

The Interactive Effects of Incubation Temperature and Organic Contaminants on Shorebird Embryo Development

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By

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Abstract

Understanding the effects of multiple, possibly interactive, stressors on wildlife is an emerging issue in ecotoxicology. In particular, how changes in temperature will alter organisms' responses to chemical contaminants may be of particular concern for arctic-breeding shorebird populations, which have declined in recent decades, concomitant with changes in spring temperatures and continued long-range transport and release of persistent organic pollutants (POPs). During incubation, for example, changes in temperature and exposure to environmental contaminants can both negatively affect avian development and may have interactive effects on the embryo that are more detrimental than either effect individually (cumulative effects hypothesis). Specifically, incubation temperature may influence the rate of development and how contaminants are metabolized, resulting in potentially multiplicative effects on embryo growth and development.

To test the cumulative effects hypothesis and assess contaminant and temperature conditions during incubation, I conducted captive (Chapter 2) and field studies (Chapter 3) using shorebirds as model organisms. In the former, I conducted a controlled egg injection and incubation study on wild killdeer (*Charadrius vociferous*) eggs exposed to concentrations of a known endocrine disruptor, PCB-126 (0, 44 or 89 ng/g) and incubated at either low (36°C), intermediate (37.5°C), or high (39°C) temperatures. In the latter study, I analyzed POPs in semipalmated sandpiper (*Calidris pusilla*) eggs from three arctic sites and examined relationships between egg POPs, ambient temperature, nest-cup temperature, and chick measures at hatch. My captive study results showed the most severe effects (yolk distention, first detection of heartbeat and incubation length, righting reflex) in birds incubated under suboptimal low or high incubation temperatures combined with high PCB-126 exposure, indicating that shorebird embryos may be more tolerant of contaminant exposure under intermediate temperatures. Field results indicated relatively low ubiquitous levels of POPs – namely organochlorines (mean \pm standard deviation, 29.33 ± 37.08 ng/g), brominated flame retardants (2.44 ± 6.56 ng/g), and polychlorinated biphenyls (26.59 ± 54.25 ng/g) – in semipalmated sandpiper eggs. At the nest level, incubation temperature was positively correlated with ambient temperature, suggesting incubating adults cannot fully buffer the effects of temperature fluctuations and embryos may be susceptible to suboptimal incubation temperatures. Collectively, these findings suggest that changing arctic conditions that alter spring temperatures

and release of historical POPs can interact to affect avian development with potential consequences for reduced fitness.

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Preface

Chapter 1 of this thesis is a general introduction and Chapters 2 and 3 are written in a manuscript style for publication in scientific journals. There is therefore some repetition of introductions, materials, and methods throughout each chapter. Chapter 4 is a conclusion to both studies, with recommendations for future research.

1 CHAPTER 1: General Introduction: Influences of Temperature and Persistent Organic Pollutants on Birds

1.1 Temperature fluctuations

Global climate change has occurred at an unprecedented rate during the last century, with polar regions experiencing warming twice the global average with a projected increase of 2.5–5.5 °C by 2099 (AMAP, 2016; ACIA, 2004; Walsh, 2014). Concurrent with higher mean temperatures, scientists forecast increased variability in daily temperatures (Easterling et al., 1997). This has led to repercussions on a global scale, such as disturbances to the sensitive cryosphere (seasonal snow cover, glaciers, ice caps, permafrost, and sea, river and lake ice), which is arguably one of the most important factors affecting arctic wildlife survival (AMAP, 2016; Barron et al., 1995; Walsh, 2014). These environmental changes have already begun to have implications for wildlife habitat and populations. In birds, climate change is influencing the advancement in the timing and duration of migration and breeding (Gaston et al., 2005; Laaksonen et al., 2006; Smith et al., 2010; Liebezeit et al., 2014), trophic mismatch (McKinnon et al., 2012), clutch size (Lepage et al., 2000), and chick size (Andrew et al., 2017).

In particular, temperature is one of the most important physicochemical forces acting on living organisms, especially stenothermal ectotherms, such as avian embryos (*in ovo*), which operate within a narrow range of environmental conditions to develop and survive (Schmidt-Nielsen, 1997). Avian embryos are highly susceptible to temperature changes because they are unable to regulate their own body temperature and rely entirely on the microenvironment around the egg, which is controlled by ambient temperature and heat transfer from incubating adults (Amininasab et al., 2016; Bertin et al., 2018). Thus, inclement temperature can affect survival, breeding, or decrease reproductive success by affecting incubation of eggs or growth and survival of offspring (Elkins, 2004; Vali, 2012). Changes in incubation temperature can be caused by several factors: nest initiation date, nest attentiveness, clutch size, parental condition, individual behavior, and microclimate conditions (Boersma, 1982; Durant et al., 2013b; Kwon et al., 2018), such that thermal conditions during development are rarely constant. For example, field measurements have shown that embryos are exposed to substantial daily thermal fluctuations when adult birds leave the nest (foraging, anti-predator response), and thus eggs are

frequently subject to temperatures above and below their ideal temperature range (Grant, 2012). In addition, Schneider and McWilliams (2007) found that plovers were unable to maintain constant incubation temperatures when ambient temperatures were below $<20^{\circ}\text{C}$.

Deviations from a species' ideal incubation temperature is a concern because even brief exposures to high and low temperatures can have long-term effects on the development of the embryo (Ardia et al., 2010), and fluctuations of even 1°C can influence embryonic development, incubation length, growth, survival, hatchling weight, and subsequent offspring behaviour and fecundity (Bertin et al., 2018; DuRant et al., 2013). For example, in wood ducks (*Aix sponsa*) incubation temperatures below the optimum resulted in increased incubation length and reduced survival (Hepp et al., 2006), as well as higher baseline and stress-induced corticosterone concentrations and lower growth rates (DuRant et al., 2009). In contrast, high incubation temperatures accelerated the developmental process in wood ducks (Hepp et al., 2006), but reduced survival and body mass in blue tits (*Cyanistes caeruleus*; Nord & Nilsson, 2016), highlighting the importance of maintaining incubation temperatures within an optimal range for proper embryonic growth and development (DuRant et al., 2013).

1.2 Distribution of persistent organic pollutants (POPs)

During the last half of the 20th century, the global environment has become contaminated with persistent organic pollutants (POPs), which are recalcitrant, lipid-soluble chemical contaminants that were produced commercially in large quantities for diverse industrial applications (Tanabe, 1988). Long-term (multi-annual) global changes in levels of POPs in environmental media and biota are determined by changes in three main factors i) deliberate emissions/release through industrial applications, agriculture, consumer products, as well as, inadvertent formation of by-products of incomplete combustion or industrial processes, ii) redistribution of contaminants previously accumulated in the environment (surface soils and surface waters) and iii) environmental transport pathways, and processes that affect these, both in abiotic systems (e.g. ice and precipitation, winds and ocean currents) and in biological systems (ecosystem and food web structure, etc.; AMAP 2016). Many POPs are referred to as legacy POPs, production and use of which are regulated, banned, or have been voluntarily withdrawn. However, persistent organic pollutants exhibit physicochemical properties such as low water solubility, strong lipophilicity, and resistance to metabolic degradation that ensure the rate of

their reduction in the environment is slow. Therefore, today they can be detected in virtually every environmental matrix worldwide, including undeveloped polar regions, sometimes decades after production and use have been banned (Letcher et al., 2010; Melnes et al., 2017; Safe, 1994). The primary source of exposure to POPs for wildlife are from foods or prey items located in freshwater and marine environments. Many POPs have considerable accumulation potential in organisms and can bioaccumulate in sediments and soil and biomagnify in the food chain, where they can result in a large variety of chronic health effects, such as endocrine disruption (Frye et al., 2012), organ abnormalities (Bosveld et al., 2005; Powell et al., 1997), morphological abnormalities (Ferne et al., 2003), changes in immune function (Lavoie & Grasman, 2007a) and behaviour (DeWitt et al., 2005; Eng et al., 2012), and reductions in survival (Barron et al., 1995a), growth (Rattner et al., 2013), and hormone secretion (Cesh et al., 2010; Katarzyńska et al., 2015).

1.2.1 Polychlorinated biphenyls: structure and properties

Polychlorinated biphenyls (PCBs) are a class of POPs that – despite many bans in sales and use since the 1970s – remain of particular scientific and public concern due to their ubiquity, persistence, lipophilicity, and the wide array of adverse health effects documented in laboratory and wildlife species. This complex group is a family of aromatic hydrocarbons that consist of two linked benzene rings (biphenyl) substituted with 1-10 chlorine positions. The carbon positions are numbered 1 to 6 on one ring, and 1' to 6' on the other. Positions 2,2',6, and 6' are called “ortho”, positions 3,3',5 and 5' are named “meta” and 4 and 4' are called “para” (Figure 1.1). Different patterns of chlorine substitution make up 209 congeners. For a given PCB congener, the name lists the numbers sequentially (e.g. the PCB congener with chlorines on carbons 2,4,5, and 3',4' is identified as 2,3',4,4',5). PCB congeners of the same chlorination are divided into groups referred to as homologs or isomers (i.e. homologs with different substitution patterns) from mono- to decachlorobiphenyls. More than 60% of the PCBs are tetra- to hexachlorophenyls. Congeners with a high degree of chlorination are more resistant to biotransformation and elimination from the body and therefore have longer relative residence times in biological tissues. Less chlorinated congeners tend to have shorter residence times because they are biotransformed and eliminated faster. Specifically, congeners with chlorine atoms in the 2,5- and 2,3,6-positions are more susceptible to biotransformation, whereas

congeners with chlorine bonds in the 2,3,4- 2,4,5- 3,4,5- and 2,3,4,5-positions are more persistent.

PCBs are often divided into two distinct classes of coplanar, (non-ortho substituted) and non-coplanar (ortho-substituted) congeners (Katarzyńska et al., 2015). Coplanar PCBs have chlorines in the non-ortho positions resulting in the two phenyl rings assuming a planar state which results in a rigid structure and are structurally similar to dioxins and thus are called dioxin-like compounds (DLCs). In non-coplanar PCBs, chlorination of the ortho positions does not favor a planar (i.e flat) alignment of the phenyl rings. Coplanar PCBs (e.g. PCB-126) are known developmental and immune system toxicants, which have been shown to have many adverse effects on wildlife: organ and body malformations (Ludwig et al., 1996; Zhang et al., 2002), immunotoxicity (Riecke et al., 2003), endocrine disruption (Ottinger et al., 2007), edema (Powell et al., 1997), and mortality (Barron et al., 1995), as well as causing behavioural (Bustnes et al., 2001; Frye et al., 2012) and developmental deficits (Ferne et al., 2006; Sagerup et al., 2009).

1.3 Flame retardants: structure and properties

Flame retardants are a diverse group of chemicals, which can be categorized based on chemical structure and properties (i.e. whether they contain bromine, chlorine, phosphorus, nitrogen, metals, or boron). Those containing bromine (i.e. brominated flame retardants) are the most abundantly used flame retardants and are applied to a broad range of combustible materials, such as plastics, textiles (e.g. furniture), and surface finishes (e.g. electronics) to inhibit or delay the spread of fire by releasing halogens (Bergman et al., 2012; Darnerud, 2003; De Wit, 2002). Additive BFRs are not covalently bound products to which they adhere, resulting in the chemicals separating from the surface and leaching into the environment (De Wit, 2002). Polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), and hexabromocyclododecanes (HBCDs) are among the most abundant BFRs detected in the environment and are found in almost all biotic and abiotic matrices worldwide (Costa et al., 2008). Brominated flame retardants have been shown to reduce brood size in peregrine falcons (*Falco peregrinus*; Johansson et al., 2009), induce changes in thyroid, vitamin A, glutathione homeostasis, oxidative stress, immunomodulation, and growth in American kestrels (*Falco*

sparverius; Fernie et al., 2005a; Fernie et al., 2005b; Fernie et al., 2006), and have varying effects on reproduction of osprey (*Pandion haliaetus*; Henny et al., 2011; Henny et al., 2009).

1.3.1 Organochlorine pesticides: structure and properties

Organochlorine (OC) pesticides, such as dichlorodiphenyltrichlorethane (DDT), hexachlorobenzene (HCB), and dieldrin, are synthetic pesticides commonly used all over the world. They were used in large amounts for agricultural production, which led to increased pollution of environmental compartments (soil, water, and air), as well as accumulation in humans and wildlife. One of the most widely known organochlorine pesticides is DDT, an insecticide that was widely used during World War II – with about 100,000 tons per year produced in the late 1950s in the United States (Connell, 2005). DDT and its metabolite DDE have well-established adverse effects on reproduction, due to its estrogen agonistic and neurotoxic activities (WHO, 1979). For example, DDT from urban and agricultural pesticides caused reproductive failure in western grebes (*Aechmophorus occidentalis*; Rudd & Herman, 1972), ospreys (Spitzer et al., 1978), peregrine falcons (*Falco peregrinus*; Cade et al., 1971), and bald eagles (Grier, 2006).

1.4 Avian species sensitivity to dioxin-like contaminants

Dioxin-like contaminants (DLCs) are a family of compounds encompassing several POPs, which share common structures that cause adverse biological effects (Tilley & Fry, 2015). the term “dioxins” may be used to refer to compounds (e.g. 2,3, 7,8-TCDD) which are not intentionally generated, however many are by-products of industrial or combustion processes. Species sensitivity to DLCs is dependent on many factors, namely species, age, and sex, which can influence the exposure deposition, depuration, and circulation of contaminant uptake and thus toxicological effects (Giera et al., 2011; Maervoet et al., 2005), and the toxic effects of individual dioxins among avian species can vary 1000-fold (Farmahin et al., 2012). For example, trace levels of 3,3',4,4',5-Pentachlorobiphenyl (PCB-126) decreased pipping and hatching success in American kestrels and common terns (*Sterna hirundo*), but not chickens (*Gallus gallus*; Hoffman et al., 1998; McKernan et al., 2009).

Variation in avian sensitivity to DLCs is largely mediated by the aryl hydrocarbon receptor (AhR), a ligand-activated nuclear transcription factor (Eng et al., 2014; Farmahin et al.,

2013; Head et al., 2008). AhR1 is a highly transcriptionally active AhR paralog that consists of three major domains—the DNA binding domain, the ligand-binding domain (LBD), and the transactivation domain (Karchner et al., 2006), and different degrees of sensitivity are associated with identity of the amino acids within the LBD at sites 324 and 380 (Eng et al., 2014; Farmahin et al., 2013; Head et al., 2008). The sequences of the AhR1 LBD in over 70 avian species have been determined, resulting in three identified groups of sensitivity: high sensitivity (type 1, Ile324_Ser380), moderate sensitivity (type 2, Ile324_Ala380), and low sensitivity (type 3, Val324_Ala380 (Farmahin et al., 2013; Head et al., 2008; Karchner et al., 2006). For shorebirds, the AhR1 LBD type are known only for a few species, including the spotted sandpiper (*Actitis macularius*; Type 2) and killdeer (*Charadrius vociferous*; Type 3; Farmahin et al., 2013).

1.5 Vulnerability of early life stages

In birds, many components critical to normal physiology and behaviour are formed during early life stages, making these stages the most vulnerable to both natural and anthropogenic stress. Inter-tissue distribution kinetics of PCBs in birds is relatively rapid and completed within a little over a week following exposure to a contaminated diet (Daley et al., 2013). This rapid distribution of contaminants is a concern for developing birds because embryogenesis is a critical period of growth when an embryo can be highly susceptible to external stressors. Organogenesis (the process of organ growth) is an important period during development that occurs on day four of incubation, which suggests contaminant exposure to a developing embryo when organs are being formed is a critical window (Cohen-Barnhouse et al., 2011).

Circulating hormones, namely the pituitary growth hormone, corticosterone, sex steroids, and thyroid hormones (TH), influence development, growth, physiology, and behaviour and have the ability to mediate stress through a multifaceted response to environmental and physiological stimuli (Astier, 1980). DLCs are endocrine-disrupting chemicals, which are a group of xenobiotics that alter hormone-dependent processes and disrupt endocrine gland function by increasing metabolism and excretion of TH (Fernie et al., 2003; Giera et al., 2011; Katarzyńska et al., 2015; Melnes et al., 2017). In addition, due to their estrogenic potency, exposure to DLCs, namely PCBs (non-ortho-substituted) during early life stages can alter pituitary, gonadal, and thyroidal function, as well as hormone secretion and size and shape of the hypothalamic-

pituitary-thyroid, which controls egg incubation, pipping, and hatch (Durant et al., 2013a; Rattner et al., 2013; Verreault et al., 2006). For example, black guillemots (*Cephus grylle*) breeding near PCB-contaminated Saglek Bay in Labrador had enlarged livers, elevated ethoxyresorufin-O-deethylase (EROD) activity, and increased liver lipid levels, which correlated to PCB levels in tissues (Fisk et al., 2005).

In precocial birds, the thyroid gland emerges on day two, secreting two hormones thyroxine (T₄), and triiodothyronine (T₃), which increase during late stages of incubation and are thought to be important for embryonic growth, tissue growth and differentiation, organ maturation and hatching (Durant et al., 2014). Exposure to these contaminants also alter body temperature regulation, which is especially a concern for precocial birds whose survival is dependent on their ability to produce endogenous heat and limit heat loss to maintain stable body temperature at hatch (Whittow & Tazawa, 1991). Furthermore, wood ducks incubated at low temperatures had lower concentrations of T₃ and exhibited reduced physiological performance (e.g. slower growth and poor thermoregulatory ability). These studies indicate thyroid hormone can act as an underlying mechanism for the effects on thermoregulation, metabolism, growth and immune responses to temperature and contaminant exposure.

1.6 Temperature and contaminant interactions

An important consequence of climate change is increased exposure to contaminants from altered environmental distribution mechanisms (Macdonald et al., 2003). For example, increased ambient temperature can change environmental partitioning of contaminants through mechanisms of increased volatility, wet deposition, and enhanced degradation. Climate change (loss of permafrost, change in the seasonal cycle of snow or ice) may also alter soil and water concentrations or re-release POPs into the environment, making them bioavailable (Macdonald et al., 2005; Noyes et al., 2009). At a finer scale, changes in ambient temperature can influence the cycle of contaminant uptake, accumulation, elimination, and detoxification within individuals (Patra et al., 2015). Maternal contaminant transfer and uptake in eggs is highly influenced by the quantity and metabolism of lipids to an egg, and eggs incubated at higher temperatures use lipids more rapidly than those incubated at low temperatures (Drouillard et al., 2003; Ikonopoulou et al., 2013). Chronic exposure to some contaminants can reduce the upper thermal tolerance for organisms, such that some contaminants become more toxic at elevated temperatures, increasing

the risk of toxic interactions during global warming (Patra et al., 2015). Thus, as temperature, metabolism, and toxicity increase, species living close to their thermal tolerance may experience adverse effects compared to those who are living within their normal thermal range. Although there is considerable evidence for single stressor effects, such as temperature or contaminant exposure, on developing avian species, rarely do single stressors occur in the wild, and the complex effects of combined temperature and contaminant stress in free-living species has not been studied in free-ranging avian species.

