How can birds live long and hard?
Patterns in the physiology and behaviour of aging birds

by

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Abstract

As animals age, they are expected to invest more energy in reproduction as they have fewer subsequent chances to reproduce (the "restraint" hypothesis). Conversely, the oldest animals may show restraint in reproduction because even a small increase in energy expended during reproduction may lead to death. Alternatively, both young and very old animals may lack the ability to maintain high levels of energy expenditure (the "constraint" hypothesis), leading to reduced reproductive success. Many studies have observed an increase in reproductive success with age followed by a reduction at the end of life, but fewer studies have examined the proximate mechanisms, which provide a context for understanding ultimate causes. I examined over 30 behavioural and physiological metrics of aging in two species of free-living long-lived seabirds (thick-billed murres *Uria lomvia* and black-legged kittiwakes *Rissa tridactyla*) and a short-lived passerine (tree swallows *Tachycineta bicolor*). For all species, reproductive success was high at intermediate ages. In support of the "restraint" hypothesis, when birds were stressed, glucocorticoid hormones, which direct energy away from reproduction and towards survival, were higher in young birds (swallows) and both young and very old birds (kittiwakes and murres). When birds were handicapped older birds expended more energy. When challenged exogenously, there was no change in hormone levels with age, implying that they were "choosing" to be restrained. Resting metabolic rate (RMR) declined linearly with age in both seabird species. T3, which I show is indicative of RMR in birds, also declined with age, demonstrating that the reduction in metabolism was strategic and not due to changing body composition. In contrast, daily energy expenditure in both seabird species during breeding was constant with age while antioxidant capacity

became elevated during middle age, and increased further with age. Several measures of behavioural performance did not vary with age. I conclude that hormonal cues lead to greater investment in an adult's energy stores over its offspring's energy reserves (restraint hypothesis) at the start of life. At the end of life, both hypotheses were supported; energy expenditure was constrained by senescence, leading to increased restraint in investing additionally in offspring.

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Chapter One. Introduction

I have looked upon those brilliant creatures,
And now my heart is sore.
Alls changed since I, hearing at twilight,
The first time on this shore,
The bell-beat of their wings above my head,
Trod with a lighter tread.

Unwearied still, lover by lover,
They paddle in the cold
Companionable streams or climb the air;
Their hearts have not grown old;
Passion or conquest, wander where they will,
Attend upon them still.

-Yeats, *The Wild Swans At Coole*

Senescence, used here as synonymous with aging, is the progressive decline or deterioration in individual performance with age that irreversibly leads to death (Medawar 1947, 1952; Hamilton 1966; Rose 1991; Kirkwood & Austad 2000). Senescence was seldom studied in an ecological context until the last decades of the 20th century (Monaghan *et al.* 2008). Prior to that period, it was widely believed that most wild animals died before they reached the age at which senescence occurred, and it was therefore considered to be virtually impossible to study senescence in the wild (Monaghan *et al.* 2008). Recently, it has become well-established that both survival and reproductive success usually increase at the start of life and then eventually decline at the end of life in wild animals (Clutton-Brock 1988, Newton 1989, Forslund & Pärt 1995, Reid *et al.* 2003; Jones *et al.* 2008; Ricklefs 2008, 2010; Vleck *et al.* 2007, 2011). Changes in reproductive success and potential survival occur at different ages (Ricklefs 2008; Holmes & Martin 2009).

The first studies in this field measured variation in survival or reproductive success with age (Clutton-Brock 1988, Newton 1989, Forslund & Pärt 1995, Jones *et al.* 2008; Ricklefs 2008, 2010). These studies were careful to distinguish actuarial senescence (a decline in survival with age) from reproductive senescence (a decline in reproduction with age) (Kirkwood & Austad 2000). While either component can contribute equally to reductions in evolutionary fitness with age, they can be caused by different underlying mechanisms. Although senescent declines in both the survival and reproduction of wild animals are well documented, the causes underlying these declines remain elusive (Nisbet 2001; Kirkwood 2002). Such studies cannot answer the question "Why do we die?" (Pearl 1922). To answer the question of proximate mechanism (which contributes to

understanding the ultimate explanation for senescence), one must examine somatic senescence, the progressive deterioration of somatic tissue with age. Somatic senescence has been much less explored. Presumably, declines in the soma underlie both actuarial and reproductive senescence, although somatic senescence may also be neutral in terms of its effect on selection (Monaghan *et al.* 2008; Vleck *et al.* 2007, 2011). One goal of the current thesis is to examine somatic senescence in wild birds, within the context of both actuarial and reproductive senescence.

WHY STUDY AGING IN THE NATURAL ENVIRONMENT?

Aging is a defining theme of the 21st century for Western countries. In 2011, 14% of Canadians were over 65; by 2036 24% of Canadians are predicted to be over 65 (Statistics Canada 2011). These demographic challenges play a pivotal role in my society, affecting social security, and investment in health care, and are regarded as a driving force leading to the Great Recession in 2008-11. Despite the importance of aging, most of what we know about aging patterns comes from short-lived fruit flies, round worms and laboratory rats (Ricklefs 2008; Monaghan *et al.* 2008). There is an urgent need for the study of aging in long-lived animals across a wide taxonomic cross-section of species.

According to the August Krogh principle, for any question in physiology there is an ideal organism to study that question. Therefore, comparative physiology has proven instrumental in uncovering fundamental physiological principles that are then applied to medicine. There is no reason to believe that aging should be any different. Specifically, modern molecular gerontology focuses on the discovery of "immortality" genes (genes in which mutation induces a modification in longevity) and several research programmes work towards the possibility that genetic modification can enhance longevity in humans

(Finch 2012; Boehm *et al.* 2012). However, similar genetic modifications occur via natural selection, and yet most mortality in long-lived animals is associated with senescence, suggesting that natural selection has promoted lifespan extension via genetic modification in long-lived animals. Understanding why natural selection is unable to further extend lifespan may therefore aid in understanding the limitations implicit in genetic modification of lifespan in humans. Accordingly, a second goal of the current thesis is to increase the basic knowledge of the comparative physiology of aging, so that such knowledge may inform medical applications in future.

In particular, the thesis aims to fill the gap in the literature of studies that examine the proximate causes of senescence in a natural setting (Nussey *et al.* 2008). Specifically, I respond to the recent appeal that more studies examine multiple traits simultaneously when examining senescence in wild animals (Nussey *et al.* 2008).

EVOLUTIONARY ECOLOGY OF SENESCENCE: ULTIMATE EXPLANATIONS FOR AGING PATTERNS

Not all animals senesce or at least not all senesce at the same rate. While some worms complete their entire life cycle in less than a week, bristlecone pines can live thousands of years, and there are reports of a nearly 500-year old quahog clam and hydroid colonies that live indefinitely, genetically unchanged (Vaupel *et al.* 2004; Boehm *et al.* 2012). A cell culture created from a uterine cancer in 1951 (HeLa) is still in use today and likewise essentially immortal (Baudisch 2008). Why does such extensive variation exist? There is a rich history of different evolutionary explanations for these patterns (Table 1.1).

Although the majority of this thesis will focus on proximate mechanisms, such as physiological pathways, it is important to provide an evolutionary foundation for

examining those proximate mechanisms. The question "Why do we age?" has been discussed since Antiquity, and was clearly a question that perplexed Darwin himself; he felt that understanding the mechanism of inheritance was essential to understanding "those characters which are transmitted through the early years of life, but are expressed only at maturity or during old age" (Darwin 1871). The first modern theory of senescence, attributed to Wallace (Weismann 1889) argued that senescence occurred so that individuals could make resources available to their offspring. By dying, old birds reduced competition for space and resources with their offspring. Those arguments have been refuted as, regardless of the benefit to the offspring (which for many animals, such as a bird in a colony with millions of individuals, with be highly diluted), an individual that does not senesce and therefore continues to reproduce will usually have higher evolutionary fitness than one that does not senesce (Medawar 1952). Nonetheless, the discovery of "immortality" genes—genes that when expressed or present can dramatically alter lifespan—and increasing evidence that some animals show negligible or negative senescence (decreased mortality with age) has led to the revival of interest in programmed or adaptive theories of aging in recent years (Longo *et al.* 2005; Mitteldorf & Pepper 2009).

