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The Impact of Toxic Elements and Persistent Organic Pollutants on Avian Influenza Prevalence in Arctic Seabirds

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Cover Photo: Blomstrandhalvøya black-legged kittiwake colony (Megan Lee)

Abstract

As climate change alters northern environments, the dynamics of Arctic disease are predicted to undergo shifts. These shifts may also be influenced by the immunomodulatory properties of anthropogenic contaminants present in the Arctic. This study aimed to investigate the relationship between the prevalence of a climate-sensitive disease and concentrations of pollutants in an Arctic seabird.

We collected plasma samples from black-legged kittiwakes *(Rissa tridactyla)* breeding at Kongsfjorden, Svalbard. These samples were analyzed for toxic elements and screened for the avian influenza virus (AIV). Samples taken in previous years from the same kittiwake population, which had been already been analyzed for toxic elements and/or persistent organic pollutants (POPs), were also screened for the virus. Logistic regression models were developed to relate concentrations of contaminants to the probability of AIV infection in these birds. Samples from Svalbard glaucous gulls and Norwegian raptors were also screened for AIV.

Evidence of AIV infection was found in black-legged kittiwakes in 2014 and 2015, but not in 2017. The best-fit logistic regression model created with data from 2015 included arsenic, selenium, and mercury as significant explanatory variables. Arsenic and selenium increased the probability of infection while mercury decreased it. Further models created from combined 2014 and 2015 data did not show any significant influence of POPs over the probability of infection. AIV was also found in glaucous gulls, but not in raptors.

To our knowledge, this is the first evidence of AIV on Svalbard. The presence of the virus in multiple avian species emphasizes the need for broader screening efforts. Out of all studied contaminants, arsenic and selenium seem to pose the greatest risk for increasing AIV infection in kittiwakes. Mercury seems to decrease this risk, perhaps due to an antagonistic effect with selenium. This study did not find any influence of POPs over AIV infection, possibly because kittiwakes face relatively low levels of POP exposure.

Further research is needed to establish baseline knowledge about AIV on Svalbard and to clarify the relationships between pollutants and infection. This information is necessary to detect and predict shifts in AIV dynamics that may put wildlife at risk.

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List of Abbreviations

AIC	Akaike information criterion
AIV	avian influenza virus
As	arsenic
Cd	cadmium
CHL	chlordane
***β*-HCH**	*beta*-hexachlorocyclohexane
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
HCB	hexachlorobenzene
Hg	mercury
ICP-MS	inductive coupled plasma mass spectrometry
LOD	limit of detection
OCP	organochlorine pesticide
Pb	lead
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
POP	persistent organic pollutant
***p,p'*-DDE**	*para,para*-dichlorodiphenyldichloroethylene
RBC	red blood cell
Se	selenium
ww	wet weight

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1. INTRODUCTION

1.1. Climate Change and its Effect on Arctic Disease Dynamics

In no place on Earth are the effects of climate change more visible than in the Arctic. Although warming trends have been observed globally, the average annual temperature increase since 1980 has been twice as high in the Arctic as in the rest of the world (AMAP, 2011). A variety of environmental responses have already been observed, including permafrost thawing, sea ice retreat, and the northward movement of the tree line (AMAP, 2009). As increasing temperatures reshape the physical environment of the Arctic, local ecosystems are likely to be impacted in a wide variety of ways. Novel shifts in Arctic disease dynamics are one predicted effect of warming trends (Burek et al., 2008, Harvell et al., 2002, Hueffer et al., 2013, Bradley et al., 2016).

1.1.1. Mechanisms and Possible Effects of Changing Disease Dynamics

There are several mechanisms by which Arctic host-pathogen relationships could be altered by climate factors. Firstly, the impacts of climate change on wildlife ecology may increase disease transmission rates (Burek et al., 2008, Harvell et al., 2002). Climate-driven habitat loss could facilitate transmission by increasing population densities. For example, a decline in suitable sea ice may lead to increased crowding at seal haul-outs (Burek et al., 2008). Other ecological shifts—such as host range expansions, altered feeding patterns, and behavioral adaptations—may expose species to new or altered pathogen transmission routes and thereby increase rates of infection (Burek et al., 2008).

Climate change may also create environmental conditions that promote pathogen survival and development (Burek et al., 2008, Harvell et al., 2002, Hueffer et al., 2013). This is especially true for vector-borne diseases, which are carried by parasites that are typically temperature-limited (Bradley et al., 2016, Harvell et al., 2002). Warmer, wetter conditions in the Arctic could limit winter die-offs of parasite species and infected hosts; increase rates of parasite development and replication; and allow vector species to survive areas that were previously too harsh (Bradley et al., 2016, Harvell et al., 2002, Hueffer et al., 2013).

Finally, host ability to respond to disease may be impacted by climate-induced stressors. Poor nutrition is a known cause of immunosuppression in many mammals, and Arctic species are likely to face nutritional stress as food web relationships shift (Burek et al., 2008). Changes in

exposure to toxicants may also impact the capabilities of the host immune system (Bradley et al., 2016, Burek et al., 2008), a possibility which will be further addressed in Section 1.2.

One or more of these mechanisms could shift host-pathogen relationships to increase disease rates, which can ultimately result in population declines and contribute to extinctions (Descamps et al., 2012, Harvell et al., 2002). Another possible outcome is the expansion of diseases into ranges where they have not previously been detected (Bradley et al., 2016, Burek et al., 2008, Harvell et al., 2002). Evidence linking disease expansion to climate variables has been found in Hawaii, where shifting environmental conditions promoted the spread of avian malaria into regions where the disease had previously been limited by cool temperatures (Atkinson et al., 2014). Range expansion may be a particular threat to Arctic species, as the introduction of a pathogen into an immunologically naïve population can cause serious disease outbreaks (Burek et al., 2008).

1.1.2. The Significance of Arctic Avian Species

Climate change is expected to affect the ecology, distribution, and migratory behaviors of wild birds (Gilbert et al., 2008). Shifts in migration patterns would provide a mechanism by which pathogens could be transported to new areas (Reed et al., 2003) and redistributed among avian populations (Gilbert et al., 2008). High densities and diversities of avian species along flyways and pre-migration staging areas provide opportunities for pathogen transmission (Gilbert et al., 2008).

Arctic birds are highly migratory. Among western European birds, 83% of breeding species at 80°N are only present in summer months and just four species (the northern fulmar *Fulmarus glacialis*, glaucous gull *Larus hyperboreus*, ivory gull *Pagophila eburnea*, and ptarmigan *Lagopus mutus*) do not migrate to more southerly latitudes in the winter (Newton and Dale, 1996). This high degree of contact with temperate populations make birds likely carriers of disease to northern environments.

Evidence suggests that shifts in Arctic avian disease are already occurring. Ticks infected with Lyme disease-causing *Borrelia* spirochetes were recently found on seabirds in Northern Norway (Larsson et al., 2007), while the discovery of *Plasmodium* in blood from birds resident in Alaska shows that avian malaria occurs as far north as 64°N in North America (Loiseau et al., 2012). Changes in disease dynamics may have serious consequences for bird populations. Research on a colony of common eiders (*Somateria mollissima*) in Arctic Canada demonstrated

that serious epidemics of avian cholera, when occurring at rates that have been observed in nature, have the potential to cause colony extinction (Descamps et al., 2012).

However, population level effects of climate-driven disease shifts are difficult to predict. This is in part because the impacts of climate change on avian ecology and host-pathogen dynamics are multifaceted and interrelated, but also because baseline data on disease prevalence in Arctic bird species is lacking (Harvell et al., 2002, Hueffer et al., 2013). This knowledge is necessary to detect changes in disease patterns and accurately determine the risks faced by wild bird populations.

For this study, I focused on the avian influenza virus (AIV) as the pathogen of interest. This virus was chosen for its dependence on birds as reservoir hosts, its potential to impact Arctic species, and its predicted sensitivity to climactic shifts.

1.1.3. Avian Influenza

Avian influenza is so-called because wild aquatic birds serve as its main reservoir of genetic diversity, carrying all known glycoprotein-classified subtypes of the virus in all possible combinations (Alexander, 2007, Boyce et al., 2009, Olsen et al., 2006).

AIV causes little to no disease in wild birds (Alexander, 2007, Boyce et al., 2009, Olsen et al., 2006). This seems to be an adaptation to the migratory life histories of its hosts, as acute symptoms would interfere with migration and lower the chances of viral transmission over long distances (Gilbert et al., 2008). Geese and ducks have the highest infection rates (Alexander, 2007). The predominating viral subtype in gulls, H13, is not one of the dominant subtypes in waterfowl and is not often found in waterfowl or other birds (Kawaoka et al., 1988, Olsen et al., 2006, Webster et al., 1992). The limited ability of gull viral subtypes to replicate in ducks (Olsen et al., 2006) also suggests that gulls and shorebirds act as a reservoir for a separate AIV gene pool.

Although AIV is not likely to cause disease in wild birds, it can cause destructive symptoms in mammals (Webster et al., 1992). When carrier species come into contact with vulnerable hosts, disease outbreaks can occur (Burek et al., 2008), so increasing rates of infection among Arctic avian species may put Arctic mammalian species at greater risk. AIV has the potential to cause lethal disease in seals (White, 2013) and has previously been detected at low levels in belugas and ringed seals from Arctic Canada (Nielsen et al., 2001). The virus was detected in the lungs of two harbor seals during a recent die-off in Sweden (Zohari et al., 2014).

Phylogenetic analysis demonstrated a close relationship to strains detected in Eurasian birds, suggesting that transmission had occurred via exposure to an infected bird or its droppings.

Climate change is highly likely to affect the distribution and migration patterns of the wild birds that compose the AIV reservoir (Gilbert et al., 2008). This may result in new interactions between separate avian populations, providing new opportunities for viral redistribution and possibly facilitating recombination between diverse strains. Abrupt emergences of new AIV strains (i.e. antigenic shifts) have been implicated in several influenza pandemics in humans (Reed et al., 2003). Furthermore, novel climate-affected interactions between carrier birds and uninfected avian and non-avian species may facilitate the transmission of the virus to naïve populations.

To my knowledge, no evidence of avian influenza has previously been found on Svalbard. However, the virus has been isolated from black-legged kittiwakes *(Rissa tridactyla)* in Northern Norway (Toennessen et al., 2011).

1.2. The Immunomodulatory Effects of Pollution

The presence of anthropogenic pollution in the Arctic is a well-documented phenomenon (AMAP, 2009, AMAP, 2004, Hung et al., 2016). Polar regions act as sinks for slow-degrading contaminants transported from sources at lower latitudes by the movement of air, water, and migratory animals (AMAP, 2009). Such contaminants include heavy metals (e.g. mercury, lead, and cadmium), other toxic elements (e.g. arsenic), and persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs).

