Carryover Effects of Winter and Pre-breeding Conditions on Reproduction in Northern Common Eiders *Somateria mollissima borealis* Nesting in Arctic Canada

by

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Dedication

This thesis is dedicated to the eiders. For providing a fantastic example of hardiness.

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Abstract

Life-history stages are conceptually and temporally separated, yet interactions between these stages still occur. The overwintering phase of the annual cycle has become increasingly recognized for its selective pressures which can generate carryover effects. Migratory animals like the northern common eider provide a good model system to explore carryover effects because individual experiences can vary throughout different stages of their life history such as during overwintering, breeding, and migration. The overarching goal of my thesis is to investigate the underlying mechanisms that influence breeding including the potential for carryover effects of environmental factors across multiple seasons. To achieve this goal, my work has four objectives: 1) establish a minimally invasive method to assign common eiders to overwintering sites following arrival to breeding grounds, 2) use this method to gain insight into when common eiders form breeding pairs, 3) characterize changes in male physiology and condition throughout the pre-breeding period and how male physiological state influences female reproduction, and 4) link winter and spring environmental conditions to breeding decisions. In Chapter Two, I use stable isotopes of carbon to assign an individual to their overwintering site. In Chapter Three, using the stable isotopes of carbon in claws and blood to represent locations in winter and spring, respectively, I show some pairs form on the wintering grounds, but the majority of pairs form during spring, which may have fitness benefits in some years. In Chapter Four, using a suite of physiological traits, I demonstrate male mate guarding behaviours and energetic costs are primarily aimed towards securing paternity, rather than benefiting female reproductive traits. In Chapter Five, I use a path analysis to demonstrate both spring and winter conditions can impact breeding, but eiders are likely able to buffer for poor winter conditions during spring to invest in reproduction. Collectively, my thesis shows, although there are apparent benefits to the timing of pairing, and winter conditions impact arrival body mass, ultimately female reproductive decisions are influenced by spring breeding conditions. Truly, conditions during the pre-breeding period are the most important factor impacting reproductive investment via the ability for females to accrue fat stores.

List of Abbreviations and Symbols Used

Abbreviation	Description
AD	Arrival date
AIC	Akaike Information Criterion
ANOVA	Analysis of variance
BOH	Beta-hydroxybutyrate
BP	Breeding propensity
CI	Confidence interval
C:M	2:1 chloroform:methanol solution
COE	Carryover effect
COEI	Common eider
CORT	Baseline corticosterone
df	Degrees of freedom
DFA	Discriminant function analysis
EBI	East Bay Island
ECCC	Environment and Climate Change Canada
EIA	Enzyme immune-assay
GLIER	Great Lakes Institute of Environmental Research
GLMM	Generalized linear mixed models
GRLD	Greenland
IgY	Immunoglobulin-Y
K	Centers of the clusters formed with k-means cluster analysis
LAY	Laying
n	Sample size
NA, na	Not applicable
NAO	North Atlantic Oscillation
NB	Non-breeders
NFLD	Newfoundland
NOAA	National Oceanic and Atmospheric Administration, USA
NSERC	Natural Sciences and Engineering Research Council of Canada

Abbreviation	Description
NU	Nunavut
PCA	Principal component analysis
PR	Pre-recruiting
R	Pearson correlation; from -1 to 1, where +/- 1 indicates the strongest correlation and 0 the weakest
R^2	Coefficient of determination; proportion of the variance in the dependent variable that is predicted by the independent variable
RFG	Rapid follicle growth
rpm	Rotations per minute
R_{sample}	Ratio of the isotopes (i.e. C^{13}/C^{12}, N^{15}/N^{14} and H^2/H^1) in samples
$R_{standard}$	The ratios of isotopes in the international standards
SD, s.d.	Standard deviation
SE	Standard error
SEM	Structural equation modelling
ST	Spring temperature
TRIG	Triglycerides
Temp	Temperature
VLDL	Very-low-density lipoprotein
VTG	Vitellogenin
WT	Winter temperature
δ	Delta notation
$\delta^{13}C$	Delta notation, [(Rsample/Rstandard)-1] ×1000 (‰) where R is the ratio of ^{13}C to ^{12}C
$\delta^{15}N$	Delta notation, [(Rsample/Rstandard)-1] ×1000 (‰) where R is the ratio of ^{15}N to ^{14}N
δ^2H	Delta notation, [(Rsample/Rstandard)-1] ×1000 (‰) where R is the ratio of ^2H to ^1H
ΔAIC	Difference in AIC between models
‰	Unit; parts per mil

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Chapter 1: Introduction

The life-history cycle of an individual generally encompasses several stages, broadly, beginning from its initial recruitment into a population via birth or hatching into the juvenile stage, through maturation, into reproductive adulthood, and then senescence. Although different species exhibit different variations of this life-history cycle, two biological traits that influence an individual's lifetime reproductive fitness are recruitment and adult survival (Stearns 1976; Molles and & Cahill 2008, p. 234). Recruitment influences the number of young an individual produces during each breeding attempt or eventually recruits back into a breeding population (Stearns 1976; Molles and & Cahill 2008, p. 234). Adult survival directly influences the number of reproductive opportunities an individual may have: the longer an individual survives, the more reproductive opportunities that individual should have.

Reproduction requires energy, which for birds can involve investment across numerous stages, including migration or travel to breeding areas, courtship and pair bonding, pre-laying foraging activity, and onwards towards egg production, incubation, and rearing chicks (Christensen 2000; Descamps *et al.* 2011; Williams 2012 Chapters 5 and 6). As a result, the ability to fuel reproduction plays a major role in influencing patterns and degrees of reproductive investment (Drent & Daan 1980; Rowe *et al.* 1994; Hennin *et al.* 2016a; Lamarre *et al.* 2017). Additionally, interactions between events occurring during the non-breeding or overwintering period can also affect the energetic state or body condition of individuals and affect their reproductive decisions. For example, the ability to forage and quality of diet during pre-breeding can have impacts on physiological condition

and reproductive success (Marra *et al.* 1998; Sorensen *et al.* 2009; Descamps *et al.* 2011). Understanding the factors that influence the ability of individuals to successfully fuel reproduction and optimize reproductive decisions that maximize fitness is crucial to furthering our understanding of how these factors impact populations (Marra *et al.* 2015) The overarching goal of my thesis is to increase the general understanding of the underlying mechanisms that influence reproduction including how winter and spring conditions may impact breeding decisions.

1.1 Importance of carryover effects

Even though many life-history stages are conceptually and temporally separated, interactions between these different stages can still occur. The overwintering phase of the annual cycle has become increasingly recognized for its influence on the fitness of individual animals, because investment can be shaped by the selective pressures operating at this time, which can generate carryover effects (Greenberg & Marra 2005; Williams 2012). Stated simply, carryover effects occur when the conditions and activities during one life stage influences some aspect of performance during a subsequent stage (O'Connor *et al.* 2014). Carryover effects can be in both positive and negative directions (Williams 2012, p. 260); good conditions in one stage can have positive effects on a subsequent stage and bad conditions can negatively impact subsequent stages. However, carryover effects are difficult to study because it can be hard to follow individuals across multiple life stages (Williams 2012, p. 247).

Much research on carryover effects has been directed at the links between the non-breeding and the breeding period, often within the context of seasonal migrations (Norris

& Taylor 2006; Ockendon *et al.* 2013; O'Connor *et al.* 2014)**.** For example, in migratory birds, the timing of arrival at the breeding areas and their body mass at that time are key traits known to affect breeding phenology and success (Bêty *et al.* 2003; Gunnarsson *et al.* 2005; Love *et al.* 2010; Hennin *et al.* 2016a). The completion of migration between wintering and breeding locations is linked to the availability of energetic resources (Tamisier *et al.* 1995; Johnson *et al.* 2016). The relative amounts of somatic energy that individuals can accrue prior to migration, the rates at which they expend it during migration, and the ability for individuals to refuel during migration (Smith & McWilliams 2014), can have major implications for breeding decisions and investment (Martin 1987; Oosterhuis & Van Dijk 2002; Crossin *et al.* 2012a, 2013; Hennin *et al.* 2018), as well as success (e.g. chick fledging; Williams 2012, p. 224-225).

Individuals that migrate long distances can use locally foraged resources for egg production on arrival (Ockendon *et al.* 2013), while some may use resources gained during migration (Williams 2012, p. 267). For example, pectoral sandpipers *Calidris melanotos* that arrive to breeding areas early fuel egg production using resources accrued at staging areas prior to arrival; however, later-arriving individuals use locally foraged resources to fuel egg production (Yohannes *et al.* 2010). In this case, for pectoral sandpipers, their resource allocation strategy was actually influenced by winter moulting sites, individuals wintering closer to breeding habitats were able to top off their resources during migration enough to fuel reproduction, whereas those travelling further, the ones arriving later, needed to top up resources at arrival (Yohannes *et al.* 2010). Likewise, the condition that individuals are in prior to migration can allow them to arrive in better condition. American redstarts *Setophaga ruticilla* wintering in higher quality habitats tend to depart earlier, and

subsequently arrive earlier and in better condition at their breeding grounds (Marra *et al.* 1998).

Carryover effects can also occur over shorter time spans. For example, when foraging opportunities at breeding areas are favourable, both Cassin's auklets *Ptychoramphus aleuticus* and yellow warblers *Setophaga petechia* are able to accrue high degrees of pre-breeding body condition, irrespective of their arrival condition, which then positively predicts fitness metrics like lay date and egg/clutch size (Sorensen *et al.* 2009; Drake *et al.* 2013).

The breeding strategy of an individual can vary based on the energetic resources they use to support their reproduction. Capital breeders use their reserved energy stores (endogenous resources) to fund their egg production and incubation periods (Drent & Daan 1980; Meijer & Drent 1999). The cost of storing all energy stores on the body is quite high, so not many species are considered to be purely capital breeders. In contrast, income breeders use the energy derived from foraging on the breeding grounds (exogenous resources) to fund egg production, laying, and incubation (Drent & Daan 1980; Meijer & Drent 1999). Mixed or capital-income breeders use a combination of exogenous and endogenous resources.

Each of these reproductive strategies may influence carryover effects in different ways. A capitally breeding individual from a poor overwintering site may be more affected by having fewer stored resources available upon arrival to their breeding site than an individual / species which is an exclusively income breeder and more reliant on local energy sources just prior to breeding (Williams 2012, p. 261). However, the opposite could also be true where an individual from a capital breeding species could be at an advantage

in years in which they can arrive in a breeding area with sufficient energetic resources to invest in their reproduction.

The mechanisms behind capital and income breeders' decision making may be quite different. Drent & Daan (1980) suggest capital breeders will not exhibit carry-over effects, as they can simply use their own stored capital to fuel reproduction and capital breeding is speculated to be more common in larger species because they are better able to transport large amounts of energy stores (Hobson & Jehl 2010). For example, tundra swans *Cygnus columbianus* with shorter and slower migrations to their breeding grounds produced eggs with a larger amount of capital stores than those with longer or faster migrations (Nolet 2006). Similarly, pink-footed geese *Anser brachyrhynchus* may experience a lower pre-breeding body condition after a winter with adverse environmental conditions, but are able to compensate during the spring and so winter conditions do not affect their breeding condition (Clausen *et al.* 2015). Mixed-breeding strategists and the extent to which they use their capital stores or income-based energy (Jaatinen *et al.* 2016; Williams *et al.* 2017) should, perhaps, be in and of itself considered a carry-over effect as being able to adapt and use income strategies to develop some of their clutch is advantageous for maintenance of their overall condition and future survival.

Resource allocation is an important driver of carryover effects, but some carryover effects may also be driven by physiological systems, where mechanisms may link multiple life-history stages (Williams 2012, p. 268-274). For example in macaroni penguins *Eudyptes chyrsolophus*, vitellogenin (VTG; a yolk precursor, one of two primary sources of yolk protein and lipid; Williams 2012, p. 18) is required for egg development, therefore individuals with lower VTG are potentially less reproductively ready (Crossin *et al.* 2010).

In macaroni penguins, the early stages of egg development overlap with the end of migration. As such, individuals arriving to the breeding colony following migration with lower VTG and with a shorter period between arrival and laying tended to have more dimorphic eggs; consequently the carryover effect of having a large overlap between migration and egg development is more dimorphic egg size (Crossin *et al.* 2010). Similarly, in black-browed albatross *Thalassarche melanophris*, hormonal profiles of individuals arriving to the breeding colony were predictive of their reproductive decisions; deferring albatrosses arriving to the breeding colony had low progesterone, testosterone, VTG and body mass (Crossin *et al.* 2012a). These hormonal profiles were attributed to potential carryover effects of a stressful winter at sea (Crossin *et al.* 2012a).

Understanding the links and carryover effects between periods of the annual cycle in birds is a key step towards an understanding of population-level processes (Marra *et al.* 2015). However, one of the challenges with exploring carryover effects is being able to determine where individuals were located at various stages, to gain insight into their experiences at each stage, without impacting the animal's behavior.

1.2 Using stable isotopes for spatial tracking

Studies of carryover effects that include migration and the impacts of environmental factors, require the spatial tracking of individuals during multiple life-history stages. Tracking individuals inherently includes numerous challenges most crucially, obtaining a sufficient sample size without impacting the organism's behaviours. Stable isotopes are a useful tool because when predators consume prey the stable isotope signatures of their prey are incorporated and reflected in their tissues (Hénaux *et al.* 2012)

and collection of samples is relatively non-invasive. Stable isotopes have been widely used to estimate habitat quality without knowing site-specific information (Marra *et al.* 1998; Sorensen *et al.* 2009) to estimate carryover effects. Stable isotopes can also be used as a tool to infer the geographic locations of where these tissues were grown (Hobson 1999).

Carbon and nitrogen stable isotopes are commonly used to indicate diet (Thompson & Furness 1995; Forero & Hobson 2003; Phillips & Gregg 2003; Ronconi *et al.* 2010; Steenweg *et al.* 2011). Nitrogen-15 is enriched in predators compared to their prey, thus can indicate trophic level while carbon-13 remains relatively constant, but rather changes with distance from shore or along a latitudinal gradient (Cherel *et al.* 2008; Steenweg *et al.* 2011). Carbon and nitrogen have also been used to assign birds within marine environments as nitrogen and carbon deposition can vary due to ocean currents (Lovvorn *et al.* 2003). In addition, hydrogen stable isotopes are deposited via precipitation and as a result isotopic gradients called isoscapes occur (Mehl *et al.* 2005; Bowen *et al.* 2005). Hydrogen isoscapes have been widely used to assign migrating birds to terrestrial overwintering sites (Lott *et al.* 2003; Hobson *et al.* 2004; Yerkes *et al.* 2008; Haché *et al.* 2012; Hénaux *et al.* 2012; Macdonald *et al.* 2012), but because of the dynamic nature of the marine environment, hydrogen isoscapes are less straight forward to use in marine birds (Bond & Jones 2009).

Stable isotopes have been used to delineate habitat and geographical location of overwintering sites in terrestrial habitats via differences between deposits of isotopes and the use of isoscapes and are generally minimally invasive to collect (Hobson 1999; Bowen *et al.* 2005; Hobson *et al.* 2012). Furthermore, in marine birds, Mehl *et al.* (2005) differentiated between two groups of king eiders *Somateria spectabilis* wintering in the

arctic between Alaska and Greenland using stable isotopes of nitrogen. More recently, Yerkes *et al*. (2008) also used a combination of stable isotopes to infer associations between overwintering sites, pre-breeding habitat, length of migration and arrival conditions of northern pintails *Anas acuta*. Individuals wintering inland near fresh water arrived at breeding sites in better condition than those wintering coastally, regardless of whether they staged in similar habitats.

Stable isotopes have been widely used to estimate habitat quality without knowing site specific information (Marra *et al.* 1998; Sorensen *et al.* 2009) to estimate carryover effects. These studies show that not only can stable isotopes be used to indicate winter habitat quality but can be also be used to delineate wintering locations. Stable isotopes can then be used to investigate winter location to then estimate pre-breeding experiences and how these factors may influence reproductive investment and success.

1.3 Common eiders as a model species

Common eiders *Somateria mollissima* are the largest sea ducks in the Northern Hemisphere. They spend the majority of their lives at sea, inhabiting marine coastal areas (Robertson 2018) across polar and temperate regions and in Europe and North America. In Canada, many of the common eider subspecies are migratory and generally migrate from their breeding grounds to fall moulting areas and then continue to their overwintering areas (Goudie *et al.* 2000). Migrating eiders undertake a spring migration foraging along the way (Mosbech *et al.* 2006) and arrive to staging areas in the spring to develop energy reserves to fuel egg formation, laying, and incubation. They do not feed during incubation and thus are dependent on energy stores that they accrue during staging to support successful

reproduction (Goudie *et al.* 2000; Sénéchal *et al.* 2011b). Studies have shown that female body mass may decrease by up to 45 % during the 24-26 day incubation period (Parker & Holm 1990; Goudie *et al.* 2000). So, it is important that females accrue sufficient body reserves during the pre-breeding period as this can ultimately affect their decision to breed (Drent *et al.* 2003; Gaston *et al.* 2005; Love *et al.* 2010); breeding propensity in each year can have implications for lifetime fitness. Male common eiders do not aid in incubation or chick rearing, so a few days after a female has laid the final egg in her clutch males depart for moulting areas. Ducklings fledge the nest to a nearby body of water soon after the last egg has hatched. Adult females and their ducklings form large groups, where they share protection of their collective young (Goudie *et al.* 2000). Female eiders and their young migrate onwards to overwintering areas following moult (Robertson 2018).

Migratory animals like the northern common eider *S. m. borealis* provide a good model system to explore carryover effects because individual experiences can vary throughout the different stages of their life history such as during overwintering, breeding, and migration. Migration can provide a backdrop against which inter-individual variation in fitness related traits and processes can be ideally observed; conditions in different locations will be different, so if some individuals within a study population migrate to distinct areas, individual variation in fitness could be attributed to these differences in experiences. For instance, in common eiders, one breeding colony at East Bay Island overwinters in two distinct areas: Western Greenland and Newfoundland, Canada. Current rough estimates indicate that 60% - 90% of East Bay eiders migrate to Disko Bay and the Nuuk region of Western Greenland with the rest migrating along the Labrador and Newfoundland coasts, Canada (Mosbech *et al.* 2006; Steenweg *et al.* 2017). The resultant

winter migrations can be highly variable ranging up to 3000 km from breeding grounds (Mosbech *et al.* 2006). Winter sea ice conditions are an important driver of the timing of migration, of migration routes, and of winter foraging destinations (Mosbech *et al.* 2006). Eiders depend on open areas in the sea ice for foraging during the overwintering period (Robertson & Gilchrist 1998), if there is a reduction in sea ice, potential foraging areas can increase and eiders could potentially arrive at breeding areas in better condition (Jean-Gagnon *et al.* 2018). Given that these overwintering areas are so far apart, sea ice conditions can be entirely different. As a result, eiders arriving at the breeding colony will have experienced distinctive wintering conditions.

After the overwintering period, common eiders nesting at East Bay Island, arrive in staging areas near the breeding colony approximately one month before they lay their eggs. During this time females feed extensively and develop somatic energy reserves to support reproductive development and investment.

Up to this point studies have mainly focused on the effects of pre-breeding conditions on reproductive output. Some work has investigated the impacts of winter climatic variables of survival (Guéry *et al.* 2017) and arrival body mass (Descamps *et al.* 2010) in common eiders; however, these studies treated all eiders from one colony as the same population of overwintering eiders, and it has been established that eiders from the same breeding colony overwinter in different locations. Further, these studies did not combine both spring and wintering conditions. This omission is mainly due to the difficulty of tracking individuals without impacting their reproductive decisions. As such, there is a lack of information examining how different wintering and spring conditions may impact breeding decisions on an individual level. Because the colony of common eiders breeding

at East Bay Island overwinter in at least two distinct areas, this acts as two treatment groups, providing an ideal scenario to test the effects of differing wintering experiences on reproduction. In addition, stable isotopes have the potential to be a useful tool in delineating different overwintering populations.

In birds like the common eider which have a mixed capital-income breeding strategy, factors that influence body mass are important in determining their propensity to breed. Common eiders optimize their reproductive decisions (i.e. the decision to breed and number of eggs to form) based on their body mass and arrival date by determining whether they have enough resources to dedicate to egg production (Descamps *et al.* 2011; Hennin *et al.* 2018). Female common eiders use stored resources brought from the wintering grounds, topped up by local foraging on the breeding grounds during the pre-breeding period to produce their eggs and successfully complete their 24-day incubation fast (Sénéchal *et al.* 2011b). In consequence, arrival date at the breeding colony, body mass, and breeding decisions are likely affected by foraging conditions experienced during the winter, migration (Oosterhuis & Van Dijk 2002; Mosbech *et al.* 2006) and spring (Jean-Gagnon *et al.* 2018).

Other factors that may influence arrival body mass and breeding decisions is the timing of pairing or investment by male mates. The phenology of pair formation can vary significantly depending on the species because of differences in life history and parental investment (Rohwer & Anderson 1988). As a result, little is known about how the timing of pairing can influence reproduction. Generally, male waterfowl species do not help their female mate during incubation or during chick rearing, instead, they may invest during the pre-breeding phase via defense of their female mates from extra pair copulations to allow

for undisturbed foraging (e.g. in snow geese, Choinière and Gauthier 1995; in common eiders, Christensen 2000) or even to support foraging to maximize their female's reproductive success (Rodway 2007). Males should benefit reproductively from mate guarding from both paternal endurance and female condition perspectives. Because pre-breeding condition influences fitness proxies like lay date, clutch size, and hatching date (Descamps *et al.* 2011; Hennin *et al.* 2016a, 2018), the timing of pairing, and how males influence their female mate's breeding investment, should be key to both male and female reproductive fitness. However, although studies have documented foraging dynamics (Hario & Hollmén 2004; Steele *et al.* 2007) and energy acquisition (body mass) (Hipes & Hepp 1995), few studies have examined the energetic changes in male birds in relation to female breeding investment, and whether male energetics and investment in guarding influences the fitness of their female mates. The lack of studies examining energetic investment by males is likely because studying pairing phenology and male investment in any seasonally-breeding seabird system remains difficult since individuals tend to overwinter in remote locations and spend the pre-breeding period offshore (Merkel *et al.* 2006), limiting the possibility of directly observing winter and spring behaviour amongst males and females.

There are many possible influences on reproductive decisions for many species, including common eiders. Given that common eiders are a long-lived species they possess the flexibility to defer reproduction in a given year, in favour of self-maintenance should they not acquire enough somatic stores to invest in reproduction. These somatic resources and resulting reproductive investment could depend on timing of pairing, investment of the male mate, or spring and wintering conditions. So far, these questions remain unanswered.

1.4 Aims of this thesis

The overwintering period comprises a large portion of the common eider's life history and can have impacts on eider body condition (Jamieson *et al.* 2005; Descamps *et al.* 2010), with the pre-breeding period well known to affect eider condition (Parker & Holm 1990; Love *et al.* 2009, 2010; Merkel 2010). Studies investigating carryover effects have not yet linked both winter and spring environmental factors to body mass, breeding phenology and behaviours. Studies linking breeding parameters to both winter and spring conditions are lacking mostly due to the difficulty of tracking individuals through time and space, especially during the non-breeding season (Crossin *et al.* 2014). The overarching goal of my thesis is to investigate underlying mechanisms that influence breeding. This includes the role of the timing of pairing, male body condition, the over-wintering residencies and the pre-breeding period in generating carryover effects onto reproductive decisions.

My work has four specific objectives: 1) to establish a minimally invasive and effective method to assign common eiders to their overwintering sites after arrival to their breeding grounds, 2) to use this method to gain insight into when common eiders form breeding pairs, 3) to characterize changes in male physiology and body condition throughout the pre-breeding period to determine how male physiological state might influence female reproduction, and 4) to link winter and spring environmental conditions to breeding decisions in common eiders. Often work investigating questions relating to carryover effects can be limited by sample size; specifically, studies investigating

carryover effects are often limited by comparing only a few years, however, my work covers three wintering locations over four years.

In Chapter Two, I test the utility of several stable isotopes (13-carbon, 15-nitrogen and 2-hydrogen) in inferring overwintering sites for common eiders arriving at their breeding colony. To infer overwintering sites, I compare the stable isotope values in arriving common eider blood and claws to those obtained from individuals on the wintering grounds in Greenland and Newfoundland. The goal of this work is to identify which tissues and stable isotopes are best used to identify winter migration and residency patterns in the absence of telemetric methods.

With this newly developed stable isotope method, in Chapter Three I use stable isotope values in claws and blood of paired individuals, reflecting their winter and spring locations respectively, to determine when common eiders formed their pairs. I then use this information to investigate whether pairing phenology impacts female common eider body condition gain or breeding propensity. Because the first two chapters of my thesis were published earlier on in my PhD and I only collected samples from males beginning in my second year of field work, these two chapters contain two years of data (2014 and 2015 for Chapter Two; 2015 and 2016 for Chapter Three).

Building on the information of how the timing of pairing may or may not affect female body mass or breeding propensity, in Chapter Four, I focus on the energetic costs and reproductive benefits of mate-guarding behaviours in male common eiders. Males exhibit extensive mate guarding behaviors throughout the reproductive period, and the consequences of these behaviors are mostly unknown. I specifically examined the variation in male physiological state using both energetic and hormonal measures in relation to his

mate's breeding stage. I then investigated whether variation in these traits were predictors of two important performance traits in this species: pre-laying female fattening rate and lay date. This chapter was precipitated by observations in the field during my first field season, and so, the data associated with this chapter includes the last three years of field work.

In Chapter Five, I use a four-year data set of stable isotopes to infer the overwintering origins of female common eiders arriving to the breeding colony. I examine the impacts of winter and spring temperatures on arrival date, baseline corticosterone (energetic management), body mass and ultimately breeding propensity. This work aims to examine whether winter temperatures have a greater impact on female reproduction than spring temperatures, or whether spring acts as a buffer for poor winter conditions. I restrict this analysis to only females, because based on my findings in Chapter Four, male eiders did not particularly impact female body condition or her reproductive investment.

Finally, in the general discussion (Chapter Six), I summarize my key findings. I examine how these findings contribute to the general understanding of common eider reproduction and suggest how these methods may be used to explore the impacts of anthropogenic influences in marine birds. Further, I provide suggestions on how stable isotopes could be applied in future research to further our understanding of migration in marine birds more generally.

1.4.1 Thesis structure

I have chosen to present my thesis research as four independent research manuscripts (two of which are published articles; Chapter Two in *Ecology and Evolution* and Chapter Three in the *Journal of Ornithology*). As a result, there is some repetition between the articles, mainly in the background information and methods sections. Each

chapter has several contributing authors, nevertheless, for each I was the lead author; I developed the ideas with input from my supervisory committee, led the research and analyses, and handled the writing components of each including edits from co-authors and throughout publishing. Because of their involvement, I would like to acknowledge the guidance and support my co-authors have provided in all steps of the process from advancing these ideas, to providing advice on writing and analyses, and eventually publishing. For the two chapters which are published, copyright release information is included in Appendix 1. To increase the ability for others to reproduce my data analyses, I have included the commented R Code used for each chapter in separate appendices.

Chapter 2: Stable Isotopes Can Be Used to Infer the Overwintering Locations of Pre-Breeding Marine Birds in The Canadian Arctic

Abstract

Although assessments of winter carryover effects on fitness-related breeding parameters are vital for determining the links between environmental variation and fitness, direct methods of determining overwintering distributions (e.g. electronic tracking) can be expensive, limiting the number of individuals studied. Alternatively, stable isotope analysis in specific tissues can be used as an indirect means of determining individual overwintering areas of residency. Although increasingly used to infer the overwintering distributions of terrestrial birds, stable isotopes have been used less often to infer overwintering areas of marine birds. Using Arctic-breeding common eiders, I test the effectiveness of an integrated stable isotope approach (13-carbon, 15-nitrogen and 2-hydrogen) to infer overwintering locations. Knowing the overwinter destinations of eiders from tracking studies at the study colony at East Bay Island, Nunavut, I sampled claw and blood tissues at two known overwintering locations, Nuuk, Greenland and Newfoundland, Canada. These two locations yielded distinct tissue-specific isotopic profiles. I then compared the isotope profiles of tissues collected from eiders upon their arrival at the breeding colony and used a k-means cluster analysis approach to match arriving eiders to an overwintering group. Samples from the claws of eiders were most effective for determining overwinter origin, due to this tissue's slow growth rate relative to the 40-day turn-over rate of blood. Despite taking an integrative approach using multiple isotopes, k-means cluster analysis was most effective when using 13-carbon alone to assign eiders to an overwintering group.

My research demonstrates that it is possible to use stable isotope analysis to assign an overwintering location to a marine bird. There are few examples of the effective use of this technique on a marine bird at this scale; I provide a framework for applying this technique to detect changes in the migration phenology of birds' responses to rapid changes in the Arctic.

2.1 Introduction

The non-breeding phase of the annual cycle is increasingly being recognized for its impacts on individual fitness in animals, as many physiological, behavioural, and life-history related traits that influence breeding phenology and investment are shaped by the selective pressures operating at this time, which can generate carryover effects (Greenberg & Marra 2005; Williams 2012). The study of carryover effects - especially how variation in overwintering experiences can impact subsequent reproductive performance, population processes, and fitness - is a burgeoning field of research that is central to testing hypotheses of behavioural, evolutionary, and physiological ecology (O'Connor *et al.* 2014)**.** Individual variation in overwintering location impacts foraging activity and physiological condition at arrival on the breeding grounds, and accordingly, an individual's preparedness for breeding (Marra *et al.* 1998; Sorensen *et al.* 2009; Descamps *et al.* 2011). Since arrival body condition and timing on the breeding grounds are key traits known to impact the breeding phenology and success of migratory birds (Bêty *et al.* 2003; Gunnarsson *et al.* 2005; Love *et al.* 2010; Hennin *et al.* 2016a), determining how an individual's winter experience affects these traits is a key step in understanding population level processes.

Analyses of naturally-occurring biochemical markers in tissues is a common means for discerning the overwintering activity and locations of migratory species in terrestrial-based habitats, especially stable isotope ratios of 2-hydrogen (deuterium, δ^2H), 13-carbon ($\delta^{13}C$), and 15-nitrogen ($\delta^{15}N$) (Hobson, 1999; Norris *et al*., 2005; Yerkes *et al*., 2008). Isotopic signatures reflect the environment in which a given tissue (and by extension the individual) grows (Bearhop *et al.* 2002; Bond & Jones 2009) because these stable isotopes are integrated within an individual's tissues through consumption of locally acquired water and food. Therefore, by matching tissue specific isotopic signatures to terrestrially delineated isotopic landscapes called isoscapes, stable isotopes have been successfully used to determine the overwintering or breeding areas of many terrestrial birds, (Hobson 1999; Haché *et al.* 2012). Isoscapes of δ^2H are generated as a result of predictable, regionally-generalized patterns of precipitation (Mehl *et al.* 2005; Bowen *et al.* 2005) and have proven useful for inferring the terrestrial overwintering grounds of various migrant bird species (Hobson *et al.* 2004; Yerkes *et al.* 2008; Haché *et al.* 2012; Hénaux *et al.* 2012). Unlike deuterium, $\delta^{13}C$ and $\delta^{15}N$ isoscapes are generated by landscape-scale processes related to nitrogen cycling in the soil ($\delta^{15}N$) and the plant types present ($\delta^{13}C$) (Rubenstein & Hobson 2004; Bond & Jones 2009). Recently, $\delta^{13}C$ and $\delta^{15}N$ have been combined together to infer the overwintering locations of migratory shorebirds sampled at coastal stopover sites along western Africa and Europe (Catry *et al.* 2016). Thus, it is possible to use multiple isotopes to assign individuals to a more specific location. For example, δ^2H, $\delta^{13}C$ and $\delta^{15}N$ were used to determine the staging and overwintering areas of Alaskan northern pintails (*Anas acuta;* Yerkes *et al*. 2008), as well as the natal origins of five different species of European bat species (Popa-Lisseanu *et al.* 2012).

The ability to apply stable isotope analysis to marine species is generally more challenging than terrestrial systems due to the highly dynamic nature of marine systems, which results in less predictable isoscapes, making isotope ratios collected from seabirds more difficult to define and interpret (Bond & Jones 2009). To overcome the lack of reliable isoscapes in the marine environments, integrative ecologists have begun comparing the isotopic values from tissues in the species of interest with those occupying lower trophic positions (Mehl *et al.* 2005) as a proxy for the isotopic signal of the environment. For example, $\delta^{15}N$ was used to infer the overwintering locations of breeding king eiders *Somateria spectabilis*, by matching $\delta^{15}N$ values in eider feathers, which were grown in winter, to signatures in copepod prey collected from two known overwintering areas located ~3000 km apart (Mehl *et al.* 2005). A limitation to this approach arises when comparing dissimilar tissues types (e.g., blood versus feathers), or when making inter-specific comparisons, since tissue- or species-specific discrimination factors are required to accurately relate the isotopic signatures of an animal to that of its prey (Bearhop *et al.* 2002; Bond & Diamond 2011). This is because the incorporation of isotopes into different tissues varies as a function of their structural composition and/or turnover rate, and because incorporation rates can also vary among species with differing metabolic rates, energetic requirements, and life histories (Bearhop *et al.* 2002; Haché *et al.* 2012). Discrimination factors that account for these factors are required to make meaningful interpretations; however, they are often not available or quantifiable for a given study. A potential solution for determining the non-breeding, winter location of a species is to characterize the isotopic signatures using specific tissues from individuals collected at the known wintering areas (Norris *et al.* 2005). This method has the advantage of negating the need for discrimination

factors as well as providing a baseline wintering reference signature to which samples collected from other individuals at a different time and location (e.g., on the breeding grounds) can be compared.

In this study, I identify wintering $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ isotope values for the Northern common eider *Somateria mollissima borealis* (Fig 1.; hereafter "eider"), a migratory sea duck which spends a majority of its life on the ocean, with the aim to assign overwintering locations to individuals sampled at arrival on breeding grounds. Previous satellite tracking studies at the breeding colony of my study site (Mitivik Island, East Bay Island, Nunavut, Canada) have indicated that eiders migrate to, and spend their winter in, two general locations: off the south-western coast of Greenland near Nuuk and northwards towards Disko Bay, and along the coast of Newfoundland and Labrador, Canada (Mosbech *et al.* 2006). Beginning in late April, eiders leave their overwintering areas for the staging areas in and around Northern Hudson bay, arriving there in late May travelling between 60-130 km per day (Mosbech *et al.* 2006). Eiders move to the breeding colony in early- to mid-June when the ice has begun to clear from the head of the bay (F. Jean-Gagnon, unpublished data). The two wintering locations are markedly distinct with regards to their geology, community composition and hydrology, and thus provide an amenable system in which to test predictions about isotopic differentiation in eider tissues and make inferences about geographic distribution during the non-breeding period in winter. Specifically, I first predicted that the $\delta^{15}N$ signatures of eiders overwintering in Greenland would be greater than in birds from Newfoundland due to known differences in local circulation and nutrient enrichment patterns between the Labrador and West Greenland currents, and greater $\delta^{15}N$ enrichment in Greenland (Rubenstein & Hobson 2004). Second, because a large proportion

of adult eiders in Greenland spend most of the winter in fjords while eiders in Newfoundland move along coastal areas and offshore islands, and $\delta^{13}C$ differs with distance from shore and as a function of latitude (Rubenstein & Hobson 2004; Cherel *et al.* 2008), I predicted that $\delta^{13}C$ would be higher in birds overwintering in Greenland (Graham *et al.* 2010). Finally, as precipitation is directly related to $\delta^{2}H$ levels (Mehl *et al.* 2005; Bowen *et al.* 2005), I predicted that the substantial freshwater inputs from melting glaciers in Greenland would result in lower $\delta^{2}H$ levels in Greenland compared to Newfoundland (Bowen 2010).

