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# Energetic physiology mediates reproductive decisions in a long-lived, capital-income breeding seaduck

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# ENERGETIC PHYSIOLOGY MEDIATES REPRODUCTIVE DECISIONS IN A LONG-LIVED, CAPITAL-INCOME BREEDING SEADUCK

By

**Holly L. Hennin**

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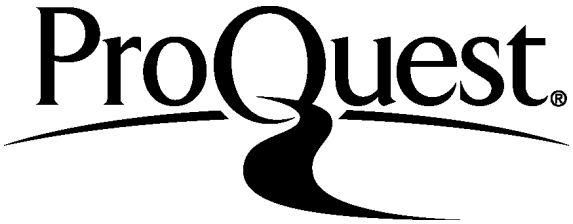

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## DECLARATION OF CO-AUTHORSHIP/PREVIOUS PUBLICATION

### I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows:
This thesis incorporates the results from a collaboration with researchers at Environment Canada (Dr. H.G. Gilchrist; chapters 2,3 and 5), Université du Québec à Rimouski (Dr. Joël Bêty and Dr. Pierre Legagneux; chapters 2,3 and 5), Simon Fraser University (Dr. Tony Williams; chapters 1, and 2), US Geological Survey Patuxent Wildlife Research Center (Dr. Alicia Wells-Berlin; chapter 4), Carleton University (Dr. R. Mark Forbes, chapter 5), University of Saskatoon (Dr. Catherine Soos and Dr. N. Jane Harms, chapter 5), and University of Windsor (Tyne M. Baker; chapter 1), all under the supervision of Dr. Oliver P. Love. In all cases, the key ideas, primary contributions, experimental designs, data analysis, interpretations, and writing were performed by the author with input from each of the co-authors on writing and idea development in their respective chapters. In chapter 2, all coauthors provided feedback on writing, Dr. Legagneux aided in statistical analyses and graphing results, with aid from Drs. Love, Bêty, and Williams on interpretations. In chapter 3, Dr. Legagneux aided in statistical analyses and graphing results, and all other co-authors aided in refinement of writing and development of ideas. In chapter 4, Dr. Wells-Berlin along with U.S. Geological Survey aided in providing the infrastructure to conduct the experiment, with input on writing from Drs. Wells-Berlin and Love. In chapter 5, Drs. Love, Gilchrist, Bêty, and Legagneux provided feedback on writing and refinement of ideas, Drs. Soos and Forbes provided funds to perform the experiment, and Dr. Harms aided in performing and teaching the manipulation techniques for the experiment.

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I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

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This thesis includes three original papers that have been previously published/submitted for publication in peer reviewed journals, as follows:

| **Thesis Chapter** | **Publication title/full citation** | **Publication status** |
|---|---|---|
| Chapter 2 | Pre-breeding energetic management in a mixed-strategy breeder | Published in *Oecologia* |
| Chapter 3 | Energetic physiology mediates individual optimization of breeding phenology in a migratory Arctic seabird | Resubmitted to *American Naturalist* |
| Chapter 4 | Baseline glucocorticoids are drivers of body mass gain in a diving seabird | Published in *Ecology and Evolution* |

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## ABSTRACT

Life history decisions are constrained by the allocation of limited resources to multiple functions, generating life history trade-offs. Individuals better able to acquire or manage endogenous resources are expected to optimize these trade-offs more efficiently, have higher performance and therefore achieve higher fitness. However, we still know little about how and why different individuals modulate energetic management to optimize breeding decisions. Physiology mediates the relationship between the individual and its environment, and therefore energetic physiology in particular is thought to be a prime candidate for regulating life history decisions. Baseline corticosterone is a hormone that mediates energetic balance and represents an individual's energetic demand. Plasma triglycerides are an energetic metabolite representing an individual's relative fattening rate and therefore their ability to meet energetic demands during costly life history stages. Together these two traits can represent an individual's "energetic physiology". My thesis uses a combination of correlative and manipulative techniques to determine the role of energetic physiology in mediating variation in a key life history decision: breeding phenology. To field-test predictions within the framework of the Physiology/Life History nexus I worked with a wild population of Arctic-nesting common eiders (*Somateria mollissima*), a diving seaduck with a mixed capital-income breeding strategy, at East Bay Island, NU, Canada. I found that baseline corticosterone and plasma triglycerides increase across the pre-laying period, likely to support the high energetic demands for investing in reproduction. Further, these traits interact to optimize the timing of breeding on an individual basis. Using a captive seaduck system to prepare for field experiments, I confirmed that I could experimentally elevate corticosterone within a baseline range and produce a concomitant increase in body mass. Using this experimental approach, I elevated baseline corticosterone in wild female eiders and confirmed that elevations of baseline corticosterone resulted in earlier laying, shorter delays before laying following migratory arrival and higher reproductive success. This thesis provides important mechanistic details of how variation in energetic physiology can drive individual variation in reproductive decisions, as well as

offering a robust test of components of the Physiology/Life History Nexus framework in a free-living system.

# DEDICATION

For my parents who instilled a love of the outdoors and wildlife in me from a young age and never stopped encouraging me.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# CHAPTER 1 :

# THE ROLE OF ENERGETIC PHYSIOLOGY IN LIFE HISTORY DECISIONS[1]

## *The Evolution of Life Histories: Constraints Generating Variation*

Evolutionary ecologists have long appreciated and tried to quantify the substantial diversity in life histories across taxa (Stearns 1977; Ricklefs 1977). Indeed, a primary goal in the study of life history theory is to both characterize and determine how and why this variation exists both across and within species in response to environmental variation (Ricklefs 1991; Brommer 2000; Stearns 2000). Species differ in the degree to which life history traits such as size at birth, growth rates, age at reproductive maturity, and investment in reproduction impact fitness (Stearns 1992). Moreover, because energetic resources and time are limiting factors in the lives of all organisms, each of these traits competes for the same, restricted energetic and temporally constrained resources, often resulting in species-specific life history trade-offs (Stearns 1989; Stearns 1992; Roff 1992). As such, species have evolved varying strategies for optimally allocating energy to key life history traits to minimize the costs and trade-offs constraining individuals, thereby maximizing benefits and fitness (Brommer 2000).

Within species, morphs or sub-populations can exhibit substantial variability in life-histories. For example, although r- vs. K-selection is described as a species-level life history strategy, empirical evidence also demonstrates a diversification of strategies within a species at the individual-level. In fruit flies, a typically r-selected species, individuals have been found to exhibit different strategies for allocating energy, demonstrating the same r-/K-continuum at a finer scale (Stearns et al. 2000). To reinforce

[1] This chapter the result of joint research with O.P. Love.

the presence of this variation, researchers directly selected for earlier-laying individuals over multiple generations, resulting in a shift towards individuals emerging earlier and at smaller sizes as adults, with corresponding earlier ages at peak fecundity and shorter life spans (Stearns et al. 2000). Similarly, in garter snakes (*Thamnophis elegans*) two eco-types have been identified depending on resource availability; individuals with continuous access to prey and high predation rates exhibited r-selected traits (rapid growth, early maturation, low adult survival), whereas individuals in populations with unreliable access to prey but low predation rates demonstrated more K-selected life history traits (slow growth, delayed age at maturity, high adult survival; Bronikowski and Arnold 1999). Finally, in side-blotched lizards (*Uta stansburiana*) depending on density-dependent effects, orange coloured morphs have demonstrated more r-selected (shorter lived) traits, having large clutches with small egg size compared to yellow-morph females which appear more K-selected (longer lived) with smaller clutches and larger eggs (Sinervo et al. 2000). This strong impact of environmental variation in influencing life history strategies and traits within species can be driven by developmental plasticity, phenotypic flexibility and even fixed phenotypes (Piersma and Drent 2003; Nussey et al. 2007; Williams 2008).

Individuals must also optimize the life history decisions they make based on a combination of environmental variation, variation in their current state and within the context of the constraints that drive the evolution of life histories and trade-offs at the species level (McNamara et al. 1995; McNamara and Houston 1996; Ricklefs 2000). Optimization models have been useful in trying to identify mechanisms at the heart of individual variation in life history decisions because they formalize these relationships

into testable predictions for examining the variation seen in life history traits across individuals (e.g., Parker and Maynard Smith 1990; Rowe and Ludwig 1991; Rowe et al. 1994; Kisdi et al. 1998). For example, the timing of reproduction (i.e., breeding phenology) is an important investment decision because it is often linked to expected current reproductive output, offspring survival, and impacts future reproductive investment and survival (Perrins 1970; Lepage et al. 2000; Satake et al. 2001). Theoretical optimization models predict that individuals in differing states will make different decisions resulting in differing fitness consequences (McNamara and Houston 1996; McNamara 1997). Specifically, individuals that are in better condition and in more favourable environments (e.g., more resources, less predation risk) are predicted to produce more offspring within a breeding season (Stearns 1992; Rowe et al. 1994; Kisdi et al. 1998; Morris 1998) impacting realized fitness (McGraw and Caswell 1996). Although optimization models have generated field-testable predictions that have been examined empirically in a number of taxa (Sinervo and Doughty 1996; Gotthard et al. 1999; Bêty et al. 2003; Dickerson et al. 2005; Varpe et al. 2007), the phenotypic traits used as proxies of individual 'state' have often been broad metrics generally related to performance (e.g., body mass, body condition). While useful, these traits often add fairly little additional predictive power in describing variation in the reproductive decision of interest (Descamps et al. 2011) and importantly they provide no additional underlying mechanistic insight into the central regulators driving variation in individual quality or state. To fill these mechanistic gaps, integrative ecologists have proposed examining finer-scale regulators thought to be at the heart of life history decisions and trade-offs, and which ultimately generate fitness variation across individuals (Williams 2008).

### *The Physiology/Life History Nexus*

One of the primary goals of evolutionary physiology is to determine the mechanisms underlying variation in life histories across species (West et al. 1997; Enquist et al. 2003; Brown et al. 2004), as well as investment in life history traits within species (Stearns 2000; Moore and Hopkins 2009). Almost 15 years ago Ricklefs and Wikelski (2002) proposed the framework of the Physiology/Life History Nexus which suggests that physiological traits are phenotypes arising from genotypes which interact with the environment across an individual's life to influence organismal performance, shaping both the production and selection of offspring phenotypes, and thus life history traits. Physiological traits are of particular interest as mechanistic drivers of life history variation given that they: i) exhibit substantial amounts of individual variation in their production (Ketterson and Nolan 1999; Williams 2008), ii) have pleiotropic influences on multiple endogenous systems (Ketterson and Nolan 1999; Harshman and Zera 2007; Zera et al. 2007), and iii) are produced through genes interacting with the environment (Ketterson and Nolan 1992; Ketterson and Nolan 1999; Zera and Harshman 2001; Ricklefs and Wikelski 2002; Harshman and Zera 2007; McNamara and Houston 2008; Moore and Hopkins 2009). Systems as diverse as immune function (Møller and Saino 2004; Knowles et al. 2010), metabolism (Humphries and McCann 2014; McKechnie 2015), oxidative stress (Bize et al. 2008), developmental hormones (Zera and Harshman 2001; Harshman and Zera 2007), and reproductive hormones (testosterone: Reed et al. 2006; estradiol: Williams 2001) have all been proposed as potential regulators of life history decisions and trade-offs.

### *Measuring and Manipulating Energetic Physiology*

*i) Energetic Metabolites*

Although single measures of body mass can be informative for assessing the current state or condition of an individual, using this technique to determine the trajectory (i.e., rate) of mass change of an individual requires multiple captures, something which is often highly difficult and negatively impacts free-living species. Energetic metabolites have been critical in providing researchers with a proxy to assess rates of body mass change from metabolites obtained in a single blood sample (Jenni-Eiermann and Jenni 1994; Cerasale et al. 2006). In particular, plasma energetic metabolites have been identified as highly useful metrics for quantifying both fasting (β-hydroxy-butyrate - BOH) and feeding (triglycerides) in individuals.

Fasting individuals use endogenous fat stores or protein catabolism to support their energetic demands and as a result begin producing BOH, a ketone synthesized from the free fatty acids produced during catabolism (Robinson and Williamson 1980; Jenni-Eiermann and Jenni 1994). Not only does plasma BOH characterize decreases in body mass effectively, it can do so far better than other commonly used metabolites such as plasma glucose, which often simply correlates with body mass itself, rather than predicting changes in body mass (Jenni-Eierman and Jenni 1991; Jenni-Eiermann and Jenni 1994; Williams et al. 1999). Plasma BOH has been employed as a useful physiological marker of fasting in a number of species (Jenni and Schwilch 2001; Anteau and Afton 2008; Price et al. 2013; but see McGuire et al. 2009). In contrast, following recent foraging and digestion (i.e., feeding) individuals synthesize generic very low-

density lipoprotein (VLDL; i.e., triglycerides) in the liver (Walzem 1996; Gibbons et al. 2006). Triglycerides (TRIG) are then transported through the blood stream to be deposited to adipose tissues (Jenni and Jenni-Eiermann 1996). As such, when corrected for individual body mass, a static measure of plasma TRIG represents the instantaneous rate at which an individual is fattening (Williams et al. 1999). Therefore, during life history stages where individuals are predicted to be gaining in condition rather than mobilizing stored resources, plasma TRIG can act as an informative metabolite for assessing positive changes in individual body mass without recapturing and further disturbing individuals. Studies have verified that TRIG provides a robust measure of physiological fattening rate in a variety of migratory species that require the deposition of endogenous lipid stores (birds: Jenni-Eiermann and Jenni 1994; Williams et al. 1999; Seaman et al. 2005; Cerasale and Guglielmo 2006; Anteau and Afton 2008; Dietz et al. 2009; marine turtles: Price et al. 2013; bats: McGuire et al. 2009).

*ii) Baseline Glucocorticoids*

Glucocorticoids (GCs) are a group of lipophilic hormones found in all vertebrate species that are key in maintaining homeostasis (McEwan and Wingfield 2003; 2010) and promoting gluconeogenesis (Dallman et al. 1993). There are two primary GCs across all vertebrate taxa: cortisol, secreted largely by mammals and fish, and corticosterone, secreted largely in reptiles, amphibians and birds (Romero 2002). The production of GCs occurs through the hypothalamic-pituitary-adrenal (HPA) axis (HP-interrenal axis in fish; Crespi et al. 2013). The hypothalamus is stimulated by either an external (e.g., light levels) or internal (e.g., low fat stores) cue, resulting in the secretion of corticotropin

releasing factor (CRF). The CRF acts on the pituitary gland to stimulate the release of adrenocorticotropic hormone (ACTH) which then stimulates the production of GCs in the adrenal (or interrenal) tissue. Once released, GCs are transported through the blood stream to act on the intra-cellular receptors (Breuner and Orchnik 2001; Lattin et al. 2016) of multiple tissues to influence protein and carbohydrate metabolism (Romero 2004; Landys et al. 2006).

Plasma GCs play two key roles across an organism's lifespan. They are most often studied and recognized for their role in the *stress response*, in which GC concentrations rise dramatically following an unpredictable perturbation in the environment (e.g., intra-species competition, interaction with a predator, inclement weather; Romero 2004; Koolhaas et al. 2011). This is an adaptive response meant to stimulate internal biological responses to promote the survival of the individual through the perturbation (e.g., increased mobilization of resources, altered behaviour; Sapolsky et al. 2000). However, when these stress-induced levels of GCs are sustained chronically they can induce negative effects such as suppressed immune function (Sapolsky et al. 2000) or increased oxidative damage (Constantini et al. 2011).

Comparatively less research has been conducted in ecological systems examining the influences of GCs at *baseline* levels, which play a critical role in mediating daily and annual energetic demands (Dallman et al.1993; Romero 2002; Landys et al. 2006; Crespi et al. 2013). Further, there is substantial inter-individual variation in baseline GCs, even within the same life history stage, largely thought to be driven by individuals being under varying energetic constraints and demands (Williams 2008). There also exists predictable variation in baseline GCs across different life history stages, with higher baseline levels

being secreted during more energetically demanding stages (Romero 2002; Landys et al. 2006; Crespi et al. 2013). Taken together, baseline GCs are increasingly thought of as playing regulatory roles in modulating variation in life history decisions because they influence energetics (Dallman et al. 1993; Romero 2002; Landys et al. 2006; Crespi et al. 2013) and mediate transitions between life history stages across multiple taxa (Wada 2008; Crespi et al. 2013). For example, there is a growing body of evidence suggesting that baseline GCs act as a preparatory mechanism for energetically demanding life history stages (e.g., reproduction; Love et al. 2014; Hennin et al. 2015) likely resulting from the links between GC secretion, foraging behaviour and gains in body mass (Landys et al. 2004; Lõhmus et al. 2006; Angelier et al. 2007; Holberton 1999; Holberton et al. 2007; Crossin et al. 2012; Hennin et al. 2016). Further, maternally-derived GCs are known to have carry-over effects on offspring phenotype and performance across taxa (Schreck et al. 2001; Love et al. 2005; Meylan and Clobert 2005; Sheriff et al. 2009; Monclús et al. 2011) and some components of GC physiology are known to be heritable (i.e., stress response: Pottinger and Carrick 1999; Evans et al. 2006; Malisch et al. 2007; Hazard et al. 2008; Baugh et al. 2012; Jenkins et al. 2014), implying that aspects of GCs can be acted on by selection and play a role in the evolution of life histories. Despite the potential for baseline GCs to play a central role in mediating life-history variation, relatively little direct work has been conducted in free-living systems to confirm these predictions (Love et al. 2009; 2013; Crossin et al. 2015).

*iii) Phenotypic Engineering of Energetic Physiology*

Although correlative studies are informative for generating predictive relationships between physiological traits and life history decisions, they do not provide causality or test the capacity of these mechanisms to evolve (McNamara and Houston 1996). Specifically, studies that manipulate physiological traits ("phenotypic engineering") are useful in examining the underlying causal relationships between physiology, life history decisions and fitness because they shift individuals outside their current optimum phenotypic trait value to test for the costs and benefits of the new phenotype (Ketterson et al.1996; Sinervo and Basolo 1996; Williams 2008). The latter is especially important for examining *how* and *why* a given physiological phenotype has evolved *via* the optimization of multiple trade-offs (Reed et al. 2006; McGlothlin and Ketterson 2008; McGlothlin et al. 2010). Steroid hormones in particular are thought to be highly useful for examining the evolution of complex life history decisions because they are: i) plastic and respond to environmental variation and therefore link environmental variation with phenotypic variation (Zera and Harshman 2001), ii) often programmed through maternal effects (e.g., Sheriff and Love 2013), iii) have a heritability component through genetic inheritance (Zera et al. 2007), and iv) impose constraints on individuals generating trade-offs (Ketterson and Nolan 1992; Williams 2008). Although manipulating energetic physiology in free-living systems and following individuals to estimate fitness is often difficult (Crossin et al. 2015), there are some notable studies that have significantly increased our appreciation for how these physiological traits regulate life history decisions. For example, baseline elevations of corticosterone in free-living macaroni

penguins (*Eudyptes chrysolophus*) increased foraging rates, individual condition, and investment in offspring quality (Crossin et al. 2012). In side blotched lizards, females with experimentally elevated levels of baseline corticosterone increased investment in egg quality, implicating its role in driving trade-offs between egg quality and quantity (Sinervo and DeNardo 1996). Finally, in European starlings (*Sturnus vulgaris*), females with experimentally elevated baseline corticosterone demonstrated sex-biased investment decisions, investing relatively more in daughters given the energetic costs associated with rearing larger sons (Love et al. 2005, Love and Williams 2008). Therefore, there appears to be good causal evidence for baseline GCs playing roles in energetic physiology and mediating optimized individual investment decisions.

***Study System and Female Avian Reproductive Physiology***

Common eiders (*Somateria mollissima*) are a long-lived, circumpolar Arctic diving seaduck that overwinter in polynyas or along the coast of Western Greenland, Labrador and Newfoundland, as well as the northern United States (Mosbech et al. 2006). They feed predominantly on bivalves, gastropods and amphipods (Sénéchal et al. 2011), diving up to 11 meters in oceanic waters, often against currents, to forage (Heath et al. 2007). Males and females pair for breeding often on the wintering grounds or during migration. They come to land to reproduce in the summer, usually in large colonies. Females have high natal site fidelity (Cooch 1965; Reed 1975; Wakeley and Mendall 1976; Anderson et al. 1992) and are often found grouped and nesting with closely related females (McKinnon et al. 2006). Males and females are sexually dimorphic with males being largely white with black markings on the wings and head and green colouration on the

neck, while females are brown with black barring. Additionally, females experience delayed maturation where individuals will not begin breeding until 2-3 years of age (Milne 1963).

As in all migratory birds, after arrival on the breeding grounds, the timing of laying is thought to be refined by continual changes in day length, increasing temperature, elevated resources availability, and social cues (Williams 2012). Previous research in mixed strategy (capital-income; Stephens et al. 2009) breeding species such as common eiders, in which females use a combination of endogenously stored resources and locally-acquired resources from foraging to fuel follicle production (Sénéchal et al. 2011), has shown that condition at and timing of arrival on the breeding grounds interact to influence reproductive decisions and therefore reproductive success (Kilpi et al. 2001; Descamps et al. 2011). Furthermore, the ability to gain endogenous fat stores at high rates on the breeding grounds is as important as their arrival condition given that females must accumulate and acquire enough fat stores for follicle growth, and still have enough remaining somatic stores to complete their 24 day incubation fast (Korschgen 1977; Parker and Holm 1990; Erikstad and Tveraa 1995; Bottitta et al. 2003). Females must also invest in reproduction as early as possible given the restricted time frame of an Arctic summer, otherwise risking offspring survival (Love et al. 2010; Descamps et al. 2011). This makes the rapid accumulation of resources an important factor affecting reproductive success through its impacts on the timing of reproductive investment.

Once a threshold body condition has been met, internal and external cues work together to activate the HPG (hypothalamic-pituitary-gonad) axis in females *via* conserved mechanisms present across all avian species. In birds this process starts as the

hypothalamus stimulates the production of GnRH (gonadotropin-releasing home) and a reduction in GnIH (gonadotropin-inhibiting hormone), where GnRH activates the production of follicle stimulating hormone (FSH; thought to influence follicle development) and luteinizing hormone (LH; stimulates production of steroids from ovary and ovulation of follicles) from the pituitary (Williams 2012). Luteinizing hormone signals the ovary to produce estrogens, which stimulate sexual behaviour. Estrogens also travel to the liver, which then stimulates a mechanistic shift from producing generic VLDL (very low-density lipoprotein; used for somatic fat stores) to the production of yolk-targeted VLDL (VLDLy) and vitellogenin, precursors of lipids and proteins to egg yolks, respectively (Walzem 1996; Walzem et al. 1999). The secretion of estrogen has been shown to be tightly coupled with the production of these two yolk precursors and the growth of yolks in the ovary (e.g., Challenger et al. 2001; Salvante and Williams 2002; Gorman et al. 2009) thus initiating the rapid follicle growth (RFG) period in which females quickly grow their yolks in preparation for ovulation. Yolks are grown in a hierarchy, each taking several days to grow, and generally, the first grown follicle is ovulated first (Astheimer and Grau 1990; Ojanen 1983; Williams 2012). Once a follicle has finished growing, it is ovulated and brought into the infundibulum at the top of the oviduct where the egg is fertilized, transported through the magnum where the albumin and membrane are formed, then to the shell gland where the formation of the shell occurs followed by laying (Williams 2012). In eiders, the RFG period is predicted to take approximately 6 days, conservatively (Alisauskas and Ankney 1992), and an additional 28 hours is needed for ovulation, shell formation, and laying (Watson et al. 1993). Female eiders lay four eggs on average (range: 1-6; Love et al. 2010; Descamps et al.

2011) in an open-nest cup on the ground lined with down plucked from her breast and eider hens are the sole incubators of the clutch. Once a female has completed laying her clutch, the production of yolk precursors cease (Challenger et al. 2001; Salvante and Williams 2002; Gorman et al. 2009) and the reproductive tract regresses in size (Vézina and Williams 2003).

Female eiders incubate their clutch for ~24 days until their ducklings hatch (Korschgen 1977; Parker and Holm 1990; Erikstad and Tveraa 1995; Bottitta et al. 2003), leaving the nest infrequently to drink water, otherwise risking predation of their unguarded clutch (Bolduc and Guillemette 2003). Since common eiders are long-lived (average of 10 years, maximum of 20 years; Coulson 1984) individuals have the flexibility within their life history to skip reproduction for a season if they are in poor arrival body condition or if conditions at the breeding site are unfavourable for gaining subsequent body condition (Goudie et al. 2000). Females can also abandon their clutches before hatching if their internal lipid resources are depleted prior to duckling hatching (Bustnes and Erikstad 1991), and these individuals will often stay on the breeding grounds, congregating around hens with hatched ducklings (termed a "crèche"). This behaviour is thought to help enhance the survival of the ducklings from predators (Gorman and Milne 1972), and is likely why more closely genetically related females are found within a crèche (McKinnon et al. 2006). Once ducklings hatch, females wait approximately 24 hours before leaving the nest, taking them to a freshwater pond near the nest initially, and then to the ocean to forage. Common eider ducklings are precocial and are therefore capable of foraging independently. During this time females will recover

from their incubation fast and undergo a full body moult. In the fall, females and ducklings will migrate to the wintering grounds.

The combination of a short breeding season, meeting an energetic threshold requirement for reproduction (Sénéchal et al. 2011), a mixed capital-income breeding strategy (Sénéchal et al. 2011) and a long incubation period (Bottitta et al. 2003) place common eider hens under a strong set of energetic demands and constraints. As such, the timing of investment is a key reproductive decision in this species that can impact individual reproductive success (Love et al. 2010; Descamps et al. 2011). Although the influence of coarse measures of condition (i.e., body mass) have been shown to influence the optimization of reproductive timing and investment (Bêty et al. 2003; Descamps et al. 2011), the energetic mechanisms underlying individual variation in the ability to optimize reproductive investment are still unknown in both common eiders and generally across species.

### *Thesis Objectives*

The overall objective of my thesis is to examine whether individual variation in energetic physiology plays a causal role in driving individual variation in a key life history decision: breeding phenology. To accomplish this objective my thesis mainly focuses on Arctic-nesting common eiders at East Bay Island, Nunavut, Canada (Fig. 1.1), as a model organism. I focus on the pre-breeding stage of reproduction in females, and specifically within pre-recruiting females since these individuals have not yet committed to reproduction (i.e., recruiting follicles). As such, pre-recruiting females have the flexibility to "decide" (in an optimization sense) whether or not to breed in a given year, and if so,

when to optimally time egg-laying to maximize their own fitness. Theoretical models predict that in eiders the timing of laying will be driven by an individual's ability to gain quickly in condition on the breeding grounds (Rowe et al. 1994) to both aid in follicle recruitment and for somatic fattening (Sénéchal et al. 2011), and optimally manage their energetics to complete reproduction within the temporally-constrained breeding period of the Arctic. To characterize energetic physiology, I focus on two traits: baseline corticosterone, which mediates daily energetics of an individual (indicating an individual's energetic demands), and triglycerides, which are a proxy for physiological fattening rates.