It was Medawar (1952) who first described the evolutionary paradox of senescence. All else being equal, within any group of animals, those individuals with genes that promote longevity would tend to live longer, and have more offspring in subsequent generations. The "longevity genes" would therefore become fixed in the population, and therefore, over time, a Darwinian demon--an animal that lives forever--should be

selected. Since no such demon exists, Medawar (1952) argued that there must be fundamental physiological or genetic constraints that limit lifespan.

The first step for resolving Medawar's paradox was John Haldane's reflections on the evolutionary implications of Huntington's disease, a rare disease caused by a dominant mutation that caused (at that time) irreversible brain disease and death, but that usually does not appear until an individual is at least 70 years old. Haldane reasoned that Huntington's disease does not occur at younger ages because such mutations would be quickly selected against. This was the first suggestion that the force of selection declines with age. Since that discovery, there have been three non-exclusive theories advanced to explain senescence from a non-adaptive perspective: mutation accumulation, antagonistic pleiotropy and disposable soma (Table 1.1). All three non-programmed theories rely heavily on the fact that Fisher's reproductive value (or residual reproductive fitness), and consequently the force of selection, declines with age (Medawar 1952; Williams 1957).

According to mutation accumulation theory, the weakening in the force of selection with age due to few individuals surviving to that age allows deleterious alleles to accumulate over generations. Thus, although selection will always favour an individual that lives longer, random mutations will counteract that trend. Because of the weakening force of selection with age, the random mutations tend to accumulate in old individuals. Most physiological or behavioural parameters relevant to aging are likely controlled by gene networks, and the same logic can be applied to complex gene networks. Medawar was clearly inspired by Haldane's work on Huntington's disease, and there is also now evidence in support of the mutation accumulation theory from laboratory experiments

showing that mutations in a single gene can confer large differences in longevity (Kenyon *et al.* 1993; Nemoto & Finkel 2004).

Antagonistic pleiotropy occurs when genes (or gene networks) with positive early effects but negative effects later in life are selected because they have a net positive contribution to fitness, in the light of the low probability of surviving to be affected by late effects (Williams 1957, 1966). For example, if energy resources are finite, then allocation of energy to current reproduction reduces energy allocation to survival (Kirkwood and Austad 2000). There is compelling evidence from both the laboratory and the wild that reproduction can be associated with mortality (the cost of reproduction). For instance, *inr* and *chico/irs* genes decrease fecundity but increase longevity in *Drosophila* while the *isp1* gene increases lifespan but limit juvenile growth in *C. elegans* (Johnson & Sinclair 1999; Guarente & Kenyon 2000). Similarly, reproduction at early ages in the wild can be associated with increased senescence later in life (Reznick & Endler 1982; Nussey *et al.* 2008).

The disposable soma theory is sometimes considered to be a sub-theory of antagonistic pleiotropy. The theory states that there is tradeoff in the allocation of energy between reproduction and soma. Hamilton (1966) suggested that senescence is the inevitable accumulation of reproductive events that each diminishes the residual reproductive value of an individual. Kirkwood & Holliday (1979) expanded on this idea by positing that due to the decreasing reproductive value with age, investment in reproduction over soma should increase with age. The disposable soma theory also overlaps with the mutation accumulation theory as it suggests that species with different rates of extrinsic mortality, as measured by lifespan, are expected to have different

optimal investment in somatic maintenance and repair, with the disposable soma essentially being shed once most genes have already made it to the next generation (Kirkwood & Rose 1991). There is now ample evidence in wild populations that reproduction increases senescence, and not just via pleiotropy, but due to the accumulation of wear-and-tear associated with each reproductive event (Reid *et al.* 2003; Reed *et al.* 2008).

Another theory, closely related to the disposable soma theory, is allocation theory (Stearns 1992). Like the disposable soma theory, it posits a tradeoff in the allocation of energy between reproduction and soma. However, rather than placing the emphasis on reductions in the force of selection with age (and on progressive deterioration of the soma), allocation theory considers aging to be largely adaptive, with different amounts of energy allocated to different components at different times in the organism's lifespan. The proponents of allocation theory cite the presence of "immortality genes", the ability of genes to control lifespan during caloric restriction and the wide variability in mortality-age trajectories among taxa, as support for this theory (Vaupel *et al.* 2004; Baudisch & Vaupel 2013).

One difficulty in testing the evolutionary explanations for aging is that all five theories of aging are not mutually exclusive. In that sense, all five theories are likely to be correct in at least some cases or supported by some data, but that does not mean that any of the other theories are incorrect. While I will continue to consider the ultimate explanations for aging, I focus for the remainder of the thesis on more proximate mechanisms. Understanding the proximate physiological mechanisms underlying aging provides insight into the evolutionary forces that underly observed patterns in aging.

Because most mechanisms that enhance survival, such as somatic maintenance, or reproduction require metabolic resources, and because these resources are finite, it is likely that tradeoffs in the allocation of energy drive the evolution of senescence. The hypothalamic-pituitary-adrenal axis is closely associated with activity and energy expenditure, and energy expenditure and immunity can represent tradeoffs associated with this axis; increased energy expenditure to augment current reproductive success can result in reduced immunity and decreased survival (Ricklefs & Wikelski 2002; Reed *et al.* 2008). Longer-lived species will presumably have multiple mechanisms to delay or reduce senescence, and this is likely reflected in their manifestation of these physiological tradeoffs (Kirkwood 2002).

AGING IN FLYING ANIMALS: ABSENCE OF REDUCTIONS IN CONDITION WITH AGE

Birds and bats have received special attention because they have a longer maximum lifespan and slower physiological aging than expected from their body size and metabolic rate (Nisbet 2001; Holmes & Ottinger 2003; Møller 2008). As noted by Yeats in his poem “The Wild Swans at Coole”, birds do not show the progressive deterioration with age that is so pronounced in mammals. Indeed, a number of flying "Methuselahs" have been recorded, such as a 41 year-old 7-g bat and a 91 year-old parrot (Munshi-South & Wilkinson 2009). Long-lived seabirds especially have low rates of extrinsic mortality (e.g. predation, disease, starvation, weather-related stress; Gaston 2004). Evolutionary theory predicts that species with low mortality rates should benefit from evolving mechanisms that prevent age-related cellular damage. At the same time, many of these species, especially penguins and Charadriiforms (gulls, terns and auks) have very high levels of

energy expenditure. For example, arctic terns (*Sterna paradisea*) fly each year between the Polar Regions while auks have among the highest mass-corrected metabolic rates of any homeotherm (Croll & McLaren 1993; Nisbet 2001; Gaston 2004). Thus, I should expect that these animals will have evolved mechanisms to reduce the impact of oxidative stress on the disposable soma.

Not only do birds live a long time despite the metabolically expensive requirements of flight, but many of the longest-lived seabirds live in the Polar Regions. In general, some of the longest-lived warm-blooded animals live in the Polar Regions. For example, the longest-lived wild birds (albatrosses, Ricklefs, 2008) and mammals (bowheads/*arviq*, George *et al.* 2011) live at high latitudes. Similarly, the longest-lived animal, the quahog clam *Arctica islandica*, is found in Arctic waters (Strahl *et al.* 2007). These animals, like the phoenix, keep the fire of life alive for decades or centuries, returning each spring from the ashes of an Arctic winter. How they do so is the focus of several sections of the current thesis.

THE EVIDENCE FOR AGING IN THE WILD

In most animals, reproductive success and survival increase at the start of life and decrease at the end of life (Ricklefs 2000, 2008). The ability to detect the latter defines measurable senescence for a particular species (e.g. Jones *et al.* 2008), and it is likely that claims that specific groups (e.g. deep water fish, sharks, turtles, seabirds) do not senesce (e.g. Caillet *et al.* 2001; Miller 2001) are either from studies that are too short in duration, have inadequate statistical power, or employ incorrect methodology (Calder 1984; Jones *et al.* 2008). Similarly, early (but persistent—e.g. Reeder & Kramer 2005) beliefs that senescence cannot be detected in the wild because few animals live to an age where they

experience senescence have been generally discredited (Angelier *et al.* 2007a,b; Hindle *et al.* 2009a,b). Senescence and its onset time are part of a suite of interconnected life history traits that occur upon a slow-fast continuum. Animals with slower life histories--smaller clutch size/litters, longer lifespan, later age at maturity--senesce later than animals with faster life histories, and there is no difference along this continuum between birds and mammals. Thus, slower rates of senescence in birds compared to mammals may represent reduced predation due to flight capabilities or less-leaky mitochondria, but that is only because those factors are the cause of an overall slower life history for birds compared to mammals (Jones *et al.* 2008).

