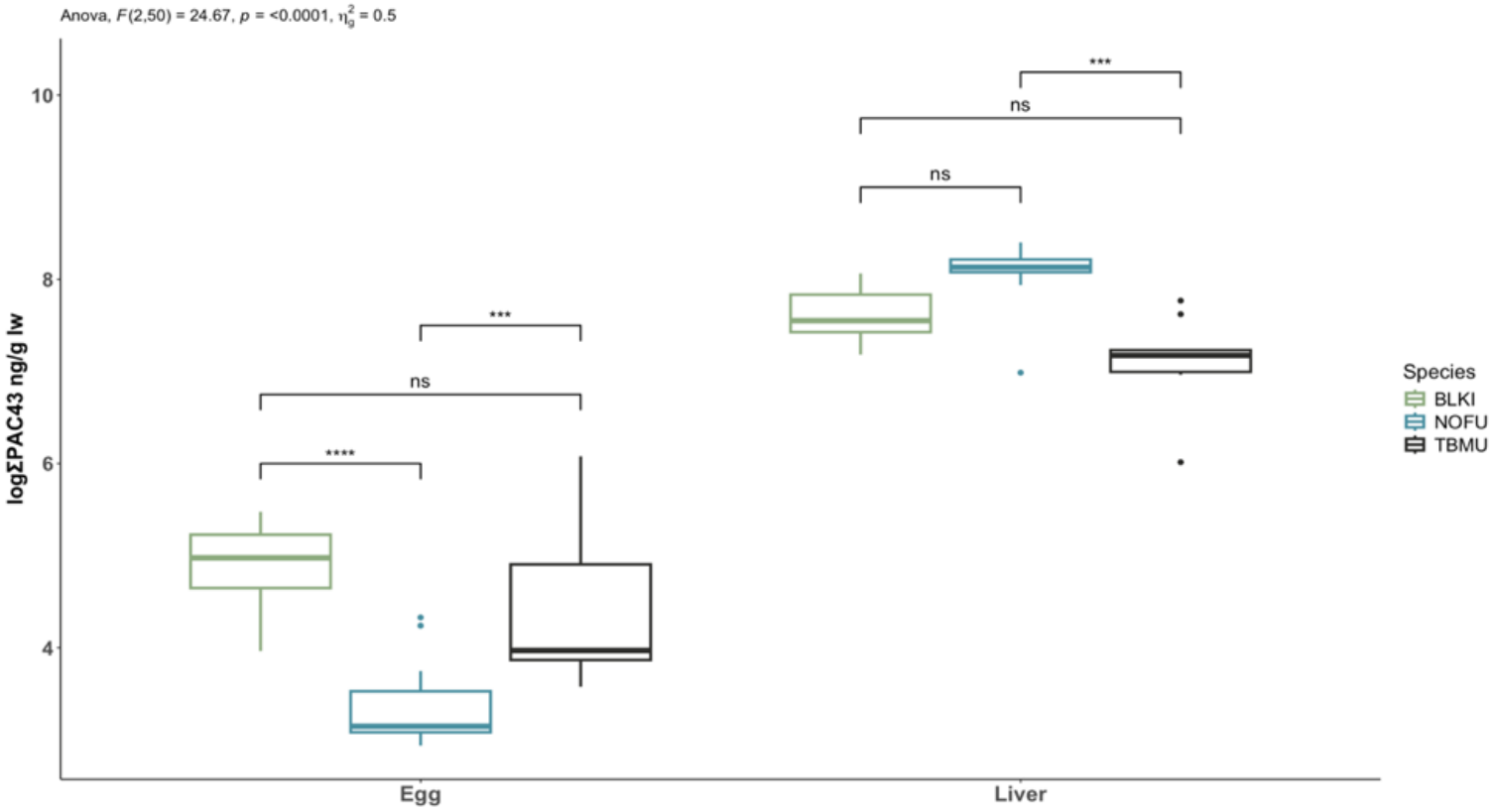

Figure 4.1 Box plots of homocyclic PAC concentrations in three seabird species-black-legged kittiwakes (*Rissa tridactyla*, BLKI), northern fulmars (*Fulmarus glacialis*, NOFU), and thick-billed murres (*Uria lomvia*, TBMU)-from Prince Leopold Island in 2023.