In my first data chapter (Chapter 2), I characterize the temporal secretion profiles of energetic physiological traits and investigate potential thresholds these traits may pose to reproductive investment at a population scale. My second data chapter (Chapter 3) builds on the trends observed in the first data chapter, using individual variation in energetic physiology to test predictions on breeding phenology using a condition-dependent individual optimization model (Rowe et al. 1994). My third data chapter (Chapter 4) uses the tractability of a captive system to examine the mechanistic link between experimentally elevated baseline corticosterone and changes in body mass gain (i.e., fattening rate). My fourth data chapter (Chapter 5) combines knowledge gained from Chapters 2-4 to manipulate baseline corticosterone in free-living eider hens to determine the causal role of baseline glucocorticoids in influencing breeding phenology and success. Finally in my Discussion chapter (Chapter 6), I aim to highlight the relative importance of my thesis in: i) building upon previous work examining energetic physiology and the mechanisms underlying breeding decisions in mixed-strategy

breeding birds, and ii) testing the broader framework of the Physiology/Life History Nexus (Ricklefs and Wikelski 2002).

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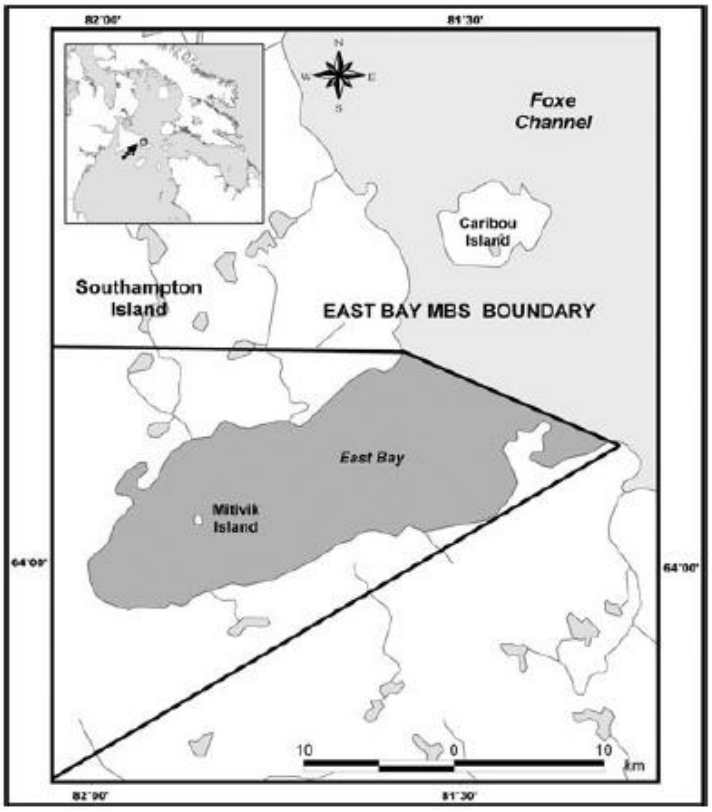


**Figure 1.1** - Map of Mitivik (East Bay) Island, Nunavut Canada located in the East Bay Island Migratory Bird Sanctuary. From: Mallory ML, Fontaine AJ (2004) Key marine habitat sites for migratory birds in Nunavut and the Northwest Territories. CWS, Occasional Paper No. 109, ISBN: 0-662-34046-9

# CHAPTER 2 :

# PRE-BREEDING ENERGETIC MANAGEMENT IN A MIXED-STRATEGY BREEDER[2]

## *Introduction*

Reproduction is an energetically demanding life history stage in which individuals must balance energetics between somatic needs and reproduction. After arriving at the breeding grounds from migration, individuals better able to optimally manage energetic allocation based on intrinsic (i.e., arrival condition) and extrinsic factors (e.g., resource availability) prior to reproduction are predicted to maximise fitness (Drent and Daan 1980; Stearns 1992; Rowe et al. 1994; McNamara and Houston 1996; Kisdi et al. 1998). In highly seasonal environments the timing of breeding is critical because of the trade-off between delaying reproduction to gain in body condition and reproductive investment, against the declining survival probability of later produced offspring (Drent and Daan 1980; Rowe et al. 1994; Bêty et al. 2003). Although these relationships have been broadly tested empirically (Lepage et al. 2000; Bêty et al. 2003; Descamps et al. 2011; Sénéchal et al. 2011), the underlying mechanisms mediating the rapid and required change in body condition for reproduction, and trade-off between self maintenance and reproductive investment, have not been well-characterized (Williams 2005; Williams 2012a).

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A number of physiological traits have recently been identified for their potential preparative and mediatory role in reproductive investment decisions (Williams 2012a). Glucocorticoids (GCs) such as corticosterone and cortisol are a group of hormones that mediate daily and annual energetic management (Romero 2002; Landys et al. 2006) and preparation for energetically demanding life history stages (Love et al. 2014). Baseline GCs have been suggested as mediators of life history decisions (e.g., Love et al. 2013, 2014) given that they are elevated during energetically demanding life history stages (Dallman et al. 1993; Romero 2002; Landys et al. 2006; Crespi et al. 2013). Further, increases in baseline GCs are thought to stimulate foraging behaviour (Astheimer et al. 1992; Dallman et al. 1993; Angelier et al. 2008; Kitaysky et al. 2010; Lynn et al. 2010), which may influence an individual's ability to accumulate resources prior to reproductive investment (Holberton 1999; Kitaysky et al. 1999; Holberton et al. 2007). However, due to the logistical constraints of capturing free-living birds prior to breeding, the role GCs play in pre-breeding energetic management and endogenous resource accumulation are largely unknown.

Similarly, integrative ecologists have begun quantifying resource accrual (rate of condition gain) by measuring plasma levels of generic very low-density lipoprotein (VLDL). Generic VLDL is a type of lipoprotein assembled in the liver from triglycerides, cholesterol, and apolipoproteins which transports triacylglycerides throughout the body following feeding in vertebrates (Gibbons et al. 2004), and is directly related to the rate of condition gain in many species (Seaman et al. 2005; Cerasale and Guglielmo 2006; Williams et al. 2007; Anteau and Afton 2008). In oviparous vertebrates, the period of rapid follicle growth (RFG) in which follicles increase in size quickly in preparation for

ovulation (Drent and Daan 1980; Rowe et al. 1999; Bêty et al. 2003; Williams 2012a) results in a mechanistic shift from the production of generic VLDL to VLDLy (yolk-targeted VLDL). At this time the production of vitellogenin (VTG), an egg yolk precursor comprised of lipoproteins and phosphoproteins that make up most of the protein content of yolk in nearly all oviparous species (Robinson 2008) also increases dramatically (Walzem et al. 1999; Challenger et al. 2001; Salvante and Williams 2002; Gorman et al. 2009). The secretion trends in VLDL and VTG have been well-documented in income-strategy breeding species (European starlings (*Sturnus vulgaris*) - Challenger et al. 2001; zebra finches (*Taeniopygia guttata*) - Salvante and Williams 2002) in which follicles are fuelled entirely by resources from current foraging (Stephens et al. 2009). However, little is known about these dynamics in species using a mixed, capital-income breeding strategy in which individuals use largely endogenous, stored resources and some resources from current foraging to fuel follicle production (Stephens et al. 2009). Since the energetic demands and resource allocation strategies differ widely within the income-capital spectrum, there are likely also fundamental differences in the pre-breeding roles of GCs, VLDL and VTG in individuals reliant on a capital or partial-capital strategy.

Here we examine the temporal dynamics of plasma baseline GCs (corticosterone; CORT), VLDL and VTG during pre-breeding by sampling individual female Arctic-breeding common eiders (*Somateria mollissima*) up to three weeks before a given individuals' investment in reproduction. Females in this species are ideal for examining the physiological mechanisms which mediate pre-breeding resource allocation decisions because they arrive in varying condition after migration, and therefore must both recover

from migration while rapidly accumulating enough body stores to fuel egg production (Descamps et al. 2011; Sénéchal et al. 2011) and simultaneously fatten to sustain a long incubation period without feeding (24 days - Korschgen 1977). Arctic-breeding eiders are further constrained by the short polar breeding season within which they must successfully time reproduction to maximize reproductive success (Love et al. 2010). Our goals were to: i) characterize the post-migration, pre-breeding energetic dynamics of plasma CORT, VLDL (i.e., generic VLDL and VLDLy - see methods), and VTG at the population scale, and ii) examine the relationships between body mass and these physiological traits to determine potential physiological thresholds associated with reproductive status. We predicted that baseline CORT would be significantly elevated both prior to recruiting follicles and RFG compared to arrival to match the energetic demands of depositing fat stores and follicle recruitment, with an associated increase in VLDL. Based on previous work in various avian species (Challenger et al. 2001; Salvante and Williams 2002; Gorman et al. 2009), we predicted a positive, rapid elevation in plasma VTG confined to the RFG period only. Finally, Sénéchal et al. (2011) found that a body mass threshold drives the initiation of follicle growth and the RFG period. Assuming that the secretion of the physiological traits we have chosen mediate the gain in body condition, we predicted strong non-linear relationships (with potential thresholds) between baseline CORT/VLDL/VTG and body mass, providing evidence that they represent relevant underlying mechanisms involved in RFG initiation and reproductive readiness.

## *Materials and Methods*

### *Study System and Reproductive Stage Assessment*

Data were collected from 2003-2013 (except 2005) from the largest known nesting colony of common eiders in the Canadian Arctic (up to 9000 pairs annually) located on Mitivik Island (64°02'N, 81°47'W), a small (800 m × 400 m), low-lying (<8 m elevation) island in the East Bay Migratory Bird Sanctuary, Nunavut, Canada. Females migrate from wintering grounds off the coasts of Greenland and Newfoundland, Canada in May, arrive in early June and lay between mid-June and early July (Mosbech et al. 2006).

Each year, eiders were captured using flight nets from mid-June to early-July overlapping with the eider's timing of arrival at the breeding grounds at Mitivik Island (Descamps et al. 2011). Following capture, a random sample of females (n = 799 across 10 years) were measured and weighed (tarsus - mm; body mass - g), banded, and given a unique combination of temporary plastic nasal tags, attached with UV degradable monofilament which fall off at the end of the season and do not impact survival (H.G. Gilchrist *unpublished*). Subsequent breeding behaviour of individuals (e.g., laying date, clutch size, reproductive success) was monitored consistently across years from seven permanent observation blinds using spotting scopes and entering the colony 2 to 3 times each season to check nests.

Individuals were considered to be non-breeders if they were captured, but were never found to have laid an egg in the colony. Pre-breeding individuals were defined as being caught in flight nets without having yet initiated laying. Accurate breeding state of all individuals was determined *via* consistent, twice daily nest tracking on the island.

When we were uncertain of an individual's laying date the nest was visited to determine clutch size and the duration of incubation *via* egg candling. With the knowledge of a female's clutch size, incubation duration in days, and that female eiders lay one egg every 28 hours (Watson et al. 1993), we were able to back-calculate the original laying date. Knowing the laying date, we were then able to ascertain the reproductive stage of the female at capture. Pre-breeding birds were divided into two categories based on follicle recruitment at the time of capture: RFG or pre-recruiting females. Individuals were identified as "pre-recruiting" if they were captured 8 days or longer before their lay date (Alisauskas and Ankney 1992; Watson et al. 1993). The length of the RFG period for this species has been estimated theoretically (Alisauskas and Ankney 1992; Robertson 1995) to be 6-9 days in length with a 28 hour delay prior to laying for albumen and shell formation (Watson et al. 1993) giving a range of 7 to 10 days. We thus conservatively classified individuals as "RFG" if females were caught between 1 to 7 days prior to laying. Individuals were therefore considered to be "laying" if they were caught 0 to 4 days into laying (based on an average clutch size of 4 in this colony), and "incubating" if they were captured and sampled 5 days or more post-laying based on back-calculations of laying date (as above). Although we categorize and present data for non-breeders, incubating, and laying hens, we only include these groups of females for reference to pre-breeding birds.

*Blood Sampling*

In 2003 and 2004 a maximum of 300 µL of blood was collected within 10 min of capture for VLDL and VTG measures from each female by puncturing the tarsal vein (2003: 26

G needle and heparinized 75 µL capillary tubes; 2004: heparinized vacutainer with a 26 G butterfly needle). From 2006-2013, blood samples were collected within 3 minutes of capture to obtain baseline CORT (Wingfield 1982; Romero and Reed 2005; O. Love *unpublished*) and plasma VLDL using a 23 G thin wall, 1 inch needle attached to a heparinized 1mL syringe. Blood samples were kept at 4°C and centrifuged at 10,000 rpm for 10 min within 6 hours of collection. Plasma was separated from red blood cells and stored at -20°C for further analysis.

*Physiological Assays*

Baseline plasma corticosterone was analysed using a previously-validated enzyme-linked immunoassay (EIA; Assay Designs, Ann Arbor, MI, USA) run in triplicate at a 1:20 dilution with 1.5% of kit-provided steroid displacement buffer (Love and Williams 2008). Each plate was run with a kit-provided standard curve by serially diluting a 200,000 $pg.mL^{-1}$ CORT standard and a control of laying hen plasma (Sigma-Aldrich Canada, Oakville, Ontario, Canada). Assay plates were read on a spectrophotometer plate reader at 405nM, and the mean inter- and intra-assay coefficient of variation across all plates was 8.54% and 5.87%, respectively.

Samples were analysed for zinc (Zn) using a commercially-available kit (Zinc Kit; Wako Chemicals, Virginia, USA) as a measure of the plasma concentration of the yolk precursor vitellogenin (VTG) developed for chickens (Mitchell and Carlisle 1991), and validated in waterfowl (Gorman et al. 2009) and seabirds (Vanderkist et al. 2000; Crossin et al. 2010). Each plate was run with a kit-provided Zn standard (2 $\mu g.mL^{-1}$) and a control sample of laying-hen plasma (Sigma-Aldrich, USA). Samples were read on a

spectrophotometer plate reader, and the inter- and intra-assay coefficients of variation were 3.11% and 3.57% for total Zn, respectively, and 12.49% and 9.17% for depleted Zn, respectively.

Very Low-Density Lipoprotein (VLDL) was quantified using a commercially-available and previously-validated kit which measures plasma triglycerides (TRIG; Sigma Aldrich, U.S.A., #TR0100-1KT; Williams et al. 2007). Samples were run with spectrophotometer plate reader for the concentration of total and free glycerol which when subtracted, provide the TRIG concentration ($mmol.L^{-1}$). Each plate was run with a control of laying hen plasma (Sigma-Aldrich, USA) and a standard curve based on a serial dilution of the glycerol standard (2.54 $mmol.L^{-1}$). Inter- and intra-assay coefficients of variation for total TRIG were 11.27% and 4.42%, and for free glycerol 5.51% and 6.29%, respectively. The TRIG values from the assay indicated generic VLDL, which we corrected for body mass to obtain estimated fattening rate in pre-recruiting birds (Williams et al. 2007), and a proxy for the amount of VLDLy in RFG stage birds (Vanderkist et al. 2000; Crossin et al. 2010).

*Statistical Analyses*

To characterise the secretion dynamics of each physiological trait we determined potential breakpoints in the relationship between physiological traits or body mass using segmented linear regression (Muggeo 2003) in pre-recruiting and RFG females. This analysis identifies sudden, significant positive or negative changes (i.e., breakpoints) in a series of data points, allowing us to detect if and when there were changes in the secretion patterns of CORT, VLDL, VTG or in body mass (dependent variables) across

the pre-breeding period (with pre-laying interval as the independent variable). This procedure identifies and estimates breakpoints by iteratively fitting a model with a linear predictor. For each iteration, a standard linear model is fitted and the breakpoint value is updated until algorithm convergence occurs. This procedure was performed for each dependent variable separately. All segmented models were fitted using the Segmented R package (Muggeo 2003; R Core Team 2014).

To describe physiological trait dynamics, we first tested for the presence of several non-linear relationships between body mass and CORT, VLDL or VTG for all females to determine which best characterised the relationship between body mass and each physiological trait. Specifically, we compared Gompertz, logistic, Weibull, quadratic and linear (used as a null model here) models with year included as a random factor. For each dependent variable (CORT, VLDL or VTG), we ranked the five models using Akaike Information Criterion (AIC; Burnham and Anderson 2002) to determine which model best described the relationship between each physiological trait and body mass. A linear model best described the relationship between body mass and CORT (the null model ranked at $\Delta$AIC = 1.83 compared to the logistic model). Logistic regressions best explained the relationship between body mass and VTG ($\Delta$AICc = 1.02 compared to the Gompertz model and $\Delta$AIC = 5.31 compared to the null model) and body mass and VLDL ($\Delta$AIC = 6.27 compared to the Gompertz model and $\Delta$AIC = 20.1 compared to the null model). For these non-linear relationships between VLDL or VTG and body mass, we used the *lavielle* function of the R package *adehabitatLT* (Calenge 2012) to identify segments (thresholds) on the fitted logistic relationships. This function performs a non-parametric segmentation using the penalised contrast method of Lavielle (1999) with the

goal of determining the threshold values of body mass at which VTG and VLDL start changing in preparation for, or as a result of, reproduction. For both VLDL and VTG, we then obtained the body mass corresponding to the different identified sections. Previous research in this colony has shown that correcting body mass for body size (i.e., "body condition") accounts for only 1% of the variation in body mass and body mass alone is a good proxy of body reserves, performing even better than body mass adjusted for wing (Descamps et al. 2010). As such, we used uncorrected body mass in all our analyses. All values are presented as mean ± s.e.m.

## ***Results***

### *Physiology and Body Mass Dynamics Across Breeding Stages*

On average across the nine-year sampling period, females arrived on 174 ± 0.6 days (Julian date) and laid on 180 ± 0.8 days (Julian date). Although there was variation in arrival and laying dates across years, all mean arrival and laying dates were within 7 and 8 days of each other, respectively (Table 2.1).

Body mass increased slightly, but was consistently high throughout the pre-recruiting and RFG periods (Fig. 2.1A), and we detected no break points for body mass. Plasma VTG was low 20 days prior to laying, it increased steadily throughout the remaining pre-recruiting period, with the highest observed values between 14 and 7 days prior to laying (Fig. 2.1B), although no breakpoints were found for VTG. Baseline CORT was relatively low in the pre-recruiting period, with a sharp increase starting 8 days prior to laying (breakpoint value: 8.0 ± 3.4 days), one day before the predicted RFG period (Fig. 2.1C). Plasma VLDL was low at the beginning of the pre-recruiting period,

but henceforth increased rapidly, reaching the highest observed values 5.4 days prior to laying during the predicted RFG period (breakpoint value: 5.4 ±1.0 days), before declining in concentration again until laying (Fig. 2.1D).

*Physiological Thresholds and Reproductive Stage Change Points*

For VLDL the segmentation procedure indicated three different ranges: i) low range values (2.85 ± 0.39 mmol.$L^{-1}$), ii) an increase phase, and iii) high range values (13.11 ± 0.34 mmol.$L^{-1}$; n = 112; Fig. 2.2A; Table 2.2). In relation to body mass, the low range values of VLDL occurred between 1410 g - 1776 g, the increasing phase between 1777 g - 2004 g, and the high range values from 2005 g - 2430 g (Fig. 2.2A). Similar to VLDL, VTG also showed three distinct ranges: i) low range values (1.05 ± 0.27 ug Zn.$mL^{-1}$), ii) an increase phase, and iii) high range values (3.61 ± 0.15 ug Zn.$mL^{-1}$; n = 67; Fig 2.2B; Table 2.2). In relation to body mass, the low range values of VTG occurred between 1410 g - 1690 g, the increasing phase between 1691 g - 1894 g, and the high range values from 1895 g - 2430 g (Fig. 2.2B). Since a linear model best described the relationship between CORT and body mass, we were unable to test for the presence of thresholds. However, a mixed model regression showed that body mass significantly predicted baseline CORT levels (ß = 0.006 ± 0.001, $F_{1,552}$ = 20.36, p < 0.0001, conditional $R^2$ = 0.08; Fig. 2.2C).

***Discussion***

Given the difficulty in capturing individuals prior to reproductive investment, data on the pre-breeding mechanisms that mediate eventual reproductive decisions are extremely rare. As such, little is known about the pre-breeding dynamics of physiological traits

governing the energetics of reproductive investment. We found that Arctic-breeding female common eiders exhibited consistent and dramatic increases in baseline CORT prior to and throughout the period of follicle recruitment. As predicted, plasma VLDL (physiological fattening rate) subsequently increased throughout the pre-recruiting period and predictably declined in the middle of the RFG period. In contrast to previous studies in income breeding species, elevated VTG in the mixed capital-income strategy eiders was not confined solely to the RFG period, but rather began increasing more than two weeks in advance of laying and over a week prior to the predicted RFG period. Further, the secretion of VTG and VLDL across the pre-breeding period appeared to be uncoupled in relation to RFG. Specifically, elevations in VLDL were confined to the RFG period, whereas VTG was elevated throughout both the pre-recruiting and RFG periods. The secretion of VTG and VLDL were related to key changes in body mass that likely represent a threshold for shifting breeding stages. Finally, the secretion of baseline CORT was linearly related to body mass, indicating that it may be causally linked to increases in the rate of condition gain and follicle recruitment prior to laying. This study is one of the first to illuminate the potential mechanisms and responses underlying the decision to invest in reproduction, providing foundational information for future correlative and manipulative work examining how energetic physiology and environmental variation influence fitness in vertebrates.

*Determining the RFG Period in Common Eiders*

Empirically determining an accurate RFG period and validating physiological markers of reproductive timing for common eiders is important given this species' emerging use as a

general model to examine the physiological, phenological and reproductive responses of seabirds to environmental variation across its circumpolar breeding range (Love et al. 2010; Mallory et al. 2010). Currently, the length of the RFG period for common eiders has only been estimated indirectly and theoretically, and without consensus (Alisauskas and Ankney 1992; Robertson 1995). Previous research in waterfowl (Gorman et al. 2009) and in income-breeding passerines (Challenger et al. 2000; Salvante and Williams 2002) have shown undetectable VTG concentrations and generally low levels of VLDL in pre-breeding, non-breeding and wintering birds with a rapid increase and then decline of VTG and VLDLy within a clearly defined, easily identifiable RFG period (Challenger et al. 2001; Salvante and Williams 2002; Gorman et al. 2009; Palm 2012). We found that on a population level, female eiders have unprecedented, consistent elevations of plasma VTG far earlier than predicted (18 days prior to laying rather than 6-9 days) and that only VLDL demonstrated a breakpoint (decrease) prior to laying. This data suggests that eiders may initiate VTG production up to 2 weeks prior to laying, although they may not initiate rapid follicular growth or uptake of VLDL to yolks until shortly prior to laying (continued discussion below). Captive studies with the ability to repeatedly sample individuals and monitor follicular growth on a daily basis will help to further determine the follicle growth patterns and the potential adaptive reasons for this earlier than expected elevation of VTG in common eiders.

*Yolk-Precursor Secretion and Thresholds*

We found that both VTG and VLDL demonstrated threshold relationships with body mass, where females around 1700-1800 g were in an increasing phase, gaining in mass

(increasing VLDL) and fuelling follicle development (increasing VTG) until reaching a mass plateau around 1900-2000 g. This is consistent with the body mass thresholds reported by Sénéchal et al. (2011) in which females that were 2000 g or heavier were developing follicles and females under 1800 g tended to be non-follicle recruiting. Females around 1800 g may commit to breeding, initiate investment and begin secretion of VTG and VLDL through their continued foraging and gaining in body mass until they reach approximately 2000 g. After achieving a body mass of 2000 g, then females initiate follicle development with peak secretion of VTG and VLDL. It is important to note that the thresholds for VTG are about 100 g lighter than thresholds for VLDL which support our results demonstrating that VTG secretion is elevated prior to VLDL secretion (Fig. 2.1). Although extrinsic energetic mechanisms acting as cues for initiating reproduction have been suggested and empirically tested broadly (i.e., food availability, energetic balance), our current understanding of the underlying (i.e., physiological) mechanisms influencing food intake, gain in condition, and initiation of reproductive state are poorly understood (Williams 2012b). As such, this is one of the first studies examining the underlying mechanisms influencing individual variation in the rate of condition gain and energetic management in pre-breeding birds. Continued investigations of energetic management and yolk-precursors interacting with body mass in other long-lived, capital-income strategy species may further our understanding of the specific follicle recruitment strategies, and the associated trade-offs and constraints which may be dependent on life history and reproductive strategy.

*Pre-Breeding Energetics*

Resource accrual and allocation during the pre-breeding period is critical in mixed capital-income breeding species such as common eiders to meet the multiple energetic requirements for reproductive investment (i.e., fattening and depositing protein stores for incubation and egg production; Bottitta et al. 2003; Sénéchal et al. 2011). As a mixed (i.e., capital-income) strategy breeder, after arriving at the breeding grounds female eiders must recover in condition from migration, then deposit substantial endogenous resources (i.e., body fat and protein) to fuel eventual long incubation bouts, and divert resources towards egg production. While we know that female eiders spend an increasing amount of time diving during the pre-breeding period (52% more time foraging compared to annual average) declining after laying the first egg (Rigou and Guillemette 2010), we currently do not know the mechanisms mediating this increase in foraging rate. As we predicted, plasma CORT increased significantly throughout the pre-breeding period, peaking just prior to the predicted RFG period when female eiders are in peak energetic demand for reproductive investment (Sénéchal et al. 2011). Given that CORT levels have been linked to foraging behaviour (Astheimer et al. 1992; Dallman et al. 1993; Angelier et al. 2008; Kitaysky et al. 2010; Lynn et al. 2010; Crossin et al. 2012) and preparation for energetically demanding life history stages (Love et al. 2014), we suggest that this dramatic increase in baseline CORT may be the mechanism stimulating foraging and facilitating resource accrual during this energetically demanding life history stage. This idea is further confirmed *via* the positive relationship we detected between baseline CORT and body mass. Considering that this increase in baseline CORT occurs for much

longer than one week after arrival on the breeding grounds, it is most likely that the elevation in CORT is related to reproduction, rather than post-migratory condition gains. Alternatively, elevations in baseline CORT may be a response from increased foraging rather than driving foraging behaviours. To determine whether baseline CORT is in fact causally driving pre-breeding fattening rates and reproductive investment, future studies will need to examine the relationship between baseline CORT, fattening rate and reproduction using a combination of correlative and manipulative approaches (e.g., Hennin et al. 2012).