Several factors confound the measurement of senescence. First, if only high quality individuals survive to later cohorts, a reduction in reproductive success or survival with age could be masked by the fact that only high quality individuals survive to show that trait. This has been partially overcome by following individuals throughout their lives, rather than using an actuarial or life-table approach (Reid *et al.* 2003; Jones *et al.* 2008). Second, if environmental conditions change over the course of the study, then this can mimic or mask senescence, especially for long-lived animals where the only members of the old age classes will necessarily be in the most recent years (Crespin *et al.* 2006; Lewis *et al.* 2006; Jones *et al.* 2008). The latter problem has usually been accounted for by introducing a year term in mixed models (Crespin *et al.* 2006), but this method usually assumes linearity when effects may be nonlinear and, where there is substantial unexplained variance associated with the year term some of the variation will likely be ascribed to senescence. Third, most individuals in wild populations die from other (extrinsic) causes before they reach the age where senescent changes become detectable,

so enormous sample sizes are needed. Other methodological problems are associated with life tables, especially cross-sectional studies, which make some strong assumptions about mortality rates. A recent study used individual-based data to calculate a novel statistic, the individual fitness contribution, which considers both reproduction and survival (Jones *et al.* 2008).

Animals typically die much sooner in the wild than in captivity. This no doubt represents reduced extrinsic mortality risk. However, longevity in the wild is also underestimated because marking methods reduce survival (i.e. flipper-banded penguins have lower survival than microchipped individuals; Stonehouse 1999). Most bird monitoring schemes require colour bands, and animals that are different in any way are often targeted by predators; I encountered this problem first-hand when working with a colour-marked black-legged kittiwake (*Rissa tridactyla*) population at Prince Leopold Island, where 40 birds out of 30 000 were marked. The following year, four out of a dozen dead falcon-killed kittiwakes were colour-banded. Similarly, known-age populations are likely intensively-studied in other aspects. Recording devices increase energy costs while reducing foraging success and survival (Paredes *et al.* 2005; Elliott *et al.* 2007, 2008) and blood-sampling can decrease survival by 33% (Brown & Brown 2009). Thus, the uncertainty principle of wildlife biology is clearly at work in studies of senescence in wild animals.

Both the mutation accumulation and antagonistic pleiotropy theories of aging assume that there is a genetic basis for aging. In invertebrates, variation at a single gene locus can increase lifespan (e.g. *daf*-2 in *C. elegans*; Walker & Lithgow 2003), but the mechanisms are complex; the same genetic mutation that extends lifespan in the laboratory may

reduce it in the wild. Moreover, there are numerous other proteins—apart from the proteins coded by the gene—that have different abundances in the mutants. Genetic control is likely even more complex in vertebrates; lifespan is not heritable within captive populations of many birds, suggesting that extrinsic factors are more important than intrinsic factors even in captive situations (Ricklefs & Cadena 2008).

A significant challenge in measuring senescence in wild populations is determining whether causes of mortality are intrinsic or extrinsic. Furthermore, the intrinsic and extrinsic factors can and will interact; reduced immunocompetence (intrinsic) may only cause mortality in the presence of an epidemic (extrinsic; e.g. Wilcoxen *et al.* 2010). More broadly, causes of mortality are seldom known for wild animals and intrinsic causes of mortality (cancer, heart failure, diabetes) are especially poorly documented (Ricklefs 2008; Holmes & Martin 2009). Indeed, several studies have demonstrated a link between aging and environmental conditions (Coulson *et al.* 2001; Bonenfant *et al.* 2008; Hamel *et al.* 2009). Thus, the relationships between reproductive success/survival and age will be strongest in years of poor environmental conditions, and the effect of environmental conditions on reproductive success and survival are primarily on marginal birds, such as very young or very old individuals. For example, young albatrosses have lower reproductive success than older albatrosses, but only in poor years (Nevoux *et al.* 2007). Thus, although birds, for example, may have low intrinsic mortality rates (slower senescence), the reason they have evolved these low intrinsic mortality rates is because they have low extrinsic mortality rates (Munshi-South & Wilkinson 2009).

THE PHYSIOLOGY OF AGING

The Fast and the Fur-less. The rate of living theory, first advocated at the start of the last century, stated that life expectancy was determined by the rate of energy metabolism (Rubner 1908; Pearl 1922). A simple encapsulation of this concept is the notion is that all animals have the same number of heartbeats in their lifetime; a mouse with a typically high heart rate and an elephant with a typically low heart rate have roughly the same number of heartbeats over a typical lifespan because the elephant lives, on average, much longer than the mouse. Thus, in the rate of living theory, all animals have a similar amount of total lifespan energy allocation, a so-called life history invariant, and they either expend small amounts over a long time or large amounts over a short time. Although more recent inter-specific comparisons across and within classes show strong relationships between lifespan and both body size and metabolic rate, they also reveal considerable variation in lifespan between animals with similar metabolic rates (Speakman *et al.* 2003; Speakman 2005). For example, birds and bats live much longer than typical mammals for their metabolic rate. While it is clear the rate of living is not the main variable governing lifespan, the actual mechanism involved is not well understood.

The free radical or oxidative stress theory provides a mechanism for the observed patterns described under the rate of living hypothesis (Harman 1956; Costantini 2008). Furthermore, although the rate of living hypothesis in its purest form—that cumulative energy expenditure of an organism determines lifespan—is largely discredited, the oxidative stress theory remains a major mechanism determining lifespan through mechanisms outlined in the next paragraphs. Reactive oxygen species are produced (especially) during mitochondrial oxidative phosphorylation and, because they are highly

reactive, cause damage to macromolecules (Halliwell & Gutteridge 1999). The damage can be cumulative and lead to the deterioration of cells and organs that is essentially the aging process. Thus, animals with high levels of energy metabolism (oxidative phosphorylation) often have higher rates of damage (senescence). The membrane pacemaker theory specifically posits lifespan is controlled by cell membrane composition; membrane fatty acid composition correlates with maximum lifespan in birds and mammals because long-lived animals have membranes with fatty acids less susceptible to oxidative stress (Hulbert 2006, Hulbert *et al.* 2007, 2008).

Oxidative stress can play a particularly important role in longevity via damage to telomeres (short, repeated sequences of DNA that cap eukaryotic chromosomes), causing telomeres to shorten due to the inability of DNA polymerase to completely replicate the DNA (Haussman *et al.* 2007). Once telomeres shorten to a critical length, cells enter a terminally non-divisive state, which is linked to organismal senescence (Wu 2003; Haussman *et al.* 2007); telomere shortening rates correlate with lifespan (Haussman *et al.* 2007).

Three major ecological stressors have been postulated to connect energy metabolism with damage (Costantini 2008). First, investment in reproduction leads to increased energy metabolism, potentially resulting in tradeoffs between early-life reproduction and lifespan (Rose & Bradley 1998; Zera & Harshman 2008; Reed *et al.* 2008). Second, immune responses can increase oxidative damage either through increased oxygen consumption or because heterophils, macrophages and lymphocytes produce prooxidants to kill pathogens (Sheldon & Verhulst 1996; Norris & Evans 2000). Third, prolonged exercise (e.g. migration) results in increased metabolic rate and potential damage

(Costantini *et al.* 2008). The oxidative stress theory is closely linked to total metabolic rate because it is assumed that the production of reactive oxygen species forms a fixed percentage of total oxygen consumption (Melov *et al.* 2001), although that assumption is often false as the proportion of electron flow directed towards radical production varies among tissue types (Barja *et al.* 1994; Herrero & Barja 1997; Gredilla *et al.* 2001; Barja 2004).