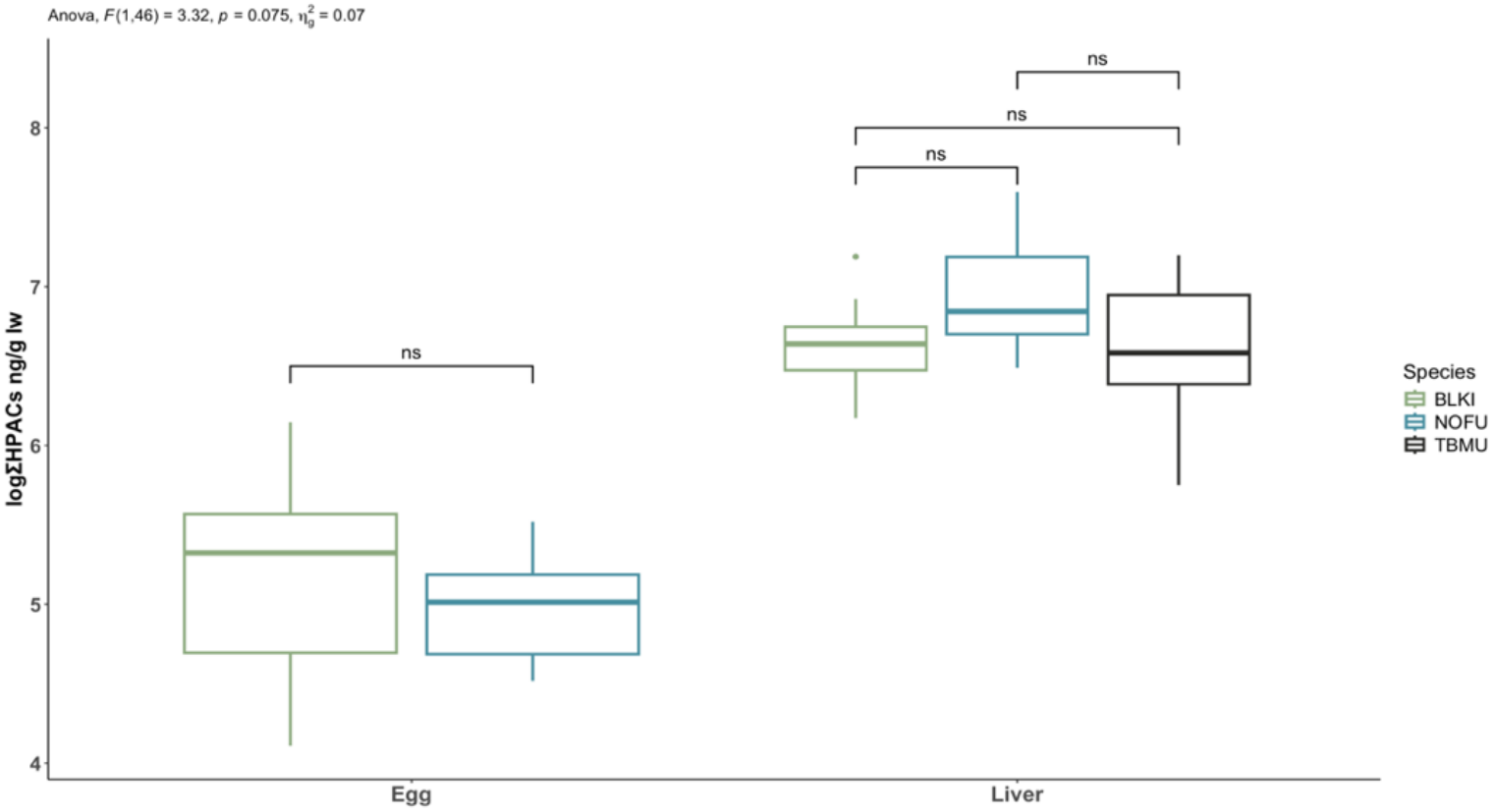

Figure 4.2 Box plots of heterocyclic PAC concentrations in three seabird species-black-legged kittiwakes (*Rissa tridactyla*, BLKI), northern fulmars (*Fulmarus glacialis*, NOFU), and thick-billed murres (*Uria lomvia*, TBMU)-from Prince Leopold Island in 2023.