In addition to increases in baseline CORT throughout the pre-breeding period, there was a relative increase in VLDL up until 5.4 days prior to laying at which time the mean concentration decreased. This decline in plasma VLDL may be driven by the transition between females achieving the minimum amount of endogenous fat stores they require for reproduction and the initiation of allocating fewer exogenous resources (and potentially some endogenous resources) to rapid follicular growth in preparation for ovulation. This result is again in stark contrast to the secretion patterns previously found in other avian species (Challenger et al. 2001; Salvante and Williams 2002; Gorman et al. 2009), where VTG and VLDL secretion are tightly coupled. Since pre-breeding females are within a life history stage in which the accumulation and management of fat stores is critical to successfully complete reproduction, females may deposit their less critical and possibly less required protein stores (VTG) to follicles much earlier than other species. Early protein deposition may initiate early follicle recruitment while females are still acquiring endogenous fat stores for themselves, thereby potentially uncoupling the secretion of VTG and VLDLy. Once females have accumulated enough endogenous fat

for reproduction, they may forage at a low rate to ensure their endogenous stores remain as full as possible, requiring only minimal rates of fat deposition to maintain body stores and therefore decreased plasma VLDL. As such, early protein deposition to follicles may be an adaptive strategy in mixed-strategy breeders for optimizing reproductive investment and success within stochastic polar environments.

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**Table 2.1 -** Julian arrival and laying dates of female common eiders from East Bay, Nunavut from 2003-2013.

| | Julian Arrival Date | | | | | Julian Laying Date | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Year** | **N** | **Min** | **Max** | **Mean** | **SE** | **N** | **Min** | **Max** | **Mean** | **SE** |
| 2003 | 65 | 165 | 183 | 172.3 | 0.61 | 53 | 171 | 191 | 180.6 | 0.67 |
| 2004 | 91 | 165 | 189 | 178.5 | 0.78 | 51 | 176 | 199 | 186.2 | 0.74 |
| 2005[a] | 276 | 163 | 178 | 167.8 | 0.3 | 161 | 164 | 194 | 178.0 | 0.5 |
| 2006 | 144 | 161 | 173 | 167.2 | 0.31 | 121 | 157 | 198 | 175.3 | 0.57 |
| 2007 | 135 | 169 | 174 | 172.3 | 0.14 | 94 | 164 | 192 | 183.0 | 0.53 |
| 2008 | 91 | 165 | 178 | 173.3 | 0.30 | 63 | 168 | 195 | 178.4 | 0.62 |
| 2009 | 103 | 169 | 189 | 178.8 | 0.56 | 55 | 175 | 196 | 183.7 | 0.68 |
| 2010 | 86 | 165 | 183 | 176.2 | 0.58 | 43 | 170 | 192 | 178.0 | 0.79 |
| 2011 | 39 | 168 | 180 | 173.1 | 0.59 | 35 | 168 | 193 | 181.0 | 0.94 |
| 2012 | 18 | 169 | 180 | 175.2 | 0.85 | 13 | 173 | 187 | 178.0 | 1.11 |
| 2013 | 27 | 167 | 84 | 174.5 | 0.89 | 19 | 170 | 189 | 179.9 | 1.24 |

[a]Indicates that physiological data were not available for this year.

**Table 2.2 -** Parameter estimates for the logistic equation (Verhulst model) with year considered as a random factor. Estimate, SE, t and p values are given for each parameter of the equation for both VLDL (N = 656) and VTG (N = 122).

| | VLDL (df = 644) | | | | VTG (df = 118) | | | |
|---|---|---|---|---|---|---|---|---|
| | Estimate | SE | t value | p | Estimate | SE | t value | p |
| a | 12.15 | 1.73 | 7.03 | <0.0001 | 3.78 | 0.27 | 13.88 | <0.0001 |
| b | 1877.38 | 19.99 | 93.92 | <0.0001 | 1823.24 | 39.33 | 46.35 | <0.0001 |
| c | 120.31 | 14.81 | 8.12 | <0.0001 | 125.36 | 36.69 | 3.42 | <0.001 |

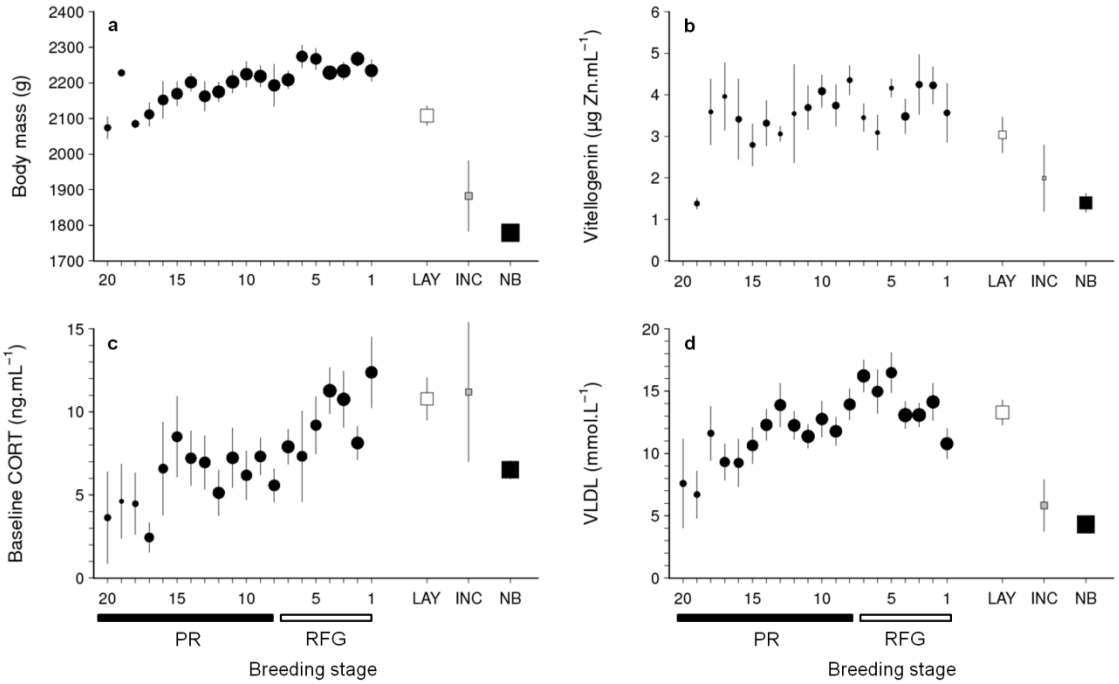


**Figure 2.1** - Changes in body mass (A), vitellogenin-VTG (B), baseline CORT (C) and total VLDL (D) across breeding stages of female common eiders. The black rectangle represents the duration of the pre-recruiting (PR) stage and open rectangle denotes the rapid follicle growth (RFG) stage. Values are means ± s.e.m. provided for each day prior laying (black dots), during laying (LAY, white squares), incubation (INC, gray squares) and in non-breeding (NB, black squares) periods. Symbol sizes are proportional to log (N).

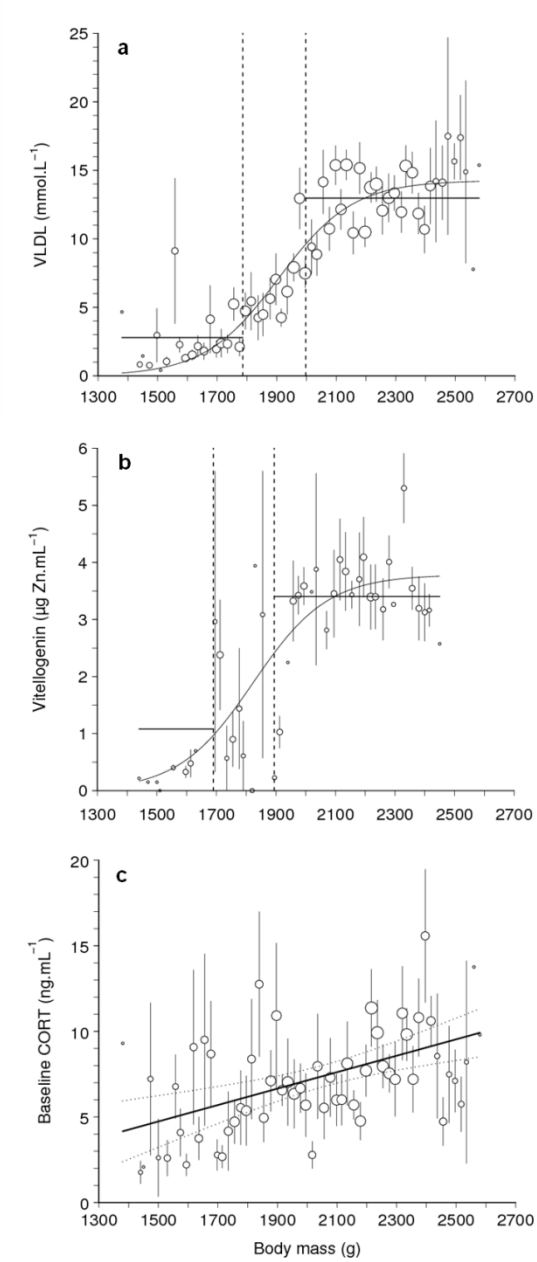


**Figure 2.2** - Relationship between total VLDL (A), vitellogenin-VTG (B) and baseline CORT (C) with body mass at capture. Logistic regression are fitted (solid black lines) as well as sections (dashed vertical lines) with horizontal black lines indicating the mean for the identified sections. Values are means ± s.e.m. Dot sizes are proportional to log(N).

# CHAPTER 3 :

# ENERGETIC PHYSIOLOGY MEDIATES INDIVIDUAL OPTIMIZATION OF BREEDING PHENOLOGY IN A MIGRATORY ARCTIC SEABIRD[3]

## *Introduction*

Life history trade-offs are driven by the allocation of limited resources to multiple life history traits and decisions (Stearns 1992; McNamara and Houston 1996). As such, because individual state is predicted to be indicative of the amount of resources available to allocate to multiple life history traits and decisions, it is thought to be a primary driver regulating individual optimization of life history trade-offs (Stearns 1992; Kisdi et al. 1998; Moore and Hopkins 2009). In avian species breeding in temporally constrained, seasonal environments, the timing of reproduction is an important fitness-related decision influencing the quantity and quality of an individual's investment (Perrins 1970; Smith 1993; Lepage et al. 2000; Garant et al. 2007; Brommer and Rattiste 2008), and therefore its subsequent reproductive success (Lepage et al. 2000; Love et al. 2010; Descamps et al. 2011). Individuals that can arrive earlier and in greater condition are predicted to invest in reproduction earlier, produce more offspring with higher survival and achieve higher reproductive success (Rowe et al. 1994; Kisdi et al. 1998; Morris 1998). The link between earlier reproduction and higher reproductive investment is thought to result from the trade-off between the benefit of delaying reproduction to invest in additional offspring, against the declining value of offspring as the breeding season progresses

[3] This chapter is resubmitted to *American Naturalist* and is the outcome of joint research with J. Bêty, P. Legagneux , H.G. Gilchrist, T.D. Williams and O.P. Love

(Drent and Daan 1980; Rowe et al. 1994; Morris 1998; Lepage et al. 2000; Bêty et al. 2003).

To develop testable hypotheses for the assumed relationships between timing of and body condition at arrival on the breeding grounds, timing of laying, and investment in reproduction (i.e., clutch size), Rowe et al. (1994) formalized a condition-dependent individual optimization model largely centered around migratory birds breeding in seasonal environments (Fig. 3.1 - based on Figs. 1-5 of Rowe et al. 1994). The model predicts that individuals arriving from migration to the breeding grounds earlier and in greater body condition have the potential to acquire the local resources they need to fuel reproduction earlier and therefore initiate reproduction earlier, a key fitness-related parameter in many avian species (Drent and Daan 1980; Rowe et al. 1994; Lepage et al. 2000; Bêty et al. 2003; Descamps et al. 2011). The model's ultimate goal is to examine the mechanisms behind the well-known seasonal decline in clutch size in many avian species, where earlier laying dates should be associated with greater investment in reproduction (i.e., clutch size) (Drent and Daan 1980; Rowe et al. 1994; Bêty et al. 2003; Descamps et al. 2011). Independent of arrival date and condition, Rowe and colleagues also made a second set of important predictions that to date have received far less attention in the literature. They predicted that once on the breeding grounds individuals with the ability to gain in condition faster (i.e., more optimal energetic management), and therefore fuel investment in reproduction sooner, should be able to lay earlier even after controlling for variation in arrival date and body condition (Figs. 4, 5 of Rowe et al. 1994). The biological implications of this prediction suggests that even females arriving in lower condition or at a later date, but able to efficiently manage their energetics (i.e.,

quickly accumulate resources needed for reproduction), may be able to lay earlier and invest more in reproduction than previously appreciated (Fig. 3.1, scenario A vs. B and C vs. D). Although there have been some robust, empirical tests of this optimization model with regards to arrival date and body condition (i.e., body mass; Bêty et al. 2003; Descamps et al. 2011), a substantial amount of variation in reproductive decisions remains unexplained. Unfortunately, it has been difficult to test empirically for the effects of individual variation in the pre-breeding rates of condition gain directly since this requires the capture of free-living individuals twice to assess condition gain (e.g., before birds initiate follicle recruitment and immediately after they commence laying), while mitigating the well-known effects of capture stress on breeding activities (Love et al. 2004; Buttler et al. 2011; Legagneux et al. 2012).

Measures of energetic physiology are prime candidates for assessing individual variation in body condition because they are thought to be proximate mechanisms underlying individual variation in reproductive decisions and can require the capture of an individual only once (Zera and Harshman 2001; Ricklefs and Wikelski 2002; Harshman and Zera 2007; Zera et al. 2007). Furthermore, there is a substantial amount of inter-individual variation in the production of and sensitivity to the production of physiological traits and therefore physiological phenotypes, which may play important roles in key life history decisions (Ketterson and Nolan 1999; Williams 2008). Plasma levels of triglycerides (TRIG) and baseline glucocorticoids (GCs) in particular have recently been identified for their potential role in influencing reproductive decisions (Williams 2012; Love et al. 2014; Hennin et al. 2015) and may therefore be useful for empirically testing predictions of condition-mediated models in free-living species.

Plasma TRIG is a metabolite present in very-low density lipoprotein which increases following foraging and is the primary method in which fat is deposited to adipose tissues (Gibbons et al. 2006). Plasma TRIG titres have been shown to correlate positively with increases in body mass in bats (McGuire et al. 2009), turtles (Price et al. 2013), and many free-living and captive avian species including warblers (e.g., Jenni-Eiermann and Jenni 1994; Jenni and Jenni-Eiermann 1996; Jenni and Schwilch 2001), shorebirds (Williams et al. 1999; Cerasale and Guglielmo 2006; Lyons et al. 2008), and ducks (Anteau and Afton 2008). In many avian species, plasma TRIG has been useful for characterizing the accumulation of fat stores (Jenni-Eiermann and Jenni 1994; Cerasale and Guglielmo 2006) and rate of gain in condition (Williams et al. 1999). However, once oviparous females begin growing their follicles in preparation of ovulation (known as the Rapid Follicular Growth – RFG – phase), females undergo a mechanistic shift from producing TRIG for somatic fattening to producing yolk-targeted very low-density lipoprotein (VLDLy) for lipid deposition to the yolks of eggs (Salvante and Williams 2002; Salvante et al. 2007; Williams 2012). Therefore, to use plasma TRIG as a relevant measure of physiological fattening, it is critical to appreciate the exact breeding stage of a given individual.

Largely studied for their role in the stress response, glucocorticoids (GCs) are a group of pleiotropic hormones which, at baseline levels, play very important roles in managing daily and seasonal energetics (Dallman et al. 1993; Romero 2002; Landys et al. 2006; Crespi et al. 2013), largely by maintaining homeostasis through their role in gluconeogenesis (Sapolsky et al. 2000; McEwan and Wingfield 2003). In response to predictable changes in life history, baseline GC levels are often elevated to support the

energetic demands of those stages (e.g., reproduction) (Dallman et al. 1993; Romero 2002; Landys et al. 2006; Crespi et al. 2013). Recent correlative and manipulative studies examining baseline corticosterone (CORT; the primary avian glucocorticoid) have indicated that it increases prior to reproduction to potentially fulfill a preparatory role for the energetically demanding stage ahead (Love et al. 2014; Hennin et al. 2015), likely due to the relationship between baseline CORT and energetic management, foraging behaviour and fat deposition (Landys et al. 2004; Lõhmus et al. 2006; Angelier et al. 2007a; Holberton 1999; Holberton et al. 2007; Crossin et al. 2012; Hennin et al. 2016). Indeed, experimental increases in baseline levels of CORT have been shown to directly, causally influence increases in body mass likely *via* increased foraging behaviour and fat deposition (Hennin et al. 2016). Nonetheless, since baseline GCs have pleiotropic effects on other endocrine pathways (e.g., the hypothalamic-pituitary-gonadal axis; Miller et al. 2009), elevations even within a baseline range can potentially impact the functioning of other traits and endocrine pathways. Therefore, baseline CORT is a prime candidate for influencing changes in condition and overall energetic management in individuals that are preparing to invest in reproduction given that individuals must manage elevations carefully to optimize impacts across endocrine systems. By sampling individuals prior to committing to reproductive investment and measuring both the rate of instantaneous condition gain (plasma TRIG) and energetic management (baseline plasma CORT), we have the potential to quantify the mechanisms mediating the ability of individuals to optimize energetic management and mechanistically test Rowe and colleagues' condition-dependent individual optimization model.

Here we use data on plasma TRIG and baseline CORT collected from over 350 pre-breeding Arctic-breeding female common eiders (*Somateria mollissima*) across 8 years to test empirically whether individual variation in pre-breeding energetic physiology (fattening rates and energetic management) influences the timing of breeding within the context of the condition-dependent individual optimization model (Rowe et al. 1994; Bêty et al. 2003). Capital breeding birds use only endogenous fat stores to fuel reproduction, whereas income breeders use solely resources acquired from local, immediate foraging (Stephens et al. 2009). Common eiders have a mixed, capital-income breeding strategy (Sénéchal et al. 2011) using a combination of endogenous, stored resources and resources acquired from current, local foraging to grow their follicles and therefore must feed intensively during the pre-breeding period. As such female common eiders are an ideal system for examining the energetic mechanisms mediating reproductive decisions because they: i) must reach a body mass threshold to initiate follicle recruitment (Sénéchal et al. 2011; Hennin et al. 2015), ii) must acquire enough endogenous resources to fuel a 24 day incubation period in which they fast (Bottitta et al. 2003), otherwise risking nest abandonment (Bustnes and Erikstad 1991), iii) reproduce within a highly seasonal environment and therefore must optimally time reproduction to maximize reproductive success (Love et al. 2010), and iv) have previously been shown to be a model species for testing predictions of state-based optimization models (e.g., the causal relationship between pre-breeding body condition and laying date was showed experimentally; Descamps et al. 2011).

To test empirically whether the rate of condition gain measured through energetic physiology influences breeding phenology, we separated the model into two testable

components as predicted by Rowe et. al (1994) (Fig. 3.1): i) the delay before laying (time required to sufficiently gain in condition and reach the optimal combinations of laying date and breeding investment), and ii) the relative laying date. Although the relative laying date and the delay before laying metrics are related, they describe different, important aspects of reproduction as well as testable components of the optimization model. Relative lay date specifically indicates the timing of reproduction, whereas the delay before laying indicates the amount of time individuals require to gain in condition after migration, meet the minimum body mass threshold required to initiate reproduction, grow follicles, and ultimately begin laying their clutch. Based on Rowe and colleagues' condition-dependent individual optimization model, two potential predictions of the way in which variation in energetic physiology could influence the optimization of breeding phenology decisions can be made, after controlling for the known influence of arrival date and body condition (i.e., body mass). First, we predicted that individuals with higher fattening rates (higher plasma TRIG) and high signals of energetic demand (high CORT to induce foraging) would result in higher rates of condition gain and therefore earlier breeding phenology (a shorter delay prior to laying and earlier relative laying dates). Alternatively, given that individuals must balance the benefits and costs of elevated baseline GCs carefully (Love et al. 2014; Crossin et al. 2015), we could also predict that individuals able to manage their energetic physiology more efficiently (i.e., the ability to maximize physiological fattening while minimizing elevations of baseline CORT) may in fact exhibit the earliest breeding phenology. The ultimate goal of Rowe and colleagues' condition-dependent individual optimization model is to predict both timing of breeding and reproductive investment (i.e., clutch size). Due to logistical constraints of acquiring

accurate clutch size data in the field, we are currently unable to test specific predictions related to the second component of the model. However, the negative association between laying date and clutch size was previously confirmed at our study site (Descamps et al. 2011).

## *Materials and Methods*

### *Study Site and General Field Methods*

Our study site is located in the East Bay Migratory Bird Sanctuary on Mitivik Island, Nunavut, Canada (64°02'N, 81°47'W), and is Canada's largest colony of Arctic-nesting common eiders (up to 9000 pairs annually). Females winter along the coast of Newfoundland and Labrador, Canada or off of the Western coast of Greenland (Mosbech et al. 2006), initiate spring migration in mid-May, arrive on the breeding grounds in mid-June and initiate laying in late June and early July (Love et al. 2010; Hennin et al. 2015). From 2006 to 2013, we captured pre-breeding females (N = 366) opportunistically using flight nets early in the season during mid-June to early July as they flew over the colony. Since we targeted the timing of capture to coincide with the timing of arrival on the breeding grounds (Love et al. 2010), we use individual capture date as a useful proxy for their timing of arrival at the colony (Descamps et al. 2011; see Discussion).

Within 3 minutes of an individual hitting the flight net, a blood sample was taken from the tarsal vein of each female using a 1mL heparinized syringe and 23 G thin-wall, 0.5 inch needle to obtain baseline physiology (Romero and Reed 2005). Across all years, blood samples were collected at all times of day. Although baseline CORT and TRIG have been shown to exhibit diel variation in secretion in temperate breeding species (e.g.

Dallman et al. 1993; Jenni and Jenni-Eiermann 1996), common eiders at our study site do not exhibit these diel trends during the breeding season (Steenweg et al. 2015), allowing us to include all collected blood samples in analyses. Blood samples were transferred to a heparinized collecting tube, centrifuged for 10 min at 10,000 rpm, and the separated plasma and red blood cells were stored at -20˚C in the field (-80˚C in the lab) until further analysis. After sampling, body mass (g) and tarsus length (mm) were collected, females were banded, and given a unique combination of plastic, coloured nasal tags for future identification. Nasal tags were attached with UV degradable monofilament to ensure that the tags would fall off at the end of the breeding season, prior to fall migration. Although wing-bar measurements have been used to age hens in other species and populations (e.g. Carney 1992), this technique is unreliable in hens nesting at Mitivik Island (H.G. Gilchrist, *unpublished*) and as such we were unable to assign age accurately to our hens.

Breeding activities of the individuals within our eider colony are monitored yearly using spotting scope-based observations from seven permanent blinds. Consistent behavioural observations are collected using consistent protocols and trained observers. Using these observation techniques, the nests of nasal-tagged hens were monitored twice daily to collect accurate laying dates (transformed into relative laying dates; individual Julian lay date relative to the median laying date of the colony that year; Lepage et al. 2000) which allowed for the calculation of the delay before laying (number of days between arrival and laying dates).

*Physiological Assays*

Plasma triglycerides (TRIG) were measured using a commercially available kit (Sigma-Aldrich, Ontario, Canada; Williams et al. 1999) optimized for use in pre-breeding common eiders (Hennin et al. 2015). Following dilution, samples were added to 96-well microplates with Reagent A to measure free glycerol, followed by Reagent B to measure total glycerol. After the addition of each reagent, the plates were shaken for 10 min at 37°C, then read using a plate reader at 540 nM wavelength. The amount of triglycerides ($mmol.L^{-1}$) in the plasma was calculated by subtracting the amount of free glycerol (first read) from the amount of total glycerol (second read). Each plate was run with a commercially available internal plasma control (Sigma-Aldrich Canada, Oakville, Ontario, Canada) and a serially diluted standard curve of glycerol standard (2.54 $mmol.L^{-1}$). Inter- and intra-assay coefficients of variation were 11.27% and 4.42% for total TRIG, and 5.51% and 6.29% for free glycerol, respectively.

Baseline plasma corticosterone (CORT) was measured using a commercially available, previously validated enzyme immunoassay kit based on competitive binding (EIA; Assay Designs, Ann Arbor, MI, USA) and optimized for common eiders (Hennin et al. 2015). Samples were run un-extracted and in triplicate at a 1:20 dilution with 1.5% steroid displacement buffer. Each assay plate was run with a standard curve by serially diluting a 200,000 $pg.mL^{-1}$, kit-provided corticosterone standard and a corticosterone-spiked control sample, and read at 405 nM using a plate reader (for details see Hennin et al. 2015). The inter- and intra-assay coefficient of variation across all plates was 8.54% and 5.87%, respectively.

*Statistical Analyses*

Analyses for the delay before laying and relative laying date were constrained to include only pre-recruiting females. Females were classified as pre-recruiting if they were caught eight days or longer before laying since these birds would not have yet begun recruiting follicles (Alisauskas and Ankney 1992; Hennin et al. 2015). Birds caught within seven days of laying have already committed to follicle recruitment (i.e., investment in eggs; Hennin et al. 2015) and instead would be categorized to the Rapid Follicle Growth (RFG) stage. In instances where females were recaptured in different years (only 32 in over 350 birds included in the current study), we included only the first instance of her capture in which we were able to assay baseline CORT and TRIG. All subsequent captures were excluded to prevent statistical bias.

We tested the effect of four (independent) covariates using generalized linear mixed models (GLMM): female body mass at arrival ("Mass"), relative arrival date ("Arrival"; date individual arrived relative to the colony median arrival date for that year), mass-corrected TRIG ("FatRate") which is an index of fattening rate in birds (Williams et al. 1999), and baseline CORT ("CORT"; log transformed) on delay before laying and relative laying date of individuals. Since the relationship between fattening rate and baseline CORT may vary depending on current energetics and breeding stage (Hennin et al. 2015), we also examined the potential for an interaction between fattening rate and baseline CORT in our analyses. Due to the inclusion of mass-corrected TRIG in our analyses to represent fattening rate, we did not include interactions between body mass and energetic physiology. Finally, we included year as a random factor in all

analyses to account for annual variation in reproductive decisions and physiology, in addition to other extrinsic factors. Our analyses were conducted in R (R Core Team 2014) using the unrestricted maximum likelihood method of the 'lme4' package (Bates et al. 2012). We then used Akaike Information Criterion adjusted for small sample size (AICc) and Akaike weights to select the most parsimonious model for each independent variable. The model with the lowest AICc value was considered to be the most parsimonious, and models found to be within 2 ΔAICc units were considered competing models (Burnham and Anderson 2002). Finally, we used model averaging (multimodel inference) to estimate parameters as it reduces bias and increases precision (Burnham and Anderson 2002). All values are presented as mean ± s.e.m. unless otherwise stated.