1.6.1 Shorebirds as a model study species

The most recent State of Canada's Birds 2019 Report indicates that migratory shorebird populations have declined by almost half since 1970, experiencing some of the most substantial declines of any guild (Morrison et al., 2006, Andres et al., 2012). In particular, 60% of shorebirds breeding in the arctic are facing major population declines with three out of the six species currently listed under the Species at Risk Act (SARA) as Special Concern, Threatened, or Endangered, (Buff-breasted Sandpiper (*Tryngites subruficollis*), Eskimo Curlew (*Numenius borealis*) and Red Knot (*Calidris canutus*; Government of Canada 2019). Several potential threats have been investigated, for instance the loss of winter and breeding habitat, decreased availability of food, disease and higher predation rates (NABCI, 2012). However, potential threats related to increasing contaminant exposure simultaneous with fluctuations in ambient temperature are unknown. This is surprising because shorebirds are vulnerable to elevated concentrations of POPs when foraging in wetlands, estuaries, and agricultural fields where contaminants may disproportionately accumulate in sediments and biota (Braune & Noble, 2009). Furthermore, arctic shorebirds are susceptible to unpredictable temperatures and weather during breeding which may result in variation in incubation temperatures. Therefore, I propose that shorebirds are an ideal study group given their high risk of contaminant exposure, variable climate conditions they experience, and the relevance to conservation. In my captive study (Chapter 2), I used killdeer (*Charadrius vociferous*) as a representative of the Charadriidae family, because it is not feasible to collect live eggs from arctic breeding birds. In addition, killdeer are suitable model species because they have a large breeding range and are abundant in North America. Killdeer also have similar life history traits as many arctic shorebirds and can be successfully reared in a captive setting (Malone & Proctor, 1966). To analyze the potential

interactive effects of contaminants and climate change on free-living arctic shorebirds (Chapter 3), I used Semipalmated sandpipers (hereafter “sandpipers”; *Calidris pusilla*), because they are one of the most broadly distributed shorebird species in North America, breeding in areas (the sub-arctic to mid-arctic across northern Canada and Alaska) where contaminants are known to accumulate (AMAP, 2016; Andres et al., 2012; Gratto-Trevor et al., 2012).

1.6.2 Study sites

Each of my two studies took place in different study sites. Chapter 2 research took place in southern Saskatchewan at the Facility for Applied Avian Research at the University of Saskatchewan. The survey area for killdeer egg collection was over 360 km² with a focus on Porter Lake basin (52.1954082, -106.2772211). Porter Lakes basin has been listed as a Globally Significant Important Bird Area for shorebirds based on the high number of Hudsonian Godwits (*Limosa haemastica*; IBA website). The basin is surrounded by primarily cropland and native prairie with a gravel dirt road intermittently washed away from flooding on the east side of the basin. It is a shallow saline lake, which makes it susceptible to drought creating mudflats from the receding shoreline during periods of lower water levels. The exposed mudflats and cropland attract many shorebird species including killdeer, Hudsonian godwits, American avocets (*Recurvirostra americana*), and spotted sandpipers (*Actitis macularius*).

To evaluate the levels and types of POPs in arctic-nesting shorebirds in Chapter 3, I analyzed data collected at three field sites across Inuit Nunangat and Alaska, which span the range of breeding sandpipers in North America (Table 3.1, Figure 3.1). The Colville River Delta (hereafter Colville), Alaska, United States (70°42'N, 150°68'W) is the largest river delta on Alaska's North Slope, consisting of diverse habitats such as wet meadows, tundra, and freshwater lakes critical for a range of species. The Karrak Lake Research Station (hereafter Karrak), Nunavut, Canada (67°00' N, 100°30' W) is located within the Ahiak Migratory Bird Sanctuary, which covers an area of 62,920 km², making it the largest protected area in Canada. The sanctuary includes extensive wetland habitat that provides vital habitat for many migratory species. Coats Island (hereafter Coats), Nunavut, Canada (62°79' N, 82°28' W) is comprised of mostly low-lying sedge tundra, wetlands, and raised beaches with diverse populations of seabird and shorebird species. Due to logistic constraints, potential effects of POPs levels and changes in ambient temperature on incubation duration and chick size were assessed only at Karrak in 2017.

1.6.3 Study objectives

The complex nature of interactions between temperature and the toxicity of a wide range of contaminants present challenges for wildlife populations that are important considerations in ecological risk assessments. Although extensive research on avian species has been done in controlled settings, a more complete study that integrates field and captive studies can help better interpret possible interactive effects that might be expected in wild species. For example, captive ecotoxicity studies are valuable tools for avian research that help document direct effects under controlled conditions. However, they commonly experiment on a single domestic species, exposed to one particular type of chemical under ideal environmental conditions, which do not accurately reflect the environmental stressors that wild populations endure. Field research provides complementary information on dynamics affecting wildlife populations by measuring contaminant concentrations in the environment or tissues of biota, or the environmental conditions they experience in the wild. Therefore, it is important to supplement field research with captive studies to allow direct manipulation under controlled setting, allowing easy interpretation of direct effects and drawing on the knowledge of realistic field conditions. To my knowledge, the study of the interaction between temperature and contaminants is unknown in any shorebird species. Consequently, the cumulative effect of multiple stressors on developing shorebirds cannot be predicted based on existing scientific knowledge. The broad goals of my research were therefore to provide an improved understanding of the current thermal and toxicological stresses that wild arctic breeding shorebird populations are facing, as well as the interactive effects of these stressors on shorebird growth and development.

My objectives were:

- I. To determine the effects of varying incubation temperatures and 3,3',4,4',5-pentachlorobiphenyl (PCB-126), a known endocrine and thyroid disrupting compound, on incubation duration, hatchability, developmental and growth of wild shorebirds reared in a captive setting (Chapter 2).
- II. To assess levels and types of POPs in shorebirds and to evaluate potential effects of contaminant exposure and changes in ambient temperature on wild birds in northern areas (Chapter 3).

1.7 Tables and figures

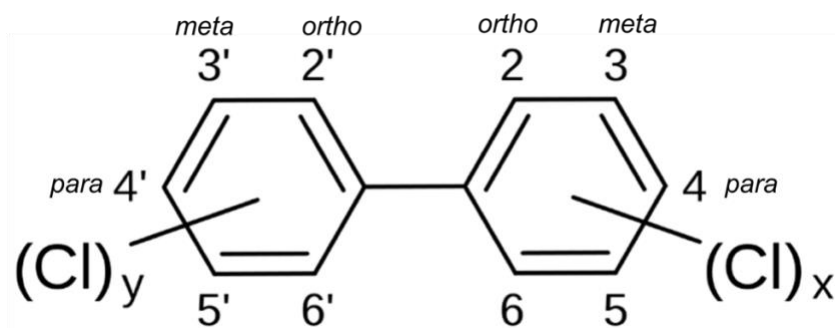


Figure 1.1: Generalized molecular structure of a polychlorinated biphenyl (PCB) showing the 10 chlorine substitution positions on the biphenyl ring (i.e. 2,2',6,6' (ortho), 3,3',5,5' (meta) and/ or 4,4' (para)). Different patterns of chlorine substitution make up 209 congeners which are divided into groups called homologs (10 groups total).

2 CHAPTER 2: The Interactive Effects of Incubation Temperature and 3,3',4,4',5-pentachlorobiphenyl (PCB-126) exposure on Killdeer (*Charadrius vociferus*) Embryo and Chick Development

2.1 Abstract

In birds, many components critical to normal physiology and behaviour are a product of the embryonic and early post-hatch developmental environment. Therefore, an individual's early life stage is highly vulnerable to both natural and anthropogenic stress, such as temperature and exposure to environmental contaminants. For example, incubation temperature may influence the rate of development and contaminant metabolism and absorption in body tissues, resulting in potentially multiplicative impacts on embryo development (cumulative effects hypothesis). I tested the hypothesis that early contaminant exposure and temperature stress can both negatively affect avian development and may have interactive effects that are more detrimental than either stressor individually. I conducted a controlled egg injection and incubation study on wild killdeer (*Charadrius vociferous*) eggs exposed to concentrations of a known endocrine disruptor, 3,3',4,4',5-pentachlorobiphenyl (PCB-126; 0, 44 or 89 ng/g) and incubated at either low (36°C), intermediate (37.5°C), or high (39°C) temperatures. My results indicated low temperature treatments had earlier detection of heart beat, longer incubation length, lower growth rate post-hatch, and higher post-hatch mortality compared to birds incubated under intermediate temperatures. High incubation temperatures resulted in shorter incubation length, earlier detection of heart rate and faster righting time. As predicted, embryo and chick mortality was greater in the high PCB dosed birds incubated at intermediate and high temperatures. Incidence of distended yolk sacs (%) also increased with PCB exposure in all temperature groups, with the largest increase in the high temperature group. Overall, my results show low incubation temperature can cause greater adverse effects than PCB-126 exposure, but that negative effects of PCB-126 exposure are exacerbated by high incubation temperatures. These findings suggest that shorebird embryos may be more tolerant of PCB exposure when incubated at 37.5°C, rather than at temperatures either below or above the apparent optimum.

2.2 Introduction

Global annual mean temperatures increased by 0.9 °C during 1976–2019 (NOAA, 2019) and are projected to further increase (2.5–5.5°C) by the end of the century, with many regions experiencing asymmetrical warming and increased fluctuations in temperature (Millett et al., 2009; Zongxing et al., 2012; IPCC, 2014). In particular, wildlife in northern areas are experiencing disproportionate effects of global climate change, including annual average air temperatures that have increased twice as fast as elsewhere in the world (Walsh et al., 2014). This is a concern because temperature plays a critical role in animal development and is arguably one of the most important physicochemical forces acting on living organisms (Pearson & Dawson, 2003; Post & Forchhammer, 2007; Simmonds et al., 2017). In wild birds for example, changes in ambient temperature can influence the intensity of incubation effort and average incubation temperature (Amininasab et al., 2016; Conway, 2002). Incubation temperature influences incubation length, growth, survival, hatchling weight, and subsequent offspring behavior and fecundity (DuRant et al., 2013b). Therefore, maintaining incubation temperature within an optimal range is vital for proper embryonic development, and any deviation can cause stress and possible mortality (Durant et al., 2013ab). For example, subtle temperature differences (<1.0°C) in wood ducks (*Aix sponsa*) during incubation had adverse consequences for many physiological traits such as growth and increased baseline and stress-induced corticosterone concentrations that can later impact fitness (Durant et al., 2009). Relative to southern-nesting species, arctic-nesting bird species may be particularly affected by changes in incubation patterns, because once the parent leaves the nest, eggs are exposed to extreme daily thermal fluctuations, that match ambient temperature fluctuations (AMAP, 2016; ACIA, 2004; Walsh, 2014)

Simultaneous with ongoing temperature fluctuations, wildlife are experiencing exposure to many environmental contaminants. In particular, persistent organic pollutants (POPs) have a long half-life in the environment due to their strong lipophilic properties and resistance to metabolic degradation (Barrie et al., 1992; Hung et al., 2010). Many POPs are highly lipid soluble and have considerable accumulation potential in organisms and, in some cases, can biomagnify in the food chain, causing a variety of health effects in free-living animals, including reductions in survival, growth and fertility, gonadal abnormalities and behaviour and immune dysfunction (Barron et al., 1995; Frye et al., 2012; Grasman & Whitacre, 2001; Norstrom et

al.,1988). In particular, polychlorinated biphenyls (PCBs) are endocrine-disrupting POPs, which at sub-lethal levels can interfere with the endocrine system and alter thyroid hormone production and regulation, which control many aspects of physiology, for example basal metabolic rate, thermoregulation, differentiation of organ systems, growth, hatching, lipogenesis, and brain and nervous system development (Verreault et al., 2004). The coplanar PCB congeners share structural similarities and a common mechanisms of action as dioxin-like contaminants and can bind with high affinity to the aryl hydrocarbon receptor (AhR), a ligand-activated nuclear transcription factor that affects toxicity and species sensitivity (Farmahin et al., 2013; Head et al., 2008). This complex group is a family of aromatic hydrocarbons that consist of two linked benzene rings (biphenyl) substituted with 1-10 chlorine positions. The carbon positions are numbered 1 to 6 on one ring, and 1' to 6' on the other. Positions 2,2',6, and 6' are called "ortho", positions 3,3',5 and 5' are named "meta" and 4 and 4' are called "para" (Figure 1.1).

In particular, early life stages are highly vulnerable to both natural and anthropogenic stress, including incubation temperature and POPs, and cumulative impacts during these stages may have an important influence on avian development (Harris & Elliott, 2011; Ottinger et al., 2007). Changes in temperature can influence the toxicokinetics (chemical absorption, distribution, metabolism, and excretion) of contaminants in wildlife, possibly causing a magnified response to chemical exposure under variable climate conditions (Holmstrup et al., 2010; Hooper et al., 2013; Stahl et al., 2013). Stress during the embryonic and early post-hatch developmental environment has been shown to impair traits related to fitness, such as post-hatching growth rates, behavior, fear response, and locomotion, and precocial birds may be particularly affected, because they must acquire much of their own resources after hatching without the parents' help (DuRant et al., 2013ab). Therefore, optimal early development in precocial offspring may be crucial for offspring post-hatch growth and survival.

Few studies have evaluated the combined effects of contaminant exposure and changing incubation conditions on development of wild, precocial offspring. Here, I studied killdeer (*Charadrius vociferous*), a shorebird (Family: Charadriidae) model, to represent arctic migrant shorebirds that may be disproportionately affected by temperature fluctuations during incubation as well as contaminant exposure when foraging in wetlands and estuaries on breeding grounds where contaminants accumulate in sediments and biota (Braune & Noble, 2009; Hargreaves et al., 2010). Therefore, my objectives were to use a multi-stressor approach to test (i) the

individual and (ii) combined responses of contaminant exposure and changing incubation temperature on embryonic and post-hatch development in an avian model. I accomplished this using a controlled egg injection and incubation study on eggs exposed to a known endocrine disruptor, PCB-126 (0, 44 or 89 ng/g) and incubated at either low (36°C), intermediate (37.5°C), or high (39°C) temperatures.

2.3 Methods

2.3.1 Killdeer egg collection

It was not feasible to collect live eggs from arctic breeding birds; therefore, I chose to use killdeer as a representative of the Charadriidae family in the captive study because they are a precocial, abundant shorebird in North America that has similar life history traits as many arctic shorebirds and have been successfully reared in a captive setting (Malone & Proctor, 2017). Killdeer commonly inhabit a wide variety of aquatic and industrial habitats in our study area. Northern populations of killdeer breed from mid-April to August and can lay up to three clutches of 3-4 eggs per breeding season (Bunni, 1959). Killdeer nests were searched for in areas of known and suspected breeding sites based on a pilot study (Vien & Morrissey, unpublished data) and habitat preferences (rocky substrate in close proximity to water bodies) within southern Saskatchewan, Canada. Although the search area was over 360 km², the majority of nests were found near Porter Lake (52.1954082, -106.2772211) and the Bradwell Reservoir (51.9034173, -106.2531949) in small groups along roads parallel to lakes or drainage systems.

Once I found a killdeer nest, eggs were floated and candled to determine the number of days incubated (Ackerman & Smith 2010; Lokemoen & Koford 1996; Brua & Machin 2000). If eggs were incubated less than three days (typically nests contained fresh and unincubated eggs), they were collected and transported in a small padded container to the Facility for Applied Avian Research (FAAR) at the University of Saskatchewan. I placed eggs in Brinsea Egg Incubators (Brinsea Products, Western-super-mare-UK) less than five hours after collection. Killdeer egg collection and research was in accordance with permits issued by Environment and Climate Change Canada (SK-SC004 and 15-SK-SC004A). All experimental procedures conform to the Canadian Council on Animal Care (CCAC) guidelines and were approved by the University of Saskatchewan's University Animal Care Committee (UACC; Permit 20120021).

2.3.2 Treatment groups and egg injection with PCB-126

I randomly assigned eggs from each nest to 1 of 6 treatments composed of three concentrations of PCB-126 (0, 44 or 89 ng/g) and three incubation temperatures; low (36°C), intermediate (37.5°C), or high (39°C). I dissolved PCB-126 in dimethyl sulfoxide (DMSO) to produce a control (0 ng/g) and two dose levels: low PCB, (44 ng/g egg) and high PCB (89 ng/g egg). Selected PCB-126 dose levels were to mimic toxicologically relevant exposures that produce sub-lethal effects, while not producing overt signs of toxicity. Accordingly, our exposure level did not exceed the predicted PCB-126 LD₅₀ for type-3 species (89 ng/g, 95% CI = 29-297 ng/g (Farmahin et al., 2013; Table 2.1). Higher concentrations have been tested on other precocial, type-3 species; Common tern (*Sterna hirundo*, LD₅₀=104 ng/g) and Double-crested-cormorant (*Phalacrocorax auratus*, LD₅₀=177 ng/g; Powell et al., 1998; Hoffman et al., 1998) but not shorebird species. Dosing solutions were formulated based on an injection volume of 0.5 µL/g egg, and mean concentrations of PCB-126 for all dosing solution batches were confirmed by SGS AXYS Analytical Services Ltd and measured as < detection (0 PCB ng/g, DMSO only), 30 ng/g (Low PCB, nominal= 44 ng/g) and 83 ng/g (High PCB, nominal = 89ng/g; Sidney, BC, CA).

Egg injection prior to incubation allows the compound to partition into the matrices of the egg components and thus mimic maternal deposition (Dean et al., 2018). Organogenesis (the process of organ growth) is an important period during development that occurs on day four (HH stages 22-25) of incubation (Hamburger & Hamilton, 1951). Killdeer have an incubation length of ~22-24 days, so I injected on days 1-3 to mimic exposure during the first critical sensitive period from maternal transfer (DeWitt et al., 2005; Romanoff, 1972). Injection sites were cleaned with 70% ethanol and a dry cloth. An injection hole was drilled with a Dremel tool (0.08 mm) until the eggshell was thin enough for a syringe to pierce through. I placed each egg with the air cell (rounded side) down, which allows egg yolk to float upwards (pointed side up). A sterile 27-gauge (2") bevelled needle (Hamilton syringes, Gastight 1700 Series) was pushed through the membrane from the rounded bottom end, upwards, trapping the yolk in the upper pointed end. This allowed the needle to successfully pierce the egg yolk for administering the PCB 126 dose. The injection site was sealed with paraffin wax or super glue.

2.3.3 Incubation and animal husbandry

Incubation of killdeer eggs followed protocol from the Brinsea handbook (Brinsea Products, Western-super-mare-UK), and temperatures were selected from a previous pilot study that determined optimal killdeer incubation temperature based on survival (37-38°C; Vien & Morrissey, unpublished data), with temperatures greater and/or lower than 37.5°C considered as suboptimal. I placed eggs horizontally in incubators set to 36°C, 37.5°C and 39°C, maintained at 75-80% humidity, automatic turning of eggs every 45 minutes, and a single cooling period 20 minutes a day. I weighed eggs to the nearest 0.1g every 4 days and presence of a heart beat and heart rate (beats per minute) was recorded daily using the Buddy Digital Egg Monitor (Vetronic Services, Abbotskerswell, Devon, UK). Near the end of incubation, I inspected eggs once or twice daily for “starring” (cracks in shell, a sign of chick emergence), then removed the egg from the turning racks and placed it into individual plastic mesh holding cages (8 x 8 cm) within the same incubator. This allowed for individual identification after hatch. Newly hatched killdeer remained in the incubator until feathers were dry.

Up to 3-5 chicks were placed in custom-made 3 x 18 cm brooding boxes that contained rocks, sticks, and a heat lamp for enrichment. Chicks were fed small live prey (small mealworms), along with *ad libitum* ground cat food (Hills Science Diet- kitten (36% protein), organic turkey starter (26 % protein), OPTIMUM fish food (40% protein), and fresh water. On day 15, chicks were moved into a larger indoor pen (3 x 18 cm) with a heat lamp and more enrichment (logs, water pools, textured floor mats). At 30 days of age, chicks were moved to an outdoor pen (4 x 2 m) and maintained for weekly mass measurements and future long-term studies.

2.3.4 Chick growth and behaviour

Chick mass (± 0.01 g) was measured daily until day 30 after hatch, and morphometric measurements (right and left tarsus length (± 0.1 mm), total head (± 0.1 mm), and culmen (± 0.1 mm)) were measured with digital calipers on days 1, 7, 15, 21, 29. Wing length (± 0.5 mm) was measured on days 7, 15, 21, 29. Birds were visually inspected daily for teratogenic malformations (deformities caused by environmental factors) including wry neck (twisted or tilted neck), splayed legs (feet pointing outward and inability to walk), wasting syndrome

(decreases in body weight, which ultimately leads to death), yolk distention and gastroschisis (protruding bowel).