Studies using stable isotopes to assign overwintering sites for birds often use isotopes obtained from feathers samples (Mehl *et al.* 2005; Hobson *et al.* 2012; Garcia-Perez & Hobson 2014). However, eiders undergo a near-complete post-breeding molt in the fall, before migrating to their overwintering locations, meaning these signatures would reflect molting rather than wintering sites. After investigating differences in isotopic values of common eider populations, my second goal was to test whether blood or claw (toenail) tissues would be best used to infer overwintering origin of arriving eiders.

Our third goal was to determine if I could assign unknown individuals arriving on the breeding grounds to specific wintering locations using a k-means clustering method. Specifically, I tested several k-means clustering analyses using different combinations of the stable isotope data, to best classify the known winter eider samples to their correct overwintering location. Subsequently, I included samples collected from pre-breeding eiders in these clustering analyses to assign overwintering locations to pre-breeding eiders based on their isotopic signatures. With these results I then discuss the resulting proportions of pre-breeding eiders assigned to either the Greenland or Newfoundland overwinter

groups and compare these to the proportions expected from previous telemetry studies in this system.

2.2 Methods

2.2.1 Study system

To characterize the isotopic makeup of each overwintering location, eiders were sampled on their overwintering grounds (Figure 2). Eiders in Newfoundland (Change Islands; 49°57'N, -54°27'W) were collected by hunters and submitted to Environment and Climate Change Canada (Mt. Pearl, NL) between 23 December 2013 and 17 January 2014 for a contaminants study. Additional eiders were collected from Newfoundland (Sunnyside; 47°48'N, -53°53'W), when several died after striking light standards at a coastal industrial site on 01 Apr 2016 and were submitted to Environment and Climate Change Canada. In Greenland (Qussuk Fjord, Nuuk; 64°76'N, -51°01'W), local fishermen collected eiders from fisheries by-catch between 15 April and 22 April 2014 and submitted them to the Greenland Institute of Natural Resources. Any eiders showing signs of decomposition or oiling were not sampled for this study. All eider carcasses were frozen at -20°C until dissection. Additional details about these collections, including samples sizes and the types of tissues collected, are summarized in Table 1. Arriving, pre-breeding eiders used to assign to wintering groups were captured at their breeding colony, East Bay Island (EBI), East Bay Migratory Bird Sanctuary, Nunavut, Canada (Figure 1; 64°02'N, 81°47'W) using flight nets during the pre-breeding period (11 June to 01 July 2014 and 19 June to 04 July 2015).

2.2.2 Tissue sample collection

The time-period reflected by a specific tissue depends on the tissue's turnover or growth rate (Oppel & Powell 2010; Steenweg *et al.* 2011; Hénaux *et al.* 2012). In birds weighing ~1.5 kg, whole blood and red blood cells have a turnover rate of approximately 3-4 weeks (Hahn *et al.* 2012). Claws, however, do not have a turnover rate because they are metabolically inert and growth is continuous, and, for a mature duck, a typical claw will represent ~90 to 110 days of growth (Hopkins III *et al.* 2013), as the tip of the claw is filed down by natural abrasion. Therefore, in eiders sampled on their wintering grounds, the base of the claw should reflect the most recent growth and the most accurate wintering signature. For eiders collected at the breeding grounds, the full claw length, from tip to base, would reflect growth over the previous 3 months, which overlaps with their time spent within their core overwintering areas (Mosbech *et al*. 2006).

For claw samples collected from overwintering birds, total claw length was measured for each individual, and samples were clipped from the base of the claw on the middle toe of the left foot and stored in a paper envelope. For birds captured at the breeding site, the middle toe claw of the left foot was measured from base to tip to the nearest millimeter, and the distal 2 mm of the claw was clipped and stored in a small paper envelope for further analysis.

I also collected blood samples from wintering eiders to compare isotope levels between locations, and within locations across years. Blood samples for wintering birds were collected from Newfoundland (Change Islands) and Nuuk, Greenland. Frozen whole blood was removed from the heart atrium or ventricle and stored in an Eppendorf tube for further analysis. For pre-breeding eiders at EBI, fresh 1ml blood samples were taken from

the tarsal vein using a heparinized 23-gauge needle and syringe, then stored in heparinized Eppendorf tubes and kept cool to approximately 4°C. This blood was collected as part of another project and therefore all samples were centrifuged at 10 000 rpm for 10 min, and red blood samples were separated from plasma, unlike in the wintering birds. Red blood cells were stored at -80°C until prepared for analysis.

2.2.3 Laboratory analyses

To prepare samples for stable isotope analysis, blood samples were oven-dried at 50°C for 30 hours (winter eiders) or freeze-dried for 30 hours (breeding site samples). Although samples were dried using different methods, these two drying techniques have been shown to have no effect on stable isotope analysis results (Hobson *et al.* 1997). All blood samples were lipid extracted to reduce the effect additional lipids may have on the $\delta^{13}C$ signatures (Mazerolle & Hobson 2005), making whole blood and red blood cell samples comparable, and I removed surface oils from claw samples. To extract lipids and remove surface oils, all dried blood and claw samples were soaked in 2:1 chloroform:methanol solution (C:M) for 24 hours and then centrifuged for 10 minutes at 10 000 rpm. The C:M was siphoned off using a pipette, then samples were rinsed again with C:M, centrifuged for an additional 10 minutes, and the C:M was siphoned off once more. Samples were then left open under a fume hood for 24 hours to allow any left-over C:M to evaporate. All blood samples were ground with a mortar and pestle into a powder, and claw samples were snipped into tiny pieces. Subsamples were weighed to 0.3-0.5 mg and folded into a tin capsule for $\delta^{13}C$ and $\delta^{15}N$ analysis, and for $\delta^{2}H$ analysis subsamples were calibrated in the lab for 48 hours, weighed to 0.1-0.2 mg, desiccated in an oven at 100ºC for one hour and crushed into a silver capsule. Results of stable isotope analyses are

reported in δ units where $\delta = [(R_{sample}/R_{standard})-1] \times 1000$. R_{sample} are the ratio of the isotopes (i.e. C^{13}/C^{12}, N^{15}/N^{14} and H^{2}/H^{1}) in samples, $R_{standard}$ are the ratios of isotopes in the international standards, unique for each element (for carbon: Vienna Pee Dee Belemnite, nitrogen: Atmospheric Air, hydrogen: Vienna Standard Mean Ocean Water). Standards were run every five samples and duplicates were analyzed for every nine samples, with precisions of 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ analysis and 3‰ for $\delta^{2}H$ (Norris *et al.* 2005; Macdonald *et al.* 2012). Stable isotope analyses were performed at the Queen's Facility for Isotope Research, Queen's University (Kingston, Ontario, Canada) using a Costech ECS 4010 for $\delta^{13}C$ and $\delta^{15}N$ analysis and a Thermo-Finnigan thermo-combustion elemental analyzer for $\delta^{2}H$ analysis coupled to a Thermo-Finnigan DELTA^{plus}XP Continuous-Flow Isotope Ratio Mass Spectrometer.

2.2.4 Data analyses

To address my overarching goal and test my initial hypotheses that $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values in blood and claws will differ between Greenland and Newfoundland samples, I ran separate two-factor ANOVA models to compare $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values from wintering birds, using location and sex as factors. I ran a one-factor ANOVA for each isotope in eiders collected at East Bay in 2014 and 2015 to test for differences between years. There was no discernable annual variation in claw isotopes, thus I pooled these tissues from 2014 to 2016 for the wintering birds.

To address my second goal - to determine whether blood or claws are the best tissue to use for these analyses - I assessed whether stable isotopes of these samples overlapped for overwintering and arriving eiders. The turnover-rate for blood in birds of this size (Bearhop *et al.* 2002; Steenweg *et al.* 2011; Hahn *et al.* 2012) may be too rapid to be used

in this circumstance. If this is the case, I would perform my k-means cluster analyses using the stable isotope signatures from claws.

To address my third goal of determining which isotopes are best included in the k-means clustering algorithm for later assigning an arriving eider to its overwintering site, I tested a k-means clustering method for its ability to correctly classify known winter-sampled eiders to their correct overwintering location based on the stable isotope signatures in claws. This k-means approach is a centroid based partitional clustering method, where the centroids are the arithmetically calculated centers of the clusters and where of k is the number of clusters. The initial centroids for each cluster can either be randomly selected or pre-assigned from the data (Tan *et al.* 2006). Each of the remaining data points are iteratively assigned to the cluster to minimize the sum of squared error of each centroid (Tan *et al.* 2006). This method has been used previously on stable isotopes and other biomarker data to classify passerines (Garcia-Perez & Hobson 2014) and marine mammals into discrete groups (Pomerleau *et al.* 2014). I defined the starting centroids from the means of isotope values obtained from individuals in each overwintering location because previous studies indicate that eiders breeding at East Bay Island overwinter in two distinct regions (Mosbech *et al.* 2006; Figure 2). The western Greenland overwintering group primarily overwinters near Nuuk, however, some migrate 600 km further north to Disko Bay. Because exploratory plots indicated a third potential group, I ran cluster analyses with both 2 and 3 clusters for both years, using the mean of the third group as the starting centroid for this new cluster. I conducted cluster analysis for each year separately because the presence of this third group varies between years.

Having pre-defined the starting centroids for the cluster analysis, I then ran the known identity, winter-sampled individuals through the cluster analysis to determine if the analysis could correctly classify individuals to their original location/group. I elected to test k-means cluster analysis using all the stable isotopes (1) $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$, (2) $\delta^{13}C$ and $\delta^{15}N$ together and then (3) only $\delta^{13}C$ as two-way ANOVA results indicated that there was only a significant difference between groups in $\delta^{13}C$ but not $\delta^{2}H$ or $\delta^{15}N$ (Table 2). I used the sum of the squared error to measure the quality of each clustering method (Tan *et al.* 2006), used the number of misclassified winter birds as a measure of its accuracy, and plotted the results to determine if the clustering was realistic. All data analyses were conducted using R version 3.3.1 (2016-09-28) using packages cluster (Maechler *et al.* 2016) and MASS (Venables & Ripley 2002). All code for analyses are available in Appendix 2.

2.3 Results

The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ signatures from blood samples of the two groups of winter-caught eiders (Greenland and Newfoundland) were significantly different ($P < 0.001$ for each) but did not differ by sex (Table 2). Likewise, claw $\delta^{13}C$ signatures differed significantly between the two groups (Table 2; $P < 0.001$), although $\delta^{15}N$ and $\delta^{2}H$ signatures were not significantly different (Table 2; $P = 0.70$ and $P = 0.80$, respectively) and sex was not a significant factor (Table 2). Moreover, although I did not detect any annual variation in $\delta^{13}C$ signatures of claws from birds captured at arrival in 2014 and 2015 ($F_{1, 222} = 1.90$, $P = 0.17$), both $\delta^{15}N$ and $\delta^{2}H$ signatures differed significantly across years ($\delta^{15}N$: $F_{1, 222} = 6.29$, $P = 0.013$; $\delta^{2}H$: $F_{1, 217} = 6.36$, $P = 0.012$).

Stable isotope signatures in the blood samples of eiders arriving at the breeding colony overlapped with those from wintering eiders from Newfoundland, but not with the wintering eiders from Greenland (Table 3; Figure 3). Moreover, signatures from pre-breeding birds were comparatively enriched in $\delta^{15}N$ and depleted in $\delta^{2}H$ (Table 3; Figure 3) suggesting they partially reflected signatures acquired during the migration period. Consequently, I used the stable isotope signatures from claws of pre-breeding eiders to conduct k-means cluster analysis.

Tests of k-means cluster analyses using the stable isotopes found in the claws of winter-caught eiders minimized SSE and resulted in fewer misclassified wintering eiders when using the stable isotope $\delta^{13}C$ alone (2014: 0 and 2015: 2 misclassified) as opposed to integrating $\delta^{13}C$ and $\delta^{15}N$ (2014: 0 and 2015: 3 misclassified), or $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values (2014: 29 and 2015: 28 misclassified; Table 4). For 2014 pre-breeding eiders, the most parsimonious representation of groupings included 3 clusters (8 vs. 0 misclassified for 2 vs. 3 clusters, respectively), where for the 2015 pre-breeding eiders, 2 clusters yielded the best results (2 vs. 16 misclassified for 2 vs. 3 clusters, respectively; Table 4, Figure). These two models suggest that in 2014 a total of 79 individuals overwintered in Nuuk, Greenland, 13 in Newfoundland and another 15 near Disko Bay, Greenland, while in 2015 it was estimated that 102 eiders overwintered in Nuuk, Greenland and 13 in Newfoundland.

2.4 Discussion

Supporting my first goal, isotopic signatures were indeed significantly different between the two wintering groups. In contrast to a study on American redstarts (*Setophaga ruticilla*; Norris *et al.* 2005), blood samples did not reflect overwintering sites, but rather

signatures probably reflected spring migration likely due to the slow nature of eider migration to the breeding grounds at East Bay Island. Cluster analysis of stable isotope values in the claws of eiders, however, was more successful in differentiating between wintering locations, and using this technique I was then able to infer the overwintering locations of eiders arriving at their breeding colony, which supports my second goal. Concerning my third goal, $\delta^{13}C$ alone, and not the integration of $\delta^{13}C$ with $\delta^{15}N$ and $\delta^{2}H$, was most useful for differentiating wintering locations, likely because these areas differ in habitat features known to affect $\delta^{13}C$ values (marine coastal vs. inland fjord), and in latitude (Rubenstein & Hobson 2004; Cherel *et al.* 2008; Graham *et al.* 2010).

K-means cluster analysis was effective for determining both the number of clusters to include for each year (2014 or 2015) and their respective centroids. Moreover, the method revealed a novel overwintering group and generated very few misclassified winter birds. Discriminant function analysis (DFA) is sometimes similarly used in this circumstance, but is not recommended with the use of spatial data whereas clustering methods are (Zuur *et al.* 2007). In comparison to DFA, k-means cluster analysis uses starting centroids to form the clusters, rather than in DFA which provides limits from which the groups or clusters are formed. Secondly, because winter samples from the third inferred group from Disko Bay were unavailable, I did not have the boundaries to include in the DFA. Including the mean of this group as a starting centroid for k-means was a way to manage this issue. Nonetheless, one advantage of DFA over K-means cluster analysis is that DFA can assign a percentage of confidence to each individuals' assignment; however, given the infrequent misclassification of winter birds, I am confident that K-means cluster analysis of $\delta^{13}C$ adequately classified arriving eiders into their known overwintering

groups. Overall, this work demonstrates that it is possible to not only back-assign individual eiders to their overwintering grounds using samples collected upon arrival at their breeding grounds, but also to detect potentially novel wintering grounds.

Although blood samples were obtained in January and April from Newfoundland and Greenland, respectively, I expected differences in isotopes to be applicable as samples were obtained within 4 months of each other, and the differences between locations would be bigger than between the different times. The differences between the two overwintering areas in blood and claws were similar, although a bit larger for claws. As such, I am confident that the differences in blood were due to their geographic location rather than the differences in timing.

The ability to back-assign seabirds and sea ducks arriving at the breeding colony to their over-wintering locations is a major advance for studies of marine birds. This method has the potential to significantly impact future studies investigating the carry-over effects of migration behaviors on individual and population level processes in a number of ways. First, future studies using these techniques can cost-effectively and relatively non-invasively determine overwintering location, and subsequently determine how variation in overwintering environmental conditions carryover to affect important reproductive parameters. Second, once established as a baseline, this method can then be used to delineate populations and monitor any fluctuations in these populations. For example, previous satellite tracking research (2001-2003) from this colony indicated that approximately 40% of eiders overwintered in Newfoundland, Canada and 60% in Greenland (Mosbech *et al.* 2006). I found that in both years only about 10% of the eiders overwintered in Newfoundland, with different proportions arriving from Nuuk and Disko

Bay, Greenland, in each year. In 2014, 14% of the eiders were detected to occupy a third overwintering cluster (Table 4). I suggest that these individuals are likely from Disko Bay, because more northern marine areas are enriched in 13-carbon (Hobson 1999; Rubenstein & Hobson 2004; West *et al.* 2006), it is likely that $\delta^{13}C$ is higher in Disko Bay compared to Nuuk as seen in shrimp *Pandalus borealis* and copepods *Calanus finmarchicus* (Hansen *et al.* 2012). While the western Greenland breeding population has increased by 12% per year (Merkel 2010) since the implementation of hunting quotas, the Newfoundland population has been potentially impacted by the same Avian Cholera outbreak that affected East Bay Island and other colonies along the Hudson Strait (Iverson *et al.* 2016). This shift in proportion of eiders overwintering in Greenland versus Newfoundland may be influenced by spill over from the Greenland population. Alternatively, the changes in proportions may be more reflective of the relatively small sample size (n = 25) from the satellite tagging study compared to this study. This emphasizes the importance of using isotopic methods to track changes in population demographics and migration patterns. This could be especially important as climate change pushes animals out of traditional ranges or to become non-migratory; new isotopic signatures in breeding individuals could indicate novel overwintering sites and direct future winter sampling.

2.4.1 Implications for future applications

Using stable isotope analysis as an indirect tracking method, rather than direct tracking methods using instruments (e.g., satellite or GPS telemetry, geolocation, etc.), has the potential to provide meaningful location data while also allowing for larger sample sizes at substantially lower costs and simultaneously reducing the impact on the individual animals being tracked (Bowlin et al. 2010). Although the use of stable isotopes to infer

overwintering origin is quite common in terrestrial species (Rubenstein & Hobson 2004; Hobson *et al.* 2004, 2012b; Yerkes *et al.* 2008; Haché *et al.* 2012; Miller *et al.* 2012; Popa-Lisseanu *et al.* 2012; Garcia-Perez & Hobson 2014), individuals are often assigned by comparing isotope signatures in feathers to established carbon- or hydrogen-based isoscapes. It is difficult to tailor this method for use in marine species, however, the more infrequently used approach of sampling individuals from their overwintering and breeding sites, then testing different tissues and isotope combinations can be applied across taxa. Alternatively, I suggest that this method could also be easily adapted to infer breeding areas to flocks of overwintering birds, if samples are collected at appropriate times.

Oppel & Powell (2008) provide an example of using head feathers from king eiders in the western Arctic to assign individuals to overwintering areas. In marine birds where specific feather molt schedules may be unknown and in those species that undergo a near-complete full-body molt in the fall (Goudie *et al.* 2000) claws are well suited tissues to sample to reflect overwintering signatures in both winter- and pre-breeding-caught individuals. Claws have a growth rate that is useful for both time-periods, sampling is entirely non-invasive and will not impact flight like sampling flight feathers may (Swaddle *et al.* 1996), and they are easily collectable and simple to include in sampling protocols. In addition, this method allows one to avoid having to use discrimination factors, which are required when comparing different tissues, and therefore reduces the ambiguity in my location estimations (Bearhop *et al.* 2002; Bond & Diamond 2011). The use of 13-carbon in claws also simplifies the technique, as studies looking at 2-hydrogen need to consider the exchange of water within tissues (Hobson *et al.* 1999).

For studies on marine birds especially, which are often long-lived and faithful to both their overwintering and breeding sites (Robertson & Cooke 1999; Mallory *et al.* 2010a; Reed *et al.* 2015), I suggest that researchers combine their tracking studies with analysis of multiple stable isotopes so that they can ground truth stable isotope tracking methods simultaneously with telemetry tracking. In addition, researchers could use isotopes as a way of increasing their sample size to test whether the individuals they have specifically tagged are representative of the overall population. This would be especially useful in population delineation studies, a focus in marine bird research and amongst wildlife managers (Gilliland *et al.* 2009; Boyd *et al.* 2015). The only drawback of the aforementioned tracking study of this breeding colony (Mosbech *et al.* 2006) is that the eiders were not sampled for stable isotopes in conjunction with satellite tracking, and so I could not completely validate my winter assignment for arriving eiders. Ideally tracked birds would be sampled for stable isotope analysis to test for differences between isotopic signatures in relation to where the tracking devices indicate they spend the period of time of interest to validate isotopic approaches. Of course, this method requires that individuals are faithful to overwintering areas.

I recognize that isotopic baselines can have some isotope-specific temporal variation (Rubenstein & Hobson 2004; Bowen 2010). For instance, atmospheric $\delta^{13}C$ can fluctuate by as much as 0.75 ‰ in higher latitudes within one year and has shown a total decrease of 0.25 to 0.5 ‰ over a ten year period depending on the latitude (Bowen 2010) as a result of ever increasing CO^2 emissions (West *et al.* 2006). In turn, these fluctuations in atmospheric $\delta^{13}C$ can affect patterns reflected in the oceans. Consequently, I suggest

future studies aiming to assign individuals to an overwintering location re-sample reference winter locations at least every five to ten years to account for these variations.

In summary, following my study design, I recommend that researchers test the effectiveness of several stable isotopes to determine the best combination for their system. Nevertheless, using these types of isotopic assignment methods, could replace costly (in terms of funds and impacts on individual birds) device-based tracking studies as a viable solution for increasing sample size, delineating populations and monitoring more populations or species simultaneously. Indeed, these methods should be readily transferable to other life-history periods (i.e. breeding locations of groups of wintering birds). Further exploratory studies are needed to investigate the feasibility with pelagic seabirds; however, these methods are applicable to other sea ducks and more broadly to coastally feeding seabirds and shorebirds, and other marine animals.

The work presented in Chapter Two also appears in: Steenweg RJ, Crossin GT, Kyser TK, Merkel FR, Gilchrist HG, Hennin HL, Robertson GJ, Provencher JF, Mills Flemming J and Love OP (2017) Stable isotopes can be used to infer the overwintering locations of prebreeding marine birds in the Canadian Arctic. Ecol Evol 1–11. doi: 10.1002/ece3.3410

Student Contribution to Paper: RJS collected and processed samples from all field sites, conducted isotope analyses and data analyses, and wrote the manuscript. GTC helped with data analyses and manuscript writing. TKK provided facilities for isotope analyses and provided feedback for interpretation of the data. FRM collected samples from GRLD and helped with development of ideas. HGG collected samples from EBI and helped with

conception of ideas. HLH collected samples from EBI, helped with interpretation of data, and helped with sample processing. GJR collected and provided samples from NFLD. JFP collected and provided samples from NFLD. JMF provided guidance for data analyses and interpretation. OPL collected samples from EBI, came up with initial idea for work, and helped extensively with manuscript writing. All authors critically revised and approved the final version of the manuscript submitted.

Table 2. 1. Summary of samples collected from each location, the time period that tissues will reflect isotopically, and samples sizes (N).

Location	Tissue	Date of Collection	Time Period Reflected	N	N Males	N Females
Newfoundland	claws	April 2016	winter	24	8	16
	whole blood	Dec 2013 to Jan 2014	winter	35	30	5
Nuuk, Greenland	claws	April 2014	winter	33	6	29
	whole blood	April 2014	late winter	34	6	29
East Bay Island	claws	June to July 2014	winter	109	0	109
	red blood cells	June to July 2014	spring migration	108	0	108
	claws	June to July 2015	winter	115	43	72
	red blood cells	June to July 2015	spring migration	125	51	74

Table 2. 2. Two-way ANOVA model results for isotope signatures in eiders sampled from the two overwintering areas for both whole blood and claws. Significant relationships are bolded.

Tissue	Isotope	Means ‰ (SD)		df	F value	Overall P	Location P	Sex P
		Nuuk, Greenland	Newfound-land					
Blood	$\delta^{13}C$	-18.55 (0.90)	-20.05 (0.51)	2, 67	35.89	**<0.001**	**<0.001**	0.792
	$\delta^{15}N$	10.19 (0.43)	10.88 (0.60)	2, 67	15.64	**<0.001**	**<0.001**	0.709
	$\delta^{2}H$	-78.85 (6.91)	-71.26 (7.33)	2, 67	11.6	**<0.001**	**<0.001**	0.171
Claws	$\delta^{13}C$	-18.12 (0.60)	-20.55 (0.58)	2, 55	119.6	**<0.001**	**<0.001**	0.632
	$\delta^{15}N$	12.98 (0.77)	13.14 (0.52)	2, 55	0.352	0.704	0.414	0.987
	$\delta^{2}H$	-49.97 (9.27)	-50.33 (7.04)	2, 55	0.221	0.803	0.785	0.522

Table 2. 3. Summary of stable isotope signatures in eider blood and claws from individuals sampled during the pre-breeding period at East Bay Island.

		2014			2015		
Tissue	Isotope	Means δ‰ (SD)	Min δ‰, Max δ‰	N	Means δ‰ (SD)	Min δ‰, Max δ‰	N
Blood	$\delta^{13}C$	-18.17 (1.59)	-19.77, -12.91	108	-18.87 (0.52)	-20.28, -17.32	125
	$\delta^{15}N$	12.38 (0.66)	10.60, 14.25	108	12.91 (0.92)	10.92, 15.21	125
	$\delta^{2}H$	-80.02 (6.61)	-94.99, -64.93	107	-86.10 (6.57)	-101.23, -70.06	121
Claws	$\delta^{13}C$	-17.92 (1.46)	-20.43, -13.91	109	-18.13 (0.78)	-20.28, -15.68	115
	$\delta^{15}N$	12.90 (0.73)	11.47, 14.69	109	13.20 (1.07)	10.93, 16.31	115
	$\delta^{2}H$	-42.30 (10.73)	-63.11, -11.75	106	-45.76 (9.72)	-67.70, -20.83	112

Table 2. 4. Assessment of strength of each k-means cluster analysis using total variance explained and number of misclassified winter birds. Those resulting in the fewest misclassified winter birds are bolded. Final centroid refers to the mean (or center) of the clusters formed by the k-means cluster analysis, which differ from the starting centroids used to guide the beginning of the analysis.

Year	K	Stable Isotopes Included	Between Sum of Squares	Total Sum of Squares	Total Variance Explained (%)	Final centroids (δ^{13}C ‰, δ^{15}N ‰, δ^{2}H ‰)			Number of Misclassified Winter Birds
						1	2	3	
2014	3	δ^{13}C, δ^{15}N, δ^{2}H	14770.32	18794.45	78.59	-18.83, 12.74, -56.70	-18.50, 12.87, -45.54	-17.56, 13.24, -32.07	29
	3	δ^{13}C, δ^{15}N	322.26	459.60	70.16	-18.15, 12.90	-20.20, 13.02	-14.81, 13.04	0
	3	**δ^{13}C**	321.79	382.35	**84.16**	-18.16	-20.23	-14.81	**0**
	2	δ^{13}C	196.29	382.35	51.34	-17.24	-19.45	NA	8
2015	2	δ^{13}C, δ^{15}N, δ^{2}H	10726.89	15361.29	69.83	-18.51, 12.88, -53.24	-18.41, 13.61, -36.42	NA	28
	2	δ^{13}C, δ^{15}N	135.92	362.01	37.54	-17.93, 13.23	-19.93, 12.90	NA	3
	2	**δ^{13}C**	133.88	208.94	**64.08**	-17.97	-20.09	NA	**2**
	3	δ^{13}C	176.74	208.94	84.59	-18.65	-20.50	-17.51	16

Figure 2. 1. A pair of common eiders on East Bay Island, Nunavut (Photo: R. Steenweg).

Figure 2. 2. Map of eider migration from the breeding colony at East Bay Island to overwintering areas in Greenland and Newfoundland. Winter sampling sites are denoted with red stars and East Bay Island with an orange star.

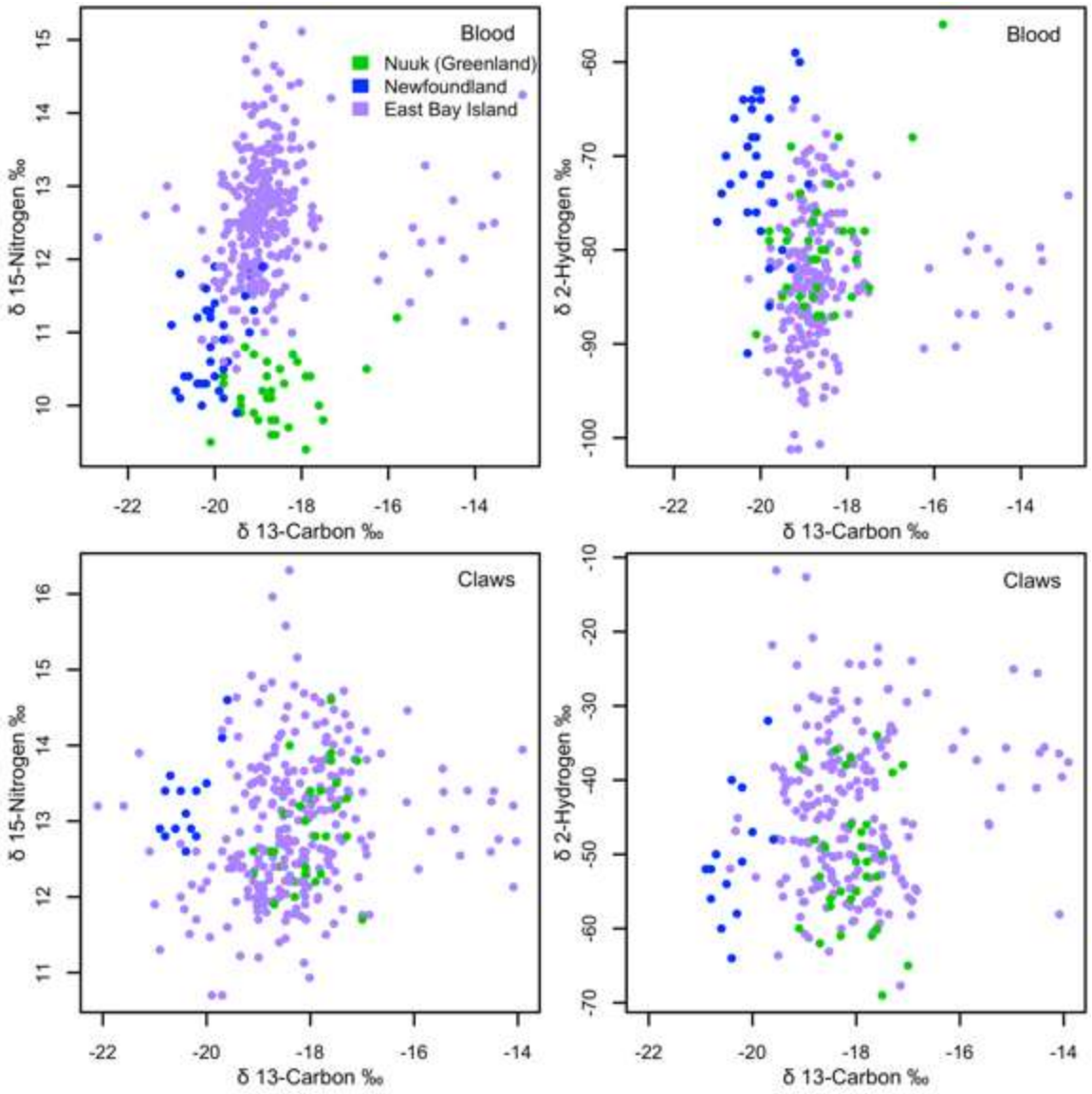

Figure 2. 3. Scatterplot of stable isotope data for winter (Nuuk, Greenland and Newfoundland)- and pre-breeding (East Bay Island)-caught eiders for both blood and claw tissues.

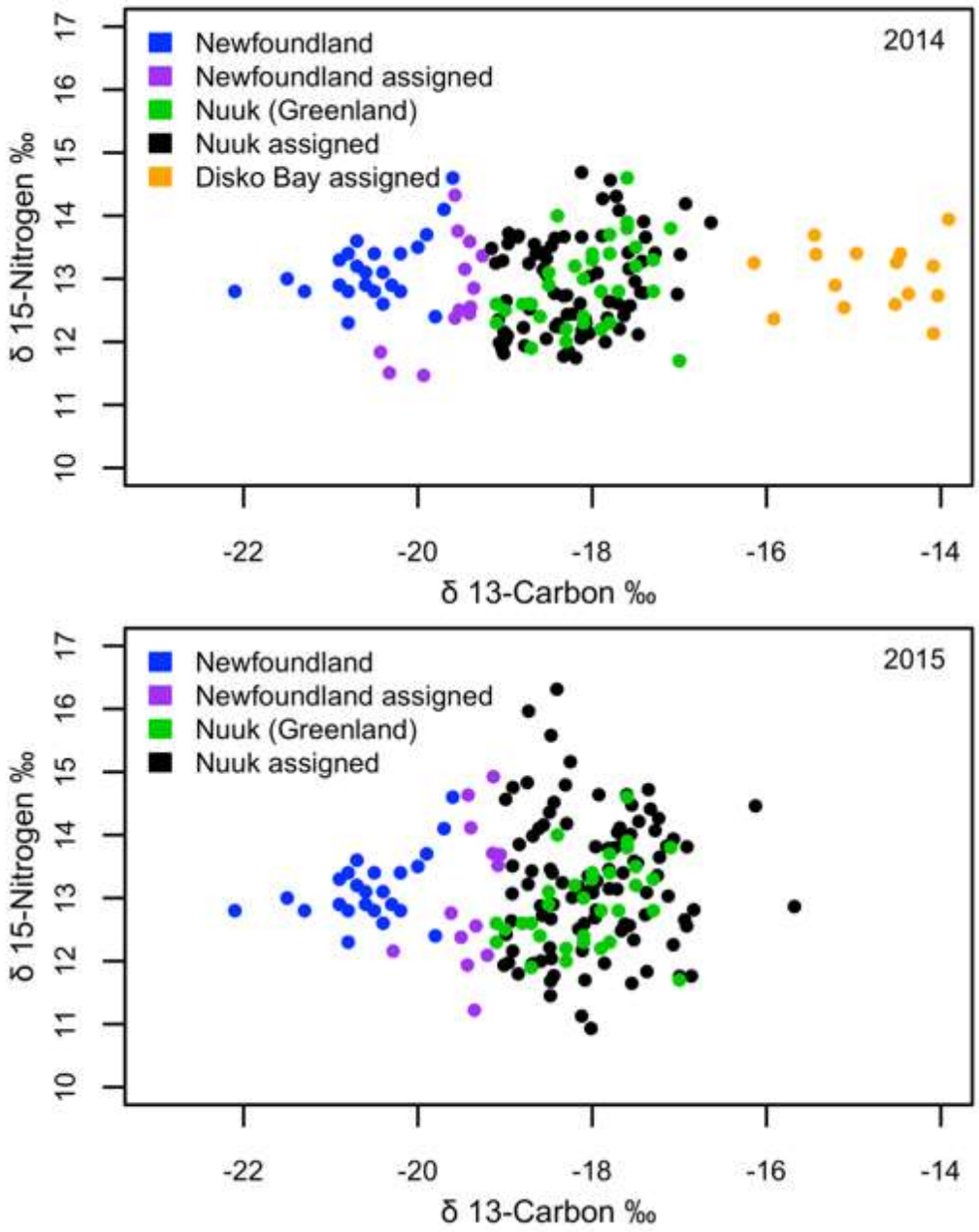

Figure 2. 4. Scatterplot of the results of one-dimensional k-means cluster analysis using the stable isotopes of carbon found in claws obtained from eiders during the pre-breeding periods in 2014 and 2015 and from their overwintering sites in Newfoundland, Canada and Nuuk, Greenland. 'Assigned' refers to pre-breeding eiders assigned to their respective overwintering areas. Results are plotted against nitrogen for ease of visualization.