1.2.1. Immune Effects of Heavy Metals and Toxic Elements

Previous research on free-living birds has demonstrated altered humoral immunity in birds living close to areas that are highly contaminated with heavy metals, such as metal smelters (Eeva et al., 2005, Snoeijs et al., 2004). However, other studies have found no immunological impacts of heavy metal exposure at Arctic-relevant concentrations. Of particular interest is the finding that barnacle goslings (*Branta leucopsis*) exposed to a contaminated mining area on Svalbard did not show an altered immune response after an acute stressor as compared to control goslings (de Jong et al., 2017).

Arsenic (As): Sources of arsenic include anthropogenic activities known to occur on Svalbard, such as mining and fossil fuel combustion, as well as the use of pesticides, wood

preservatives, and pigments (Lage et al., 2006, Sanchez-Virosta et al., 2015). Studies on humans and laboratory animals have noted a wide range of toxic effects, most notably increased oxidative stress, carcinogenicity, and genotoxicity. Experiments on birds have been more limited, but have reported negative effects on growth, liver damage, and altered blood biochemistry, while correlative studies on wild avian species have found decreased breeding performance, increased mortality, and evidence of oxidative stress near arsenic-polluted areas (Sanchez-Virosta et al., 2015). Arsenic-driven immunomodulation has been observed in birds, rodents, and humans (Lage et al., 2006). One study found altered white blood cell profiles and macrophage activity in avocets (*Recurvirostra americana*) hatched from eggs collected at ponds contaminated with arsenic, selenium, and boron (Fairbrother et al., 1994). Notably, the most severely affected chicks came from the pond with very high arsenic concentrations.

Cadmium (Cd): Released into the ocean by mining activities (Muir et al., 1992), cadmium is known to bioaccumulate in marine food webs (Scheuhammer, 1987). It is present in Kongsfjorden black-legged kittiwakes (Øverjordet et al., 2015b) and has been found in Arctic seabirds at levels high enough to cause kidney damage (AMAP, 2004). Immunosuppressive effects have been reported in laboratory rodents (Dan et al., 2000), although several studies have found no significant relationships between cadmium and immune endpoints in birds (Wayland et al., 2003, Sant'Ana et al., 2005, Finkelstein et al., 2007).

Mercury (Hg): The Arctic acts as a sink for mercury, which can be carried over long distances in the atmosphere and deposited on the snow in a bioavailable form (AMAP, 2004). Most of the anthropogenic mercury present in the Arctic originated at lower latitudes, with the largest global source being coal combustion (AMAP, 2011). Once deposited, it may be transformed by microbes into organic methylmercury, which is highly toxic and readily bioaccumulates and biomagnifies up food chains. Mercury occurs in high concentrations in Arctic marine top predators (AMAP, 2011) and has been measured in tissues of Kongsfjorden black-legged kittiwakes (Øverjordet et al., 2015b). In addition to its well-established neurotoxic and teratogenic effects (AMAP, 2011), evidence suggests that mercury can impact the immune system. Weakened immune responses have been observed in both captive common loons (*Gavia immer*) experimentally dosed with methylmercury (Kenow et al., 2007) and free-living tree swallows (*Tachycineta bicolor*) living at mercury-contaminated sites (Hawley et al., 2009).

Impaired macrophage phagocytosis was also seen in black-footed albatrosses (*Phoebastria nigripes*) with high blood mercury concentrations (Finkelstein et al., 2007).

Lead (Pb): After release during combustion processes, lead enters the atmosphere as an aerosol and can reach the Arctic via the movement of air masses (Sturges and Barrie, 1989). Past and current use of lead shot and leaded gasoline in Arctic regions have also constituted local sources (AMAP, 2004). Once in the biota, lead is typically stored in the bones and the kidneys. Lead accumulation can be especially high during processes that remobilize skeletal calcium, such as when a breeding female bird is producing eggshell (Scheuhammer, 1987).

Immunomodulatory effects of lead have been noted in birds, but the exact nature of these effects is unclear, with some studies reporting immunosuppression (Rocke and Samuel, 1991, Snoeijs et al., 2005) while others observe stimulation (Nain and Smits, 2011). Sex-specificity is commonly reported, though whether males or females are more greatly affected is not consistent (Snoeijs et al., 2005, Rocke and Samuel, 1991, Scheuhammer, 1987). Previous research suggests possible interactive effects between lead toxicity and the presence or severity of disease. One study associated lead shot ingestion with mortality from coccidiosis in geese (Locke and Bagley, 1967) while another found that dosing with lead decreased mortality in birds challenged with an *E. coli* infection (Nain and Smits, 2011).

Selenium (Se): At moderate levels, selenium has established benefits to the immune system. It has been shown to increase resistance to the coccidiosis disease in chickens (Colnago et al., 1984) and may help mitigate the immunosuppressive effects of stress in common eiders (Wayland et al., 2002). Deficiency in this nutritionally essential element has been associated with immune dysfunction in both laboratory animals and humans (Beck, 2007) and has been specifically shown to cause more severe symptoms in mice infected with the avian influenza virus (Beck et al., 2001). Additionally, selenium is known to have a protective effect against mercury toxicity (Cuvin-Aralar and Furness, 1991). However, excessive selenium can induce immunotoxic effects (Janz et al., 2010), including altered immune parameters (Fairbrother and Fowles, 1990) and increased mortality from viral infection (Fairbrother et al., 2004).

1.2.2. Immune Effects of Persistent Organic Pollutants

Arctic wildlife is exposed to complex mixtures of POPs (AMAP, 2004). Experimentally administered POP cocktails have been shown to impair the immune systems of captive glaucous gull chicks (Sagerup et al., 2009). In free-living animals, POP-related immunomodulation was

suspected or implicated in several marine mammal die-offs during the 1980s and 1990s (Ross, 2010), suggesting possible links between POPs and infectious disease.

Commonly-studied organochlorine pesticides include hexachlorobenzene (HCB); *beta*-hexachlorocyclohexane (*β*-HCH); chlordanes such as *oxy*-chlordane, *cis*- and *trans*-chlordane, and *cis*- and *trans*-nonachlor; and metabolites of dichlorodiphenyltrichloroethane (DDT) such as *para,para*-dichlorodiphenyldichloroethylene (*p,p'*-DDE). The possible immune effects of these compounds have been well-studied in Arctic mammalian predators (Kirkegaard et al., 2005). However, less research has been done on Arctic birds. Positive relationships between levels of OCPs and white blood cell counts have been seen in glaucous gulls breeding on the Arctic island Bjørnoya (Bustnes et al., 2004). A similar relationship was previously seen in Caspian terns (*Hydroprogne caspia*) and herring gulls *(Larus argentatus)* living around the Great Lakes (Grasman et al., 1996).

Once used as coolant fluids and released as industrial by-products, PCBs exist as 209 different congeners (AMAP, 2004). In addition to altered immune endpoints observed in PCB-exposed birds (Smits et al., 2002, Grasman and Fox, 2001), previous research has directly linked PCB exposure to increased mortality in ducklings infected with a viral disease (Friend and Trainer, 1970). Correlations have also been found between certain PCB congeners and higher burdens of parasitic nematodes in Arctic glaucous gulls (Sagerup et al., 2000).

Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants of which 209 congeners exist. Ubiquitous in the environment and particularly accumulative in aquatic ecosystems, PBDEs have been shown to negatively impact the growth of experimentally exposed birds (Fernie et al., 2006). Altered immune function has been observed in both *in vitro* mammalian cell lines (Frouin et al., 2010) and captive birds (Fernie et al., 2005).

1.3. Aims and Objectives

This study aimed to identify relationships between the prevalence of AIV infection and concentrations of pollutants in an Arctic seabird. Due to its abundance and pan-Arctic distribution (Strøm, n.d.), the black-legged kittiwake *(Rissa tridactyla)* was chosen as a model organism, using a well-studied population in Kongsfjorden, Svalbard.

The primary objectives of this study were to:

1. assess levels of heavy metals and toxic elements in kittiwake blood
2. determine the prevalence of AIV in the local kittiwake population
3. investigate correlations between presence or absence of the pathogen and pollutant levels in blood in order to identify possible drivers of avian immunomodulation by contaminants
4. screen other available Arctic and/or Norwegian bird samples for AIV in order to contribute to the baseline knowledge of the prevalence of this disease in wild birds

It was hypothesized that individuals with a higher pollutant burden would be more likely to be infected with avian influenza.

2. MATERIALS AND METHODS

2.1. Collection of Samples

2.1.1. Study Species and Location

This study was predominantly carried out on black-legged kittiwakes (*Rissa tridactyla*, hereafter referred to as "kittiwake") from breeding colonies in Kongsfjorden, Svalbard (Figure 1). Influenced by both Atlantic and Arctic water masses as well as input from its tidal glaciers, the Kongsfjorden marine ecosystem is likely to be sensitive to climate change as both of these factors are expected to vary as temperatures increase (Hop et al., 2002).

Figure 1. Map of Kongsfjorden, Svalbard, with locations of Ny-Ålesund and the Blomstrandhalvøya and Krykkjefjellet black-legged kittiwake breeding colonies marked. Satellite imagery from TopoSvalbard (Norwegian Polar Institute, retrieved April 2018).

The black-legged kittiwake is a medium-sized gull with highly pelagic habits (Strøm, n.d.). Its circumpolar distribution and status as the most numerous gull in the world (Strøm, n.d.) make it a good representative species for Arctic seabirds. During the breeding season, kittiwakes nest in dense colonies, which can consist of tens of thousands of individuals. On Svalbard, they may share ecological interactions with other bird and mammal species during this time. Eggs, chicks, and occasional adults are preyed upon by the Arctic fox *(Vulpes lagopus),* glaucous gull, great skua *(Stercorarius skua),* and Arctic skua *(Stercorarius parasiticus),* while Brünnich's guillemots *(Uria lomvia)* are known to nest in mixed colonies alongside kittiwakes (Strøm, n.d.).

2.1.2. Sampling Methods

Field sampling occurred in July 2017 at the Blomstrandhalvøya breeding colony (Figure 1). Both male and female kittiwakes were captured, and in several cases, sampled individuals constituted both members of a breeding pair. Birds were sampled at two time-points, approximately one week apart. The first samples were taken between July 11 and 15, while second samples were taken on July 21. After the first sampling, birds were marked using colored markers and nest locations were recorded. Efforts were made to recapture the same individuals for the second sampling period, but this was not always possible. Paired blood samples were obtained from 12 birds. A further 2 birds were only sampled at the first time-point and 1 bird was only sampled at the second time-point. Although 15 birds were sampled for blood, 17 birds were sampled in total. This figure includes an additional 2 birds from which only feather and swab samples were obtained. The feathers and swabs were ultimately not used in this study.