Proper locomotion is incredibly important for precocial species which are independent once hatched. Righting reflex represents a measure of fearfulness and behavioural inability (tonic immobility) of motor reflexes and coordination at hatch. To investigate effects of stressors on motor activity, I performed two sets of righting reflex trials following method of Smith et al. (2011) on days 1 and 7. These trials were video recorded for ease of scoring and quality assurance. I restrained chicks on their back and released them once they stopped moving (~1-5 sec). I recorded the time it took chicks to right themselves and repeated for a total of five consecutive trials. If the chick made no attempt at moving after 30 seconds, it was righted by hand and recorded as 30 seconds. I tested each chick five times with 15 seconds between trials. Due to observable fatigue, the 5th trial was dropped and the average of 4 trials was used. Following the test series, chicks were placed back in their home cage/pen.

2.3.5 Statistical analyses

Statistical analyses were performed using R version 3.5.2 (R Core Team 2018). Prior to analyses, I thoroughly examined the data and plotted to look for errors or unusual patterns and tested for normality using the Shapiro-Wilk test. Incubation length, clutch initiation date, egg mass, body mass and morphometrics were log₁₀ transformed to meet assumptions of normally-distributed residuals.

Egg stage analyses

I used linear models to determine differences in fresh egg mass, age of injection and clutch initiation date among groups. I also used linear mixed effects models to determine the effects of treatment group (PCB-126 and incubation temperature) on length of incubation and first detectable heartbeat. The models were formulated with treatment group as a fixed effect, fitted with the restricted maximum-likelihood method. Although treatments were randomized among eggs within a nest, Nest ID (n = 21) was considered a random intercept to control for genetic or other similarities among individuals from the same nests. Significant differences between treatment groups were compared following the Tukey–Kramer honestly significant

difference (HSD) *post-hoc* test, with p values adjusted for unbalanced multiple comparisons, using the “glht” function in the “multcomp” package (Hothorn et al., 2008).

Chick stage analysis

For each bird, on day 1,7,15,21,29 post-hatch I estimated the effects of treatment (PCB-126 and incubation temperature) on hatchling biometrics (mass, total head, culmen, tarsus, and wing lengths/asymmetry). Additionally, I estimated the effects of treatment on time to right on days 1 and 7. On day 1, I estimated fluctuating asymmetry (i.e. deviation from perfect bilateral symmetry), and body condition, using scaled mass index (SMI; Peig & Green, 2009), which corrects body mass for body size using residuals from an ordinary least squares (OLS) regression of body mass against length. I used total head to represent length because it had the strongest correlation with mass relative to other body morphometrics. I formulated the models with treatment group, egg mass, chick mass, and injection age as fixed effects, fitted with the restricted maximum-likelihood method. Nest ID was considered a random intercept to control for relatedness within nests. Significant differences between treatment groups were compared following the Tukey–Kramer honestly significant difference (HSD) *post-hoc* test, with p values adjusted for unbalanced multiple comparisons, using the “glht” function in the “multcomp” packages in R (Hothorn et al., 2008).

To evaluate the impact of treatment group on individual growth rates (indexed as body mass, right tarsus, total head and culmen length), I used Gompertz growth models (Olshansky & Carnes 1997), which are equivalent to a lag-1 autoregressive regression model with log-transformed index values. Repeated measures across individuals were accounted for by using a nested random effects structure of Chick ID within the random effect Nest ID.

For binary response data (egg survival, yolk distention, chick survival, teratogenicity), I used generalized linear models (GLM) for analyses to evaluate the effect of treatment group. Egg survival was determined as percentage of fertilized eggs that hatched in each treatment. For yolk distention and egg survival, these models were specified with a binomial distribution and logit-link function, but chick survival and teratogenicity showed near-perfect separation (fitted probabilities reached 0% or 100% for some groups), which can cause estimation problems with common binomial regression packages (Gelman, 2008). To account for the separation, I

used an implementation of binomial regression fit using a Bayesian framework with uninformative priors, which uses a “Cauchy” prior to regularize the coefficients and pull them just slightly towards zero (“arm” package, Gelman, 2008). This allows unbiased estimation of binomial regression parameters from small datasets exhibiting perfect separation. The criterion for significance of all analyses was set at $p < 0.05$.

2.4 Results

2.4.1 Killdeer embryo survival and incubation patterns

A total of 71% (52/73) of eggs successfully hatched. Treatment had no effect on egg hatchability and survival ($F_{8,60}=1.043$, $p=0.42$; Table 2.2). For eggs that failed to hatch, the incubation day at which the embryos died did not vary by treatment group ($p=0.6$).

There was no difference in fresh egg mass ($p=0.5$), age (day) of injection ($p=0.9$) or clutch initiation date ($p=0.4$) among treatment groups. Temperature treatment significantly affected incubation duration ($F_{8,38}=32.47$, $p<0.001$), with lower temperatures inducing slower development ($\beta=-0.02$, $p<0.001$, Figure 2.1b, Table 2.2). Eggs incubated at low temperature (36°C) hatched an average of five days later than eggs incubated at high temperature (39°C), but PCB exposure did not significantly affect incubation duration ($\beta=-0.0002$, $p=0.6$).

Treatment did exert a strong effect on first detectable heartbeat ($F_{8,35}=3.97$, $p<0.001$), with suboptimal temperatures (36 and 39°C) having earlier detectable heartbeat than embryos incubated at 37.5°C, regardless of PCB treatment (Figure 2.1a). In the 37.5 °C incubated chicks, increasing PCB dose did not result in earlier detection of the first heartbeat ($p=0.45$).

2.4.2 Killdeer post-hatch survival, growth and behaviour

The majority of chicks 80% (39/49) that hatched survived to day 30. Treatment had a significant effect on post-hatch survival rate ($F_{8,40}=3.57$, $p=0.003$; Table 2.2), with lower temperatures having lower survival ($\beta=1.03$, $p=0.02$). There was 100% post-hatch survival in all intermediate 37.5°C temperature groups, as well as in the 0PCB-39°C and in the LowPCB-39°C groups. Reduced post-hatch survival was observed in all three 36°C temperature groups, and in the HighPCB-39°C group (Table 2.2).

Body mass at hatch (day 0) did not differ among treatment groups ($F_{9,37}=1.39$, $p=0.23$). Additionally, treatment did not have an effect on body condition (SMI) at hatch ($F_{7,33}=1.32$, $p=0.27$). However, body mass at day 29 was significantly lower in 0PCB-36°C and HighPCB-36°C on days 7 ($F_{7,32}=2.37$, $p=0.04$), 15 ($F_{7,32}=2.38$, $p=0.04$) and 29 ($F_{7,32}=3.12$, $p=0.01$) compared to 0PCB-37.5°C. The 0PCB-36°C treatment group also had a significantly lower body condition at day 29 ($p=0.02$) compared to 0PCB-37.5°C treatment group (Figure 2.2b). Additionally, right tarsus length was significantly shorter in 0PCB-36°C and HighPCB-36°C groups on day 7 ($F_{7,32}=2.79$, $p=0.02$). Right wing chord was significantly shorter in HighPCB-36°C on day 15 ($F_{7,33}=2.51$, $p=0.03$) and day 29 ($F_{7,33}=4.795$, $p<0.001$; Table 2.4 and Table 2.5). Total head length was not significantly different among treatment groups on day 1, ($F_{7,33}=1.33$, $p=0.29$) 7 ($F_{7,33}=0.94$, $p=0.50$), 15 ($F_{7,33}=1.9$, $p=0.14$), or 29 ($F_{7,33}=1.9$, $p=0.14$). Culmen length also was not significantly different among treatment groups on day 1, ($F_{7,33}=1.55$, $p=0.22$) 7 ($F_{7,33}=0.66$, $p=0.70$), 15 ($F_{7,33}=1.30$, $p=0.32$), or 29 ($F_{7,33}=0.92$, $p=0.51$). Fluctuating asymmetry of right wing chord and/or tarsi as an indicator of developmental stress did not vary among treatment groups at day 29 (Table 2.2 & 2.3).

Treatment did have a strong effect on overall growth based on body mass ($F_{9,1220}=72.41$, $p<0.0001$) with lower incubation temperatures resulting in a decreased growth rate (Figure 2.3). HighPCB-36°C ($\beta=-5.69$, $p=0.01$) and 0PCB-36°C ($\beta=-5.42$, $p=0.01$) had significantly lower mass growth than 0PCB-37.5°C treatment group. In addition, HighPCB-36°C had significantly lower growth rates for tarsus ($\beta=-0.01$, $p<0.05$) and wing chord ($\beta=-0.05$, $p<0.001$) compared to 0PCB-37.5°C treatment groups (Figure 2.3).

In the righting reflex tests, all treatment groups took longer to right themselves with age ($p=0.02$). Treatment group did significantly affect righting time ($F_{8,31}=2.32$, $p=0.04$) with 0PCB-36°C ($\beta=0.33$, $p=0.002$) groups having longer righting time and HighPCB-39°C ($\beta=-0.26$, $p=0.02$) group having shorter righting time (Figure 2.1d).

2.4.3 Teratogenicity and yolk distention

A total of 14% (7/49) of hatched chicks had morphological deformities consisting of either wry neck ($n=1$), splayed legs ($n=3$), wasting syndrome ($n=2$) and gastroschisis ($n=1$). HighPCB-36°C (57%, $p=0.01$; $n=7$) produced significantly more abnormalities when compared to PCB-37.5°C (0%; $n=8$). In addition, the last surviving chick from LowPCB-36°C also had a

deformity. Distended yolks were observed in 80% of chicks that died post-hatch. The High PCB-39°C treatment group exhibited significantly greater frequency of yolk distention ($\beta = 2.18$, $p = 0.01$; Figure 2.4).

2.5 Discussion

Increasing concerns over the ongoing effects of anthropogenic stressors, specifically changing ambient temperature and toxic contaminant exposure on wildlife, has led to an urgent need to understand the cumulative effects of these stressors. Here, I present new experimental insights on how PCB-126 exposure and varying incubation temperatures can alter embryonic and post-hatch development in a wild shorebird. In the current study, *in ovo* injection of PCB-126 caused varying levels of toxicity under the three incubation temperatures, with birds incubated at intermediate temperature (37.5°C) exhibiting higher tolerance to PCB exposure. I found there were no consistent treatment-related effects on key developmental outcomes. Results showed that treatment had no effect on embryonic survival but did significantly affect post-hatch survival. As hypothesized, interactive effects were clearly observed in HighPCB-39°C, which exhibited a greater occurrence of distended yolks and shorter righting reflex time. Temperature significantly affected incubation length, growth and morphometrics regardless of PCB exposure. These results suggest that there is substantial variation between embryonic and post-hatch responses to the combination of contaminant and temperature stress.

2.5.1 Consequences of incubation temperature variation in the wild

Incubation temperatures of wild birds can vary greatly, due to changes in foraging conditions for incubating adults, nest attentiveness, and microclimate conditions (Boersma, 1982; Kwon et al., 2018). For example, Grant et al. (1982) reported mean egg temperatures during incubation of multiple ground nesting shorebird species; ranging from 34.1-38.1°C with fluctuations influenced by large variation in ambient temperatures of 30.3-43.7°C. Therefore, incubation temperatures presented in my study are well within incubation temperatures experienced in some wild species. In addition, climate change is not only affecting ambient temperatures but also increasing the frequency of fluctuations in temperatures due to adverse weather (Millett, Johnson, and Guntenspergen 2009; Zongxing et al., 2012; IPCC, 2014). This is

a concern because adverse effects occurred when incubated under consistent high and low incubation temperatures but research also shows that brief exposures to high and low temperatures can also have long-term effects on the development of the embryo (Ardia et al., 2010; Grant, 2012). In my study, low temperatures led to greater mortality, earlier detection of heartbeat, longer incubation length, and reduced growth rate often regardless of PCB exposure. In our motor reflex assay, low temperature groups took a longer time to right themselves demonstrating greater tonic immobility. Whether this impairment is due to cerebellar dysfunction or due to damage in the spinal region was not determined. Similarly, Hopkins et al. (2011) observed Wood ducks (*Aix sponsa*) incubated under low temperatures had reduced locomotor activity possibly due to changes in muscle fiber morphology.

These findings indicate single stressors alone may result in adverse effects. Studies have shown that low incubation temperatures can increase metabolic rate and heart rate (i.e sustained hypermetabolism; Ar & Tazawa, 1999; Mortola, 2006; Nord, 2011), which could explain earlier detection of heartbeat in 36°C treatment groups compared to 37.5°C. Sustained hypometabolism may reduce yolk lipid uptake and increase energy needed for development. This may result in chronic stress and subsequent low embryonic and post-hatch survival (Carol 1987; Feast 1998; Nord & Nilsson 2011; Olson et al., 2006). In contrast, earlier detection of heartbeat in high temperature groups was also observed in my study. This may possibly have been caused by accelerated growth rate and decreased incubation length *in ovo*.

Wild birds can influence incubation temperatures through varying attentiveness, shading the eggs, sitting on the eggs, and belly soaking. However, many factors can alter the adults behaviour, including ambient weather, food availability and POPs, which have been shown to have a negative correlation with nest temperature and parental attentiveness though the underlying mechanisms are relatively unknown (Bustnes et al., 2001; Verboven et al., 2009). Thus, it is likely that maintaining optimal incubation conditions may become increasingly difficult for arctic breeding birds if changes in climate and pollution progress.

2.5.2 Consequences of increased PCB exposure in the wild

Global environmental change is expected to increase partitioning of POPs to the atmosphere and thus increase long-range transport of these contaminants to arctic locations (Hooper et al., 2013; Rubach et al., 2011; Wania 2006). Although increases in atmospheric

concentrations of these pollutants may be offset by enhanced degradation and reduced production (Hung et al., 2010; Stern et al., 2009), concentrations similar to those used in the present study have been found in arctic shorebirds (Lunny et al., unpublished; Saalfeld et al., 2016). My results show varying responses to PCB exposure during embryonic development and post-hatch development. PCBs are highly lipophilic and thus transfer to the embryo via yolk uptake, which may be slow during development, resulting in PCB loads still present in yolk sacs at hatch (de Roode & van den Brink, 2002). Consequently, exposure to lipophilic compounds may have greater effects post-hatch after the yolk is fully absorbed (Dean et al., 2018). By comparison, my results indicated no effect on embryonic survival but showed lower post-hatch survival in high PCB dosed groups incubated at 36°C (43%) and 39°C (72%) relative to intermediate temperatures, highPCB-37.5°C (100%). Although the predicted LD₅₀ values for PCB-126 exposure in ovo to type-3 bird species (e.g. killdeer) ranges between 29-297 ng/g (Farmahin et al., 2013), mortality was not observed in the HighPCB-37.5°C treatment group. This suggests that killdeer are relatively tolerant to PCB-126 when incubated under intermediate temperatures and that killdeer LD₅₀s may be closer to the upper type-3 species sensitivity range (Table 2.1). The greater survival in intermediate temperatures indicates that birds incubated in ideal conditions may be able to tolerate higher doses of PCBs than birds incubated at suboptimal temperatures.

Although PCBs have been shown to increase incubation length (Lavoie & Grasman, 2007ab), my results were not consistent with this effect, possibly because doses were too low or temperature effects on incubation masked this. Another indicator of PCB toxicity is the frequency of deformities which were more prevalent in the HighPCB groups. Similar deformities were observed in wild great cormorants (another type-3 species) and Caspian terns (*Hydroprogne caspia*) exposed to PCB mixtures in the upper Great Lakes (Ludwig et al., 1996). Deformities observed in my study were induced from the individual PCB-126 congener at doses orders of magnitude higher than concentrations detected in the Great Lakes wild bird eggs. Nevertheless, my results imply exposure to sublethal concentrations have the potential to cause physiological stress which may limit the ability of species to cope with other stressors, such as changes in temperature.

2.5.3 Possible interactive effects in the wild

I predicted that the high PCB-39°C treatment group would exhibit increased toxicity due to rapid growth and metabolic uptake compared to the high PCB-37.5°C and high PCB-36°C treatment groups (Noyes, 2009; Patra, 2015). This was partially observed in the results for combined mortality of both embryo and hatchlings (%), distended yolks (%) and righting reflex responses, with HighPCB-39°C showing the most frequent adverse effects. The decreased survival in birds incubated at suboptimal temperatures (39°C and 36°C) may be a result of lower toxic tolerance when incubated at sub-optimal incubation temperatures. In addition, 80% of chicks that died had distended yolks. Under normal circumstances the yolk-sac is pulled from the egg cavity into the abdomen of a chick as an extension of the intestine before hatching but certain factors, such as stress and natural and experimental infection can lead to slow yolk absorption (Leeson et al., 2012). Similarly, increased distention was observed in chickens exposed to DLCs via air-cell injection (Brunström & Andersson, 1988; Brunström & Darnerud, 1983; Powell et al., 1997). My results also indicated the high dose birds exhibited reduced tonic immobility during my righting reflex assays. Similarly, a study on white leghorn chickens exposed to TMPP (tri (methylphenyl)phosphate; a non-halogen-containing OPFR) found that increased motor activity was likely associated with erratic and anxious behavior (Bradley et al., 2015).

The increase in toxicity in high temperatures can be explained by an increase in the rate of contaminant uptake due to increased metabolic rate and decreased oxygen solubility and thus rapid uptake and metabolism. (Noyes et al., 2009; Patra et al., 2015; Schiedek et al., 2007). By contrast, at low incubation temperatures, uptake of PCB-126 may have been reduced thereby leading to reduced exposure. Ultimately, potential changes in avian development as a result of temperature and toxicant interactions will be influenced by highly variable conditions in the wild. Based on this study, two scenarios emerge as being of greatest concern: 1) under low temperatures which can dramatically alter normal embryonic growth and development and 2) under high temperatures where contaminants are more toxic and thus wildlife are at increased risk of adverse interactions in a warming climate. This is especially a concern for sensitive species living at the edge of their tolerance range, such as arctic populations, and may have difficulty acclimating to climate change and release of legacy pollutants.

2.5.4 Conclusion

Arctic breeding populations are threatened by many stressors that will likely become more detrimental in the future. My results suggest that, the ability of arctic species and populations to tolerate changes in temperatures, as anticipated under climate change, may be impaired with toxicant co-exposures. Specifically, experiencing these multiple stressors early in life may translate to a decreased survival post-hatch, increased distended yolk deformities and changes in locomotor activity. However, I cannot presume that developmental effects to stressors in a captive study directly correspond to responses seen in the wild. Therefore, further field investigations to obtain information on multiple stressors and avian responses in an ecologically realistic setting is important.

2.6 Tables and figures

Table 2.1: Predicted and reported avian in ovo LD50 values for PCB-126 adapted from Eng et al. (2014).

Species	AhR1 subtype ^a	LD ₅₀ (ng/g)	Injection location	Vehicle	Reference
Chicken (average)	1A	1.1^b	Variable	Variable	Head et al., 2008
Chicken	1A	0.4	Air cell	Corn oil	Hoffman et al., 1998
Chicken	1A	0.6	Yolk	Emulsion ^c	Head et al., 2006
Chicken	1A	2.3	Yolk	Triolein	Powell et al., 1996
Genotype prediction	1	1.5 95% CI: 0.86 - 2.8	n/a	n/a	Farmahin et al., 2013
Gray catbird	1C	1.6	Yolk	DMSO	Eng et al., 2017
European starling	1B	5.6	Yolk	DMSO	Eng et al., 2014
Bobwhite quail	2G	24	Air cell	Corn oil	Hoffman et al., 1996
Genotype prediction	2	26 95% CI: 11 - 66	n/a	n/a	Farmahin et al., 2013
Wild turkey	2A	29 ^d	Yolk	Emulsion	Brunstrum et al., 1989
American kestrel	3B	65	Air cell	Corn oil	Hoffman et al., 1998
Genotype prediction	3	89 95% CI: 29 - 297	n/a	n/a	Farmahin et al., 2013
Common tern	3B	104	Air cell	Corn oil	Hoffman et al., 1998
Double-crested cormorant	3B	177	Yolk	Triolein	Powell et al., 1998

^abased on the amino acid sequence of the ligand-binding domain ⁵.