While the rate of living theory states that higher rates of metabolism should be negatively linked to lifespan, the uncoupling to survive theory states the reverse: that higher rates of metabolism should be positively linked to lifespan (Brand 2000). The uncoupling to survive theory notes that the production of reactive oxygen species at complex III in the electron transport chain depends on the time that a reduced ubisemiquinone Q exists adjacent to the positively charged side of the mitochondrial membrane, the location where it is capable of donating an electron to an oxygen atom. Since greater uncoupling and higher metabolic rates should lead to lower concentrations of reactive oxygen species and longer lifespans, this has been called the uncoupling to survive hypothesis. Both lead to greater oxygen consumption and predict a positive association between rates of energy metabolism and longevity. Although larger species have slower mass-specific metabolic rates (Rubner 1908), the relationship is confounded because both traits covary with body mass. The correlation between metabolic rate and longevity may be a spurious association that arises because of the effect on both of body size differences between different species. Furthermore, the relationships disappear or are somewhat weaker once corrected for phylogeny (i.e. each species is not an independent datapoint, as assumed by the regression of basal metabolic rate on lifespan; Promislow &

Harvey 1990; Speakman *et al.* 2002; Speakman 2005). When daily energy expenditure is used, smaller animals expend significantly more energy per lifetime than larger animals, but the latter relationships are biased by the larger sample sizes for smaller animals, meaning greater opportunity for measuring a greater lifespan.

Several intraspecific studies provide conflicting evidence. Restricting temperature, for ectotherms, or activity, for most animals, can lower metabolic rate and increase lifespan (Yan & Sohal 2000; Ragland & Sohal 1975) while increasing metabolic rate directly can shorten lifespan (Daan *et al.* 1996; Wolf & Schmidt-Hempel 1989). Meanwhile, intraspecific comparisons in mice and dogs show a positive relationship between mass-corrected metabolic rate and lifespan (Liang *et al.* 2003; Speakman *et al.* 2003). In particular, within a single strain of mouse, smaller mice have higher mass-corrected metabolic rates, live longer and have higher mitochondrial uncoupling (Speakman *et al.* 2004; Speakman 2005).

Energy metabolism does not only vary with lifespan across taxa or individuals within a taxon, but also possibly within the lifespan of an individual. Indeed, if the rate of living hypothesis holds, then one would expect individuals to adjust their metabolism strategically based on future prospects, either showing metabolic restraint near death to increase longevity or increased metabolism associated with terminal investment. In contrast, reduced metabolism with age could be due to physiological deterioration of mitochondria while increased metabolism with age could be due to increased energetic needs of repair (Moe *et al.* 2007, 2010; Shoyama *et al.* 2011).

Declines in basal metabolic rate (BMR) with age have been described for many mammals (Benedek *et al.* 1995; Ryan *et al.* 1996; Piers *et al.*1998; Greenberg 1999;

Hunter *et al.* 2001; Even *et al.* 2001; Miyasaka *et al.* 2003; Speakman *et al.* 2003) and a short-lived bird (Broggi *et al.* 2007), but BMR does not appear to vary with age in invertebrates (Promislow & Haselkorn 2002; Sukhotin *et al.* 2002; Chappell *et al.* 2003), mole-rats (O'Connor *et al.* 2002) or seabirds (Blackmer *et al.* 2005; Moe *et al.* 2007). The absence of a decline in BMR in long-lived rodents and birds has been used as evidence for the disposable soma theory, either because the somatic maintenance and repair mechanisms have prevented damage to macromolecules underlying the metabolic pathways, or because the absence of a decline in BMR reflects the energetic costs of having a high degree of somatic maintenance into old age (Moe *et al.* 2007). Despite the importance of metabolic rate adjustment in the aging process, change in metabolic rate, either through changes in basal metabolic rate, aerobic scope or sustained metabolic rate, with age has not been exhaustively studied in long-lived animals with exceptionally high metabolic rates, such as bats, seals and charadriiform seabirds. The latter group includes extreme breath-hold divers, which are expected to be especially prone to oxidative damage due to periods of hypoxia (Hindle *et al.* 2009b).

Immunocompetence is an energetically-expensive activity and increasing energy consumption during reproduction reduces immunocompetence (Deerenberg *et al.* 1997; Nordling *et al.* 1998). Under the rate of living hypothesis, one would predict that immunocompetence would decrease with age due to both a preference for early-life genes associated with reproduction/energy consumption over late-life genes associated with immunocompetence, and a generally reduced ability to create complicated cells late in life due to somatic deterioration. Indeed, immunocompetence does appear to decline with age in short-lived animals (Saino *et al.* 2003; Cichon *et al.* 2003), but not in long-lived

seabirds (Apanius & Nisbet 2006). In general, short-lived species invest less in immune defense and rely on less expensive, nonspecific immune functions rather than costly acquired defenses against particular pathogens.

Two studies of short-lived birds, a swallow and a flycatcher, have shown declines in antibody-mediated immune responses with age (Saino *et al.* 2003; Cichon *et al.* 2003) while one study on a short-lived swallow species and one study on a long-lived tern species found no correlation between these variables (Palacios *et al.* 2007; Apanius & Nisbet 2003, 2006). All studies of cell-mediated immunity reveal a decline with age, including three studies of short-lived finches and swallows, one study of medium-lived ruffs and one study of long-lived storm-petrels (Lozano & Lank 2003; Haussmann *et al.* 2005; Palacios *et al.* 2007). The rate of decline in cell-mediated response was proportionate to lifespan, averaging a 57% decrease over 80% of maximum lifespan (Haussman *et al.* 2005). Thus, long-lived birds (1 out of 1 study showed no variation with age) do appear to maintain specific defenses further into old age than short-lived birds (2 out of 3 studies showed declines with age) while nonspecific defenses appear to decline at a rate proportional to lifespan, but clearly more information is needed. Thus, while reduced immunocompetence (possibly alongside traits such as muscular senescence and telomere shortening) are potential byproducts of oxidative stress—in other words they *are* the disposable soma—more information is needed on whether immunity deteriorates with age in long-lived birds with high metabolic rates, or, if not, how they are prevented from deteriorating.

An endocrine basis for the end-of-time: the role of three endocrine axes in senescence. The endocrine system is particularly sensitive to aging. Corticosterone and prolactin, the

former of which may act directly to reduce the latter (i.e. Angelier *et al.* 2007a,b), are two hormones that are particularly associated with aging. Glucocorticoids, especially cortisol in mammals and corticosterone in birds, are associated with stress and the fight-or-flight response (Wingfield & Kitaysky 2002) while prolactin is associated with parental behaviour (Chastel *et al.* 2005). Corticosterone and prolactin responses to stressful conditions can reflect parental breeding investment, with higher baseline or stress-induced corticosterone often serving as the proximate mechanism behind young birds' "willingness" to sacrifice current reproduction for future survival in response to a stressor (Wingfield & Kitaysky 2002; Chastel *et al.* 2005). At the other end of the lifetime, senescence also heightens sensitivity of the hypothalamo-pituitary-adrenal axis to stressful events (Sapolsky *et al.* 1986; Van Cauter *et al.* 1996; Wilkinson *et al.* 1997; Boscaro *et al.* 1998; Bergendahl *et al.* 2000; Angelier *et al.* 2007a, b) by attenuating the glucocorticoid negative feedback (Wilkinson *et al.* 1997; Boscaro *et al.* 1998; Gust *et al.* 2000). Similarly, the cumulative cost of many previous breeding attempts can induce a reduction in prolactin levels and a lower ability to secrete or synthesize prolactin (Buntin 1996; Wang & Buntin 1999; Duckworth *et al.* 2003; Angelier *et al.* 2007a, b) or elevated levels of corticosterone itself could lead to reduced prolactin (Angelier *et al.* 2007a, b).