Birds were captured from their nests using a nylon lasso on the end of an extending pole. Biometric measurements (weight, head length, wing length, tarsus length) were taken. Only weight was measured at both the first and second time-points; head, wing, and tarsus lengths were assumed to remain unchanged between samplings. The relative biometric measurements of individuals in a breeding pair were used to estimate sex, as male kittiwakes are generally heavier than female kittiwakes during the summer months (Barrett et al., 1985). Eight male birds and nine female birds were sampled. Field data is shown in Appendix 1.

Blood samples were taken from the brachial vein using a heparinized syringe. We collected 70 to 400 µL of whole blood per individual. These were collected into BD Vacutainer cell preparation tubes (Fisher Scientific) and kept in foil-wrapped containers at ambient temperature until centrifugation for 15 minutes at 1800 rpm. The separated plasma was then conserved and stored at -20 °C.

Feathers samples, cloacal swabs, and oropharyngeal swabs were also taken. Swabs were taken in duplicate and stored in clean plastic tubes. Ten feathers were plucked from the upper back and placed in a clean envelope for elemental analysis. All samples were stored at -20 °C until further use.

2.1.3. Additional Avian Sample Sets

In addition to the samples I helped to collect, I also carried out pathogen screening on several sets of avian plasma samples that remained from previous projects. Quantification of various pollutants in these samples had already been carried out by others.

Most extensively, I made use of kittiwake plasma collected from Kongsfjorden in 2015 and 2014. The 2015 samples (n=25) were collected from two Kongsfjorden breeding colonies, Blomstrandhalvøya and Krykkjefjellet (Figure 1). All sampled birds were adult females. Levels of toxic elements (As, Cd, Hg, Pb, and Se) were analyzed in red blood cells while selected organochlorine pollutants (*β*-HCH, *p,p'*-DDE, HCB, *cis*- and *trans*-nonachlor, *oxy*-chlordane, 16 PCB congeners, and 4 PBDE congeners) were measured in plasma. Methodology for the sample collection and pollutant analysis can be found in Castaño-Ortiz (2016).

The 2014 kittiwake samples (n=16) were collected at both Blomstrandhalvøya and Krykkjefjellet (Figure 1) from both male and female birds. Plasma samples were analyzed for PCBs, PBDEs, selected organochlorine pesticides, and phosphorus flame retardants (PFRs). Further details on sample collection and pollutant analysis can be found in Svendsen (2015).

Three sample sets from other Norwegian avian species were also screened for AIV: glaucous gull samples collected from Adventfjorden, Svalbard in 2017 by Åse-Karen Mortensen and Torunn Slettemark Hovden; white-tailed eagle (*Haliaeetus albicilla*) samples collected from two locations in mainland Norway in 2016 for the NewRaptor project; and northern goshawk (*Accipiter gentilis*) samples collected from two further Norwegian locations for the same project. Glaucous gull samples were taken from adult birds while both sets of raptor samples were from nestlings.

2.2. Quantification of Heavy Metals and Toxic Elements

Concentrations of metals and other elements were measured in blood and feather samples from 2017. The general procedure consisted of an acid digestion of samples followed by inductive coupled plasma mass spectrometry (ICP-MS). I participated in the preparation of feather samples and data analysis but was not present for the sample digestion and ICP-MS procedure. This was carried out by Silje S. Lundgren and Syverin Lierhagen at the Department of Chemistry at NTNU.

2.2.1. Digestion Procedure for Blood

Plasma aliquots (1 mL) were transferred to perfluoroalkyl (PFA) tubes and digested in an equal volume of 0.6M HNO_3 in a microwave digestion chamber (Milestone UltraCLAVE). The sample was then diluted to approximately 15 mL for introduction to the ICP-MS.

2.2.2. Preparation and Digestion of Feathers

Feathers samples were washed sequentially in the following solutions: 1) acetone (suprapure); 2) MilliQ water; 3) acetone (suprapure); 4) HNO_3 (2% v/v); 5) MilliQ water. Washes were carried out by transferring samples to centrifuge tubes, filling with the appropriate solution until feathers were just covered, and then shaking for 5 minutes. Excess solution was removed, and after acetone and HNO_3 washes, feathers were flushed twice with MilliQ water before the next solution was added. Samples were subsequently freeze-dried.

For each sample, approximately 50 mg of feather material was added to 3 mL of 50% (v/v) HNO_3 in PFA vessels for microwave digestion (Milestone UltraCLAVE). Samples were diluted to 30 mL, and then 15 mL aliquots were transferred to metal-free polypropylene centrifuge tubes (VWR) for analysis.

2.2.3. Elemental Analysis

The analysis was conducted on an Element 2 ICP-MS (ThermoScientific). For each element analyzed, two separate limits of detection (LODs) were calculated. One was based on the instrument detection limit while the second was defined as 3 * the standard deviation of the blank. The highest value between these two LODs was used as the actual LOD.

2.2.4. Quality Assurance

Repeating tests were performed at semi-regular intervals to ensure precise measurements. No significant deviations were detected. Repeated measurements were averaged to represent the sample concentration.

2.3. Extraction of Swab Samples

A QIAmp DNA Microbiome kit (QIAGEN) was used to extract DNA from cloacal swab samples. The intention was to use pathogen-specific primers and polymerase chain reaction (PCR) techniques to screen swab extracts for bacterial pathogens. However, analysis of extraction products by NanoDrop 2000c spectrophotometer (ThermoScientific) revealed that no

DNA was present. This may have been due to a lack of DNA on the swabs, a fault in the kit, or human error while carrying out the procedure. Because of time limitations, it was decided not to attempt the extraction again using the duplicate swab samples.

2.4. Avian Influenza Virus Screening

Plasma samples were screened for antibodies associated with avian influenza infection. This was done using enzyme-linked immunosorbent assay (ELISA) principles, which are designed to detect the presence of antibodies. Samples are incubated in wells coated with viral antigens before the addition of an enzyme-linked conjugate. In a sample where antibodies are present, the antigen binding sites are blocked, allowing the conjugate to be washed away. In samples without antibodies, the conjugate binds to the well and develops color when the appropriate enzyme substrate is added.

Plasma samples were screened for avian influenza virus using an Influenza A virus antibody test kit (IDEXX). For 2017 kittiwake samples, only samples from the first time-point were used (n=12). Additional screening was conducted on plasma from 2015 kittiwakes (n=25), 2014 kittiwakes (n=16), white-tailed eagles (n=43), northern goshawks (n=29), and glaucous gulls (n=15). Absorbance values were visualized using a Cytation 5 Imaging Reader (BioTek). Percent prevalence of the AIV virus was calculated for each sample set.

2.5. Statistical Methods

All statistical analyses were carried out using the statistical software RStudio (Version 1.0.143). Figures were generated using the ggplot2 add-on. For all statistical tests, the significance level (α) was set at 0.05.

2.5.1. Avian Influenza Prevalence by Colony and Sex

A two-tailed binomial exact goodness-of-fit test was used to assess whether there was a significant difference between the number of infected kittiwakes observed at Blomstrandhalvøya vs. Krykkjefjellet in 2015. This method was chosen over a Chi square goodness-of-fit test due to the low sample size. The same test was also used to check for a significant difference between the number of males and females among infected glaucous gulls.

2.5.2. Levels of Heavy Metals and Toxic Elements in 2017 Kittiwakes

Five elements (As, Cd, Hg, Pb, and Se) known to be immunologically active were selected for investigation in this study. Concentrations measured in each plasma and feather sample can be found in Appendix 2.

Concentrations in paired plasma (n=12) samples were compared to determine whether levels had changed between the first and second time-points. This was done using paired t-tests, except in the case of selenium levels in plasma, for which a paired Wilcoxon signed-rank test was used instead due to the non-normality of the differences. Measurements below the LOD (of which there were only 2) were set to the half the value of the LOD.

Further analysis was carried out using plasma samples from the first time-point (n=14) only. This was done to: 1) obtain a single value for each sample without averaging concentrations that had been found to be significantly different between time-points; 2) maximize the sample size, as more plasma samples had been obtained at the first time-point than at the second time-point; 3) eliminate values below the LOD, which had only been found at the second time-point, and 4) facilitate a more accurate comparison with samples from Castaño-Ortiz (2016), which had been collected on dates similar to the first time-point.

Summary statistics were performed for each element (Appendix 3) and then visualized as boxplots. The lower and upper bounds of the boxes in these plots represent the 25th percentile (Q1) and 75th percentile (Q3) respectively, while the middle band represents the median value (Q2). The "whiskers" extend to the highest and lowest values falling within 1.5 times the value of the interquartile range. More extreme values are represented by dots. In cases where a logarithmic axis was used, the statistical values shown were calculated before log-transformation.

Although elements were also quantified in feathers, these results will not be presented, as they do not fall within the aims of this thesis. Feathers were originally collected with the goal of performing of viral diversity analysis in feather stems, but this was ultimately not possible due to time limitations. While elemental concentrations in feathers and blood may correlate during feather growth (Bearhop et al., 2000, Markowski et al., 2013), previous research suggests that the migratory nature of kittiwakes and the timing of molt mean that feather concentrations do not correlate with local contamination in Kongsfjorden (Svendsen et al., 2018). Thus, this possibility

was not investigated. Results of feather analysis can be found in Appendices 2 (raw data) and 3 (summary statistics).

2.5.3. Logistic Regression Models of AIV Prevalence

Statistical modeling was used to determine which pollutants and biological factors (e.g. mass, sex) contributed significantly to the probability of a kittiwake being infected with AIV. Because the dependent variable (infection status) was binary and categorical (i.e. the only two possible outcomes were "infected" or "not infected"), we selected a logistic regression model for this purpose. A logit link was used to account for the small sample size.

Because the inclusion of too many independent variables can result in an overfitted model, a limited number of possible parameters were chosen for each initial model based on their toxicological relevance. All pollutant concentrations were assessed for collinearity beforehand. Collinear pollutants were included in the models as a sum. After the creation of the initial model, backwards selection was conducted based on the Akaike information criterion (AIC) values in order to obtain the final best-fit model.