^baverage calculated based on a review of several egg injection studies ¹.

^cemulsion of lecithin, peanut oil and water.

^dderived by linear interpolation between the LOAEL (20 ng/g; 36% mortality), and the LD₁₀₀ (60 ng/g; 100% mortality).

Table 2.2: Sample size (n), egg, chick and total survival (%) and Arithmetic mean (\pm SD) incubation duration (days) and first detectable heartbeat (HB, days) of killdeer incubated at three different temperatures (36, 37.5, and 39°C) and dosed with three different PCB-126 concentrations (0 PCB ng/g (DMSO), Low PCB (45 ng/g) and High (89 ng/g)). Sample sizes for HB are denoted in brackets.

Temperature (°C)	36			37.5			39		
PCB Dose (ng/g)	0	Low	High	0	Low	High	0	Low	High
Eggs (n)	10	4	9	8	5	10	9	4	11
Egg survival (%) ^a	60	25	77	87.5	80	70	88	50	70
Chick survival (%) ^b	50	0	43	100	100	100	100	100	72
Total survival (%) ^c	30	0	34	87.5	80	70	88	50	45
Incubation duration (days)	29.2 (0.98)	31	29.3 (1.38)	26.4 -0.53	25.8 (0.97)	26.6 (0.97)	24.3 (1.03)	24.5 (0.07)	23.8 (0.89)
HB [n days]	12 -2.12 [5]	9 [1]	13.3 (1.40) [6]	17.6 (1.72) [5]	17.5 (0.5) [2]	14.8 (1.42) [5]	9.9 (1.16) [6]	12 [2]	10.14 (.5) [7]

^a Egg survival=survival from clutch initiation date (CID) to hatch

^b Chick survival=survival from hatch to day 30

^c Total survival=survival from CID to day 30

Table 2.3: Sample size (n), total number of deformities observed at hatch (n), yolk distention at hatch (%) and arithmetic mean (\pm SD) of mass at hatch (g), and time to right (sec) for killdeer chicks incubated at three different temperatures (36, 37.5, and 39°C) and dosed with one of three different PCB-126 concentrations (0 PCB ng/g (DMSO), low PCB (45 ng/g) and high (89 ng/g)).

Temperature (°C)	36			37.5			39		
PCB dose (ng/g)	0	Low	High	0	Low	High	0	Low	High
Chicks (n)	6	1	7	7	4	7	8	2	7
Total deformities	1	1	4	0	0	0	0	0	1
Yolk distention (%)	33	100	43	14	50	57	25	50	86
Mass at hatch (g)	10.6 (0.81)	11.1	10.8 (1.12)	11.1 (0.79)	11.42 (0.39)	10.6 (1.16)	10.57 (0.40)	10.85 (0.35)	10.74 (0.67)
Time to right (sec)	32.1 (21.6)	5.9	18.7 (12.42)	19.8 (18.55)	11 (7.97)	20.9 (31.59)	11.9 (6.85)	7.1 (4.27)	5.2 (1.63)

Table 2.4: Sample size and arithmetic mean (\pm SD) of morphological measurements (mm) for killdeer chicks at hatch (day 1) incubated at three different temperatures (36, 37.5, and 39°C) and dosed with one of three different PCB-126 concentrations (0 PCB ng/g (DMSO), low PCB (45 ng/g) and high (89 ng/g)).

Temperature (°C)	36			37.5			39		
PCB Dose (ng/g)	0	Low	High	0	Low	High	0	Low	High
Chicks (n)	6	1	6	7	4	7	8	2	5
Tarsus length (mm)	23.4 (1.5)	23.55	24.1 (1.0)	23.56 (0.9)	24.3 (1.4)	23.1 (1.0)	23.2 (1.4)	24.9 (0.5)	23.4 (1.2)
Culmen (mm)	9.2 (1.1)	8.0	9.4 (0.7)	9.0 (0.6)	9.0 (0.6)	8.7 (0.5)	8.6 (0.7)	8.0 (0.1)	8.7 (1.0)
Total head (mm)	27.4 (1.2)	26.0	27.7 (0.9)	26.8 (1.0)	27.9 (0.7)	27.0 (0.8)	27.0 (0.9)	28.1 (0.1)	27.1 (0.9)
Tarsus asymmetry (mm)	0.4 (0.4)	0.1	0.6 (0.4)	0.4 (0.2)	0.7 (0.1)	0.4 (0.3)	0.5 (0.4)	0.1 (0)	0.6 (0.3)

Table 2.5: Arithmetic mean (\pm SD) morphological measurements in killdeer at post-hatch(day 29) incubated at three different temperatures (36, 37.5, and 39°C) and dosed with three different PCB-126 concentrations (0PCB ng/g (DMSO), Low (45 ng/g) and High (89 ng/g)).

Temperature (°C)	36			37.5			39		
PCB Dose (ng/g)	0	Low	High	0	Low	High	0	Low	High
Chicks (n)	4	0	4	7	4	7	8	2	5
Tarsus length (mm)	36.8 (1.4)	NA	37.3 (1.9)	38.5 (1.3)	39.9 (1.6)	38.1 (0.8)	37.6 (1.4)	39.6 (0.0)	38.3 (0.9)
Wing length (mm)	115.7 (9.6)	NA	104.8 (23.3)	131.1 (5.6)	131.4 (5.2)	132.1 (3.1)	129.5 (4.7)	132.5 (10.6)	131.2 (3.0)
Culmen (mm)	17.1 (1.7)	NA	18.2 (1.8)	18.6 (1.2)	19.2 (0.7)	18.2 (1.2)	18.0 (0.9)	18.7 (2.9)	18.3 (1.3)
Total head (mm)	44.9 (3.4)	NA	44.4 (3.3)	46.4 (2.1)	18.6 (0.3)	46.2 (2.2)	45.6 (2.0)	48.5 (3.0)	45.2 (2.9)
Wing asymmetry (mm)	1.6 (1.2)	NA	1 (0.8)	0.6 (0.8)	1.4 (1.3)	1.1 (1.5)	0.4 (0.5)	1.5 (0.7)	1.5 (1)
Tarsus asymmetry (mm)	0.26 (0.2)	NA	0.4 (0.4)	0.9 (0.5)	0.5 (0.4)	0.4 (0.2)	0.6 (0.6)	0.3 (0.3)	0.2 (0.1)

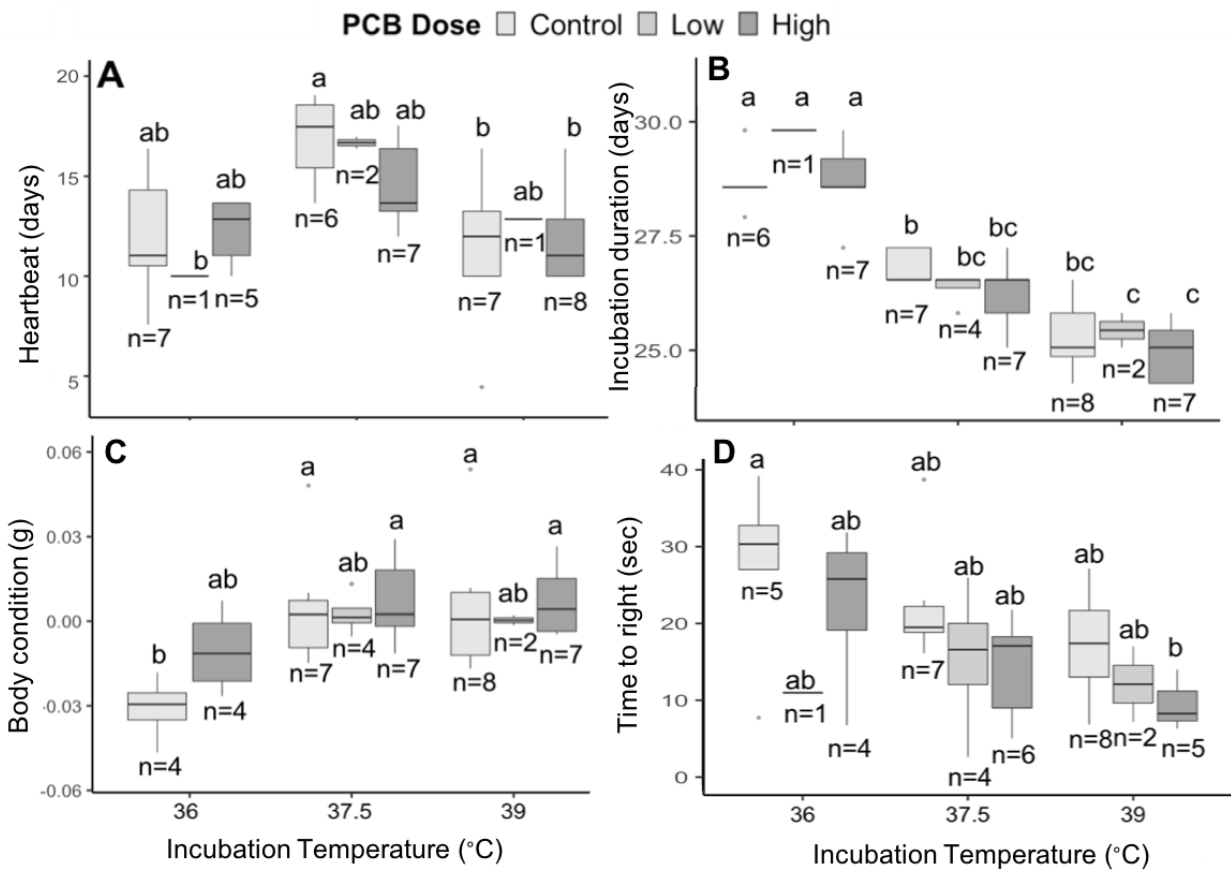


Figure 2.1: Differences among treatment groups (\log_{10}) in A) embryonic first detectable heartbeat (days), B) incubation length (days), C) body condition at day 29 (g) and D) righting ability on day 1 (sec). Killdeer eggs were experimentally incubated at one of three different temperatures (36, 37.5, and 39°C) and exposed to one of three different PCB-126 concentrations (0 PCB ng/g (DMSO), low (44 ng PCB/g) and high (89 ng PCB/g)). Sample size for each treatment group is shown below boxes. Different letters denote significant differences at $\alpha=0.05$ level among treatment groups (Tukey HSD test).

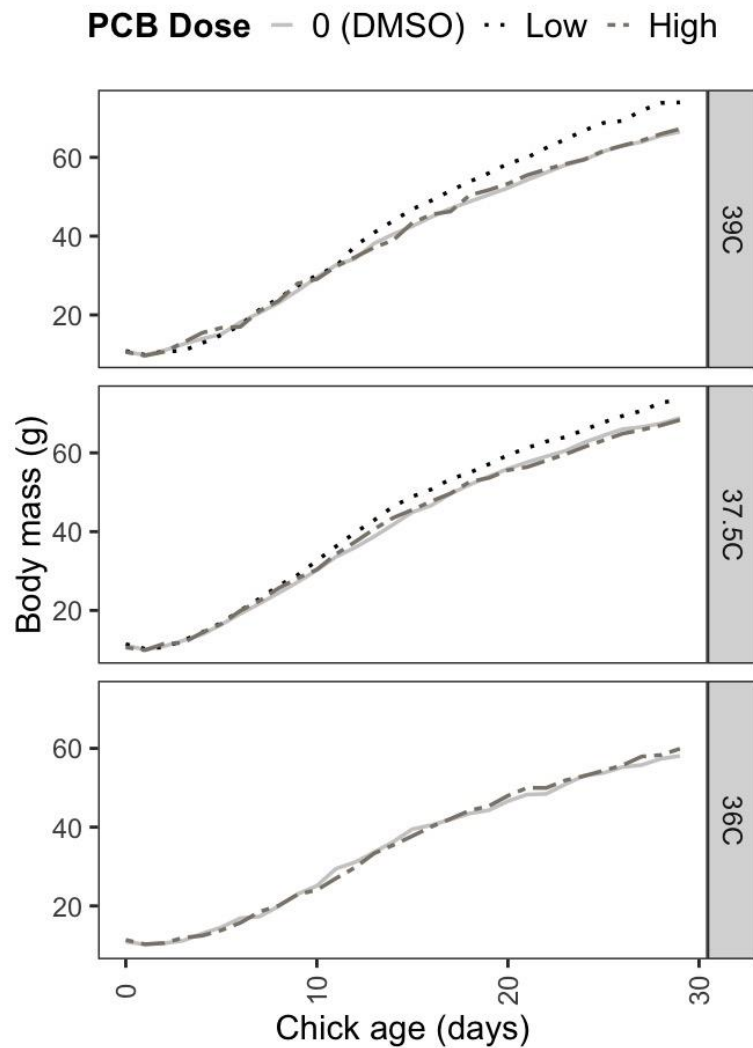


Figure 2.2: Growth (\log_{10} (g)) of killdeer hatchlings from day 1 to 29 post-hatch. Eggs were experimentally incubated at one of three different temperatures (36, 37.5, and 39°C) and exposed to one of three different PCB-126 concentrations (0 PCB ng/g (DMSO), low (44 ng PCB/g) and high (89 ng PCB/g)).

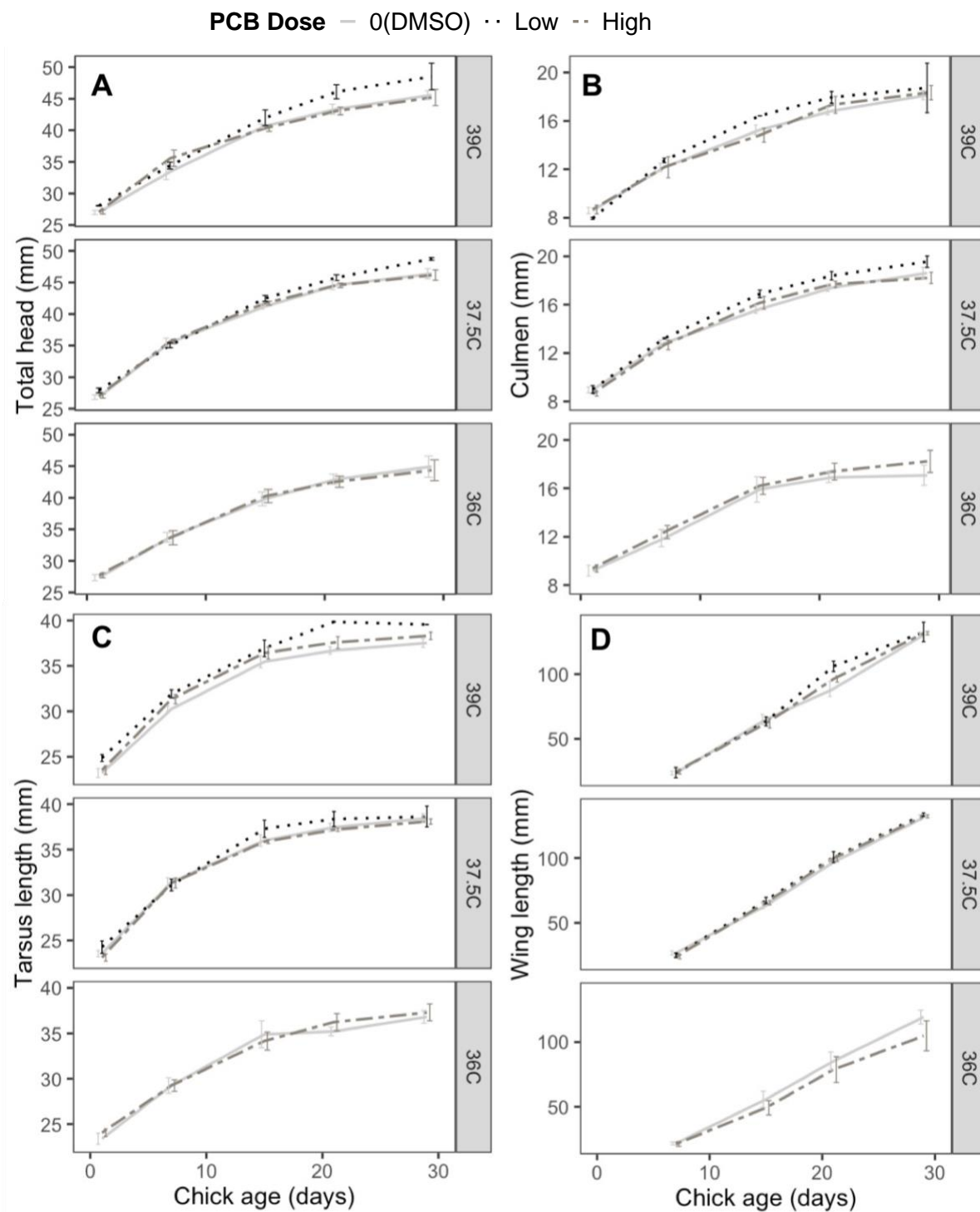


Figure 2.3: Morphometric measurements (\log_{10}), A) total head (mm), B) culmen (mm), C) right tarsus length (mm), D) and right wing length (mm) of killdeer hatchlings on day 1, 7, 15 and 29 post-hatch. Eggs were experimentally incubated at one of three different temperatures (36, 37.5, and 39°C) and exposed to one of three different PCB-126 concentrations (0 PCB ng/g (DMSO), low (44 ng PCB/g) and high (89 ng PCB/g)). Error bars represent SEM (Standard error of the mean).

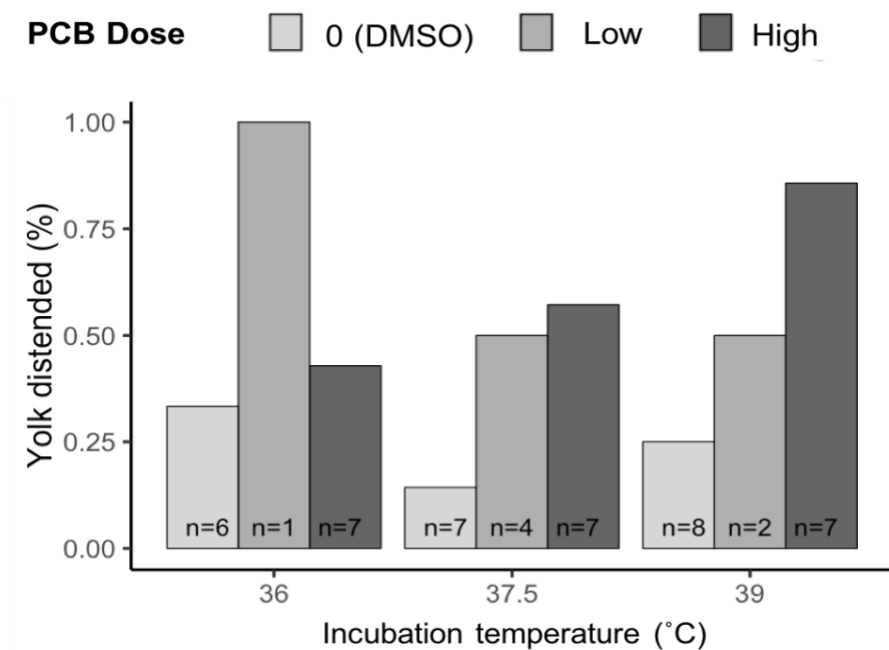


Figure 2.4: Percent of killdeer hatchlings born with distended yolk sacs that were incubated at one of three different temperatures (36, 37.5, and 39°C) and injected with one of three different PCB-126 concentrations (0 PCB (DMSO only), low (45 ng/g) and high (89 ng/g)).