Chapter 3: Stable Isotopes of Carbon Reveal Flexible Pairing Strategies in A Migratory Arctic Bird

Abstract

Many birds change their partners every year and pairing may occur before arrival on the breeding grounds. Early pairing strategies can benefit mates by strengthening pair-bonds and increasing the rate of pre-breeding resource acquisition, leading to increased reproductive output and success, especially for migratory species breeding in seasonally-constrained environments like the Arctic. Despite the theorized and documented advantages of early pairing, fairly little is known about pairing phenology in many species. Here, I test the use of a stable isotope (carbon $\delta^{13}C$) method to assign geographic origin of paired birds to examine pairing phenology in Arctic-breeding common eiders *Somateria mollissima borealis*. During two consecutive years, I captured paired individuals upon their arrival at breeding grounds approximately 2-3 weeks before laying. Pairs with similar $\delta^{13}C$ in claws indicates they paired during winter while similar blood values (with no similarity in claws) would reveal pairs formed much later during the pre-breeding period near or on the breeding grounds. While a large proportion of pairs (43%) appeared to pair on wintering grounds, an almost equal number (52%) likely paired within 1-month prior to arrival on the breeding grounds. The remaining 5% did not have an obvious pairing time. Despite this variability in pairing phenology, I found no significant differences in body mass between females or males which paired in winter or spring. In the year characterized with more challenging winter conditions, pairs formed in spring tended to have a higher breeding propensity than those formed in winter, although there were no detectable links to body

mass. Delaying pairing until spring may be advantageous for Arctic-breeding eiders, although a specific mechanism is unknown. Future research focusing on the energetic costs and benefits for male eiders during these periods would help further understand pairing phenology and potential impacts of males on female breeding decisions.

3.1 Introduction

The ability for individuals to optimally time life history events can be a key driver of variation in fitness. For example, in seasonally-breeding birds, migration and reproduction are often timed to match peak resource and territory availability on the breeding grounds (Drent *et al.* 2003; Descamps *et al.* 2011), with the aim of ensuring an optimal laying date that maximizes the probability of offspring survival (Love *et al.* 2010; Rockwell *et al.* 2012; Hennin *et al.* 2016a). An important potential driver of (or constraint on) variation in the phenology of seasonal breeding is the location of mate pairing given its potential influence on the timing of arrival at breeding grounds during spring migration, and by extension the timing of laying with correlated effects on clutch size, hatching dates, and fledging dates (Spurr & Milne 1976; Bluhm 1988; Hennin *et al.* 2018).

Within the waterfowl family (*Anatidae*), a key factor affecting the timing of pair formation is whether pairing provides some discernible benefit to a mate (e.g. by conferring a competitive advantage to resource accrual and foraging success), or by providing mate defense benefits to the guarding sex (Fowler 1995; Robertson *et al.* 1998). Since waterfowl populations often have male-biased sex ratios (Rohwer & Anderson 1988), females can afford to be choosey in their selection of a mate (Rodway 2007) and sexual selection is expected to favour pair formation as early as possible before breeding (Bluhm 1988;

Rohwer & Anderson 1988). As such, in a number of species, pair formation can even occur as early as immediately following the previous breeding season (e.g., American widgeon *Anas Americana*, Mini *et al*. 2014; Harlequin ducks *Histrionicus histrionicus*, Robertson *et al*. 1998). Although the phenology of seasonal pair formation varies significantly across species and can be driven by differences in life history, breeding strategies, and levels of parental investment (Rohwer & Anderson 1988), fairly little is known in regards to the influence of pairing date and location on downstream performance or fitness traits. Males of most waterfowl species typically do not engage in incubation or offspring-rearing behaviours, they can nonetheless invest during pre-breeding *via* the defense of their female mates which maximizes pre-laying foraging success (Rodway 2007). Given the known influence of pre-breeding body mass on fitness proxies such as lay date, clutch size, and hatching date (Descamps *et al.* 2011; Hennin *et al.* 2016a, 2018), the timing of pairing, and how it influences breeding investment, may be key to both male and female fitness. However, in highly seasonal environments such as the Arctic, individuals might be reluctant to pair in winter due to potentially greater resource limitations and because there is considerable variation in ultimate overwintering destination, the choice of which is linked to individual pre-migratory condition (Bottitta *et al.* 2003). Unfortunately, studying pairing phenology in any seasonally-breeding seabird system remains difficult since individuals tend to overwinter in remote locations and spend the pre-breeding period offshore (Merkel *et al.* 2006), limiting the possibility of directly observing winter and spring behaviour amongst males and females.

Here I apply a recently developed method for determining the overwinter location of common eiders, based on stable isotope analysis (Steenweg *et al.* 2017), to examine the

phenology of pair formation and its subsequent downstream effects on breeding decisions in Arctic-nesting common eiders *Somateria mollissima borealis*. I examine these questions within an Arctic-breeding colony (East Bay Island, Nunavut, Canada), which uniquely enabled us to simultaneously capture both mates prior to breeding, assess their wintering location and arrival body mass, and then later observe breeding behaviours (breeding propensity and lay date) and reproductive investment (clutch size). Previous remote tracking of eiders breeding at this colony revealed two primary overwinter locations: Southwest Greenland and Newfoundland, Canada (Mosbech *et al.* 2006), characterized by different winter climatological conditions (Descamps *et al.* 2010). Common eiders arriving at the breeding colony at East Bay have isotopic values in their claw tips that indicate their wintering grounds in either Newfoundland or Western Greenland, 90-110 days before sampling (Steenweg *et al.* 2017). Stable isotope ratios in different tissues reflect assimilated diet based on location, diet, and the growth or turnover rate specific to each tissue (Hobson 1999; Clark *et al.* 2006). Values in red blood cells (herein: blood), which has a quicker turnover rate, reflect their more recent spring-staging period 30-40 days before sampling (Oppel & Powell 2010; Steenweg *et al.* 2017). I aimed to use the tissue-specific turnover rates of stable isotopes in claws and red blood cells to examine whether eiders form their pairs in the winter or spring. A strong correlation between paired members in claw isotopic values would suggest pairing occurred in winter, while a strong correlation in blood (and corresponding weak correlation in claws) would suggest pairing in spring.

3.2 Methods

3.2.1 Study area and sample collection

Common eiders (hereafter eiders) breeding on East Bay Island, (Migratory Bird Sanctuary, Nunavut, Canada, 64°02'N, 81°47'W) overwinter off the coast of either Newfoundland, Canada, or Western Greenland (Mosbech *et al.* 2006) and arrive to the staging areas in the spring in late May to early June; they arrive to the breeding colony in early June, before laying in late-June (Jean-Gagnon *et al.* 2018). During the spring staging and pre-breeding periods in mid-May to late-June female eiders must forage locally to both invest in reproduction (Sénéchal *et al.* 2011b; Hennin *et al.* 2015), and to obtain sufficient energetic stores to maintain a fasting incubation (Bottitta *et al.* 2003). I captured female eiders and their paired mates (given that male eiders closely follow their female mate up to and including the end of the egg-laying period; Hario and Hollmén 2004) using flight nets during the pre-breeding period from 19-June to 04-July-2015 (n = 40 pairs) and 21-June to 30-June-2016 (n = 29 pairs). I obtained both blood and claw samples from 65 of these pairs (2015: 38 pairs; 2016: 27 pairs, 130 individuals total). I collected blood samples from the tarsal vein using a heparinized 23-gauge, 1 inch, thin wall needle and syringe, stored samples in a heparinized Eppendorf tube and kept cool. Blood was centrifuged at 10,000 rpm for 10 min, and red blood samples were separated from plasma and stored at -80°C until further analysis (Hennin *et al.* 2015). I collected claw samples by clipping the distal 2 mm from the middle toe of the left foot and stored in a small paper coin envelope (Steenweg *et al.* 2017). Prior to release, all individuals were weighed, measured and banded with metal and alpha-numeric darvic bands. In addition, females were outfitted with uniquely colored and shaped temporary plastic nasal tags attached through their nostrils with UV-degradable monofilament. Unique nasal tags enable the assignment of laying date

and breeding investment via observation of individuals via multiple permanent blinds and spotting scopes (Descamps *et al.* 2009; Love *et al.* 2010).

3.2.2 Assignment of wintering location using stable isotopes

$\delta^{13}C$ in claws reflect approximately 90-110 days prior to sampling (Hopkins III *et al.* 2013). Recently stable isotopes in claws has been used successfully to infer the winter location of individuals (blackcaps: Rolshausen et al. 2010; common eiders: Steenweg et al. 2017). Specifically, eiders have been successfully assigned to their wintering locations upon arrival at their breeding sites by comparing stable isotope values in claws collected from wintering birds to those collected from eiders arriving to their breeding grounds (Steenweg et al. 2017). In addition, since $\delta^{13}C$ in red blood cells reflects approximately 30-40 days prior to sampling (Hahn *et al.* 2012), they can be used to infer a spring location in my study since they coincide with the spring-staging periods (Steenweg et al. 2017). Here I use stable isotopes in claws and blood in arriving eiders during the pre-breeding period to reflect values from their winter and spring periods, respectively.

Blood samples were freeze-dried for 30 hours and lipid-extracted to reduce the impact of lipids on $\delta^{13}C$ values and surface oils were removed from claw samples. To extract lipids and remove surface oils from blood and claw samples, 2:1 chloroform:methanol solution was added to vials containing the samples, vortexed for 15 s and soaked for 24 hours. Vials were centrifuged at 10,000 rpm for 10 min, the supernatant was then siphoned off with a pipette, samples were rinsed again with the chloroform:methanol solution, vortexed for 15 s, centrifuged and the remaining supernatant was again siphoned. Samples were then dried under a fume-hood for 24 hours. Subsamples of blood and claws were weighed to 0.30-0.50 mg and folded into a tin capsule.

Stable isotope analysis was conducted at the Queen's Facility for Isotope Research, Queen's University (Kingston, Ontario) using a Costech ECS4010 coupled to a DELTA^{plus}XP Continuous-Flow Isotope Ratio Mass Spectrometer. All stable isotope results are reported within accuracy of 0.1‰ based on analyses of the international standard Vienna Pee Dee Belemnite and in house keratin (COW1: -13.17‰ ±0.21, UC1: -25.7‰ ±0.14) run alternately every five samples. Duplicates were run every nine samples with an accuracy 0.2‰. All $^{13}C/^{12}C$ are reported in delta notation (δ) in parts per mil (‰).

3.2.3 Data analysis

I used two methods to determine the relative phenology of pair-formation in eiders. First, I used linear regression analyses to investigate the strength of the relationship between the stable isotope values of paired females and males using claw (winter) and blood (spring) samples. Secondly, to identify overwintering locations, I used a k-means cluster analysis (Garcia-Perez & Hobson 2014; Pomerleau *et al.* 2014) of $\delta^{13}C$ obtained from claws of pre-breeders, and set the starting centroid values to those obtained from eiders captured and sampled at each overwintering location (Greenland and Newfoundland; Steenweg *et al.* 2017). I then calculated the 'ordinal distance' between the $\delta^{13}C$ values in claws (winter) and blood (spring) by taking the absolute value of the numerical difference between isotopic values in each pair. I assumed individuals overwintering or migrating in proximity to each other will have similar isotopic values. As a proxy for similarity, I use the standard deviation of $\delta^{13}C$ values acquired from previously published values of the claw (0.59‰) and blood (0.86‰) samples obtained from individuals in their overwintering areas (Steenweg *et al.* 2017; this study). $\delta^{13}C$ claws values of paired individuals within one standard deviation (s.d.) were considered

isotopically similar and I assumed those pairs wintered in the same location. If $\delta^{13}C$ claw values from paired individuals differed by more than one s.d. I considered those individuals to winter in separate locations even if their general wintering area (inferred from $\delta^{13}C$ values) was similar. Although eiders breeding at East Bay Island generally overwinter in either Newfoundland or Western Greenland, they may nevertheless cover large areas within each region (Mosbech *et al.* 2006). Therefore, individuals of the same pair may winter in the same general region but spend time in geographically distant portions of that region. Paired individuals could potentially experience different ecological conditions and consume different prey items with dissimilar isotopic values. There is variability in stable isotope values in coastal habitats. Individuals with dissimilar isotopic values are unlikely to have been geographically close together even if they were foraging on similar prey, as the stable isotopes of carbon change with latitude (Graham *et al.* 2010). In addition, I conducted a sensitivity analysis by considering different threshold values with ordinal distances of 0.5 and 1.5 times the standard deviation. I then used a linear model to examine relationships among eiders who paired in the winter (claw $\delta^{13}C$ values) and during spring-staging (blood $\delta^{13}C$ values). I only included pairs in these analyses with claw or blood $\delta^{13}C$ values within the ordinal distance window as I could not be confident in the timing of pairing of the others.

Finally, to examine links between pairing phenology, female and male body mass and reproductive parameters I used logistic regression analyses to examine relationships between the timing of pairing (spring versus winter), male and female arrival body mass, and included year. I subsequently used logistic regression analyses to examine the role that the timing of pairing and female body mass play in predicting the subsequent breeding

propensity (females re-sighted breeding in the colony). I used linear models to examine the relationship between the timing of pairing and the interval between arrival to the colony and laying (pre-laying interval) and lay date of female eiders. Since body mass, timing of arrival and seasonal conditions are known to collectively impact reproductive decisions (Descamps *et al.* 2011; Harms *et al.* 2015; Hennin *et al.* 2016b, 2018), my null model consisted of body mass, capture date and year. I tested for additional effects of timing of pairing and interaction with year. If a significant interaction was found between year and pairing phenology, I broke down the interaction and provided the model summary for each year separately. All data analyses were conducted using R version 3.4.3 (2017-11-30) using packages MASS package (Venables & Ripley 2002) and cluster (Maechler *et al.* 2016). All R code used for analyses is available in Appendix 3.

3.3 Results

3.3.1 Patterns in pairing phenology

Linear models of the $\delta^{13}C$ values in claws and blood indicated that pairs had more similar isotopic values during the spring ($R = 0.66$, $F_{(1,62)} = 50.25$, $P < 0.001$) compared to the winter ($R = 0.35$, $F_{(1,67)} = 10.79$, $P = 0.001$, Figure 1). Linear models of $\delta^{13}C$ values between individuals that paired in the winter and in the spring were statistically significant for 0.5, 1, and 1.5 times the standard deviation ($P < 0.001$ for all, Table 1) indicating that all of the three standard deviation windows resulted in significant relationships between paired individuals. The strength of correlation was highest for 0.5 times the standard deviation (winter: 0.98, spring: 0.83) compared to 1 times the standard deviation (winter: 0.88, spring: 0.71, Figure 2) and 1.5 times the standard deviation (winter: 0.77, spring:

0.70) indicating that 0.5 times the standard deviation shows the strongest relationship, but that any of the three standard deviation windows could be used. Using 0.5, 1, and 1.5 times the standard deviation resulted in 11, 3, and 1 pairs without a pairing time, respectively. Because using 0.5 times the standard deviation resulted in more pairs without a pairing time, and decreased sample size, I used 1 times the standard deviation for subsequent analyses.

To determine the timing of pairing, I used an ordinal distance of 1 times the standard deviation in $\delta^{13}C$ between pairs (Figure 3). Using this ordinal distance, 43% of pairs were isotopically similar on the wintering grounds (n = 17 and 11 in 2015 and 2016, respectively), and 52% of the pairs were isotopically similar only at the spring staging area (n = 18 and 16 in 2015 and 2016, respectively). Only three pairs total (i.e., 5%; n = 3 in 2015, n = 0 in 2016) show no isotopic similarity in either period and were removed from subsequent analyses.

3.3.2 Links between pairing phenology and breeding

I estimated that overall 52% (n = 34) of the sampled eiders paired in the spring, and 43% (n = 28) paired in the winter. Of those, in 2015, 63% (n = 10) of the breeding eiders paired in the spring and in 2016, 50% (n = 4) of breeding birds paired in the spring (Table 2). Logistic regressions indicated that the timing of pairing was not significantly related to female or male body mass (female: timing of pairing: P = 0.52, year: P= < 0.001, df = 2, 58; male: timing of pairing: P = 0.54, year: P = 0.003, df = 2, 58). The timing of pairing had a near-significant relationship with breeding propensity, and female body mass significantly predicted breeding propensity in 2015 (P = 0.07 and P = 0.01, respectively, df = 3, 30; Table 3), but neither were significant in 2016 (P = 0.53, P = 0.63, respectively,

df = 3, 23). However, the timing of pairing showed no significant link to the pre-laying interval or lay date (Table 4).

3.4 Discussion

Using a recently-validated isotopic method for determining the overwinter location of eiders (Steenweg *et al.* 2017) I show that eiders breeding at East Bay Island in the Canadian Arctic exhibit variable pairing strategies, with some birds pairing on the overwintering grounds in Western Greenland and Newfoundland, others during spring staging and very few individuals with unknown timing of pairing, potentially having paired on the breeding grounds. Of the eiders with conclusive pairing phenology, 55% of pairs adopted a spring-pairing strategy (2015: 51%, 2016: 59%), while 45% (2015: 49%, 2016: 41%) adopted a winter-pairing strategy. These findings provide new information about the breeding biology of Arctic breeding eiders, elucidates mixed pairing strategies amongst breeding eiders, and suggests that variation in pairing strategies could have downstream impacts on breeding.

3.4.1 Patterns of pairing phenology in northern Common eiders

Previously, little was known about the timing and variability of pair-formation in Arctic eiders, other than they were expected to pair in the spring (Goudie *et al.* 2000). Since many populations of eiders winter and breed in remote areas, it is not possible to directly observe when pairing occurs compared to in other duck species (Heitmeyer 1995). My results indicate that it is 1) possible to infer the timing of pairing using stable isotope analysis and 2) Arctic breeding eiders appear to employ both spring and winter pairing strategies.

Differences in pairing strategies may be driven by age or experience. Within eiders in Scotland, younger females formed pairs later in the winter compared to older females which paired in the fall (Spurr & Milne 1976). Later-formed pairs typically laid later in the summer or not at all, whereas earlier formed pairs laid throughout the season and were more successful. For this temperate population, later pairings were mostly made up of younger birds and females pairing early probably benefited from male assisted winter foraging compared to unpaired females (Spurr & Milne 1976). Similarly, the differences in pairing strategies in East Bay eiders could be driven by age or experience – but with few known-age birds within this study colony, it is not possible to answer this question – or the benefits of winter pairing may differ between years.

3.4.2 Links between pairing location/phenology and breeding decisions

The skewed sex ratios (biased toward males) of most waterfowl species and populations favours female choice (Blums & Mednis 1996) where benefits to the female outweigh the costs to the male (Rohwer & Anderson 1988). For example, if paired female eiders have better access to limited resources in winter when paired, pair formation should occur as soon as possible (early winter, Bluhm 1988). My data suggests that eiders were able to pair during winter, and nearly half of the birds that I studied did (Table 2). Previous work has shown that North Atlantic Oscillation (NAO) values during the winter are associated with survival in Arctic breeding eiders; common eider populations have higher survival and body mass during years with a lower NAO (Guéry *et al.* 2017). The winter of 2015 was characterized as a harsher winter in Greenland with a more positive North Atlantic Oscillation value, with higher incidences of storms versus 2016 (NOAA 2017). In 2015, females pairing in spring tended to be more likely to breed than females that paired

in winter. Although this trend was only near-significant ($p = 0.07$), 55% of the spring-paired birds bred that year, compared to 35% of the winter-paired birds. In 2016 where the NAO index indicated lower incidences of storms, the proportions were reversed; 25% of the spring paired birds bred compared to 36% of the winter paired birds, suggesting some disadvantage to pairing in harsh winters.

Newfoundland and Western Greenland are both influenced by the NAO, but in opposite ways (Descamps *et al.* 2010; NOAA 2018). In years when the NAO is in a strong positive phase, such as in 2015 (1.17 average between February and April; NOAA 2017), there tends to be below-average temperatures in Greenland with high incidences of storms, and above-average temperatures in Newfoundland, with most of the eiders wintering in Greenland (90%, Steenweg *et al.* 2017). These cold temperatures and frequent winter storms may have constrained eiders ability to optimally thermoregulate, contributing to declining body condition through the winter (Merkel *et al.* 2006). In any given year, the body mass of eiders returning to East Bay for the spring staging and pre-breeding period can be highly variable (body mass ranges from 1700 to 2600 g; Descamps *et al.* 2011), which is a testament to the energetic demands that Arctic winters and subsequent migration can impose. However, my data suggests that the timing of pairing does not appear to directly link to a female's body mass at arrival to the breeding grounds, the interval between arrival and laying, nor lay date. The differences in pairing phenology between 2015 and 2016 may be driven by differences in winter climate and how individual eiders deal with it. Given that eiders wintering in Greenland tended to pair more in springtime, and that pairing in spring nearly increased the likelihood of breeding that year, I can speculate that

pair formation in winter is likely detrimental to winter survival and/or foraging success during such conditions, and that perhaps eiders are better off unpaired in harsh winters.

Further, because Arctic winters may be harsh and stormy, in some years it may be costly for males to invest energy into mate defense, eiders may require calm conditions in order to engage in courtship behaviors, and the benefits of defense may be low. In geese, females that are paired in the winter have more foraging opportunities to facilitate the accumulation of energy stores (Choinière & Gauthier 1995). Conversely, this may not be possible for diving ducks as the defense of underwater resources is near impossible. It has been suggested that female migratory waterfowl in the north may not have an advantage to gain more nutrition when paired during the winter (Rodway 2007). In addition, the potential for mate loss due to accidental separation during migration could also affect the male's readiness to pair early. It appears that sometimes it may not be advantageous for pairs to form until their arrival to the spring-staging areas. Given this high variability in individual body condition across years, it may be strategic for eiders to delay pair formation until spring in years with harsh winters, which would allow them to assess the condition of potential mates who survived the winter, rather than committing to one individual who could be in poor condition by the end of migration. In contrast to 2015, the NAO in 2016 was in a weakly positive phase (0.90; NOAA 2017), which resulted in milder conditions in both Greenland and Newfoundland. In turn, relatively more eiders may have opted for Newfoundland, which suggests either competitive exclusion in Greenland when conditions are milder, and/or previously unavailable foraging opportunities in Newfoundland. Whatever the case, the consequence of the 2016 winter saw the formation of pairs in both winter and spring, which were equally as likely to breed in that year.

3.4.3 Implications of pairing decisions (in a changing world)

In eiders, only females are philopatric to their breeding sites (Robertson & Gilchrist 1998; Mckinnon *et al.* 2006; Lehikoinen *et al.* 2008). The demographics of the East Bay eider population are changing; the population of East Bay eiders has decreased from 8000 nesting pairs in 2006 to 1300 pairs in 2016 (H.G. Gilchrist, unpublished data), due to an avian cholera outbreak (Iverson *et al.* 2016) and several years of sustained polar bear predation of nests (Iverson *et al.* 2014; Dey *et al.* 2017), reducing local recruitment into the population. Currently about 90% of the breeding population overwinters in Western Greenland (Steenweg *et al.* 2017), whereas in early 2000 approximately 60% of the population were overwintering there (Mosbech *et al.* 2006). While recruitment into the breeding population is low in the East Bay colony, eider populations in Western Greenland are increasing by about 12% per year (Merkel 2010), and most Greenland-breeding eiders also overwinter in Greenland (Mosbech et el. 2006). Since male-male competition in ducks tends to be high due to their skewed sex ratios, winter pair formation, with males following their female mates to the breeding colony, may be a way in which the East Bay colony population is able to persist even though there has been little to no natal breeding recruitment. Genetic tools could be used to assess the demographic changes within the colony to determine how the colony continues to persist following multiple years of egg loss and whether the timing of pair formation has changed as a result of these natural perturbations.

Furthermore, the differences between years indicate that there may be carry-over effects of winter condition to the timing of pairing and subsequently to breeding decisions. The earlier eiders lay, the more likely they are to be successful (Descamps *et al.* 2011;

Hennin *et al.* 2016a) and lay date is mediated by the ability for females to accrue energy stores (Hennin *et al.* 2015), and therefore females may need to be paired by a certain date in order for males to defend them while foraging in spring. The costs and benefits of mate-defense during either period are thus far unknown. Further studies investigating the cost-benefit trade-offs of paired individuals should elucidate how the timing of pairing is beneficial to both male and female eiders.

The work presented in in Chapter Three also appears in: Steenweg RJ, Legagneux P, Crossin GT, Gilchrist HG, Kyser TK and Love OP (2019) Stable isotopes of carbon reveal flexible pairing strategies in a migratory Arctic bird. J Ornithol 160:607–616. doi: 10.1007/s10336-019-01661-y

Student Contribution to Paper: RJS collected and processed samples from all field sites, conducted isotope analyses and data analyses, and wrote the manuscript. PL collected samples from EBI, helped refine ideas and with data analyses. GTC helped with manuscript writing. TKK provided facilities for stable isotope analyses. OPL collected samples from EBI and helped refine ideas. All authors critically revised and approved the final version of the manuscript, with the exception of TKK who had passed away by that time.

Table 3. 1. Results of sensitivity analysis comparing the relationship in stable isotope values between paired male and female eiders. These relationships were tested for 1, 0.5, and 1.5 times the standard deviation (ordinal distance). Significant relationships are bolded.

Time period paired	Ordinal distance	Variable	Estimate (SE)	t	F (df)	p	R
Winter	0.5	Intercept	-1.03 (0.87)	-1.18	398.6 (1, 13)	0.26	0.98
		δ^{13}C in claws	0.94 (0.04)	19.97		**<0.001**	
Spring	0.5	Intercept	-3.35 (1.70)	-1.97	85.73 (1, 37)	0.056	0.83
		δ^{13}C in blood	0.83 (0.09)	9.26		**<0.001**	
Winter	1	Intercept	-2.05 (1.65)	-1.24	98.49 (1, 26)	0.225	0.88
		δ^{13}C in claws	0.88 (0.09)	9.92		**<0.001**	
Spring	1	**Intercept**	-7.13 (1.98)	-3.61	36.45 (1, 34)	**<0.001**	0.71
		δ^{13}C in blood	0.63 (0.10)	6.04		**<0.001**	
Winter	1.5	**Intercept**	-5.20 (1.79)	-2.90	54.69 (1, 36)	**0.006**	0.77
		δ^{13}C in claws	0.71 (0.10)	7.40		**<0.001**	
Spring	1.5	**Intercept**	-7.67 (2.20)	-3.48	26.32 (1, 25)	**0.001**	0.70
		δ^{13}C in blood	0.59 (0.11)	5.13		**<0.001**	

Table 3. 2. The breeding decisions of eiders which formed their pairs on the overwintering grounds (winter) and in spring staging areas (spring), results obtained from using the ordinal distance of less than 1 standard deviation.

	Breeding Decision				
	Non-breeding		Breeding		
Year	Proportion paired in spring (n)	Proportion paired in winter (n)	Proportion paired in spring (n)	Proportion paired in winter (n)	Total
2015	0.42 (8)	0.58 (11)	0.63 (10)	0.37 (6)	35
2016	0.63 (12)	0.37 (7)	0.50 (4)	0.50 (4)	27

Table 3. 3. Results of logistic regression models investing effects of the timing of pair formation on the breeding decision of pairs. Null model includes female mass and capture date (see methods for details). Because of a nearly significant effect of the year:pairing interaction, I analyzed years separately. Significant relationships are bolded.

Year	Response	Variable	Estimate (SE)	z	df	p
2015	Breeding	Intercept	11.57 (14.86)	0.778	(5, 55)	0.44
and	Propensity	Timing of pairing	-1.34 (0.81)	-1.65		0.09
2016		**Female mass**	0.005 (0.002)	2.49		**0.01**
		Year	-2.67 (0.97)	-2.74		**0.006**
		Capture date	-0.13 (0.08)	-1.52		0.13
		Timing of Pairing:Year	2.11 (1.23)	1.71		0.09
2015	Breeding	Intercept	-1.35(17.2)	-0.08	(3, 30)	0.94
	Propensity	Timing of pairing	-1.60 (0.90)	-1.78		0.07
		Female mass	0.008 (0.003)	2.52		**0.01**
		Capture date	-0.09 (0.10)	-0.89		0.37
2016	Breeding	Intercept	68.88 (45.18)	1.52	(3, 23)	0.13
	Propensity	Timing of pairing	0.59 (0.95)	0.62		0.53
		Female mass	0.002 (0.003)	0.48		0.63
		Capture date	-0.41 (0.25)	-1.66		0.09

Table 3.4. Results of linear models investing effects of the timing of pair formation on the interval between arrival and laying (pre-laying interval) and lay date of the female. Models include female mass as it has been established to have a significant effect on reproductive decisions in eiders, capture date, year, and the timing of pairing and year interaction. Significant relationships are bolded.

Year	Response	Variable	Estimate (SE)	t	df	p
2015 and 2016	Pre-laying Interval	Intercept	113.91 (35.91)	3.17	5, 18	0.01
		Timing of pairing	-0.73 (1.96)	-0.37		0.71
		Female mass	-0.002 (0.006)	-0.37		0.72
		Year	-1.14 (2.63)	-0.43		0.67
		Capture date	-0.59 (0.20)	-2.89		**0.01**
		Timing of pairing:Year	0.18 (3.66)	0.05		0.96
2015 and 2016	Lay Date	Intercept	113.91 (35.91)	3.17	5, 18	0.65
		Timing of pairing	-0.73 (1.96)	-0.37		0.71
		Female mass	-0.002 (0.006)	-0.37		0.72
		Year	-1.14 (2.63)	-0.43		0.67
		Capture date	-0.41 (0.20)	2.04		0.06
		Timing of pairing:Year	0.18(3.66)	0.05		0.96

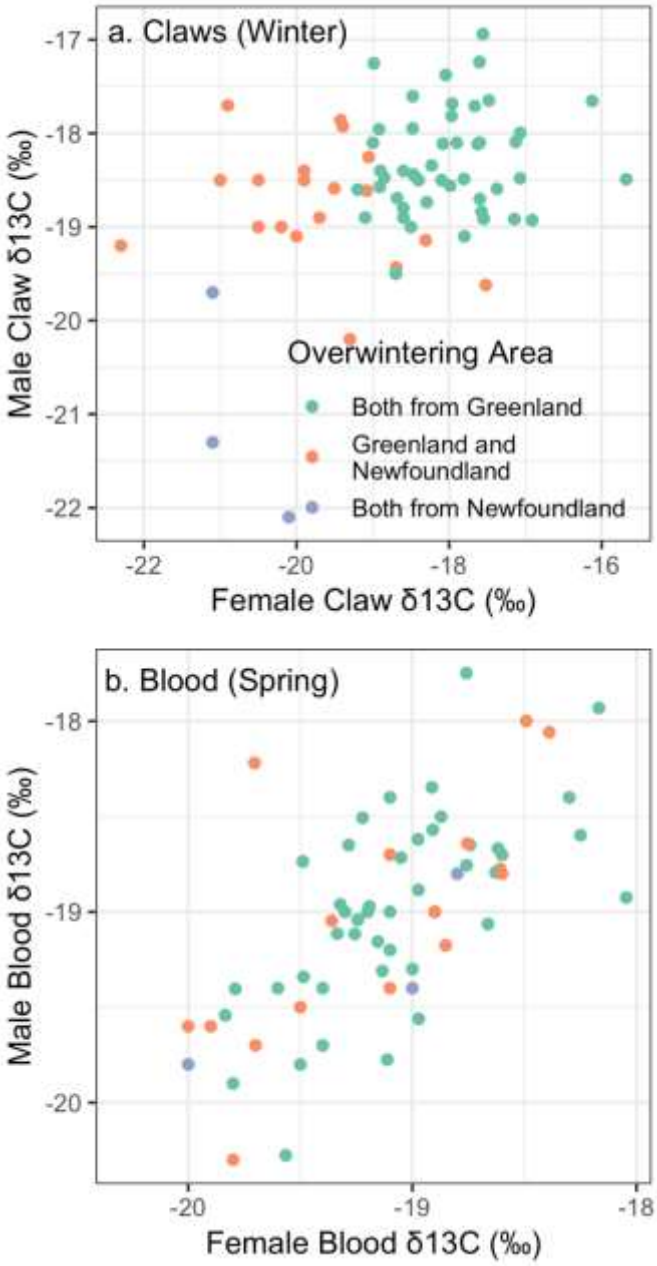

Figure 3. 1. Stable isotope values in the claws and blood of eider pairs used to assign individuals to overwintering areas (see Methods). Claws represent winter values while blood represent more recent spring values.

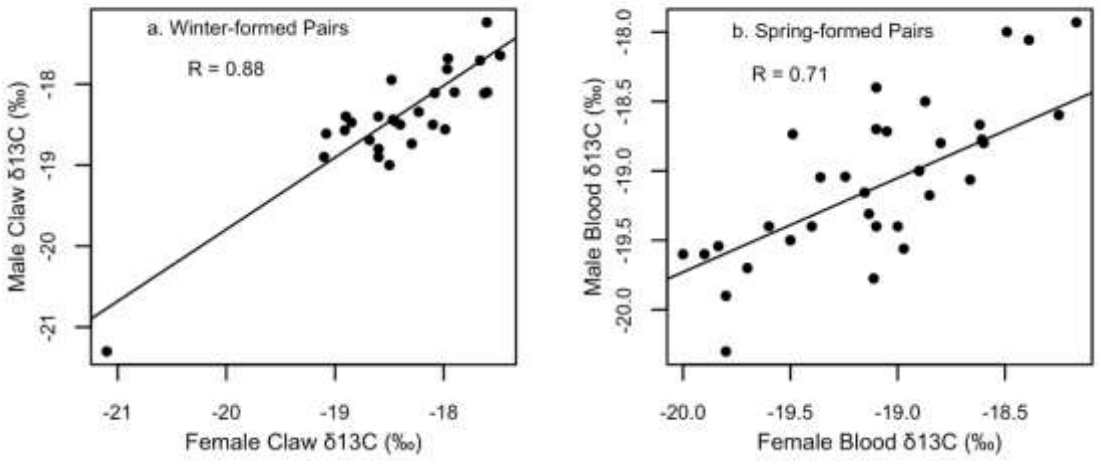

Figure 3. 2. Correlation of the stable isotopic values of common eider pairs inferred to have formed in the winter (a) and in the spring (b) shows a strong relationship between individuals for both spring and winter formed pairs. The lowest isotopic value for winter is from a pair that migrated from Newfoundland and therefore have much lower δ13-carbon as compared to the pairs which were inferred to have wintered in Greenland.