2.5.3.1. Modeling with 2015 Kittiwakes

The first model (hereafter referred to as Model A) was developed using data from the 2015 kittiwake samples. The following independent variables were initially included in the model: As; Hg; Pb; Se; *p,p'*-DDE; sum of all other POPs (ΣPOPs); and mass. The four elemental pollutants were chosen because all are known to be immunologically active and were found in high concentrations in the kittiwakes. POP concentrations were included as a sum due to the high degree of collinearity between most of the compounds. The only non-collinear compound, *p,p'*-DDE, was treated as a separate variable.

Once the best-fit model was obtained, a modeled dataset was generated for each variable in the final model individually. This data was plotted to visualize the effect of that variable on the probability of infection. Shaded areas in these plots represent confidence intervals. Dots along the borders represent the actual concentrations of the modeled element measured in study birds, with the upper border displaying infected individuals and the lower border displaying uninfected individuals.

2.5.3.2. Modeling with Combined 2014 and 2015 Kittiwakes

Three further models (hereafter referred to as Models B, C, and D) were created using the organic pollutants that had been analyzed in both 2014 and 2015 kittiwakes (HCB, *p,p'*-DDE, *oxy*-chlordane, *cis*- and *trans*-nonachlor, PCBs, and PBDEs).

Because some PCB congeners were analyzed in the 2014 samples but not in the 2015 ones and vice versa, only congeners analyzed in both years were included. Therefore, the ΣPCB value used in these models is not equivalent to the one used in Model A, when only data from 2015 was under consideration. The list of congeners included in each model can be found in Appendix 4. Although *β*-HCH was analyzed in both years, it was not included in analysis because in 2014, >50% of the samples were below the LOD.

Pollutant concentrations for the 2014 birds were reported in pg/g ww while 2015 birds were reported in pg/mL. These were assumed to be comparable units as the density of blood is approximately equal to that of water, 1 g/mL (Vitello et al., 2015).

High degrees of collinearity were found between concentrations of nearly all POPs, making it implausible to separate out the effects of individual compounds. For this reason, Model B initially included ΣPOPs as an independent factor, as well as sex, colony, mass, and year.

Despite strong collinearities, we decided to create an additional model (Model C) with the pollutants separated into five different independent factors: HCB, *p,p'*-DDE, the sum of chlordanes (ΣCHL), ΣPCBs, and ΣPBDEs. Although we did not expect the model to accurately reflect the influences of each pollutant group on AIV infection, we found precedent in previous literature (Bustnes et al., 2004) to attempt analysis of highly-correlated pollutant groups in order to elucidate which compounds in the POP mixture may be important to investigate further. Sex, colony, mass, and year were also included in the initial model.

Model D was constructed using the same independent variables as Model C, but with slightly altered data: one suspected outlier with a very high PBDE concentration and one individual with a PBDE concentration below the LOD were eliminated for Model D, as they were suspected to be unduly influencing Model C.

2.5.4. Comparison of Toxic Elements in 2017 and 2015 Kittiwakes

It was not possible to investigate factors affecting AIV infection in 2017 kittiwakes, as no AIV was detected in these samples. Consequently, we wished to instead assess whether burdens

of certain elements (As, Hg, and Se) varied between 2015 (a year in which AIV was detected) and 2017 (a year in which no AIV was detected).

Differences in pollutant analysis methodology prevented a direct comparison between the two sample sets. Elemental concentrations had been measured in the plasma for 2017 birds, but in the red blood cells (RBCs) for 2015 birds. To circumvent this, I searched the literature for previous studies that had investigated the distribution of the elements of interest between plasma and RBCs (Table 1). Using previously established ratios, I calculated the hypothetical RBC concentrations in 2017 birds based on the measured plasma concentrations. These values were then used as a proxy to compare the burdens of each element in 2015 and 2017 kittiwakes.

Table 1. Plasma:RBC ratios used to calculate theoretical RBC concentrations of As, Hg, and Se from actual plasma concentrations measured in 2017 kittiwakes.

Element	Plasma:RBC Ratio	Species	Reference
As	1:3	Human	Iriyama et al., 2012
Hg	1:11	Iceland gull (*Larus glaucoides*)	Bond and Robertson, 2015
Se	1:2	Hens	Oishi et al., 1986

Several limitations of this method must be noted. First of all, the plasma:RCB ratio of a given element can vary depending on factors such as the concentration in the diet (Oishi et al., 1986) and the age of the individual (Bond and Robertson, 2015). Secondly, it was not possible to find ratios specific to black-legged kittiwakes, and while I attempted to choose studies on the most closely-related species possible, interspecific variation may limit the accuracy of these values. This is particularly true in the case of arsenic, for which the most relevant study I could find concerned a human leukemia patient (Iriyama et al., 2012).

Proxy RBC values from 2017 (n=14) and measured RBC concentrations from 2015 (n=14) were compared using unpaired t-tests (Se and As) or a Wilcoxon rank sum test (Hg, due to non-normality of 2015 data). Molar ratios of Se:Hg were also calculated and compared between years using a Wilcoxon rank sum test. Since all 2017 birds had been sampled at Blomstrandhalvøya, only 2015 birds from that colony were included in the analysis to increase comparability.

3. RESULTS

3.1. Levels of Heavy Metals and Toxic Elements in 2017 Kittiwakes

The five selected elements (As, Cd, Hg, Pb, and Se) were detected above the LOD in the majority of plasma samples. One second time-point sample was found to be below the LOD for Cd and a separate second time-point sample was below the LOD for Hg.

Based on 12 paired samples, no significant differences in Se, Hg, Pb, or Cd concentrations were found in plasma between the first and second sampling time-points (all $p>0.05$). Concentrations of As in plasma showed a significant decrease ($p=0.028$) from the first time-point to the second (Figure 2).

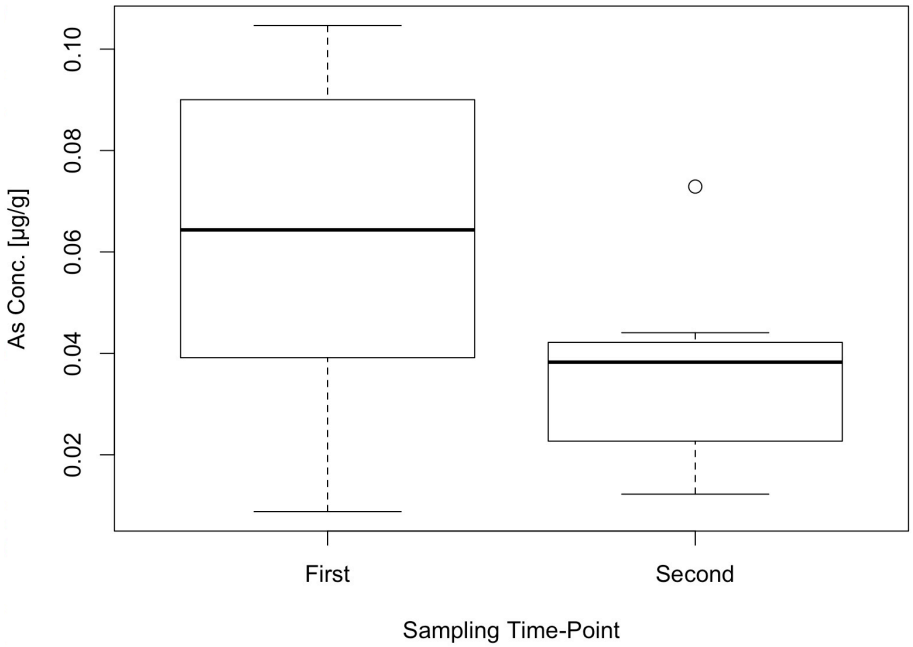

Figure 2. Concentrations of arsenic in plasma of black-legged kittiwakes at first and second sampling times (approx. 1 week apart). Difference is significant ($p=0.028$).

Further analysis was done with first time-point measurements only (n=14), for which all samples measured above the LOD for all elements. Of the five elements, Se was the most abundant, followed by As (Figure 3). Hg and Pb were found in similar concentrations and Cd was least abundant. Se was well in excess of Hg, with a 175:1 molar ratio of Se:Hg.

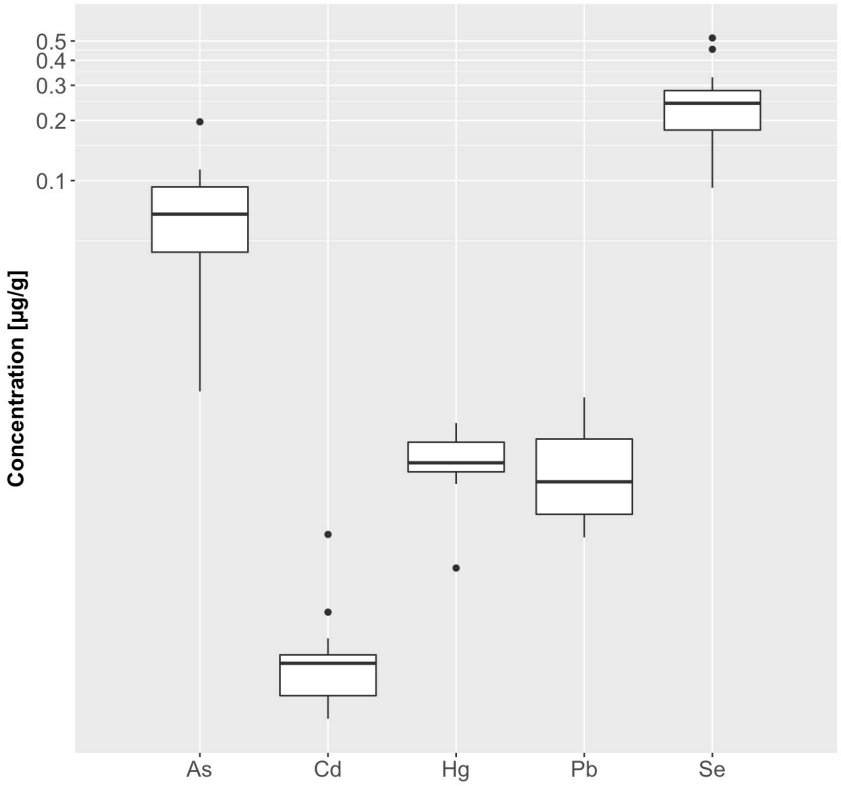

Figure 3. Concentrations of As, Cd, Hg, Pb, and Se in the plasma of black-legged kittiwakes (n=14).

3.2. Prevalence of Avian Influenza in Avian Plasma Sets

Plasma samples from four avian species collected in different years from various regions of Norway were screened for AIV antibodies. A summary of the results can be found in Table 2.