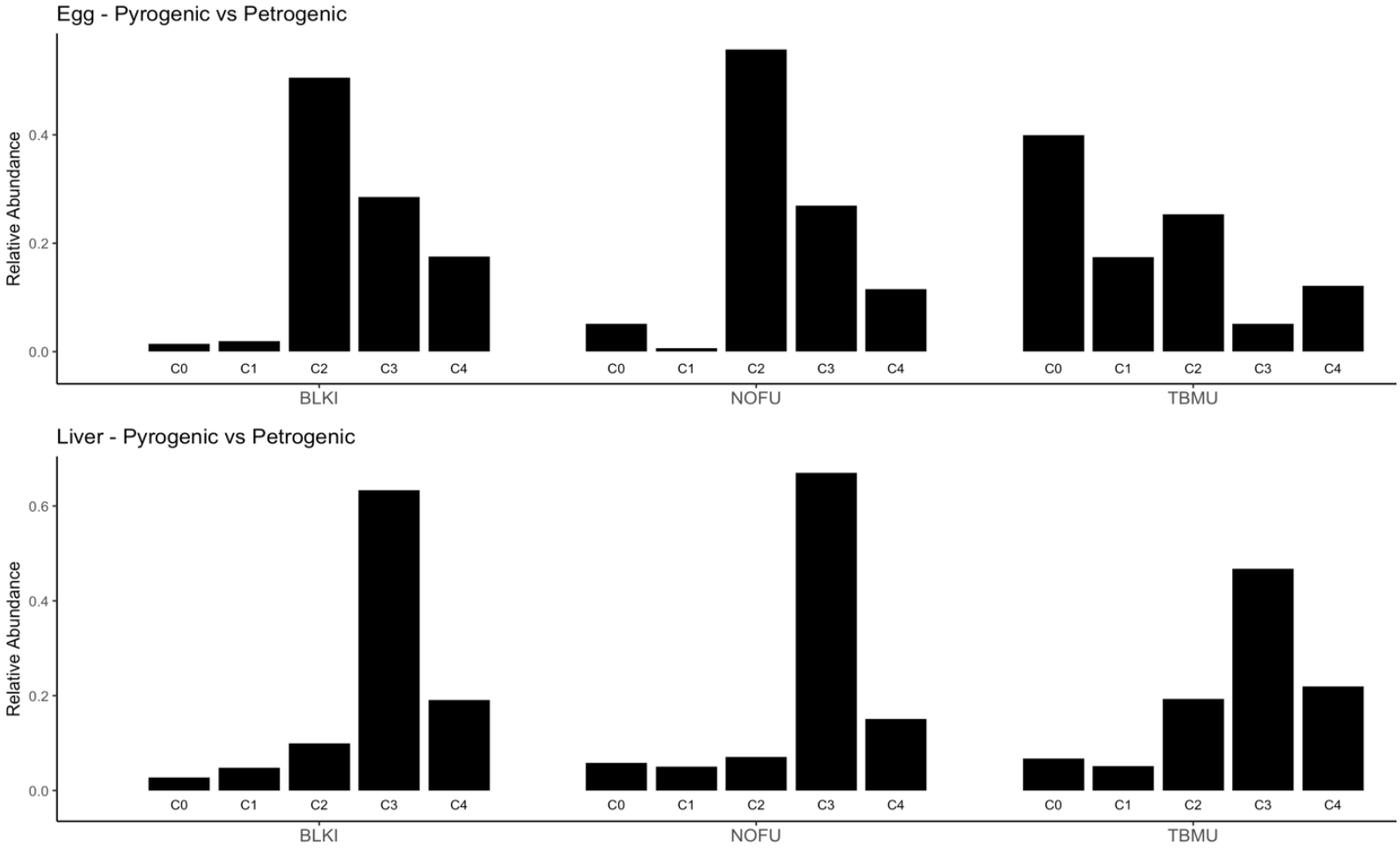

Figure 4.3 Relative abundance of parent (C0) and alkylated (C1-C4) polycyclic aromatic compounds in eggs and livers of three seabird species-black-legged kittiwakes (*Rissa tridactyla*, BLKI), northern fulmars (*Fulmarus glacialis*, NOFU), and thick-billed murres (*Uria lomvia*, TBMU)-from Prince Leopold Island in 2023.