***Results***

For the delay before laying, body mass was present in all competing models, with the interaction between fattening rate and baseline CORT being present in the top two models (Table 3.1). Females with heavier body mass at arrival, and those with higher fattening rates combined with lower levels of baseline CORT had the shortest delays before laying (Table 3.2; Fig. 3.2A, 3.2B). For relative laying date, both body mass and relative arrival date were present in all competing models, and the interaction between baseline CORT and fattening rate was present in the top model (Table 3.1). According to the parameter estimates, individuals with a heavier body mass at arrival laid earlier, and those with higher fattening rates combined with lower levels of baseline CORT had the earliest laying dates (Table 3.2; Fig. 3.2C, 3.2D).

### *Discussion*

We examined a highly relevant, but previously-untested, component of the Rowe and colleagues' condition-dependent individual optimization model (Rowe et al. 1994) – the impact of individual variability in rate of gain in condition on breeding phenology. We predicted that after controlling for the known influences of arrival date and body condition (i.e., body mass) that both pre-breeding physiological fattening and baseline levels of plasma CORT (ability to manage energetics) would enhance our ability to predict reproductive phenology parameters associated with the condition-dependent individual optimization model. We predicted that the earliest laying dates and shortest delays before laying would result from higher physiological fattening rates coupled to either higher or lower baseline CORT, the former prediction being related to elevated baseline CORT's known role in mediating foraging, and the latter in relation to individual efficiency in optimizing the cost/benefit ratio of elevated baseline CORT levels. We found support for the second prediction; female common eiders with higher fattening rates and lower baseline CORT (better energetic physiology efficiency) had the shortest delays before laying and the earliest relative laying dates. As such, being able to fatten at a high rate (high TRIG) in preparation for reproduction while maintaining low energetic costs (low baseline CORT; i.e., higher efficiency) appears to provide the optimal physiological phenotype that maximizes investment in reproduction with regards to reproductive phenology. Nonetheless, females unable to maintain high physiological fattening rates appear to be able to achieve moderate reproductive phenology by compensating with higher baseline CORT, which could drive increases in absolute foraging rates (see Introduction). Regardless of the interaction with baseline CORT, and

as predicted by Rowe and colleagues' condition-dependent individual optimization model (Fig. 3.1), low fattening rates (low TRIG) consistently resulted in the longer delays before laying and the latest relative laying dates, especially when combined with low energetic demand (baseline CORT). It therefore appears that variation in the timing of arrival and arrival body mass can be fine-tuned by individually-optimized strategies for acquiring resources on the breeding grounds and hence driving the accrual of endogenous fat stores in preparation for reproduction.

*A Novel Test of the Individual Optimization Model*

Previous empirical work testing predictions of the condition-dependent individual optimization model we also examine here (Rowe et al. 1994), have focused upon the impacts of arrival condition (measured through body mass) and arrival date on breeding phenology. Both correlative and manipulative studies in free-living species have confirmed that individuals arriving with heavier body mass, at an earlier date, or a combination of both, appear to reach their optimal combination of reproductive phenology and investment earlier and thus initiate breeding the earliest (Bêty et al. 2003; Descamps et al. 2011). Given a number of logistical constraints of working in free-living systems, a key additional prediction made by Rowe and colleagues remained largely untested in the wild, namely that a faster gain in condition will positively influence the timing of breeding (reproductive phenology). While food supplementation studies can influence fattening rates and reveal some of the relationships between individual condition, and reproductive timing and investment (Ruffino et al. 2014), other parameters such at territory quality and competition can be influenced simultaneously, often making

results difficult to interpret (Williams 2012). By employing highly relevant metrics of energetic physiology we confirmed this long-held prediction; after controlling for arrival date and body mass, females with higher instantaneous physiological fattening (i.e., greater rate of condition gain and slope – higher plasma TRIG/physiological fattening rate), regardless of their energetic demand (baseline CORT), had a shorter delay before laying as well as an earlier relative laying date. These results have important implications for how life history investment decisions evolve. At the simplest level, our results suggest that certain physiological/behavioural phenotypes may enable individuals to gain condition quickly on the breeding grounds following arrival from migration.

Alternatively, individual females may have differing degrees of physiological/behavioural flexibility enabling them to adjust their foraging behaviour (either the rate at which they forage, or the locations that they forage within, or both) based on a combination of intrinsic and extrinsic conditions to optimally adjust their breeding phenology independently of their arrival date and "condition". To test for differences between the two potential mechanisms, physiological manipulations in free-living individuals will be necessary. We were unable to test the impact of variation in the rate of condition gain on reproductive investment (i.e., clutch size) due to the difficulty of acquiring accurate estimates of clutch size for most females nesting at our study site within the current 8-year dataset (2006-2013). However, the links between pre-breeding body mass, arrival date and the seasonal decline in clutch size have already been demonstrated in mixed strategy Arctic-breeding species including common eiders (Bêty et al. 2003; Descamps et al. 2011). What remains to be examined then is whether higher

pre-breeding fattening rates *per se* and optimization of energetic management may result in larger clutch sizes as predicted by Rowe et al. (1994).

*Influence of Energy Acquisition on the Optimal Timing of Reproduction*

The impact of a high fattening rate on both the delay before laying and laying date was dependent on an individual's ability to manage its energetics (i.e., baseline CORT levels). There may be two potential ways in which these physiologically mediated effects on laying decisions could arise, both operating through differences in individually-optimized breeding strategies across females (with associated downstream costs and benefits). Firstly, it is well known that the energetic physiology of long-lived vertebrates changes over the lifespan of an individual (Monclús et al. 2011; Elliott et al. 2014). Age-related changes in baseline CORT (Heidinger et al. 2006; Monclús et al. 2011; Riechert et al. 2012) and physiological sensitivity to the effects of baseline CORT (Peiffer et al. 1991; Reul et al. 1991; Perlman et al. 2007) have also been documented in a number of vertebrate species. Consequently, age-related variation may directly shape the relationship between energetic physiological traits and reproductive decisions (Heidinger et al. 2006; Monclús et al. 2011; Riechert et al. 2012). We are currently unable to reliably determine age in our study species, however an ability to do so in the future, or work in a colony of known-age birds would allow further testing of the relationships between age-related changes in the reproductive sensitivity to variation in baseline CORT.

Secondly, independent of age-related effects on energetic physiology, inherent individual variation in the secretion of baseline CORT, behavioural (i.e., foraging) sensitivity to baseline CORT (Angelier et al. 2007b; Crossin et al. 2012), the acquisition

and assimilation of energetic resources (Heath et al. 2010; Rigou and Guillemette 2010), or the ability to optimally mitigate the costs of migration (Crossin et al. 2010) could all work together to fine-tune general optimal investment strategies (Williams 2008; Williams 2012). For example, those individuals able to maintain baseline GCs at lower levels given similar life history costs may have the highest inherent 'quality', exhibiting greater energetic physiology efficiency, and therefore gain higher fitness benefits (Angelier et al. 2010). Alternatively, inter-individual variation in the ability to manage energetic physiology under varying environmental conditions (i.e., physiological flexibility) could result in different individual-based optimization strategies within the same life history stage (Angelier et al. 2007b; Schultner et al. 2013; Love et al. 2014). For example, we found that while having high energetic physiology efficiency (high mass-corrected TRIG (fattening rate) and low baseline CORT) may be optimal for an individual if it is achievable, females may still be able to optimize laying decisions *via* higher physiological fattening rates coupled with elevated baseline CORT, although this strategy would be predicted to come at a hypothesized cost given the impacts of elevated GCs on oxidative damage (Constantini et al. 2011), ultimately leading to reduced life-spans (Bize et al. 2008). Within this framework, it is possible that late laying birds with low fattening rates and low CORT may be adopting a slower pace-of-life strategy resulting in a lower intra-annual probability of reproductive success, potentially smaller amount of within-season investment (i.e., smaller clutch sizes), but benefiting *via* a longer lifespan as a result (Comendant et al. 2003; Lancaster et al. 2008; Descamps et al. 2011; Palacios et al. 2012). While biologically valid and relevant, these hypotheses remain to be tested in free-living species since they necessitate the difficulties of

hormonally manipulating pre-breeding females and then consistently following short- and long-term benefits/costs to reproduction or survival within females across multiple years. Captive studies involving the pre-breeding manipulations of baseline CORT would also be helpful to minimize the impacts of external sources of variation potentially influencing the interaction between baseline CORT and TRIG, while still moving individuals outside of their optimal physiological phenotype to examine underlying costs on a per-individual basis.

*Conclusions and Future Research*

The inclusion of physiological traits that represent fattening rate (plasma TRIG) and energetic demand (baseline CORT) during the pre-recruiting period allowed us to investigate a previously untested component of the condition-dependent individual optimization model first proposed by Rowe et al. (1994). Although the influence of these physiological traits on reproductive decisions was more complex than originally predicted, their inclusion enhanced the predictive capacity of the model and improved our interpretation of how variation in individual physiological phenotypes influences reproductive decisions that impact fitness. To determine whether these mechanisms have a causal effect on breeding phenology and breeding investment, and to examine whether variation in their physiological control mechanisms drive investment trade-offs, future research should aim to manipulate baseline CORT or plasma TRIG levels within biologically-relevant levels in free-living individuals during the critical pre-breeding stage (Crossin et al. 2015). Being able to couple these metrics with measures of foraging behaviour (i.e., foraging rates and profitability) and monitoring long-term success (i.e.,

survival) would greatly improve our appreciation for how underlying mechanisms enable individually-optimized life history strategies to evolve.

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**Table 3.1 -** Results of model selection on the delay before laying and relative laying date of pre-recruiting female common eiders breeding at Mitivik Island. Log-likelihood, number of parameters (k), ΔAICc, AICc weight ($w_i$) and are provided for each competing model. Year was included as a random factor. A model with an interaction also included corresponding parameters. Competing models (< 2.0 $\Delta AIC_c$ *)* have $w_i$ values with an asterisk (*).

| | log likelihood | k | $\Delta AIC_c$ | $w_i$ |
|---|---|---|---|---|
| Delay before laying: | | | | |
| Mass + Arrival + CORT*FatRate | -511.23 | 8 | 0.00† | 0.23* |
| Mass + CORT*FatRate | -512.39 | 7 | 0.15 | 0.21* |
| Mass | -516.16 | 4 | 1.28 | 0.12* |
| Mass + Arrival | -515.25 | 5 | 1.56 | 0.10* |
| Mass + FatRate | -515.46 | 5 | 1.99 | 0.08* |
| Mass + Arrival + FatRate | -514.58 | 6 | 2.37 | 0.07 |
| Mass + CORT | -515.94 | 5 | 2.96 | 0.05 |
| Mass + Arrival + CORT | -515.09 | 6 | 3.38 | 0.04 |
| Mass + Arrival + CORT + FatRate | -514.40 | 7 | 4.17 | 0.03 |
| CORT*FatRate | -518.82 | 6 | 10.84 | 0.00 |
| Arrival + CORT*FatRate | -517.98 | 7 | 11.32 | 0.00 |
| Null | -523.89 | 3 | 14.66 | 0.00 |
| CORT | -523.01 | 4 | 14.99 | 0.00 |
| FatRate | -523.89 | 4 | 15.32 | 0.00 |
| Arrival | -523.32 | 4 | 15.60 | 0.00 |
| Arrival + FatRate | -522.64 | 5 | 16.35 | 0.00 |
| Relative lay date: | | | | |
| Mass + Arrival + CORT*FatRate | -516.93 | 8 | 0.00‡ | 0.26* |
| Mass + Arrival | -520.22 | 5 | 0.12 | 0.25* |
| Mass + Arrival + FatRate | -519.28 | 6 | 0.36 | 0.22* |
| Mass + Arrival + CORT | -520.21 | 6 | 2.22 | 0.09 |
| Mass + Arrival + CORT + FatRate | -519.27 | 7 | 2.50 | 0.07 |
| Arrival + CORT*FatRate | -525.31 | 7 | 14.57 | 0.00 |
| Arrival | -529.29 | 4 | 16.15 | 0.00 |
| Arrival + FatRate | -528.42 | 5 | 16.51 | 0.00 |
| Mass + CORT*FatRate | -555.81 | 7 | 75.57 | 0.00 |

| | log likelihood | k | $\Delta AIC_c$ | $w_i$ |
|---|---|---|---|---|
| Mass | -559.23 | 4 | 76.01 | 0.00 |
| Mass + FatRate | -558.71 | 5 | 77.10 | 0.00 |
| Mass + CORT | -558.97 | 5 | 77.60 | 0.00 |
| CORT*FatRate | -562.99 | 6 | 87.78 | 0.00 |
| Null | -566.74 | 3 | 88.95 | 0.00 |
| FatRate | -566.23 | 4 | 90.03 | 0.00 |
| CORT | -566.74 | 4 | 91.04 | 0.00 |

†$AIC_c$ value = 1039.25
‡$AIC_c$ value = 1050.66

**Table 3.2** - Summary of model-averaged parameter estimates and unconditional standard errors for parameters included in the delay before laying and relative laying date analyses.

| | β | SE | Min 95% CI | Max 95% CI |
|---|---|---|---|---|
| Delay before laying: | | | | |
| Intercept | 23.91 | 2.90 | 18.22 | 29.61 |
| Arrival | -0.10 | 0.07 | -0.24 | 0.04 |
| Mass | -5.12 | 1.33 | -7.73 | -2.50 |
| CORT | -0.11 | 0.25 | -0.60 | 0.38 |
| FatRate | -0.05 | 0.04 | -0.14 | 0.04 |
| CORT*FatRate | 0.09 | 0.04 | 0.02 | 0.16 |
| Relative lay date: | | | | |
| Intercept | 18.58 | 2.93 | 12.80 | 24.32 |
| Arrival | 0.78 | 0.08 | 0.63 | 0.94 |
| Mass | -5.67 | 1.32 | -8.30 | -3.08 |
| CORT | 0.05 | 0.25 | -0.40 | 0.54 |
| FatRate | -0.06 | 0.05 | -0.20 | 0.03 |
| CORT*FatRate | 0.08 | 0.00 | 0.01 | 0.15 |

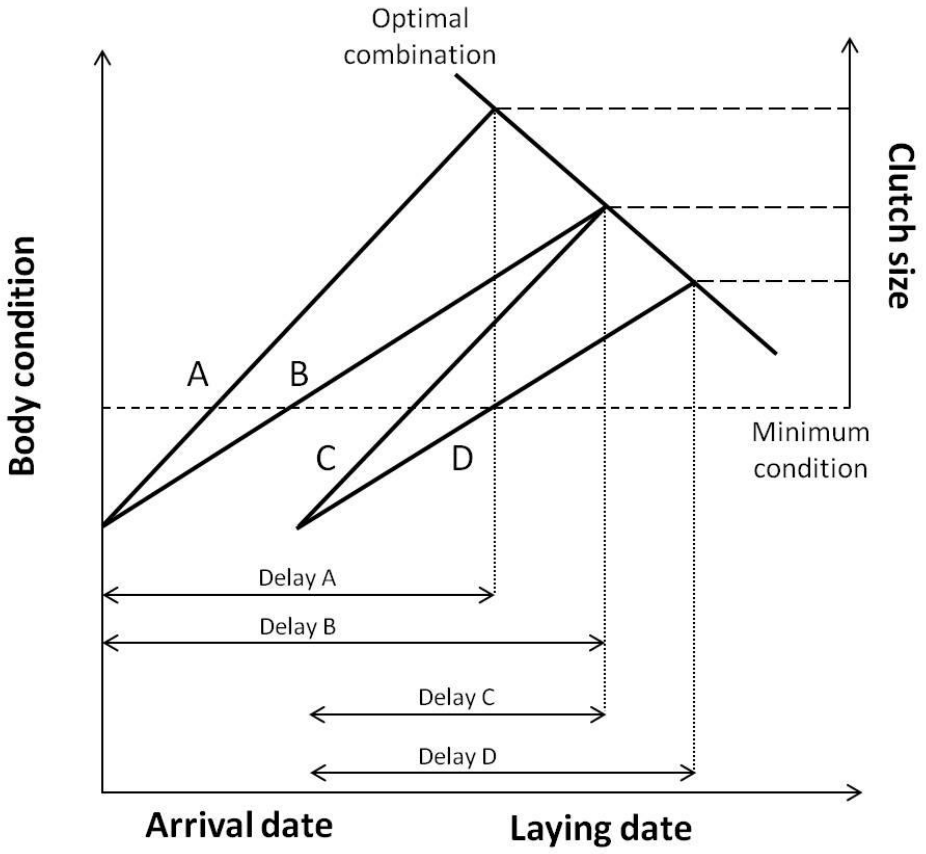


**Figure 3.1** - Condition-dependent individual optimization model (adapted from Bêty et al. 2003). Short-dashed horizontal lines indicate the body condition threshold at which individuals can invest in and commit to reproduction. Solid lines represent individual gains in condition leading up to their optimal combinations of laying date and breeding investment (represented by the slanted bold line). Vertical dotted lines indicate predicted laying dates and the horizontal long dashed lines indicate predicted investment (clutch size). The letters represent the potential outcomes for individuals with varying fattening rates (slopes). Individuals with a higher rate of condition gain during pre-breeding should meet the optimal combinations earlier (shorter delay) and lay earlier (A vs. B and C vs. D; Rowe et al.1994) and potentially mitigate the effects of late arrival (B vs. C) or poorer body condition after arrival on the breeding grounds.

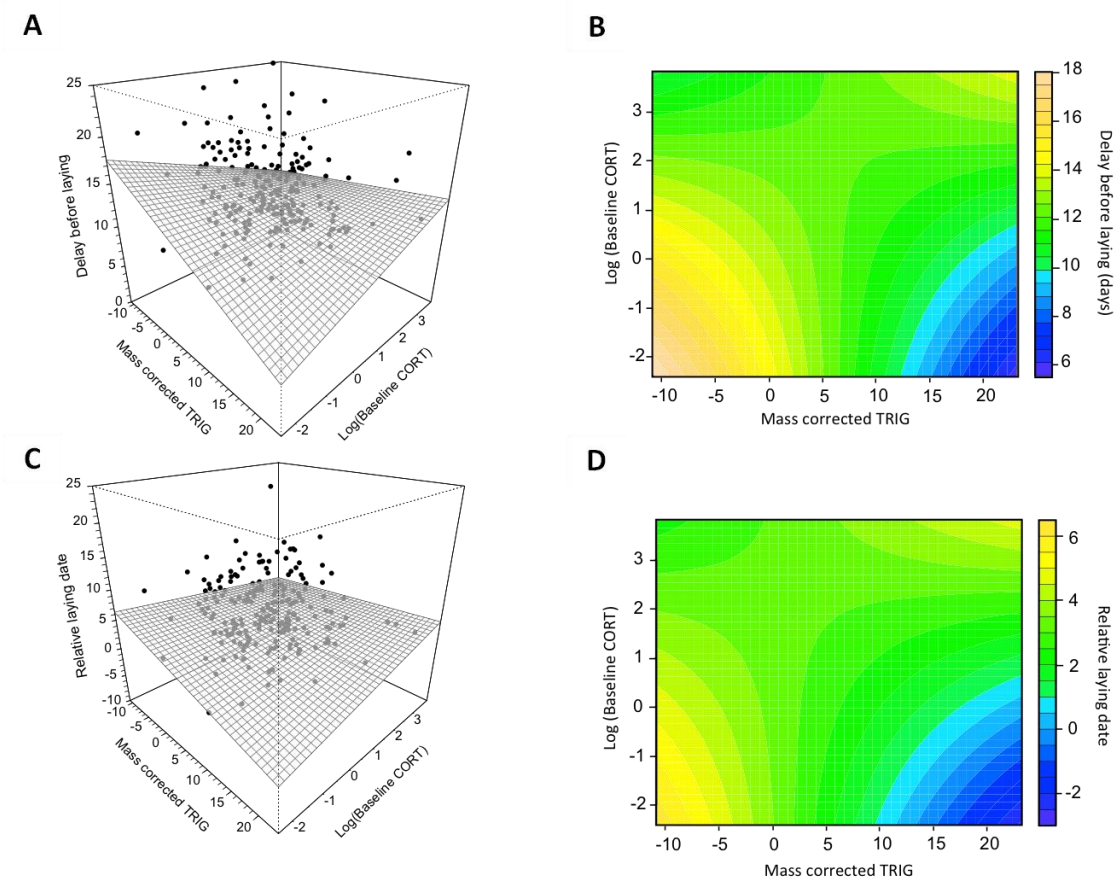


**Figure 3.2** - Interaction between baseline CORT (log-transformed) and fattening rate (residuals between plasma triglycerides and body mass) on the delay before laying (A, B) and on relative lay date (C, D). The interactions are illustrated with 3d graphs (A, C) and 2d graphs (B, D) with the grid on the 3d plot and the contour plot both representing the same model-corrected values (corrected for relative arrival date and body mass). The dots in the 3d plot represent the raw values to compare to the model-corrected values (grid). The 2d contour plots represent the distribution of the delay before laying or relative lay date (each colour represents 0.5 days) in relation to baseline CORT and fattening rate.

# CHAPTER 4 :

# BASELINE GLUCOCORTICOIDS ARE DRIVERS OF BODY MASS GAIN IN A DIVING SEABIRD[4]

## *Introduction*

Variation in individual state (e.g., body condition/energetic stores) is the source of numerous life history trade-offs (Stearns 1992) and can act as both a constraint, and a driver, of fitness-related life history decisions (McNamara and Houston 1996). During energetically demanding life history stages (i.e., migration, reproduction), individuals with greater energetic stores (i.e., higher body condition) are predicted to complete these stages with greater success (Stearns 1992; Rowe et al. 1994; McNamara and Houston 1996; Kisdi et al. 1998). For example, individuals with higher body mass are often able to arrive earlier on the breeding grounds, and reproduce earlier and with greater success (Lepage et al. 2000; Bêty et al. 2003; Gladbach et al. 2010; Descamps et al. 2011). Although individual variation in the rate at which accumulating these energetic stores (i.e., lipids) is theorized to have a major impact on reproductive timing and investment (Bêty et al. 2003; McNamara and Houston 2008), there is scant empirical data on the underlying mechanisms driving the procurement and accumulation of endogenous resources (Hennin et al. 2015).

Baseline glucocorticoids (GCs – corticosterone/cortisol) are hormones found in all vertebrates that mediate variation in energetic demand (Dallman et al. 1993; Landys et al. 2006). As such, baseline GCs experience daily and seasonal variation, with higher

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levels being associated with more energetically demanding life history stages (Romero 2002; Landys et al. 2006; Crespi et al. 2013). Baseline GCs are elevated when resources are scarce or individuals are in a negative energetic state (Love et al. 2005; Kitaysky et al. 2007; Jenni-Eiermann et al. 2008), presumably to stimulate foraging behaviour and subsequent resource acquisition (Lõhmus et al. 2006; Crossin et al. 2010). In individuals preparing for energetically demanding life history stages, elevated baseline GCs within biologically-relevant levels result in higher foraging rates (Astheimer et al. 1992; Kitaysky et al. 1999; Lõhmus et al. 2006; Angelier et al. 2007; Crossin et al. 2012), increased rates of condition gain (Crossin et al. 2012), and larger lipid stores (Holberton 1999; Holberton et al. 2007), all of which can positively influence fitness (Crossin et al. 2010). Nonetheless, it is important to remember the dual nature of elevated GCs (Lõhmus et al. 2006), especially when manipulating them in wild organisms (Crossin et al. 2015). When GCs are experimentally elevated outside the baseline range - such as during an acute stress response or *via* pharmacological manipulation for example - they promote the *expenditure* of lipid stores to fuel survival-related behaviours (Wingfield et al. 1998; Breuner and Hahn 2003), thereby, resulting in a *negative* proximate impact on body condition (Criscuolo et al. 2005; Bourgeon and Raclot 2006; Jenni-Eiermann et al. 2008; Angelier et al. 2009; Angelier et al. 2010). As such, while lower, biologically-relevant elevations of GCs in a baseline range are expected to have *positive* impacts on individual state (Crossin et al. 2015; Hennin et al. 2015), greater elevations are expected to induce an emergency life history stage or stress response, and have a short-term *negative* impact on individual state (Wingfield et al. 1998).

We manipulated baseline corticosterone (CORT; the primary avian GC) in a captive colony of white-winged scoters (*Melanitta fusca deglandi*), a northern-breeding, diving seaduck. Our overall goal was to examine whether variation in baseline GCs acts as a causal mechanism for positive impacts on individual state in a species that gains lipid stores prior to migration and commencing breeding. Our specific aims were to: i) examine whether a biologically relevant exogenous increase of CORT within a baseline range resulted in positive changes in body mass (i.e., increases in body fat), ii) determine whether this effect was due to either positive effects of elevated baseline CORT (i.e., *via* predicted increases in resource-acquisition) or the often presumed "inhibitory" effects of elevated baseline CORT (i.e., *via* negative feedback through the hypothalamic pituitary adrenal (HPA) axis), and iii) determine whether increases in body mass were reflected by an increase in plasma triglycerides (a measure of physiological fattening rate). For the latter, we focused on plasma triglycerides (TRIG) since they are a measure of the lipids synthesized by the liver used for depositing fat stores endogenously (Jenni and Schwilch 2001; Zajac et al. 2006; Cerasale and Guglielmo 2006; Anteau and Afton 2008) and when corrected for body mass, indicate an individual's physiological fattening rate (Williams et al. 2007).

## ***Materials and Methods***

### *Study Site and Colony*

Work was conducted between January and April 2013 using a captive colony of adult white-winged scoters (hereafter scoters; male: n = 5; female: n = 8) housed at the Patuxent Wildlife Research Center (PWRC) in Laurel, MD, USA. Birds were kept in

mixed-sex, outdoor pens covered with shade cloth. Pens were 11.5 $m^2$ with gravel substrate and a conical pond (2.1 m diameter, ~1 m deep at centre) with continual flowing freshwater. Experimental pens were separated from each other by at least two non-experimental pens to reduce researcher influences on GCs and bird behaviour. Birds were maintained on an *ad libitum* diet of Mazuri sea duck diet pellets (PMI Nutrition International) to ensure that no variation in body mass or fattening rates could be attributable to differences in diet composition (Seaman et al. 2005; Cerasale and Guglielmo 2006). All maintenance and experimental procedures were approved through both the PWRC (ACUC approval for "Corticosterone, energetics and individual state in diving seaducks") and University of Windsor (AUPP #12-15: "Mechanisms behind variation in individual state in diving seaducks) animal care and use committees.