Table 2. Prevalence of the avian influenza virus (AIV) in six sets of avian plasma samples collected from around Norway. Locations marked with an asterisk (*) are on Svalbard.

Species	Year	Location	n	AIV Prevalence
Black-legged Kittiwake	2017	Kongsfjorden*	12	0%
Black-legged Kittiwake	2015	Kongsfjorden*	25	28%
Black-legged Kittiwake	2014	Kongsfjorden*	16	12.5%
Glaucous Gull	2017	Adventfjorden*	15	33%
White-Tailed Eagle	2016	Steigen / Smøla	43	0%
Northern Goshawk	2016	Trondheim / Tromsø	29	0%

No AIV antibodies were detected in any of the plasma samples from the 2017 kittiwakes (n=12). 28% of the kittiwake samples from 2015 (n=25) tested positive for AIV antibodies. This included birds from both Blomstrandhalvøya (n=5) and Krykkjefjellet (n=2). The difference between the number of infected birds found at each colony was not significant (p=0.431).

Evidence of AIV infection was found in 12% of the kittiwake samples from 2014 (n=17), again including both birds from Blomstrandhalvøya (n=1) and Krykkjefjellet (n=1). Samples from this year included birds of both sexes; both of the infected birds were male.

33% of the glaucous gulls (n=15) collected in 2017 tested positive for AIV. This included 1 female bird and 4 male birds. The difference between sexes was not significant (p=0.375).

None of the white-tailed eagle (n=43) or northern goshawk (n=29) samples contained AIV antibodies.

3.3. Modeling of AIV Prevalence in 2015 Kittiwakes

Binary logistic regression models were created to determine which dependent variables significantly explained the prevalence of AIV infection in Kongsfjorden kittiwakes (Table 3).

Table 3. Summary of the four models developed to investigate which factors contributed to the probability of AIV infection in black-legged kittiwakes. All models were logit-linked binary logistic regressions with AIV infection as the dependent variable.

Model	Dataset Used	n	Independent Vars. (initial)	Independent Vars. (final)
A	2015	25	As, Hg, Pb, Se, *p,p'*-DDE, sum of all other POPs, mass	As, Hg, Se
B	2014 + 2015	41	ΣPOPs, sex, colony, mass, year	year, sex
C	2014 + 2015	41	HCB, *p,p'*-DDE, ΣCHL, ΣPCB, ΣPBDE, sex, colony, mass, year	year, ΣPBDE
D	2014 + 2015, PBDE outliers removed	39	HCB, *p,p'*-DDE, ΣCHL, ΣPCB, ΣPBDE, sex, colony, mass, year	year

The first model (Model A) was created using data from 2015 kittiwakes only. The initial model included the following independent variables: mass; Hg; Se; Cd; As; *p,p'*-DDE; and the sum of the other POPs. After backwards selection, the best-fit model included As, Se, and Hg. There is some debate as to whether *p*-values provide any information of worth when performing stepwise selection. Nevertheless, the *p*-values for each variable in the final model are presented alongside their average concentrations in RBCs (Table 4).

Table 4. Independent variables in remaining Model A after AIC-based backwards selection with final *p*-values and average concentrations in kittiwake RBCs.

Independent Variable	*p*-value	Avg. Conc. (ng/g dw)
As	0.037	1197.04
Hg	0.051	388.62
Se	0.037	49031.13

Plots of modeled data show that increasing concentrations of Se and As have positive effects on the probability of AIV infection, while higher Hg concentrations lowers infection probability (Figure 4, A-C). Modeling the data using the Se:Hg molar ratio corroborates the patterns seen with the elements individually, as an increasing Se:Hg ratio increases infection probability (Figure 4, D).

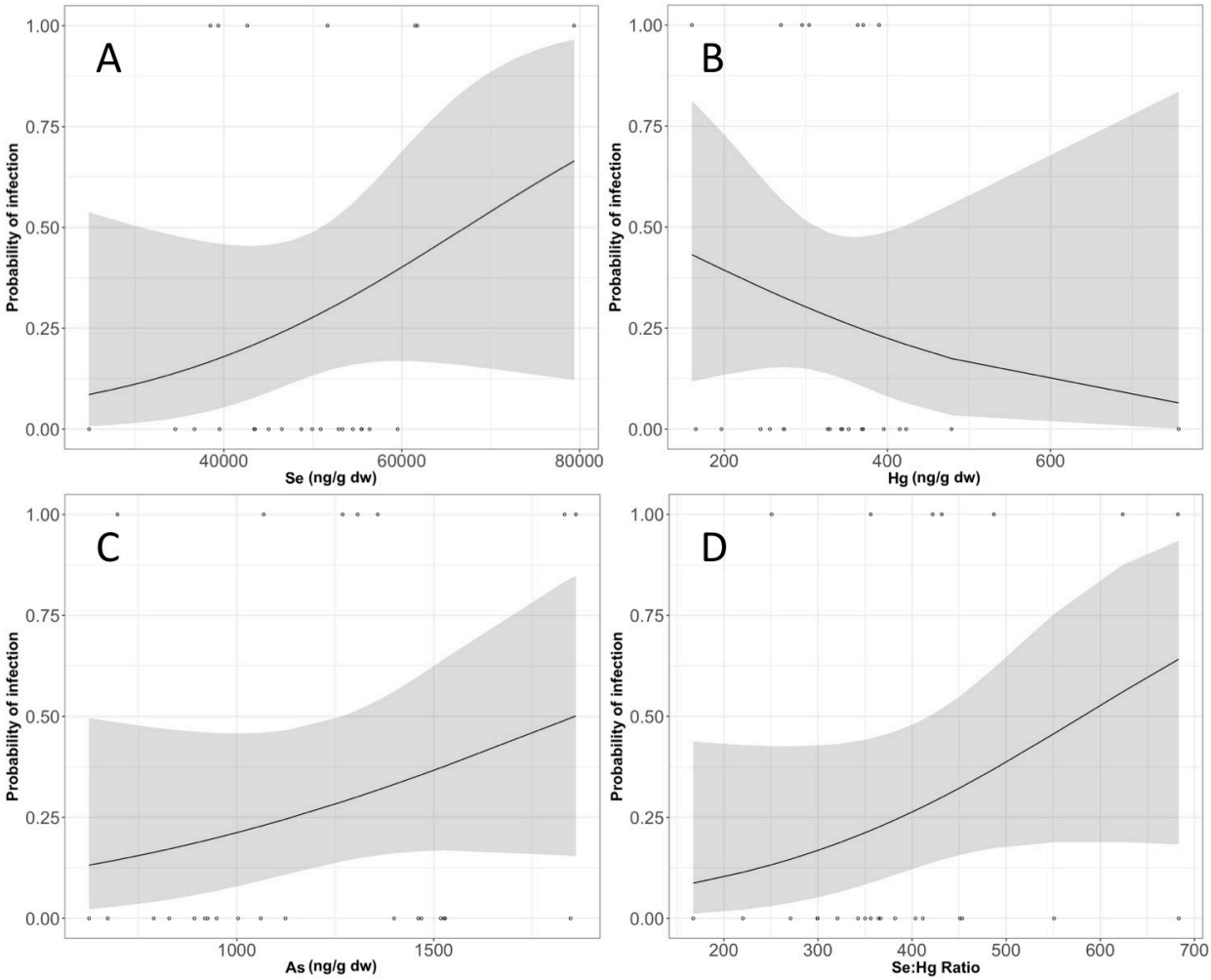

Figure 4. Modeled data (Model A) showing the probability of AIV infection in black-legged kittiwakes with increasing Se (A), Hg (B), As (C), and molar ratio of Se:Hg (D).

3.4. Modeling of AIV Prevalence in Combined 2014 and 2015 Kittiwakes

Data from 2014 and 2015 kittiwakes was combined into a new data set (Figure 5). Three further models (Models B, C, and D) were generated from this combined sample (Table 3).

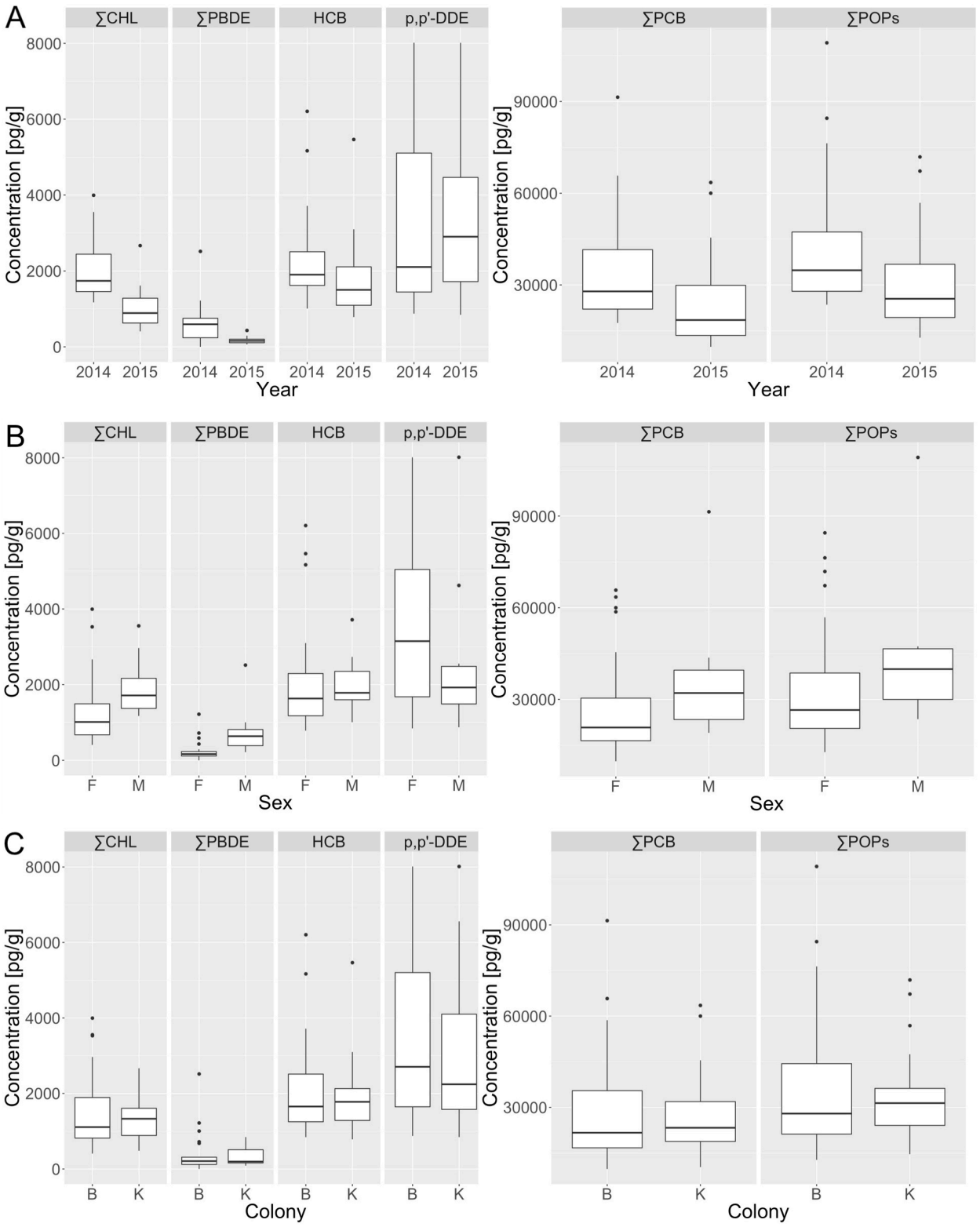

Figure 5. Distributions of concentrations of POPs in the combined 2014/2015 kittiwake data set (n=41) separated by year (A), sex (B), and colony (C).