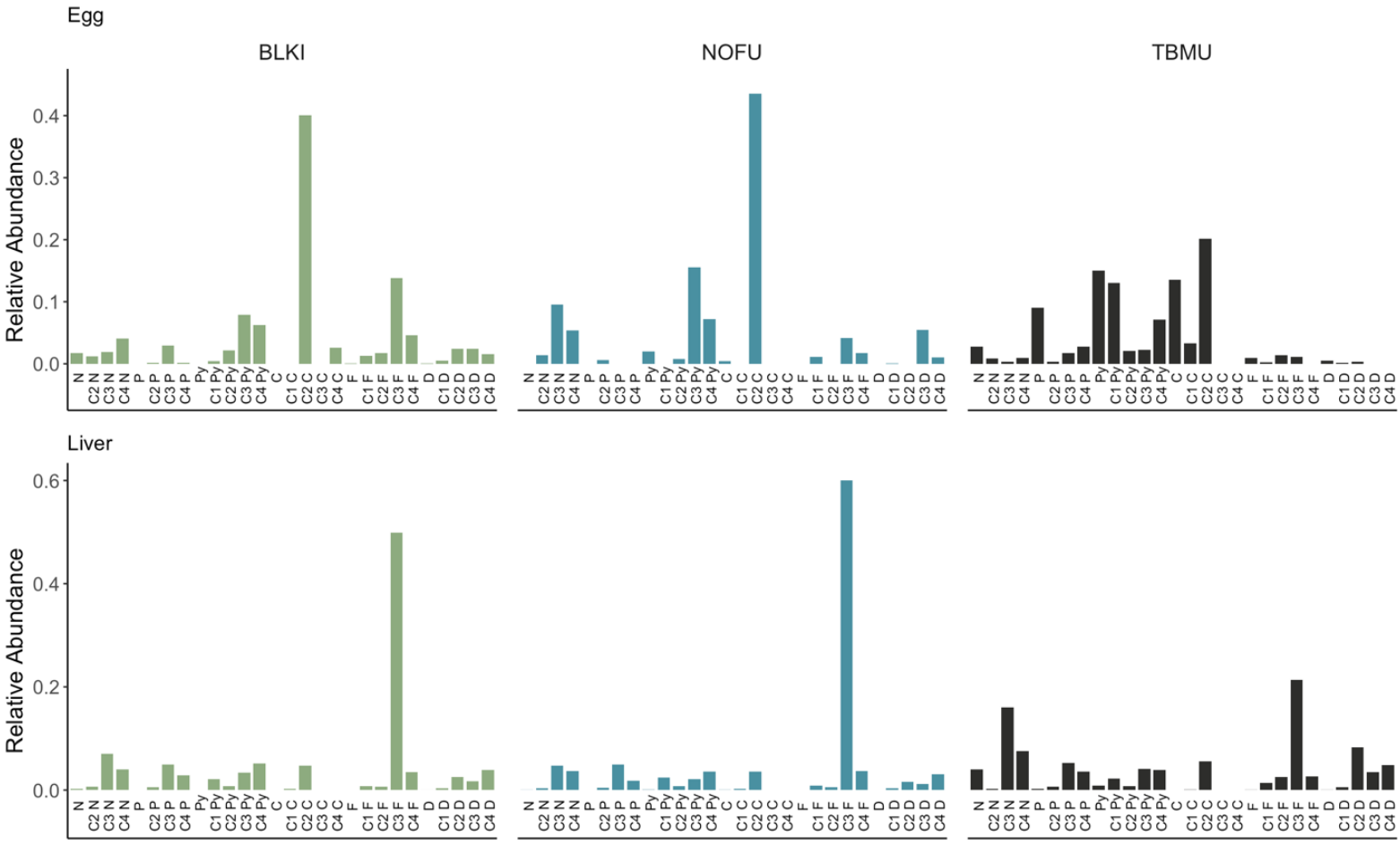

Figure 4.4 Relative abundance of unsubstituted (C0) and alkylated (C1-C4) polycyclic aromatic compounds in eggs and livers of three seabird species-black-legged kittiwakes (*Rissa tridactyla*, BLKI), northern fulmars (*Fulmarus glacialis*, NOFU), and thick-billed murres (*Uria lomvia*, TBMU)-from Prince Leopold Island in 2023. Relative abundance is presented for naphthalene (N), phenanthrene (P), pyrene (Py), chrysene (C), fluorene (F) and dibenzothiophene (D).