3 CHAPTER 3: Evaluation of Organic Contaminant Profiles and Nest Temperature on Embryonic Development in an Arctic-breeding Shorebird

3.1 Abstract

Relative to more temperate areas, regions at high latitudes have recently exhibited a faster rate of climate change, including increased temperatures and melting ice reserves, with rapid changes expected to continue into the future. Such changes have influenced the production, use, release, and fate of persistent organic pollutants (POPs), leading to changes in exposure of wildlife in northern areas. This is especially a concern for shorebird populations, which have declined in recent decades, concomitant with changes in spring temperatures and continued long-range transport of POPs to northern latitudes. To evaluate potential effects of contaminant exposure and changes in ambient temperature on wild birds breeding in the north, I followed semipalmated sandpiper (*Calidris pusilla*) nests from three arctic sites and examined egg contaminant concentrations, nest-cup temperatures, incubation duration, nest success and hatchling size.

My results indicated low levels of POPs in shorebird eggs (relative to other arctic species), with variation among all three sites in polychlorinated biphenyls (PCBs; 12.88 ± 3.37 (range=1.2-338 ng/g), but not organochlorines ($\mu=19.00 \pm 2.33$ (range=5.1-181 ng/g) or brominated flame retardants ($\mu = 1.05 \pm 2.74$ (range=0.19-39 ng/g). Multivariate analyses indicated temporal and spatial differences in the concentration and composition of PCB congeners, signifying possible differences in exposure across sub-populations. At the nest level, incubation temperature was positively correlated with ambient temperature, but nest temperature and PCB exposure did not significantly influence incubation length or fledgling mass. Closer examination with larger sample sizes of possible interactive effects are needed to better understand how climate change might alter the effects of contaminant exposure on wildlife.

3.2 Introduction

Persistent organic pollutants (POPs) are a group of strongly lipophilic chemicals that are resistant to metabolic degradation (Barrie et al., 1992; Hung et al., 2010). Due to long range atmospheric transport and release from melting ice stores, POPs continue to be detected in polar regions decades after the United Nations Environmental Programme ratified the Stockholm Convention to eliminate and reduce their emissions (Letcher et al., 2010; Melnes et al., 2017). Although there are many structural forms of POPs, several forms are highly toxic and have been linked to a variety of chronic health effects in wildlife, including reductions in survival and growth, as well as changes in body condition and immune function (Barron et al., 1995; Frye et al., 2012; Grasman & Whitacre, 2001; Norstrom et al., 1988). For these reasons, levels of POPs and congener profiles in arctic biota are regularly assessed as part of ongoing environmental monitoring efforts, but such programs tend to focus on freshwater fish species, seabirds, and marine mammals (Mallory et al., 2006; Reiner et al., 2017; Verreault et al., 2018). Therefore, the levels and types of POPs in non-focal species are not well documented and their potential impacts on these populations are poorly understood.

Persistent organic pollutants such as polychlorinated biphenyls (PCBs), organochlorines and brominated flame retardant chemicals represent an environmental concern for the health of arctic wildlife. In particular, polychlorinated biphenyls (PCBs) are a class of POPs that – despite bans in sales and use since the 1970s – remain of particular scientific and public concern, due to their ubiquity, persistence, lipophilicity (Barrie et al., 1992; Hung et al., 2010), and a wide array of adverse health effects documented in captive and free-living species (Barron et al., 1995; Frye et al., 2012; Grasman & Whitacre, 2001; Norstrom et al., 1988). This complex group is a family of aromatic hydrocarbons that consist of two linked benzene rings (biphenyl) substituted with 1-10 chlorine positions. Different patterns of chlorine substitution make up 209 congeners with congeners of the same chlorination divided into groups referred to as homologs (i.e., the mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, and decachlorobiphenyl homologs). Homologs with a high degree of chlorination are more resistant to biotransformation and elimination from the body and therefore have longer relative residence times in biological tissue, compared to less chlorinated congeners/homologs, which are biotransformed and eliminated more quickly, such

that annual differences in total PCB concentrations across avian populations might be partially explained by differences in the homolog/congener profiles they are exposed to.

Organochlorine (OC) pesticides, such as dichlorodiphenyltrichlorethane (DDT), hexachlorobenzene (HCB), and dieldrin, are synthetic pesticides commonly used all over the world. They were used in large amounts for agricultural production, which led to increased pollution of environmental compartments (soil, water, and air), as well as accumulation in humans and wildlife. For example, DDT from urban and agricultural pesticides has caused reproductive failure in western grebes (*Aechmophorus occidentalis*; Rudd & Herman, 1972), ospreys (*Pandion haliaetus*; Spitzer et al., 1978), peregrine falcons (*Falco peregrinus*; Cade et al., 1971), and bald eagles (Grier, 2006).

Finally, flame retardants are a diverse group of chemicals with the most abundant group containing bromine (i.e. brominated flame retardants). BFRs are applied to a broad range of combustible materials, such as plastics, textiles (e.g. furniture), and surface finishes (e.g. electronics) to inhibit or delay the spread of fire by releasing halogens (Bergman et al., 2012; Darnerud, 2003; De Wit, 2002). Brominated flame retardants have been shown to reduce brood size in peregrine falcons (*Falco peregrinus*; Johansson et al., 2009), induce changes in thyroid, vitamin A, glutathione homeostasis, oxidative stress, immunomodulation, and growth in American kestrels (*Falco sparverius*; Fernie et al., 2005a; Fernie et al., 2005b; Fernie et al., 2006), and have varying effects on reproduction of osprey (*Pandion haliaetus*; Henny et al., 2011; Henny et al., 2009).

Simultaneous with ongoing contaminant exposure, wildlife in northern areas are also experiencing disproportionate effects of global climate change, including annual average air temperatures that have increased twice as fast as elsewhere in the world (Walsh et al., 2014). The effects of these changes on arctic wildlife are pervasive and have been observed in many aspects of species' biology (Pearson & Dawson, 2003; Post & Forchhammer, 2007; Simmonds et al., 2017). For example, in birds, recent evidence suggests that changes in ambient temperature influence incubation temperature and duration (Amininasab et al., 2016; Conway, 2002) – central aspects of avian reproduction that directly affect embryonic development, as well as growth and survival of offspring (Durant et al., 2013a). In addition, changes in ambient temperature can affect the absorption, distribution, metabolism, and excretion of toxic chemicals in wildlife, such that toxicants, like POPs, may have varying effects based on different exposure

scenarios (Holmstrup et al., 2010; Hooper et al., 2013; Stahl et al., 2013). However, to my knowledge, the potentially important combined effects of contaminants and changes in ambient temperature on incubation patterns and offspring characteristics have not been evaluated simultaneously in wild birds.

To better understand the levels and types of POPs in non-focal arctic bird species and to assess the extent to which contaminant exposure and changes in ambient temperature might affect embryonic development in northern areas, I collected eggs from arctic-nesting semipalmated sandpiper (*Calidris pusilla*, hereafter sandpiper) and monitored contaminant levels, ambient temperature, length of incubation, and temperatures in the nest as well as chick mass at hatch. Specifically, my objectives were (i) to evaluate variation in POPs levels and congener profiles among three arctic sites, (ii) to examine the relationship between ambient temperature and nest temperature, and (iii) to investigate the relationships between contaminant loads, nest temperatures, incubation patterns, and chick mass at hatch. Shorebirds, the dominant avifauna in many arctic ecosystems, are ideal models for my research, because they often forage in areas (wetlands, estuaries) where contaminants accumulate (Braune & Noble, 2009; Hargreaves et al., 2010). Many shorebird populations also appear to be declining (Gratto-Trevor et al., 2010; Andres et al., 2012), so from a conservation standpoint, potential links between contaminants in sandpiper eggs, changes in climate, and health of offspring warrant further investigation.

Although the specific patterns of variation in POP profiles are difficult to predict, based on expected variation in deposition patterns and climate patterns, I predicted that overall POPs concentrations, as well as congener and homolog profiles, would vary spatially and temporally (Schmutz et al., 2009). I also predicted that nest temperatures would co-vary with ambient temperatures, with increasing nest temperatures leading to shorter duration of incubation and increased mass of chicks. Finally, I predicted that these patterns would be reversed for nests with higher concentrations of POPs, which should have longer incubation duration, but smaller chicks.

3.3 Methods

3.3.1 Study species

Sandpipers, which primarily breed in the sub-arctic to mid-arctic across northern Canada and Alaska (Andres et al., 2012; Gratto-Trevor et al., 2012), are a small, socially monogamous, migratory shorebird, with a breeding and brood-rearing window that spans ~2 months, from mid-May to mid-July. Clutch size is ~4 eggs, and eggs are incubated nearly 100% of the time by both adults until hatch – an incubation period ranging between 19 and 22 days (Ashkenazie & Safriel, 1979). Chicks are precocial, grow rapidly, and fledge at approximately 16 days of age (Gratto et al., 1985; Sandercock, 1998).

3.3.2 Site descriptions

To evaluate the levels and types of POPs in arctic-nesting shorebirds, I analyzed data collected at three field sites across Inuit Nunangat and Alaska, which span the range of breeding semipalmated sandpipers in North America (Table 3.1, Figure 3.1). The Colville River Delta (hereafter Colville), Alaska, United States (70°42'N, 150°68'W) is the largest river delta on Alaska's North Slope, consisting of diverse habitats such as wet meadows, tundra, and freshwater lakes critical for a range of species. The Karrak Lake Research Station (hereafter Karrak), Nunavut, Canada (67°00' N, 100°30' W) is located within the Ahiak Migratory Bird Sanctuary, which covers an area of 62,920 km², making it the largest protected area in Canada. The sanctuary includes extensive wetland habitat that provides vital habitat for many migratory species. Coats Island (hereafter Coats), Nunavut, Canada (62°79' N, 82°28' W) is comprised of mostly low-lying sedge tundra, wetlands, and raised beaches with diverse populations of seabird and shorebird species. Due to logistical constraints, potential effects of POPs and changes in ambient temperature on incubation duration and chick size were assessed only at Karrak in 2017.

3.3.3 Field methods

Based on protocols described by the Arctic Shorebird Demographics Network (Brown et al., 2014), sandpiper eggs were collected at all three sites in accordance with permits issued by the U.S. Fish and Wildlife Service (MB332384C-0), Alaska Department of Fish and Game (17-157), Environment and Climate Change Canada (NUN-MBS-17-04, NUN-SCI-17-01, NUN-

SCI-15-02), the Nunavut Department of the Environment (2016-024, 2017-041), and the University of Saskatchewan Animal Research Ethics Board (AUP # 20170047).

Investigators visually or audibly located displaying and nesting sandpiper adults by walking transects (varying in distance and number, depending on field location) and by dragging a rope along the ground between two people to flush nesting birds within defined study plots. If a nest was found mid-incubation, eggs were floated, and clutch initiation date and hatch date were estimated (Liebezeit et al., 2014), with incubation duration calculated as the difference between clutch initiation date and observed hatch. On the first day of incubation one egg was collected and stored at 4°C in a dark container until shipment to the National Wildlife Research Centre in Ottawa for analyses of persistent organic pollutants (see complete list of analytes in Table S3.1). Over two years (2016, 2017), a total of 39 sandpiper eggs were collected from three arctic breeding sites (Figure 3.1). Nests were monitored continuously after eggs were seen starting or pipping, and to assess chick size, immediately following hatch, all chicks were weighed (\pm 0.01 g; n = 19 chicks).

In 2017, daily weather conditions at Karrak were measured with a locally established field camp weather station that provided data on temperature, relative humidity, wind speed/direction, and cloud cover twice a day at consistent times and location between 18-May and 18-July. Nest temperatures (n = 14 nests) were recorded every 10-15 minutes using temperature data loggers (high-resolution Thermochron iButtons, model DS1921H), with a minimum accuracy of $\pm 1^\circ\text{C}$ and an operational range of 15 to 46°C (Maxim Integrated Products Inc.). Prior to deployment, to reduce the risk of eggs cracking, iButtons were secured to one of three different sizes of screws and padded with foam. Placement of iButtons (in the position of the missing egg, previously removed for contaminant analyses) at a given nest usually took under a minute. Following deployment, nests were monitored until one adult returned. If an adult did not resume incubating within 30 minutes of deployment, iButtons were removed. Because temperatures reported by loggers depend on parental brooding position, as well as time spent off the nest, clutch size, and arrangement of eggs (Hope et al., 2018), these measurements likely represent nest-cup temperatures rather than true incubation temperatures. However, such an index (i.e., relative incubation temperature) is relevant to my study, as my goal is to describe the range of variation in nest temperatures as a possible response to variation in ambient temperatures – not to make precise inferences about actual incubation conditions.

3.3.4 Laboratory methods

On arrival at the lab, egg samples were homogenized and frozen at -20C until analyzed. Lipids were removed from homogenized tissue with the aid of accelerated solvent extraction, chemicals were separated from the remaining lipids and biogenic compounds by gel permeation chromatography, and any residual lipids were removed using solid phase extraction. The purified sample extracts were then analyzed using a capillary gas chromatograph coupled with a mass selective detector. As presented, results are recovery corrected and also corrected for background contamination by subtracting values of the associated method blank. All concentrations are presented as ng/g wet weight (ww). Congeners that could not be separated chromatographically and were co-eluted during the separation process are summed together (ECCC Test Report KG01-2017).

3.3.5 Statistical analyses

I conducted all statistical analyses using R version 3.5.2 (R Core Team 2018). Prior to analyses, all data were thoroughly examined by producing plots to look for errors, unusual patterns, or correlations and tested data for normality (Shapiro-Wilk test, $p > 0.05$). Because contaminant concentrations, nest temperature, ambient temperature, and morphological measurements were not normally distributed, I logarithmically (\log_{10}) transformed all data. Further, site and year were partially confounded in my data (only the Karrak site has data from both years), therefore, I considered site and year as a combined variable (site-year) in each analysis.

To assess the proportional contribution of each POP compound class to the total contaminant load (Σ POPs, see Table S1) across sites, I used a one-way ANOVA, followed by Wilcoxon *post-hoc* test. To test for differences in concentrations of total compound classes across site-years, I used a one way ANOVA and a Tukey–Kramer honestly significant difference (HSD) *post-hoc* test, with p values adjusted for unbalanced multiple comparisons (“glht” function in the multcomp package; Hothorn et al., 2008). The proportional contributions of different PCB congener homolog groups (Σ tri, tetra, penta, hexa, hepta, octa, nona, and deca) to the total PCB load (Σ PCBs) at each site were analyzed using a one-way ANOVA followed by Wilcoxon *post-hoc* test to test differences across site-years. To visualize and test for differences

among the various PCB congeners (expressed as proportion of Σ PCBs to remove artifacts related to concentration differences between samples) across site-years, I conducted a principal component analysis (PCA), using centered data (singular value decomposition to shift variables to be zero-centered) for the ten PCB congeners with the highest quality of representation.

To evaluate the relative support for a relationship between nest temperature and ambient temperature, I used general linear mixed models and an information-theoretic approach (Anderson & Burnham, 2004; Burnham & Anderson, 2002; Zuur et al., 2010). I developed an *a priori* set of candidate models, with daily average nest temperature as a response variable, daily average ambient temperature (amb. temp) as a predictor variable (fixed effect), and a random effect of Nest ID to account for repeated measurements within nests. To test for curvilinear relationships, quadratic (amb.temp²) and cubic measures (amb.temp³) of daily average ambient temperature were also included as potential predictors. Models were then ranked based on Akaike's Information Criterion, corrected for small sample size (AIC_c), and model selection tables were generated using the package MuMIn (Barton, 2018), with inference concerning fixed effects based on precision (95% confidence interval) of regression coefficients (β) estimated by the restricted maximum likelihood method.

To test for effects of nest temperature and POPs on (i) incubation length and (ii) chick mass at hatch, I used general linear mixed effects models and an information-theoretic approach, as described for nest temperature. However, due to limited overlap between nests that had temperature and contaminant measurements, I was not able to test the effects of temperature and contaminants in the same models. Instead I constructed two model sets for each response variable, one assessing nest temperature effects (temperature models), and the other assessing effects of Σ PCBs (contaminant models), which showed the greatest variation among the POPs compounds. In addition to key predictor variables related to my hypotheses of interest, in both model sets for incubation length, I also included clutch initiation date (CID, fixed effect) as a potential predictor variable, and in both model sets for chick mass, I used Nest ID as random effect (intercept only) to account for dependency among chicks from the same nest. Incubation length was included as a possible covariate only in the contaminant models for chick mass.

3.4 Results

3.4.1 Levels and types of POPs in sandpiper eggs

Across site-years, of all POPs analyzed, only 26% of compounds were found in >50% of samples, and only one compound (hexachlorobenzene) was detected in 100% of samples. Conversely, 18% of compounds (α -, β -, γ -hexachlorocyclohexane, β -TBECH / BDE-15, tetrabromoethylcyclohexane, syn-isomer and anti-isomer, BDE190, HBB, α -, γ - chlordane, 1,2,4,5.tetrachlorobenzene, and PCB-17/18, 33 and 177) were not found in any sandpiper eggs. The primary BFR congeners were BDE-47 (mean \pm standard deviation, range = 0.81 ± 2.11 , 0 – 18.0), BDE-99 (0.49 ± 1.80 , 0 – 10.2), and BDE-85 (0.30 ± 0.27 , 0 – 1.4), whereas the most commonly detected OC compounds were p,p'-DDE (11.75 ± 16.39 , 0 – 68.7), dieldrin (4.90 ± 13.95 , 0 – 86.7), and hexachlorobenzene (4.77 ± 3.04 , 0.9 – 16.7). Together, Σ OCs and Σ PCBs consistently comprised the greatest proportion of the total POPs load – for 2016 sites (Karrak and Coats), Σ PCBs was the dominant contributor to Σ POPs ($F_{3,35}=14.89$, $p<0.001$), while for 2017 sites (Colville and Karrak), Σ OCs had the greatest contribution ($F_{3,35}=11.56$, $p<0.001$; Figure 3.3). Across site-years, I detected no significant differences in total concentrations of Σ BFRs or Σ OCs; however, for Σ PCBs, results indicate substantial interannual variation at the Karrak site, with generally lower levels in 2017 (Table 3.2, Figure 3.2). The primary PCB congeners were PCB-153 (5.32 ± 11.83 , 0 – 74.0), PCB-138 (2.91 ± 7.37 , 0 – 46), PCB-118 (2.40 ± 8.91 , 0 – 55.9), and PCB-180 (2.05 ± 3.60 , 0 – 21.5). Compound and PCB homolog summaries are grouped together in Table 3.3 and Table 3.4.

For PCBs, the proportional contribution of different homolog groups varied among site-year combinations, with significant differences observed among the more chlorinated compounds: the relative proportion of five ring (i.e. penta-) compounds was highest at Karrak in 2017 ($F_{3,33}=9.29$, $p<0.01$), whereas for both eight ring (i.e. octa) and nine ring (i.e. nona) compounds, relative proportions were higher at both sites (Karrak and Coats) in 2016 compared to 2017 sites ($F_{3,33}=10.18$, $p<0.001$, $F_{3,33}=18.32$, $p<0.001$ respectively; Tables 3.4, 3.5, Figure 3.4). Six ring (i.e. hexa) compounds were the predominant homolog with the order of PCB abundance that followed hexa- differing among sites.

For the nine most commonly observed PCB congeners, 65% of the overall variation in proportional contributions was explained by the first two principal components (Table 3.5).

Principal component 1 (PC1) is characterized by more negative loadings for PCBs 153, 138, 118 and more positive loading for PCB 74. PC2 is characterized by a more negative loading for PCB 70 and more positive loadings for PCB 206 and 74, with separations among samples primarily related to relative contributions of the dominant congener, PCB 153. Observed differences among principal components show that 2016 sites at Karrak and Coats tended to be distinct from 2017 sites at Karrak and Colville, with Karrak-2016 samples having higher proportions of PCBs 206, 183, and 194, relative to all other site year combinations, and Coats-2016 tending to have higher proportions of PCB 153, relative to Karrak-2016 (Figure 3.6).