The hypothalamo-pituitary-adrenal axis has been suggested to relate directly to senescence through the concept of allostatic load (Wingfield 2003). Old rats with cognitive impairment showed an increased recovery time of the hypothalamo-pituitary-adrenal axis following a stressor and reduction in glucocorticoid receptor mRNA expression in the hippocampus (Bizon *et al.* 2001). Thus, it appears that repeated stress results in increased glucocorticoid levels that in turn cause hippocampal cell death and a

reduction in glucocorticoid receptors, leading to elevated glucocorticoid levels and cognitive impairment (glucocorticoid cascade hypothesis; Sapolsky *et al.* 1986). A related idea, allostatic load, refers to the cumulative cost to the body of allostasis, which is the ability of the body to maintain stability in response to an environmental perturbation or stress (Wingfield 2003). In response to stress, allostatic load may increase to the point where glucocorticoid levels surge and an emergency life-history stage is entered called allostatic overload. Type I allostatic overload occurs when energy demand exceeds supply, while Type 2 occurs from social dysfunction when energy consumption is sufficient (Wingfield 2003). High glucocorticoid levels can be pathological, and the cumulative effects of allostasis can lead to death, so the rate of senescence experienced by an organism is proportional to its lifetime allostatic load (Wingfield 2003). Thus, the hypothalamo-pituitary-adrenal axis through allostasis provides a mechanism for senescence whereby repeated environmental perturbations, potentially exacerbated during energetically-demanding periods such as migration or reproduction, cause somatic deterioration that accelerates mortality.

Other impacts of aging include the deregulation of the hypothalamo-pituitary-adrenal axis. Basal glucocorticoid levels may or may not increase with age (Dellu *et al.* 1996; Meaney *et al.* 1988; Sapolsky *et al.* 1983 Goncharova & Lapin 2002; Sonntag *et al.* 1987), however, the negative feedback system regulating the glucocorticoid response to stress is generally impaired in older animals such that the recovery time following a stressor, in terms of time to return to basal glucocorticoid levels, increases with age (e.g., Bizon *et al.* 2001; Dellu *et al.* 1996; Goncharova & Lapin 2002; Sapolsky *et al.* 1983). This impairment of the negative feedback system is associated with cognitive impairment

in mammals (Issa *et al.* 1990; Meaney *et al.* 1988). Nonetheless, there are few studies of free-living or middle-aged animals, and middle-aged animals may be more relevant in a free-living context because few animals live to old age (Reeder & Kramer 2005).

Like corticosterone, testosterone is a steroid hormone, but from the androgen group, and can be associated with senescence. It is the principal male sex hormone, and is associated with aggressiveness in some birds (Dufty 1989; Redpath *et al.* 2006). Testosterone is often negatively related with survival (Dufty 1989; Wingfield *et al.* 2001; but see Brown *et al.* 2005), and in some cases testosterone causes immunosuppression (Duffy *et al.* 2000). Thus, testosterone appears to increase investment in current reproduction over survival (Marler & Moore 1988). For example, red grouse with experimentally-elevated testosterone have lower survival rates but higher reproductive success (Redpath *et al.* 2006).

Like the hypothalamic-pituitary-adrenal axis, the hypothalamic-pituitary-thyroid axis works through a negative feedback loop. Depending on blood thyroid hormone levels, the hypothalamus releases thyroid-releasing hormone (TRH), which acts on the pituitary gland, stimulating release of thyroid-stimulating hormone, TSH. TSH in turn stimulates increased production of thyroxine (T_4), which is finally deiodinated to become triiodothyronine (T_3), a compound that has roughly four times greater affinity for the thyroid hormone receptor than T_4 (McNabb 1995). Thyroid hormones have different effects depending on the growth stage of the organism; in young animals thyroid hormones help regulate growth, such as the postnatal growth of the central nervous system (McNabb 1995). In older organisms, thyroid hormones are closely linked with cell metabolism, increased sodium-potassium ATPase production and macromolecular

synthesis and degradation; there is usually a strong correlation between thyroid hormone levels and basal metabolic rate (McNabb 1995).

The primary relationship between the hypothalamic-pituitary-thyroid axis and senescence is likely a reduction in basal metabolic rate with age, although TRH is also degraded more quickly in older animals. The reduction is at least partially due to a reduction in the sensitivity of cellular metabolism to T_4 with age (Zitnik & Roth 1981; Gambert 1982). T_4 can also lead to cardiac hypertrophy in aging mammals (Zitnik & Roth 1981), so the hormone may be related to age-related changes in cardiac dysfunction. Thyroid hormones are also involved in body changes resulting from fasting, as age-related responses to fasting depend on thyroid hormone levels (Gambert 1982). Other aspects, such as protein synthesis, are apparently less affected by senescence (i.e. Biggs & Booth 1990). Interestingly, T_4-binding globulin is one of the few compounds upregulated during senescence in rats, prior to terminal illness (Savu *et al.* 1991).

The endocrine system also plays a strong role in age-related declines in fertility, which is accompanied by a decrease in reproductive behaviour (Ottinger *et al.* 1983; Sherman & Morton 1988) and neuroendocrine alterations that affect hypothalamic function (reviewed in Ottinger 1992). Specifically, altered neuroendocrine and behavioural responses precede a measurable decline in reproductive performance, whereas loss of fertility and egg production are accompanied by falling plasma steroid concentrations (Ottinger 1992). Although fertility is difficult to measure in wild populations, where low hatching success may be due to a variety of factors (Briskie & Mackintosh 2004), endocrine measures may provide more practical indicators of change in reproductive ability. For example changes

in androgen, estradiol and luteinizing hormone were not observed during aging of wild, long-lived common terns (Nisbet *et al.* 1999).

BEHAVIOUR AND THE AGING ANIMAL

Once physiological deterioration reaches a critical level, an animal's behaviour will be impacted. Except for the cases where physiological deterioration occurs rapidly and fatally, such as the case of heart attacks in otherwise healthy organisms, senescence prior to death is likely associated with behavioural changes. Thus, behavioural senescence should be detected at a later age than physiological senescence, assuming that the change in behaviour results from the change in physiology. This may be why senescent declines in reproductive success have been widely described (reviews: Ricklefs 2008; Holmes & Martin 2009), but detection of the behaviours underlying these patterns remain elusive—assuming there is a behavioural component independent of a decline in fertility. Many studies on birds have implied that the decline in reproductive success is due to a reduction in nest attentiveness, nest defense capabilities, attractiveness such that old birds may only be able to get young, inexperienced partners, or foraging abilities (see next paragraph), but a connection between declining reproductive success in old age and behaviour has seldom, if ever, been demonstrated (Ottinger 1992; Holmes & Martin 2009). Alternatively, the declines may represent changes in territory quality, with older birds driven to more marginal sites or to become non-breeders (Crespin *et al.* 2006; Kim *et al.* 2007).

While age-related improvements in foraging behaviour at the start of life are well documented (Martin 1995), broad statements that senescent animals have lower foraging efficiency (i.e. Mangel 2005) are not. It is often assumed that reduced reproductive

success among older birds represents reduced foraging efficiency (e.g. Rockwell *et al.* 1993), but it has seldom been demonstrated. Nonetheless, models suggest a close link between foraging and evolution of survival rates in honeybees (Vischer & Dukas 1995; O'Donnell & Jeanne 1995). Deterioration in muscle capabilities in older animals (e.g. Hindle *et al.* 2009a,b) implies reduced foraging behaviour in old age. For instance, reductions in muscle mass, cross-sectional area and force generation per unit of cross-sectional area, as well as altered collagen isoform type and increased collagen content and cross-linking have been observed in many aging mammals (Kovanen & Suominen 1989; Brooks & Faulkner 1994; Gosselin *et al.* 1998; Thompson 1999; Hindle *et al.* 2009a,b). Nonetheless, it is possible that increased experience or higher activity-specific metabolic rates may counteract physiological deterioration, leading to no difference in activity budgets for older animals relative to younger ones. Indeed, the only study that documented old age-related reductions in foraging behaviour so far has been a study that showed old (35+ years old) male, but not female, grey-headed albatrosses (*Thalassarche chrysostoma*) make longer foraging trips and have lower daily mass gain, than experienced middle-aged individuals (Catry *et al.* 2006). Clearly, more information on the effect of old age on foraging behaviour is needed—are male albatrosses a statistical fluke?

Predator avoidance also an integral component of foraging behaviour, and would be expected to be under even stronger selection for improved performance, given that success is the difference between life and death. Furthermore, it may be more difficult to mitigate physiological deterioration via increased effort, as is possible in foraging behaviour, because predator avoidance depends critically on physiological condition,

such as burst speed It has long been shown that old prey are more likely to be selected by predators (Vucetich & Peterson 2009), which would provide an explanation for why survival declines in old age for those animals.