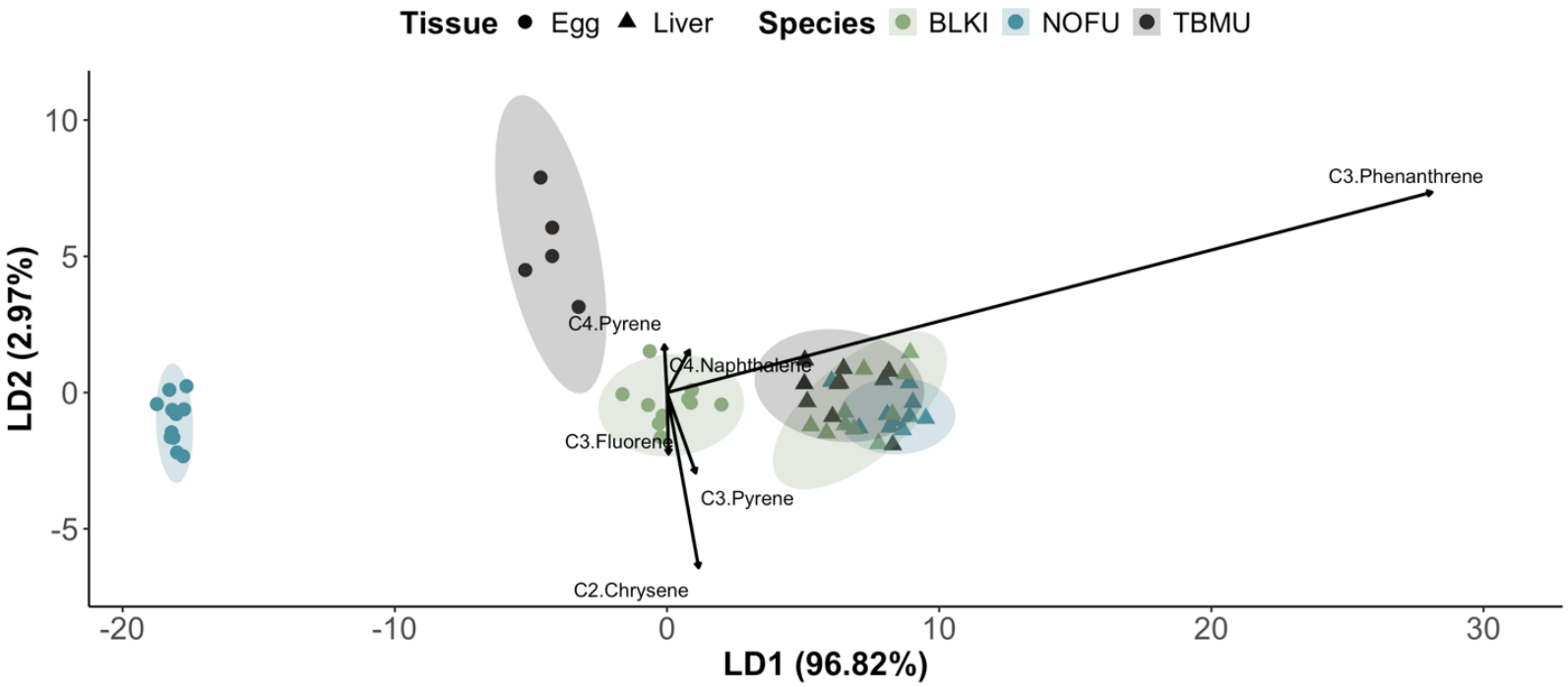

Figure 4.5 Linear discriminant analysis for homocyclic PACs in black-legging kittiwake (BLKI), northern fulmar (NOFU) and thick-billed murre (TBMU) livers and eggs from Prince Leopold Island in 2023.