3.4.2 Relationships between temperature, contaminants, incubation, and chick mass

In 2017, spring melt occurred early (day 158, 9 days earlier than 2016) at Karrak Lake and ambient temperatures during the spring nesting period recorded mild cool temperatures (24-hour mean = 5.9°C) that tended to increase as the nesting season progressed (Figure 3.7). Daytime ambient temperatures ranged between 0 and 23°C and night-time values from -1 to 21°C. Average temperatures in sandpiper nests (n = 14 nests) ranged from 22.8 to 35.1°C, with a daily mean of 30.1°C, and duration of incubation ranged from 17 – 24 days (mean = 20.5 days).

Temperatures in the nest bowl were positively related to ambient temperatures in a non-linear fashion ($\beta_{\text{amb.temp}} = -0.17$; 95% CI: -0.31 to -0.03; $\beta_{\text{amb.temp}}^2 = 0.16$; 95% CI: 0.07 to 0.24), with nest temperatures increasing more quickly once a presumed threshold of ambient temperature was reached (Figure 3.8). Although I cannot rule out a possible additional quadratic term $\text{amb. temperature}^3$ ($\Delta\text{AIC} = 2.1$), Akaike weights (w_i) provide an evidence ratio of only 23% in favor of including this variable.

Although sample size was small, average daily nest temperature was not a significant predictor of either incubation length or chick mass at hatch (Table 3.6). The only significant factor influencing incubation length was CID, but this effect was very small ($w_i = 0.81$; $\beta_{\text{CID}} = -0.005$; 95% CI: -0.01 to -0.002, Table 3.6). In assessing chick mass at hatch, the best-supported model ($w_i = 0.83$) was the statistical null (random effects only) as both PCBs and nest temperature predictors were not supported (Table 3.6 and 3.8). Specifically, ΣPCBs were not significantly related to incubation length ($w_i = 0.04$, $\beta_{\Sigma\text{PCBs}} = 0.07$; 95% CI: -0.02 to 0.16) or chick mass at hatch ($w_i = 0.22$, $\beta_{\Sigma\text{PCBs}} = -0.02$; 95% CI: -0.06 to 0.02).

3.5 Discussion

I examined variation in the concentrations and types of POPs in the eggs of an arctic-nesting shorebird and characterized temperatures in the nest-cups to test for relationships between ambient temperature, nest temperature, contaminants, incubation, and mass of chicks. I found evidence for a non-linear relationship between ambient temperature and nest temperature, but possibly due to limited samples size, I did not detect effects of nest temperature or contaminant levels on incubation length or chick mass at hatch. This study provides an important baseline for future evaluation of trends in POPs in arctic-breeding shorebirds and emphasizes the suitability of this abundant widespread species as a biomonitor for changes in contaminants and arctic climate conditions.

3.5.1 Persistent organic pollutant levels and concerns

PCB, OC, and BFR concentrations were similar to levels found in other shorebird species (Saalfeld et al., 2016), with levels well below known toxicological thresholds for all contaminants (Fisk et al., 2005; Hoffman et al., 1996; Rattner et al., 2000). This is likely due to the relatively low trophic level of this species, which forages primarily on insects (Hoffman et al., 1996; Hargreaves et al., 2010). In addition, all three arctic sites are remote from industrial sources. However, if PCBs increase in the arctic, future populations may be at risk, because PCB loads in the highest percentiles observed in my study (mean 26.5, range=0 to 338 ng/g) are approaching the median lethal dose (300-400 ng/g) in chickens (Carro et al., 2013). In addition, decreased growth was found in chickens exposed to PCB mixtures equal to the average concentrations found in my sandpiper eggs (20 ng/g). However, chickens are highly sensitive to POPs, and it is not clear whether similar effects would be observed in wild shorebirds at these levels.

PCBs were the dominant POPs group in 2016 whereas OCs were the dominant group in 2017. This difference may be attributed to large-scale variation in deposition patterns, or possibly related to different spring weather conditions such as precipitation, and advanced snow melt date (Table 3.1; Noyes et al., 2009). The variation in dominant POP compound classes indicates that temporal replication in sampling is needed to properly assess contaminant profiles.

My results indicate high abundance of OCs, particularly DDT, dieldrin and chlordanes in eggs, perhaps due to their former large scale agricultural applications, which involves dispersion

over large areas and higher tendency for long-term persistence and long-range atmospheric transfer (AMAP, 2016). Organochlorine levels are similar to those found in Alaskan shorebird species (Saalfeld et al., 2016), but much lower than levels reported in seabird species (Bustnes et al., 2001; Peck et al., 2016; Verreault et al., 2018).

In addition, levels of BFRs found in the sandpiper eggs were low relative to levels measured in other arctic species and orders of magnitudes lower than the levels of OCs and PCBs (Verboven et al., 2009). However, many of these compounds continue to be produced and applied in large volumes, which may lead to a build up in arctic environments. Negative impacts have been reported: male zebra finches exposed to 50.7 -173.8 ng/g of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) reduced their song frequency (Eng et al., 2012), and American kestrels exposed to 285-1104 ng/g of polybrominated diphenyl ether mixture DE-71 had longer incubation periods (Sullivan et al., 2013).

3.5.2 OC, BFR and PCB congeners/compounds of significance

My data on proportional occurrence of PCB homologs, indicated high proportions of hexachlorobiphenyls (PCB 153), which is consistent with previous work on other species in the Canadian high arctic (Daelemans et al., 1992; Furgal et al., 2005; Peck et al., 2016). This is likely because the resistance of PCBs to degradation increases with higher chlorination and therefore more-chlorinated compounds, like PCB-153 and other hexachlorobiphenyls bioaccumulate more readily and are therefore more likely to become enriched with increasing trophic level, relative to the lighter chlorinated congeners that more rapidly degrade (Wania, 2006; Willman et al., 1999).

All eggs collected had detectable levels of HCB, which is released into the environment as a by-product of the manufacture of chlorinated compounds, such as pesticides. The high prevalence of HCB is likely due to suspected increases in atmospheric concentrations linked to the decline in sea-ice cover and volatilization from environmental sinks in the warming arctic (AMAP, 2016; Hung et al., 2010). Thus, in coming years, HCB concentrations are expected to continue to increase in Arctic biota and sediment. In addition, I found a particularly strong occurrence of p'p'-DDE, which may be due to both dietary accumulation and DDT metabolism (Borgå et al., 2004). It is the most persistent metabolite of p,p-DDT and has ongoing use for malaria control in the southern hemisphere (Connell et al., 1999).

Finally, congener profiles of BFRs in my samples were similar to those found in arctic seabirds, with BDE-47, 85, 153 and -99 being most dominant (Braune et al., 2001; Braune & Noble, 2009). This is because lower-brominated congeners are believed to have a long range transport potential comparable to that of PCBs (De Wit, 2002; Verreault et al., 2005). Latitudinal gradients have been observed in soil, and air, with a general increase with higher latitudes (De Wit et al., 2006). However, latitudinal trends also tend to be congener specific.

In general, my results suggest no geographic latitudinal or longitudinal pattern in any compound class groups. In addition, the remote characteristics of my study sites and the persistent characteristics of most of POP congeners/compounds observed in eggs indicate the deposition of these contaminants is from long-range atmospheric transport from sources outside the arctic. However, differences in compounds and concentrations of POPs between years may suggest patterns of contaminant release that can be driven by spring melt (Noyes et al. 2009).

The PCA grouped sites reasonably well, demonstrating similarity among PCB congener patterns for sites sampled in the same year. Most contaminants arrive to arctic locations via air or ocean currents, which transport them from source locations in the tropics and temperate regions (Braune et al., 2009). Another possible explanation for the variation in congener profiles across site-years may be related to the resources that are allocated to egg production (Gao et al., 2009; Morrissey et al., 2004). Arctic breeding shorebirds are known to be primarily income breeders (Gratto & Cooke, 2007; Yohannes et al., 2019), such that eggs should reflect local contamination, with variation across site-years being dependent on spatial and temporal patterns of POPs across arctic breeding areas. Yet, my results indicate greater temporal (i.e. annual) than spatial variation in congener profiles, suggesting that sandpipers may not be demonstrating a purely income breeding strategy like previously thought and that POPs exposure may also be occurring outside of breeding areas. Sandpipers from sites with different migratory routes and breeding locations (Coats and Karrak), appear to share similar contaminant profiles (Brown et al., 2017). Further research is needed to determine the origin of these contaminants and to better understand when during their annual cycles birds are most at risk of exposure.

3.5.3 Ambient temperature, nest temperature, contaminants, and development

Changes in ambient temperature may affect nest temperature through both indirect (altered nest attentiveness) and direct (microclimate) mechanisms (Boersma, 1982; Kwon et al., 2018). In the case of bi-parental incubators, like sandpipers, incubation constancy is near 100% (Sandercock, 1988), such that microclimate may have a stronger effect. External ambient effects on nest temperature are likely to be exacerbated in this ground-nesting species because nests are scrapes on frozen, rocky, and shrubby substrates that offer little thermal resistance against ambient climate conditions (Ar & Sidis, 2002). Like sandpipers, plovers were also unable to maintain constant incubation temperature when ambient temperatures were $<20^{\circ}\text{C}$ (Schneider & McWilliams, 2007). Further research is needed to understand how nest temperatures will be affected by long-term changes in climate, because even brief exposures to high and low temperatures can have consequences for the development of avian embryos (Bertin et al., 2018; DuRant et al., 2013ab). Although varying nest temperature did not appear to influence development in this study, what duration and magnitude of sub-optimal incubation temperatures are necessary to cause developmental effects remain unknown .

My study was only able to coarsely assess the effects of temperature and POPs in isolation, and not when combined, they are more likely to have adverse health effects (Sagerup et al., 2009), due to changes in detoxification and metabolism. For example, maternal contaminant transfer to eggs is highly influenced by quantity of lipids in each egg, and hatchlings incubated at high temperatures utilize lipids more rapidly than those incubated at low temperatures (Drouillard et al., 2003; Ikonopoulou et al., 2013). These interactions are likely to result in magnified toxicological responses in eggs incubated at higher temperatures. Furthermore, past research has shown a negative correlation between POP loads in parents and parental attentiveness (Bustnes et al., 2001; Verboven et al., 2009), which may be especially detrimental when nests are exposed to unpredictable weather conditions. As pollutant exposure and global warming continue to progress in northern areas, changes in parental care and incubation conditions may reflect additive stress on the embryo and its subsequent survival.

3.6 Tables and figures

Table 3.1: Summary of study site characteristics at three geographically distinct arctic locations (Karrak, Coats Island, and Colville). Snow melt date was determined when all snow depths reached 0 cm in a predetermined area and are reported as Julian date. Total precipitation and average temperature were recorded from May 27 (Julian day 147) to July 16th (Julian day 197) for both 2016 and 2017.

Location	Year	Lat Long Coordinates	Snow Melt Date	Tot. Prec. (mm)	Ave. temp °C	Distance to ocean (km)
Colville	2017	62°79'N, 82°28'W	171 (June 20)	3.6	6.04	~5
Karrak	2016	67°00'N, 100°30'W	167 (June 15)	62.3	7.20	~50
	2017	67°00'N, 100°30'W	158 (June 7)	45.2	6.33	~50
Coats Island	2016	70°42'N, 150°68'W	183 (July 7)	0	7.46	~20

Table 3.2: Comparison of major groups (Σ BFRs = total brominated flame retardants; Σ OC = organochlorines, Σ PCB = polychlorinated biphenyls) of persistent organic pollutant concentrations represented as arithmetic mean \pm SD (geometric mean [range]) (ng/g) from semipalmated sandpiper eggs collected from three arctic sites (Karrak, Coats Island, Colville).

Site	Year	n	Lipid (%)	Σ BFR	Σ OC	Σ PCB
Colville	2017	10	10.8	1.7 \pm 1.4 (1.4 [0.8-5.3])	23.3 \pm 56.8 (24.6 [8.4-181.4])	41.7 \pm 104.6 (9.7 [1.4-338.0])
Karrak	2016	10	12.3	0.8 \pm 0.7 (0.8 [0.1-2.3])	15.6 \pm 9.3 (13.4 [5.6-33.8])	19.5 \pm 11.7 (17.1 [10.4-38.8])
	2017	11	10.5	2.3 \pm 4.6 (1.0 [0.3-16.2])	37.8 \pm 40.5 (23.1 [5.7-124.6])	8.4 \pm 13.3 (5.3 [1.2-47.5])
Coats Island	2016	8	11.3	05.6 \pm 13.6 (1.2 [0.4-39.1])	17.5 \pm 6.4 (16.4 [8.2-28.4])	41.5 \pm 17.4 (37.8 [14.7-46.1])

*For detailed list of congeners within each compound class (Σ BFR, OC, PCB) see Table S3.1

Table 3.3: Comparison of persistent organic pollutant concentrations represented as arithmetic mean \pm SD (geometric mean [range]) (ng/g) from semipalmated sandpiper eggs collected from three arctic sites (Karrak, Coats Island, Colville). *NA indicates concenger/compound group was not detected in sample.

Compounds*	Colville- 2017	Karrak-2016	Karrak-2017	Coats Island- 2016
n	10	10	11	8
% lipid	10.8	12.3	10.5	11.3
Σ BDE	4.2 \pm 0.1 (0.4 [0.2-0.8])	8.5 \pm 0.2 (0.8 [0.2-2.4])	24.8 \pm 0.8 (0.9 [0.3-16])	43.9 \pm 2 (1.2 [0.3-38.7])
Σ CBz	87.3 \pm 4.3 (8.1 [5.2-17.5])	52.1 \pm 2.1 (5.0 [3.4-8.1])	28.5 \pm 1.2 (2.4 [1.1-5.3])	40.5 \pm 2.2 (4.4 [0.9-8.9])
Σ CHL	158.8 \pm 16.9 (8.1 [2.3-123.3])	16 \pm 0.9 (2.5 [0.9-7.2])	51.7 \pm 2.3 (4.8 [1.6-25])	14.9 \pm 0.6 (1.9 [0.6-5.2])
Σ DDT	140.3 \pm 14.1 (6.2 [0.9-70.9])	71.5 \pm 5 (5.2 [2.2-23.1])	193.4 \pm 14.4 (9.6 [0.5-88.3])	69.6 \pm 4.4 (8.1 [3.9-12.8])
Σ Mirex	22.7 \pm 2.5 (4.0 [0.5-11.5])	0.7 \pm 0.2 (0.7 [NA])	4.7 \pm 0.8 (4.7 [NA])	NA
Σ PCB	41.7 \pm 104.6 (9.7 [1.4-338.0])	19.5 \pm 11.7 (17.1 [10.4-38.8])	8.4 \pm 13.3 (5.3 [1.2-47.5])	41.5 \pm 17.4 (37.8 [14.7-46.1])
Σ TBECH	1.8 \pm 0.6 (1.8 [NA])	*NA	0.4 \pm 0.1 (0.4 [NA])	*NA
BB101	5.5 \pm 0.4 (0.4 [0.1-1.2])	*NA	0.1 \pm 0 (0.04 [0.03-0.1])	*NA
BTBPE	*NA	*NA	*NA	0.3 \pm 0.1 (0.3 [NA])
Dieldrin	23.7 \pm 2.3 (2.4 [1.2-7.4])	15.7 \pm 4 (6.3 [3.2-12.5])	137 \pm 25.1 (4.4 [1.1-86.7])	14.8 \pm 1.7 (2.8 [1.7-4.4])
HBCD	5.5 \pm 1.5 (0.7 [0.2-4.8])	*NA	0.2 \pm 0.1 (0.2 [NA])	0.5 \pm 0.1 (0.2 [0.2])
HCH	*NA	*NA	*NA	*NA
Σ DP	*NA	*NA	*NA	*NA
HBB	*NA	*NA	*NA	*NA

CBz=chlorinated benzenes, CHL=chlorinated hydrocarbons,

Σ DDT = dichlorodiphenyltrichloroethane

Σ TBECH = 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane, BB101 = brominated biphenyl 101

BTBPE = 1,2-Bis(2,4,6-tribromophenoxy)ethane, HBCD = hexabromocyclododecane,

HCH = hexachlorocyclohexane, DP = dechlorane Plus, HBB = hexabromobenzene

*For detailed list of compounds within each Σ groupings see Table S3.1

Table 3.4: Comparison of PCB homolog concentrations represented as arithmetic mean \pm SD (geometric mean [range]) (ng/g) from semipalmated sandpiper eggs collected from three arctic sites (Karrak, Coats Island, Colville). *NA indicates concenger/compound was not detected in sample.

PCB Homologs*	Colville-2017	Karrak-2016	Karrak-2017	Coats Island-2016
n	10	10	11	8
% lipid	10.8	12.4	10.5	11.3
Σ Tri	0.1 \pm 0.2 (0.3 [0.2-0.5])	0.1 \pm 0.1 (0.3 [0.2-0.4])	0.1 \pm 0.2 (0.3 [0.2-0.6])	0.3 \pm 0.3 (0.4 [0.2-0.9])
Σ Tetra	2.3 \pm 5.6 (3.0 [1.3-18.1])	3.6 \pm 3.4 (3.6 [1.1-10.2])	1.0 \pm 2.0 (3.0 [1.4-6.2])	4.4 \pm 1.9 (4.0 [2.1-7.8])
Σ Penta	13.3 \pm 38.1 (3.2 [0.7-121.5])	0.8 \pm 0.8 (1.0 [0.3-2.1])	2.6 \pm 5.0 (1.6 [0.7-17.2])	6.7 \pm 4.3 (5.3 [1.7-13.6])
Σ Hexa	24.0 \pm 54.6 (9.9 [2.1-177.8])	9.9 \pm 6.9 (8.1 [2.9-22.5])	4.6 \pm 6.6 (3.6 [0.5-23.5])	23.0 \pm 10.7 (20.6 [8.1-43.1])
Σ Octa	0.5 \pm 1.5 (1.7 [0.6-4.8])	2.4 \pm 3.1 (3.1 [1.5-5.9])	*NA	3.3 \pm 1.2 (3.1 [1.5-4.6])
Σ Nona	0.2 \pm 0.8 (2.4 [NA])	2. \pm 1.4 (2.5 [1.5-5.3])	0.1 \pm 0.3 (1.1 [NA])	3.1 \pm 1.9 (2.6 [1-5.9])
Σ Deca	1.3 \pm 4.1 (12.9 [NA])	0.4 \pm 0.7 (1.3 [1.1-1.8])	*NA	0.9 \pm 1 (1.6 [1.1-2.4])

*For detailed list of individual compounds within each Σ homolog group see Table S3.1

Table 3.5: Summary of PCA analysis and axis loadings (PC1 and PC2) for the nine most common PCB congeners found in semipalmated sandpipers eggs at three arctic sites (Karrak, Coats Island, Colville). Coefficients of the linear combinations are shown.

	PC1	PC2
Standard deviation	0.23	0.16
Proportion of Variance	0.42	0.23
Cumulative variance	0.42	0.65
	Loadings	
PCB congener	PC1	PC2
70	0.2	-0.31
74	0.33	0.82
118	-0.15	0.17
138	-0.39	-0.17
153	-0.76	0.05
180	-0.1	-0.15
183	0.11	-0.13
187	0.1	-0.09
206	0.18	0.29

Table 3.6: Summary of model parameters and regression coefficients predicting incubation length and mass at hatch for 14 semipalmated sandpiper nests sampled at Karrak in 2017. Fixed effects included average nest temperature and Σ PCB exposure. Parenthesis indicate random effect.

Response variable	Model parameters	Estimate	Std. Error	r ²	p
Incubation length	avg. nest temperature	-0.48	0.23	0.28	0.08
Mass at hatch	avg. nest temperature + (Nest ID)	0.19	0.20	0.08	0.33
Incubation length	Σ PCB	0.07	0.04	0.16	0.11
Mass at hatch	Σ PCB+ (Nest ID)	-0.02	0.02	0.10	0.23

Table 3.7: Summary of model selection for general linear models assessing incubation duration (\log_{10} , days) and mass at hatch (\log_{10} , g) for semipalmated sandpiper nests at Karrak in 2017 (n=14). Fixed effects included nest temperature (\log_{10} , °C), clutch initiation date (CID. Parenthesis indicate random effect.