Improvements in young birds: increased foraging success, differential selection or increased investment in reproduction? Most studies of age-related processes in birds focus on the first few years of life, likely because it is easier to obtain large sample sizes in those periods. It is instructive to review knowledge about the processes at work at that stage. Many studies of both birds and mammals show that in comparison to older birds, first-time breeders are less likely to breed successfully (Forslund & Part 1995; de Forest & Gaston 1996; Newton 1998; Reid *et al.* 2003), are more likely to skip breeding the following year (Weimerskirch 1990; Viallefont *et al.* 1995), and more likely to die after first breeding (Clutton-Brock *et al.* 1996; Reid *et al.* 2003). Most studies that separate age from experience, as measured by the number of reproductive attempts, show that it is experience, rather than age, that is the pertinent variable for explaining variation in reproductive success (Crespin *et al.* 2006). Studies usually invoke one of three explanations for improvements in reproductive success/survival in the first few years of life: (1) improved foraging efficiency, as adult birds almost always have better foraging abilities than young birds, although it is unclear that there are any improvements after birds reach reproductive maturity; (2) differential selection allowing only successful individuals to survive into older cohorts; and, (3) increased investment in current reproduction by older individuals.

Aging interacts with environmental factors. For example, inexperienced black-browed albatrosses (*Thalassarche melanophrys*) have much lower survival than experienced

albatrosses, but only during challenging environmental conditions, which are abnormally warm years for this species; however, while inexperienced birds have lower reproductive success than experienced birds, the proportional reduction is similar across all environmental conditions (Nevoux *et al.* 2007). The authors argue that this suggests that improvements occur through a selection process whereby only good birds survive to old age, rather than through improvements in foraging abilities or increased investment in current reproduction (Nevoux *et al.* 2007).

INDIVIDUAL HETEROGEITY AND AGING: TWO SIDES OF THE SAME PROCESS

A small number of individuals are responsible for most of the genetic content of the subsequent generation across a wide variety of socially monogamous bird species where roughly 20% of individuals produce roughly 80% of a population's lifetime reproductive success; the distribution is even more skewed in non-monogamous species (Newton 1989). Thus, even though lifespan sometimes trades off with reproductive success (Ricklefs & Cadena 2007; Reed *et al.* 2008), the most productive animals also often live the longest (e.g. Newton 1989; Mauck *et al.* 2004) because they exhibit consistently high-quality behaviours or physiological condition. For example, phenotypic traits, such as body condition, stress response or behaviour, correlate with lifetime reproductive success as shown in the 2009 special issue of *Behaviour* (**142**(9-10)) dedicated to the subject and several other related studies (e.g. Milner *et al.* 1999; Kruuk *et al.* 2002; Blums *et al.* 2005; Reid *et al.* 2005; Lewis *et al.* 2006). These studies suggest that high quality individuals may sustain high energy expenditure or low stress for their entire lifespan because they have better genes (Hare & Murie 1992; Hamel *et al.* 2009).

Individual heterogeneity plays an important role in studies of aging. Ideally, studies of aging would occur longitudinally, so that individual heterogeneity could be accounted for through paired or nested statistical design. However, because some aspects require destructive sampling, few animals live to old age, and, thus, require enormous initial sample sizes. Because most studies are too short—a four-year Ph.D. is probably on the longer end of the distribution of study duration—virtually all studies of physiological or behavioural senescence in wild animals are cross-sectional in nature. Because studies are cross-sectional, it is important to understand the between-individual variation in the parameters studied. Age is often an important component in individual heterogeneity. In some cases, early-life reproduction apparently reduces survival (Reed *et al.* 2008) while in other cases early-life reproduction correlates positively with survival because high quality individuals reproduce often and survive long (Mauck *et al.* 2004). Few studies have been able to quantify behavioural or physiological changes over time within individuals and their consequent impact on variation among individuals, which is the raw material for natural selection (Williams 2008). There is, therefore, an ongoing need to examine individual variation, rather than just average values across individuals (Williams 2008).

STUDY SPECIES

Charadriiform seabirds. Perhaps because they are easily caught at the breeding colonies, and it is easy to obtain large sample sizes, seabirds have been the focus of many long-term banding studies (Gaston 2004). There are four seabird orders which represent two relatively distant clades in the avian tree of life (Kaiser 2007): clade 1 consists of Procellariforms (albatrosses and petrels), Sphenisciforms (penguins) and Pelecaniforms

(a poorly-defined group that may or may not be monophyletic, including pelicans, cormorants, boobies and frigatebirds) while clade 2 consists of the Charadriiforms (gulls, terns, auks and shorebirds—the last being primarily non-seabirds). Senescence patterns are probably different among each group, but seabirds share many similar life history characteristics (seabird syndrome; Gaston 2004) and therefore may share similar adaptations for longevity. Because some populations of seabirds do not show senescent patterns in telomeres, immunity or reproductive/survival parameters, seabirds have been suggested as model animals for the study of longevity (Nisbet 2001). Some seabirds do show reduced reproductive performance and/or survival among the oldest birds, including short-tailed shearwaters (Wooler et al, 1990; Bradley et al, 1995), wandering albatrosses (Weimerskirsch, 1992), southern fulmars (Jones *et al.* 2008) and little blue penguins (Dann & Cullen, 1990). However, northern fulmars and common terns show no decline in reproductive performance or survival even among birds that had bred for more than 30 years (Ollason & Dunnet, 1988 Jones *et al.* 2008).

Charadriiform seabirds are of special interest because mass-corrected field metabolic rates are up to an order of magnitude higher than for procellariform seabirds (Videler 2006). Furthermore, gulls (family Laridae) have the highest variation of any bird family in residual longevity after accounting for body mass (Munshi-South & Wilkinson 2009). Terns, black-legged kittiwakes and common murres have been studied in detail for senescence-related patterns (Nisbet 2001; Coulson & Fairweather 2001; Crespin *et al.* 2006; Reed *et al.* 2008). The kittiwake study showed that senescence was due to reduced reproductive success in the year before death (Coulson & Fairweather 2001). Although using birds banded as adults may still allow for detection of senescence, assuming that

age at first banding is constant with constant variance, these problems illustrate the difficulty of using such data for determining an age for onset of senescence and, likely, difficulties in determining what is an appropriate value for age at first banding. Also, the populations' reproductive success has collapsed near the end of the study, due to a decline in the sandlance fishery (Crespin *et al.* 2006). Crespin *et al.* (2006) used linear models to separate the effect of year from the effect of senescence, but it is possible that the effects are strongly non-linear and interactive. There are also a number of long-term colour-marked gull populations, but I know of no detailed investigation of their aging.

Passerine birds. It has proven much more difficult to measure senescence in passerines in the wild because the required sample sizes are enormous. Indeed, the only studies that have shown senescence are for barn swallows, collared flycatchers and purple martins (Holmes & Martin 2009; Stutchbury *et al.* 2009); I know of no studies on oscine passerines.

CHAPTER OUTLINE

The current thesis provides an overview of the patterns of aging in four species: thick-billed murres, black-legged kittiwakes and tree swallows, with particular emphasis on the two long-lived seabirds (murres and kittiwakes). The current chapter (Introduction) provides a background in terms of both the ultimate explanations and detailed proximate mechanisms from laboratory models that are beyond what can be accomplished in field measurements. In Chapter Two, I examine energy expenditure in murres, with particular emphasis on whether there is a tradeoff between energy expenditure during reproduction and survival, and whether energy expenditure changes with age. In Chapter Three, I document the use of thyroid hormones as indices of resting metabolic rate in wild birds,

which are then used in subsequent chapters. In Chapter Four, I look at patterns in aging relative to dive performance in murres. In Chapter Five, I look at patterns in a variety of baseline behavioural and physiological parameters relative to age in both murres and kittiwakes. In Chapter Six, I examine some of the same parameters relative to age in murres and kittiwakes, but in response to a stressor. Chapter Seven examines variation with age in some of the same behavioural and physiological parameters in short-lived tree swallows (*Tachycineta bicolor*). I then synthesize the results of these six data chapters in Chapter Eight.

Table 1.1. Evolutionary explanations for senescence.