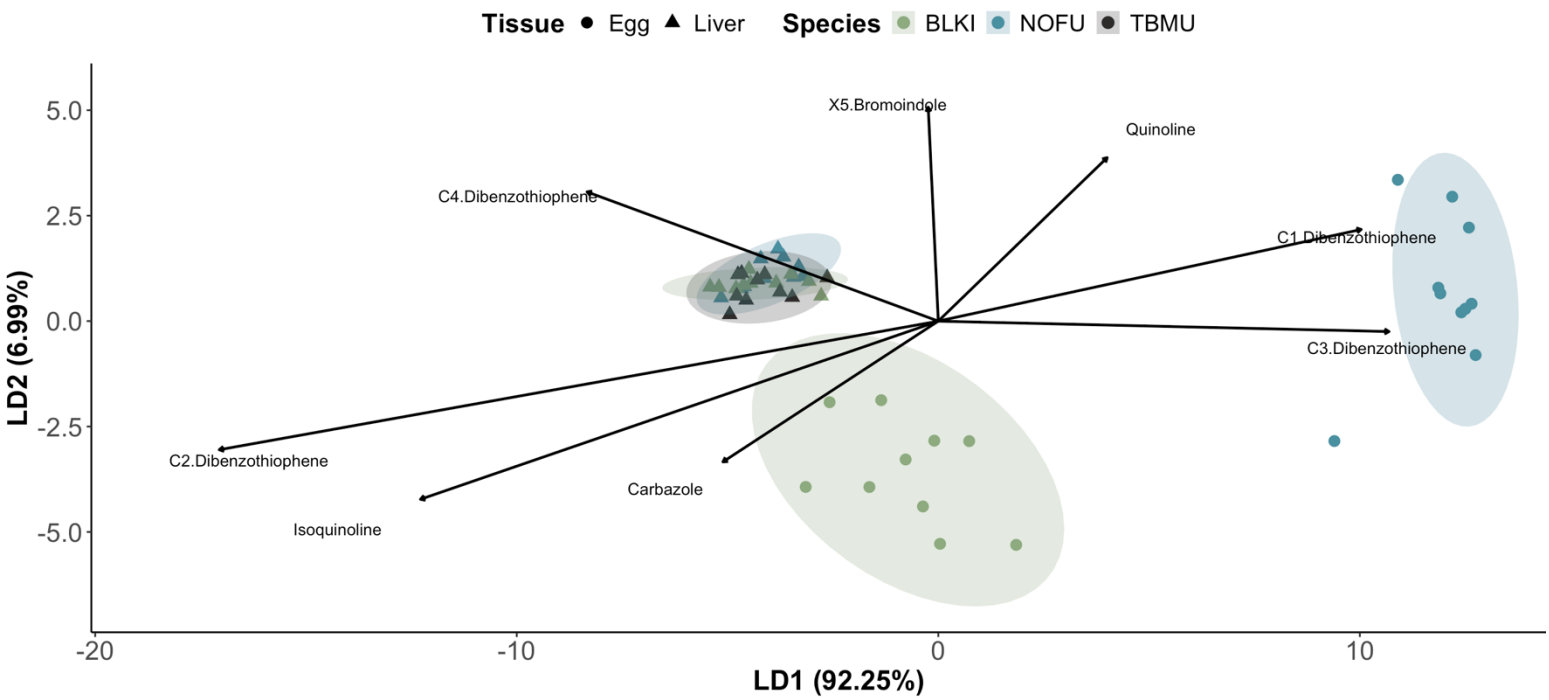

Figure 4.6 Linear discriminant analysis for heterocyclic PACs in black-legging kittiwake (BLKI), northern fulmar (NOFU) and thick-billed murre (TBMU) livers and eggs from Prince Leopold Island in 2023.