*Experimental Design, Blood Sampling and Corticosterone Manipulation*

Three separate 21-day trials were performed. Individuals were assigned a randomized implantation schedule *via* random number generator across trials for each of our three treatment pellets (Innovative Research of America, Sarasota, FL, USA) for a repeated-measures design: control pellet (15 mg containing cholesterol), a low dose of CORT ("low CORT"; 15 mg pellet of CORT in a cholesterol matrix) and a high dose of CORT ("high CORT"; 35 mg pellet of CORT in a cholesterol matrix). The experimental manipulation was designed to elevate corticosterone levels within a biologically relevant baseline range (wild white-winged scoters: 6.64 ± 1.19 $ng.mL^{-1}$; range 0.51-46.7 $ng.mL^{-1}$; Palm et al. 2013), and not to elevate levels to those seen during an acute stress response. Indeed we found that the plasma levels of our manipulated birds fell well within this

baseline range (range 1.00-33.4 ng.mL$^{-1}$; see Results). Although pellets are designed to last 21 days in mammals, based on previous studies in birds we expected them to last approximately 14 days or less given their higher basal body temperature (see Bonier et al. 2007; Müller et al. 2009a; Müller et al. 2009b). We therefore sampled individuals every 3 days for a total of 14 days (with the exception of day 5 of the experiment; see *ACTH Challenge* details). Birds were given a week of rest before initiating the next trial.

Blood sampling took place between 0800 h and 1200 h to control for any potential diel variation in baseline corticosterone. Individuals in a pen were sampled together within 3 minutes of researchers being in sight of the pens to obtain conservative baseline samples (Romero and Reed 2005) and then weighed (g). Blood samples were collected by puncturing the tarsal vein using a 26 G needle and 75 uL heparinized capillary tubes. Blood samples were placed in heparinized storage tubes and centrifuged at 10,000 rpm for 10 minutes. Plasma was separated from the red blood cells and stored separately at -80°C until further analysis. Tarsus measurements (mm) were taken on the first day of sampling.

On the first day of an experimental trial, birds were implanted with treatment pellets at the base of the thigh in an area of loose skin. The leg in which birds were implanted alternated after each trial. After sanitizing the implant area with betadine, the implant site was anesthetized locally with 0.36 mg.kg$^{-1}$ dose of 5 mg.mL$^{-1}$ bupivacaine (Hopsira, Montréal, QC, Canada) using a 25 G needle. After the local anesthetic had taken effect (approx. 5 minutes) and the area was re-sterilized with betadine, a small incision slightly larger than the pellet was made in the skin using a #15 scalpel blade, and a pocket in the subcutis was made for the pellet by gently separating the skin from the

muscle. After the appropriate treatment pellet was inserted under the skin, the wound was closed using 2-3 sutures of UV degradable monofiliament (PDS* II (polydioxanone) suture; Ethicon, Markham, ON, Canada). The surgical area was re-sanitized and the bird was released back into its pen. The surgical site was monitored throughout the trial to ensure that it was healing properly and that there was no infection. In 7 instances (5 individuals in trial 2, and 3 in trial 3), individuals rejected the implanted pellets, encapsulating and extruding the pellets. As a result, the dosage of CORT to the individual is ambiguous and unstandardized. Therefore the data from those individuals within that trial were excluded.

*Adrenocorticotropic Hormone (ACTH) Challenge*

On the fourth and fifth days of the experiment for each trial, birds underwent an ACTH hormonal challenge to test the responsiveness of the HPA axis (Noirault et al. 1999; Faure et al. 2003; Nilsson et al. 2008). Following a blood sample to assess baseline CORT, birds were injected with either 100 $IU.kg^{-1}$ of porcine ACTH (Sigma, St. Louis, MO, USA) dissolved in 0.5 mL of lactated Ringers solution (Sigma, St. Louis, MO, USA) (high CORT: n = 4, control CORT: n = 5), or 0.5 mL of lactated Ringers solution (hereafter referred to as "saline") as a control (high CORT: n = 4, control CORT: n = 3) into the breast muscle using a 25 G needle-equipped syringe. Birds received either ACTH or saline solution on day 4 and the opposite injection on day 5 to also obtain individual variation in responses to the injections. Birds were then placed in individual carrier crates in a darkened, outdoor area and blood sampled 30 and 60 minutes post-injection before being released back into their pens. Blood samples were centrifuged at 10,000 rpm for 10

minutes and the plasma and red blood cells were stored separately at -80°C until further analysis.

*Physiological Assays*

Baseline corticosterone (CORT) was measured using a commercially available, enzyme immunoassay kit based on competitive binding and previously-validated in diving seaducks (EIA; Assay Designs, Ann Arbor, MI, U.S.A.) (Hennin et al. 2015) and was optimized for white-winged scoters (Palm et al. 2013). Samples were un-extracted and run in triplicate at a 1:40 dilution with a 1.5% steroid displacement buffer. Each plate included a CORT-spiked control sample and a standard curve produced by serially diluting a kit-provided, 200,000 $pg.mL^{-1}$ CORT standard, and plates were read at 405 nM (for details see Hennin et al. 2015). The inter- and intra-assay coefficient of variation across all plates was 2.66% and 7.78%, respectively.

Plasma triglycerides (TRIG) were measured using a commercially available kit (Sigma-Aldrich, Oakville, ON, Canada) previously validated in diving seaducks (Hennin et al. 2015). We used a 1:2 dilution for samples before adding them to a 96-well microplate in duplicate. Each plate included a serially diluted standard curve of glycerol standard (2.54 mmol $L^{-1}$) and a control of laying hen plasma (Sigma-Aldrich Canada, Oakville, ON, Canada). Reagent A was first added to measure free glycerol, followed by Reagent B to measure total glycerol. After the addition of each reagent, the plates were left to shake for 10 minutes at 37°C, then read using a plate reader at 540 nM wavelength. The amount of triglycerides ($mmol.L^{-1}$) was calculated by subtracting the amount of free glycerol found in the first plate read from the amount of total glycerol found in the

second plate read. Inter- and intra-assay coefficients of variation were 7.61% and 8.32% for total TRIG, and 7.27% and 4.68% for free glycerol, respectively. Final TRIG values were corrected for body mass to obtain residuals, indicating fattening rates (Williams et al. 2007).

*Statistical Analyses*

We tested for differences in baseline CORT and size-corrected body mass between treatments and between sexes at the start of the overall experiment (beginning of trial 1) using an analysis of variance (ANOVA). To test for trial-induced changes in baseline CORT and potential seasonal effects, we ran an ANOVA with trial number as an independent variable to test for differences across trials in baseline CORT. Due to lower than expected blood sample numbers, in this analysis we were only able to include one of an individual's measures from each of the three trials (no repeated measures) while maintaining balanced sample sizes across trials. Data points used in the analyses were selected randomly using a random number generator. Data from all trials were included in the analysis since the influence of CORT pellets within a focal trial did not influence baseline CORT values in subsequent trials (see Results). We ran a general linear mixed-effects model with individual as a random effect to test for trial-induced changes in size-corrected body mass at the start of each trial (comparing day 1 measures for all 3 trials). Size-corrected body masses appeared to demonstrate a trend towards increasing in the second and third trials compared to the first trial (trial: $F_{2,8.48} = 3.78$, $p < 0.06$; trial 1: 1152.1 ± 42.8 g, n = 9, trial 2: 1213.0 ± 42.5 g, n = 8, trial 3: 1216.2 ± 44.0 g, n = 6). We, therefore, suspected that the washout period between trials was too short, potentially

biasing the treatment's apparent impact on body mass in subsequent trials. As such, to be as conservative as possible we excluded trials 2 and 3 from analyses involving body mass and fattening to prevent any potential biases.

The peak concentration of plasma CORT due to the treatment pellets was predicted to be approximately 4 days post-implantation (Müller et al. 2009a). To test whether the low CORT and high CORT treatments elevated baseline CORT differentially compared to the control group, we ran two one-tailed t-tests comparing the control to each treatment group separately on day 4. The low CORT treatment did not significantly differ from the control group on day 4 ($t = 0.73$, $df = 5.78$, $p < 0.25$, control: $n = 4$; low CORT: $n = 4$; Fig. 4.1A), indicating it was unsuccessful in elevating CORT. Further we found only a moderate effect size (Cohen's $d = -0.52$, $r = -0.25$). We therefore excluded the low CORT group from all subsequent analyses since we were aimed to test the influence of baseline elevations of CORT on changes in mass. We performed a general linear mixed-model analysis to examine the responsiveness of the HPA axis to the ACTH challenge, with individual included as a random effect. We also included the fixed effects of treatment group, sampling time (initial baseline sample, 30/60 min post injection), ACTH treatment, and an interaction between ACTH treatment and sampling time (with a Tukey's post-hoc test). We found no effect of sex on differences in response to the ACTH trials ($F_{1,7.85} = 0.35$, $p = 0.57$) and therefore pooled sexes for ACTH trial analysis.

The change in body mass was calculated as the difference between the body mass on a given experimental day and that at the start of the trial, making the comparison of the relative changes across individuals and sexes possible. Change in body mass was analysed using a mixed-effects generalized linear model including individual as a random

effect, and as fixed effects CORT treatment group, experimental day, sex, and the interaction between CORT treatment and experimental day. We ran a Tukey's post-hoc test to test for differences between groups in the interactive effect. Changes in physiological fattening rate (TRIG) were analysed using a general linear model including experimental day and treatment group as independent variables. Since it is likely that impacts of elevated baseline CORT on plasma TRIG are temporally delayed after foraging, we ran two models: one to compare day 1 one and 4 (expected day of peak CORT secretion) and a second to compare days 1 and 7 (next sampling day after peak secretion). We included only one of an individual's measures across trials to maintain balanced sample sizes across trials. Due to low sample sizes we were unable to include sex as a variable in fattening rate analyses. Baseline CORT values were log transformed for normality in analyses, and back-transformed for figures and to report means and standard errors. All data met the assumptions for a parametric test. All analyses were run using JMP 10.0 (SAS Institute, Cary, California, USA). Results are presented as means ± s.e.m. unless otherwise stated.

## *Results*

### *Changes in Baseline Corticosterone and HPA Axis Activity*

There were no differences between the three treatment groups ($p > 0.77$; Fig. 4.1A) or between sexes ($p > 0.59$) in their initial baseline CORT values at the start of the overall experiment ($F_{2,6} = 0.23$, $p > 0.80$, $n = 9$), and no differences in baseline CORT on the first day of each trial ($F_{2,9} = 0.42$, $p > 0.66$, $n = 9$; trial 1: $4.72 \pm 1.44$ ng.mL$^{-1}$, $n = 6$; trial 2: $2.90 \pm 1.55$ ng.mL$^{-1}$, $n = 4$; trial 3: $4.95 \pm 1.87$ ng.mL$^{-1}$, $n = 2$). However, baseline CORT

was significantly elevated in the high CORT treatment group compared to control birds by day 4 of the experiment ($F_{2,7} = 12.66$, $p < 0.005$, n = 10 (control = 6, high CORT = 4); Fig. 4.1A), with a relatively large effect size (Cohen's d = -2.14, r = -0.73). Further, males had higher baseline CORT levels than females (males: 13.48 ± 1.28 $ng.mL^{-1}$, n = 5; females: 4.82 ± 1.31 $ng.mL^{-1}$, n = 5; $p < 0.03$). As predicted, individuals administered ACTH had significantly higher CORT levels 30 and 60 minutes post injection compared with saline injections (sampling time-ACTH treatment interaction: Table 4.1, 4.2; Fig. 4.1B). However, both treatment groups responded to the ACTH challenge similarly (Table 4.1; Fig. 4.1B), indicating that the exogenous CORT treatment had no significant effect on the responsiveness of the HPA axis.

*Changes in Body Mass and Fattening Rate*

We found no difference in size-corrected body mass between experimental groups at the beginning of the first trial (t = 0.10, df = 6.81, p = 0.93, n = 11; control: 0.15 ± 1.18, n = 6; treatment: 0.37 ± 1.92, n = 5). However, we detected a significant 2-way interaction between treatment and experimental day on the change in body mass where individuals in the high CORT (n = 5) treatment had larger positive changes in body mass compared to control (n = 5) individuals (Table 4.1, 4.2; Fig. 4.2A). Surprisingly, we did not detect a treatment effect on physiological fattening rates (TRIG corrected for body mass) between control and high CORT groups when comparing either day 1 to 4 (control: n = 3; high CORT: n = 4; Table 4.1,4. 2, Fig. 4.2B) or day 1 to 7 (control: n = 3; high CORT: n = 3; Table 4.1, 4.2, Fig. 4.2B).

## *Discussion*

We successfully experimentally elevated baseline corticosterone (CORT) in captive white-winged scoters within a biologically relevant, baseline range without inhibiting the activity of the HPA axis. Although we only produced an ephemeral increase in baseline CORT, peaking on experimental day 4 with levels appearing to return to baseline levels between day 7 and 10, individuals demonstrated a consistent and continual increase in body mass over the two-week period of implantation. As such, we have discovered a direct and positive causal relationship between elevated baseline CORT and increases in body mass in a diving seaduck. Importantly, this baseline CORT-mediated increase in body mass was due to a positive effect of elevated baseline CORT (i.e., likely *via* predicted increases in resource-acquisition) and not an "inhibitory" effect of elevated exogenous baseline CORT resulting in negative feedback of the HPA axis. Nonetheless, this treatment-related change in body mass was not mirrored by an increase in the physiological fattening rate (see below).

### *Corticosterone Secretion Dynamics*

Only the high CORT treatment pellets significantly elevated CORT in our captive white-winged scoters, although these levels were well within the expected natural baseline range for this species in the wild ($6.64 \pm 1.19$ ng.mL$^{-1}$; range 0.51-46.7 ng.mL$^{-1}$) (Palm et al. 2013), indicating that our detected levels were within a biologically relevant, baseline range. We found that male white-winged scoters had higher baseline plasma CORT than females at the peak of CORT release from the pellet. Pellets implanted in individuals

were consistent in size and thus slightly variable across individuals. It is likely that males, which are larger on average than females, had a slightly higher metabolic rate than females, given the inherent positive relationship between body mass and metabolic rate (e.g., Nagy 2005). As such, if males had a higher metabolic rate, and individuals were administered an un-scaled dosage of CORT, males may have metabolized the pellets more quickly, thereby exposing themselves to greater concentrations of CORT from the pellets than females. Although the pellets are manufactured to elevate CORT over a period of 21 days in mammals, we found a peak in CORT secretion 3 days post-implantation (i.e., on experimental day 4) with a rapid tapering off of CORT beginning approximately one week post-implantation. Studies in other avian species using this manipulation technique have found similar secretion trends in which there is a peak in plasma CORT concentrations shortly after implantation (1-3 days; Müller et al. 2009a) which slowly tapers off until returning to baseline levels (within approximately 7 days post implantation; Bourgeon and Raclot 2006; Bonier et al. 2007; Almasi et al. 2008; Müller et al. 2009b).

There are three potential reasons for the rapid tapering off of CORT in these secretion profiles. First, it is possible that individuals were preventing the release of CORT either through encapsulation of the pellet or through clearing CORT from the circulatory system. We monitored the implantation site throughout each experimental trial to ensure the site was healing and for the presence of the pellets. Any individuals that had encapsulated pellets during the trial were removed from analyses due to the ambiguity in the nature of CORT secretion from the pellets while encapsulated. Second, through negative feedback individuals could have begun down-regulating the endogenous

production of CORT due to the rapid influx of exogenous CORT (Goutte et al. 2011). To test for this impact, we intentionally timed our ACTH trials to closely match the expected maximal secretion of CORT from the pellets to examine whether the HPA axis was still active and fully responsive. If the exogenous CORT was inhibiting the HPA axis, then the high CORT birds should have exhibited a significant depression in endogenous CORT secretion in response to the ACTH challenge. However, high CORT birds challenged with ACTH showed CORT responses that were not significantly different from those of ACTH-injected control birds. This indicates that the HPA axis of high CORT birds was still active and able to secrete endogenous CORT normally. The final and most likely explanation for the relatively ephemeral nature of the CORT pellets is that they are primarily designed for use in mammals and therefore pellets were likely metabolized more quickly in avian species which exhibit higher relative metabolic rates (Müller et al. 2009a). Again, this assumption is supported by recent work using these pellets in a number of avian species (Bourgeon and Raclot 2006; Bonier et al. 2007; Almasi et al. 2008; Müller et al. 2009b).

*Linking Elevated Baseline Corticosterone to Changes in Body Mass*

We found that individuals with exogenously elevated baseline CORT increased in body mass throughout the trial period. Although a number of studies have examined relationships between elevated baseline CORT and foraging behaviours (Astheimer et al. 1992, Dallman et al. 1993; Breuner et al. 1998), here we confirm that this CORT-mediated effect on body mass was due to a direct *positive* effect of elevated baseline CORT, presumably *via* the previously-observed increase in resource-acquisition. It is key

to note that we witnessed changes in mass in the high CORT birds, particularly later in the trial period, and no changes occurred in our control birds across the trial, indicating that these results were not due to seasonal influences but rather due to the treatment. Importantly, CORT-mediated increases in body mass did not occur as a result of the elevated exogenous baseline CORT inhibiting the HPA axis, thereby reducing endogenous production of CORT with an associated downstream positive impact on body mass. Rather, individuals responded directly to the elevation of baseline CORT itself, with subsequent increases in body mass. It is well known that relationships between elevated baseline CORT and changes in body mass depend heavily on the interactions between life history stage, life history strategy, and the dosage of CORT administered in a given species (Crossin et al. 2015). For instance yellow-rumped warblers (*Setophaga coronata*) exposed to longer day lengths (i.e., simulating spring migration) exhibited elevated baseline CORT secretion with a temporally-paired increase in body mass (Holberton 1999). Similarly, an experimental reduction in baseline CORT levels in dark-eyed juncos (*Junco hyemalis*) resulted in less body mass gain than individuals with higher, normal baseline levels (Holberton et al. 2007). Finally, in wild macaroni penguins (*Eudyptes chrysolophus*), individuals with experimentally elevated baseline CORT exhibited increased foraging behaviour and higher body mass gain (Crossin et al. 2010). Conversely, incubating common eiders (*Somateria mollissima*) with experimentally elevated CORT outside the normal baseline range (i.e., into the range of an acute stress response) lost significant amounts of body mass (Bourgeon and Raclot 2006), underscoring the dual nature of CORT secretion (Landys et al. 2006) and that the direction of this relationship is highly context dependent (Crossin et al. 2015). Although

our results indicate that CORT acts as a 'direct' mechanism driving an increase in body mass, CORT can also influence or be influenced by the secretion of other hormones (e.g., ghrelin, thyroid hormone, insulin) or neuropeptides (e.g., neuropeptide Y) which can have downstream consequences for foraging (Landys et al. 2006; Cornelius et al. 2013). As such, CORT may be a component of a complex set of mechanisms which can influence body mass, the strength of which may be dependent on life history stage, individual condition and environmental signals (Landys et al. 2006; Cornelius et al. 2013).

Previous research examining the relationship between plasma TRIG and mass gain has found that elevated TRIG positively indicates an individual's body mass gain ("physiological fattening rate"; Jenni and Schwilch 2001; Cerasale and Guglielmo 2006; Anteau and Afton 2008; but see Dietz et al. 2009). We therefore predicted that if individual changes in body mass occurred in direct response to exogenous baseline CORT elevation, we would have also detected a corresponding increase in plasma TRIG. Interestingly, we detected no difference in plasma TRIG secretion between our control and high CORT groups on day 4 or day 7 post-implantation, nor were there any apparent trends in the data. Similar non-trends have been detected in red knots (*Calidris canutus*) (Dietz et al. 2009) and western sandpipers (*Calidris mauri*) (Seaman et al. 2005); however in the latter case this may have been due to methodological differences used to stimulate foraging and, therefore, changes in body mass (Seaman et al. 2005). Although we did not fast individuals to stimulate body mass gain, this lack of a trend may result from the time lag between foraging, digestion and circulating triglycerides, with the concentration of plasma TRIG increasing throughout the day (Jenni and Jenni-Eiermann

1996). Therefore, we may not have been able to detect a difference in our birds since they were sampled prior to feeding, exhibiting no relationship to treatment group despite the increased signal of energetic demand (i.e., elevated baseline CORT). Indeed, treatment differences in fattening rates may have been more detectible post-feeding in the early afternoon or evident through differences in foraging rates or amount of food consumed. Future studies seeking to establish causal links between baseline GCs, fattening rates and changes in body mass should ideally account for changes in foraging behaviour if possible.

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**Table 4.1** - Summary of fixed effects for ACTH trials, change in body mass and fattening rate (plasma TRIG) analyses in response to an exogenous elevation of baseline corticosterone in captive white-winged scoters. Bolded values indicate significant effects.

| Analysis | Variable | F | df | p |
|---|---|---|---|---|
| ACTH Trial | CORT Treatment | 0.3 | 1, 40.58 | 0.59 |
| | ACTH Treatment | 4.84 | 1, 38.41 | **0.03** |
| | Sample Time | 28.7 | 2, 30.83 | **0.0001** |
| | Sample Time*ACTH Treatment | 8.88 | 2, 30.83 | **0.0009** |
| Change in body mass | Experiment Day | 8.68 | 4, 21.35 | **0.0003** |
| | Treatment | 60.17 | 1, 3.43 | **0.0003** |
| | Sex | 1.15 | 1, 3.42 | 0.35 |
| | Experiment Day*Treatment | 4.02 | 4, 21.35 | **0.01** |
| Fattening rate 1-4 | Experiment Day | 2.69 | 1 | 0.18 |
| | Treatment | 0.21 | 1 | 0.67 |
| Fattening rate 1-7 | Experiment Day | 1.11 | 1 | 0.37 |
| | Treatment | 3.89 | 1 | 0.14 |

**Table 4.2 -** Parameter estimates for ACTH trial, change in body mass, and fattening rates (mass-corrected plasma TRIG) between experimental day 1 to 4 and 1 to 7 for white-winged scoters. Bolded values indicate significant effects.

| Analysis | Parameter | Estimate | SE | df | t | p |
|---|---|---|---|---|---|---|
| ACTH Trial | Intercept | 1.22 | 0.08 | 7.21 | 15.99 | **0.0001** |
| | CORT Treatment (Control) | -0.03 | 0.05 | 40.58 | -0.55 | 0.59 |
| | ACTH Treatment (ACTH) | 0.09 | 0.04 | 38.41 | 2.2 | **0.03** |
| | Sample Time (T0) | -0.37 | 0.05 | 30.83 | -7.53 | **0.0001** |
| | Sample Time (T30) | 0.15 | 0.05 | 30.83 | 3.04 | **0.005** |
| | Sample Time (T0)*ACTH Treatment (ACTH) | -0.21 | 0.05 | 30.83 | -4.21 | **0.0002** |
| | Sample Time (T30)*ACTH Treatment (ACTH) | 0.1 | 0.05 | 30.83 | 2.01 | **0.05** |
| Change in body mass | Intercept | 32.15 | 3.75 | 3.35 | 8.56 | **0.002** |
| | Experiment Day 1 | -31.10 | 7.18 | 21.21 | -4.33 | **0.0003** |
| | Experiment Day 4 | -12.80 | 7.18 | 21.21 | -1.78 | 0.09 |
| | Experiment Day 7 | -1.41 | 7.49 | 21.93 | -0.19 | 0.85 |
| | Experiment Day 10 | 16.84 | 7.18 | 21.21 | 2.34 | **0.03** |
| | Treatment (Control) | -28.43 | 3.67 | 3.43 | -7.76 | **0.003** |
| | Sex (Female) | -3.93 | 3.67 | 3.43 | -1.07 | 0.35 |
| | Experiment Day 1*Treatment (Control) | 28.17 | 7.18 | 21/21 | 3.92 | **0.0008** |
| | Experiment Day 4*Treatment (Control) | -1.53 | 7.18 | 21.21 | -0.21 | 0.83 |
| | Experiment Day 7*Treatment (Control) | -9.48 | 7.49 | 21.93 | -1.27 | 0.22 |
| | Experiment Day 10*Treatment (Control) | -6.56 | 7.18 | 21.21 | -0.91 | 0.37 |

| Analysis | Parameter | Estimate | SE | df | t | p |
|---|---|---|---|---|---|---|
| Fattening Day 1-4 | Intercept | -0.45 | 0.12 | NA | -3.83 | **0.02** |
| | Experiment Day 1 | -0.19 | 0.12 | NA | -1.64 | 0.18 |
| | Treatment (Control) | 0.05 | 0.12 | NA | 0.46 | 0.67 |
| Fattening Day 1-7 | Intercept | -0.15 | 0.16 | NA | -0.92 | 0.43 |
| | Experiment Day 1 | -0.18 | 0.17 | NA | -1.05 | 0.37 |
| | Treatment (Control) | -0.34 | 0.17 | NA | -1.97 | 0.14 |

**Table 4.3** - Summary of sample sizes collected for corticosterone and mass-corrected triglycerides (fattening rate). All three trials of data were use for corticosterone analyses and presentation. Only the first trial was included in analyses and presentation for fattening rate (see Methods).

| | Corticosterone | | | Fattening Rate | |
|---|---|---|---|---|---|
| **Experimental day** | **Control** | **Low CORT** | **High CORT** | **Control** | **High CORT** |
| 1 | 5 | 6 | 3 | 3 | 3 |
| 4 | 6 | 7 | 4 | 2 | 1 |
| 7 | 4 | 4 | 3 | 1 | 2 |
| 10 | 1 | 3 | 4 | 3 | 1 |
| 13 | 4 | 1 | 4 | 1 | 2 |

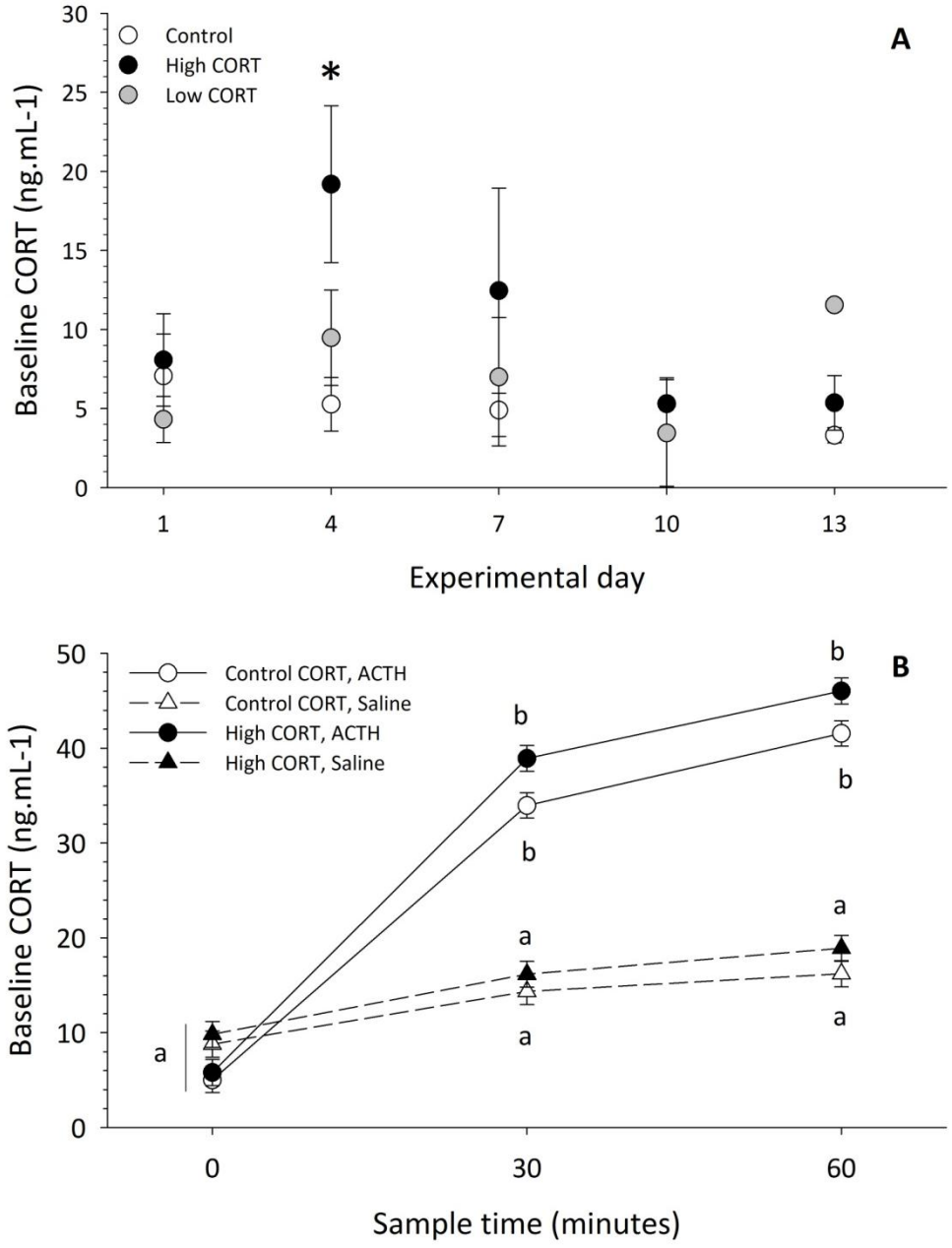


**Figure 4.1** - Influence of treatment (exogenous baseline CORT elevation vs. control) (A) and ACTH injection (B) on baseline levels of corticosterone in captive white-winged scoters. Data included in analyses are presented as the model-corrected least square means and standard errors. Values presented for CORT are back transformed from a log transformation. Asterisk indicates a significant difference between the high CORT group and control group only. Refer to Table 4.3 for sample sizes (A).