Theory	**Explanation**	**Sources**
Programmed aging	Rates of senescence are genetically programmed and adaptive. Potential adaptive benefits include a shorter mean life span that causes a quicker generational turnover and therefore a quicker diffusion of favorable genes within a species creating a competitive advantage and increased genetic variability due to more individuals being present.	Weismann 1882; Longo *et al.* 2005; Mitteldorf & Pepper 2009
Mutation accumulation	Senescence is due to the effects of harmful mutations accumulating over evolutionary time, which are manifest at older ages when, in the wild, survivors are very few or absent (or after the age of final reproduction) and consequently selective forces are too weak to eliminate the mutations.	Haldane 1941; Medawar 1946, 1952
Antagonistic pleiotropy	Senescence is also due to the effects of harmful mutations accumulating over evolutionary time, but which have a net positive effect because of their effects early in life. Senescence therefore results from selection for mutations that are beneficial in youth but deleterious later in life.	Williams 1957
Disposable soma	Organisms only have a limited amount of energy that can be allocated to either reproductive or non-reproductive tissues (soma). Senescence is the result of natural degrading processes that result in accumulation of damage, and, the damage can be repaired only at the expense of reproductive effort.	Kirkwood & Holliday 1979; Kirkwood & Austad 2000
Allocation theory	Organisms only have a limited amount of energy that can be allocated to either reproductive or non-reproductive tissues (soma). Selection programs individual investment in reproduction *vs.* non-reproduction. The declining selection force with age is relatively unimportant compared to the advantages and disadvantages of reproducing at a particular age.	Stearns 1992; Vaupel *et al.* 2004; Baudisch & Vaupel 2013

Table 1.2. Examples of studies that examined reproductive (R) or actuarial (A) senescence in wild populations of birds or mammals using a cross-sectional (C) or longitudinal (L) design. Unless otherwise indicated, "A" refers to a decline in survival with age. The table is not exhaustive, but is meant to provide examples of those approaches used to date.

Species	Trait	Design	Reference
Red deer *Cervus elaphus*	R (fecundity)	L	Nussey *et al.* 2007
Fallow deer *Dama dama*	R (probability of a reproductive attempt per breeding season)	L	McElligott *et al.* 2002
Barn swallow *Hirundo rustica*	R (reproductive success, tail streamer length)	L	Møller & De Lope 1999
European roe deer *Capreolus capreolus*	R (young produced, males)	C	Gaillard *et al.* 2000a, 2003; Yoccoz *et al.* 2002
Moose *Alces alces*	R (litter size, offspring mortality; females)	C	Ericsson *et al.* 2001
Red deer *Cervus elaphus*	R (decrease as of age 9/10; steeper decline in males than in females)	L	Nussey *et al.* 2009b
Red deer *Cervus elaphus*	R (harem size, rut length decreases after age 9 or 10)	L	Nussey *et al.* 2009
Blue-footed booby *Sula nebouxii*	R (colour intensity of ornaments, secondary sexual characteristics, decreases with age)	C	Velando *et al.* 2009
Barn Swallow *Hirundo rustica*	R (external rectrice length, secondary sexual character)	C	Møller & De Lope 1999
Red deer *Cervus elaphus*	R (energy reserves during reproduction decreases with age in males)	C	Yoccoz *et al.* 2002
Polar bear *Ursus maritimus*	R (energy reserves during reproduction decreases with age in females)	C	Derocher & Stirling 1994
Boreal owl *Aegolius funereus*	R (clutch size declines in old males)	L	Laaksonen *et al.* 2002
Mute swan *Cygnus olor*	R (clutch size declines after 12 years)	L	McCleery *et al.* 2008
Wandering Albatross *Diomedea exulans*	R (modification of the egg morphology with the age of the female; quadratic curve)	C	Weimerskirch *et al.* 1992
Blue footed booby *Sula nebouxii*	R (decrease in egg size with age)	C	Beamonte Barrientos *et al.*

			2010
Reindeer *Rangifer tarandus*	R (birth weight of young decreases with female age)	L	Weladji *et al.* 2002, 2009
Grey seal *Halichoerus grypus*	R (birth weight of young decreases with parent`s age)	C	Bowen *et al.* 2006
Barn swallow *Hirundo rustica*	R (phenotypic quality of young decreases with parent age after accounting for other factors)	C	Saino *et al.* 2002
Horse *Equus caballus*	R (time spent protecting young decreases with age)	C	Cameron *et al.* 2000
Wandering albatross *Diomedea exulans*	R (more females produced by old parents; quadratic pattern)	C	Weimerskirch *et al.* 2005
Viviparous lizard *Lacerta vivipara*	R (offspring dispersal decreases with parents' age	L	Ronce *et al.* 1998
Mute swan *Cygnus olor*	R (lay date delayed after 12 years; accounted for individual heterogeneity)	L	McCleery *et al.* 2008
Snow petrel *Pagodroma nivea*	R (delayed in older individuals; quadratic pattern)	C	Goutte *et al.* 2010
Black-legged kittiwake *Rissa tridactyla*	R (hatching success)	L	Thomas & Coulson 1988
Glaucous-winged gull *Larus glaucescens*	R (hatching success, clutch size)	C	Reid 1988
Snow petrel *Pagodroma nivea*	R (Reduced reproductive attempts birds 34 years or older)	L	Berman *et al.* 2009
Common tern *Sterna hirundo*	R (decline in feeding rate among old males)	C	Galbraith *et al.* 1999
Western gull *Larus occidentalis*	R (hatching asynchrony, egg size)	L	Sydeman & Emslie 1992
Blue-footed booby *Sula nebouxii*	R (males, reproductive success)	C	Velando *et al.* 2006
Southern Fulmar *Fulmarus glacialis*	R (breeding probability and success; decline occurred after 21 years of age)	L	Berman *et al.* 2009
Common tern *Sterna hirundo*	R (Fewer young produced; controls for	L	Rebke *et al.* 2010

	individual heterogeneity)		
Grey-headed albatross *Thalassarche chrysostoma*	R (males; compared two age classes)	C	Catry *et al.* 2006
Mew gull *Larus canus*	R (reduced reproductive success only in the last year of life)	L	Rattiste 2004
Black-browed albatross *Thallasarche melanophris*	R (reproductive success)	C	Angelier *et al.* 2007
Short-tailed shearwater *Puffinus tenuirostris*	A + R (young fledged)	L	Bradley *et al.* 1989; Wooler *et al.* 1990
Little penguin *Eudyptila minor*	A + R (young fledged)	L	Dann & Cullen 1990
Wandering albatross *Diomedea exulans*	A + R (reduced fledging success and foraging success with age in males)	C	Weimerskrich 1992 Weimerskirch *et al.* 2005 Lecomte *et al.* 2010
Common guillemot *Uria aalge*	A + R (decline in annual survival late in life depends on investment at the start of life)	L	Reed *et al.* 2008; Crespin *et al.* 2006; Lewis *et al.* 2007
Common eider *Somateria mollissima*	A	L	Coulson 1984
Boreal chickadee *Parus montanus*	A, only in females	L	Orell & Belda 2002
Barn Swallow *Hirundo rustica*	A	L	Møller & De Lope 1999
Guppy *Poecilia reticulata*	A	L	Bryant & Reznick 2004
Viviparous lizard *Lacerta vivipara*	A	L	Ronce *et al.* 1998
Sheep *Ovis aries*	A	L	Catchpole *et al.* 2000
Red deer *Cervus elaphus*	A	L	Moyes *et al.* 2009
European roe deer *Capreolus capreolus*; Bighorn sheep *Ovis canadensis*; Pyrenean chamois *Rupicapra pyrenaica*	A (steeper decline in males)	L	Gaillard *et al.* 1993; 2004 Loison *et al.* 1999; See also Gaillard *et al.* 2000a, 2003 (review for ungulates)
Common eider *Somateria mollissima*	A	L	Coulson 1984
European shag *Phalacrocorax aristotelis*	A	L	Harris *et al.* 1994

California gull *Larus californicus*	A	L	Pugesek *et al.* 1995
Purple martin *Progne subis*	A (Reduced survival in birds 5+ years of age)	L	Stutchbury *et al.* 200
Buller's albatross *Thalassarche bulleri*	A (birds with 25+ y of reproduction have higher mortality than those with fewer than 25 years)	C	Sagar *et al.* 2000

Chapter Two. Age-related variation in energy expenditure in a long-lived bird within the envelope of an energy ceiling

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My contribution: I conducted all the field work (with field assistants, and in 2009 in collaboration with M. LeVaillant, and with logistical help from T. Gaston; except for the 1987 and 1988 data, which were obtained by D. Croll, and the 2004 data, which were obtained by T. Gaston) and laboratory work (with laboratory assistants, except for the doubly-labelled water analyses which were conducted in J. Speakman's laboratory), completed all statistical analyses and wrote the paper (with comments from all authors). Y. Ropert and A. Kato (along with other collaborators) helped me design the experiments and supplied loggers.