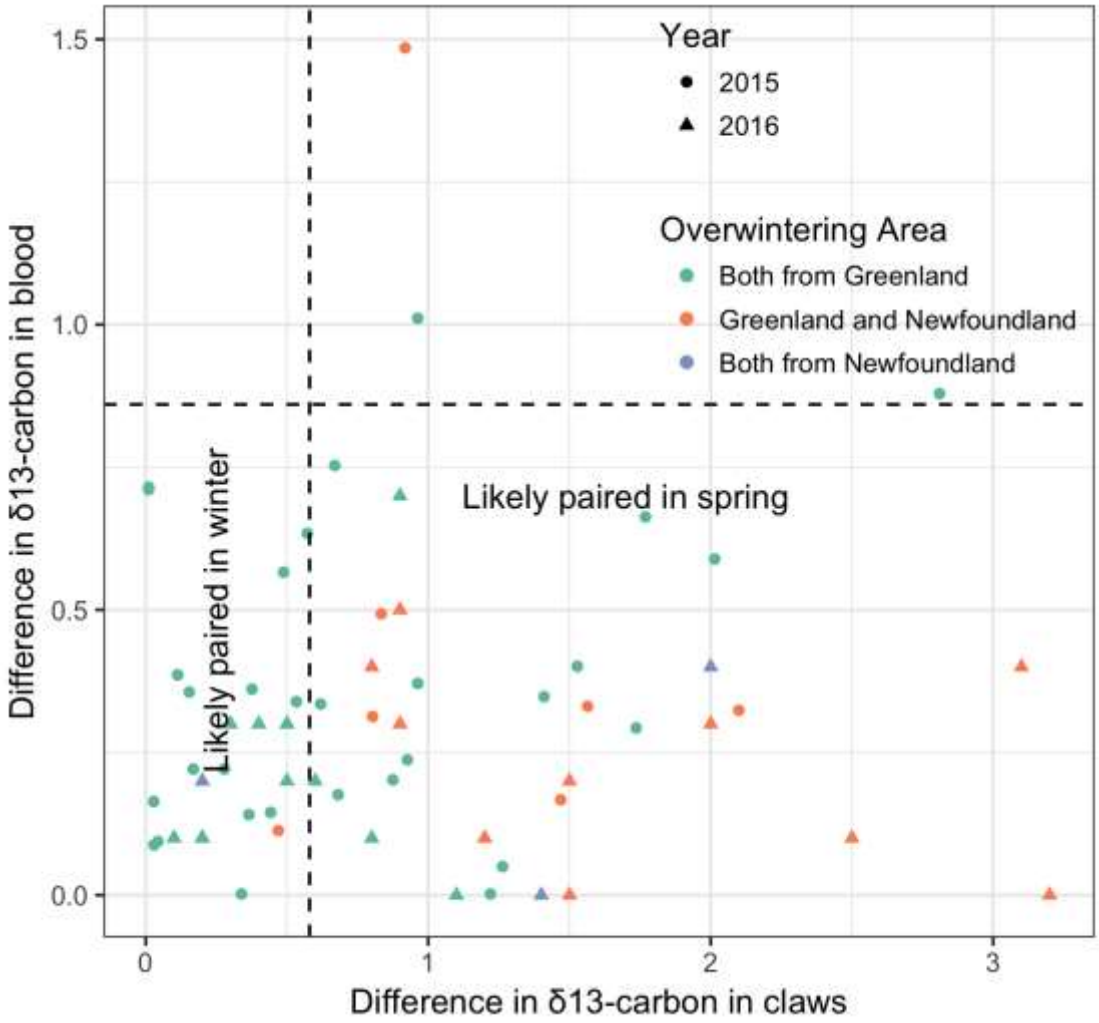

Figure 3. 3. The difference in stable isotopic values within pairs in both blood and claws. The vertical dotted line is drawn at the first standard deviation of the stable isotope values in the claws of known overwintering eiders, the horizontal dotted line is at the first standard deviation of the stable isotopic values in the blood of eiders.

Chapter 4: Examining Male Physiological State Suggests Limited Energetic Costs or Fitness Benefits to Mate Guarding in An Arctic-Breeding Seabird

Abstract

The benefits of mate-guarding behaviours by males are fairly well established and can include both benefits to females (facilitating foraging and reproductive output), and benefits to males (protecting paternity and preventing extra-pair copulation). Despite the recognition of a theoretical cost to mate-guarding for males, few studies have examined whether i) male physiological state declines as reproductive investment by their mate increases, and ii) males compensate for energetic costs and gain fitness benefits via indirect effects on their mate. Using morphological and physiologic metrics, I examined these questions in common eiders where males exhibit extensive mate-guarding behaviors throughout the reproductive period. I assessed male physiological state using energetic metabolites (beta-hydroxybutyrate [indicator of fasting] and triglycerides), immunoglobulin-Y (immunity marker), and energetic (corticosterone) and reproductive (testosterone) hormones and determined whether variation in these metrics were predictors of two important female breeding performance traits in this species: fattening rate and lay date. Although not statistically significant, male body condition tended to be lower when females were at their most fertile and testosterone was elevated. Beta-hydroxybutyrate, triglycerides, corticosterone, and immunoglobulin-Y did not correlate with female breeding stage. Variation in male physiological state did not transfer any benefits to female fattening rates nor relative lay date. Mate guarding in common eiders may have evolved to

reduce loss of paternity; males may pay some energetic costs, but do not transfer fitness benefits to their mate. This is supported by elevated male testosterone levels when females are most fertile, suggesting male vigilance is aimed at securing paternity and discouraging extra-pair copulations.

4.1 Introduction

In avian mating systems extra-pair copulations and paternities tends to be high, even amongst socially monogamous species (Birkhead & Møller 1996). In response to this, many mate guarding behaviors have evolved in males as a means to minimize the likelihood of extra-pair copulations, thus protecting individual paternity and fitness (Hario and Hollmén 2004; Schubert *et al.* 2009). Nevertheless, breeding is known to be energetically expensive, and there is some evidence that males can at times increase the intensity of their mate guarding efforts (Schubert *et al.* 2009), which further increases energetic costs and leads to reductions in body condition (e.g. geese, Choinière and Gauthier 1995; wood ducks, Hipes and Hepp 1995; harlequin ducks, Squires *et al.* 2007; Esler and Bond 2010). Additionally, males will engage in many high energy use behaviors to protect their mates such as male-male combat (chasing or pecking) and vigilance (scanning for interloping males) (Steele *et al.* 2007). Presumably, guarding behaviors by males during the pre-breeding period have evolved to maximize their fertilization success and thus fitness (Hario & Hollmén 2004), although maintaining such vigilance is expected to exact costs by limiting the time available for foraging and self-maintenance (Squires *et al.* 2007).

In addition to the direct benefits to paternity, the reproductive benefits of male mate guarding may also include an increase in his mate's ability to meet her own energetic requirements for the initiation or maintenance of breeding (Bond *et al.* 2007; Squires *et al.* 2007), with possible downstream effects on the likelihood of successfully laying or incubating eggs, and fledging chicks (Christensen 2000; Guillemain *et al.* 2003). Because female birds must invest in energy rich eggs (Martin 1987; Choinière & Gauthier 1995), and the decision to lay is condition-dependent in many species and consequently dependent on resource acquisition before breeding initiation (i.e., in mixed- or capital-breeding species; (Parker & Holm 1990; Bêty *et al.* 2003; Hennin *et al.* 2016a) the initiation of breeding requires some minimal level of energetic investment to support laying (Bêty *et al.* 2003; Sorensen *et al.* 2009; Hennin *et al.* 2015) and incubation (Hennin *et al.* 2015). To some degree, a female's ability to accrue energetic resources via foraging may depend upon (or benefit from) her mate's ability to shield her from interloping males seeking extra pair copulations, thus allowing her to forage undisturbed (e.g. snow geese, Choinière and Gauthier 1995; common eiders, Christensen 2000). Therefore, despite the energetic costs of mate guarding, males should benefit in a fitness sense by helping to maximize both their own paternity and female investment in body condition and reproduction. As such, a male's physiological state should decline as his mate progresses from pre-breeding to follicle recruitment and through to laying. Further, I would expect these costs to show benefits, with males showing the greatest energetic costs to also show the greatest benefits to female body condition gain or breeding performance. However, although studies have documented foraging dynamics (Hario & Hollmén 2004; Steele *et al.* 2007) and energy acquisition (body mass) (Hipes & Hepp 1995), few studies have been capable of quantifying these

energetic costs in males in relation to female investment in breeding, and whether these costs of mate guarding generate the expected fitness benefits.

Using an integrative approach, I examine the variation in a suite of physiologic metrics indicating physiological state (i.e. energetic and reproductive state) in male common eiders relative to their female mate's relative investment in reproduction (i.e., her breeding stage). I then ask whether variation in these state-based metrics predict key metrics for female reproduction (e.g., fattening rate and laying date). Common eiders are an excellent study species to investigate the physiologic mechanisms underlying male mate guarding behavior given that females use a mixed, capital-income breeding investment strategy (Hario & Hollmén 2004) where variation in female body condition (i.e. body mass) is a positive predictor of reproductive investment (Love *et al.* 2010; Descamps *et al.* 2011; Hennin *et al.* 2015, 2016a; Legagneux *et al.* 2016). Nonetheless, how and why male mate-guarding contributes to female reproduction (e.g. laying date) remains largely unknown. I assessed male physiological state (i.e. size corrected body mass, workload, and reproductive state) using several physiological metrics while controlling for the reproductive stage of his mate.

To assess energetic state in males, I measured baseline levels of corticosterone (CORT), circulating triglycerides (TRIG), and beta-hydroxybutyrate (BOH) (Jenni-Eiermann & Jenni 1994; Angelier *et al.* 2007; Williams *et al.* 2007). I chose baseline CORT for its known role in modulating foraging behavior and resource acquisition in breeding female birds (Angelier *et al.* 2007; Crossin *et al.* 2012b; Hennin *et al.* 2016a). Consequently, elevated CORT can indicate an increased demand for resources (Love *et al.* 2014). I also examined variation in TRIG as a measure of physiological fattening rates

(Williams *et al.* 2007; Hennin *et al.* 2016b). Conversely, because BOH is a by-product of lipid hydrolysis (Williams *et al.* 1999) it increases during mass loss and fasting as well as during periods of high workload (Jenni-Eiermann & Jenni 1994). I measured variation in immunoglobulin Y (IgY), the primary avian anti-body, as reduced levels have been shown to be a responsive physiological marker of declining body condition (Bourgeon *et al.* 2010). Finally, I measured variation in testosterone, given its well-recognized role in courtship behavior and reproductive development in male birds (Fusani 2008), as a potential indicator of defense behaviors associated with pair-bonds. In females, I recorded her reproductive stage of breeding investment (pre-recruitment, rapid follicle growth, laying), her plasma TRIG levels as a measure of fattening rate, as well as her eventual lay date. Based on the hypothesis that male common eiders face energetic costs to facilitate their partners' access to high quality foraging opportunities and therefore investment in reproduction, I made multiple predictions. I first predicted that (1) male common eiders with higher body condition would be paired with females with higher body condition, indicating that males would be better prepared to invest in female condition gain and males with higher body condition likely out compete males with lower body condition for these females. Subsequently, as males work progressively harder to defend their mates, they should have fewer opportunities to forage for themselves resulting in reductions in TRIG and IgY, and elevations in BOH. Given that the mate-guarding period overlaps with the time when females are preparing physiologically for laying and at their most fertile, my second prediction was that (2) as the breeding season progresses, mate vigilance should increase, resulting in a decline in male physiological state manifested by increasing baseline CORT and BOH, and declining TRIG and IgY. From a reproductive point of view,

because testosterone is well known as a mediator of courtship behaviour and male aggression (Fusani 2008), I expected male testosterone levels to peak during the height of their mate's fertility (i.e., rapid follicle growth and laying stages). Finally, I expected variation in male physiological state to predict his mate's body condition and her resulting ability to invest in breeding. I predicted that (3) male physiological state would be a positive predictor of variation in female reproductive investment measured as both higher physiological fattening rates (i.e., plasma TRIG levels) and earlier relative lay dates.

4.2 Materials and methods

4.2.1 Study site and species

Work was conducted at a long-term study site (East Bay Island, Nunavut, Canada) where research on arrival and breeding common eiders has been conducted since 1997 (Steenweg *et al.* 2015; Hennin *et al.* 2018; Jean-Gagnon *et al.* 2018). Pre-breeding eiders were captured via flight nets upon their arrival at a breeding colony from mid-June to early-July 2015-2017 (n = 105 total pairs, n = 44 breeding pairs). Male-female pairs travel together during the pre-breeding staging period; therefore, eiders were considered paired when captured together, simultaneously, in the net. Only known pairs of eiders (e.g. when a male was caught following a female into the net) were considered for this study.

Eider pairs were extracted from the net as quickly as possible and blood samples were collected from the tarsal vein using a heparinized 1-mL syringe from both sexes within 3 minutes of capture to ensure baseline physiology status (Romero & Reed 2005). Blood samples were transferred to a 1.5 mL heparinized tube and stored at approx. 4-6 °C and centrifuged within 8 hours of collection at 14 000 rpm for 10 minutes to separate

plasma from red blood cells. Plasma and red blood cell fractions where then frozen at -80 °C until analysis. Each eider was then weighed (g) and banded with a unique alpha numeric full darvic and half darvic plastic bands, as well as a USGS Bird Banding Lab steel band. Female eiders were outfitted with uniquely coloured and shaped plastic nasal tags, threaded through their nostrils with UV degradable monofilament, for easy individual identification on the breeding colony; nasal tags are not permanent and fall off by the autumn. Pairs were then released simultaneously, within an hour of capture.

Using spotting scopes, the breeding colony was surveyed twice-daily from six permanent observation blinds from early June until mid-July to obtain lay dates for all nasal-tagged females. In cases where lay date was not determined via daily observation, but had clearly occurred due to the presence of eggs, nests were visited so that lay date could be determined via egg candling (Weller 1956). Based on breeding physiology and ecology data from this colony (Hennin *et al.* 2015), lay date information was then used to assign individual females to one of three breeding stages at capture – pre-recruiting stage (PR): meaning that females had not yet made the decision to invest in reproduction; rapid-follicle growth stage (RFG): meaning that females were within 7 days of laying and had already begun to recruit yolk follicles; or laying: meaning that females had already begun laying a clutch of eggs. Finally, females that were initially captured, but which did not subsequently lay, were classified as non-breeders (NB)(Hennin *et al.* 2015).

4.2.2 Measurement of physiological metrics

All assays for physiological metrics were conducted at the Love Lab, University of Windsor. Baseline corticosterone (CORT) and plasma triglyceride (TRIG) measures in common eiders breeding in the Arctic have been previously shown to be unaffected by

changes in daylength or tidal cycles during the pre-breeding period (Steenweg *et al.* 2015). Therefore, I did not account for time of day of sample collection for analyses of physiological metrics. Baseline corticosterone (CORT) in plasma was measured using a commercially available enzyme immune-assay (EIA; Assay Designs, Ann Arbor, MI, USA) validated in common eiders breeding at East Bay (Hennin *et al.* 2015). All samples were run in triplicate at 1:20 dilution with 1.5% steroid displacement buffer by volume, in random order and in a 96-well plate. Each plate included a control of serially diluted laying hen plasma (Sigma–Aldrich Canada, Oakville, Ontario, Canada) and a kit-provided standard curve (200,000 pg/mL). Plates were read at 405 nM using a Biotek Powerwave HT microplate reader. The inter- and intra-plate coefficients of variation were 9.96% and 19.26%, respectively.

Circulating plasma triglycerides (TRIG) were measured using a commercially available assay kit (Sigma Aldrich, USA, #TR0100-1KT) validated in common eiders at East Bay (Hennin *et al.* 2015). Samples were run in duplicate with a control of laying chicken hen plasma (Sigma–Aldrich Canada, Oakville, Ontario, Canada) and a standard curve based on a serial dilution of the glycerol standard (2.54 mmol/L; Sigma Aldrich, USA). Samples were run either at a 1:2 or 1:10 dilution (Williams *et al.* 1999) added to 96-well microplates with Reagent A to measure free glycerol, followed by Reagent B to measure total glycerol and then the plates were shaken for 10 min at 37 °C. Samples were read at 540 nm using a Biotek Powerwave HT microplate reader for the concentration of total and free glycerol. Final TRIG concentration (mmol/L) was obtained by subtracting the free glycerol value from the total glycerol value. Plasma TRIG levels were corrected for body mass to estimate physiological fattening rate in females (Williams *et al.* 1999).

Inter- and intra- plate coefficients of variation were 9.88% and 9.32% for total TRIG, respectively.

Plasma hydroxybutyrate (BOH) was measured using a commercially available kinetic assay kit (K-HDBA, Megazyme Kit (Sigma, Lamarre *et al.* 2017). All samples were run in triplicate at 1:63 dilution, in random order, in a 96-well plate. Each plate included a control of serially diluted standards. All samples were reacted with 20 µL reagent buffer and 2 µL dehydrogenase and absorbance was read at 492 nm every 3 mins for 30 min using a Biotek Powerwave HT microplate reader. The inter- and intra-plate coefficients of variation were 6.11% and 4.11%, respectively.

Plasma Immunoglobulin-Y (IgY) was measured using an in-house enzyme-linked immunosorbent assay (ELISA) and has been previously validated in common eiders (Sigma CLS 3370; Bourgeon *et al.* 2006, Provencher *et al.* 2016). Samples were run in duplicate and diluted at 1:32000 in buffer solution, in random order and in 96-well plates with 2 controls per 47 samples. ELISA plates were filled with 100 µL of diluted samples and incubated at 37 °C for 1 hour and then kept at 4 °C for approximately 24 hours. Plates were emptied and rinsed with 200 µL of PBS-Tween solution. 100 µL of milk solution (fat-free powdered milk in PBS-Tween solution) was added to the plates and incubated at 37 °C for 1 hour. Wells were emptied and rinsed with PBS-Tween solution. 100 µL of chicken anti-body solution was added to the plate and incubated at 37 °C for 1 hour. Wells were emptied and 100 µL of revealing solution was added to the plate and incubated at 37 °C for 1 hour. A single plate was read at 405 nm using a Biotek Powerwave HT microplate reader, and the intra-plate coefficient of variation was 5.25%.

Testosterone was measured in plasma using a competitive assay (Cayman's Testosterone EIA Kit#582701, Crossin *et al.* 2017). Samples were run in triplicate in random order and diluted to 1:200 with buffer solution in a 96-well plate. Samples were measured against controls of chicken plasma and a standard curve based on a serial dilution of the stock solution. 50 µL EIA Antiserum was added to 50 µL of diluted samples and standards and plates were incubated at 26 °C, shaking at 500 rpm for 2 hours in the dark. Wells were dumped and rinsed with 200 µL of wash buffer 5 times. 200 µL was added to each well, incubated at 26 °C, shaking at 500 rpm for 1 hour in the dark. Plates were read at 412 nm using a Biotek Powerwave HT microplate reader, if levels were below 0.3 ng/mL, then plates were incubated for an additional 30 minutes and read again. Inter- and intra-plate coefficients of variation were 6.74% and 5.78%, respectively.

4.2.3 Statistical analyses

Male body condition index

Studies assessing whether using body mass alone as an appropriate index of body condition in male eiders currently do not exist. For female common eiders breeding at East Bay Island using size-corrected body mass as opposed to body mass alone as a measure of body condition only improves the predictive capacity for variation in body fat by 3% (Descamps *et al.* 2010) and so I use body mass as a measure of female body condition. To better assess the use of mass versus mass corrected for body size (wing chord, tarsus, or head size) as an index of body condition of male common eiders I used dissection data from 15 male common eiders collected as fisheries bycatch and submitted to the Greenlandic Institute of Natural Resources in Nuuk, Greenland, where 60-90% of breeding eiders from East Bay overwinter (Mosbech *et al.* 2006; Steenweg *et al.* 2017). As per

Descamps *et al.* (2010), I performed linear regressions with body size (wing chord, tarsus, or head size) as the independent variable and body mass as the dependent variable to establish body mass residuals to account for allometric differences. I compared the variation in body fat accounted for by body mass and residual body mass (corrected by each body size measure from the linear regressions). For the measure of body fat I used the mass of abdominal fat, which is highly correlated to the mass of total body fat (Jamieson *et al.* 2006). Wing chord, tarsus and head size account for much of the variation in both total fat mass and percent fat in wintering male common eiders (Supplementary Table 1)r results indicate that correcting body mass for head size accounts for 24 and 26% more variation in fat mass and percent fat, respectively, than body mass alone (Supplementary Table 2). Therefore, I used mass corrected for head size for use as a proxy for body condition in further analyses.

Physiological indices of male state

I ran a principal component analysis on all five physiological metrics obtained from males (n = 39) and body condition to obtain a composite metric representing male physiological state. All physiological metrics met the requirements for parametric analyses, with the exception of plasma CORT which was log-transformed to ensure normality (logCORT). I then used the principal components with eigenvalues greater than 1 as the composite metric of male physiological state. The first two principal components explained 29.1 and 19.7% of the variance in male state-based metrics, with eigen values of 1.74 and 1.18, respectively. For the first principal component, body condition and testosterone were both highly positively correlated (with correlation values of 0.75 and 0.64, respectively;

Supplementary Table 3) with BOH being highly negatively correlated (-0.73). Plasma TRIG and logCORT loaded less onto this principal component (0.35 and -0.26, respectively). For the second principal component logCORT was highly positively correlated (0.70) and IgY was negatively correlated (-0.79). I used individual based loadings of the first two principal components as the indices of male physiological state (as male state components 1 and 2) to examine relationships with female reproduction.

Changes in male physiology across breeding stages

I used linear models to assess my first prediction (1) that male common eiders in higher body condition (i.e. size corrected body mass) would be paired with females in higher body condition (i.e. mass for females; Descamps *et al.* 2010). I used general linear models to assess my second (2) prediction that as a consequence of a male's investment in reproduction, male body condition, physiological metrics (logCORT, TRIG, BOH, Testosterone and IgY), and my combined indices of male physiological state would change across the female's pre-breeding period (PR, RFG, LAY – see specific trait-based predictions in the Introduction). I used breakpoint analyses to detect changes in male body condition, each physiological trait and my combined indices of male physiological state throughout the pre-breeding period. This analysis detects significant positive or negative changes between data points (break points) by iteratively fitting linear models with linear predictors (Muggeo 2003). The breakpoints are identified and updated with each iteration until the algorithm converges.

Male physiological state and its relationship with female breeding parameters

I used linear models to test the first component of my third prediction (3a) that female fattening rate (i.e. body condition gain) would be predicted by male physiological state (PCA index of male state). I included relative capture date (median capture date minus the capture date of the individual) and year as fixed effects because these have been previously shown to influence eider lay date at this colony (Descamps *et al.* 2011). For the examination of female fattening rate, I restricted my analyses to pre-recruiting females (PR, n = 7) since during the PR period females have not yet committed to investing in breeding. Once females enter the RFG period, females have committed to reproduction and her follicles are rapidly growing, so fattening rates cannot be calculated. Fattening rates were obtained by correcting plasma TRIG for body mass, where the residuals indicate fattening rate (Williams *et al.* 2007; Hennin *et al.* 2016b). Due to the small sample size of PR females, to preserve degrees of freedom, I restricted the fattening rate analysis to be in relation to the male physiological state components and did not run an additional analysis with all physiological metrics. To address the second component of my third prediction (3b) I used linear models to test whether female relative lay date would be predicted by male physiological state, I included relative capture date, female mass, and year as fixed effects.

All analyses were conducted using R version 3.4.3 (R Core Team 2017), breakpoint analysis were conducted using the R package *segmented* (Muggeo 2003, 2008). All R code used for analyses is available in Appendix 4. In the interest of moving away from exclusively relying on p-values to discuss significance (Wasserstein *et al.* 2019) I discuss my results in relation to the strength of the relationship by using p-values as a continuous value and assessing their weight (Amrhein *et al.* 2019; McShane *et al.* 2019). Although I

interpret $P < 0.05$ as indicating a strong and significant relationship, I still consider $P < 0.15$ as showing a marginally significant relationship when supported by estimates indicating a biologically meaningful and reasonable difference. One incubating female, males with missing mass or head size measurements, and individuals with corticosterone values higher than three times the standard deviation were removed from the analyses to avoid values that did not represent baseline.

4.3 Results

4.3.1 Changes in male physiology across breeding stages

Supporting my first prediction (1) that male common eiders in higher body condition would be paired with females in higher body condition; male body condition and female body mass were positively related ($F_{(1,42)} = 8.85$, $P = 0.005$, $R^2 = 0.17$, Estimate = 0.49 ± 0.16 (SE)). My second prediction (2), that as a consequence of a male's investment in female reproduction, male physiological state would change across the breeding period, was marginally supported by multiple metrics. While the model predicting male body condition was not statistically significant ($F_{(4,39)} = 1.76$, $P = 0.16$, $R^2 = 0.15$, Table 1, Figure 1a), with a moderately strong correlation of 0.39, variation in male body condition was somewhat explained by female breeding stage, where male body condition was marginally significantly lower during the female laying period compared to during the pre-recruiting period (Estimate = -151.41 ± 77.82 (SE), $P = 0.06$). The model predicting male baseline logCORT was statistically significant ($F_{(5,31)} = 3.22$, $P = 0.02$, $R^2 = 0.34$, Figure 1b), although this was driven by effects of year, with birds in 2017 having significantly lower body condition (Estimate = -0.46 ± 0.17 (SE), $P = 0.01$), and no breakpoints were detected

across the pre-breeding period. The model predicting variation in male TRIG was not statistically significant ($F_{(5,34)} = 1.16$, $P = 0.35$, $R^2 = 0.15$; Figure 1c), however, breakpoint analyses indicated that TRIG values declined until 7 (± 3) days before their paired female began laying, after which secretion began to increase. The model predicting male BOH was statistically significant ($F_{(5,34)} = 2.97$, $P = 0.03$, $R^2 = 0.30$, Figure 1d), but was driven primarily by effects of body mass (Estimate = -0.009 ± 0, $P = 0.05$) and year with birds in 2017 having significantly lower BOH (Estimate = -0.345 ± 0.17, $P = 0.05$). The model predicting male IgY ($F_{(5,34)} = 3.10$, $P = 0.02$, $R^2 = 0.31$, Figure 1e), was statistically significant, but was only driven by effects of year with individuals in 2016 having lower IgY in 2016 (Estimate = -0.34 ±.0.11, $P = 0.004$). No breakpoints detected for either trait. Models predicting male testosterone were marginally significant ($F_{(5,28)} = 1.77$, $P = 0.15$, $R^2 = 0.24$, Figure 1f), with a correlation of 0.49, 24% of the variation in Testosterone was explained by the model. Testosterone was marginally significantly higher in both RFG and laying stages than in PR (RFG: Estimate = 4.49 ± 2.34 (SE), $P = 0.06$ and PR: Estimate = 4.48 ± 2.51 (SE), $P = 0.08$, respectively; Table 1). No breakpoints were detected. The model predicting the first component of the index of male physiological state was not statistically significant ($F_{(4,39)} = 2.31$, $P = 0.08$, $R^2 = 0.19$, Figure 1g). However, the model predicting the second component of the index of male physiological state was significant ($F_{(4,39)} = 5.16$, $P = 0.002$, $R^2 = 0.35$, Figure1h), driven by positive effects of year in 2016 (Estimate = 0.19 ± 0.42, $P = 0.007$). No breakpoints were detected for either physiological state components.

4.3.2 Male physiological state and its relationship with female breeding parameters

The first part of my third prediction (3a) that male physiological state would influence female fattening rate (i.e. condition gain) was not supported. The model predicting fattening rate was marginally significant ($F_{(5,1)}$ = 129.00, P = 0.07, R^2 = 1.00; Table 2); however, the strength of this relationship was only driven by the effects of relative capture date (Estimate = -1.09 ± 0.08 (SE), P = 0.04) and year (2017; 11.86 ± 0.86, P = 0.05). The second part of my third prediction (3b) that male physiological state would influence relative lay date was not supported. The model predicting relative lay date using male physiological state was significant ($F_{(10,20)}$ = 0.270, P = 0.03, R^2 = 0.57) and my model predicting relative lay date with male physiological state was also significant ($F_{(6,37)}$ = 2.39, P = 0.05, R^2 = 0.28); however, again, both of these relationships were primarily driven by effects of relative capture date (Table 3).

4.4 Discussion

In this study I examined the variability in male common eider state (body condition and physiological metrics) relative to their mate's body condition and breeding stage. I used male physiological metrics indicating energetic state, workload, and reproductive state to establish whether male common eiders facilitate their partners' reproductive output via their effort, or whether males adopt physiological costs to help ensure their paternity of offspring. I found that male and female body condition was positively correlated, suggesting females with relatively high body condition tended to pair with males also in relatively high body condition. I provide evidence that some male physiological metrics (male body condition and testosterone) changed in relation to female pre-breeding stages. However, I found no evidence to support the idea that male effort (represented by a

combined metric of male physiological state) conferred any benefit to female fattening rates or relative lay date. Alternatively, my combined results indicating that male and female body condition were significantly positively related and that male testosterone was elevated during the most fertile female periods of breeding support the concepts of assortative mating (Cooke *et al.* 1976; individuals chose mates of similar phenotypes to their own) and maximization of paternity, respectively.

4.4.1 Variation in male physiology across breeding stages

Male eiders showed a marginal decrease in body condition across the entire breeding period examined in this study. Although this relationship was not statistically significant, males sampled when their female mate was in the laying stage had a lower body condition compared to those in the pre-recruiting stage, a trend which I feel warrants further exploration. This possible seasonal decrease in body condition suggests males could be expending energy and working harder to guard their mates as breeding progresses, which may consequently limit foraging opportunities for themselves and require them to fuel all of their mate-guarding activities with stored somatic reserves. Although some studies have suggested that male vigilance positively influences female foraging (Christensen 2000), given I found a complete lack of relationship of male physiological state to female fattening rate or advancement of laying in eiders at East Bay Island, male eiders may instead favour mate vigilance to ensure their paternity, supporting previous results from common eiders breeding in the Northern Baltic Sea (Hario & Hollmén 2004).

Correspondingly, male testosterone levels lend additional support to this assumption since the highest levels of testosterone I measured occurred during the RFG and laying period when female eiders are at their most fertile. According to Fusani (2008),

testosterone has a threshold effect on the activation of courtship behaviour, rather than a linear or correlative relationship with any metric of courtship intensity (Foerster & Kempenaers 2005; Fusani 2008). As such, testosterone is expected to activate courtship behaviour in an all-or-nothing manner. Elevated testosterone in male eiders during the RFG and laying stages suggests that the highest degree of male vigilance was exhibited during the stages of the female cycle when copulation is most likely when females were most fertile. In this case, elevated testosterone in males would facilitate courtship behaviours as well as gamete production to ensure their chances of successful copulation (Wingfield *et al.* 2001; Fusani 2008). Comparable to increases in courtship activity seen in male canvasbacks *Aythya valisineria* with high testosterone (Bluhm *et al.* 1984), the increase in testosterone in male common eiders during the female's fertile period could also serve to increase mate guarding behaviors to defend their female mate from extra-pair copulations. In contrast, if increased male vigilance had evolved to ensure females had uninterrupted foraging or better access to foraging opportunities (as proposed by Christensen 2000), testosterone should have been elevated during the pre-recruitment stage when accumulation of fat stores is most important for females, but it was not.

4.4.2 Male physiological state and its relationship to pre-breeding females

Although females in relatively good body condition tended to pair with males in relatively good body condition, my data suggest that male physiological state has no impact on female condition gain, as indicated by the lack of relationship between male physiological state and female fattening rates. In surface-dabbling ducks, males' vigilance is associated with an increase in their mate's access to resources (Rohwer & Anderson 1988). Similarly, in terrestrially foraging greater snow geese *Chen caerulescens*, males

used more energy reserves than females during the pre-laying period, and so females paired with males in better condition were more likely to breed (Choinière & Gauthier 1995). In contrast, however, in my study when male eider body condition and physiology were combined as an overall index of male physiological state, I could not find a relationship to proximate or ultimate benefits to females measured here as increases in female fattening rates or advancements in laying date. Unlike in other waterfowl, it does not appear that male eiders aid females in gaining body condition prior to breeding. However, because it was only possible to sample male eiders once during the breeding season, I rely on detecting change in male physiological state over time by measuring across individuals. It is possible that these small trends over time are masked by a lack of detection changes across individuals, masked by individual variation.

Variation in male physiological state did not appear to transfer any detectable benefits to female eiders during the pre-breeding period (i.e. advanced lay date or increased fattening rates). The last male that copulates with a female is most likely to secure paternity (Møller & Birkhead 1991). It is most likely that it is advantageous for males to closely follow their female mate to defend them from extra pair copulations during this period. Indeed, my state-based data suggest that it is likely that male eiders are simply ensuring that they are the last to copulate with their paired female.

Instead of choosing mates based on the perceived benefits of enhanced state or condition, an alternative explanation for mate selection drivers in common eiders at East Bay is that they exhibit an assortative mating strategy by choosing mates of similar body condition to their own. Assortative mating can be based on any number of traits including age (Ludwig & Becker 2008), size (Delestrade 2001), or coloration (Cooke *et al.* 1976), as

long as mated pairs are showing positively correlated phenotypes (Kopp *et al.* 2018). When choosing mates they may self-reference and select others that match their own traits or phenotypes (Kopp *et al.* 2018). Since male and female common eiders are sexually dimorphic, they may choose their mate based directly on body condition (size), or perhaps additional traits which are correlated with body condition or overall state. For instance, in some breeding populations (although not at East Bay), the degree of feather whiteness in female eiders correlates with reproductive quality (Hanssen *et al.* 2006). Regardless of the underlying mechanism, using body condition as a basis of choosing a mate may be advantageous since elevated body condition allows females to invest in their clutch earlier (Descamps *et al.* 2011; Hennin *et al.* 2018), while elevated male body condition should allow males to defend and invest in their paternity (supported by results herein). Therefore, for males, choosing females in good body condition is likely beneficial for future reproductive investment, while for females it is beneficial to select a mate in good body condition since he is more likely to pass on this same ability to gain or maintain high condition to their offspring.

Table 4. 1. Summary of parameter estimates of fixed effects from linear models investigating variation in male common eider physiology in relation to breeding stage of female common eiders (breeders only), year, and male body condition. Significant relationships are bolded.