Due to high degrees of collinearity between the majority of pollutants, Model B was initially created using the sum of all POPs (ΣPOPs), as well as year, mass, colony, and sex. Backwards selection resulted in a final model containing only year and sex.

Model C was created to explore possible contributions of separate pollutant groups. It initially included year, mass, colony, sex, HCB, *p,p'*-DDE, ΣCHL, ΣPCB, and ΣPBDE. The final model included year and ΣPBDE. Upon plotting of modeled data, it was observed that the data seemed to be highly influenced by a single infected bird, which had PBDE concentrations twice as high as the next-most PBDE-polluted individual (Figure 6).

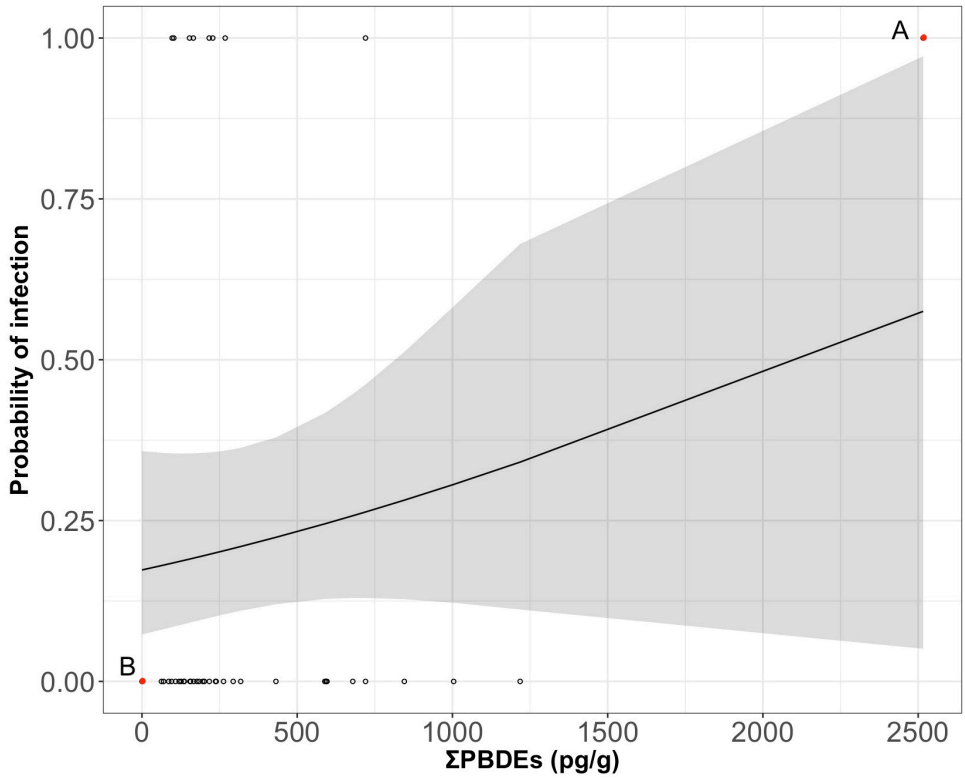

Figure 6. Modeled data (Model C) showing the probability of AIV infection in black-legged kittiwakes with increasing PBDE concentrations. One infected bird with a high PBDE burden (A) appeared to be highly influencing the trend and was removed for the next model, along with an uninfected bird with a reported concentration of zero (B).

To investigate the influence of this outlier, another model (Model D) was created with this individual removed. Another individual with a reported PBDE concentration of zero was also removed, as this value was presumably below the limit of detection but not necessarily equal

to zero and therefore could have decreased the accuracy of the model. Model D was initially created with the same parameters as Model C. The final model contained only year.

Final independent variables from Models B, C, and D can be found in Table 5, along with associated *p*-values.

Table 5. *P*-values for each independent variable remaining the final Models B, C, and D after backwards selection. All models were created from combined 2014/2015 kittiwake data with AIV infection as the dependent variable.

Model B		**Model C**		**Model D**	
Variable	*p*-value	Variable	*p*-value	Variable	*p*-value
Year	0.062	Year	0.022	Year	0.099
Sex	0.152	ΣPBDE	0.03		

3.5. Comparison of Toxic Elements in 2017 and 2015 Kittiwakes

Using plasma:RBC ratios obtained from previous literature (Table 2), hypothetical RBC concentrations were calculated from the plasma concentrations of As, Hg, and Se in 2017 kittiwakes. The average values of these proxy concentrations were significantly lower than the actual RBC concentrations in 2015 kittiwakes for all three elements (Table 6).

Table 6. Average RBC concentrations (ng/g ww) of As, Hg, and Se of kittiwakes sampled in 2017 (n=14, theoretical estimates calculated from plasma concentrations, reported with one significant figure) and 2015 (n=14, measured concentrations). *P*-values represent comparisons within elements and between years, using either an unpaired t-test (As and Se) or a Wilcoxon rank sum test (Hg, Se:Hg ratio).

	As	**Hg**	**Se**	**Se:Hg Ratio**
2017	200	40	500	30
2015	968.87	281.45	43002.25	421.64
***p*-value**	7.601e-9	4.985e-8	7.266e-10	4.985e-8

Selenium levels in 2015 were approximately 84 times higher than in 2017. Mercury concentrations were approximately 6 times higher and arsenic approximately 4 times higher. The Se:Hg ratio was also about 14 times higher in 2015.

4. DISCUSSION

4.1. Heavy Metals and Toxic Elements in 2017 Kittiwakes

Analysis of elemental pollutants in Svalbard kittiwakes has been carried out in a variety of tissues, which makes comparison between studies difficult. Castaño-Ortiz (2016) found higher levels of all five elements in RBCs from Kongsfjorden kittiwakes than what was measured in plasma in the present study. Another study also found higher levels of mercury in RBCs from Kongsfjorden kittiwakes (Goutte et al., 2015).

Levels of cadmium, mercury, and selenium were much higher in liver and muscle from Svalbard kittiwakes (Øverjordet et al., 2015a, Øverjordet et al., 2015b). However, the hepatic Se:Hg ratio found by Øverjordet et al. (2015a) was much lower (between 17.1 to 25.6) than the ratio we found in plasma (175). This may be because the majority of Hg found in blood binds to RBCs (Bond and Robertson, 2015) and so would not have been measured in plasma.

Levels of cadmium, mercury, and selenium in our samples were lower than previously measured in whole blood from Canadian common eiders (Wayland et al., 2001). Our plasma concentrations of mercury were also lower than those measured in the plasma of Canadian Iceland gulls (Bond and Robertson, 2015).

Plasma concentrations did not vary between the first and second time-points for any of the heavy metals or for selenium. This study's original motivation for sampling at two time-points was to investigate differences in plasma concentrations of POPs, which are known to remobilize as kittiwakes utilize lipid stores during the breeding period (Bustnes et al., 2017). Metals, by contrast, are not lipophilic and thus remain unaffected by lipid dynamics, so this lack of change is unsurprising.

Arsenic levels decreased in plasma from the first time-point to the second. In previous studies, arsenic concentrations in blood have been sensitive to temporal factors such as diet (Sanchez-Virosta et al., 2015). Because the excretion rate of arsenic is high (Albert et al., 2008a), it is likely to reflect short-term fluctuations in exposure to a greater degree than other elements.

4.2. Prevalence of AIV in Kongsfjorden Kittiwakes

AIV was detected in Kongsfjorden kittiwakes in both 2014 and 2015, but not in 2017. To our knowledge, this study is the first to report the presence of AIV on Svalbard.

The lack of infected birds among the 2017 samples could be explained by several possibilities. First, the sample size from 2017 (n=12) was slightly smaller than in either 2014 or 2015 (n=16 and n=25 respectively). Assuming the size of the breeding colonies and number of infected birds was roughly similar between years, the smaller number of samples would decrease the probability of detecting AIV. Alternately, AIV may have actually been less prevalent in 2017 than in previous years. In either case, it is likely that AIV was still present 2017 and was simply not detected.

4.2.1. Assessing Patterns of AIV Prevalence

This study specifically aimed to address the relationship between AIV infection and pollutant levels. It was not intended as an epidemiological study. Thus, the inferences on the patterns of AIV in Kongsfjorden kittiwakes presented here are limited.

We assessed percent prevalence of AIV in the three years. However, these values should not be treated as reliable estimates for AIV prevalence in the Kongsfjorden kittiwake population for several reasons. Firstly, the sample sizes in all years were very small compared to overall size of the colonies, so samples are not necessarily representative of the colonies as a whole. Furthermore, it is known or assumed that sampled birds were collected from nests located in close proximity of each other and in some cases, individual birds constituted a nesting pair. Individuals with infected mates or neighbors may face a greater risk of exposure to infective feces, possibly increasing their own likelihood of contracting AIV. Thus, it is not certain if the samples can be considered fully independent of one another.

The number of infected birds at Krykkjefjellet and at Blomstrandhalvøya did not vary significantly in either 2014 or 2015. The two colonies presumably have a high degree of contact with one another, as both feed in the same fjord and may spend the winter in the same area.

However, it is also possible that the populations remain separate while in Kongsfjorden. Kittiwakes feed in the upwelling zones at tidal glacier fronts (Strøm, n.d.), and the proximity of each breeding colony to a different tidal glacier may prevent mixing during the breeding season. Differences in AIV prevalence between colonies may exist and may have been overlooked due to the small sample size of this study.