Model predicting sandpiper incubation duration						
	df	logLik	deviance	AICc	delta	weight
Incubation length~ CID	2	21.2	0.004	-31.6	0	0.81
Incubation length~1	2	16.5	0.01	-27	4.56	0.08
Incubation length~ ave. nest temperature	3	18.9	0.008	-26.4	5.16	0.06
Incubation length~ ave. nest temperature + CID	4	21.8	0.004	-10.1	5.55	0.05
Model predicting sandpiper mass at hatch						
	df	logLik	deviance	AICc	delta	weight
Mass at hatch~ 1 + (Nest ID)	3	27.3	-54.6	-45.2	0	0.83
Mass at hatch~ ave. nest temperature+ (Nest ID)	4	27.8	-55.7	-41.1	4.23	0.10
Mass at hatch~ CID + (Nest ID)	4	27.3	-54.6	-40.0	5.23	0.06
Mass at hatch~ ave. nest temperature + CID + (Nest ID)	5	27.8	-55.7	-33.8	11.47	0

Table 3.8: Summary of model selection for general linear models assessing incubation duration (\log_{10} , days) and mass at hatch (g) of semipalmated sandpiper nests at Karrak in 2017 (n=14). Parenthesis indicate random effect. Fixed effects included Σ PCB (\log_{10}) and clutch initiation date (\log_{10} , CID).

Model predicting sandpiper incubation length						
	df	logLik	deviance	AICc	delta	weight
Incubation length~ CID	3	-17.36	36.59	44.1	0	0.89
Incubation length~1	2	-21.9	36.07	49.4	5.27	0.06
Incubation length~ Σ PCB	3	-20.37	84.03	50.2	6.03	0.04
Incubation length~ Σ PCB + CID	4	-16.69	74.89	55.4	11.21	0
Model predicting sandpiper mass at hatch						
	df	logLik	deviance	AICc	delta	weight
Mass at hatch~ 1 + (Nest D)	3	40.03	-80.0	-72.5	0	0.51
Mass at hatch~ Σ PCB+ (Nest ID)	4	40.83	-81.6	-70.8	1.66	0.22
Mass at hatch~ incubation duration + (Nest ID)	4	40.22	-80.4	-96.6	2.89	0.12
Mass at hatch~ CID+ (Nest ID)	4	40.15	-80.3	-69.3	3.02	0.11

Table S3.1: A suite of 77 POPs were analyzed at the National Wildlife Research Centre, which were divided into three compound classes: flame retardants (BFRs, n=22), and organochlorines (OCs, n=20) and polychlorinated biphenyls (PCBs). Congeners and compounds within each compound class were divided into groups for simple interpretation during analysis. Congeners which could not be separated chromatographically and were co-eluted during the separation process are summed together and identified with a dash.

BFRs		Ocs		PCBs			
Group		Group		Homolog group		Homolog group	
ΣBDE	BDE17	ΣCBz	1,2,4,5-Tetrachlorobenzene	ΣTrich	PCB17/18	ΣHexa	PCB156
	BDE28		1,2,3,4-Tetrachlorobenzene		PCB28/31		PCB158
	BDE47		Pentachlorobenzene		PCB33	ΣHepta	PCB170
	BDE49		Hexachlorobenzene	ΣTetra	PCB44		PCB171
	BDE66	α-Hexachlorocyclohexane	PCB49		PCB177		
	BDE85	β-Hexachlorocyclohexane	PCB52		PCB180		
	BDE99	g-Hexachlorocyclohexane	PCB70		PCB183		
	BDE100	Octachlorostyrene	Octachlorostyrene		PCB74		PCB187
	BDE138	ΣCHL	Heptachlor Epoxide	Σpenta	PCB87	ΣOcta	PCB194
	BDE153		Oxychlordane		PCB95		PCB195
	BDE154/BB153		trans-Chlordane		PCB99		PCB199
	BDE183		cis-Chlordane		PCB101		PCB205
	BDE190		trans-Nonachlor		PCB105	ΣNona	PCB206
	BDE209		cis-Nonachlor		PCB110		PCB208
Σ Dechlorane plus	syn-isomer	Dieldrin	Dieldrin		PCB118	ΣDeca	PCB209
	anti-isomer	ΣDDT	p,p-DDD	ΣHexa	PCB128		
ΣTBECH	α- TBECH ¹		p,p-DDE		PCB138		
	β-TBECH/BDE15		p,p-DDT		PCB149		
BB100	brominated biphenyl 101	ΣMirex	Photomirex		PCB151		
HBCD	hexabromocyclododecane		Mirex		PCB153		
BTBPE	BTBPE ²						
HBB	Hexabromobenzene						

¹ TBECH= tetrabromoethylcyclohexane

² BTBPE= 1,2-bis-(2,4,6-tribromophenoxy)ethane

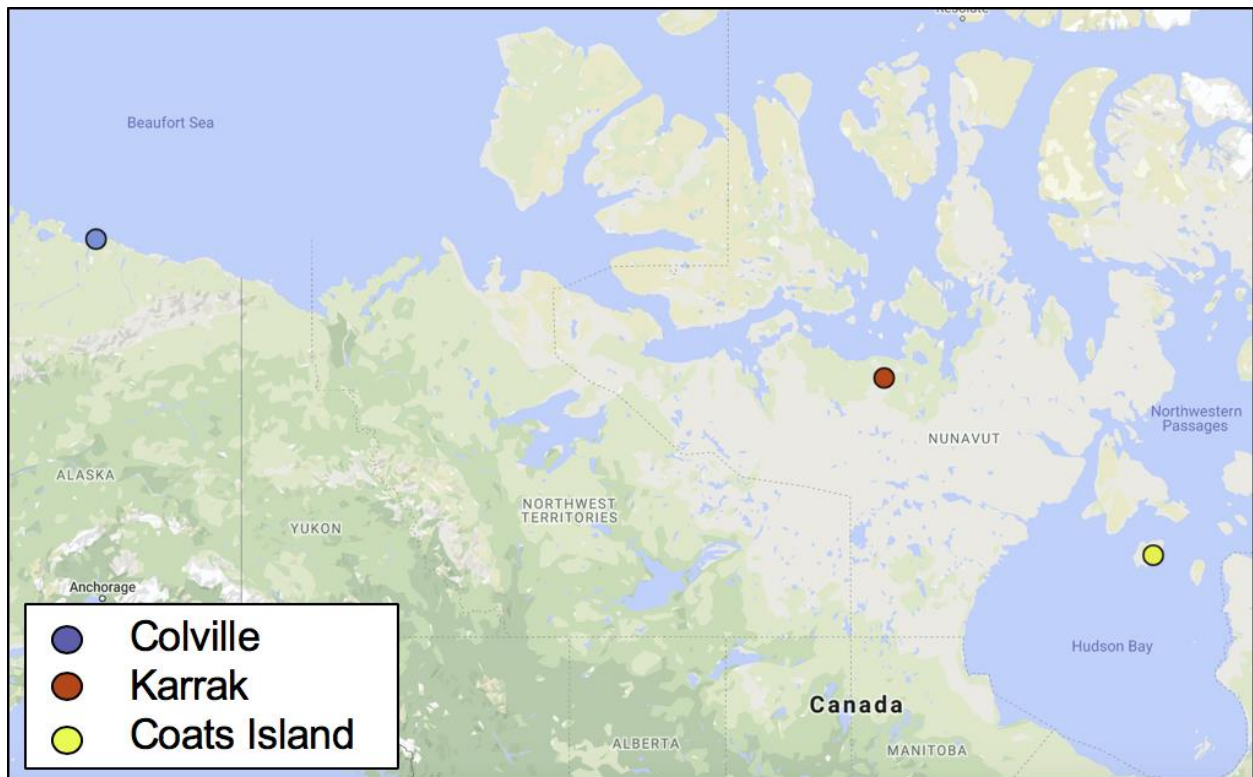


Figure 3.1: Locations of semipalmated sandpiper egg collections at three geographically distinct arctic study sites in 2016 and 2017: Colville ($70^{\circ}42'N$, $150^{\circ}68'W$, $n=10$), Karrak ($67^{\circ}14'N$, $100^{\circ}30'W$, $n=10$ (2016), $n=11$ (2017)) and Coats Island ($62^{\circ}79' N$, $82^{\circ}28' W$, $n=8$).

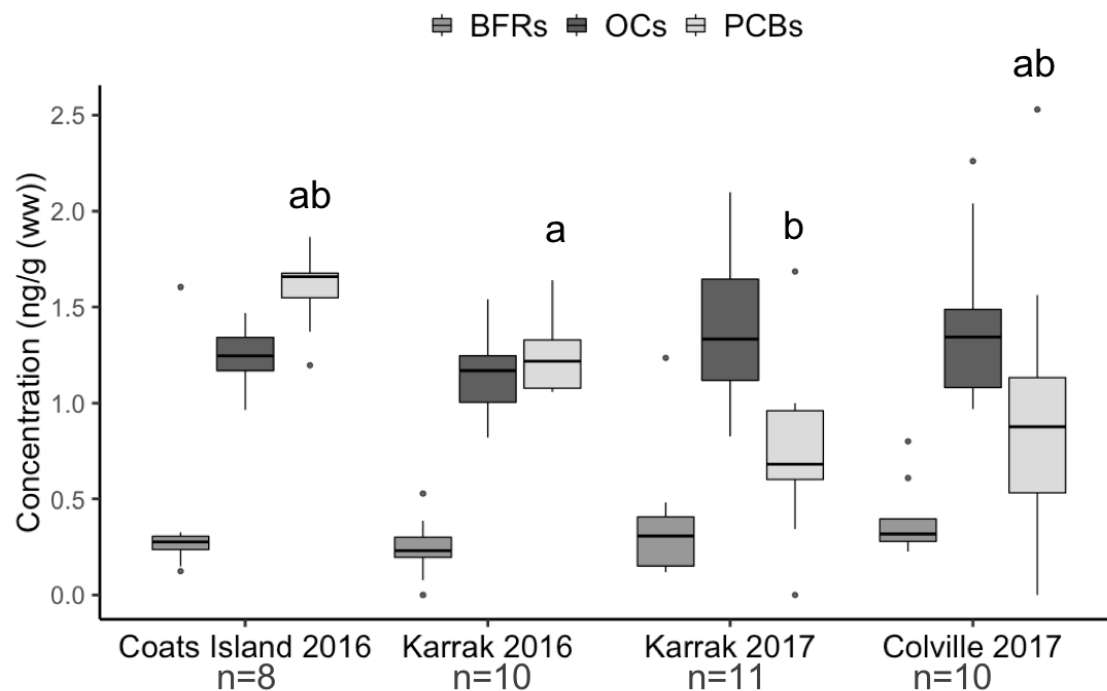


Figure 3.2: Boxplot of sum contaminant class (BFR, OC, PCB) concentrations (log10, ng/g (ww)) detected in semipalmated sandpiper eggs collected from three different arctic sites (Coats Island, Karrak, Colville,) in 2016 and 2017. The black line represents the median, the box represents the interquartile range containing 50% of the values, and whiskers mark the 1.5 fold interquartile range and dots represent outliers where concentration was beyond 1.5 quartile. Different letters denote significant differences in PCB concentrations at the $\alpha=0.05$ level among sites (Tukey HSD test). No significant differences were found among OCs and BFRs.

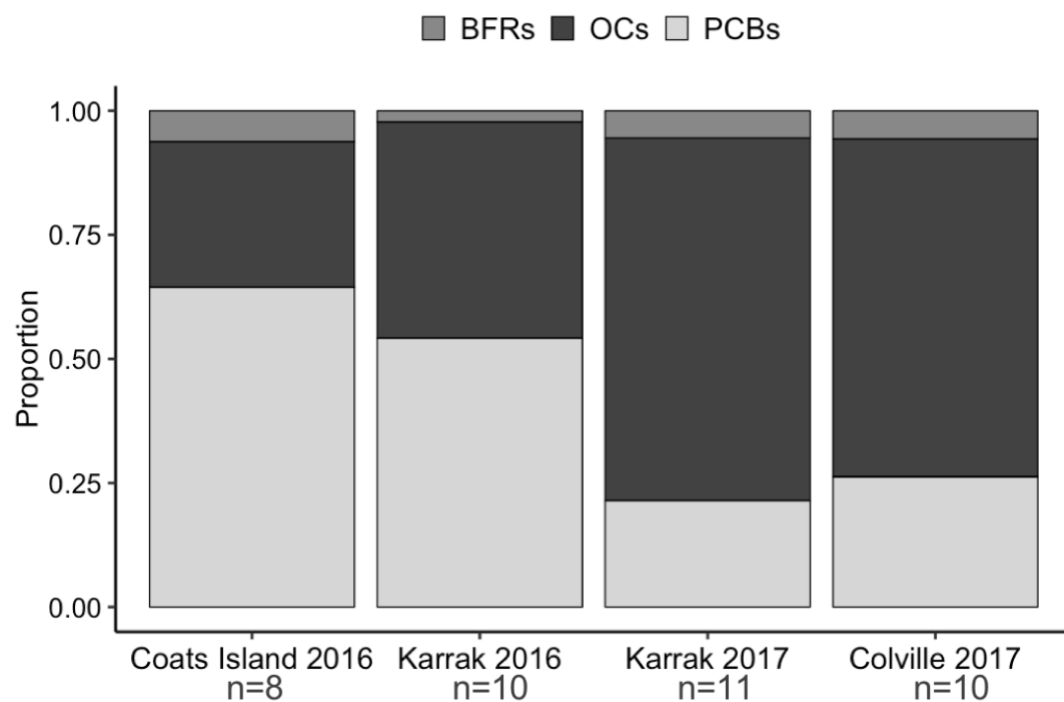


Figure 3.3: Bar graph depicting proportion of compound groups (BFR, OCs and PCBs) relative to the overall total POPs in semipalmated sandpiper eggs at three arctic sites. Karrak was sampled in 2016 and 2017, whereas Coats Island and Colville were only sampled once in 2016 and 2017, respectively.

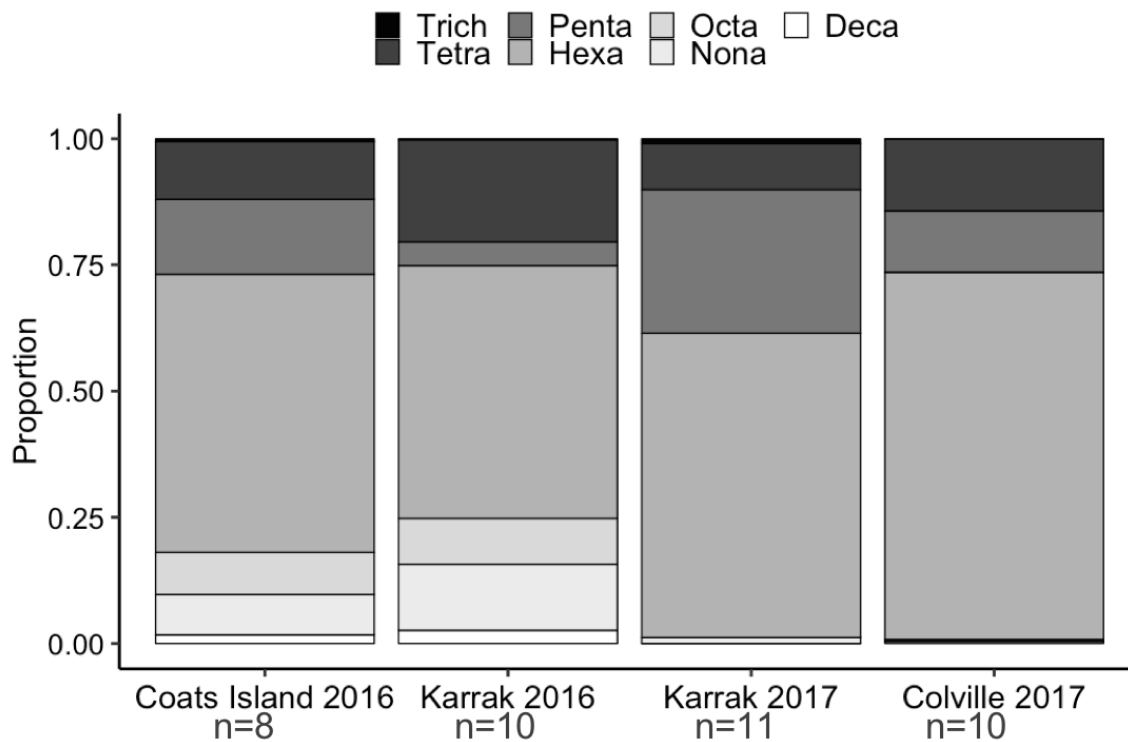


Figure 3.4: Bar graph depicting proportion of PCB congeners by homolog group (degree of chlorination) to the total PCB load in semipalmated sandpiper eggs at three arctic sites. Karrak was sampled in 2016 and 2017, whereas Coats Island and Colville were only sampled once in 2016 and 2017, respectively

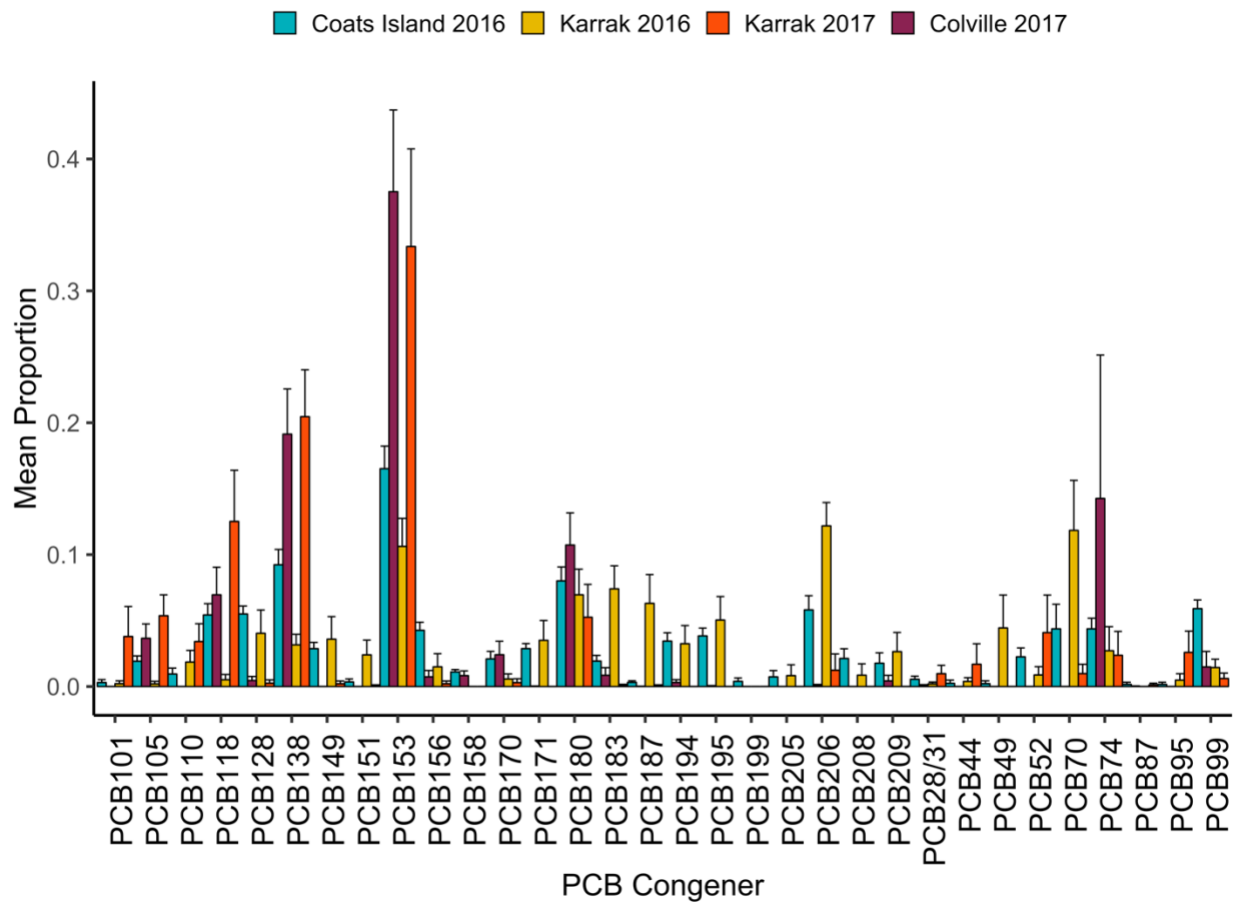


Figure 3.5: Mean (+SE) proportion of individual PCB congeners to the total ΣPCB load in semipalmated sandpipers eggs at three study sites. Graph shows similar PCB profiles within years. Karrak was sampled in 2016 and 2017, whereas Coats Island and Colville were only sampled once in 2016 and 2017, respectively.