Response	Variable	Estimate	± SE	*t*	*p*
Body condition	Intercept	36.9	65.81	0.56	0.58
	Breeding stage (RFG)	-70.87	63.60	-1.11	0.27
	Breeding stage (LAY)	-151.41	77.82	-1.95	0.06
	Year (2016)	36.08	65.73	0.55	0.59
	Year (2017)	89.98	50.58	1.78	0.08
logCORT	Intercept	0.65	0.24	2.71	**0.01**
	Breeding stage (RFG)	-0.30	0.22	-1.43	0.16
	Breeding stage (LAY)	0.27	0.26	1.02	0.31
	Male body condition	0.001	0.00	-1.12	0.27
	Year (2016)	0.09	0.19	0.49	0.63
	Year (2017)	-0.46	0.17	-2.69	**0.01**
TRIG	**Intercept**	0.80	0.15	5.17	**<0.001**
	Breeding stage (RFG)	-0.26	0.14	-1.91	0.06
	Breeding stage (LAY)	0.07	0.16	-0.41	0.69
	Male body condition	0.0002	0.00	0.70	0.49
	Year (2016)	0.03	0.13	0.25	0.80
	Year (2017)	-0.08	0.11	-0.71	0.48
BOH	Intercept	0.94	0.25	3.82	**<0.001**
	Breeding stage (RFG)	0.13	0.22	0.61	0.55
	Breeding stage (LAY)	-0.13	0.26	-0.49	0.63
	Male body condition	-0.009	0.00	-2.07	**0.05**
	Year (2016)	-0.13	0.20	-0.66	0.51
	Year (2017)	-0.35	0.17	-2.08	**0.05**
IgY	**Intercept**	0.63	0.13	4.71	**<0.001**
	Breeding stage (RFG)	-0.15	0.12	-1.29	0.21
	Breeding stage (LAY)	-0.10	0.14	-0.69	0.50
	Male condition	0.0002	0.00	-0.85	0.40
	Year (2016)	-0.34	0.11	-3.08	**0.004**
	Year (2017)	-0.02	0.09	-0.24	0.82
Testosterone	**Intercept**	2.78	2.58	1.08	0.29
	Breeding stage (RFG)	4.49	2.34	1.92	0.06
	Breeding stage (LAY)	4.48	2.51	1.79	0.08
	Male body condition	0.01	0.004	1.26	0.22
	Year (2016)	2.84	1.93	1.47	0.15
	Year (2017)	1.70	1.61	1.06	0.30
Male State Component 1	Intercept	0.83	0.48	-1.75	0.09
	Breeding stage (RFG)	0.26	0.48	0.55	0.59
	Breeding stage (LAY)	0.59	0.61	0.97	0.34
	Year (2016)	0.80	0.51	1.56	0.13
	Year (2017)	1.10	0.39	2.79	**0.009**
Male State Component 2	Intercept	-0.22	0.39	-0.57	0.58
	Breeding stage (RFG)	0.10	0.39	0.25	0.81
	Breeding stage (LAY)	0.01	0.49	0.02	0.98
	Year (2016)	1.19	0.42	2.86	**0.007**
	Year (2017)	-0.57	0.32	-1.78	0.08

Table 4. 2. Summary of parameter estimates of fixed effects of linear models investigating variation in fattening rate in pre-recruiting female eiders in relation to male physiological state, year and relative capture date. Significant relationships are bolded.

Response	Variable	Estimate	± SE	*t*	*p*
Female fattening rate	**Intercept**	-6.48	0.35	-18.26	**0.03**
	Male State Component 1	0.28	0.30	0.92	0.53
	Male State Component 2	2.34	0.73	3.20	0.19
	Year 2016	3.91	0.65	5.99	0.10
	Year 2017	11.86	0.86	13.78	**0.05**
	Relative capture date	-1.09	0.08	-14.21	**0.04**

Table 4. 3. Summary of parameter estimates of fixed effects from linear models of analyses investigating variation in relative lay date in relation to male common eider physiology and male physiological state, including year, female mass and capture date. Significant relationships are bolded.

Response	Variable	Estimate	± SE	*t*	*p*
Relative Lay Date	Intercept	1.05	13.58	0.08	0.94
	Body condition	0.01	0.004	1.32	0.20
	logCORT	2.61	1.74	1.5	0.15
	TRIG	-0.40	3.09	-0.13	0.90
	BOH	0.25	2.01	0.12	0.90
	IgY	5.85	3.32	1.76	0.09
	Testosterone	-0.29	0.18	-1.67	0.11
	Year 2016	4.79	2.61	1.83	0.08
	Year 2017	2.85	2.13	1.34	0.20
	Female mass	-0.002	0.005	-0.38	0.71
	Relative capture date	0.79	0.26	3.02	**0.01**
Relative Lay Date	Intercept	-2.09	9.1	-0.23	0.82
	Male state component 1	0.34	0.61	0.56	0.58
	Male state component 2	-0.88	0.71	-1.23	0.23
	Year 2016	1.82	1.85	0.98	0.33
	Year 2017	-0.90	1.48	-0.61	0.55
	Female mass	0.001	0.004	0.2	0.85
	Relative capture date	0.52	0.21	2.41	**0.02**

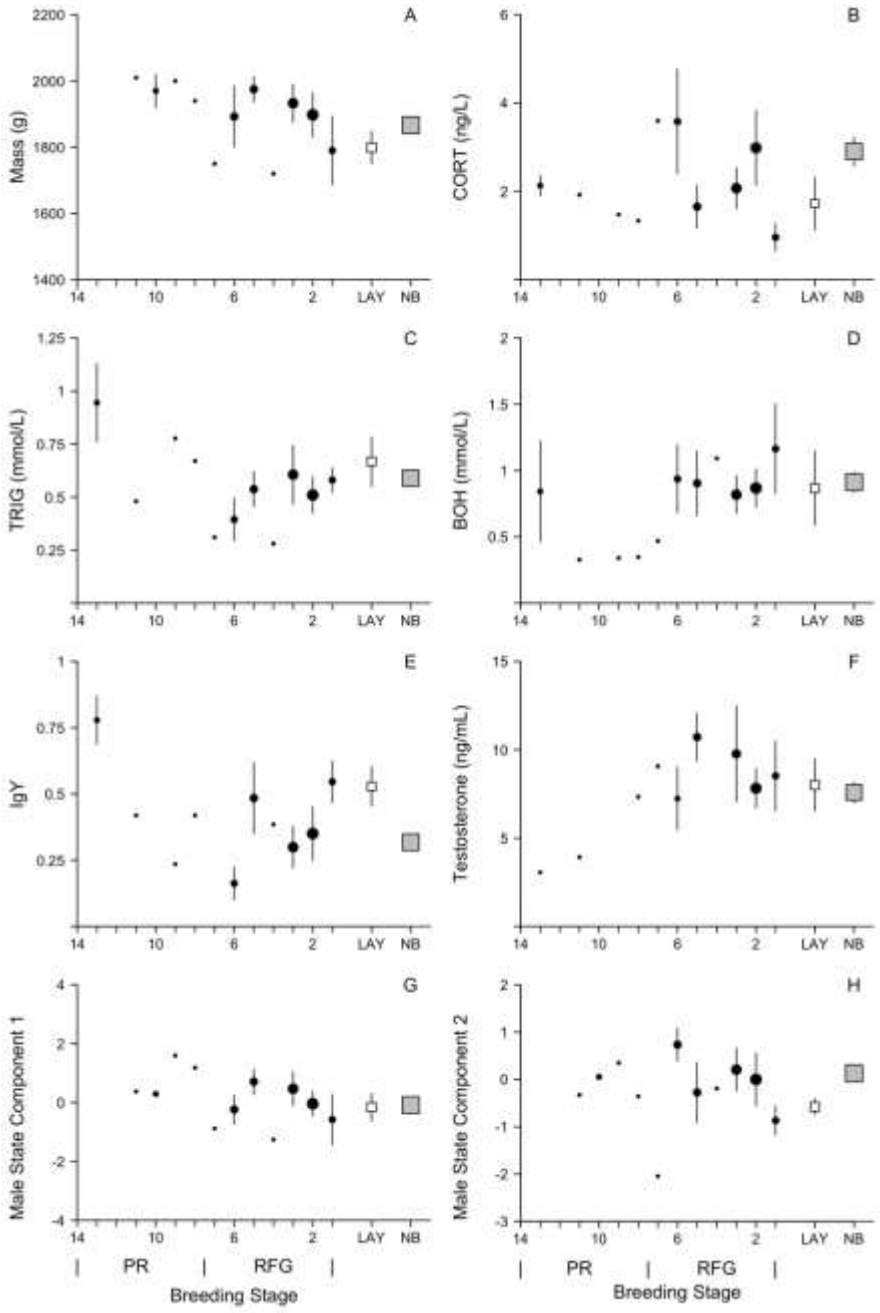

Figure 4. 1. Variation of male physiologic metrics and metric of male physiological state in relationship to their paired female mate's breeding stage (PR: pre-recruiting, RFG: rapid follicle growth). Values are mean ± SE provided over the pre-laying interval (black dots), laying (LAY, white square) and non-breeding (NB, grey square). Symbol sizes are proportional to log(n).

Supplementary Table 4. 1. Effects of wing chord, tarsus and head size on male common eider body mass overwintering in Greenland.

Dependent Variable	Predictor	Slope (SE)	p-value	R^2
Body mass	Wing chord	6.42 (4.48)	0.18	0.14
	Tarsus	25.69 (17.33)	0.16	0.14
	Head	22.62 (14.55)	0.14	0.16

Supplementary Table 4. 2. Male common eider body fat and body fat percentages versus residuals of morphometric measurements and mass from eiders overwintering in Greenland.

Dependent Variable	Predictor	Slope (SE)	p-value	R^2
Fat mass	$Residuals^{wing\ chord}$	37.62 (10.16)	0.002	0.51
Fat mass	$Residuals^{tarsus}$	41.77 (8.7)	0.0003	0.64
Fat mass	$Residuals^{head}$	45.33 (6.99)	<0.0001	0.76
Fat mass	Mass	40.74 (10.85)	0.002	0.52
% Fat	$Residuals^{wing\ chord}$	80884 (25540)	0.007	0.44
% Fat	$Residuals^{tarsus}$	93638 (21670)	0.0008	0.59
% Fat	$Residuals^{head}$	101999 (18103)	<0.0001	0.71
% Fat	Mass	88045 (27226)	0.007	0.45

Supplementary Table 4. 3. Results of principal component analysis as an index of male physiological state.

Component	Eigen value	% variance explained	Variable	Correlation	p-value
First	1.75	29.11	Body condition	0.76	<0.0001
			Testosterone	0.64	<0.0001
			Triglycerides	0.35	<0.0001
			logCORT	-0.26	<0.0001
			BOH	-0.73	<0.0001
Second	1.18	19.65	logCORT	0.70	<0.0001
			MIgY	-0.79	<0.0001
Third	0.97	16.10	Triglycerides	0.89	<0.0001
			BOH	0.33	0.0006
			logCORT	-0.20	0.03

Chapter 5: Favourable Spring Conditions Can Buffer the Impact of Winter Carryover Effects on A Key Breeding Decision in An Arctic-Breeding Seabird

Abstract

The availability and investment of energy among successive life-history stages is a key feature of carryover effects. In migratory organisms, examining how both winter and spring experiences carryover to affect breeding activity is difficult due to the challenges in tracking individuals through these periods without impacting their behaviour, thereby biasing results. Using common eiders, I examined whether spring conditions at an Arctic breeding location (East Bay Island, Nunavut, Canada) shared by all birds can buffer the impacts of wintering conditions on body mass and breeding decisions in birds that winter at different locations (Nuuk and Disko Bay, Greenland, and Newfoundland, Canada; assessed by analyzing stable isotopes of winter-grown claw samples). Specifically I used path analysis to examine how wintering and spring environmental conditions interact to affect breeding propensity (a key reproductive decision influencing lifetime fitness in female eiders) within the contexts of the timing of colony arrival, pre-breeding body condition (i.e. body mass), and a physiological proxy for foraging effort (baseline corticosterone; CORT). I demonstrate that warmer winter temperatures predicted lower body mass at arrival (likely an artifact the quality of the actual wintering location itself rather than temperatures), whereas warmer spring temperatures predicted earlier arrival dates and higher arrival body mass. Both higher body mass and earlier arrival dates of eider hens increased the probability that they would nest (i.e. higher breeding propensity).

However, effects of both winter and spring temperatures on CORT, and its downstream effects on breeding propensity, were not significant. Overall, I demonstrate that favourable pre-breeding conditions in Arctic-breeding common eiders can apparently compensate for the impact that unfavourable wintering conditions can have on breeding investment, perhaps because of greater access to foraging areas prior to laying.

5.1 Introduction

Across a diversity of species, energetic constraints play important roles in investment decisions at all stages of their annual cycles (Schultz *et al.* 1991; Barnes & Partridge 2003; Coma & Ribes 2003; Lamarre *et al.* 2017; Festa-Bianchet *et al.* 2019). The accumulation and careful management of energetic resources is critical for fueling transitions between key events or life-history stages (such as between migration and reproduction) (Schultz *et al.* 1991; Alerstam 2006; Drent *et al.* 2006). Defined as carryover effects, wherein the previous experience of an individual explains its current performance (*sensu*: O'Connor et al. 2014), these impacts can be driven by multiple factors including the availability of energy and nutrients (van Noordwijk & de Jong 1986; Shertzer & Ellner 2002; Barnes & Partridge 2003; Harrison *et al.* 2011; Williams 2012 pp. 247–259). Importantly, since these effects have the potential to impact variation in individual state and performance at subsequent life history stages (Shertzer & Ellner 2002), they also have the potential to impact investment in downstream events such as breeding decisions (Burnett *et al.* 2017).

Carryover effects are often found in, or exaggerated in, migratory species, since the ability to successfully migrate between wintering and breeding locations is linked to the

availability of resources to meet energetic demands (Tamisier *et al.* 1995; Johnson *et al.* 2016). It is possible that the extent to which individuals can accumulate and maintain energetic stores can have significant implications for reproduction, especially with respect to breeding decisions and investment (Martin 1987; Oosterhuis & Van Dijk 2002; Crossin *et al.* 2012a, 2013; Hennin *et al.* 2018), and breeding success (Williams 2012 pp. 224–225; Burnett *et al.* 2017). Indeed, individuals in higher quality wintering habitats often arrive to the breeding site earlier, arrive in higher body condition, lay earlier and have higher reproductive output/success (Norris *et al.* 2004; Sorensen *et al.* 2009; Drake *et al.* 2013). An important mechanism underlying wintering habitat quality, and the carryover effects on subsequent reproduction, is food availability (Shertzer & Ellner 2002; Brown & Sherry 2006; Ballesteros *et al.* 2013) which, for some species, can be influenced by variation in abiotic factors such as temperature (Lehikoinen *et al.* 2006; Williams *et al.* 2015).

Despite evidence indicating that habitat quality on the wintering grounds carries over to impact subsequent reproduction (Norris 2005; Rockwell *et al.* 2012; Szostek & Becker 2015; Imlay *et al.* 2019), in some species breeding parameters can be more heavily influenced by conditions in their immediate, pre-breeding environment (Harrison *et al.* 2011; Van Oudenhove *et al.* 2014). For instance, in multiple migratory bird species, the temperatures experienced after arrival on the breeding grounds were more important drivers of lay date than carryover effects of precipitation (as a proxy for resource abundance) on the wintering grounds (Love *et al.* 2010; Ockendon *et al.* 2013; Senner *et al.* 2014; Ramírez *et al.* 2017; Jean-Gagnon *et al.* 2018). Despite the negative influences of low quality wintering habitat on timing of arrival on the breeding grounds, body condition and investment in important reproductive metrics (e.g., timing of arrival,

breeding propensity, reductions in clutch size and breeding success), favourable conditions during migration and spring arrival on breeding grounds can improve foraging conditions to buffer these negative carryover effects (Perrins 1970; Rowe *et al.* 1994; Bêty *et al.* 2003; Descamps *et al.* 2011).

Here I examine the carryover effects of winter and how variation in subsequent spring conditions may influence reproductive parameters of common eider ducks nesting at a colony in Arctic Canada. Eiders breeding at this colony are an ideal system to test these questions for several reasons. First, birds migrate thousands of kilometers from overwintering sites off the coasts of Western Greenland and Newfoundland to breed in the Eastern Canadian Arctic (Mosbech *et al.* 2006). Importantly, the winter weather driven by the North Atlantic Oscillation (NAO, NOAA 2017) typically generates different and opposite environmental conditions at these overwintering locations (Mosbech *et al.* 2006; Descamps *et al.* 2010; Steenweg *et al.* 2017), with the potential to generate different carryover effects on eider reproduction. Second, as mixed breeding strategy (capital-income) birds, female common eiders use stored resources brought from the wintering grounds, topped up by local foraging on the breeding grounds to invest in reproduction and fuel egg production (Sénéchal *et al.* 2011a). Eiders also need to build enough reserves to successfully complete a 24-day incubation fast (Bottitta *et al.* 2003). Therefore, resources brought from the wintering grounds, as well as the ability to quickly gain the necessary body condition on the breeding grounds, should both contribute to predicting variation in the decision to breed (Sénéchal *et al.* 2011a; Descamps *et al.* 2011; Hennin *et al.* 2018). It is logical that breeding decisions should be affected by environmental conditions both on the wintering grounds (Descamps *et al.* 2010) and also on the breeding grounds (Love *et*

al. 2010; Jean-Gagnon *et al.* 2018), which can be further modified by individual variation in the ability to gain in condition on the breeding grounds (Hennin *et al.* 2016a, 2018). Since common eiders are a long-lived species, females not able to achieve the threshold minimum body condition required to invest in reproduction should have the flexibility to defer reproduction to following years in favour of current self-maintenance (Legagneux *et al.* 2016). Third, I have used a non-invasive method (stable isotopes in winter-grown claws; Steenweg *et al.* 2017) to assign the overwintering location of migratory females from samples collected at arrival on this breeding colony. This method allows us to compare wintering and pre-breeding spring conditions on reproductive performance without the deployment of bio-logging devices, which have the potential to bias results through impacts on bird behaviour, foraging, reproduction, and survival (Burger & Shaffer 2008). Finally, using individually unique, visual identifiers (i.e., alpha-numeric bands/rings and temporary nasal-tags) it is possible to accurately track the females nesting at this colony within a breeding attempt. As a result, it is possible to relate both wintering and spring conditions to key traits that impact reproduction (i.e., body condition) and individual variation in breeding propensity, a critical breeding decision that is a significant driver of variation in lifetime reproductive output (Reed *et al.* 2015).

I use a path analysis approach (e.g. Descamps *et al.* 2011; Harms *et al.* 2015; Provencher *et al.* 2016; Hennin *et al.* 2018) to examine the possible influences of winter and spring environmental conditions (temperature) on individual variation in: the timing of arrival on breeding grounds, pre-breeding body condition (body mass), a physiological indicator of foraging effort that affects condition gain (baseline corticosterone; CORT), and breeding propensity. Given that eiders are mixed-strategy breeders, I predicted that

wintering conditions experienced by female eiders could generate carryover effects onto breeding, with conditions during the pre-breeding staging period having the potential to allow individuals to compensate for possible energetic shortfalls extending from winter conditions. Specifically, since eiders are diving sea ducks and are reliant on open water for foraging opportunities, I predicted that colder winter temperatures would negatively affect the timing of arrival and body mass at arrival on the breeding grounds (Descamps *et al.* 2010), and subsequently have negative downstream impacts on breeding propensity. However, if female eiders experience favorable spring environmental conditions upon arrival at the breeding grounds (i.e., warmer conditions with more open water for foraging), individuals may be able to buffer against or compensate for winter-derived energetic shortfalls. Overall, I predicted that warmer spring temperatures, earlier arrival dates and higher pre-breeding body mass would buffer wintering carryover effects leading to positive effects on breeding propensity.

5.2 Methods

5.2.1 General field methods and sampling

I tested my questions using female common eiders at a long-term monitored breeding colony on East Bay (Mitivik) Island within the East Bay Migratory Bird Sanctuary, Nunavut (64°02'N, 81°47'W). Females were captured during the pre-breeding period (mid-June to early July) from 2014 to 2017 (n = 247 individuals; Table 1) using large flight nets. All birds were blood sampled from the tarsal vein within 3 minutes of initial capture to obtain baseline physiological metrics (Hennin *et al.* 2015). I then measured body mass to the nearest 10 g, to assess arrival body condition (Descamps *et al.*

2011), and collected the distal 2 mm from the middle toe claw on the left foot, for analysis of stable isotopes to assign winter location (Steenweg *et al.* 2017). Birds were banded with field-readable full and ½ darvic alpha-numeric plastic bands and a USGS Bird Banding Lab metal band. Each female was also given a combination of uniquely colored and shaped plastic nasal tags threaded through their nostrils with an ultra-violet degradable monofilament that allow for individual identification on the colony, but which fall off prior to fall migration. I obtained breeding propensity data for all captured females by surveying the colony twice-daily for nasal-tagged females from seven permanent observation blinds from early June to mid-July. Individual females were categorized as non-breeders (n = 160) if they did not return to the colony to lay, given the high site fidelity of this colony (Jean-Gagnon *et al.* 2018) and as breeders (n = 86) if they were observed incubating eggs. Females were considered to be in the laying (LAY, n = 21) or incubating (INC, n = 6) stages if caught once they had already begun laying or known to be incubating, determined through twice daily plot monitoring efforts. I removed laying and incubating hens from this analysis because their body mass would be influenced by the development and laying of eggs at this time, hence would not be an accurate representation of body condition (Descamps *et al.* 2011).

5.2.2 Assignment of wintering location and environmental conditions

I used stable isotopes from claws collected at arrival on the breeding grounds to infer individual overwintering area (see Steenweg *et al.* 2017; Chapter 2, for analytical details), and assigned each female to one of three (see below; data analysis) wintering ground locations. Briefly, I removed surface oils from claw samples by placing claw subsamples into vials and adding a 2:1 chloroform:methanol solution, vortexing them for

15 seconds and letting them sit for 24 hours. I then centrifuged vials at 10, 000 rpm for 10 minutes and siphoned off the supernatant with a pipette. I then rinsed samples with the chloroform:methanol solution, vortexed them for 15 seconds, and then centrifuged and siphoned the supernatant from the samples once more. I then dried samples in a fume-hood for 24 hours. Subsamples of claws were weighed to 0.30-0.50 mg, then placed into tin capsules to be analyzed for stable isotopes of carbon (^{13}C and ^{12}C).

Samples collected from 2014-2016 were analyzed at Queen's University, and from 2017 at the Great Lakes Institute of Environmental Research (GLIER) at the University of Windsor. To ensure that these two labs were consistent and comparable in their carbon isotopic measurements, I re-analyzed 10 randomly selected samples at GLIER that I had previously analyzed at Queen's. These pairs of samples were within 0.4 ± 0.8 (s.d.) of each other, indicating each sample was sufficiently homogenous and the results of the two labs were indeed comparable. All stable isotope results are reported within an accuracy of 0.1‰ based on analyses of the international standard Vienna Pee Dee Belemnite and in-house keratin (COW1: -13.17‰ ±0.21, UC1: -25.7‰ ±0.14) run alternately every five samples. To assess accuracy of my measurements, duplicates were run every nine samples with an accuracy 0.2‰. All $^{13}C/^{12}C$ are reported in delta notation (δ) in parts per mil (‰).

To establish the general wintering conditions for each individual eider, I generated data on winter conditions for each year by averaging temperatures from January to March in each of the three common eider overwintering areas: Nuuk and Disko Bay in Greenland (Cappelen 2018), and Cartwright in Newfoundland Canada located 45 Km from the nesting colony (Environment and Climate Change Canada 2018). I generated data on spring conditions by averaging the temperature for May from the nearest weather station to the

breeding colony located at Coral Harbour, Southampton Island, Nunavut, Canada (Environment and Climate Change Canada 2018) (Table 1).

5.2.3 Physiological indicator of foraging effort - baseline corticosterone

I included baseline corticosterone (CORT) measured from plasma samples collected at capture in my models given that elevations in baseline CORT have been linked to increases in foraging behaviors, condition gain, and energetic demand during the pre-breeding period (Holberton 1999; Angelier *et al.* 2007; Crossin *et al.* 2012b; Love *et al.* 2014; Hennin *et al.* 2016a). Baseline CORT was measured using an enzyme immunoassay (EIA; Assay Designs, Ann Arbor, MI, USA) previously validated in common eiders breeding at East Bay (Hennin *et al.* 2015). All samples were run in triplicate at 1:20 dilution with 1.5% steroid displacement buffer by volume, in random order and in a 96-well plate. Each plate included a control of laying hen plasma (Sigma–Aldrich Canada, Oakville, Ontario, Canada) and a kit-provided, serially diluted standard curve (200,000 pg/mL). Plates were read at 405 nM. The inter- and intra-plate coefficients of variation were 9.96% and 19.26%, respectively.

5.2.4 Data analysis

To determine overwintering sites of individual arriving common eiders, I used a k-means cluster analysis of the stable isotope data derived from claws of common eiders arriving to the breeding colony (as per Steenweg *et al.* 2017; Chapter 2). The k-means cluster analysis was informed by using the previously published starting centroids calculated from the means of the stable isotope data obtained from wintering common eiders on their wintering sites and I included the winter common eider data in my cluster analysis (Steenweg *et al.* 2017).

I used piecewise structural equation modelling (R package *piecewiseSEM* Version 2.0.1; Lefcheck *et al.* 2019) to test whether environmental variables (winter and/or spring temperatures) directly predicted my response variable (breeding propensity), or whether these relationships were mediated through effects on other variables (i.e., arrival date, CORT, and/or body mass) (Shipley 2013; Lefcheck 2016). This approach allowed us to determine direct and indirect correlations between spring and winter conditions, arrival date, CORT, body mass, and how these together influenced breeding propensity (Lefcheck 2016; Hennin *et al.* 2018).

I constructed nine separate conceptual path models, each with biologically feasible linkages among the variables (Figure 1). These models were then converted to a set of conditional dependencies that were then analysed as generalized linear mixed models with sample number as a random intercept (to account for shared variance in sampling order). I used Gaussian models with identity function (normally distributed data for spring and winter temperatures, arrival date, baseline CORT, and body mass) and standardized the data to allow effects to be compared across the multiple responses and a binomial model with logit function (binomial data; breeding propensity) (Lefcheck 2020). I ranked each model with Akaike Information Criterion (AIC) within *piecewiseSEM* to assess the strongest candidate models. I calculated path coefficients and P-values for these top models. All data analyses were conducted in R version 3.6.2. All R code used for analyses is available in Appendix 5.

5.3 Results

Analyses generated two competitive models within two ΔAIC values of each other (Table 2). The two highest ranked models had similar structures (Figure 2), including negative linkages between unfavourable winter conditions and light body mass, as well as significant positive linkages between warm spring conditions and both early arrival date and heavy body mass.

Contrary to my predictions, colder winter temperature predicted higher body mass, with no effects on arrival dates. Overall, warmer spring temperatures on the breeding grounds predicted earlier arrival dates and higher body mass. Birds with higher body mass were more likely to breed as were those that arrived at the colony earliest (i.e., a predicting higher breeding propensity). Neither of the top models included direct effects of either spring or winter conditions on breeding propensity, these effects were mediated through body mass and arrival date. Nor did the top models include links between spring or winter conditions on CORT, nor CORT on breeding propensity.

5.4 Discussion

I used a multi-year data set to examine the relative contributions of winter and spring environmental conditions on key arrival traits and a key reproductive decision to assess the relative impacts of carryover effects in Arctic-breeding female common eiders. I found that spring conditions had a greater overall influence than winter temperatures on all metrics (2 - 3 times greater); spring temperatures influenced timing of arrival, and body mass, while winter temperatures influenced body mass. Overall, my results indicate that females arriving under favourable spring conditions are able to mitigate negative carryover

effects of challenging winter conditions likely via positive effects on pre-breeding foraging. These results are of particular importance for Arctic-breeding common eiders since both arrival body mass and the subsequent gain in condition on the breeding grounds are both predictors of reproductive decisions and success.

5.4.1 Impacts of spring conditions on condition-dependent breeding decisions

Previous research in this species demonstrated that harsh wintering conditions negatively impact arrival body mass in females (Descamps *et al.* 2010), and that arrival body mass is critical in predicting timing of reproduction, clutch size and hatching success (Descamps *et al.* 2010; Hennin *et al.* 2016a, 2018). However, my path analytical approach suggests that females can overcome these negative impacts of wintering conditions to maintain reproductive investment. I found that warmer spring temperatures advanced arrival dates and increased body mass which then had positive influences on breeding propensity. Female body mass was a key intrinsic variable linking extrinsic environmental conditions (temperatures) to breeding propensity which is consistent with other studies demonstrating the key role body mass plays in mediating reproduction in common eiders (Descamps *et al.* 2010; Hennin *et al.* 2016a, 2018).

It is likely that the strong relationship between spring temperatures on the breeding grounds and subsequent positive effects on reproductive decisions is mediated by local sea ice conditions on the breeding grounds (Jean-Gagnon *et al.* 2018). In years with warmer spring temperatures, there is more available open water and birds also lay earlier (Jean-Gagnon *et al.* 2018), presumably via more extensive foraging opportunities that enable females to bolster their ability to accrue body mass (i.e., fat) to support clutch formation and egg laying. My results also help to mechanistically bolster previous work at this colony

linking warmer spring conditions and earlier breeding phenology and breeding success (Love *et al.* 2010), positive links between elevated pre-breeding fattening rates and earlier lay dates (Hennin *et al.* 2016a), and the importance of elevated body condition in driving the seasonal decline in clutch size in common eiders (Descamps *et al.* 2011).

Interestingly, variation in baseline CORT did not emerge as a significant predictor of breeding propensity. I had anticipated that variation in baseline CORT would be a significant physiological mediator linking winter and/or spring temperatures to breeding propensity, via its role as a metabolic regulator of daily activity, foraging behaviour and body condition gain (Crossin *et al.* 2012b; Hennin *et al.* 2016b). Despite the lack of an apparent significant impact in this study, baseline CORT has been shown to be an important regulator in the energetics of pre-laying eiders (Hennin *et al.* 2015; Hennin 2016). Female common eiders have been shown to increase baseline CORT secretion as they transition from the pre-recruiting to the rapid follicle growth period (Hennin *et al.* 2015) and manipulations of baseline CORT elevated body condition gains in a captive diving sea duck (Hennin *et al.* 2016b). CORT has also been shown advance lay dates and increase breeding success in common eiders (Hennin 2016). In my analysis I needed to include both breeding and non-breeding birds to examine impacts of carryover effects on the probability of breeding within a given year. It is possible that the role of baseline CORT as physiological/energetic mediator may have been diminished by including non-breeding birds, since non-breeders may have little to no need to meet body condition thresholds for breeding. While elevated baseline CORT may not impact the decision of whether to breed or not, it may nonetheless play an important role in driving variation in other important reproductive metrics (Holberton 1999; Angelier *et al.* 2007; Crossin *et al.* 2012b; Hennin

et al. 2016b). For example, in female common eiders rapid fat gain is of critical importance to initiate laying, and the accumulation of energy stores during the pre-breeding period allows them to reach the threshold mass required for reproduction (Hennin *et al.* 2015). The sooner common eiders can accumulate sufficient energy reserves, the earlier they can lay and the larger their investment (i.e., clutch size) can be (Sénéchal *et al.* 2011a; Descamps *et al.* 2011; Hennin *et al.* 2018). In my study, this is supported by the significant, direct relationship between arrival date and breeding propensity in which females that arrived at the breeding grounds earlier were more likely to breed.

Winter temperatures had a nearly significant negative relationship with body mass in female eiders. It may be that in years with colder winter temperatures, common eiders could arrive at the breeding grounds with higher body condition. However, this contradicts previous studies in this species at this breeding colony demonstrating that colder temperatures in winter result in low arrival body condition (Descamps *et al.* 2010). It may not be temperature *per se* that is important, but the actual wintering location itself that is important in predicting body condition. The East Bay breeding population winters in different locations (Newfoundland versus Southwestern Greenland; Mosbech *et al.* 2006; Steenweg *et al.* 2017) that are differentially affected by wintering conditions. In addition, eider diet in Newfoundland contains a higher proportion of mussels (Newfoundland: Goudie and Ankney 1986; Greenland: Merkel *et al.* 2007), which is a preferred diet item due to their higher energy content (Goudie & Ankney 1986; Guillemette 1998; Larsen & Guillemette 2000; Merkel *et al.* 2007). Furthermore, many wintering sites in Newfoundland are closer to the eventual breeding colony than Southwestern Greenland (Mosbech *et al.* 2006). Since the energetic costs of flight in common eiders are high

(Pelletier *et al.* 2008), eiders wintering in different locations may face different costs of migration, have differing quality of prey sources at those wintering sites to fuel migration, and likely a differing ability to carry fat stores with them from the breeding grounds, impacting arrival body condition. Ultimately, although my study is an initial step towards assessing their potential for carryover effects in common eiders, the effects of wintering location are likely very complex.

5.4.2 Impacts and mechanisms of variation in seabird carryover effects

My findings suggest that female common eiders are able to buffer winter carryover effects if they encounter favourable (i.e., warm) spring conditions during their pre-breeding period, which can last upwards of one month after arrival on the breeding grounds (Mosbech *et al.* 2006). In fact, the positive effect of spring temperatures on arrival body mass is more than twice that of winter conditions. During the spring, birds may be able to compensate for the energetic shortfalls resulting from conditions on their overwintering grounds (Merkel et al. 2006; but see Jamieson *et al.* 2005), or the energetic costs stemming from spring migration. Wintering conditions may prevent individuals from forming pairs prior to arrival and recent data suggest that birds may also use this post-arrival spring period for pair formation (Steenweg *et al.* 2019). These results underscore the importance of early timing of arrival to the breeding grounds during the pre-breeding period for proximate energy gain, finding a mate, investment in breeding, and ultimately for fitness benefits.

Winter carryover effects often occur or have the strongest effects in species with a minimal pre-breeding period (i.e. interval between arrival and breeding) and can be further impacted by breeding strategies (i.e., more capital or income based resource use; Meijer and Drent 1999). The pre-laying period is important for gaining sufficient body condition

to fuel egg development across multiple species (i.e. macaroni penguins *Eudyptes chrysolophus*; Crossin *et al.* 2010 and white-winged scoters *Melanitta fusca*; Gurney *et al.* 2014). Overall, common eiders have a relatively long pre-laying period (up to 20 days; Hennin *et al.* 2015), so should have the flexibility to overcome potential carryover effects. In support of this, I found that female eiders are indeed capable of overcoming wintering carryover effects. There are likely individual-based differences in the ability for individuals to compensate for the effects of challenging wintering conditions [e.g., overwintering location (Descamps *et al.* 2010), foraging and assimilation ability (Bond & Esler 2006; Rigou & Guillemette 2010; Heath *et al.* 2010), and physiological fattening rates (Hennin *et al.* 2018)]. Carryover effects may also be exhibited in the common eider's reliance on more capitally (endogenous) or income (exogenous) based energetic reserves. Income breeders are largely affected by prey source availability on the breeding grounds, accordingly they exhibit little to no winter carryover effects at this level (Guillemain *et al.* 2008; Senner *et al.* 2014). However, many birds likely use a combination of capital and income based resources to fuel egg growth by topping up resources as needed by local foraging during pre-breeding (Sénéchal *et al.* 2011b; Descamps *et al.* 2011; Clausen *et al.* 2015; Provencher *et al.* 2016a). The relative contribution of income vs capital stores to follicle growth may depend on wintering conditions and its effects on arrival body condition (Descamps *et al.* 2010). Winter conditions may impact the availability of capitally based stores to allocate to reproduction and potentially result in a greater reliance on income based resources (Sénéchal *et al.* 2011b). The opposing wintering conditions that females at this colony are exposed to at their different overwintering locations (Steenweg *et al.* 2017) may indeed impact their arrival body condition with downstream consequences

for reproductive decisions (Descamps *et al.* 2011; Hennin *et al.* 2016a, 2018; this study). Yet, the consistency and strength of the relationship between spring temperature and arrival condition in my competitive models indicate spring conditions (likely mediating foraging opportunities and the accumulation of capital stores) is the most critical factor affecting the ability of females to invest in reproduction (Descamps *et al.* 2011; Hennin *et al.* 2016a, 2018).