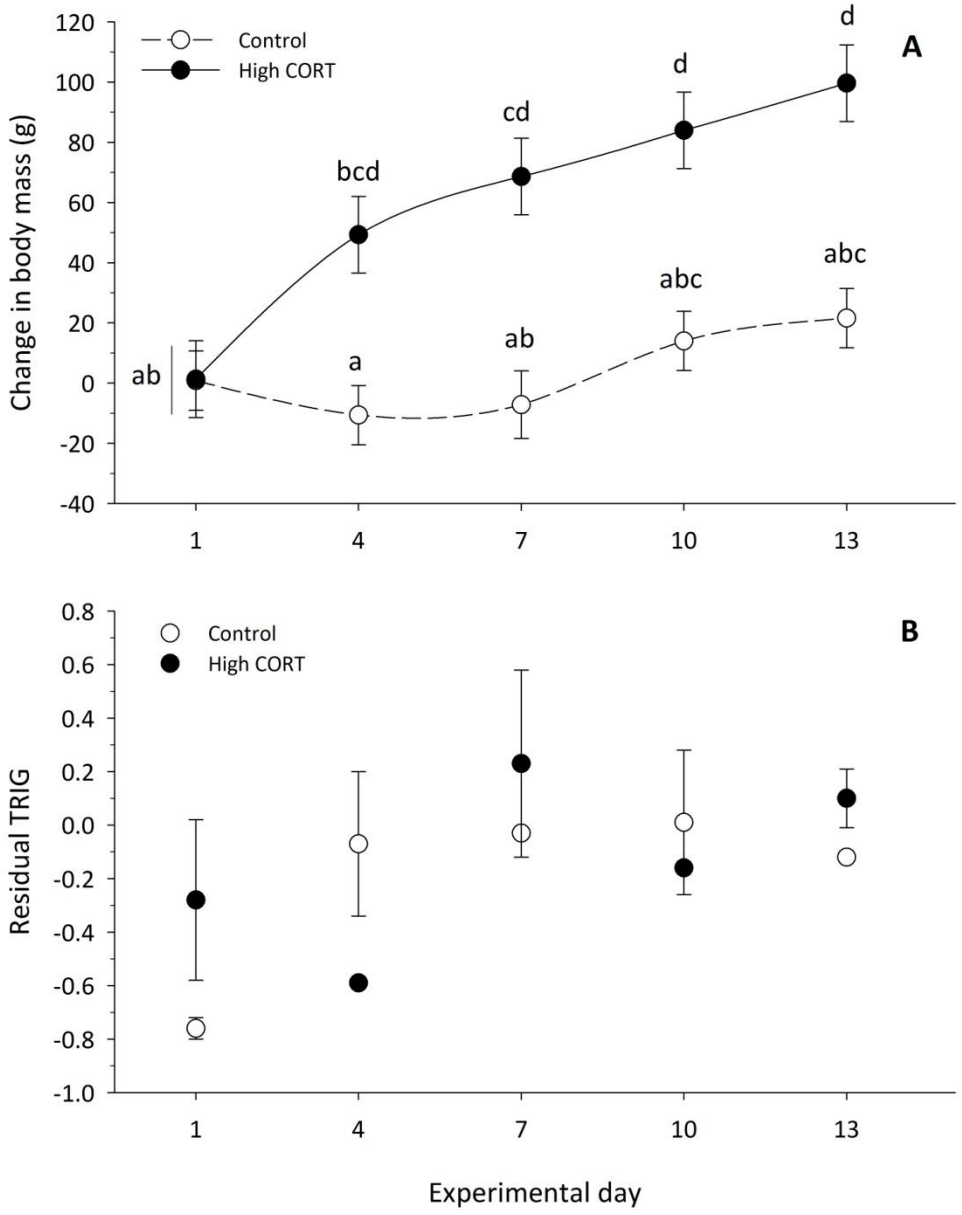


**Figure 4.2** - The impact of an exogenous elevation in baseline CORT on changes in body mass (A) and fattening rate (B) in captive white-winged scoters across experimental days. Data included in analyses are presented as the model-corrected least square means and standard errors. Letters indicate significant differences between groups. Refer to Table 4.3 for sample sizes (B).

# CHAPTER 5 :

# CORTICOSTERONE ACTS AS A CAUSAL MECHANISM DRIVING BREEDING PHENOLOGY AND REPRODUCTIVE SUCCESS[5]

## *Introduction*

Reproduction is a highly-demanding life history stage where individuals face many trade-offs for the allocation of time and energy, the outcome of which ultimately influences their fitness (Stearns 1989; 1992; Daan and Tinbergen 1997). In migratory species breeding in seasonally-constrained environments, reproductive decisions are thought to be driven by a combination of the timing of arrival and condition at arrival on the breeding grounds (McNamara and Houston 1996). Generally, individuals that arrive in greater condition and/or earlier are expected to reproduce earlier, invest more into reproduction and have a greater probability of being reproductively successful (Bêty et al. 2003; Descamps et al. 2011; Legagneux et al. *in revision*). While strong theoretical frameworks exist for predicting the impacts of variation in individual state on key investment decisions that impact fitness (Rowe et al. 1994; Bêty et al. 2003), and determining the mechanistic pathways that mediate the decisions that shape these important trade-offs is key to explaining the evolution of life-histories (Zera and Harshman 2001; Zera et al. 2007), identifying biologically appropriate and broadly relevant candidate mechanisms to empirically test these ideas is not straightforward (Williams 2012). Moreover, progress is often hampered by the complexity of measuring and manipulating these mechanisms in free-living species prior to reproductive

[5] This chapter is the outcome of joint research with P. Legagneux, J. Bêty, H.G. Gilchrist, M.R. Forbes, N. Jane Harms, C. Soos, and O.P. Love

investment, and then monitoring individuals to measure downstream impacts on relevant fitness traits (Reed et al. 2006).

Physiological traits are thought to be prime candidates for influencing the investment decisions that shape reproductive trade-offs because they link an individual to its external environment (Zera and Harshman 2001; Ricklefs and Wikelski 2002; Harshman and Zera 2007; Moore and Hopkins 2009). Indeed, physiological traits have been shown to be central in regulating a number of life history trade-offs in different taxa: juvenile hormone mediates the trade-off between investment in wing polymorphism (dispersal) and ovary size (reproductive investment) in crickets (*Gryllus spp.*; reviewed in Zera and Harshman 2001); glucocorticoids influence r vs. K strategies in side-blotched lizards (*Uta stansburiana*; Lancaster et al. 2008); testosterone mediates trade-offs between parental investment and extra-pair paternity in male dark-eyed juncos (*Junco hyemalis*; Ketterson and Nolan 1992; Reed et al. 2006). Physiological traits specifically tied to energetics (energetic management, foraging, food intake) are thought to be prime candidates for regulating investment decisions given that energetic constraints are at the heart of many allocation decisions central to reproduction (Stearns 1992; Zera and Harshman 2001; Harshman and Zera 2007).

Baseline glucocorticoids (GCs) play a critical role in mediating the behavioural and homeostatic functions required to meet energetic demands across many temporal scales (daily: Dallman et al. 1993; life history stage: Romero 2002; Landys et al. 2006; Crespi et al. 2013; lifetime: Angelier et al. 2006; Reichert et al. 2012; Elliott et al. 2014). From an energy acquisition perspective, elevated baseline GCs increase foraging behaviour (Astheimer et al 1992; Breuner et al.1998; Angelier et al. 2007), fat deposition

(Holberton 1999; Holberton et al. 2007) and body mass gain (Crossin et al. 2012; Hennin et al. 2016). Not surprisingly then, baseline GCs are elevated both prior to and during reproduction across various vertebrate taxa (Romero 2002; Moore and Jessop 2003; Landys et al. 2006; Hennin et al. 2015), and they have been causally linked (Love et al. 2014; Crossin et al. 2012; Legagneux et al. 2012) and correlated (e.g., Bonier et al. 2009; Spée et al. 2010; Ouyang et al. 2011) to investment in reproduction. Despite their strong potential role as central regulators of investment decisions in a diversity of vertebrates (see Love and Williams 2008; Love et al. 2014; Crossin et al. 2015), few studies have been able to test this assumption holistically given that a single study must be able to simultaneously combine a manipulation of baseline GCs within a biologically relevant range (i.e., not supra-physiological), do so in pre-breeding individuals of a free-living species, examine the impact of this elevation on investment decisions, and then track the downstream impact of those decisions on fitness (Crossin et al. 2015).

In the current study we focused on measuring and experimentally manipulating baseline corticosterone (CORT; the primary avian GC) in pre-laying, Arctic-nesting female common eiders (*Somateria mollissima*) to empirically test whether elevated baseline GCs are a causal driver of reproductive investment decisions and variation in fitness proxies. Arctic-nesting common eiders are useful as broad models for these questions for a number of reasons. First, they are mixed capital-income strategy breeders, using a combination of fat stores brought from the wintering grounds and acquired from foraging on the breeding grounds to fuel follicle development (Sénéchal et al. 2011). Second, because females undergo a 24-day incubation period in which they fast (Bottita et al. 2003) it is critical that they optimally manage their pre-laying energetics to accrue

enough somatic fat on the breeding grounds to successfully complete incubation. Third, they have a highly-constrained reproductive period in which only one successful breeding attempt is possible per year, and this attempt must be timed carefully to ensure that offspring have the required time to grow and leave the area before full ice-cover (Love et al. 2010; Jean-Gagnon 2015). Finally, the dynamics of pre-laying female physiology have been characterized in this population, and baseline corticosterone increases significantly during the pre-laying period indicating a possible important role in supporting the energetic demands of reproductive investment (Hennin et al. 2015). We predicted that exogenously increasing baseline corticosterone in pre-laying females would result in a shorter delay between arrival and laying, and therefore earlier laying dates given that elevated baseline corticosterone has recently been shown to increase fattening rates in another diving seaduck (Hennin et al. 2016). Finally, we predicted that elevated corticosterone would lead to higher reproductive (hatching) success, potentially *via* both direct (possible larger incubation fat stores) and indirect pathways (early laying dates are linked to higher hatching success in this population; Descamps et al. 2011).

## ***Methods***

### *Study Site and Field Methods*

Our study was conducted in 2011 and 2012 at Mitivik Island in the East Bay Migratory Bird Sanctuary, Nunavut, Canada (64°02'N, 81°47'W). Mitivik Island is a small island (400 x 800m) in the mouth of a shallow, productive bay and the location of Canada's largest known population of Arctic-nesting common eiders. Females nesting at this site over-winter off the coast of Newfoundland and Labrador in Canada or the Eastern coast

of Greenland (Mosbech et al. 2006). We timed the capture of arriving birds to coincide with arrival of birds on the breeding grounds (mid-June to early July; Hennin et al. 2015; average 173.5 ± 0.23 (mean ± s.e.m.) Julian days). Work was conducted with permission from the Animal Care Committees of the University of Windsor (AUPP #11-06: Reproductive strategies of Arctic-breeding common eiders) and Environment Canada (EC-PN-15-026).

Birds were caught using large flight nets (Love et al. 2010; Descamps et al. 2011; Hennin et al. 2015). After capture, females were blood sampled in under three minutes (i.e., Romero and Reed 2005) with a 1mL heparinized syringe and 23 G thin-wall, 0.5 inch needle to obtain baseline plasma corticosterone. Blood samples were then transferred into a heparinized tube, centrifuged for 10 min at 10,000 rpm, and plasma and red blood cells were separated and stored at -20˚C (-80˚C in the lab) until further analysis. We did not limit sampling for baseline corticosterone to a restricted time of day as pre-breeding female common eiders at our study site do not show diel variation in baseline corticosterone secretion (Steenweg et al. 2015). After females were blood sampled, body mass was collected, and females were banded and given a combination of uniquely shaped and coloured nasal tags. Nasal tags, which are important for post-release monitoring of females on the colony (see Descamps et al. 2011), were attached using UV degradable monofilament to allow the tags to fall off at the end of the breeding season. Females were observed from seven permanent blinds located around the periphery of the colony to prevent colony disturbance where trained observers monitored the colony using standardized behavioural observations. Nasal tags allowed for accurate monitoring of

hens to identify laying and incubation initiation dates. Once a nest was detected, it was monitored twice daily until either hatching or nest failure.

*Corticosterone Manipulation*

Female eiders were implanted with one of three treatment pellets (Innovative Research of America, Sarasota, FL, USA) prior to release, following sampling and banding. We selected dosages based on our previous captive validation (Hennin et al. 2016): control pellet (35 mg containing cholesterol matrix only), low corticosterone pellet (hereafter "low CORT"; 35 mg of hormone in a cholesterol matrix) or high corticosterone pellet (hereafter "high CORT"; 75 mg of hormone in a cholesterol matrix). We assigned females to treatment groups on a rotational basis to ensure that the implants were randomized, and that treatments were temporally matched within and across days. Pellets were implanted at an area of loose skin at the base of the thigh under the subcutis (see Hennin et al. 2016 for details). Briefly, the implant site was sterilized, then anesthetized locally using a 0.36 $mg.kg^{-1}$ dose of 5 $mg.mL^{-1}$ bupivacaine. Once numb (~5 min), the area was then re-sterilized, a small incision (1-1.5 cm) was created in the skin using a scalpel, and a pocket large enough to contain the pellet was created in the subcutis. After inserting the appropriate pellet, the incision was stitched shut using 2-3 sutures of UV degradable monofiliament (PDS* II (polydioxanone) suture; Ethicon Endo-Surgery Inc.) and sterilized once more before the bird was released.

Ideally, we would have liked to validate the release dynamics of the pellets in our free-living eiders to confirm: i) whether biologically-relevant corticosterone levels had been obtained, and ii) how the pellets interacted with the HPA axis of birds to influence

endogenous corticosterone secretion. Unfortunately, it is almost impossible to re-capture our manipulated free-living eiders. We therefore validated these questions in captivity using another diving seaduck with a similar life history (white-winged scoters - *Melanitta fusca deglandi*; Hennin et al. 2016). We confirmed that pellets: i) lasted no longer than two weeks, a temporal period which directly overlaps with the average delay before laying in our eider hens (12.7 ± 0.44 days), ii) did not inhibit the normal functioning of the hypothalamic-pituitary-adrenal (HPA) axis (i.e., did not reduce endogenous corticosterone production), and iii) resulted in mean elevations of baseline corticosterone of 17.51 ± 1.27 $ng.mL^{-1}$ (range 1.00 - 33.4 $ng.mL^{-1}$), which falls perfectly within the range of baseline corticosterone for our common eiders (0.19 - 37.67 $ng.mL^{-1}$; this study).

*Baseline Corticosterone Assay*

Baseline plasma corticosterone was measured using a previously validated, commercially available, enzyme immunoassay kit based on competitive binding (EIA; Assay Designs, Ann Arbor, MI, USA) and was optimized for common eiders (Hennin et al. 2015). We ran un-extracted samples in triplicate at a 1:20 dilution with 1.5% steroid displacement buffer. Each plate included a corticosterone-spiked control sample and a standard curve produced by serially diluting a kit-provided, 200,000 $pg.mL^{-1}$ corticosterone standard. Plates were read at 405 nM. The inter- and intra-assay coefficient of variation were 12.43% and 6.56%, respectively.

*Statistical Analyses*

All analyses were split between two pre-laying groups (pre-recruiting vs. rapid follicle growth) using the delay before laying (number of days between capture/arrival at the colony and laying date). Given that the period of rapid follicle growth occurs within 7 days of laying in our eiders (Hennin et al. 2015), individuals that were captured 8 days or more from laying were considered pre-recruiting females and therefore had not yet committed to investing in reproduction. Conversely, females captured within 7 days or less of laying had already committed to reproductive investment, were quickly growing follicles in preparation for ovulation (Challenger et al. 2001; Gorman et al. 2009; Sénéchal et al. 2011) and were therefore categorized as rapid follicle growth (RFG) females. We tested for differences between years in relative arrival date, body mass and baseline corticosterone using two-tailed t-tests. To ensure there were no inherent differences between our treatment groups before the experiment was initiated, we also tested for differences in capture date, body mass, and baseline corticosterone among treatment groups at implantation using a one-way ANOVA and a Tukey post-hoc test.

To determine the effect of treatment on laying phenology (the delay before laying and relative laying date) we ran separate GLMs with a normal distribution including year, body mass, arrival date, treatment group, and baseline corticosterone as independent variables. Since individual variation in baseline corticosterone at the time of manipulation is expected to interact with the CORT treatment to influence breeding decisions, we included the interaction between baseline plasma corticosterone at capture and treatment group. To determine the effect of treatment on reproductive success, we ran a GLM with

a binomial distribution including relative laying date, baseline corticosterone, treatment group, and the interaction between baseline corticosterone and treatment group as independent variables. Due to nearly complete reproductive failure of the colony from unusually high nest predation rates in 2012, we were unable to obtain reproductive success data and therefore only include 2011 data in reproductive success analyses. Baseline corticosterone values were log transformed for normality and back-transformed for reporting. Analyses met the assumptions for a parametric test and were run using JMP 12.0 (SAS Institute, Cary, California, U.S.A.). Results are presented as means ± s.e.m. unless otherwise stated.

***Results***

In pre-recruiting females we detected no differences in body mass ($t = -0.97$, $df = 18.13$, $p = 0.34$, 2011: 2234.3 ± 42.7 g, n = 30; 2012: 2165.6 ± 56.5 g, n = 9), relative arrival date ($t = 1.16$, $df = 16.22$, $p = 0.26$; 2011: -2.43 ± 0.59, n = 30, 2012: -1.22 ± 0.86, n = 9) or baseline plasma corticosterone levels ($t = -1.57$, $df = 12.48$, $p = 0.14$, 2011: 5.89 ± 1.23 $ng.mL^{-1}$, n = 30; 2012: 2.91 ± 1.49 $ng.mL^{-1}$, n = 9) between years. In RFG females, we found no differences in relative arrival date ($t = -0.71$, $df = 26.95$, $p = 0.48$, 2011: 0.85 ± 0.53, n = 41; 2012: 0.23 ± 0.70, n = 13) or body mass ($t = 0.29$, $df = 20.05$, $p = 0.78$; 2011: 2335.6 ± 28.3g, n = 41, 2012: 2252.3 ± 50.7, n = 13) between years, but we did detect a year difference for baseline corticosterone (2011: 9.87 ± 1.18 $ng.mL^{-1}$, n = 41; 2012: 6.03 ± 1.25 $ng.mL^{-1}$, n = 13; $t = -1.77$, $df = 25.60$, $p = 0.04$).

For pre-recruiting hens the treatment groups did not differ in their relative arrival date ($F_{2,36} = 0.19$, $p = 0.19$; control: -1.00 ± 0.81, n = 14; low CORT: -2.40 ± 0.96, n = 10; high CORT: -3.07 ± 0.79, n = 15), body mass ($F_{2,36} = 0.38$, $p = 0.69$; control: 2178.6

± 60.0 g, n = 14; low CORT: 2226.0 ± 71.0 g, n = 10; high CORT: 2250.7 ± 58.0 g, n = 15) or baseline corticosterone ($F_{2,36}$ = 0.28, p = 0.76; control: 5.14 ± 1.37 ng.mL$^{-1}$, n = 14; low CORT: 5.69 ± 1.36 ng.mL-1, n = 10; high CORT: 3.97 ± 1.46 ng.mL$^{-1}$, n = 15). Similarly, in RFG hens there were no differences in relative arrival date ($F_{2,51}$ = 0.47, p = 0.63; control: 0.19 ± 0.70, n = 21; low CORT: 1.15 ± 0.72, n = 20; high CORT: 0.85 ± 0.89, n = 13), body mass ($F_{2,51}$ = 0.86, p = 0.43; control: 2207.1 ± 39.4 g, n = 21; low CORT: 2280.0 ± 40.3 g, n = 20; high CORT: 2230.0 ± 50.1 g, n = 13) or baseline corticosterone ($F_{2,51}$ = 0.13, p = 0.88; control: 8.62 ± 1.25 ng.mL$^{-1}$, n = 21; low CORT: 9.52 ± 1.26 ng.mL$^{-1}$, n = 20; high CORT: 7.93 ± 1.33 ng.mL$^{-1}$, n = 13). Finally, there were no differences in the proportion of hens that decided to initiate breeding across the treatment groups ($\chi^2$ = 0.009, df = 2, p > 0.22).

In pre-recruiting hens, the delay before laying was significantly influenced by the interaction between baseline corticosterone and CORT treatment group (Table 5.1, 5.2). Females with low baseline plasma corticosterone at arrival and administered the high CORT treatment had a significantly shorter delay between arrival and laying compared to low CORT treatment hens with low baseline plasma corticosterone (Fig. 5.1A), which in turn had a significantly shorter delay compared to control hens with low baseline plasma corticosterone (Fig. 5.1A). Interestingly, for females with high baseline plasma corticosterone at arrival the CORT treatment had either no obvious effect on the delay before laying (low CORT dose), or even a possibly negative effect (high CORT group) (Fig. 5.1A). Relative laying date was also significantly influenced by the interaction between baseline corticosterone and CORT treatment in a similar manner to the delay before laying (Table 5.1, 5.2). Females with low baseline plasma corticosterone at arrival

administered the high CORT treatment had earlier relative laying dates compared to control females with low baseline plasma corticosterone at arrival (Fig. 5.1C). As with the delay before laying, this trend appeared to be reversed in females with higher plasma levels of baseline CORT. The experimental treatment also significantly influenced the reproductive success of pre-recruiting hens (Table 5.1), with low CORT females having higher reproductive success than control hens, and with high CORT females having intermediate reproductive success (Fig. 5.2A, 5.2C).

In the Rapid Follicle Growth (RFG) hens, there were no significant effects of CORT treatment or baseline plasma corticosterone (or the interaction) on the delay before laying (Table 5.3, 5.4; Fig. 5.1B), relative laying date (Table 5.3, 5.4; Fig. 5.1D), or reproductive success (Table 5.3, 5.4; Fig. 5.2B, 5.2D). Relative arrival date was the only significant predictor for the delay before laying and relative laying date (Table 5.3), with earlier arriving females having shorter delays before laying and earlier relative laying dates (Table 5.4). Likewise, variation in reproductive success for RFG hens could only be predicted by relative laying date, with earlier laying hens having higher reproductive success (Table 5.3); however this was a relatively weak relationship (Table 5.4).

### *Discussion*

We tested the hypothesis that experimentally elevated baseline glucocorticoids can positively impact reproductive decisions in pre-breeding females of a long-lived species (Crossin et al. 2015). In support of our predictions, corticosterone treatment had a positive influence on both laying phenology metrics as well as reproductive success in pre-recruiting common eider hens, but absolutely no impact in RFG hens already

committed to reproduction. Our combined results confirm recent correlative (Hennin et al. 2015) and manipulative studies (Comendant et al. 2003; Crossin et al. 2012; Love et al. 2014) indicating that baseline glucocorticoids (GCs) play a strong causal role in investment decisions and trade-offs across a diversity of vertebrate taxa. From a broader perspective, this work serves as a robust empirical test of components of the Physiology/Life History nexus framework (Ricklefs and Wikelski 2002), which predicts that GCs should play a key role in centrally regulating life history trade-offs and decisions.