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Chapter 5: Conclusions and Future Directions

5.1 Conclusions

The overall goal of my research was to contribute to ongoing environmental monitoring efforts in the Canadian Arctic using seabirds and their eggs as bioindicators of PAC contamination. This thesis presented two complementary studies: a long-term temporal and spatial analysis, and an investigation of species- and tissue-specific PAC accumulation. Together, the results from these studies revealed numerous trends in PAC concentrations and profiles, highlighting the complexity of petroleum-based contamination in this region.

The long-term temporal and spatial analysis of PACs presented in Chapter 3 revealed considerable variation in both concentrations and profiles between locations and among individual years. Interannual variation indicated fluctuations in PAC emissions, as reflected by shifts in the dominant compound groups detected over time. Spatial variation was also apparent, as PAC concentrations were significantly higher in eggs collected from seabirds breeding in the high Arctic versus the low Arctic. This long-term analysis helps address a significant knowledge gap and provides a benchmark for historic and current PAC levels to help track future trends.

The second study included in this thesis, presented in Chapter 4, focused on PAC accumulation in the livers and eggs of three species of seabirds collected from Prince Leopold Island. This analysis revealed species- and tissue-specific differences in PAC concentrations and profiles, likely reflecting differences in exposure, accumulation, and metabolism, driven by a combination of foraging ecology, breeding phenology, and physiological factors. The detection of heterocyclic compounds at concentrations comparable to those of homocyclic PACs also

highlights the importance of expanding monitoring efforts to include a wider range of PACs and better assess their potential impacts on Arctic wildlife.

While natural seeps are believed to be the predominant contributors to the overall PAC burdens in seabirds, as indicated by the high abundance of petrogenic compounds across both studies, the presence of pyrogenic PACs suggested anthropogenic activity also represents a significant source of these compounds. With expected increases in industrial activity in the Arctic in coming years, notably shipping and mining, the risk of PAC contamination with anthropogenic origins is a growing concern in this region. The work included in this thesis aimed to contribute to monitoring efforts intended to inform future decision-making regarding oil and gas contamination to ensure the protection of vulnerable Arctic ecosystems.

5.2 Future Directions

As interest in shipping and industrial development in Canada's Arctic continues to grow, ongoing monitoring of PACs will be essential to detect and assess changes in contamination that may pose risks to Arctic wildlife and their environments. Future efforts should focus on the continued collection and analysis of PACs in seabirds to build on and strengthen existing monitoring programs. Given that many of the higher PAC concentrations were observed in the last decade, continued monitoring is particularly important as climate change accelerate sea ice melt and intensifies wildfire activity-both of which are expected to elevate PAC levels in the coming years.

Future research can expand on the work from this thesis in several important areas. To better understand maternal transfer of PACs in seabirds, future studies should aim to include more direct comparisons between maternal seabird livers and their eggs. By prioritizing the collection of matching samples, additional studies can expand on the preliminary findings of Chapter 4. Given the spatial, species-specific, and tissue-specific variation we observed, future monitoring efforts should also aim to incorporate multiple locations, species, and tissue types. Broadening the range of PACs analyzed in future studies, such as including HPACs, is essential for a more comprehensive understanding of the diverse group of compounds present in the Arctic environment and should also be prioritized. Finally, there is a need for more targeted toxicological studies, particularly focusing on heterocyclic compounds and those that are consistently detected in high concentrations in Arctic biota. Toxicological studies on these compounds are essential for understanding their ecological impacts, as many have the potential to pose significant risks to marine wildlife and their environments.