For long-lived marine birds such as the common eider, breeding propensity plays a significant role in contributing to lifetime reproduction. Nonetheless, skipping or deferring breeding may be a viable tactic to deal with variable environmental conditions (Shaw & Levin 2013; Legagneux *et al.* 2016; Öst *et al.* 2018) and may result in increased chances of survival (i.e. trade-off between current and future reproduction; Shoji et al. 2015). Skipping breeding may even increase the likelihood of breeding in the following year (Legagneux *et al.* 2016; Jean-Gagnon *et al.* 2018; see also Catry *et al.* 2013, in shearwaters; Crossin *et al.* 2017, in albatrosses) or allow individuals to capitalize on years with more agreeable conditions, overall increasing their lifetime reproductive output (Coulson 1984; Reed *et al.* 2015). In common eiders, although poor spring conditions could lead to deferred or skipped breeding for some individuals in one year, it is possible that this may carryover to increase a female common eider's body condition the following winter. An increase in body condition would then increase and the likelihood of breeding in the subsequent year. Although challenging, future studies would be able to test these questions and relationships by monitoring individuals across multiple seasons and years.

Table 5. 1. Temperatures at overwintering sites in Newfoundland, Canada and Nuuk and Disko Bay, Greenland, and at the breeding site on Southampton Island, Nunavut in spring for the years 2014-2017. In years where eiders did not winter in the area, temperatures were not applicable (na). Sample size of female common eiders arriving from each overwintering site in each year is denoted by (n).

Year	Winter Temperature (°C)			Spring Temperature (°C)
	Nuuk, Greenland (n)	Disko Bay, Greenland (n)	Newfoundland, Canada (n)	Southampton Island, Nunavut (n)
2014	-7.5 (37)	-10.4 (8)	-13.6 (9)	-3.0 (54)
2015	-11.3 (55)	-17.2 (2)	-16.5 (6)	-7.7 (63)
2016	-5.3 (37)	na	-13.3 (30)	-4.9 (67)
2017	-7.4 (46)	na	-12.2 (17)	-4.2 (63)

Table 5. 2. Comparisons of the path models linking the effects of winter and spring temperatures to circulating baseline CORT, arrival date, body mass and breeding propensity in female common eiders captured at arrival during the pre-breeding season at East Bay Island. The path structure of these models are included in Figure 1 according to their model letter. This analysis includes pre-recruiting, rapid follicle growth and non-breeding birds. Incubating and laying hens were excluded as they did not truly represent 'arriving' birds.

Model Rank	Model	AIC	ΔAIC	Fisher's C	P	df
1	G	46.67	0	20.67	0.11	14
2	H	48.53	1.86	20.53	0.06	12
3	I	51.29	4.62	27.29	0.04	16
4	D	56.02	9.35	22.02	0.14	16
5	C	56.75	10.08	18.75	0.09	12
6	F	58.32	11.65	22.32	0.07	14
7	E	58.45	11.78	24.48	0.08	16
8	B	58.53	11.86	18.53	0.05	10
9	A	59.56	12.89	15.56	0.02	6

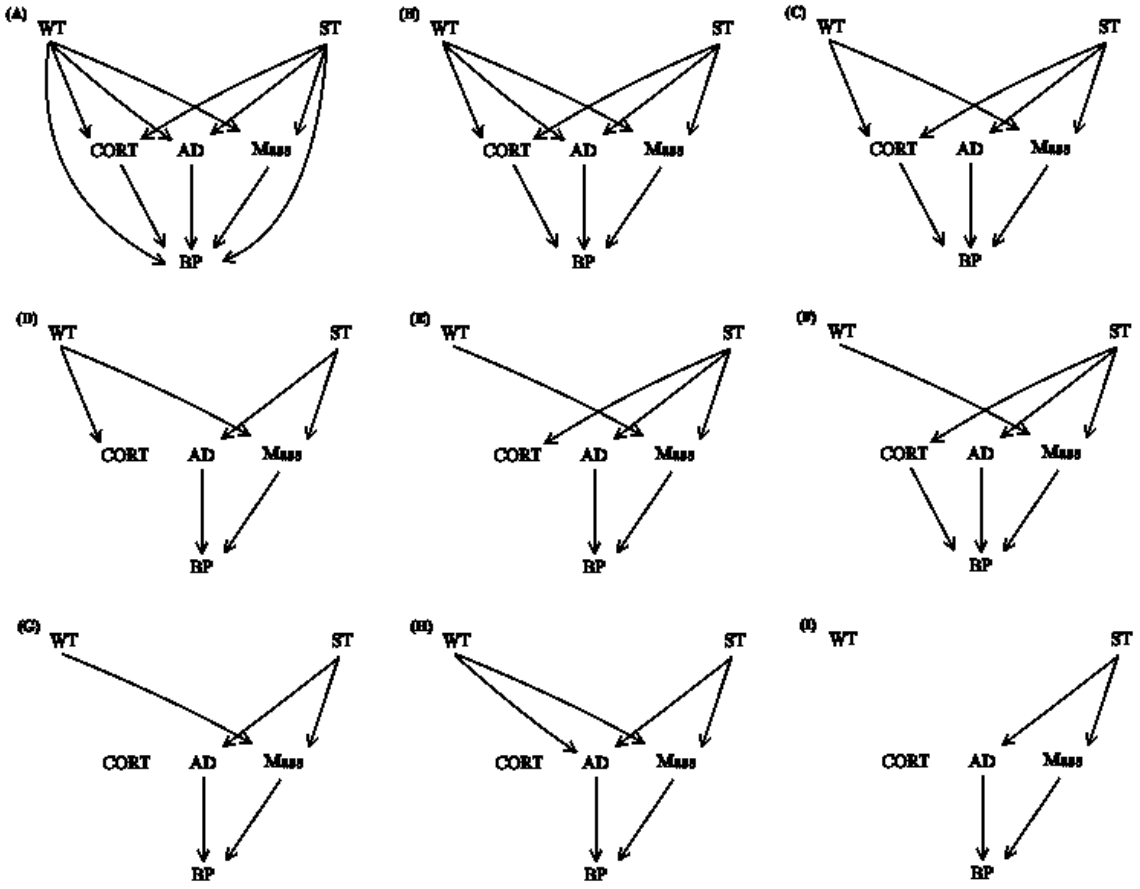

Figure 5. 1. Diagrams of the 9 hypothesized, biologically feasible path models linking environmental conditions to breeding propensity in female common eiders. The variables included in the models are winter temperature (WT), spring temperature (ST), baseline corticosterone (CORT), arrival date (AD), mass, and breeding propensity (BP).

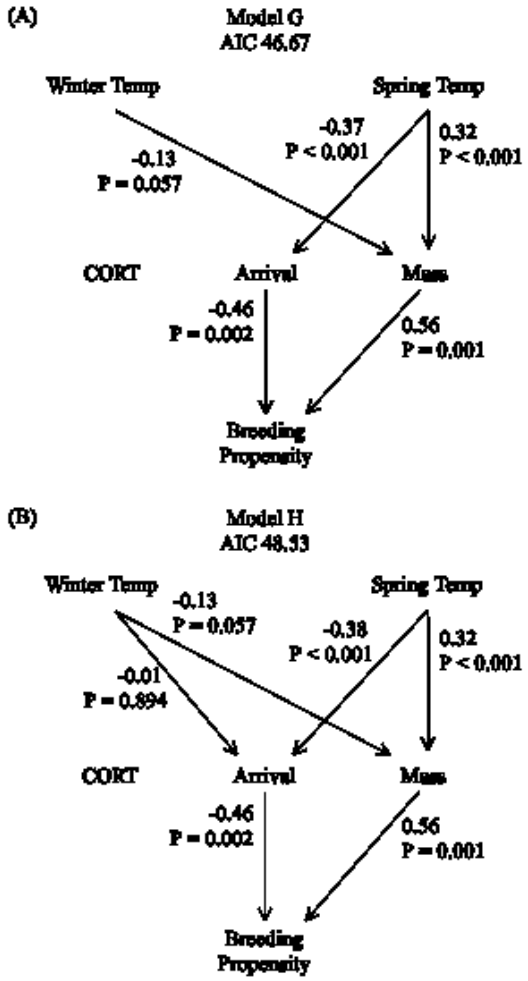

Figure 5. 2. Diagrams of the top two ranked paths as determined by AIC rank linking spring and winter temperatures to breeding propensity. Standardized path coefficients and P-values for each relationship are reported next to its corresponding arrow.

Chapter 6: General Discussion

6.1 Introduction

Carryover effects, or the interactions between different life-history stages, can occur as the experiences in one stage of life affect those in subsequent stages. Carryover effects are multidimensional; these interactions can occur in positive and negative directions (Williams 2012, p. 260) and have environmental (i.e. temperature or availability of food; Clausen *et al.* 2015; Szostek and Becker 2015; Williams *et al.* 2015) and physiological (i.e. ability to accrue and utilize resources or availability of nutrients; Catoni *et al.* 2008; Harrison *et al.* 2011; Crossin *et al.* 2012) mechanisms.

In general, ecological carryover effects are fairly well studied and publications are increasing (O'Connor *et al.* 2014). There are numerous studies available investigating the seasonal carryover effects on breeding (Paredes *et al.* 2005; Drake *et al.* 2013; Gurney *et al.* 2014; Gilmour *et al.* 2015; Salton *et al.* 2015; Shoji *et al.* 2015). The breeding period is inherently important for the recruitment of individuals into the population (Stearns 1976; Zhang *et al.* 2015), so understanding the factors that carryover from one season to another is important from a population perspective (Morrissette *et al.* 2010; Juillet *et al.* 2012). However, to gain a greater understanding of carryover effects and how individuals compensate for previous conditions, it is important to study carryover effects across multiple seasons (so much so that there has been a call for more studies across the annual cycle; Marra *et al.* 2015).

Although investigating carryover effects from one season to another (i.e. winter to migration, or pre-breeding to breeding) may detect impacts, a multi-seasonal approach

should detect interactions between multiple seasons (Van Oudenhove *et al.* 2014; Marra *et al.* 2015). In turn, multi-seasonal approaches further the understanding of how negative conditions may accumulate to have longer-term effects or even the ability for individuals to potentially capitalize on favourable conditions across multiple seasons. In Manx shearwaters *Puffinus puffinus*, for example, individuals were experimentally manipulated to have either shortened or lengthened breeding periods (Fayet *et al.* 2016). The individuals with a shortened breeding period did not show positive effects in the subsequent breeding season, but those with a lengthened breeding period spent less time on the wintering grounds, and had a delayed start to breeding the subsequent year (Fayet *et al.* 2016). This indicates that negative carryover effects of an extended breeding season persisted across multiple seasons, but positive effects did not. For three Afro-European avian migrants, carryover effects from migratory regions affected the timing of breeding more so than conditions on the breeding grounds (Finch *et al.* 2014). Both these studies underscore the importance of following individuals across multiple seasons and not just from one season to the next to understand a greater extent of carryover effects. If either of these studies only followed individuals from one life-stage to another, the carryover effects would not have been clear.

My thesis set out to explore carryover effects in Arctic-breeding common eider ducks across multiple seasons, addressing key knowledge gaps on the influences of seasonal environmental factors on reproduction. I chose to investigate these questions in common eiders for several reasons. First, common eiders are important to northern communities; eider eggs and down are collected from nests for their respective excellent nutritional and insulating properties, and individuals are hunted for sustenance and clothing

(Merkel 2004; Gilliland *et al.* 2009; Robertson 2018). Second, there are several pressures on common eiders such as hunting, cholera (Iverson *et al.* 2016), polar bear predation (Dey *et al.* 2017), parasites (Provencher *et al.* 2017; Vestbo *et al.* 2019a), trace elements (Provencher *et al.* 2016a; Mallory *et al.* 2017), and pollution (Provencher 2013). Finally, research on these species and along these topics has been wide-ranging, yet some fundamental information about what impacts common eider reproductive behaviours has been deficient.

Previously, tracking work has been limited to tagging eiders during the winter or breeding season, however, the impacts these tracking devices have on eider behaviour (mainly skipping breeding) prevented any questions related to carryover effects onto breeding from being answered (Mosbech *et al.* 2006). In addition, the sample sizes for these tracking studies were limited, in turn, the questions that were able to be addressed were also limited. Furthermore, investigating impacts on breeding in common eiders has thus far focused primarily on females. Female common eider body condition is the primary determining factor for the timing of breeding (Hennin *et al.* 2015, 2016a) and clutch size (Descamps *et al.* 2011; Hennin *et al.* 2018), and spring conditions are known to impact female body condition (Love *et al.* 2010; Jean-Gagnon *et al.* 2018). However, research investigating how pairing, male condition, or winter environmental conditions may impact reproductive decisions is lacking.

I aimed to increase the understanding of the underlying mechanisms that influence breeding including the potential for carryover effects of environmental factors across multiple seasons. I focused on developing a method to back-assign arriving eiders to their wintering areas, investigating the timing of pairing, examining changes in male physiology

and condition and its impacts on female reproduction during the pre-breeding period, and the potential for winter and/or spring carryover effects. I strived to fill these knowledge gaps with a combination of laboratory and field based work including capitalizing on methods of stable isotope analysis, integrating observations and tissue sampling of common eiders in the field, and applying my understanding of physiological traits.

My work had four particular objectives, corresponding to each of my four specific data chapters: 1) to establish a minimally invasive and effective method to assign common eiders to their overwintering sites after arrival to their breeding grounds, 2) use this method to gain insight into when common eiders form their pairs and how this may impact female pre-breeding body condition, 3) characterize changes in male physiology and condition throughout the pre-breeding period and how this might influence female reproductive decisions, and 4) to link winter and spring environmental conditions to breeding decisions in female common eiders. Often work investigating these questions can be limited by sample size; specifically, studies investigating carryover effects are often limited by comparing only a few years. My work covers three wintering locations over four years. Below I discuss the results of each of my thesis chapters, how this work informs a greater understanding of common eiders, the broader implications of this work, and research questions that warrant further investigation.

6.2 Summary of research findings

Thus far, carryover effect studies in common eiders generally investigated the effects of either winter (without differentiating between Newfoundland and Greenland eiders) or spring environmental conditions on reproduction and exclusively on female

eiders. With access to a large sample size of pre-breeding eiders at East Bay Island and across multiple years, this colony of sea ducks was an excellent study site to answer questions relating to both male and female eiders and extending carry-over effect questions to include both spring and wintering conditions.

I first set out to link common eiders to their overwintering grounds by applying the stable isotope analysis of carbon in claw tissues. I collected claw and blood samples from common eiders collected on their breeding and wintering grounds. Tissues reflect the stable isotope signatures of the environment in which they were grown. Similar to feathers, claws are an inert keratinous tissue and therefore reflect the stable isotope environment during the time in which they are grown (Oppel & Powell 2010; Steenweg *et al.* 2011; Hénaux *et al.* 2012). The total length of a claw takes about 90-120 days to grow (Hopkins III *et al.* 2013), and so the claw tips from eiders arriving on the breeding grounds that are captured in June, reflect where the eiders were in the winter. I compared the stable isotope values in these claw tips in arrival eiders to claw tips collected from eiders in their overwintering ground, at the end of their overwintering period. Indeed, the stable isotope values of carbon in claw tips from arriving eiders were similar to those collected from wintering birds. Using a cluster analysis, I provided supporting evidence that it is possible to use stable isotopes of carbon to assign an individual to their overwintering site, and successfully re-tested this with the known wintering birds. Using stable isotopes of carbon, I am now able to determine whether common eiders arriving to East Bay Island wintered in Nuuk or Disko Bay Greenland, or Newfoundland, Canada.

Using the novel findings of Chapter Two, in Chapter Three I was able to apply these new methods to resolve the timing of common eider pair formation. Currently it is

assumed that generally, ducks form their pairs six months prior to breeding (Rohwer & Anderson 1988) with northern common eiders forming their pairs in spring (H.G Gilchrist pers. comm. in Goudie *et al.* 2000). However, no specific empirical studies have confirmed the timing of pairing and it is considered to be "poorly documented in North America" (Goudie *et al.* 2000). Using the stable isotopes of carbon in claws (90 - 120 days of growth) and blood (3-4 week turnover; Hahn *et al.* 2012) to represent locations in winter and spring, respectively, I compared the values in both tissues from both the male and female caught as a pair at arrival on the breeding grounds. If the values in claws were similar then these individuals likely paired in the winter, and if the values in claws were dissimilar, but the values in blood were similar, then these pairs formed in spring. Overall, some pairs formed on the wintering grounds, but a majority of pairs formed in the spring. In the year with more challenging winter conditions, pairs that were formed in the spring were more likely to breed than those that paired in the winter likely because of the costs associated with maintaining pair bonds during migration.

Given the results of my third chapter, I next aimed to establish the costs and benefits of being paired on both males and females. To test this, in Chapter Four, I used a suite of physiologic traits to investigate the energetic costs of being paired for males, and if and/or how males confer reproductive benefits to their mate. I found male body condition declined through their female's pre-breeding period and testosterone was highest when females were most fertile and close to laying. Remarkably, I found a lack of association between male physiological state and relative lay date and female fattening rates. Due to the increases in testosterone during the female's most fertile periods, and the lack of impacts of male energetics and workloads on female fattening, these results suggest that males do not confer

benefits to females, but rather that their mate guarding behaviours and energetic investments are primarily aimed towards securing their paternity.

Finally, in Chapter Five, I was able to synthesize the results from my previous chapters to assess whether there are winter and/or spring carryover effects influencing breeding in common eiders. Given that in Chapter Four I found that male body condition did not confer a reproductive benefit to female eiders, I restricted the carryover effects questions to solely address female common eider reproductive behaviours. Using a path analysis, I found that both spring and winter conditions can impact breeding, however, the impacts of wintering conditions had less of an effect on breeding parameters than the impacts of spring conditions. Therefore, eiders are likely able to buffer for poor winter conditions during spring and still invest in reproduction.

Overall my thesis investigates behavioural and environmental impacts on reproductive decisions. Collectively, my findings show, although pairing can occur during winter and spring, males indeed expend energy and effort during the pre-breeding period, and winter conditions can affect breeding, ultimately female reproductive decisions are influenced by spring breeding conditions. This helps demonstrate that conditions during the pre-breeding period are the most important factor for influencing reproductive investment through influences on arrival body condition and the ability for females to accrue fat stores, supporting previous research focusing on spring conditions impacting reproductive parameters (Love *et al.* 2010; Jean-Gagnon *et al.* 2018).

6.3 Broader implications of work

6.3.1 Applications of stable isotope analysis

Although inferring winter origins using stable isotopes is not new in terrestrial systems (Hobson *et al.* 2004; Haché *et al.* 2012; Hénaux *et al.* 2012), and has been applied in both freshwater waterfowl (Yerkes *et al.* 2008; Hopkins III *et al.* 2013) and shorebirds (Catry *et al.* 2016), applications in marine birds are less frequent. My successful application of this method to infer wintering origins from common eiders after arrival to the breeding sites further develops essential marine applications of stable isotope analysis.

Marine birds (i.e. seabirds and sea ducks) are a valuable group with which to expand this technique given that they are often used as indicator species (Diamond & Devlin 2003; Einoder 2009; Mallory *et al.* 2010b; Velarde *et al.* 2019). Marine birds often nest in large breeding populations, however, visiting these remote colonies for field research can be expensive and logistically challenging, especially in the Arctic (Mallory *et al.* 2018). When conducting studies it is imperative to be creative, to obtain the best bang for your buck; meaning obtaining a large sample size in order to maximize benefits for the cost of the research.

Stable isotope analysis of tissues is an exemplary way to increase sample sizes when tracing seasonal movements in marine birds. With most marine birds, it would be possible to collect a suite of tissues (ex: blood, feathers, and claws), that would be associated with multiple time scales and the associated locations. In marine birds that are philopatric to breeding and wintering areas, stable isotope values in those tissues could then be combined with tracking studies to back assign values to those tracked locations. The result would be the ability to increase sample size by also assigning non-tracked individuals by tracing their origins via stable isotopes, associating the stable isotope signatures of specific tissues to locations. These tracking studies would benefit from large

sample sizes in ways that conventional radio-telemetry or GPS based tracking studies do not, and at a much lower expense. The wide implementation of this method by multiple researchers would eventually produce a suite of isotope values for marine birds in many locations. These isotope values could then be used to infer locations for less well tracked species. The limitation of this method is that it would be necessary to test multiple stable isotopes. Unlike terrestrial isoscapes, the geographic variation of stable isotopes in the ocean is less established.

Marine birds inhabit different regions throughout their annual cycle. In turn, they are susceptible to different pressures and their experiences in these locations can reflect how the environment is changing (Provencher 2014). For example, marine birds ingest plastic due to increasing amounts of plastic in the oceans (Wilcox *et al.* 2015; Avery-gomm *et al.* 2018; Provencher *et al.* 2018). As a starting point to investigate where and when individuals are most likely to be exposed to marine plastics, stable isotopes could be used to back assign individuals with high plastic loads and compare those to individuals that are less loaded with plastics. This would elucidate locations with high plastic burdens and could be used to reveal the populations or locations that are most vulnerable to these impacts.

Similar to plastics, parasites and trace elements can occur at different levels, likely also in relation to location. For example, eiders collected on their wintering grounds in Newfoundland and Greenland have different parasitic burdens (Vestbo *et al.* 2019a). However, it is unknown if birds arriving to the East Bay breeding colony that have overwintered in Greenland compared to Newfoundland would actually arrive with different parasitic loads or if the wintering birds sampled had accumulated those burdens at a

different time. Individuals may have accumulated these parasitic burdens on their own breeding grounds that are not necessarily at East Bay Island. Parasitic loads can affect reproductive investment for birds that arrive later in the breeding season (Provencher *et al.* 2017). So, information regarding where they accumulate these parasitic loads would clarify whether other eider colonies experience these effects of parasites as well or if East Bay eiders are particularly burdened by parasites. Likewise, blood lead levels in eiders increased in individuals in lower body condition, limiting investment in reproduction (Provencher *et al.* 2016a). Moreover, hepatic trace element loads and mercury levels vary among common eider nesting colonies (Akearok *et al.* 2010; Pratte *et al.* 2015). Although how hepatic trace elements and mercury vary is a little unclear; mercury levels in eggs may change with latitudes (Akearok *et al.* 2010), while other trace elements may not. It is unclear whether the origins of these burdens is during the fall, winter, or breeding seasons. Stable isotopes in a suite of tissues, indicating different areas could resolve whether parasitic, trace element, or mercury burdens are associated with wintering areas or otherwise.

Because marine birds cover a large area during their annual life-cycle, it is difficult to establish where and when they are exposed to plastics, parasites, and trace elements that can in turn impact their reproductive decisions. I recommend researchers expand their studies to incorporate isotopic analysis of tissues in order to resolve where individuals are mostly commonly exposed to these impacts. Knowing where marine birds are exposed to risks can later be used to better inform decisions on how to alleviate these impacts.

6.3.2 Carryover effects and the pre-breeding period

In common eiders, winter conditions did not impact breeding decisions as much as spring conditions, nonetheless, winter conditions did have some influence on breeding. Although, according to my research on carryover effects, wintering in Newfoundland may be more advantageous, in some years 80-90% of eiders breeding on East Bay Island overwinter in Greenland (Steenweg *et al.* 2017). As I suggest in Chapter 2, I suspect that Greenland has been acting as a source population for the East Bay colony following the decline in population as a result of extensive hunting, of an avian cholera outbreak and the continued predation from polar bears. Eiders were extensively hunted in Western Greenland until a hunting quota was initiated. The breeding population in Greenland has grown by approximately 12% per year since these quotas were implemented (Merkel 2010). However, cholera has been detected in Newfoundland and has been prevalent on East Bay Island (Iverson *et al.* 2016) but has yet to be detected in Greenland (F. Merkel pers. Comm.). Wintering populations from Newfoundland and Greenland congregate at the breeding grounds, but it appears that these interactions were not sufficient to introduce cholera to Greenland when eiders returned to wintering areas. Although stable isotope analysis of claws would have been able to answer questions regarding source and sink populations, unfortunately the long-term sample collection at this breeding site does not include claws, only feathers and blood. Yet it might still be possible to investigate potential genetic links specific to eiders in Greenland and Newfoundland, whether these winter source populations are distinct, and if this has changed throughout the history of the breeding colony. This would reveal whether eiders from Greenland acted as a source population for East Bay eiders and whether the composition of the colony has changed through time.

Common eider pre-breeding physiology and the factors that influence breeding have been well documented (physiology: Senechal et *al.* 2011; Jaatinen *et al.* 2016; Hennin et *al.* 2018, resource allocation: Parker and Holm 1990; Oosterhuis and Van Dijk 2002; Hobson *et al.* 2015, disturbance: Legagneux *et al.* 2016; Öst *et al.* 2018). My thesis further adds to this body of knowledge by describing effects of males on female condition (or lack thereof). Contrary to what seemed probable while observing male eider's mate guarding behaviours around the breeding colony, and contradicting observational studies suggesting males protect females from foraging interruptions by other males (Christensen 2000), I found that males do not impact female breeding investments or body condition. Despite male common eider energetic investments, their behaviours were likely primarily to secure paternity, as suggested by other behavioural studies (Hario & Hollmén 2004). To specifically investigate these questions, it would be useful to link male investment in mate-guarding and the paternity of eggs in the clutch. This would allow us to answer the questions of whether these behaviours make a substantial difference in the male's ability to maintain paternity.

Given that males may not positively contribute to female body condition gain and do not participate in chick rearing, based on my current results it is unclear what male traits are being favoured by females. Females are likely selecting males based on some measure of quality, whether it is via body condition, assortative mating, or some other measure, in order to mate with a male that would yield the fittest offspring. It could be possible that males expending higher energy, as shown by declining body condition, during the pre-breeding season, could be of higher quality. It's possible that, although female body condition is not impacted by males, the impacts of male body condition are later seen as an

investment in higher quality offspring. Further research investigating these concepts is required to establish what male traits result in higher quality offspring.

Further, I show that although winter conditions have some impact on pre-breeding body condition, environmental conditions during the pre-breeding period is the most important factor influencing common eider breeding propensity through its effects on body condition. It is likely that common eiders are able to compensate for poor winter conditions during spring. This is similar to geese, where in some years when individuals which were experimentally delayed during migration, were able to compensate for losses accrued during favourable pre-breeding conditions, but in more challenging years they were unable to compensate (Legagneux *et al.* 2012). Favourable spring conditions can increase an eider's likelihood of breeding; warmer temperatures mean that there are more available resources for common eiders to gain the body condition necessary to last through incubation. As a result, eiders may be particularly vulnerable to environmental changes that may occur during this period but may also benefit from seasonally increasing temperatures.

6.4 Influences of climate change on common eider populations

Although presumably warmer temperatures in spring may increase the common eiders' capacity to accrue energetic resources (Mallory *et al.* 2010a), eiders are at odds with increasing polar bear predation on their breeding colony due to reduced Arctic sea ice (Iverson *et al.* 2014; Dey *et al.* 2017). It would appear that the impacts climate change may have on common eider ducks is complicated. Colonies with a lower density of breeding

eiders are subject to lower rates of predation because it is not as profitable for polar bears to eat common eider eggs when they are dispersed (Iverson *et al.* 2014).

Climate change and reduction of sea ice may have other impacts on common eider populations. For instance, in Southwest Greenland, the annual estimate of bird casualties from collisions with shipping traffic is 2 000 birds (Vestbo *et al.* 2019b). Presumably with further reductions in sea ice, shipping traffic will increase (Smith & Stephenson 2013). Wintering populations of common eiders will be more at risk of collisions than previously.

Continued monitoring of this common eider population is required to understand how they may respond to these climatic changes. As eiders respond to these pressures (bear predation and shipping traffic) and likely begin dispersing to other smaller islands, it would be interesting to see if these dispersal patterns are indeed enough to allow common eider populations to continue in Northern Hudson Bay and the Hudson Straight and to see if their winter origination patterns persist across this geographic scale.

References

Akearok, J.A., Hebert, C.E., Braune, B.M. & Mallory, M.L. (2010). Inter- and intraclutch variation in egg mercury levels in marine bird species from the Canadian Arctic. *Science of the Total Environment, The*, **408**, 836–840.

Alerstam, T. (2006). Strategies for the transition to breeding in time-selected bird migration. *ARDEA*, **94**, 347–357.

Amrhein, V., Trafimow, D. & Greenland, S. (2019). Inferential statistics as descriptive statistics: There is no replication crisis if we don't expect replication. *The American Statistician*, **73**, 262–270.

Angelier, F., Shaffer, S.A., Weimerskirch, H., Trouvé, C. & Chastel, O. (2007). Corticosterone and foraging behavior in a pelagic seabird. *Physiological and biochemical zoology*, **80**, 283–92.

Avery-gomm, S., Borrelle, S.B. & Provencher, J.F. (2018). Linking plastic ingestion research with marine wildlife conservation. *Science of the Total Environment*, **637–638**, 1492–1495.

Ballesteros, M., Bardsen, B.J., Fauchald, P., Langeland, K., Stien, A. & Tveraa, T. (2013). Combined effects of long-term feeding, population density and vegetation green-up on reindeer demography. *Ecosphere*, **4**, 1–13.

Barnes, A.I. & Partridge, L. (2003). Costing reproduction. *Animal Behaviour*, **66**, 199–204.

Bearhop, S., Waldron, S., Votier, S.C. & Furness, R.W. (2002). Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology*, **75**, 451–8.

Bêty, J., Gauthier, G. & Giroux, J.F. (2003). Body condition, migration, and timing of reproduction in snow geese: a test of condition-dependent model of optimal clutch size. *The American Naturalist*, **162**, 110–121.

Birkhead, T.R. & Møller, A.P. (1996). Monogamy and sperm competition in birds. *Partnerships in Birds* (ed J. Black). Oxford University Press, Oxford.

Bluhm, C.K. (1988). Temporal patterns of pair formation and reproduction in annual cycles and associated endocrinology in waterfowl. *Current Ornithology Volume 5* (ed R.F. Johnston), pp. 123–186. Plenum Press, New York.

Bluhm, C.K., Phillips, R.E., Burke, W.H. & Gupta, G.N. (1984). Effects of male courtship and gonadal steroids on pair formation, egg-laying, and serum LH in Canvasback ducks (*Aythya valisineria*). *Journal of Zoology*, **204**, 185–200.

Blums, P. & Mednis, A. (1996). Secondary sex ratio in anatinae. *The Auk*, **113**, 505–511.

Bond, A.L. & Diamond, A.W. (2011). Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecological Applications*, **21**, 1017–1023.

Bond, J.C. & Esler, D. (2006). Nutrient acquisition by female Harlequin ducks prior to spring migration and reproduction: evidence for body mass optimization. *Canadian Journal of Zoology*, **84**, 1223–1229.

Bond, J.C., Esler, D. & Hobson, K.A. (2007). Isotopic evidence for sources of nutrients allocated to clutch formation by Harlequin ducks. *The Condor*, **109**, 698–704.

Bond, A.L. & Jones, I.L. (2009). A practical introduction to stable-isotope analysis for seabird biologists: approaches, cautions and caveats. *Marine Ornithology*, **37**, 183–188.

Bottitta, G.E., Nol, E. & Gilchrist, H.G. (2003). Effects of experimental manipulation of incubation length on behavior and body mass of common eiders in the Canadian Arctic. *Waterbirds*, **26**, 100–107.

Bourgeon, S., Kauffmann, M., Geiger, S., Raclot, T. & Robin, J. (2010). Relationships between metabolic status, corticosterone secretion and maintenance of innate and adaptive humoral immunities in fasted re-fed mallards. *The Journal of experimental biology*, **213**, 3810–3818.

Bourgeon, S., Martínez, J., Criscuolo, F., Le Maho, Y. & Raclot, T. (2006). Fasting-induced changes of immunological and stress indicators in breeding female eiders. *General and comparative endocrinology*, **147**, 336–42.

Bowen, G.J. (2010). Isoscapes: Spatial Pattern in Isotopic Biogeochemistry. *Annu. Rev. Earth Planet. Sci*, **38**, 161–87.

Bowen, G.J., Wassenaar, L.I. & Hobson, K.A. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, **143**, 337–48.

Bowlin, M.S., Henningsson, P., Muijres, F.T., Vleugels, R.H.E., Liechti, F. & Hedenström, A. (2010). The effects of geolocator drag and weight on the flight ranges of small migrants. *Methods in Ecology and Evolution*, **1**, 398–402.

Boyd, W.S., Bowman, T.D., Savard, J.-P.L. & Dickson, R.D. (2015). Conservation of North American sea ducks. *Ecology and conservation of North American Sea Ducks. Studies in Avian Biology* (eds J.-P.L. Savard, D. V. Derksen, D. Esler & J.M. Eadie), pp. 529–559. CRC Press, Boca Raton, FL.

Brown, D.R. & Sherry, T.W. (2006). Food supply controls the body condition of a migrant bird wintering in the tropics. *Oecologia*, **149**, 22–32.

Burger, A.A.E. & Shaffer, S.A. (2008). Application of tracking and data-logging technology in research and conservation of seabirds. *The Auk*, **125**, 253–264.

Burnett, N.J., Hinch, S.G., Bett, N.N., Braun, D.C., Casselman, M.T., Cooke, S.J., Gelchu, A., Lingard, S., Middleton, C.T. & White, C.F.H. (2017). Reducing carryover effects on the migration and spawning success of sockeye salmon through a management experiment of dam flows. *River Research and Applications*, **33**, 3–15.

Cappelen, J. (2018). *Weather observations from Greenland 1958 - 2017*.

Catoni, C., Peters, A. & Schaefer, H.M. (2008). Life history trade-offs are influenced by

the diversity, availability and interactions of dietary antioxidants. *Animal Behaviour*, **76**, 1107–1119.

Catry, T., Lourenc, P.M., Lopes, R.J., Bocher, P., Carneiro, C., Alves, J.A., Delaporte, P., Bearhop, S., Piersma, T. & Granadeiro, J.P. (2016). Use of stable isotope fingerprints to assign wintering origin and trace shorebird movements along the East Atlantic Flyway. *Basic and Applied Ecology*, **17**, 177–187.

Cherel, Y., Corre, M., Jaquemet, S., Ménard, F., Richard, P. & Weimerskirch, H. (2008). Resource partitioning within a tropical seabird community: new information from stable isotopes. *Marine Ecology Progress Series*, **366**, 281–291.

Choinière, L. & Gauthier, G. (1995). Energetics of reproduction in female and male greater snow geese. *Oecologia*, **103**, 379–389.

Christensen, T.K. (2000). Female pre-nesting foraging and male vigilance in common eider *Somateria mollissima*. *Bird Study*, **47**, 311–319.

Clark, R.G., Hobson, K.A. & Wassenaar, L.I. (2006). Geographic variation in the isotopic (dD, d13C, d15N, d34S) composition of feathers and claws from lesser scaup and northern pintail: implications for studies of migratory connectivity. *Canadian Journal of Zoology*, **1401**, 1395–1401.

Clausen, K.K., Madsen, J. & Tombre, I.M. (2015). Carry-over or compensation? The impact of winter harshness and post-winter body condition on spring-fattening in a migratory goose species. *PLOS ONE*, **10**, e0132312.

Coma, R. & Ribes, M. (2003). Seasonal energetic constraints in Mediterranean benthic suspension feeders: effects at different levels of ecological organization. *Oikos*, **101**, 205–215.

Cooke, F., Finney, G.H. & Rockwell, R.F. (1976). Assortative mating in lesser snow geese (Anser caerulescens). *Behavior Genetics*, **6**, 127–140.

Coulson, J.C. (1984). The population dynamics of the Eider Duck *Somateria mollissima* and evidence of extensive non-breeding by adult ducks. *Ibis*, **126**, 525–543.

Crossin, G.T., Cooke, S.J., Goldbogen, J.A. & Phillips, R.A. (2014). Tracking fitness in marine vertebrates: current knowledge and opportunities for future research. *Marine Ecology Progress Series*, **496**, 1–17.