It is similarly impossible to draw reliable conclusions about differences in AIV prevalence between the sexes. In 2015, all infected birds were female because only female birds were sampled. Both infected birds from 2014 were male, although both sexes were sampled. In

isolation, the 2014 samples might suggest that sex may have an impact on infection status. However, the discovery of infected females in 2015 and the absence of AIV infection among both sexes in 2017 weaken this postulation. Overall, the sample sizes are too small and there is too little consistency between the number of males and females sampled between the years to draw any conclusions about impact of sex on AIV prevalence.

This study has detected AIV in Kongsfjorden kittiwakes. The virus is prevalent at both Krykkjefjellet and Blomstrandhalvøya and among both male and female birds. However, a larger and more diverse sample of birds would be necessary accurately determine what percentage of birds carry the virus, assess the degree to which AIV prevalence varies by year, and examine whether breeding colony or sex has a significant effect on infection status.

4.2.2. AIV: A New Disease on Svalbard?

To our knowledge, this is the first time that AIV has been detected on Svalbard. However, AIV is not necessarily new to the avian population of Kongsfjorden, as it has been known to exist in other areas of the Arctic for some time. Most notably, the virus has previously been found in kittiwakes breeding in an Arctic region of mainland Norway (Toennessen et al., 2011). It has also been detected in a population of swans that breed in northern Russia (van Gils et al., 2007) and viral RNA has been found in ice from a Siberian lake frequented by migratory birds (Zhang et al., 2006). Widespread monitoring of waterfowl and shorebirds in Alaska revealed the presence of AIV, albeit at very low rates (0.06%) that were believed to be limited by the Arctic conditions (Winker et al., 2007). On one Alaskan island, AIV was detected in seven different species of migratory birds, including common guillemots *(Uria aalge)*, Brünnich's guillemots *(U. lomvia*), king eiders *(Somateria spectabilis)* and glaucous gulls (Ramey et al., 2010). These four species are all common breeders on Svalbard (Mehlum et al., 1990).

While the virus has possibly been present on Svalbard for some time, what may be shifting is the prevalence and dynamics of the disease. Increased inputs of Atlantic water and receding tidal glaciers are expected to affect the ecology of seabirds in Kongsfjorden (Hop et al., 2002). Climate-related shifts in kittiwake feeding and distribution have already been noted (Gilg et al., 2012, Grémillet and Boulinier, 2009), which could also have implications for patterns of AIV transmission. Predicted increases in temperature and precipitation (Førland et al., 2011) on Svalbard may promote the survival of shed viruses. As declining sea ice limits the availability of

seals, Svalbard polar bears (*Ursus maritimus*) have been observed to feed increasingly on bird eggs and chicks (Prop et al., 2015), putting them at greater risk of AIV infection.

AIV strains circulating in wild birds are low pathogenic viruses that are generally believed to cause no symptoms (Alexander, 2007, Boyce et al., 2009, Olsen et al., 2006). However, a study on wild Bewick's swans *(Cygnus columbianus bewickii)* found that birds carrying AIV delayed their migrations, traveled shorter distances, and consumed less food than uninfected birds, suggesting that even low pathogenic strains may have impacts on avian health (van Gils et al., 2007). Thus, shifts in AIV dynamics among Svalbard birds could potentially have implications for the avian populations themselves.

Developing more reliable estimates of AIV prevalence in Svalbard bird colonies would be very useful for detecting changes in AIV dynamics. Other potential future research directions include characterization and monitoring of possible routes of transmission, such as the amount of viral shedding that occurs via fecal matter at bird cliffs and the degree to which AIV is able to survive in bodies of water on Svalbard. Vulnerable mammalian species could also be screened for the disease.

4.3. Prevalence of AIV in Other Avian Species

4.3.1. Svalbard Glaucous Gulls

AIV was also detected in glaucous gulls (n=15) collected on Svalbard. No difference was found between the number and males and females infected, although the small sample size limits the reliability of this conclusion. The virus has been previously detected in glaucous gulls from Alaska (Ramey et al., 2010) and Iceland (Dusek et al., 2014).

The existence of AIV in multiple avian species on Svalbard further supports the hypothesis that AIV is not new to the archipelago. It also underscores the needs for screening among other birds. In particular, it would be interesting to see whether the duck and goose species abundant on Svalbard (e.g. the common eider, the barnacle goose) carry the virus. Not only do ducks and geese often carry AIV at higher rates than other aquatic birds (Gilbert et al., 2008), but Svalbard ducks and geese, as well as glaucous gulls, are vulnerable to predation by climate-stressed polar bears (Prop et al., 2015). Shifts in AIV dynamics among these species may put polar bears at greater risk of infection. Additionally, goose populations on Svalbard are

predicted to expand as result of climate change (Kéry et al., 2006, Jensen et al., 2008). If geese carry AIV, the transmission risk to other species may increase as a result of these expansions.

4.3.2. Norwegian Raptors

No AIV was detected in any of the white-tailed eagle (n=43) or the northern goshawk (n=29) samples. This is an unsurprising result considering that all raptor blood samples screened in this study came from nestlings, which should be immunologically naïve. AIV has previously been detected in northern goshawks (Gilbert et al., 2006), but to our knowledge no evidence of the virus has yet been found in white-tailed eagles.

4.4. Effects of Toxic Elements on the Probability of AIV Infection

Three elements were shown by Model A to influence the probability of AIV infection: As, Hg, and Se.

The positive effect of arsenic on the likelihood of infection was consistent with previous observations of arsenic-mediated immunomodulation, such as those summarized in Lage et al. (2006). Additionally, Fairbrother et al. (1994) reported altered white blood cell profiles, reduced phagocytosis, and increased lymphocyte proliferation in avocet chicks hatched from eggs taken from a highly-contaminated pond. However, one study on passerine birds found that dosing chicks with arsenic had no effect on several immune parameters (Albert et al., 2008b). This may be due to interspecies differences or the presence of other contaminants and stressors in the pond study.

The relationship between selenium, mercury, and probability of AIV infection showed unexpected trends. Mercury's documented immunotoxic effects (Kenow et al., 2007, Hawley et al., 2009, Finkelstein et al., 2007) and reputation as a pollutant of high concern in the Arctic (AMAP, 2011) might lead one to expect a positive relationship between infection and mercury concentrations. Meanwhile, selenium's known benefits to the immune system (Colnago et al., 1984, Wayland et al., 2002) and protective effect against mercury toxicity (Cuvin-Aralar and Furness, 1991, Khan and Wang, 2009) might be expected to help mitigate these effects. Instead, we observed the opposite: increasing selenium concentrations and decreasing mercury concentrations were associated with a higher probability of infection.

Despite its status as an essential nutrient, selenium can also be toxic in high doses and is indeed known to have a particularly narrow window between essential and toxic levels (Janz et

al., 2010). A series of studies on eider ducks indicate that selenium may benefit the immune system at dietary levels but impair immunocompetence at high doses (Janz et al., 2010). Experimental exposure to organic selenium was seen to alter several immune parameters in mallard ducks (Fairbrother and Fowles, 1990) and mallard chicks hatched from eggs taken from selenium-contaminated streams displayed increased mortality from duck hepatitis virus (Fairbrother et al., 2004).

There are a couple of possible explanations for the apparent role of mercury in decreasing the probability of AIV infection. Mercury may be stimulating the immune system rather than suppressing it, but this seems unlikely, as previous studies report immunosuppression in various avian species (Kenow et al., 2007, Hawley et al., 2009, Finkelstein et al., 2007). The effect could alternatively be attributed to mercury's interaction with selenium. Most often discussed in terms of selenium exerting a protective effect against mercury, this interaction has not yet been fully elucidated, but one common hypothesis involves the formation of a biologically inert complex between selenium and methylmercury (Khan and Wang, 2009). If this complex indeed forms, it may be reducing the effect of selenium on AIV infection. In essence, mercury could be detoxifying selenium in this system. Detoxification of selenium by mercury has been observed in one experimental study, in which dietary mercuric chloride reduced toxic effects in chicks fed selenium dioxide (Hill, 1974). Other studies have described the mutual protective effects of mercury and selenium against each other (El-Begearmi et al., 1977, Gailer et al., 2000).

Interestingly, a protective effect of arsenic against selenium toxicity has also been previously observed (Hoffman et al., 1992, Stanley et al., 1994). Our study found the opposite, with both elements having an immunotoxic effect. The fact that selenium has previously-documented toxicological interactions with both arsenic and mercury suggests that these three elements may not be exerting independent effects, but rather interacting to influence the probability of AIV infection.

4.4.1. Toxic Element Burdens and AIV Prevalence Between 2015 and 2017

RBC concentrations of all toxic elements measured in 2015 were significantly higher than those estimated from plasma concentrations in 2017. The difference was especially striking for selenium, with concentrations nearly 84 times higher in 2015, while arsenic was roughly 4 times higher. The apparent reduction in concentrations of these AIV-promoting elements in 2017 may have contributed to the absence of disease in the birds sampled that year.

The molar ratio of selenium to mercury was also much higher in 2015 than in 2017. If mercury does indeed have a protective effect against selenium toxicity in this system, as suggested in the previous section, the relative abundance of selenium compared to mercury in 2015 could also have contributed to increased AIV prevalence.

While the results of this comparison are in line with theoretical expectations, the usefulness of this analysis should be critically examined. The susceptibility of plasma:RBC ratios to factors such as diet (Oishi et al., 1986) and age (Bond and Robertson, 2015) introduces major sources of error into the calculations. Furthermore, the ratios used in the calculations were not specific to black-legged kittiwakes. Thus, the variation in AIV prevalence between years cannot be reliably linked to concentrations of toxic elements. Other factors, such as low sample size or levels of other pollutants, may have played a role in the observed difference.

4.5. Effects of POPs on Probability of AIV Infection

When both POPs and toxic elements were included in the same model (Model A), no POP-related variable was retained in the best-fit model. This suggests that POPs may not exert an important influence on the prevalence of AIV infection in kittiwakes.

Further modeling using only POPs as pollutant variables supported this idea. The sum of all POPs was dropped from the best-fit model (Model B) in favor of year and sex. When the POPs were split into three categories, the only POP category remaining in the best-fit model (Model C) was ΣPBDE, along with year. However, plotting of the modeled data suggested that the significance of this variable was largely due to a single outlier. Removal of this outlier from the data set confirmed this, as ΣPBDE was no longer retained in Model D, leaving year as the only significant variable.