Examining the role of elevated baseline GCs on reproductive investment in female vertebrates has been challenging in free-living populations for a number of reasons. Firstly, for decades the general assumption by many ecologists has been that elevated GCs generate obligate negative impacts on reproductive investment and success. This overall idea has been based upon the generalized assumption that as "stress" hormones - given their known role in the acute stress response to move organisms away from reproduction towards self-maintenance (Wingfield et al. 1998) – elevated baseline GCs should also "negatively" impact reproduction (Crossin et al. 2015). Adding to this assumption has been experimental work elevating baseline GCs to either stress-induced (e.g., Bourgeon and Raclot 2006; Angelier et al. 2009; Spée et al. 2011) or even pharmacological levels, rendering results less biologically relevant for investigations of variation in baseline physiology (Crossin et al. 2015). Secondly, it is generally very difficult to capture pre-breeding females of any free-living population whereby researchers can examine impacts of variation in phenotypic traits such as physiology on downstream reproductive investment decisions (Love et al. 2014). More often than not,

studies must focus on later stages of reproduction (e.g., pregnancy, egg-laying, incubation, brooding and offspring-rearing). While these reproductive stages are certainly important for examining investment decisions and reproductive output (e.g., Love at al. 2005; Bonier et al. 2009; Harding et al. 2009), they have not been amenable to examining the underlying key decisions of whether an individual should invest in reproduction in a given year, and if so, when to invest (Legagneux et al. *in revision*). Finally, despite strong predictions for the role of energetic physiology driving the accumulation of resources which influences reproduction in mixed capital-income breeding strategy species (Hennin et al. 2015; 2016), few studies have examined these mechanisms in these species that are so reliant on accumulating large endogenous stores of fat to fuel reproduction.

*Positive Impacts of Corticosterone on Reproduction*

Previous research has suggested that females across multiple taxa show an increase in baseline GCs during reproduction (Romero 2002; Moore and Jessop 2003; Crespi et al. 2013) and increases in baseline GCs have been proposed as preparatory mechanisms for mediating investment in reproduction (Love et al. 2013; 2014; Crossin et al. 2015). Pre-recruiting female common eider females are in a life history stage in which they must acquire substantial endogenous fat stores to both initiate and support reproduction (Bottita et al. 2003; Sénéchal et al. 2011). Nonetheless, variation in life histories and the ability to elevate GCs prior to breeding investment are both strongly expected to impact the degree and direction of the effects of elevated GCs on reproduction (Love et al. 2009; Crossin et al. 2015). As such, the positive effects on reproduction we report are suspected to be an indirect outcome of elevated baseline GCs directly driving increases in foraging

rate and therefore the rate of lipid store acquisition in this species before investment decisions have been made. Regardless of the downstream mechanism, there are four lines of evidence from our current work supporting the hypothesis that elevated baseline corticosterone plays a positive and context-dependent role in current investment in reproduction with downstream positive impacts on reproductive success.

First, experimentally elevating baseline corticosterone did not reduce the ability of birds to invest in reproduction; CORT-implanted females did not show lower breeding propensity compared with controls. This is consistent with some studies showing that elevations of GCs within the baseline range during the pre-breeding stage did not interfere with breeding propensity (Love et al. 2005; Ouyang et al. 2013). Nonetheless, additional studies have indicated that pre-breeding individuals with elevated GCs will skip reproduction altogether including black-legged kittiwakes (*Rissa tridactyla*; Goutte et al. 2010a), snow petrels (*Pagodroma nivea*; Goutte et al. 2010b) and Galápagos marine iguanas (*Amblyrhynchus cristatus*; Vitousek et al. 2010). The underlying reasons for the differences across these studies likely stems from variation in breeding strategies, with a majority of other studies focusing on species reliant on income-based strategies and therefore very different energetic demands and needs during pre-breeding.

Second, control pre-recruiting eider hens showed a negative relationship between baseline corticosterone and the delay before laying: females with high baseline plasma corticosterone invested in reproduction earlier than females with lower plasma levels. This trend supports the recently-reported temporal overlap in the increase in baseline corticosterone and progression towards laying in common eider hens in our eider population (Hennin et al. 2015; Fig. 5.1b). Further, a number of studies in reptiles and

some amphibians have shown that glucocorticoids increase during the reproductive period to support the associated energetic demands of mate attraction and reproduction (*reviewed in* Moore and Jessop 2003), implying that this trend may have broader applicability. However, in snow petrels, individuals with higher baseline corticosterone had later laying dates (Goutte et al. 2010b), and experimental elevations of corticosterone did not influence timing of reproduction at all in Florida scrub-jays (*Aphelocoma coerulescens*; Schoech et al. 2007). Again, variation in breeding strategies likely injects important context into these relationships.

Third, pre-recruiting females administered a high dose of CORT initiated laying in a shorter period of time and earlier than controls, while low CORT females appeared to be intermediate between control and high CORT groups. In addition, these positive impacts were only seen in hens with low pre-manipulation circulating baseline corticosterone, indicating a strong individually context-dependent effect of elevated baseline GCs on reproduction centred around a possible threshold. These results are supported by recent work showing that individual Florida scrub-jays in poor quality environments and high baseline plasma corticosterone had later laying dates compared to individuals with similar baseline plasma corticosterone levels in average quality environments (Schoech et al. 2009). While other studies report negative, often correlative, relationships between elevations of glucocorticoids and nest abandonment (Love et al. 2004; Ouyang et al. 2012) and investment (Sanderson et al. 2014),these studies did not focus on the pre-breeding period. Overall, little is known about graded reproductive responses to corticosterone secretion, making this a particularly rich area of focus for future research.

Finally, we found substantial, positive influences of the experimental treatment on the reproductive success of pre-recruiting females, indicating that its preparatory role in one life history stage (pre-breeding) can have positive influences in another (incubation and hatching). Although other studies report negative relationships between elevations in glucocorticoids, reproductive success (Angelier et al. 2010; Ouyang et al. 2011) and survival (Goutte et al. 2012), the current experiment adds to the small, but growing, body of evidence suggesting that elevations of baseline GCs can positively mediate reproductive investment and outcomes. For instance, European starlings (*Sturnus vulgaris*) with experimentally reduced first broods increased baseline corticosterone to support increased reproductive investment in their second brood (Love et al. 2014). Side-blotched lizards with experimentally elevated baseline corticosterone produced larger eggs and heavier clutch masses, positively affecting offspring survival (Sinervo and DeNardo 1996). Finally, female macaroni penguins (*Eudyptes chrysolophus*) with experimentally elevated baseline corticosterone foraged more, gained a greater amount of body mass and produced heavier offspring with higher survival probability (Crossin et al. 2012). Testing these questions in the wild and having the ability to follow individuals from the pre-breeding stage through to hatching and even offspring rearing is difficult, but necessary to fully test and determine the mechanistic relationships between physiological traits and fitness-related outcomes.

*Underlying Mode of Action for Corticosterone on Reproduction*

It is important to ascertain the physiological reasons underlying the positive influence of baseline corticosterone on reproduction. From a physiological point of view and based on

expectations of elevating GCs to levels outside the range of baseline levels, it could be assumed that experimentally elevated corticosterone would inhibit HPA axis function, thereby "reducing stress" and positively impacting reproduction (e.g., Goutte et al. 2011). However, in our captive validation of the implants used in this study, we found that corticosterone was elevated within a biologically relevant and baseline range both for white-winged scoters (wild birds baseline range: 0.51 - 46.7 $ng.mL^{-1}$; Palm et al. 2013; captive birds manipulated range: 0.95 - 33.35 $ng.mL^{-1}$; Hennin et al. 2016) and in wild common eiders (baseline range: 0.19 - 37.67 $ng.mL^{-1}$; this study). Secondly, to ensure baseline elevations of corticosterone were not inhibiting the function of the HPA axis, we performed an HPA axis challenge in the captive study, injecting individuals with adrenocorticotropic hormone (ACTH) to stimulate corticosterone production from the adrenal tissue (Hennin et al. 2016). All treatment birds administered ACTH increased corticosterone secretion in the same manner as control birds, indicating that the HPA axis was still fully functional and negative feedback was not artificially reducing circulating baseline corticosterone levels (Hennin at al. 2016). Finally, in response to baseline elevations of corticosterone, the captive individuals demonstrated a continual gain in body mass over time, indicating a causal role for baseline corticosterone in mediating mass gain *via* foraging rate and fat deposition (Hennin et al. 2016). The effects we saw in the current study are therefore very likely a result of baseline elevations of corticosterone producing gains in body mass, rather than a reduction of endogenous corticosterone production *via* negative feedback on the HPA axis.

From a behavioural point of view, there is growing evidence that the mechanism linking elevations of baseline corticosterone to reproductive outcomes is increases in

foraging and fattening rates. Experimental elevations of baseline corticosterone have resulted in an increase in foraging behaviours and activity (Astheimer et al. 1992, Breuner et al. 1998; Kitaysky et al. 1999; Lõhmus et al. 2006; Angelier et al. 2007), as well as body mass gain through fat deposition (Holberton 1999; Holberton et al. 2007; Crossin et al. 2012; Hennin et al. 2016). Further, in some instances increased foraging and fat deposition have then been linked to increased reproductive output (Crossin et al. 2012). Despite these strong, biologically relevant predictions, these linkages are still poorly understood, difficult to investigate and could not be examined in the present study. Future research should aim to quantify foraging metrics and resource accrual (fat deposition) as the missing piece of this puzzle linking increases in pre-breeding baseline GCs and positive impacts on reproductive investment.

While pre-recruiting hens responded reproductively to elevations in baseline corticosterone, the RFG hens appeared insensitive to the treatment, despite the RFG group having larger sample sizes than the pre-recruiting group. Due to these differential responses, our results have implications for interpreting GC-induced variation in stage-specific investment in reproduction and for characterizing the differences in investment trade-offs individuals face in these two pre-laying stages (as predicted by Crossin et al. 2015). Since pre-recruiting eider hens were not yet committed to reproduction (i.e., not yet recruiting follicles) at the time of the manipulation, they likely still had the flexibility to alter their timing of breeding. However, because RFG females had already met the required endogenous fat store threshold to initiate reproduction, it is likely that we were unable to alter the timing of laying and potentially reproductive success. Rather, in RFG hens, we may have influenced metrics associated with egg or clutch investment such as

egg mass, clutch size and therefore total clutch mass. For example, female side-botched lizards with experimentally elevated corticosterone invested in heavier eggs resulting in higher total clutch masses when controlling for clutch size (*reviewed in* Sinervo and DeNardo 1996). Common eiders demonstrate a decline in egg mass across the laying order of the clutch (O. Love *unpublished*) and a seasonal decline in clutch size (Descamps et al 2011). If the RFG hens given CORT implants forage at higher rates, but the energy cannot be directed to further increases in body lipid stores, the additional resources could be allocated to produce larger yolks and therefore larger eggs (perhaps equalizing intra-clutch variation in egg size) or perhaps even producing larger clutch sizes (as predicted by optimal investment models, Rowe et al. 1994; Bêty et al. 2003). Testing these predictions in our free-living eiders would prove challenging and potentially disruptive to the breeding colony as a whole, but nonetheless would be highly informative in testing major components of individual optimization of reproduction.

*Elevated Baseline Corticosterone and Expected Trade-offs*

We observed positive, short-term influences of baseline elevations of corticosterone on investment in current reproduction, likely mediated through its impacts on maternal condition (*via* increased foraging behaviour). However, based on expected life history trade-offs we would expect that this short-term benefit to maternal condition and current reproduction may come with cost both in the short-term (within-season) and even long-term (subsequent life history stages). In the short-term we found that, despite high CORT females laying earliest, they did not demonstrate the highest reproductive success. There are two potential underlying reasons for this result. First, it is possible that the high

CORT treatment pellets could have still been present and releasing CORT to the females once they initiated incubation. Elevated baseline corticosterone during incubation would act to metabolize lipid stores faster than is optimal, resulting in a faster loss of condition leading to an increased probability for nest abandonment (Bourgeon and Raclot 2006). However, given that: i) in captive ducks these pellets only last approximately 14 days (Hennin et al. 2016), ii) our pre-recruiting eider hens initiated incubation conservatively 12-22 days post-implantation, and iii) most of the nest abandonments in the high CORT group occurred well outside of the anticipated timeframe of influence for these pellets, we think this mechanism is highly unlikely. A second possible pathway is that eider hens administered the high CORT treatment may have foraged at such a high rate, they began mobilising endogenous lipid or protein stores to promote foraging behaviour, thereby depleting some of the remaining stores available for incubation. In support of this hypothesis, while low CORT females initiated laying slightly later than high CORT females, they may have better optimized the balance between foraging rate and the conservation of energetic stores resulting in higher reproductive success.

From a long-term trade-off perspective, despite corticosterone-treated females likely having greater rates of condition gain or even higher absolute body condition, leading to earlier laying phenology and in some instances elevated reproductive success, this may have resulted in costs to future reproduction or survival. Unfortunately, in the current eider system it is very difficult to track individual female investment decisions across years, making it virtually impossible to estimate costs to future investment. From a survival cost point of view, our group is using consistent inter-annual re-sighting observations in this long-lived species (mean lifespan 9-10 years) with which we soon

hope to be able to estimate treatment-specific survival rates for the current study. Previous studies have found that corticosterone indeed influences the trade-offs between investment in current and future reproduction (Love et al. 2005; Harms et al. 2015; Love et al. 2014), as well as current reproduction and survival (Romero and Wikelski 2001; Satterwaithe et al. 2010; Goutte et al. 2012; Harms et al. 2015). It is likely that potential costs in eiders stem from the same mechanism generating the observed benefits: foraging rate. By stimulating higher foraging rates to prepare for an earlier investment in the current reproductive attempt, CORT-implanted females may have increased workload, exposing themselves to greater amounts of oxidative damage, which can impact survival and future reproduction (e.g., Bize et al. 2008; Constantini et al. 2011). Alternatively, elevations of corticosterone have been shown to influence trade-offs between immune function and survival (e.g., Comendant et al. 2003; Lancaster et al. 2008) as well as between self-maintenance (immune system) and reproductive investment (French et al. 2007). Finally, corticosterone can be transferred from mothers to offspring (Love et al. 2013; Sheriff and Love 2013), which can impact offspring sex ratios (Love et al. 2005; Bonier et al. 2007), behaviour (Robert et al. 2009), morphometrics (McCormick 1998; Comendant et al. 2003; Meylan and Clobert 2005; Cote et al. 2006) and survival (Meylan and Clobert 2005; Cote et al. 2006; Robert et al. 2009). Since female common eiders are relatively long-lived, perhaps the costs of over investing during the short-term come not to the mothers, but rather are a hidden cost mediated by maternal effects of glucocorticoids on their offspring. All of these potential trade-offs are operating within the context of an individual's current condition. With increased investment, females may expose themselves to reduced condition perhaps post-hatching, which can have carry-

over effects on survival (Harms et al. 2015). Although the present study is an important step towards testing the traits responsible for mediating investment decisions, with downstream consequences for reproductive success, there are many opportunities to further our understanding of the underlying mechanisms linking physiology and reproductive phenology and success.

*Conclusions and Future Directions*

Determining the underlying physiological mechanisms at the heart of variation in life histories, life history decisions and therefore individual variation in fitness remains an important goal of evolutionary physiology (Zera and Harshman 2001; Ricklefs and Wikelski 2002). However, it continues to be challenging to characterize and test the physiological mechanisms influencing reproductive phenology and success due to the difficulty of capturing individuals prior to investing in reproduction and manipulating physiology within a biologically relevant range (Crossin et al. 2015). To our knowledge, our present study is one of the first in a free-living vertebrate to be able to directly manipulate corticosterone within a baseline range in individuals prior to investing in breeding, effectively testing a key energetic mechanism underlying individual variation in fitness-related reproductive decisions. Moreover, our work suggests that corticosterone acts as a causal mechanism positively influencing reproductive phenology and success, with both observed and assumed downstream costs, suggesting it is a meaningful regulator of life history trade-offs. Although we are beginning to appreciate the underlying mechanisms influencing variation in key life history decisions, our results suggest a number of opportunities for future study. Future research should characterize

the influences of elevations of baseline GCs on foraging rate and the outcomes for gain in body condition in free-living species. This has been tested before in macaroni penguins (Crossin et al. 2012), however these post-laying females had already invested in reproduction leaving a large knowledge gap regarding effects on pre-breeding individuals. Although foraging rates themselves are a likely mechanism driving the positive trends we found, there are other aspects of foraging that may also be informative. For instance, individually optimized foraging strategies (i.e., selection of foraging patches or targeted prey items) depending on variation in metabolic capabilities may differ for females of varying physiological phenotypes. Characterizing these differences will not only inform the mechanisms linking GCs to reproductive success, but also help us ascertain the different pathways by which individual optimization strategies may solve the same reproductive problems. Finally, although challenging, studies that are able to follow individuals through multiple life history stages (or years) to quantify the carry-over effects and trade-offs associated with elevations of baseline corticosterone are of the utmost importance in addressing a complex and long-standing question in the study of life history evolution: how and why physiological traits interact with environmental variation to influence individual variation in investment decisions, reproductive success and survival.

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**Table 5.1 -** Summary of fixed effects in analyses for pre-recruiting females. Bold values indicate significant effects.

| **Analysis** | **Variable** | **F** | **df** | **p** |
|---|---|---|---|---|
| Delay Before Laying | Year | 0.08 | 1 | 0.7805 |
| | Relative Arrival Date | 0.10 | 1 | 0.7575 |
| | Body Mass | 0.79 | 1 | 0.3808 |
| | Baseline CORT | 1.30 | 1 | 0.263 |
| | Treatment | 1.30 | 2 | 0.2884 |
| | Baseline CORT*Treatment | 3.45 | 2 | **0.05** |
| Relative Laying Date | Year | 0.28 | 1 | 0.5993 |
| | Relative Arrival Date | 34.71 | 1 | **<0.0001** |
| | Body Mass | 0.79 | 1 | 0.3808 |
| | Baseline CORT | 1.30 | 1 | 0.263 |
| | Treatment | 1.30 | 2 | 0.2884 |
| | Baseline CORT*Treatment | 3.45 | 2 | **0.05** |
| | **Variable** | **$\chi^2$** | **df** | **p** |
| Reproductive Success | Relative Lay Date | 0.01 | 1 | 0.93 |
| | Body Mass | 4.07 | 1 | **0.04** |
| | Baseline CORT | 0.27 | 1 | 0.60 |
| | Treatment | 7.45 | 2 | **0.02** |
| | Baseline CORT*Treatment | 2.97 | 2 | 0.23 |

**Table 5.2 -** Summary of parameter estimates for fixed effects in analyses for pre-recruiting females. Bold values indicate significant effects.

| Analysis | Variable | Estimate | SE | t | p |
|---|---|---|---|---|---|
| Delay Before Laying | Intercept | 7.42 | 4.78 | 1.55 | 0.13 |
| | Year (2011) | 0.17 | 0.62 | 0.28 | 0.78 |
| | Relative Arrival Date | -0.05 | 0.16 | -0.31 | 0.76 |
| | Body Mass | 0.00 | 0.00 | 0.89 | 0.38 |
| | Baseline CORT | -1.09 | 0.96 | -1.14 | 0.26 |
| | Treatment (Control) | 1.07 | 0.69 | 1.55 | 0.13 |
| | Treatment (High) | -0.77 | 0.65 | -1.19 | 0.24 |
| | (Baseline CORT-0.69925)*Treatment (Control) | -3.23 | 1.34 | -2.41 | **0.02** |
| | (Baseline CORT-0.69925)*Treatment (High) | 3.25 | 1.41 | 2.30 | **0.03** |
| Relative Laying Date | Intercept | 2.92 | 4.78 | 0.61 | 0.55 |
| | Year (2011) | -0.33 | 0.62 | -0.53 | 0.60 |
| | Relative Arrival Date | 0.95 | 0.16 | 5.89 | **<0.0001** |
| | Body Mass | 0.00 | 0.00 | 0.89 | 0.38 |
| | Baseline CORT | -1.09 | 0.96 | -1.14 | 0.26 |
| | Treatment (Control) | 1.07 | 0.69 | 1.55 | 0.13 |
| | Treatment (High) | -0.77 | 0.65 | -1.19 | 0.24 |
| | (Baseline CORT-0.69925)*Treatment (Control) | -3.23 | 1.34 | -2.41 | **0.02** |
| | (Baseline CORT-0.69925)*Treatment (High) | 3.25 | 1.41 | 2.30 | **0.03** |

| | Variable | Estimate | SE | $\chi^2$ | p |
|---|---|---|---|---|---|
| Reproductive Success | Intercept | 15.14 | 9.53 | 2.92 | 0.09 |
| | Relative Lay Date | -0.01 | 0.14 | 0.01 | 0.93 |
| | Body Mass | -0.01 | 0.004 | 4.07 | **0.04** |
| | Baseline CORT | 1.21 | 2.50 | 0.27 | 0.60 |
| | Treatment (Control) | 1.82 | 1.38 | 3.01 | 0.08 |
| | Treatment (Low) | -3.38 | 2.43 | 7.40 | **0.007** |
| | (Baseline CORT-0.78458)*Treatment (Control) | -1.09 | 2.59 | 0.20 | 0.66 |
| | (Baseline CORT-0.78458)*Treatment (Low) | 5.23 | 4.51 | 2.72 | 0.10 |

**Table 5.3 -** Summary of fixed effects in analyses for rapid follicle growth females. Bold values indicate significant effects.

| **Analysis** | **Variable** | **F** | **df** | **p** |
|---|---|---|---|---|
| Delay Before Laying | Year | 0.01 | 1 | 0.94 |
| | Relative Arrival Date | 4.15 | 1 | **0.05** |
| | Body Mass | 0.04 | 1 | 0.84 |
| | Baseline CORT | 0.05 | 1 | 0.82 |
| | Treatment | 0.27 | 2 | 0.77 |
| | Baseline CORT*Treatment | 1.33 | 2 | 0.28 |
| Relative Laying Date | Year | 1.25 | 1 | 0.27 |
| | Relative Arrival Date | 54.16 | 1 | **<0.0001** |
| | Body Mass | 0.04 | 1 | 0.84 |
| | Baseline CORT | 0.05 | 1 | 0.82 |
| | Treatment | 0.27 | 2 | 0.77 |
| | Baseline CORT*Treatment | 1.33 | 2 | 0.28 |
| | **Variable** | **$\chi^2$** | **df** | **p** |
| Reproductive Success | Relative Lay Date | 4.86 | 1 | **0.03** |
| | Body Mass | 0.08 | 1 | 0.77 |
| | Baseline CORT | 0.39 | 1 | 0.53 |
| | Treatment | 0.64 | 2 | 0.73 |
| | Baseline CORT*Treatment | 3.19 | 2 | 0.20 |

**Table 5.4** - Summary of parameter estimates for fixed effects in analyses for rapid follicle growth females. Bold values indicate significant effects.

| Analysis | Variable | Estimate | SE | t | p |
|---|---|---|---|---|---|
| Delay Before Laying | Intercept | 2.33 | 4.21 | 0.55 | 0.58 |
| | Year (2011) | 0.03 | 0.42 | 0.08 | 0.94 |
| | Relative Arrival Date | -0.22 | 0.11 | -2.04 | **0.05** |
| | Body Mass | 0.00 | 0.00 | 0.21 | 0.84 |
| | Baseline CORT | -0.19 | 0.86 | -0.23 | 0.82 |
| | Treatment (Control) | 0.00 | 0.47 | 0.00 | 1.00 |
| | Treatment (High) | -0.31 | 0.52 | -0.60 | 0.55 |
| | (Baseline CORT-0.94269)*Treatment (Control) | 1.70 | 1.13 | 1.50 | 0.14 |
| | (Baseline CORT-0.94269)*Treatment (High) | -0.49 | 1.26 | -0.39 | 0.70 |
| Relative Laying Date | Intercept | -2.17 | 4.21 | -0.51 | 0.61 |
| | Year (2011) | -0.47 | 0.42 | -1.12 | 0.27 |
| | Relative Arrival Date | 0.78 | 0.11 | 7.36 | **<0.0001** |
| | Body Mass | 0.00 | 0.00 | 0.21 | 0.84 |
| | Baseline CORT | -0.19 | 0.86 | -0.23 | 0.82 |
| | Treatment (Control) | 0.00 | 0.47 | 0.00 | 1.00 |
| | Treatment (High) | -0.31 | 0.52 | -0.60 | 0.55 |
| | (Baseline CORT-0.94269)*Treatment (Control) | 1.70 | 1.13 | 1.50 | 0.14 |
| | (Baseline CORT-0.94269)*Treatment (High) | -0.49 | 1.26 | -0.39 | 0.70 |

| | Variable | Estimate | SE | $\chi^2$ | p |
|---|---|---|---|---|---|
| Reproductive Success | Intercept | 0.70 | 3.49 | 0.04 | 0.84 |
| | Relative Lay Date | 0.30 | 0.16 | 3.49 | 0.06 |
| | Body Mass | 0.00 | 0.00 | 0.09 | 0.77 |
| | Baseline CORT | -0.84 | 1.35 | 0.39 | 0.53 |
| | Treatment (Control) | 0.02 | 0.59 | 0.00 | 0.97 |
| | Treatment (Low) | 0.47 | 0.72 | 0.42 | 0.52 |
| | (Baseline CORT-1.02752)*Treatment (Control) | -1.93 | 1.79 | 1.16 | 0.28 |
| | (Baseline CORT-1.02752)*Treatment (Low) | -0.93 | 1.87 | 0.25 | 0.62 |

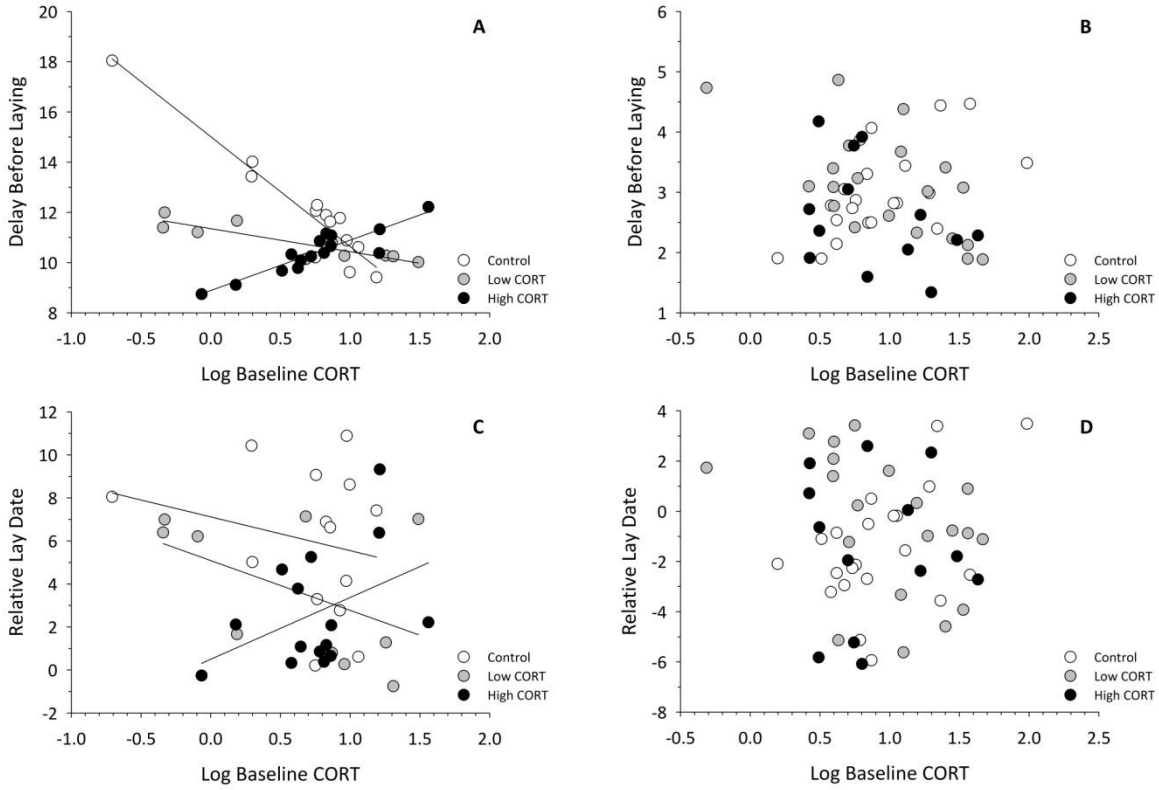


**Figure 5.1** - The model-corrected influence of the interaction between (log transformed) baseline corticosterone and treatment group on delay before laying (A and B) and relative laying date (C and D) in pre-recruiting (A, C) and rapid follicle growth (B, D) common eider hens. In pre-recruiting hens, individuals in the high CORT group with low baseline plasma CORT were able to lay in a shorter period of time (A) and lay earlier (C) relative to controls. However, these relationships were not present in RFG hens (B and D).