Crossin, G.T., Phillips, R.A., Lattin, C.R., Romero, L.M., Bordeleau, X., Harris, C.M., Love, O.P. & Williams, T.D. (2017). Costs of reproduction and carry-over effects in breeding albatrosses. *Antarctic Science*, **29**, 155–164.

Crossin, G.T., Phillips, R.A., Trathan, P.N., Fox, D.S., Dawson, A., Wynne-Edwards, K.E. & Williams, T.D. (2012a). Migratory carryover effects and endocrinological correlates of reproductive decisions and reproductive success in female albatrosses. *General and comparative endocrinology*, **176**, 151–7.

Crossin, G.T., Phillips, R.A., Wynne-Edwards, K.E. & Williams, T.D. (2013). Postmigratory body condition and ovarian steroid production predict breeding decisions by female gray-headed albatrosses. *Physiological and Biochemical Zoology*, **86**, 761–8.

Crossin, G.T., Trathan, P.N., Phillips, R.A., Dawson, A., Le Bouard, F. & Williams, T.D. (2010). A carryover effect of migration underlies individual variation in reproductive readiness and extreme egg size dimorphism in Macaroni penguins. *The American Naturalist*, **176**, 357–366.

Crossin, G.T., Trathan, P.N., Phillips, R.A., Gorman, K.B., Dawson, A., Sakamoto, K.Q. & Williams, T.D. (2012b). Corticosterone predicts foraging behavior and parental care in macaroni penguins. *The American naturalist*, **180**, E31-41.

Delestrade, A. (2001). Sexual size dimorphism and positive asortative mating in alpine choughs (*Pyrrhocorax graculus*). *The Auk*, **118**, 553–556.

Descamps, S., Bêty, J., Love, O.P. & Gilchrist, H.G. (2011). Individual optimization of reproduction in a long-lived migratory bird: a test of the condition-dependent model of laying date and clutch size. *Functional Ecology*, **25**, 671–681.

Descamps, S., Gilchrist, H.G., Bêty, J., Buttler, E.I. & Forbes, M.R. (2009). Costs of reproduction in a long-lived bird: large clutch size is associated with low survival in the presence of a highly virulent disease. *Biology letters*, **5**, 278–81.

Descamps, S., Yoccoz, N.G., Gaillard, J.-M., Gilchrist, H.G., Erikstad, K.E., Hanssen, S.A., Cazelles, B., Forbes, M.R. & Bêty, J. (2010). Detecting population heterogeneity in effects of North Atlantic Oscillations on seabird body condition: get into the rhythm. *Oikos*, **119**, 1526–1536.

Dey, C.J., Richardson, E., Mcgeachy, D., Iverson, S.A., Gilchrist, H.G. & Semeniuk, C.A.D. (2017). Increasing nest predation will be insufficient to maintain polar bear body condition in the face of sea ice loss. *Global Change Biology*, **23**, 1821–1831.

Diamond, A.W. & Devlin, C.M. (2003). Seabirds as indicators of changes in marine ecosystems: ecological monitoring on Machias Seal Island. *Environmental Monitoring and Assessment*, **88**, 153–75.

Drake, A., Rock, C., Quinlan, S.P. & Green, D.J. (2013). Carry-over effects of winter habitat vary with age and sex in yellow warblers *Setophaga petechia*. *Journal of Avian Biology*, **44**, 321–330.

Drent, R., Both, C., Green, M., Madsen, J. & Piersma, T. (2003). Pay-offs and penalties of competing migratory schedules. *Oikos*, **103**, 274–292.

Drent, R.H. & Daan, S. (1980). The prudent parent: energetic adjustments in avian breeding. *Ardea*, **68**, 225–252.

Drent, R.H., Fox, A.D. & Stahl, J. (2006). Traveling to breed. *Journal of Ornithology*, **147**, 122–134.

Einoder, L.D. (2009). A review of the use of seabirds as indicators in fisheries and ecosystem management. *Fisheries Research*, **95**, 6–13.

Environment and Climate Change Canada. (2018). Historical Data. *Government of Canada*. URL http://climate.weather.gc.ca/historical_data/search_historic_data_e.html [accessed 6 November 2018]

Esler, D. & Bond, J.C. (2010). Cross-seasonal dynamics in body mass of male Harlequin ducks: a strategy for meeting costs of reproduction. *Canadian Journal of Zoology*, **88**, 224–230.

Fayet, A.L., Freeman, R., Shoji, A., Kirk, H.L., Padget, O., Perrins, C.M. & Guilford, T. (2016). Carry-over effects on the annual cycle of a migratory seabird: An experimental study. *Journal of Animal Ecology*, **85**, 1516–1527.

Festa-Bianchet, M., Côté, S., Hamel, S. & Pelletier, F. (2019). Long-term studies of bighorn sheep and mountain goats reveal fitness costs of reproduction. *Journal of Animal Ecology*, **88**, 1118–1133.

Finch, T., Pearce-Higgins, J.W., Leech, D. & Evans, K.L. (2014). Carry-over effects from passage regions are more important than breeding climate in determining the breeding phenology and performance of three avian migrants of conservation concern. *Biodiversity and Conservation*, **23**, 2427–2444.

Foerster, K. & Kempenaers, B. (2005). Effects of testosterone on male-male competition and male-female interactions in blue tits. *Behav*, **57**, 215–223.

Forero, M.G. & Hobson, K.A. (2003). Using stable isotopes of nitrogen and carbon to study seabird ecology: applications in the Mediterranean seabird community. *Scientia Marina*, **67**, 23–32.

Fowler, G.S. (1995). Stages of age-related reproductive success in birds: simultaneous effects of age, pair-bond duration and reproductive experience. *American Zoologist*, **35**, 318–328.

Fusani, L. (2008). Testosterone control of male courtship in birds. *Hormones and Behavior*, **54**, 227–233.

Garcia-Perez, B. & Hobson, K.A. (2014). A multi-isotope (d2H , d13C , d15N) approach to establishing migratory connectivity of Barn Swallow (Hirundo rustica). *Ecosphere*, **5**, 1–12.

Gaston, A.J., Gilchrist, H.G. & Hipfner, J.M. (2005). Climate change, ice conditions and reproduction in an Arctic nesting marine bird: Brunnich's guillemot (*Uria lomvia*). *Journal of Animal Ecology*, **74**, 832–841.

Gilliland, S.G., Gilchrist, H.G., Rockwell, R.F., Robertson, G.J., Savard, J.-P.L., Merkel, F.R. & Mosbech, A. (2009). Evaluating the sustainability of harvest among northern common eiders *Somateria mollissima borealis* in Greenland and Canada. *Wildlife Biology*, **15**, 24–36.

Gilmour, M.E., Lattin, C.R., Romero, L.M., Haussmann, M.F., Mauck, R.A. & Dearborn, D.C. (2015). Finding the best predictor of reproductive performance of Leach's storm-petrels. *The Auk*, **132**, 191–205.

Goudie, I.R. & Ankney, C.D. (1986). Body size, activity budgets, and diets of sea ducks wintering in Newfoundland. *Ecology*, **67**, 1475–1482.

Goudie, I.R., Robertson, G.J. & Reed, A. (2000). Common Eider (*Somateria mollissima*). *The Birds of North America Online*. URL http://bna.birds.cornell.edu/bna/species/546

Graham, B.S., Koch, P.L., Newsome, S.D., McMahon, K.W. & Aurioles, D. (2010). Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. *Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping* (eds J.B. West, G.J. Bowen, T.E. Dawson & K.P. Tu), pp. 299–318. Springer Science + Business Media B.V.

Greenberg, R.S. & Marra, P.P. (Eds.). (2005). *Birds of Two Worlds: The Ecology and Evolution of Migration*. The Johns Hopkins University Press, Baltimore and London.

Guéry, L., Descamps, S., Pradel, R., Hanssen, S.A., Erikstad, K.E., Gabrielsen, G.W., Gilchrist, H.G. & Bêty, J. (2017). Hidden survival heterogeneity of three common eider populations in response to climate fluctuations. *Journal of Animal Ecology*, **86**, 683–693.

Guillemain, M., Caldow, R.W.G., Hodder, K.H. & Goss-Custard, J.D. (2003). Increased vigilance of paired males in sexually dimorphic species: Distinguishing between alternative explanations in wintering Eurasian wigeon. *Behavioral Ecology*, **14**, 724–729.

Guillemain, M., Elmberg, J., Arzel, C., Johnson, A.R. & Simon, G. (2008). The income-capital breeding dichotomy revisited: late winter body condition is related to breeding success in an income breeder. *Ibis*, **150**, 172–176.

Guillemette, M. (1998). The effect of time and digestion constraints in common eiders while feeding and diving over blue mussel beds. *Functional Ecology*, **12**, 123–131.

Gunnarsson, T.G., Gill, J.A., Newton, J., Potts, P.M. & Sutherland, W.J. (2005). Seasonal matching of habitat quality and fitness in a migratory bird. *Proceedings of the Royal Society Biological Sciences*, **272**, 2319–2323.

Gurney, K.E.B., Wood, C.J., Alisauskas, R.T., Wayland, M., DeVink, J.-M.A. & Slattery, S.M. (2014). Identifying carry-over effects of wintering area on reproductive parameters in white-winged scoters: an isotopic approach. *The Condor*, **116**, 251–264.

Haché, S., Hobson, K.A., Villard, M.-A. & Bayne, E.M. (2012). Assigning birds to geographic origin using feather hydrogen isotope ratios (δ2H): importance of year, age, and habitat. *Canadian Journal of Zoology*, **90**, 722–728.

Hahn, S., Hoye, B.J., Korthals, H. & Klaassen, M. (2012). From food to offspring down: tissue-specific discrimination and turn-over of stable isotopes in herbivorous waterbirds and other avian foraging guilds. *PLoS ONE*, **7**, 1–6.

Hansen, J.H., Hedeholm, R.B., Sünksen, K., Christensen, J.T. & Grønkjær, P. (2012). Spatial variability of carbon (δ13C) and nitrogen (δ15D) stable isotope ratios in an Arctic marine food web. *Marine Ecology Progress Series*, **467**, 47–59.

Hanssen, S.A., Folstad, I. & Erikstad, K.E. (2006). White plumage reflects individual quality in female eiders. *Animal Behaviour*, **71**, 337–343.

Hario, M. & Hollmén, T.E. (2004). The role of male mate-guarding in pre-laying common eiders *Somateria mollissima* in the northern Baltic Sea. *Ornis Fennica*, **81**,

119–127.

Harms, N.J., Legagneux, P., Gilchrist, H.G., Bêty, J., Love, O.P., Forbes, M.R., Bortolotti, G.R. & Soos, C. (2015). Feather corticosterone reveals effect of moulting conditions in the autumn on subsequent reproductive output and survival in an Arctic migratory bird. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20142085.

Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. & Bearhop, S. (2011). Carry-over effects as drivers of fitness differences in animals. *The Journal of Animal Ecology*, **80**, 4–18.

Heath, J.P., Gilchrist, H.G. & Ydenberg, R.C. (2010). Interactions between rate processes with different timescales explain counterintuitive foraging patterns of arctic wintering eiders. *Proceedings. Biological sciences / The Royal Society*, **277**, 3179–86.

Heitmeyer, M.E. (1995). Influences of age , body condition , and structural size on mate selection by dabbling ducks. *Canadian Journal of Zoology*, **73**, 2251–2258.

Hénaux, V., Powell, L.A., Vrtiska, M.P. & Hobson, K.A. (2012). Establishing winter origins of migrating lesser snow geese using stable isotopes. *Avian Conservation and Ecology*, **7**, 5.

Hennin, H.L. (2016). *Energetic physiology mediates reproductive decisions in a long-lived , capital-income breeding seaduck*. University of Windsor.

Hennin, H.L., Bêty, J., Legagneux, P., Gilchrist, H.G., Williams, T.D. & Love, O.P. (2016a). Energetic physiology mediates individual optimization of breeding phenology in a migratory Arctic seabird. *The American Naturalist*, **188**, 434–445.

Hennin, H.L., Dey, C.J., Bêty, J., Gilchrist, H.G., Legagneux, P., Williams, T.D. & Love, O.P. (2018). Higher rates of prebreeding condition gain positively impacts clutch size: A mechanistic test of the condition-dependent individual optimization model. *Functional Ecology*, **32**, 2019–2028.

Hennin, H.L., Legagneux, P., Bêty, J., Williams, T.D., Gilchrist, H.G., Baker, T.M. & Love, O.P. (2015). Pre-breeding energetic management in a mixed-strategy breeder. *Oecologia*, **177**, 235–243.

Hennin, H.L., Wells-berlin, A.M. & Love, O.P. (2016b). Baseline glucocorticoids are drivers of body mass gain in a diving seabird. *Ecology and Evolution*, **6**, 1702–1711.

Hipes, D.L. & Hepp, G.R. (1995). Nutrient-reserve dynamics of breeding male wood ducks. *The Condor*, **97**, 451–460.

Hobson, K.A. (1999). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, **120**, 314–326.

Hobson, K.A., Atwell, L. & Wassenaar, L.I. (1999). Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proceedings of the National Academy of Science: Ecology*, **96**, 8003–8006.

Hobson, K.A., Bowen, G.J., Wassenaar, L.I., Ferrand, Y. & Lormee, H. (2004). Using

stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. *Oecologia*, **141**, 477–88.

Hobson, K.A., Gloutney, M.L. & Gibbs, H.L. (1997). Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. *Canadian Journal of Zoology*, **75**, 1720–1723.

Hobson, K.A., Jaatinen, K. & Öst, M. (2015). Differential contributions of endogenous and exogenous nutrients to egg components in wild Baltic common eiders (*Somateria mollissima*): A test of alternative stable isotope approaches. *The Auk*, **132**, 624–633.

Hobson, K.A. & Jehl, J.R. (2010). Arctic waders and the capital-income continuum: Further tests using isotopic contrasts of egg components. *Journal of Avian Biology*, **41**, 565–572.

Hobson, K.A., Van Wilgenburg, S.L., Wassenaar, L.I. & Larson, K. (2012). Linking hydrogen (δ2H) isotopes in feathers and precipitation: sources of variance and consequences for assignment to isoscapes. *PloS ONE*, **7**, e35137.

Holberton, R.L. (1999). Changes in patterns of corticosterone secretion concurrent with migratory fattening in a Neotropical migratory bird. *General and comparative endocrinology*, **116**, 49–58.

Hopkins III, J.B., Cutting, K.A. & Warren, J.M. (2013). Use of stable isotopes to investigate keratin deposition in the claw tips of ducks. *PLoS ONE*, **8**, 1–7.

Imlay, T.L., Angelier, F., Hobson, K.A., Mastromonaco, G., Saldanha, S. & Leonard, M.L. (2019). Multiple intrinsic markers identify carry-over effects from wintering to breeding sites for three Nearctic–Neotropical migrant swallows. *The Auk*, **136**, 1–15.

Iverson, S.A., Forbes, M.R., Simard, M., Soos, C. & Gilchrist, H.G. (2016). Avian Cholera emergence in Arctic-nesting northern Common Eiders: using community-based, participatory surveillance to delineate disease outbreak patterns and predict transmission risk. *Ecology and Society*, **21**.

Iverson, S.A., Gilchrist, H.G., Smith, P.A., Gaston, A.J. & Forbes, M.R. (2014). Longer ice-free seasons increase the risk of nest depredation by polar bears for colonial breeding birds in the Canadian Arctic. *Proceedings of the Royal Society Biological Sciences*, **281**, 1–9.

Jaatinen, K., Öst, M. & Hobson, K.A. (2016). State-dependent capital and income breeding: a novel approach to evaluating individual strategies with stable isotopes. *Frontiers in Zoology*, **13**, 1–8.

Jamieson, S.E., Gilchrist, H.G., Merkel, F.R., Diamond, A.W. & Falk, K. (2005). Endogenous reserve dynamics of northern common eiders wintering in Greenland. *Polar Biology*, **29**, 585–594.

Jamieson, S.E., Gilchrist, H.G., Merkel, F.R., Falk, K. & Diamond, A.W. (2006). An evaluation of methods used to estimate carcass composition of common eiders *Somateria mollissima*. *Wildlife Biology*, **12**, 219–226.

Jean-Gagnon, F., Legagneux, P., Gilchrist, H.G., Bélanger, S., Love, O.P. & Bêty, J.

(2018). The impact of sea ice conditions on breeding decisions is modulated by body condition in an arctic partial capital breeder. *Oecologia*, **186**, 1–10.

Jenni-Eiermann, S. & Jenni, L. (1994). Plasma metabolite levels predict individual body-mass changes in a small long-distance migrant, the garden warbler. *The Auk*, **111**, 888–899.

Johnson, A.S., Perz, J., Nol, E. & Senner, N.R. (2016). Dichotomous strategies? The migration of Whimbrels breeding in the eastern Canadian sub-Arctic. *Journal of Field Ornithology*, **87**, 371–383.

Juillet, C., Choquet, R., Gauthier, G., Lefebvre, J. & Pradel, R. (2012). Carry-over effects of spring hunt and climate on recruitment to the natal colony in a migratory species. *Journal of Applied Ecology*, **49**, 1237–1246.

Kopp, M., Servedio, M.R., Mendelson, T.C., Safran, R.J., Rodríguez, R.L., Hauber, M.E., Scordato, E.C., Symes, L.B., Balakrishnan, C.N., Zonana, D.M. & Van Doorn, G.S. (2018). Mechanisms of assortative mating in speciation with gene flow: Connecting theory and empirical research. *The American naturalist*, **191**, 1–20.

Lamarre, V., Franke, A., Love, O.P., Legagneux, P. & Bêty, J. (2017). Linking pre-laying energy allocation and timing of breeding in a migratory arctic raptor. *Oecologia*, **183**, 653–666.

Larsen, J. & Guillemette, M. (2000). Influence of annual variation in food supply on abundance of wintering common eiders *Somateria mollissima*. *Marine Ecology Progress Series*, **201**, 301–309.

Lefcheck, J.S. (2016). PiecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, **7**, 573–579.

Lefcheck, J.S. (2020). Structural Equation Modeling in R for Ecology and Evolution.

Lefcheck, J.S., Byrnes, J. & Grace, J. (2019). piecewiseSEM 2.1.0.

Legagneux, P., Fast, P.L.F. & Gauthier, G. (2012). Manipulating individual state during migration provides evidence for carry-over effects modulated by environmental conditions. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 876–883.

Legagneux, P., Hennin, H.L., Gilchrist, H.G., Williams, T.D., Love, O.P. & Bêty, J. (2016). Unpredictable perturbation reduces breeding propensity regardless of pre-laying reproductive readiness in a partial capital breeder. *Journal of Avian Biology*, 880–886.

Lehikoinen, A., Christensen, T.K., Öst, M., Kilpi, M., Saurola, P. & Vattulainen, A. (2008). Large-scale change in the sex ratio of a declining eider *Somateria mollissima* population. *Wildlife Biology*, **14**, 288–301.

Lehikoinen, A., Kilpi, M. & Öst, M. (2006). Winter climate affects subsequent breeding success of common eiders. *Global Change Biology*, **12**, 1355–1365.

Lott, C.A., Meehan, T.D. & Heath, J.A. (2003). Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: influence of marine prey base. *Oecologia*, **134**, 505–510.

Love, O.P., Gilchrist, H.G., Bêty, J., Wynne-Edwards, K.E., Berzins, L. & Williams, T.D. (2009). Using life-histories to predict and interpret variability in yolk hormones. *General and comparative endocrinology*, **163**, 169–74.

Love, O.P., Gilchrist, H.G., Descamps, S., Semeniuk, C.A.D. & Bêty, J. (2010). Pre-laying climatic cues can time reproduction to optimally match offspring hatching and ice conditions in an Arctic marine bird. *Oecologia*, **164**, 277–86.

Love, O.P., Madliger, C.L., Bourgeon, S., Semeniuk, C.A.. & Williams, T.D. (2014). Evidence for baseline glucocorticoids as mediators of reproductive investment in a wild bird. *General and comparative endocrinology*, **199**, 65–9.

Lovvorn, J.R., Richman, S.E., Grebmeier, J.M. & Cooper, L.W. (2003). Diet and body condition of spectacled eiders wintering in pack ice of the Bering Sea. *Polar Biology*, **26**, 259–267.

Ludwig, S.C. & Becker, P.H. (2008). Supply and demand: causes and consequences of assortative mating in common terns *Sterna hirundo*. *Beha*, **62**, 1601–1611.

Macdonald, C.A., Fraser, K.C., Gilchrist, H.G., Kyser, T.K., Fox, J.W. & Love, O.P. (2012). Strong migratory connectivity in a declining Arctic passerine (Migratory connectivity in an Arctic passerine). *Animal Migration*, 23–30.

Maechler, M., Rousseeuw, P., Struyf, A. & Hubert, M. (2016). Finding Groups in Data: Cluster Analysis Extended. 1–80.

Mallory, M.L., Gaston, A.J., Gilchrist, H.G., Robertson, G.J. & Braune, B.M. (2010a). Effects of climate change, altered sea-ice distribution and seasonal phenology on marine birds. *A Little Less Arctic* (eds S.H. Ferguson, L.L. Loseto & M.L. Mallory), pp. 179–195. Springer Netherlands, Dordrecht.

Mallory, M.L., Gilchrist, H.G., Janssen, M., Major, H.L., Merkel, F.R., Provencher, J.F. & Strøm, H. (2018). Financial costs of conducting science in the Arctic: examples from seabird research. *NRC Rsearch Press*, **633**, 624–633.

Mallory, C.D., Gilchrist, H.G., Robertson, G.J., Provencher, J.F., Braune, B.M., Forbes, M.R. & Mallory, M.L. (2017). Hepatic trace element concentrations of breeding female common eiders across a latitudinal gradient in the eastern Canadian Arctic. *Marine Pollution Bulletin*, **124**, 252–257.

Mallory, M.L., Robinson, S. a, Hebert, C.E. & Forbes, M.R. (2010b). Seabirds as indicators of aquatic ecosystem conditions: a case for gathering multiple proxies of seabird health. *Marine pollution bulletin*, **60**, 7–12.

Marra, P.P., Cohen, E.B., Loss, S.R., Rutter, J.E., Tonra, C.M. & Eb, C. (2015). A call for full annual cycle research in animal ecology. *Biology Letters*, **11**, 20150552.

Marra, P.P., Hobson, K.A. & Holmes, R.T. (1998). Linking winter and summer events in a migratory bird by using stable-carbon isotopes. *Science*, **282**, 1884–1886.

Martin, T.E. (1987). Food as a limit on breeding birds: A life-history perspective. *Annual Review of Ecology and Systematics*, **18**, 453–487.

Mazerolle, D.F. & Hobson, K.A. (2005). Estimating origins of short-distance migrant

songbirds in North America: Contrasting inferences from hydrogen isotope measurements of feathers, claws, and blood. *The Condor*, **107**, 280–288.

Mckinnon, L., Gilchrist, H.G. & Scribner, K.T. (2006). Genetic evidence for kin-based female social structure in common eiders (Somateria mollissima). *Behavioural Ecology*, **17**, 614–621.

McShane, B.B., Gal, D., Gelman, A., Robert, C. & Tackett, J.L. (2019). Abandon statistical significance. *The American Statistician*, **73**, 235–245.

Mehl, K.R., Alisauskas, R.T., Hobson, K.A. & Merkel, F.R. (2005). Linking breeding and wintering areas of king eiders: making use of polar isotopic gradients. *Journal of Wildlife Management*, **63**, 1297–1304.

Meijer, T.H.E. & Drent, R. (1999). Re-examination of the capital and income dichotomy in breeding birds. *Ibis*, **141**, 399–414.

Merkel, F.R. (2010). Evidence of recent population recovery in common eiders breeding in western Greenland. *Journal of Wildlife Management*, **74**, 1869–1874.

Merkel, F.R. (2004). Impact of Hunting and Gillnet Impact Fishery on Wintering Eiders in Nuuk , Southwest Greenland. *Waterbirds*, **27**, 469–479.

Merkel, F.R., Jamieson, S.E., Falk, K. & Mosbech, A. (2007). The diet of common eiders wintering in Nuuk, Southwest Greenland. *Polar Biology*, **30**, 227–234.

Merkel, F.R., Mosbech, A., Sonne, C., Flagstad, A., Falk, K. & Jamieson, S.E. (2006). Local movements, home ranges and body condition of common eiders *Somateria mollissima* wintering in Southwest Greenland. *ARDEA*, **94**, 639–650.

Miller, N.G., Wassenaar, L.I., Hobson, K.A. & Norris, D.R. (2012). Migratory connectivity of the monarch butterfly (Danaus plexippus): patterns of spring re-colonization in eastern North America. *PLoS ONE*, **7**, e31891.

Mini, A.E., Harrington, E.R., Rucker, E., Dugger, B.D. & Mowbray, T.B. (2014). American Wigeon (Anas americana). *The Birds of North America (P. G. Rodewald, Ed.). Ithaca: Cornell Lab of Ornithology*.

Møller, A.P. & Birkhead, T.R. (1991). Frequent copulations and mate guarding as alternative paternity guards in birds: a comparative study. *Behaviour*, **118**, 170–186.

Molles, M.C.J. & Cahill, J.F.J. (2008). *Ecology: Concepts and Applications*, Canadian en. McGraw-Hill Ryerson.

Morrissette, M., Bêty, J., Gauthier, G., Reed, A. & Lefebvre, J. (2010). Climate, trophic interactions, density dependence and carry-over effects on the population productivity of a migratory Arctic herbivorous bird. *Oikos*, **119**, 1181–1191.

Mosbech, A., Gilchrist, H.G., Merkel, F.R., Sonne, C., Flagstad, A. & Nyegaard, H. (2006). Year-round movements of northern common eiders *Somateria mollissima borealis* breeding in Arctic Canada and West Greenland followed by satellite telemetry. *Ardea*, **94**, 651–665.

Muggeo, V.M.R. (2003). Estimating regression models with unknown break-points. *Statistics in Medicine*, **22**, 3055–3071.

Muggeo, V.M.R. (2008). Segmented: an R package to fit regression models with broken-line relationships.

NOAA. (2018). North Atlantic Oscillation (NAO). *National Centers for Environmental Information*. URL https://www.ncdc.noaa.gov/teleconnections/nao/ [accessed 9 June 2018]

Nolet, B.A. (2006). Speed of spring migration of tundra swans Cygnus columbianus in accordance with income or capital breeding strategy? *ARDEA*, **94**, 579–591.

van Noordwijk, A. & de Jong, G. (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *The American Naturalist*, **128**, 137–142.

Norris, D.R. (2005). Carry-over effects and habitat quality in migratory populations. *Oikos*, **109**, 178–186.

Norris, D.R., Marra, P.P., Kyser, T.K. & Ratcliffe, L.M. (2005). Tracking habitat use of a long-distance migratory bird, the American redstart Setophaga ruticilla, using stable-carbon isotopes in cellular blood. *Journal of Avian Biology*, **36**, 164–170.

Norris, D.R., Marra, P.P., Kyser, T.K., Sherry, T.W. & Ratcliffe, L.M. (2004). Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. *Proceedings of the Royal Society Biological Sciences*, **271**, 59–64.

Norris, D.R. & Taylor, C.M. (2006). Predicting the consequences of carry-over effects for migratory populations. *Biology Letters*, **2**, 148–151.

O'Connor, C.M., Norris, D.R., Crossin, G.T. & Cooke, S.J. (2014). Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere*, **5**, 1–11.

Ockendon, N., Leech, D. & Pearce-Higgins, J.W. (2013). Climatic effects on breeding grounds are more important drivers of breeding phenology in migrant birds than carry-over effects from wintering grounds. *Biology Letters*, **9**, 1-4 (20130669).

Oosterhuis, R. & Van Dijk, K. (2002). Effect of food shortage on the reproductive output of common eiders *Somateria mollissima* breeding at Greind (Wadden Sea). *Atlantic Seabirds*, **4**, 29–38.

Oppel, S. & Powell, A.N. (2008). Assigning king eiders to wintering regions in the Bering Sea using stable isotopes of feathers and claws. *Marine Ecology Progress Series*, **373**, 149–156.

Oppel, S. & Powell, A.N. (2010). Carbon isotope turnover in blood as a measure of arrival time in migratory birds using isotopically distinct environments. *Journal of Ornithology*, **151**, 123–131.

Öst, M., Lindén, A., Karell, P., Ramula, S. & Kilpi, M. (2018). To breed or not to breed : drivers of intermittent breeding in a seabird under increasing predation risk and male bias. *Oecologia*.

Van Oudenhove, L., Gauthier, G. & Lebreton, J.D. (2014). Year-round effects of climate on demographic parameters of an arctic-nesting goose species. *Journal of Animal Ecology*, **83**, 1322–1333.

Paredes, R., Jones, I.L. & Boness, D.J. (2005). Reduced parental care, compensatory behaviour and reproductive costs of thick-billed murres equipped with data loggers. *Animal Behaviour*, **69**, 197–208.

Parker, H. & Holm, H. (1990). Patterns of nutrient and energy expenditure in female common eiders nesting in the High Arctic. *The Auk*, **107**, 660–668.

Pelletier, D., Guillemette, M., Grandbois, J.M. & Butler, P.J. (2008). To fly or not to fly: high flight costs in a large sea duck do not imply an expensive lifestyle. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 2117–2124.

Perrins, C.M. (1970). The timing of birds' breeding seasons. *Ibis*, **112**, 242–255.

Phillips, D.L. & Gregg, J.W. (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, **136**, 261–9.

Pomerleau, C., Lesage, V., Winkler, G., Rosenberg, B. & Ferguson, S.H. (2014). Contemporary diet of bowhead whales (Balaena mysticetus) from the Eastern Canadian Arctic inferred from fatty acid biomarkers. *Arctic*, **67**, 84–92.

Popa-Lisseanu, A.G., Sörgel, K., Luckner, A., Wassenaar, L.I., Ibáñez, C., Kramer-Schadt, S., Ciechanowski, M., Görföl, T., Niermann, I., Beuneux, G., Mysłajek, R.W., Juste, J., Fonderflick, J., Kelm, D.H. & Voigt, C.C. (2012). A triple-isotope approach to predict the breeding origins of European bats. *PloS ONE*, **7**, e30388.

Pratte, I., Tomlik, M.D., Betsch, T.A., Braune, B.M., Milton, G.R. & Mallory, M.L. (2015). Trace elements in eggs of common eiders (*Somateria mollissima*) breeding in Nova Scotia, Canada. *Marine Pollution Bulletin*, 6–11.

Provencher, J.F. (2014). How arctic marine birds help researchers study a changing North. *Arctic*, **67**, 564–569.

Provencher, J.F. (2013). Parasites and pollution: Why both matter to marine bird conservation in the North. *InfoNorth*, 516–520.

Provencher, J.F., Ammendolia, J., Rochman, C.M. & Mallory, M.L. (2018). Assessing plastic debris in aquatic food webs: what we know and don't know about uptake and trophic transfer. *NRC Rsearch Press*, **14**, 1–14.

Provencher, J.F., Forbes, M.R., Hennin, H.L., Love, O.P., Braune, B.M., Mallory, M.L. & Gilchrist, H.G. (2016a). Implications of mercury and lead concentrations on breeding physiology and phenology in an Arctic bird. *Environmental Pollution*, **218**, 1014–1022.

Provencher, J.F., Forbes, M.R., Mallory, M.L., Wilson, S. & Gilchrist, H.G. (2017). Anti-parasite treatment, but not mercury burdens, influence nesting propensity dependent on arrival time or body condition in a marine bird. *Science of the Total Environment, The*, **575**, 849–857.

Provencher, J.F., Gilchrist, H.G., Mallory, M.L., Mitchell, G.W. & Forbes, M.R. (2016b). Direct and indirect causes of sex differences in mercury concentrations and parasitic infections in a marine bird. *Science of the Total Environment*, **551**–**552**, 506–512.

R Core Team. (2017). R: A language and environment for statistical computing.

Ramírez, F., Tarroux, A., Hovinen, J., Navarro, J., Afán, I., Forero, M.G. & Descamps, S. (2017). Sea ice phenology and primary productivity pulses shape breeding success in Arctic seabirds. *Scientific Reports*, **7**, 1–9.

Reed, T.E., Harris, M.P. & Wanless, S. (2015). Skipped breeding in common guillemots in a changing climate: restraint or constraint? *Frontiers in Ecology and Evolution*, **3**, 1–13.

Rigou, Y. & Guillemette, M. (2010). Foraging efforst and pre-laying strategy in breeding common eiders. *Waterbirds*, **33**, 314–322.

Robertson, G.J. (2018). *Birds of Nunavut Volume 1: Nonpasserines* (J.M. Richards & A.J. Gaston, Eds.). UBC Press, Vancouver.

Robertson, G.J. & Cooke, F. (1999). Winter philopatry in migratory waterfowl. *The Auk*, **116**, 20–34.

Robertson, G.J., Cooke, F., Goudie, R.I. & Boyd, W.S. (1998). The timing of pair formation in Harlequin ducks. *Condor*, **100**, 551–555.

Robertson, G.J. & Gilchrist, H.G. (1998). Evidence of population declines among common eiders breeding in the Belcher Islands, Northwest Territories. **51**, 378–385.

Rockwell, S.M., Bocetti, C.I. & Marra, P.P. (2012). Carry-over effects of winter climate on spring arrival date and reproductive success in an endangered migratory bird, Kirtland's warbler (Setophaga kirtlandii). *The Auk*, **129**, 744–752.

Rodway, M.S. (2007). Timing of pairing in waterfowl I: reviewing the data and extending the theory. *Waterbirds*, **30**, 488–505.

Rohwer, F.C. & Anderson, M.G. (1988). Female-biased philopatry, monogamy, and the timing of pair formation in migratory waterfowl. *Current Ornithology Volume 5* (ed R.F. Johnston), pp. 187–222. Plenum Press, New York.

Rolshausen, G., Hobson, Æ.K.A. & Schaefer, H.M. (2010). Spring arrival along a migratory divide of sympatric blackcaps (Sylvia atricapilla). 175–183.

Romero, L.M. & Reed, J.M. (2005). Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology, Part A*, **140**, 73–9.

Ronconi, R.A., Koopman, H., McKinstry, C., Wong, S. & Westgate, A. (2010). Inter-annual variability in diet of non-breeding pelagic seabirds Puffinus spp. at migratory staging areas: evidence from stable isotopes and fatty acids. *Marine Ecology Progress Series*, **419**, 267–282.

Rowe, L., Ludwig, D. & Schluter, D. (1994). Time, condition, and the seasonal decline of avian clutch size. *The American naturalist*, **143**, 698–722.

Rubenstein, D.R. & Hobson, K.A. (2004). From birds to butterflies: animal movement patterns and stable isotopes. *TRENDS in Ecology and Evolution*, **19**, 256–63.

Salton, M., Saraux, C., Dann, P. & Chiaradia, A. (2015). Carry-over body mass effect from winter to breeding in a resident seabird, the little penguin. *Royal Society Open Science*, **2**, 140390.

Schubert, M., Schradin, C., Rödel, H.G., Pillay, N. & Ribble, D.O. (2009). Male mate guarding in a socially monogamous mammal, the round-eared sengi: On costs and trade-offs. *Behavioral Ecology and Sociobiology*, **64**, 257–264.

Schultz, E.T., Clifton, L.M. & Warner, R.R. (1991). Energetic constraints and size-based tactics: The adaptive significance of breeding-schedule variation in a marine fish (Embiotocidae: Micrometrus minimus). *The American Naturalist*, **138**, 1408–1430.