While immunomodulation by POPs has been observed in wild birds, studies have mainly been conducted in birds at higher trophic levels such as glaucous gulls (Bustnes et al., 2004) or birds living in highly-contaminated areas such as the Great Lakes (Grasman et al., 1996). Indeed, the POP concentrations measured in glaucous gulls by Bustnes et al. (2004) are much higher than those from the present study (although these concentrations are not directly comparable as whole blood was used in the glaucous gull study and only plasma was used in this one.)

It is possible that there is a threshold level at which POPs begin to exert an effect on the probability of AIV infection. It would be very interesting to assess the influence of both POPs and toxic elements on AIV infection in a more highly-exposed bird, such as the glaucous gull.

(The glaucous gulls screened in this study were analyzed for per- and polyfluroalkyl substances, but not POPs and elements, and so could not be used for this purpose.)

It is of course also possible that POPs do not play any role in the dynamics of AIV infection in this system due to the nature of the virus, the specific toxic effects of POPs, or some characteristic of the physiology of kittiwakes.

No reliable conclusions can be drawn from the two variables that remained in the best-fit models (sex and year) due to the limitations of this study. Many more female birds were screened than male birds. Thus, the viral prevalence values measured in each sex are unlikely to be accurate and we cannot say that sex certainly influences AIV infection.

Similarly, the small sample sizes mean that the number of infected birds found in each year is not necessarily representative of the actual AIV prevalence in that year. However, it is worth noting that we did find evidence (discussed in Section 4.4.1) that levels of As, Hg, and Se varied between 2015 and 2017. Year may have appeared as a significant factor in these models due to similar annual changes in toxic element concentrations between 2014 and 2015. Further research would be needed to investigate this possibility.

A more homogenous sample population would be ideal for creating a more accurate model. However, since the absence of POPs from these models corroborates the absence of POPs from a model that was created with birds of a single sex from a single year (Model A), it is likely accurate that POPs play little to no role in AIV infection in the birds investigated for this study, and thus at environmental concentrations found in Kongsfjorden kittiwakes.

5. CONCLUSIONS

This study demonstrated that AIV is present among Svalbard black-legged kittiwakes and glaucous gulls. While this may not be a new development, the climate-sensitive nature of the Kongsfjorden ecosystem may alter or already be altering the dynamics of the virus. In order to detect these changes, further research is required to establish baseline data on the prevalence of AIV among local avian species. Screening of Svalbard mammals and assessment of possible transmission routes (e.g. viral shedding at bird colonies, viral presence in water) would help clarify the risk of a destructive disease outbreak.

Selenium and arsenic appear to be the most important pollutants contributing to the risk of infection with AIV in kittiwakes. These findings are supported by previous research establishing the immunotoxicity of these elements, although the mechanism by which they increase the chance of AIV infection is unknown. While mercury has been previously implicated as an immunotoxic compound, it was found to decrease the probability of infection in this system. The possibility of interactions between mercury, selenium, and arsenic and their influences on the immune system warrants further investigation.

While the immunomodulatory effects of POPs have been noted in other avian species, no category of POP appears to impact the probability of infection in this host-disease system. It is possible that kittiwakes in Kongsfjorden are not exposed to high enough concentrations of POPs to cause immune effects. Investigating possible correlations between POPs and AIV infection in more highly-exposed gulls, such as glaucous gulls, could clarify whether POPs impact Svalbard AIV dynamics by way of other species.

Many knowledge gaps still exist regarding the dynamics of AIV in the Arctic and the potential impact of immunomodulatory pollutants. This study confirms the presence of AIV on Svalbard and establishes several possible avenues for future research. Further investigation will be needed to understand and manage the disease risks faced by Arctic species as the environment around them continues to change.

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7. APPENDICES

Appendix 1. Biometric data from black-legged kittiwakes sampled at Blomstrandhalvøya in July 2017. Individuals marked with an asterisk (*) were included in element concentration calculations but not screened for AIV. Individuals marked with a cross (†) were sampled for feathers and/or swabs only and were not used in this study. $Mass_1$ denotes bird mass at the first sampling time-point while $Mass_2$ denotes mass at the second time-point, approximately 1 week later.

Bird No.	Sex	Head (mm)	Tarsus (mm)	Wing (mm)	$Mass_1$ (g)	$Mass_2$ (g)
1*	Female	89.2	33.9	312	365	N/A
2	Male	95	36	326	410	405
3	Female	89	33.5	315	355	370
4	Female	91	34	295	350	360
5*	Male	90	35	310	430	N/A
6	Female	86	33.5	300	360	320
7	Male	91.5	36	310	410	420
8	Female	92	34.5	310	385	360
9	Female	89	34.5	310	385	350
10†	Female	86	33.5	295	335	320
11	Male	94	33	305	430	410
12	Male	93	35	300	430	425
13	Male	94	35	310	405	400
14	Female	87	34	305	410	385
15†	Female	N/A	34.5	N/A	380	N/A
16	Male	94	36	295	450	410
17†	Male	92.5	36	305	N/A	415

Appendix 2. Concentrations (μg/g) of five toxic elements in black-legged kittiwake plasma (gray boxes, denoted by P) and feathers (white boxes, denoted by F), at two sampling points approximately 1 week apart.

Sample	First Time-Point					Second Time-Point				
	As	Cd	Hg	Pb	Se	As	Cd	Hg	Pb	Se
P1	0.1969	0.0017	0.0061	0.0082	0.3291	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
P2	0.0088	0.0003	0.0030	0.0024	0.1605	0.0166	0.0003	0.0018	0.0056	0.2028
P3	0.0420	0.0007	0.0036	0.0025	0.2570	0.0210	0.0007	0.0034	0.0018	0.3271
P4	0.0493	0.0004	0.0051	0.0042	0.2189	0.0441	0.0002	0.0042	0.0022	0.1893
P5	0.1137	0.0004	0.0051	0.0020	0.2657	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
P6	0.1046	0.0002	0.0038	0.0019	0.1923	0.0376	0.0003	0.0051	0.0026	0.1923
P7	0.0632	0.0004	0.0043	0.0030	0.2878	0.0432	0.0002	0.0048	0.0022	0.1891
P8	0.0703	0.0004	0.0037	0.0019	0.1747	0.0400	0.0004	0.0044	0.0050	0.1932
P9	0.0655	0.0004	0.0044	0.0016	0.4543	0.0122	**<LOD**	0.0028	0.0098	0.0567
P10	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	0.0221	0.0005	0.0034	0.0022	0.2404
P11	0.0926	0.0003	0.0034	0.0032	0.2308	0.0412	0.0002	0.0037	0.0016	0.1763
P12	0.0874	0.0003	0.0040	0.0055	0.2573	0.0244	0.0002	0.0054	0.0019	0.1420
P13	0.0363	0.0002	0.0035	0.0073	0.0921	0.0348	0.0002	0.0020	0.0056	0.0459
P14	0.0212	0.0005	0.0052	0.0052	0.5177	0.0729	0.0004	0.0034	0.0043	0.4675
P16	0.0931	0.0003	0.0011	0.0047	0.1502	0.0389	0.0006	**<LOD**	0.0068	0.0601
P17	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	0.0639	0.0003	0.0012	0.0016	0.1626
F1	0.1416	0.0124	3.2771	0.0333	2.5610	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
F2	0.1267	0.0168	7.6637	0.0688	4.5091	0.0927	0.0132	8.1474	0.0964	4.1985
F3	0.1649	0.0239	6.7301	0.0638	4.1918	0.0577	0.0102	5.0498	0.0510	3.7329
F4	0.0836	0.0203	5.0381	0.0549	3.6274	0.1050	0.0112	5.2785	0.0845	3.6150
F5	0.1264	0.0144	5.0052	0.0679	2.8220	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
F6	0.0996	0.0339	3.3623	0.1049	2.6771	0.1569	0.0343	4.6919	0.0431	2.6809
F7	0.1192	0.0120	5.5016	0.0484	2.7271	0.0833	0.0134	4.8767	0.0344	2.7050
F8	0.1685	0.0240	4.6553	0.0421	3.1237	0.1196	0.0189	4.4831	0.1243	2.8225
F9	0.1148	0.0090	5.0449	0.0517	2.8543	0.1041	0.0180	6.3817	0.0251	3.1537
F10	0.1180	0.0101	5.3164	0.0295	3.0242	0.0920	0.0153	6.0795	0.0622	3.4676
F11	0.0889	0.0133	4.0376	0.0616	4.0547	0.0645	0.0399	3.8401	0.0822	4.4232
F12	0.0968	0.0211	4.2717	0.0284	4.4402	0.1616	0.0256	5.0196	0.0340	4.7255
F13	0.1125	0.0325	4.3274	0.0145	3.6321	0.1330	0.0413	4.7244	0.0429	3.4670
F14	0.1173	0.0495	4.8821	0.0699	2.7769	0.1374	0.0460	7.2688	0.0643	3.7170
F15	0.1162	0.0202	4.9753	0.0984	3.6318	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
F16	0.1379	0.0190	7.0790	0.0467	3.4410	0.0996	0.0140	6.5175	0.0521	3.1830
F17	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	0.0865	0.0192	6.0186	0.0279	3.9697

Appendix 3. Descriptive statistics for five elements measured in feathers (n=16) and plasma (n=14) of black-legged kittiwakes sampled in 2017 at Blomstrandhalvøya. Only samples from the first sampling time-point were included in this analysis.

Element	Feather Conc. (µg/g)				Plasma Conc. (µg/g)			
	Mean	SD	Max	Min	Mean	SD	Max	Min
As	0.121	0.024	0.168	0.084	0.075	0.047	0.197	0.009
Cd	0.021	0.011	0.049	0.009	4.603e-4	3.77e-4	1.692e-3	2.02e-4
Hg	5.073	1.221	7.664	3.277	0.004	0.001	0.006	0.001
Pb	0.055	0.024	0.105	0.014	0.004	0.002	0.008	0.002
Se	3.381	0.655	4.509	2.561	0.256	0.116	0.518	0.092

Appendix 4. List of the PCB and PBDE congeners included in the ΣPCB and ΣPBDE values from the each of the four models in this study.

Model A		**Models B, C, D**	
ΣPCBs	ΣPBDE	ΣPCBs	ΣPBDE
CB 99	BDE 47	CB 99	BDE 47
CB 105	BDE 99	CB 105	BDE 99
CB 118	BDE 100	CB 118	BDE 100
CB 138	BDE 153	CB 138	BDE 153
CB 153		CB 153	
CB 156			
CB 170			
CB 171			
CB 177			
CB 180		CB 180	
CB 183		CB 183	
CB 187		CB 187	
CB 194		CB 194	
CB 196/203			
CB 199			
CB 206			