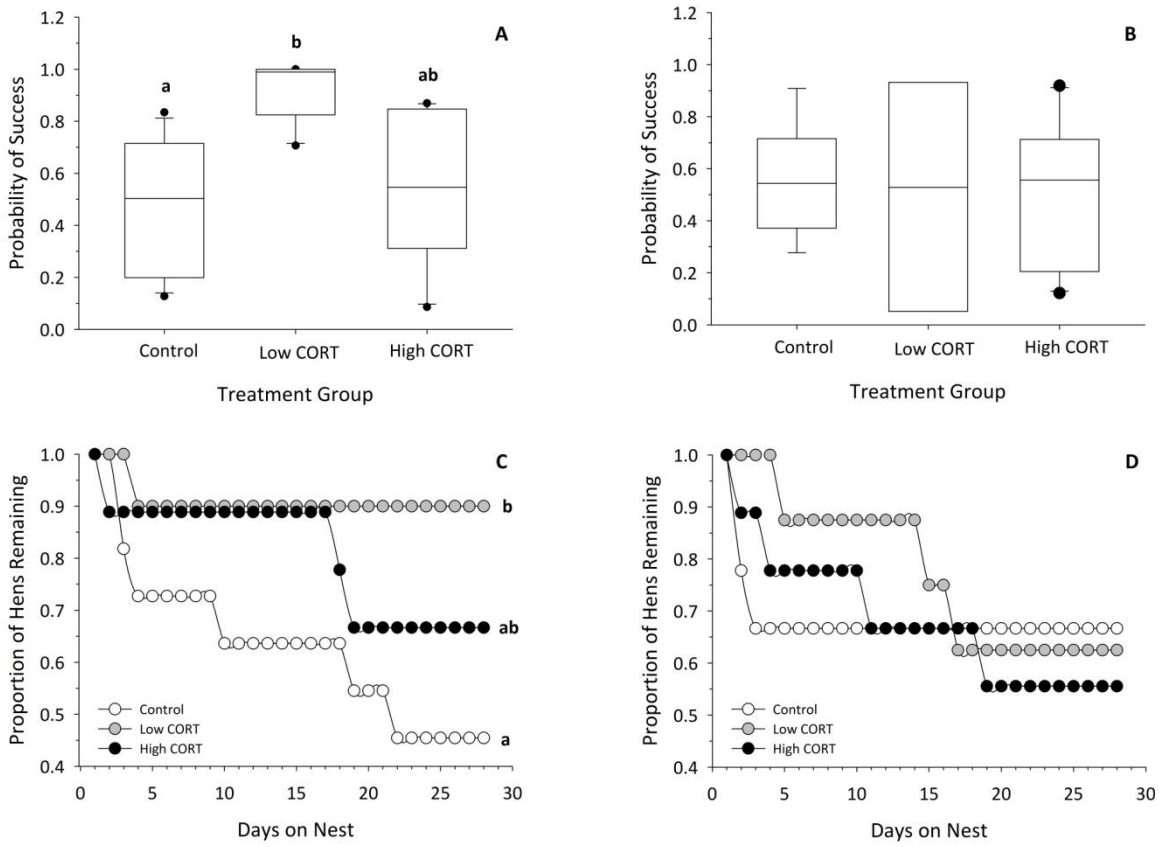


**Figure 5.2** - The model-corrected influence of treatment group on reproductive success in pre-recruiting (A, C) and rapid follicle growth (B, D) common eider hens. Pre-recruiting hens administered low CORT dosages had higher probability of reproductive success compared to control hens (A and C), however there was no effect of treatment in RFG hens (B and D). In addition to reproductive success, we show the number of hens remaining on their nest across the number of incubation days to demonstrate when females abandoned reproduction (C and D). The resultant proportion represents the model-corrected reproductive success for each treatment group.

# CHAPTER 6 :

# THE ENERGETIC PHYSIOLOGY OF LIFE HISTORY DECISIONS

Physiological traits have long been proposed as prime mechanisms for mediating underlying variation in life history traits and decisions across individuals and species (Ketterson and Nolan 1999; Zera and Harshman 2001; Ricklefs and Wikelski 2002; Harshman and Zera 2007; Zera et al. 2007; Williams 2008). Due to the importance of optimal energetic management in regulating life history decisions and trade-offs, I proposed that the use of physiological traits representative of an individual's current energetic state will be useful metrics for predicting variation in investment decisions across individuals. In this thesis, I aimed to examine the underlying mechanisms mediating the optimization of important life history decisions using energetic physiology in common eiders (*Somateria mollissima*), an Arctic-breeding diving seaduck, as a model species. I found that energetic physiology played an important role in both predicting and driving a key investment decision in this species: breeding phenology. In my first data chapter (Chapter 2) I found that baseline glucocorticoids (GCs) began increasing prior to females recruiting follicles, a pattern that likely supports the energetic demands associated with accumulating enough endogenous stores for their incubation fast. Conversely, physiological fattening declined shortly after the initiation of rapid follicle growth (RFG), likely because females had accumulated enough endogenous fat stores for incubation and were foraging only to complete follicle growth. Further, physiological fattening rates (plasma triglycerides) exhibited a potential threshold to transitioning between breeding stages. Building on these results, data from my second data chapter

(Chapter 3) suggested that baseline GCs and physiological fattening rates interact to influence individual optimization of the timing of breeding. Using a controlled captive seaduck system, my third data chapter (Chapter 4) determined that GC levels could be manipulated within a biologically-relevant, baseline range without impacting the function of the hypothalamic-pituitary-adrenal (HPA) axis. Furthermore, the elevation in baseline GCs resulted in a concomitant increase in body mass. Finally, in my fourth data chapter (Chapter 5) I performed a manipulation of baseline GCs in my focal colony of free-living common eiders and determined that experimental elevations of baseline GCs generate a graded response in pre-recruiting females with pre-existing low baseline GC phenotypes; control hens had the latest breeding phenology (longer delay before laying after arrival on the breeding grounds and later laying date), females administered the highest dose of GCs has the earliest breeding phenology and females with a low dose of GCs were intermediate in breeding phenology. However, pre-recruiting females with pre-existing high plasma GC phenotypes showed little change or potentially even negative responses to experimentally increased baseline GCs. Furthermore, pre-recruiting females administered an elevation of GCs exhibited higher reproductive success compared to control females. Finally, hens already invested in reproduction (those in the RFG stage) were non-responsive to all GC treatments. Taken together, these results collectively provide multiple lines of correlative and experimental evidence to indicate that energetic physiology acts as an important regulator of key reproductive investment decisions.

### *Interpreting Energetic Physiology: Considerations of Context*

The traits influencing variation in energetic physiology are highly labile, demonstrating both temporal and taxonomic variation (Dallman et al. 1993; Romero et al. 2002; Landys et al 2006; Crespi et al. 2013), making an individual's current state and life history context highly important when considering interpretations of how these physiological traits will impact key life history decisions. Appreciating how life history and ecological variation across species will impact differences in how energetic demand will influence life history decisions is therefore key to explaining the differences in the direction of relationships between GCs and fitness, or fitness-related metrics across species (e.g., Bonier et al. 2009; Crossin et al. 2015; Lattin et al. 2016). In particular, my thesis highlights three important contexts that are key to interpreting trends in energetic physiology: i) breeding strategies (i.e., capital vs. income breeders), and ii) life history stages (e.g., pre-breeding, follicle growth, offspring rearing within reproduction, wintering, migration) and iii) the role of GCs (driver of behaviour vs. responder to the environment).

Capital and income breeders are two ends of a continuum describing the source of resources allocated to reproductive investment that can be applied across most taxa (Stephens et al. 2009). Where capital-breeding species solely use endogenous lipid stores to support reproduction, income breeders will use only resources acquired for current foraging for this purpose (Stephens et al. 2009). It is not surprising then, that species using different strategies will be under different energetic demands and demonstrate different trends in relation to energetic physiology. For example, common eiders are a

mixed, capital-income breeding strategy species with uniparental care of offspring and must accumulate significant fat stores to complete reproduction successfully. Conversely, black-legged kittiwakes are an income breeding species with biparental care that can forage during incubation. In experimental studies during the pre-breeding period, common eiders demonstrated a positive relationship between baseline GC elevations and timing of breeding (Chapter 5), whereas black-legged kittiwakes with experimentally lowered baseline GCs had earlier timing of breeding (Goutte et al. 2011). These differing results confirm recent predictions that variation in life histories will play major roles in shaping relationships between baseline GCs and reproductive decisions (Crossin et al. 2015). As such, characterizing the degree to which individuals rely on endogenous stores can be important for interpreting the secretion trends and nature of relationships of baseline GCs to life history decisions.

It has been well-established that different life history stages impose differing energetic demands and constraints on individuals, with reproduction often being considered the most energetically demanding stage and therefore characterized by significant elevations in baseline GCs (Romero 2002; Crespi et al. 2013). Within reproductive stages, energetic demand is influenced by a number of factors including whether: i) an individual is currently investing in reproduction, ii) a species exhibits bi-parental care of offspring, or iii) young are precocial or altricial. For instance, although pre-recruiting common eider hens responded to experimental elevations of baseline GCs, hens that were already recruiting follicles did not (Chapter 5). Additionally, in incubating common eider and king eider (*Somateria spectabilis*) hens, females demonstrate positive relationships between baseline GCs and nest abandonment (Bourgeon and Raclot 2006;

Bentzen et al. 2008). Each of these trends results from the differing energetic demands specific to the stages: pre-recruiting hens must meet an energetic threshold to invest in reproduction and must fatten substantially, RFG hens have already obtained enough endogenous fat stores and therefore focus incoming energy on follicle growth, while post-laying hens need to minimise their overuse of endogenous fat stores to successfully complete incubation. Therefore although complex and often positive trends can be detected between baseline GCs and investment, it is unlikely that these trends will be consistent between life history stages or species (Crossin et al. 2015).

A final contextual consideration is the role the environment plays in mediating the potential impact of variation in energetic physiology on reproductive decisions. Although increases in energetic metabolites are generally interpreted as being responsive to changes in foraging or metabolism, elevated baseline GCs can be interpreted as both drivers of energetic intake as well as the outcome (i.e., responders) of limited resource availability in the environment. For instance, elevated baseline GCs can be negatively (Kitaysky et al. 1999; 2007; 2010; Love et al. 2005) or positively (Bronikowski and Arnold 1999) correlated with body condition or resource availability. Alternatively, elevated baseline GCs can stimulate foraging behaviour, which has been demonstrated in a number of manipulative studies both in direct manipulations of baseline GCs (Astheimer et al. 1992, Breuner et al. 1998; Lõhmus et al. 2006; Crossin et al. 2012; Hennin et al. 2016) and through environmental manipulations (Holberton 1999). Teasing apart the difference between baseline GCs as either a driver or a responder takes an appreciation for the life history stage involved, the energetic demands of the individual and the degree of environmental variation the individual is currently facing. Overall, the

work within this thesis has tried very hard to carefully control for and appreciate these many contextual implications to provide a biologically relevant interpretation of variation in energetic physiology as a positive driver of investment in reproduction. Nonetheless, it is likely the general lack of appreciation for these considerations that need to be taken into account to properly interpret variation in energetic physiology metrics that explains why we see so many contrasting results in the literature (Crossin et al. 2015). Accounting for sources of variation by truly understanding the ecology and life history of focal study species is critical to the proper use and implementation of energetic physiology, however if done correctly, energetic physiology can be a useful and informative parameter.

***Elevated Baseline Glucocorticoids: A Positive Preparatory Mechanism***

Due to its role in the acute stress response, there is often an underlying assumption that elevations in glucocorticoids are detrimental to fitness (e.g., Bonier et al. 2009). This general misinterpretation of GCs as a whole has been reinforced in the literature by numerous manipulative studies that have increased plasma GC levels to into either stress-induced or pharmacological ranges (e.g., Bourgeon and Racolot 2006; O'Connor et al. 2009; Spée et al. 2011). However, there has been increasing evidence in the literature that elevated baseline GCs in fact act as preparatory mechanisms for energetically demanding life history stages, particularly because of their role in influencing energetic management and gain in body mass (Holberton 1999; Holberton et al. 2007; Crossin et a. 2012; Hennin et al. 2016; Chapter 4). Within this thesis, I have demonstrated that as female common eiders approach their rapid follicle growth period, there is a continual increase in baseline GCs (Hennin et al. 2015; Chapter 3) and that experimental elevations of

baseline GCs within this period result in earlier breeding phenology (Chapter 5), likely mediated though the effect of elevated GCs on increases in body mass (Hennin et al. 2016; Chapter 4). My results provide further support to other studies indicating that baseline GCs can be important drivers of investment during key life history stages. For instance, experimentally reducing reproductive investment in the first clutches of female European starlings (*Sturnus vulgaris*) resulted in an increase in baseline GC secretion and an increase in investment in their second clutch, the fitness result being the same annual number of offspring as control females (Love et al. 2014). In yellow-rumped warblers (*Setophaga coronata*) exposed to increasing photoperiods to simulate spring migratory conditions individuals increased both baseline GC secretion and foraging behaviour resulting in increases in body mass (Holberton 1999). These and few other studies (i.e., Holberton et al. 2007; Crossin et al. 2012) currently represent a highly important paradigm shift for the interpretation of baseline GCs in the energetic role they play for most of an organism's life, rather than simply as a "stress hormone".

### *Individual Optimization of Reproduction*

Individual optimization of key investment decisions is an important component to life history theory because it generates a set of testable predictions for interpreting the variation in life history decisions observed across individuals (Parker and Maynard Smith 1990; Rowe and Ludwig 1991; Rowe et al. 1994; Kisdi et al. 1998). A condition dependent optimization model was developed by Rowe and colleagues (1994) to explain the optimization of the timing of reproduction and reproductive investment based on body condition (i.e., mass) and timing of arrival on the breeding grounds, and the rate at

which an individual can gain in body condition to invest in reproduction. This optimization model has only been tested twice: in snow geese (*Chen caerulescens*) and common eiders (Bêty et al. 2003; Descamps et al. 2011). Both studies aimed to determine the optimization of reproductive investment and reproductive timing based on arrival body mass and the timing of arrival on the breeding grounds (Rowe et al. 1994). However body mass offers little predictive power and much variation remained unexplained. Importantly, neither study was able to address a key prediction of the original Rowe et al. (1994) model: the rate at which individuals can gain in body mass will influence laying date. As such, one of the aims of this thesis was to explore the energetic mechanisms that mediate variation in the timing of reproduction independently of arrival condition alone. I test these predictions both correlatively (Chapter 3) and causally (Chapter 5), demonstrating that energetic physiology is indeed an important mechanism influencing the optimal timing of reproduction. Nevertheless, there were inconsistencies in the direction of the relationship that baseline GCs played, both within my studies and in relation to other studies.

Using correlative data I found that baseline GCs interacted with physiological fattening rates to influence the optimization of breeding phenology. Individuals able to manage their energetics with greater efficiency by fattening at a higher rate with a lower signal of energetic demand (lower baseline GCs) had a shorter delay before laying and an earlier laying date (earlier breeding phenology). These results support the *negative* relationship between baseline GCs and the timing of breeding that other studies have reported (e.g. Schoech et al. 2009; Goutte et al. 2010). In contrast to this result, in my experimental manipulation of baseline GCs in free-living eiders (Chapter 5) I found that

control birds demonstrated a *positive* relationship between baseline GCs and laying date. Moreover, individuals administered both low and high doses of baseline GCs invested in reproduction even earlier. These apparently contrasting trends may have stemmed from two sources. First, these two chapters included different years of data that may have differed in either the quality in wintering climate, known to influence individual arrival condition (Descamps et al. 2010), or the quality of climate on the breeding grounds, both of which could influence resource availability and therefore the ability of individuals to gain in condition (Jean-Gagnon 2015). Second, but perhaps more importantly, due to lower sample sizes the manipulative results were not able to account for the rate at which an individual was physiologically fattening. There have been very few instances of using these two traits together to examine energetic physiology as a whole, including resource use and demand during breeding stages in green turtles (*Chelonia mydas*; Jessop et al. 2004) and pied flycatchers (*Ficedula hypoleuca*; Kern et al. 2005), the incubation period for king eiders (*Somateria spectabilis*; Bentzen et al. 2008), and across the breeding and moult stages in wood thrushes (*Hylocichla mustelina*; Done et al. 2011). In each of these examples, including physiological fattening rates and other energetic metabolites with GCs enhanced the predictive capacity of the impact of an individual's current energetic state on variation in investment decisions, outcomes or carry-over effects. As such, combining both baseline GCs and fattening rate together better accounts for individual variation in flexibility of energetic physiology and how it impacts reproductive phenology.

## *Using Energetic Physiology to Test the Physiology/Life History Nexus Framework*

### *The Physiology/Life History Nexus*

The Physiology/Life History (P-LH) nexus is a theoretical framework developed by Ricklefs and Wikelski nearly 15 years ago (2002). This paper has been important in evolutionary physiology because it provides a framework linking proximate mechanisms (i.e., physiological traits) to life history traits and trade-offs. The P-LH nexus framework predicts that natural selection will shape individual life histories *via* selection for physiological phenotypes generated from individuals interacting with the environment across their life time thereby influencing reproductive decisions and outputs (i.e., "performance"; McNamara and Houston 1996; Zera and Harshman 2001; Ricklefs and Wikelski 2002). Physiological traits have therefore been proposed as ideal proximate mechanisms for testing linkages to life history variation within an evolutionary framework (Ricklefs and Wikelski 2002; Zera et al. 2007; McGlothlin and Ketterson 2008; Williams 2008). Given that allocation of limited time and energy to multiple life history traits is the underlying basis for life history trade-offs, and since energetic physiology regulates energetic balance (e.g. Dallman et al. 1993; Jenni-Eiermann and Jenni 1994), they are particularly strong candidates for mediating life history decisions. Most often, studies have focused on GCs as the primary metric of energetic physiology because they can act as both a driver and a responder to stimuli thereby having a more direct influence on behaviours and changes in energetics.

There are five principles that Ricklefs and Wikelski (2002) suggested which predict how physiology will interact with the environment to influence life history variation: 1) individuals respond to variation in their environment, 2) individuals may

switch physiological states in response to environmental stressors, 3) responses of individuals are constrained by the allocation of limited resources to multiple competing functions, 4) individuals change physiological states across their life spans, and 5) physiological states are influenced by the demography of the population. Here I discuss each of these five points in relation to my thesis results and current literature to assess the evidence for energetic physiology as a mediator of the P-LH nexus.

Previous studies have demonstrated that baseline GCs are plastic or flexible in that they will respond to environmental variation at multiple scales. At the habitat level, baseline GCs often increase in response to reduced food availability (Kitaysky et al. 2010) potentially to stimulate more profitable foraging behaviour (Angelier et al. 2008). Additionally, biotic environmental variables such as population demographics or predation pressure will alter investment decisions mediated through baseline GCs (McCormick 1998; Sinervo and DeNardo 1996; Comendant et al. 2003). At a broader scale, variation in both local weather (e.g., inclement weather; Breuner and Hahn 2003; Wingfield et al. 2011) and broader metrics of climate (e.g., climatic oscillation indices; Bechshøft et al. 2013) have also been correlated with elevated GCs and the promotion of individual survival during these more energetically challenging events. In addition to baseline levels mediating energetic demand, GCs play a role in the stress response, in which baseline levels will increase in response to an unpredictable perturbation in the environment (McEwan and Wingfield 2003; Romero et al. 2009; McEwan and Wingfield 2010), changing their physiological state in response to an environmental stressor (principle 2). The acute response to stress has been proposed as an adaptive response which alters an individual's behaviour and mobilizes endogenous resources to promote

survival mechanisms until the perturbation subsides and normal biological and behavioural functions resume (Sapolsky et al. 2000). If an individual is exposed to a chronic perturbation, and GC levels remain elevated over the longer term, the individual can enter into an "emergency-life history stage" in which resources are diverted from all other functions besides maintaining homeostasis (McEwan and Wingfield 2003; 2010). As such, at multiple scales, secretion of GCs are responsible for directly mediating an individual's interaction with the environment by adjusting physiological states in ways that impact both reproductive performance and survival.

Individuals must make a number of allocation decisions across their lifetime by facing allocation trade-offs for investment in self-maintenance vs. reproduction. In many instances, baseline GCs have been shown to influence this allocation. For instance GCs influence reproductive investment decisions in response to increased immune maintenance or responses (Wilkens and De Rijk 1997; Bowers et al. 2015). They have also been shown to negatively impact survival indirectly through their influences on oxidative stress (Constantini et al. 2011). In terms of life history decisions, GCs have been shown to mediate the timing of reproductive investment (Chapter 3; Chapter 6) and the manner in which individuals allocate resources within a reproductive attempt (e.g., Sinervo and DeNardo 1996; Love and Williams 2008). Further, GCs have been shown to regulate transitions between life history stages across many taxa (see Wada 2008), and indeed changes in circulating concentrations across life history stages support stage-specific energetic demands (Romero 2002; Landys et al. 2006; Crespi et al. 2013) linking investment decisions to changes in physiological states across an individual's lifespan (principle 3). Furthermore, individuals have been shown to alter baseline GC secretion

(Angelier et al. 2006; Heidinger et al. 2006; Monclús et al. 2011; Riechert et al. 2012; Elliott et al. 2014) and sensitivity (Peiffer et al. 1991; Reul et al. 1991; Perlman et al. 2007) across an individual's life span. Taken together, there is a substantial amount of evidence to suggest that energetic physiology plays an important role in mediating both allocation decisions and the ability to be flexible in those decisions across an individual's life span.

Finally, baseline GCs are known to be influenced by population demographics both within and across generations. Individuals living in environments with higher population densities or with higher predation rates/risk often exhibit higher baseline GC secretion to cope with those energetic demands (e.g., Sinervo and DeNardo 1996; McCormick 1998; Sheriff et al. 2009). Further, females with higher baseline GCs breeding in these environments can influence reproductive investment to promote offspring survival in environments with greater competition (Love et al. 2013; Sheriff and Love 2013). As such, not only do GCs demonstrate the capacity to interact with current population demography, but they may also prepare offspring for the potential energetic demands of the predicted, future environment (Love et al. 2013).

*Energetic Physiology/Life History Nexus: Conclusions and Future Directions*

As the field of ecological and evolutionary physiology continues to advance, it is critical that researchers aim to determine how physiological traits mediate life history decisions given their central role in evolutionary processes (Zera and Harshman 2001; Ricklefs and Wikelski 2002; Harshman and Zera 2007; Zera et al. 2007). In many capacities, energetic physiology has been a highly useful mechanism for interpreting how linkages between

the environment and the individual impact life history investment (Ketterson and Nolan 1992; Zera and Harshman 2001; Harshman and Zera 2007; Zera et al. 2007; Williams 2008; Moore and Hopkins 2009). My thesis provides an experimental test for components of the P-LH nexus relating variation in baseline GCs (representing energetic physiology) to individual variation in key reproductive decisions and success. Indeed, energetic physiology showed substantial variation across individuals and breeding stages (Chapter 2; Hennin et al. 2015; Chapter 3), which likely influenced an individual's interactions with their environment *via* changes in foraging behaviour (Chapter 4; Hennin et al. 2016), proximately impacting reproductive phenology and ultimately impacting reproductive success (Chapter 3; Chapter 5).

While this thesis was able to validate some of the principles of the P-LH nexus, two of the biggest gaps it was unable to address were the: i) role of environmental variation in shaping how energetic physiology impacts breeding decisions, and ii) the intra-individual variation in energetic physiology over time. These are often missing pieces of many free-living studies largely due to the difficulty of experimentally elevating GCs within a baseline range to properly represent energetic physiology (Crossin et al. 2015), and in recapturing long-lived, free-living organisms to determine how intra-individual variation in energetic physiology impacts future investment and survival. While many studies have been able to test components of the P-LH nexus, a single study examining each of the five principles testing whether a focal physiological trait fulfills the P-LH nexus is rare, if not non-existent.

Although difficult to accomplish without highly variable years in a short-term data set paired with a manipulation within a baseline range, long-term data sets, or highly

controlled captive populations, future research focusing on energetic physiology to test the P-LH nexus framework must make the effort to characterize individual responses to the environment. Long-term data sets in wild organisms will be particularly valuable for characterizing the long-term impacts that environmental variation can have on shaping individual variation in energetic physiology and how this variation shapes life history decisions, trade-offs, and ultimately the evolution of life histories themselves. Finally, studies that are able to follow individuals through multiple life history stages to quantify both the short-term (i.e., within a life history stage) or the long-term (across stages and potential carry-over effects) costs of elevations of energetic physiology are vitally needed to fully confirm the role of these mechanisms in mediating investment decisions. Although challenging, studies of this calibre will help to determine the mechanisms central to the evolution of life histories.

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