Sénéchal, E., Bêty, J. & Gilchrist, H.G. (2011a). Interactions between lay date, clutch size, and postlaying energetic needs in a capital breeder. *Behavioural Ecology*, **22**, 162–168.

Sénéchal, E., Bêty, J., Gilchrist, H.G., Hobson, K.A. & Jamieson, S.E. (2011b). Do purely capital layers exist among flying birds? Evidence of exogenous contribution to Arctic-nesting common eider eggs. *Oecologia*, **165**, 593–604.

Senner, N.R., Hochachka, W.M., Fox, J.W. & Afanasyev, V. (2014). An exception to the rule: carry-over effects do not accumulate in a long-distance migratory bird. *PLoS ONE*, **9**, e86588.

Shaw, A.K. & Levin, S.A. (2013). The evolution of intermittent breeding. *Mathematical Biology*, **66**, 685–703.

Shertzer, K.W. & Ellner, S.P. (2002). State-dependent energy allocation in variable environments: Life history evolution of a rotifer. *Ecology*, **83**, 2181–2193.

Shipley, B. (2013). The AIC model selection method applied to path analytic models compared using a d-separation test. *Ecology*, **94**, 560–564.

Shoji, A., Aris-Brosou, S., Culina, A., Fayet, A., Kirk, H., Padget, O., Juarez-Martinez, I., Boyle, D., Nakata, T., Perrins, C.M. & Guilford, T. (2015). Breeding phenology and winter activity predict subsequent breeding success in a trans-global migratory seabird. *Biology letters*, **11**, 20150671.

Smith, A.D. & McWilliams, S.R. (2014). What to do when stopping over: behavioral decisions of a migrating songbird during stopover are dictated by initial change in their body condition and mediated by key environmental conditions. *Behavioral Ecology*, **25**, 1423–1435.

Smith, L.C. & Stephenson, S.R. (2013). New Trans-Arctic shipping routes navigable by midcentury. *Proceedings of the National Academy of Science*, E1191–E1195.

Sorensen, M.C., Hipfner, J.M., Kyser, T.K. & Norris, D.R. (2009). Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. *The Journal of Animal Ecology*, **78**, 460–467.

Spurr, E. & Milne, H. (1976). Adaptive significance of autumn pair formation in the common eider Somateria mollissima. *Scandinavian Journal of Ornithology*, **7**, 85–89.

Squires, K.A., Martin, K. & Goudie, R.I. (2007). Vigilance behavior in the Harlequin duck (*Histrionicus histrionicus*) during the preincubation period in Labrador: Are males vigilant for self or social partner? *The Auk*, **124**, 241–252.

Stearns, S.C. (1976). Life-history tactics: A review of the ideas. *The Quarterly Review of Biology*, **51**, 3–47.

Steele, B.B., Lehikoinen, A., Öst, M. & Kilpi, M. (2007). The cost of mate guarding in the common eider. *Ornis*, **84**, 49–56.

Steenweg, R.J., Crossin, G.T., Kyser, T.K., Merkel, F.R., Gilchrist, H.G., Hennin, H.L., Robertson, G.J., Provencher, J.F., Mills Flemming, J. & Love, O.P. (2017). Stable isotopes can be used to infer the overwintering locations of prebreeding marine birds in the Canadian Arctic. *Ecology and Evolution*, 1–11.

Steenweg, R.J., Hennin, H.L., Bêty, J., Gilchrist, H.G., Williams, T.D., Crossin, G.T. & Love, O.P. (2015). Sources of diel variation in energetic physiology in an Arctic-breeding, diving seaduck. *General and Comparative Endocrinology*, **216**, 39–45.

Steenweg, R.J., Legagneux, P., Crossin, G.T., Gilchrist, H.G., Kyser, T.K. & Love, O.P. (2019). Stable isotopes of carbon reveal flexible pairing strategies in a migratory Arctic bird. *Journal of Ornithology*, **160**, 607–616.

Steenweg, R.J., Ronconi, R.A. & Leonard, M.L. (2011). Seasonal and age-dependent dietary partitioning between the great black-backed and herring gulls. *The Condor*, **113**, 795–805.

Swaddle, J.P., Witter, M.S., Cuthill, I.C., Budden, A. & McCowen, P. (1996). Plumage condition affects flight performance in Common Starlings: implications for developmental homeostasis, abrasion and moult. *Journal of Avian Biology*, **27**, 103–111.

Szostek, K.L. & Becker, P.H. (2015). Survival and local recruitment are driven by environmental carry-over effects from the wintering area in a migratory seabird. *Oecologia*, **178**, 643–657.

Tamisier, A., Allouche, L., Aubry, F. & Dehorter, O. (1995). Wintering strategies and breeding success: hypothesis for a trade-off in some waterfowl species. *Wildfowl*, **46**, 76–88.

Tan, P.-N., Steinbach, M. & Kumar, V. (2006). Chap 8 : Cluster Analysis: Basic Concepts and Algorithms. *Introduction to Data Mining*, pp. 489–567. Boston : Pearson Addison Wesley.

Thompson, D.R. & Furness, R.W. (1995). Stable-isotope ratios of carbon and nitrogen in feathers indicate seasonal dietary shifts in northern fulmars. *The Auk*, **112**, 493–498.

Velarde, E., Anderson, D. & Ezcurra, E. (2019). Seabird clues to ecosystem health. *Science*, **365**, 116–117.

Venables, W.N. & Ripley, B.D. (2002). *MASS Modern Applied Statistics with S*, Fourthn. Springer, New York.

Vestbo, S., Hindberg, C., Forbes, M.R., Mallory, M.L., Merkel, F., Steenweg, R.J., Funch, P., Gilchrist, H.G., Robertson, G.J. & Provencher, J.F. (2019a). Helminths in common eiders (Somateria mollissima): Sex, age, and migration have differential effects on parasite loads. *IJP: Parasites and Wildlife*, **9**, 184–194.

Vestbo, S., Hindberg, C., Olesen, J.M. & Funch, P. (2019b). Eiders as long distance connectors in Arctic networks. *Cross-Cultural Research*, **53**, 252–271.

Wasserstein, R.L., Schirm, A.L. & Lazar, N.A. (2019). Moving to a world beyond "p < 0.05". *The American Statistician*, **73**, 1–19.

Weller, M.W. (1956). A simple field candler for waterfowl eggs. *The Journal of Wildlife Management*, **20**, 111–113.

West, J.B., Bowen, G.J., Cerling, T.E. & Ehleringer, J.R. (2006). Stable isotopes as one of nature's ecological recorders. *TRENDS in Ecology and Evolution*, **21**, 408–14.

Wilcox, C., Van Sebille, E. & Hardesty, B.D. (2015). Threat of plastic pollution to seabirds is global, pervasive, and increasing. *Proceedings of the National Academy of Sciences*, **112**, 11899–11904.

Williams, T.D. (2012). *Physiological Adaptations for Breeding in Birds*. Princeton University Press, Princeton, NJ.

Williams, T.D., Bourgeon, S., Cornell, A., Ferguson, L., Fowler, M., Fronstin, R.B. & Love, O.P. (2015). Mid-winter temperatures, not spring temperatures, predict breeding phenology in the European starling *Sturnus vulgaris*. *Royal Society Open Science*, **2**, 140301.

Williams, T.D., Guglielmo, C.G., Egeler, O. & Martyniuk, C.J. (1999). Plasma lipid metabolites provide information on mass change over several days in captive western sandpipers. *The Auk*, **116**, 994–1000.

Williams, C.T., Klaassen, M., Barnes, B.M., Buck, C.L., Arnold, W., Giroud, S., Vetter, S.G., Ruf, T. & Williams, C.T. (2017). Seasonal reproductive tactics: annual timing and the capital-to-income breeder continuum. *Philosophical Transactions B*, **372**, 20160250.

Williams, T.D., Warnock, N., Takekawa, J.Y. & Bishop, M.A. (2007). Flyway-scale variation in plasma triglyceride levels as an index of refuelling rate in spring-migrating western sandpipers (*Calidris mauri*). *The Auk*, **124**, 886–897.

Wingfield, J.C., Lynn, S.E. & Soma, K.K. (2001). Avoiding the 'costs' of testosterone : Ecological bases of hormone-behavior interactions. *Brain, Behaviour and Evolution*, **57**, 239–251.

Yerkes, T., Hobson, K.A., Wassenaar, L.I., Macleod, R. & Coluccy, J.M. (2008). Stable isotopes (dD , d13C , d15N) reveal associations among geographic location and condition of alaskan northern pintails. *The Journal of Wildlife Management*, **72**, 715–725.

Yohannes, E., Valcu, M., Lee, R.W. & Kempenaers, B. (2010). Resource use for reproduction depends on spring arrival time and wintering area in an arctic breeding shorebird. *Journal of Avian Biology*, **41**, 580–590.

Zhang, H., Rebke, M., Becker, P.H. & Bouwhuis, S. (2015). Fitness prospects: effects of age, sex and recruitment age on reproductive value in a long-lived seabird. *Journal of Animal Ecology*, **84**, 199–207.

Zuur, A.F., Leno, E.N. & Smith, G.M. (2007). *Statistics for Biology and Health: Analysing Ecological Data* (M. Gail, K. Krickeberg, J. Sarnet, A. Tsiatis & W. Wong, Eds.). Springer Science + Business Media B.V.

Appendix 1 – Co-Authorship and Copyright Release

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Chapter 2: Stable isotopes can be used to infer the overwintering locations of pre-breeding marine birds in the Canadian Arctic

The work in this research chapter is my own and was a collaborative effort among many academic and government researchers. All of the co-authors provided feedback on the final manuscript, which has been published under the following citation:

Steenweg RJ, Crossin GT, Kyser TK, Merkel FR, Gilchrist HG, Hennin HL, Robertson GJ, Provencher JF, Mills Flemming J and Love OP (2017) Stable isotopes can be used to infer the overwintering locations of pre-breeding marine birds in the Canadian Arctic. Ecology and Evolution 1–11. doi: 10.1002/ece3.3410

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Chapter 3: Stables isotopes of carbon reveal flexible pairing strategies in a migratory Arctic bird

The work in this research chapter is my own and was a collaborative effort among a group of academic and government researchers. All of the co-authors provided feedback on the final manuscript, which has been published under the following citation:

Steenweg RJ, Legagneux P, Crossin GT, Gilchrist HG, Kyser TK and Love OP (2019) Stable isotopes of carbon reveal flexible pairing strategies in a migratory Arctic bird. Journal of Ornithology 160:607–616. doi: 10.1007/s10336-019-01661-y

Appendix 2 – R Code for Chapter 2 Data Analyses

```
rm(list=ls())
library(cluster)
library(MASS)
library(car)

#Nov 14 2016 Final Cluster Analysis of 2014 and 2015 Stable isotope data.
        rm(list=ls())
        setwd("~/Desktop/Data/Isotope Data/")
        mydata<- read.csv("~/Desktop/Data/Isotope
        Data/20161114AllIsotopesforR.csv", header=TRUE)

#One factor Anova testing differences between OW groups
#Anovas testing for differences between OW groups in blood
        blood <- mydata[c(241:275, 300:337),]
        bloodc = lm(Blood_dC ~ Location + Sex, data=blood)
        anova(bloodc)
        bloodn = lm(Blood_dN ~ Location + Sex, data=blood)
        anova(bloodn)
        bloodh = lm(Blood_dH ~ Location + Sex, data=blood)
        anova(bloodh)

#Anovas testing for differences between OW groups in Claws
        claws <- mydata[241:299,]
        clawc = lm(Claw_dC ~ Location + Sex, data=claws)
        anova(clawc)
        clawn = lm(Claw_dN ~ Location + Sex, data=claws)
        anova(clawn)
        clawh = lm(Claw_dH ~ Location + Sex, data=claws)
        anova(clawh)

#Anovas testing for differences in stable isotopes between years
        fit7 = lm(Claw_dC ~ Calendar_Year, data = mydata[1:240,])
        anova(fit7)
        fit8 = lm(Claw_dN ~ Calendar_Year, data = mydata[1:240,])
        anova(fit8)
        fit9 = lm(Claw_dH ~ Calendar_Year, data = mydata[1:240,])
        anova(fit9)

#kmeans CN winter plus 2014, using 3 clusters
#first need to remove missing data
        clawCN2014 <- mydata[-c(12,15,46,67,91,115,141,243,269),]
        clawCN2014win <-clawCN2014[c(1:107,237:290),]
        clawdCN2014 <- clawCN2014win[,5:6]
        start <- matrix(c(-18.12, -20.55, -14.82, 12.98, 13.14, 13.04), 3, 2)
```

```
start
clawdCN20143.k <- kmeans(clawdCN2014, start)
clawdCN20143.k[6]
clawdCN20143.k[2]
x<-clawdCN20143.k[1]
write.csv(x, file="2014CNK3clusters")

#kmeans CNH winter plus 2014, using 3 clusters
clawCNH2014 <- mydata[-c(12,15,46,67,85,91,115,141,243,252,269),]
clawCNH2014win <-clawCNH2014[c(1:106,233:288),5:7]
start <- matrix(c(-18.12, -20.55, -14.82, 12.98, 13.14, 13.04, -63.11, -42.30, -
38.21), 3, 3)
start
clawdCNH20143.k <- kmeans(clawCNH2014win, start)
clawdCNH20143.k[2]
clawdCNH20143.k[6]
H<-clawdCNH20143.k[1]
write.csv(H, file="2014CNHK3clusters")

#kmeans CN winters plus 2015
clawALL2015 <- mydata[-c(115, 118, 121, 124, 128, 134, 142, 147, 192,
206, 211, 239, 240, 243, 269),]
clawCN2015 <- clawALL2015[113:284,5:6]
start <- matrix(c(-18.12, -20.55, 12.98, 13.14), 2, 2)
clawCN20151.k <- kmeans(clawCN2015, start)
clawCN20151.k[6]
clawCN20151.k[3]
y<-clawCN20151.k[1]
write.csv(y, file="2015CNclusters")

#kmeans CNH winters plus 2015
clawALL2015 <- mydata[-c(115, 118, 121, 124, 128, 134, 142, 147, 168,
178, 192,204, 205, 206, 211, 239, 240, 243, 252, 269),]
clawCNH2015 <- clawALL2015[113:279,5:7]
start <- matrix(c(-18.12, -20.55, 12.98, 13.14, -63.11, -42.30), 2, 3)
start
clawCNH20151.k <- kmeans(clawCNH2015, start)
clawCNH20151.k[6]
clawCNH20151.k[3]
m<-clawCNH20151.k[1]
write.csv(m, file="2015CNHclusters")

#kmeans C winters plus 2015 2 clusters
clawALL2015 <- mydata[-c(115, 118, 121, 124, 128, 134, 142, 147, 192,
206, 211, 239, 240, 243, 269),]
clawC2015 <- clawALL2015[113:284,5]
```

```
start2 <- matrix(c(-18.12, -20.55), 2, 1)
clawC20151.k <- kmeans(clawC2015, start2)
clawC20151.k[3]
clawC20151.k[6]
a<-clawC20151.k[1]
write.csv(a, file="2015Cclusters")

#kmeans C winter plus 2015, using 3 clusters
clawALL2015 <- mydata[-c(115, 118, 121, 124, 128, 134, 142, 147, 192,
206, 211, 239, 240, 243, 269),]
clawC2015 <- clawALL2015[113:284,5]
start5 <- matrix(c(-18.56, -20.05, -15.99), 3, 1)
clawC20153.k <- kmeans(clawC2015, start5)
clawC20153.k[3]
clawC20153.k[2]
d<-clawC20153.k[1]
write.csv(d, file="2015Ck3clusters")

#kmeans C winter plus 2014, using 3 clusters
clawCN2014 <- mydata[-c(12,15,46,67,91,115,141,243,269),]
clawC2014win <-clawCN2014[c(1:107,237:290),]
clawdC2014 <- clawC2014win[,5]
start3 <- matrix(c(-18.12, -20.55, -14.92), 3, 1)
start3
clawdC20143.k <- kmeans(clawdC2014, start3)
clawdC20143.k[6]
clawdC20143.k[3]
c<-clawdC20143.k[1]
write.csv(c, file="2014CK3clusters")
clawdC20143.k[2]

#kmeans C winter plus 2014, using 2 clusters
clawCN2014 <- mydata[-c(12,15,46,67,91,115,141,243,269),]
clawC2014win <-clawCN2014[c(1:107,237:290),]
clawdC2014 <- clawC2014win[,5]
start4 <- matrix(c(-18.12, -20.55), 2, 1)
start4
clawdC20142.k <- kmeans(clawdC2014, start4)
clawdC20142.k[6]
clawdC20142.k[2]
b<-clawdC20142.k[1]
write.csv(b, file="2014CK2clusters")

clawCN2014 <- mydata[-c(12,15,46,67,91,115,141,243,269),]
clawCN2014win <-clawCN2014[c(1:107,237:290),]
clawdCN2014 <- clawCN2014win[,5:6]
```

```
clawCN2015 <- clawALL2015[113:284,5:6]
```

Appendix 3 – R Code for Chapter 3 Data Analyses

```
rm(list=ls())
library(dplyr)
library(readr)
library(cluster)
library(MASS)
library(car)
library(ggplot2)
library(gridExtra)
library(glmmTMB)
library(lme4)

setwd("~/Desktop/Data/COEI Pairs/")

#finding ordinary distance between individuals in each pair
        DIFFclaw<-abs(ALL$Claw_dC.x-ALL$Claw_dC.y)
        DIFFblood<-abs(ALL$Blood_dC.x-ALL$Blood_dC.y)
        BothCluster<-ALL$cluster.x+ALL$cluster.y
        DIFFclawN<-abs(ALL$Claw_dN.x-ALL$Claw_dN.y)
        DIFFbloodN<-abs(ALL$Blood_dN.x-ALL$Blood_dN.y)
        ALL3=cbind(DIFFclaw, DIFFblood, DIFFclawN, DIFFbloodN, BothCluster,
        data.frame(ALL)) #exported as CSV

#this has the pairing times manually added based on those differences
        ALL3<-read.csv("ALL3PairTime.csv")
        Breeding.Propensity <- ifelse(ALL3$Breeding.stage.x== "NB", 0, 1)
        ALL4=cbind(Breeding.Propensity, data.frame(ALL3))
        sum(ALL4$Breeding.Propensity == 1)
        sum(ALL4$Breeding.Propensity == 0)
        sum(ALL4$PairTime == "Spring")

#relationship in stable isotopes within pairs
        lm2 = lm(Claw_dC.y~Claw_dC.x, data=ALL3, Calendar_Year.x == 2015)
        lm4 = lm(Claw_dC.y~Claw_dC.x, data=ALL3, Calendar_Year.x == 2016)
        summary(lm2)
        summary(lm4)


#sensitivity analysis
#winter
        lm0.5=lm(Claw_dC.y ~ Claw_dC.x, data=ALL3, PairTime0.5 == "Winter")
        lm1.5=lm(Claw_dC.y ~ Claw_dC.x, data=ALL3, PairTime1.5 == "Winter")
        lm5= lm(Claw_dC.y~Claw_dC.x, data=ALL3, PairTime == "Winter")
        summary(lm0.5)
        summary(lm1.5)
```

```
summary(lm5)

#spring
        lm0.5b=lm(Blood_dC.y ~ Blood_dC.x, data=ALL3, PairTime0.5 ==
        "Spring")
        lm1.5b=lm(Blood_dC.y ~ Blood_dC.x, data=ALL3, PairTime1.5 ==
        "Spring")
        lm5b= lm(Blood_dC.y ~ Blood_dC.x, data=ALL3, PairTime == "Spring")
        summary(lm0.5b)
        summary(lm1.5b)
        summary(lm5b)


#timing of pairing in rel to female and male body mass
    ALL4$Breeding.Propensity <- as.factor(ALL4$Breeding.Propensity)
    ALL4$Year.x <- as.factor (ALL4$Year.x)
    lmmass<-glm(Mass.x ~ PairTime +Year.x, data=ALL4)
    summary(lmmass)
    lmmalemass<-glm(MALE_MATE_Mass.x ~ PairTime + Year.x, data=ALL4)
    summary(lmmalemass)

#subset both years of data
    Pairs2016 <- subset(ALL4, Year.x==2016,
    select=c(PairTime, PairTime0.5, PairTime1.5, BothCluster, DIFFclaw,
    DIFFblood, Breeding.Propensity, Mass.x, MALE_MATE_Mass.x,
    Delay_before_laying.x, cluster.x, Julian_Capture_Date.x, Lay.Date.x))
    Pairs2015 <- subset(ALL4, Year.x==2015,
    select=c(PairTime, PairTime0.5, PairTime1.5,BothCluster, DIFFclaw,
    DIFFblood, Breeding.Propensity, Mass.x, MALE_MATE_Mass.x,
    Delay_before_laying.x, cluster.x, Julian_Capture_Date.x, Lay.Date.x))

#breeding propensity as a function of timing of pairing, year, mass and capture date
    glm20fboth<-glm(Breeding.Propensity ~ PairTime*Year.x + Mass.x +
    Julian_Capture_Date.x, family= binomial, data=ALL4)
    summary(glm20fboth)
    summ(glm20fboth)

    glm20f<-glm(Breeding.Propensity ~ PairTime + Mass.x +
    Julian_Capture_Date.x, family= binomial, data=Pairs2015)
    summary(glm20f)
    summ(glm20f)

    glm20g<-glm(Breeding.Propensity ~PairTime + Mass.x + Julian_Capture_Date.x,
    family= binomial, data=Pairs2016)
    summary(glm20g)
    summ(glm20g)
```

```
#prelaying interval as a function of timing of pairing, year, mass and capture date
        lm20dboth<-lm(Delay_before_laying.x ~ PairTime*Year.x + Mass.x +
        Julian_Capture_Date.x, data=ALL4)
        summary(lm20dboth)
        summ(lm20dboth)

#laydate as a function of timing of pairing, year, mass and arrival
        lm20fboth<-lm(Lay.Date.x ~ PairTime*Year.x + Mass.x +
        Julian_Capture_Date.x, data=ALL4)
        summary(lm20fboth)
        summ(lm20fboth)
```

Appendix 4 – R Code for Chapter 4 Data Analyses

```
library(dplyr)
library(readr)
library(cluster)
library(MASS)
library(car)
library(ggplot2)
library(gridExtra)
library(segmented)
library(lme4)
library(lmerTest)
library(lsmeans)
library(MuMIn)
library(doBy)
library(jtools)
library(FactoMineR)
library(missMDA)

### test for size corrections for a better metric of male body condition ###
        rm(list=ls())
        setwd("~/Desktop/Data/COEI Pairs/")
        MBS<-read.csv("Male_Body_Size.csv")
        MBSdry<-subset(MBS, Wet==0.00) #using dry collected eiders from
        dissection data
        summary(MBSdry) #mean mass 2115 +-140.96

#corrections for wingchord
        lmBSWC<-lm(Mass~WingChord, data=MBSdry)
        summary(lmBSWC)
        BSWCres<-residuals(lmBSWC)
        BSWC<-cbind(BSWCres, MBSdry)
        testWC1<-lm(BSWCres~LegFat, data=BSWC)
        summary(testWC1)
        testWC2<-lm(BSWCres~Pfat, data=BSWC)
        summary(testWC2)
        plot(BSWCres~LegFat, data=BSWC)

#corrections for tarsus
        lmBSTS<-lm(Mass~TarTOT, data=MBSdry)
        summary(lmBSTS)
        BSTSres<-residuals(lmBSTS)
        BSTS<-cbind(BSTSres, MBSdry)
        testTS1<-lm(BSTSres~LegFat, data=BSTS)
        summary(testTS1)
        testTS2<-lm(BSTSres~Pfat, data=BSTS)
```

```
summary(testTS2)
plot(BSTSres~LegFat, data=BSTS)

#corrections for headsize
lmBSHD<-lm(Mass~HEAD, data=MBSdry)
summary(lmBSHD)
BSHDres<-residuals(lmBSHD)
BSHD<-cbind(BSHDres, MBSdry)
testHD1<-lm(BSHDres~LegFat, data=BSHD)
summary(testHD1)
testHD2<-lm(BSHDres~Pfat, data=BSHD)
summary(testHD2)

#no correction
test3<-lm(Mass~LegFat, data=MBSdry)
summary(test3)
test4<-lm(Mass~Pfat, data=MBSdry)
summary(test4)

###running analyses for 3 predictions###
rm(list=ls())

setwd("~/Desktop/Data/COEI Pairs/")
pairNM<- read.csv("RCOEIPairTESTNOManip2017.csv", header=TRUE)
FPhys<- read.csv("PhysFYearOli_2017.csv", header=TRUE)
MPhys<- read.csv("PhysMALE_YearOli_2017.csv", header=TRUE)
FemPhys <- merge(pairNM, FPhys, by="FYearOli")
MFPhys <-merge(FemPhys, MPhys, by="MYearOli")
MFPhys$Year<-as.factor(MFPhys$Year)

#remove line 96, this bird died in hand.
# 66 80, MBOH high, 87 INC hen
MFPhys4<- MFPhys[-c(9, 16, 61, 66, 72, 75, 80, 87, 96),]
MFPhys3<- MFPhys[-c(18, 66, 80, 87),]
#removed rows with INC hens, and outlying FCORT MCORT and missing Hd
measurements, cannot correct for headsize without these measurements
MFPhys2<-MFPhys[-c(2, 9, 12, 18, 16, 35, 38, 51, 59, 61, 64, 66, 72, 79, 80,
87, 95, 96),]


#correcting for head size and merge with dataset
lmHD<-lm(MALE_MATE_Mass~MALE_MATE_Head, data=MFPhys2)
HDres<-residuals(lmHD)
MFPhys2HD<-cbind(HDres, MFPhys2)
MFPhys2<-MFPhys2HD
```

```
### Principle Component Analyses For Male Phys State Index ####
        male.pca=PCA(MFPhys2[c("HDres", "MBOH", "MIgY", "MTRIG",
        "logMCORT", "MT")], scale.unit=TRUE, ncp=5, graph=T)
        pcaMFPhys<-cbind(MFPhys2, male.pca$ind)
        pcaMFPhys2<-pcaMFPhys[-c(62),] #remove INC hen
        summary(male.pca)

#MFPhys2: the data set used
#scale.unit: to choose whether to scale the data or not
#ncp: number of dimensions kept in the result
#graph: to choose whether to plot the graphs or not
#write.csv(pcaMFPhys2, file="Figure1Chp3pca.csv")
#only want to look at breeders, not NB, BP = 1
        pcabreeders<-subset(pcaMFPhys2, Breeding.propensity==1,
        select= c(coord.Dim.1, coord.Dim.2,MALE_MATE_Mass, MBOH, MTRIG,
        MIgY, logMCORT, MCORT, MT, PreLayingInterval, Julian_Capture_Date,
        Mass, FTRIG, FCORT, Year, LayDate, BreedingStage, HDres, RelLayDate,
        Relative_Capture_Date))

# use Anova NOT aov, bc aov uses different sum of squares than lm, Anova uses Type II
tests

#relevel so that referencing to PR females instead of laying in tests
        breeders$BreedingStage<-relevel(breeders$BreedingStage, ref="PR")

#prediction 1
        Masslm<-lm(Mass~HDres, data=breeders)
        summary(Masslm)
        summ(Masslm, confint = TRUE, digits = 3)

#prediction 2
        MCondlm<-lm(HDres~BreedingStage+Year, data=breeders)
        summary(MCondlm)
        summ(MCondlm, confint = TRUE, digits = 3)

        logMCORTlm<-lm(logMCORT~BreedingStage+HDres+Year,
        data=breeders)
        summary(logMCORTlm)
        summ(logMCORTlm, confint = TRUE, digits = 3)

        MTRIGlm<-lm(MTRIG~BreedingStage+HDres+Year, data=breeders)
        summary(MTRIGlm)
        summ(MTRIGlm, confint = TRUE, digits = 3)

        MBOHlm<-lm(MBOH~BreedingStage+HDres+Year, data=breeders)
```

```
summary(MBOHlm)
summ(MBOHlm, confint = TRUE, digits = 3)

MIgYlm<-lm(MIgY~BreedingStage+HDres+Year, data=breeders)
summary(MIgYlm)
summ(MIgYlm, confint = TRUE, digits = 3)

MTlm<-lm(MT~BreedingStage+HDres+Year, data=breeders)
summary(MTlm)
summ(MTlm, confint = TRUE, digits = 3)

coord.Dim.1lm<-lm(coord.Dim.1~BreedingStage+Year, data=pcabreeders)
summary(coord.Dim.1lm)
summ(coord.Dim.1lm, confint = TRUE, digits = 3)

coord.Dim.2lm<-lm(coord.Dim.2~BreedingStage+Year, data=pcabreeders)
summary(coord.Dim.2lm)
summ(coord.Dim.2lm, confint = TRUE, digits = 3)

#Prediction 3a
FFat<-lm(FTRIG~Mass, data=pcaMFPhys2)
Ffatrate<-residuals(FFat)
pcafat<-cbind(Ffatrate, pcaMFPhys2)

#only look at PR for fattening rate
pcaPR<-subset(pcafat, BreedingStage=="PR",
select = c(MALE_MATE_Mass, MBOH, MTRIG, MIgY, logMCORT,
MCORT, MT, PreLayingInterval, Julian_Capture_Date,
Relative_Capture_Date, Mass, FTRIG, FCORT, Year, LayDate,
BreedingStage, Breeding.propensity, HDres, RelLayDate, coord.Dim.1,
coord.Dim.2, coord.Dim.3, Ffatrate))

femlay4<-lm(Ffatrate~coord.Dim.1 + coord.Dim.2 + Relative_Capture_Date
+ Year, data=pcaPR)
summary(femlay4)
Anova(femlay4)
summ(femlay4)

# Prediction 3b
alllm <- lm(RelLayDate ~ HDres + logMCORT + MTRIG + MBOH + MIgY
+ MT + Year + Mass + Relative_Capture_Date, data=breeders)
summ(alllm)
summary(alllm)
Anova(alllm)
```

```
LayDatePCAlm <- lm(RelLayDate ~ coord.Dim.1 + coord.Dim.2 + Year +
Mass + Relative_Capture_Date, data=pcabreeders)
summary(LayDatePCAlm)
summ(LayDatePCAlm)
Anova(LayDatePCAlm)
```

Appendix 5 – R Code for Chapter 5 Data Analyses

```
library("piecewiseSEM")
library("nlme")
library("lme4")
library("lmerTest")
library("igraph")
library("devtools")
rm(list=ls())

mydata<-read.csv("~/Desktop/Data/COEIPairs/FemaleCOEs_2020_02_01.csv",
header=TRUE)

#removed individuals with treatments, mass with NAs, LAY, INC, and that died on
island, also removed individuals with CORT>100 (3sd)
        COEBP<-na.omit(subset
        (mydata, select=c("WinterLoc", "Mass", "Julian_Capture_Date", "NAOWin",
        "Breeding.propensity", "Year", "BreedingStage", "CORT",
        "Oli_Bloodnumber", "Bin3", "Bin4", "Bin5", "SpringBin", "CatBin5",
        "CatSpringBin", "Claw_dN", "TempMAY", "TempWin")))
        COEBP$Year <- as.numeric(COEBP $Year)
        COEBP$Oli_Bloodnumber <- as.numeric(COEBP$Oli_Bloodnumber)
        COEBP$Capture <- as.numeric(COEBP$Julian_Capture_Date)

# Construct SEM each letter relates its own specific model

        glmsemA <- psem(
          lmer(Capture~MAY + Win +(1|Oli_Bloodnumber), COEBP),
          lmer(CORT~MAY + Win +(1|Oli_Bloodnumber), COEBP),
          lmer(Mass~ MAY + Win + (1|Oli_Bloodnumber), COEBP),
          glmer(BP~Mass +Capture + CORT + MAY + Win + (1|Oli_Bloodnumber),
        family = binomial, COEBP), data=COEBP)
        summary(glmsemA)

        glmsemB <- psem(
          lmer(Capture~MAY + Win +(1|Oli_Bloodnumber), COEBP),
          lmer(CORT~MAY + Win +(1|Oli_Bloodnumber), COEBP),
          lmer(Mass~ MAY + Win + (1|Oli_Bloodnumber), COEBP),
          glmer(BP~Mass +Capture + CORT + (1|Oli_Bloodnumber), family =
        binomial, COEBP),
          data=COEBP)
        summary(glmsemB)

        glmsemC <- psem(
          lmer(Capture~MAY + (1|Oli_Bloodnumber), COEBP),
          lmer(CORT~MAY + Win +(1|Oli_Bloodnumber), COEBP),
```

```
  lmer(Mass~ MAY + Win + (1|Oli_Bloodnumber), COEBP),
  glmer(BP~Mass +Capture + CORT + (1|Oli_Bloodnumber), family =
binomial, COEBP),
  data=COEBP)
summary(glmsemC)

glmsemD <- psem(
  lmer(Capture~MAY + (1|Oli_Bloodnumber), COEBP),
  lmer(CORT~ Win +(1|Oli_Bloodnumber), COEBP),
  lmer(Mass~ MAY + Win + (1|Oli_Bloodnumber), COEBP),
  glmer(BP~Mass +Capture + (1|Oli_Bloodnumber), family = binomial,
COEBP),
  data=COEBP)
summary(glmsemD)

glmsemE <- psem(
  lmer(Capture~MAY + (1|Oli_Bloodnumber), COEBP),
  lmer(CORT~ MAY +(1|Oli_Bloodnumber), COEBP),
  lmer(Mass~ MAY + Win + (1|Oli_Bloodnumber), COEBP),
  glmer(BP~Mass +Capture + (1|Oli_Bloodnumber), family = binomial,
COEBP),
  data=COEBP)
summary(glmsemE)

glmsemF <- psem(
  lmer(Capture~MAY + (1|Oli_Bloodnumber), COEBP),
  lmer(CORT~ MAY +(1|Oli_Bloodnumber), COEBP),
  lmer(Mass~ MAY + Win + (1|Oli_Bloodnumber), COEBP),
  glmer(BP~Mass +Capture + CORT+(1|Oli_Bloodnumber), family =
binomial, COEBP),
  data=COEBP)
summary(glmsemF)

glmsemG <- psem(
  lmer(Capture~MAY + (1|Oli_Bloodnumber), COEBP),
  lmer(Mass~ MAY + Win + (1|Oli_Bloodnumber), COEBP),
  glmer(BP~Mass +Capture + (1|Oli_Bloodnumber), family = binomial,
COEBP),
  CORT~1,
  data=COEBP)
summary(glmsemG)

glmsemH <- psem(
  lmer(Capture~MAY + Win+ (1|Oli_Bloodnumber), COEBP),
  lmer(Mass~ MAY + Win + (1|Oli_Bloodnumber), COEBP),
```

```
  glmer(BP~Mass +Capture + (1|Oli_Bloodnumber), family = binomial,
COEBP),
CORT~1,
  data=COEBP)
summary(glmsemH)

glmsemI <- psem(
  lmer(Capture~MAY + (1|Oli_Bloodnumber), COEBP),
  lmer(Mass~ MAY + (1|Oli_Bloodnumber), COEBP),
  glmer(BP~Mass +Capture + (1|Oli_Bloodnumber), family = binomial,
COEBP),
  CORT~1,
  Win~1,
  data=COEBP)
summary(glmsemI)

anova(glmsemG, glmsemH)
```