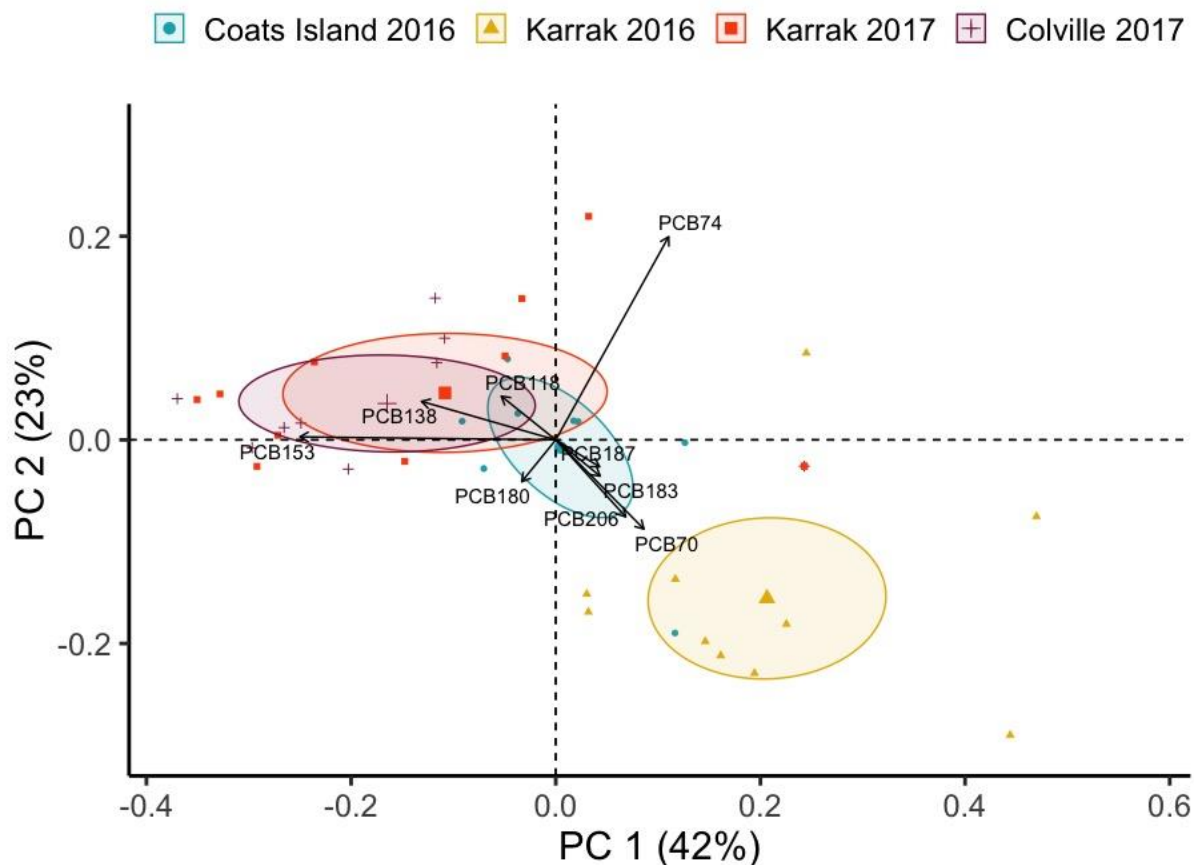


Figure 3.6: Principal components analysis (PCA) displaying proportions of the top nine contributing PCB congeners to the total Σ PCB loads in semipalmated sandpiper eggs at three sites during two years (Coats Island-2016 (n=8); Colville-2017 (n=10); Karrak-2016 (n=10); Karrak-2017 (n=11)). The horizontal axis represents the first component (PC1), the vertical axis represents the second component (PC2). The length of the arrows approximates the strength of the variables, whereas the angles between them (cosine) approximate their correlations. Points (egg ID) close together correspond to observations that have similar scores on the PCA components. The shaded ellipses demonstrate 95% confidence regions showing that Karrak 2016 was distinct from the other 3 site-years.

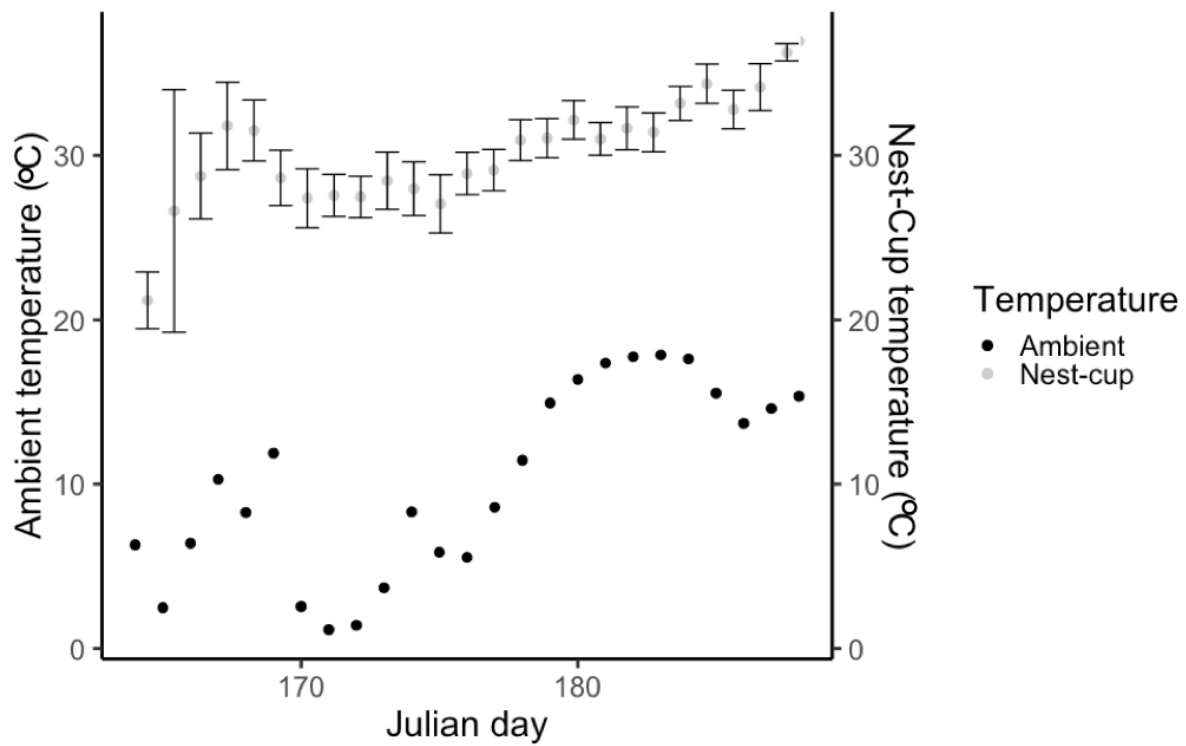


Figure 3.7: Scatterplot of mean daily ambient temperature and daily nest-cup temperatures (°C) of semipalmated sandpipers nests (n=14) at Karrak in 2017 monitored throughout the breeding season.

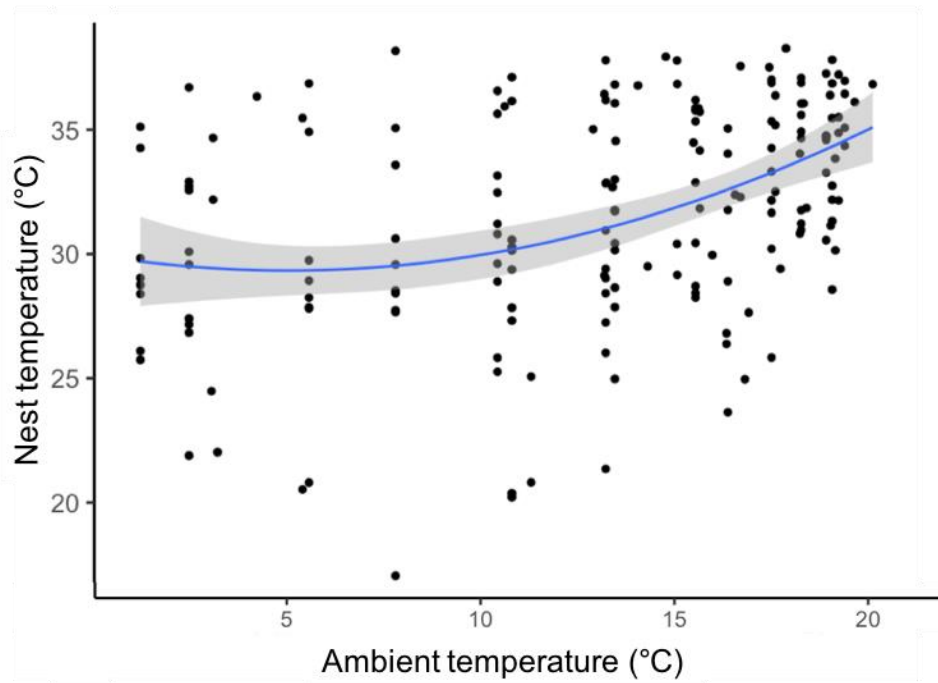


Figure 3.8: Scatter plot depicting relationship between \log_{10} ambient temperature and nest temperature (°C) of semipalmated sandpiper nests ($n=14$) monitored at Karrak in 2017. Fitted line is shown in blue and shaded areas represent 95% confidence interval.

4 CHAPTER 4: Conclusions and Recommendations for Future Research

My thesis demonstrates the importance of investigating combined thermal and toxicological stress in a wild avian species and underscores the need for continued work in ecotoxicology of shorebirds and other arctic breeding species. The broad goals of my research were to determine the possible interactive effects of contaminant exposure and incubation temperature on avian growth and development and to contribute to the understanding of how wildlife may be affected by anticipated global changes. I investigated two major questions, first “Are PCB contaminants and suboptimal temperatures adversely affecting avian development and interacting to cause a cumulative response?” I investigated this through manipulating PCB-126 egg exposure levels and incubation temperatures of a wild shorebird in a controlled captive setting and analyzed multiple developmental endpoints. Second, “What contaminant and temperatures stresses are shorebird populations facing and can they adversely affect development?” I addressed this question by analyzing ambient and nest temperature along with POP levels in eggs of wild arctic breeding semipalmated sandpipers and assessed relationships with chick mass and incubation length.

The major findings of this thesis were:

Chapter 2:

- Low temperature led to significantly longer incubation length, decreased growth and greater post-hatch mortality; whereas, high temperature led to shorter incubation length.
- High and low incubation temperature resulted in earlier detection of embryonic heartbeat compared to intermediate incubation temperature.
- Incubation temperature combined with PCB exposure had an interactive effect on frequency of yolk distention compared to temperature or PCBs alone and yolk distention was found in 80% of the birds that died.
- Incubation temperature combined with PCB exposure had an interactive effect on righting reflex time compared to temperature or PCBs alone

Chapter 3:

- Contaminants most prevalent in arctic shorebird eggs were Σ DDT (*p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE), Σ CBz (1,2,4,5-tetrachlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene), dieldrin, PCB-156 and PCB-138.
- PCB levels varied geographically and temporally in semipalmated sandpiper eggs whereas BFRs and OC did not
- Ambient temperature had a positive non-linear relationship with nest temperature, such that at higher temperatures incubating adults did not buffer the effects.
- Although sample sizes were limited, nest temperature and PCB exposure were not found to predict incubation length or hatchling mass in sandpipers.

4.1 Implications

Historically, wild species have been able to adapt to changes in the environment. However, the rapid rate of current climate change coupled with increasing bioavailability of contaminants will likely present new challenges for modern species. Some arctic birds are already responding to changes in temperatures through advanced breeding chronology. Shifts in breeding due to temperature will likely influence availability of resources, risks of incurring inclement weather, parental energetic costs, parental attentiveness, incubation length, and predation (Durant et al., 2013; Kwon et al., 2018). In addition, my field study results indicate that ambient temperatures can influence nest temperatures and suboptimal incubation temperatures can cause developmental deficiencies (captive research). In particular, cooler incubation temperatures induced longer incubation durations, earlier detection of first heartbeat, decreased post-hatch survival, and lowered growth in hatchlings. Developmental anomalies can have adverse effects on wild populations, such as higher risk of egg predation due to longer incubation duration as well as lower fitness due to poorer growth or defects (eg. yolk sac distention). Similarly, I also found that high incubation temperature treatments caused changes in detection of first heartbeat, as well as shorter incubation length. The long-term consequences of a shorter developmental period remain unknown. Although I was not able to look at longer term effects, research has shown that incubation temperatures affect both pre- and post- natal growth and physiology for up to 2 years in blue tits (Nord & Nilsson, 2011).

Furthermore, interactive effects of temperature and contaminants will disproportionately affect vulnerable species living at the edge of their homeostatic or physiological tolerance range (Gordon 2003; Noyes et al., 2009; Patra et al., 2015). For example, my captive study results indicate even birds with high tolerance to PCBs (type 3 species) will show adverse effects when exposed to sub-optimal incubation temperatures. Specifically, effects include increased frequency of yolk distention and decreased time to right when exposed to environmentally relevant PCB loads and incubation temperatures. In addition, 80% of captive killdeer that exhibited yolk distention at hatch died, indicating possible population level consequences in wild populations if exposed to these stressors. However, it should be noted that I used a single coplanar, highly toxic PCB congener (PCB-126). Nevertheless, it is possible that arctic species may experience similar developmental effects if exposed to high levels of multiple dioxin-like contaminants.

4.2 Limitations and challenges

Logistical, ethical and technical challenges associated with captive research on free-living animals and field research on wild arctic species are apparent in this study and underscore the need to combine lines of evidence. Small sample sizes coupled with high inter-individual variation in both my field and captive research reflects the challenge of collecting data on free-living species and the subsequent uncertainty in results. Therefore, integration of multiple techniques, such as modeling, experiments, and field investigations to obtain information on multiple stressors in an ecologically realistic setting is important.

Currently, semipalmated sandpiper sensitivity type to PCBs and other DLCs is unknown. An implicit assumption is that they are similar to other shorebird species (sanderling; *Calidris alba* and spotted sandpiper; *Actitis macularius* are type 2). However, there is variation among bird species even within related families. For example, spotted sandpiper is a type 2 species, while killdeer is type III (Farmahin et al., 2013). Therefore, I cannot presume that developmental effects observed in killdeer and semipalmated sandpipers directly correspond to other wild shorebirds.

The relationship between nest temperature and incubation temperature has been understudied especially in species living in extreme unpredictable environments like the arctic.

As demonstrated by this study, nest-cup temperature in this ground nesting species was influenced by ambient temperature which changed throughout the season. However, other factors can influence incubation temperature such as the time spent off the nest, clutch size, arrangement of eggs and how the bird oriented themselves on the nest. Further study is needed to more accurately characterize actual nest temperatures and how multiple factors can precisely influence the conditions that an embryo experiences in the egg. Given that I found that a difference of only 1.5°C can have consequences for embryonic growth and development, even subtle differences can be important to individual fitness in the wild.

In contaminant research, using commercial-contaminant mixtures compared to single-compound contaminants is a common debate. The composition of commercial PCB mixtures is dissimilar to the composition of PCBs in environmental media, which arises from differing physicochemical properties, degradation rates, and mixing within environmental compartments. In addition, single-congener PCBs are not environmentally relevant compared to the mixtures of dozens of chemicals found in eggs of avian wildlife. However, toxicity testing with mixtures are often impractical and disadvantageous because of the inherent complexity in interpreting the responses. Accordingly, I acknowledge that the effects of PCBs and temperature observed in this study may not be fully generalizable to other species, contaminants or conditions, but represent an important first step for identifying direct effects of multiple stressors on a developing embryo.

4.3 Recommendations for future research

My first recommendation for future work is a better understanding of age-dependent responses to incubation temperatures and contaminant exposure during critical windows of development that may alter toxicokinetic uptake and developmental responses. Past research suggests critical windows of development exist in which embryos are most susceptible to experiencing lethality or developmental change (morphological and physiological) due to exposure to environmental stress (Romanoff, 2012; Spicer & Burggren, 2003). Thus, additional research on interactive effects during other sensitive or critical stages of development (i.e before, during and after organ development), may address the variation in embryonic and post-hatch responses.

The second recommendation would be to extend this work to conduct long-term captive studies. This is because any adversity birds face during one phase (in ovo) of their annual cycle will likely manifest itself during subsequent phases of that cycle (post-hatch). Long-term analysis can help identify potential population-level effects caused during ovo and post-hatch exposure. In addition, longer-term studies of wild shorebirds remain a research priority. At least 38% of arctic-nesting shorebirds are decreasing and population trends are unknown for 25% (NABCI, 2016). Therefore, a monitoring program to assess long-term changes in shorebird populations at breeding regions and carry over effects between years is essential for tracking the risk and health of these sensitive arctic-breeding species.

Thirdly, the continuance of such a study over different climatic cycles may result in a more pronounced difference in distribution of contaminants and individual breakdown of contaminants, as well as nest/incubation temperatures during unpredictable weather conditions. This will help better understand how POPs are distributed and released during different climatic conditions and how nest temperatures will be affected by spring temperature and melt fluctuations. In addition, I recommend contaminant data are still needed to understand seasonal exposure patterns during migratory and wintering periods.

The final recommendation would be to gather similar information on other shorebird species to add to the generality of these findings. While semipalmated sandpipers are not currently of serious conservation concern in the arctic, many other arctic breeding shorebirds are experiencing even more dramatic declines. The use of semipalmated sandpipers and killdeer are amenable for research but obtaining adequate sample sizes remains challenging. Most research uses chickens and quail as a precocial model species. However, they may not be protective of free living shorebird species because their life history traits and sensitivity to stress differs (Cohen-Barnhouse et al., 2011; Head et al., 2008). Therefore, the information obtained from responses in semipalmated sandpiper and killdeer provided by this work should hopefully be more relevant to similar species of conservation concern. However, information on other shorebirds species with differing sensitivity (Type I, II and III) to PCBs and other dioxin like compounds (DLC) is lacking. In addition, although DLC toxicity has been broadly studied in birds, little is known about the specific effects associated with PCB, BFR and OC toxicity in different shorebirds, which are taxonomically and ecologically diverse.

Elucidation of the vulnerability of developing shorebirds may reveal how oviparous species may respond to multiple stressors in a changing environment. Such information will undoubtedly prove useful to researchers, managers, and policy makers when predicting and planning for the challenges to sustain wildlife populations in response to projected direct and indirect effects of climate change. In particular, this thesis has demonstrated the power of i) integrating field and captive research in the fields of developmental physiology and ecotoxicology, ii) investigating multiple stressors influencing wildlife and iii) using wild bird species as a model, to further advance emerging research of environmental stressors facing free living shorebird populations. As the human influence on natural systems increases, it will become increasingly important to understand the dynamic nature of temperature and contaminant stressors, and how developmental effects can alter future fitness.

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