Summary

1. Energy expenditure in wild animals can be limited (i) intrinsically by physiological processes that constrain an animal's capacity to use energy, (ii) extrinsically by energy availability in the environment and/or (iii) strategically based on trade-offs between elevated metabolism and survival. Although these factors apply to all individuals within a population, some individuals expend more or less energy than other individuals.
2. To examine the role of an energy ceiling in a species with a high and individually repeatable metabolic rate, I compared energy expenditure of thick-billed murres (*Uria lomvia*) with and without handicaps during a period of peak energy demand (chick-rearing, N = 16). I also compared energy expenditure of unencumbered birds (N = 260) across 8 years exhibiting contrasting environmental conditions and correlated energy expenditure with fitness (reproductive success and survival).
3. Murres experienced an energy ceiling mediated through behavioural adjustments. Handicapped birds decreased time spent flying/diving and chick provisioning rates such that overall daily energy expenditure remained unchanged across the two treatments. The energy ceiling did not reflect energy availability or trade-offs with fitness, as energy expenditure was similar across contrasting foraging conditions and was not associated with reduced survival or increased reproductive success.
4. I found partial support for the trade-off hypothesis as older murres, for which prospects for future reproduction would be relatively limited, did overcome an energy ceiling to invest more in offspring following handicapping by reducing their own energy reserves. The ceiling therefore appeared to operate at the level of intake rather than expenditure.

5. A meta-analysis comparing responses of breeding animals to handicapping suggests that my results are typical: animals either reduced investment in themselves or in their offspring to remain below an energy ceiling. Across species, whether a handicapped individual invested in its own energy stores or its offspring's growth was not explained by life-history (future vs. current reproductive potential). Many breeding animals apparently experience an intrinsic energy ceiling, but behavioural adjustments were largely independent of life history.

Introduction

Even under conditions of unlimited energy availability, energy expenditure in wild animals is limited (Drent & Daan 1980; Peterson, Nagy & Diamond 1990; Hammond & Diamond 1997). Expenditure may be limited by "decisions" linked to individual fitness, such as tradeoffs between expenditure and survival or intrinsically by the animal's ability to process energy (Speakman *et al.* 2003; Green *et al.* 2009; Welcker *et al.* 2010). For instance, digestive efficiency may limit energy intake per unit time while thermal or metabolic constraints may limit energy expenditure per unit time (Hammond & Diamond 1997; Speakman & Król 2010; Heath *et al.* 2010). Consequently, internal constraints on energy processing may impose an "energy ceiling"—a maximum limit to energy expenditure averaged over a long enough period for expenditure to be balanced by intake. The existence of such a limit has been supported in some studies of wild animals (Drent & Dann 1980; Moreno *et al.* 1999; Tinbergen & Verhulst 2000; Table 1.1), and interspecific comparisons support the idea of an intrinsic constraint as no animal is known to exceed a long-term (long enough for intake to balance expenditure) energy ceiling of 7X basal metabolic rate (Ricklefs, Konarzewski & Daan 1996; Hammond & Diamond 1997; Speakman 2000).

Whether an individual operates near its energy ceiling will depend partly on the costs and benefits of energy expenditure. For example, animals may exist below their ceiling and increase energy expenditure when energy availability or demand increases (flexible investment strategy: Speakman *et al.* 2003; Jodice *et al.* 2006; Welcker *et al.* 2009; Harding *et al.* 2009). Alternatively, animals may exist at a ceiling and hold energy investment constant in spite of increased energy availability or demand, as occurs when

parents do not decrease investment in reproduction when energy costs are increased via a handicap experiment (fixed investment strategy: Tinbergen & Verhulst 2000; Paredes, Jones & Boness 2005; Leclaire *et al.* 2011). However, behavioural adjustments to handicapping may not reflect an underlying energy ceiling because: those behaviours may cause individuals to increase or decrease daily energy expenditure, changes in behaviours may reflect reduced foraging efficiency without any change in energy costs or changes in maintenance costs may counteract the effect of changes in activity budgets (Drent & Daan 1980; Green *et al.* 2009). Likewise, an energy ceiling may be present even if one group shows behavioural changes; hunter-gatherers have the same daily energy expenditure as Westerners despite having higher activity levels, presumably due to reductions in other energy costs among hunter-gatherers (Pontzer *et al.* 2012). In support of an energy ceiling, animals that are handicapped with increased locomotory costs exhibit no change in daily energy expenditure (Table 2.1).

Although average energy expenditure is often remarkably constant across environmental conditions, there can be considerable individual variation in energy expenditure (Speakman 2000, 2008; Welcker *et al.* 2010). If energy is limited intrinsically how can some individuals have higher energy expenditure than other individuals? Why aren't all individuals limited by the ceiling? One possibility is that the variation represents error associated with short-term measurements; energy budgets may be balanced over longer time scales. Although daily energy expenditure is limited at about 7X basal metabolic rate across endotherms (Peterson *et al.* 1990; Hammond & Diamond 1997), short-term activity costs can exceed 30X basal metabolic rate (Elliott *et al.* 2013b). Another possibility is that variation in internal constraints, such as thermal

properties or digestive abilities, may limit all individuals, but that there is nonetheless variation in the internal function that causes individual variation in energy expenditure. In that case, one would expect energy expenditure to be repeatable across time. Alternatively, some individuals may invest more heavily in offspring by feeding them more often, possibly at the cost of their future survival. In that case, energy expenditure may be repeatable but also positively correlated with feeding rate and negatively correlated with survival at the level of the individual.

Auks are good models for examining the role of energy ceilings because the high activity costs in chick-rearing auks impose high daily energy expenditures that are therefore likely to be at or near an energy ceiling (Roby & Ricklefs 1986; Elliott *et al.* 2013a,b). Indeed, the high cost of provisioning in some auks (e.g. murres, *Uria* spp.) is partially responsible for their semiprecocial fledging strategy, where the single offspring leaves the breeding site while still unable to fly and completes its growth at sea (Ydenberg 1989; Houston, Thompson & Gaston 1996). Chick-rearing birds in general appear unable to work harder to produce more offspring (Lack's principle; Drent & Daan 1980).

I measured daily energy expenditure and activity budgets of a diving auk, the thick-billed murre (*Uria lomvia*), to test for evidence of an energy ceiling and the expression of either a flexible or fixed investment strategy in the presence and absence of handicaps and across years of differing prey availability. I examined mechanisms underlying a potential energy ceiling, such as whether energy was adjusted by altering behaviour (rather than maintenance costs, for example, which account for ~30% of murre daily energy expenditure during chick-rearing; Elliott *et al.* 2013b) and whether energy was

limited at the level of energy intake (e.g. digestive bottleneck) or expenditure (e.g. thermal constraint). First, I tested whether (1) daily energy expenditure was a repeatable trait of the individual. Proving that daily energy expenditure was an inherent trait of the individual was essential prior to asking whether there could be fitness consequences to energy expenditure. Next, I tested the ideas that energy expenditure was limited by (2) energy availability (environmental conditions that varied between handicapping treatments and among years), (3) an intrinsic ceiling, or (4) survival costs such that birds with high expenditure experienced increased reproductive investment but also greater mortality.

Materials and methods

I used four separate methods: (A) I attached handicaps and examined energy expenditure, activity costs, survival and adult investment in energy for its own reserves (body mass change) and offspring (energy delivered to offspring) with and without handicaps; (B) I measured energy expenditure in years of contrasting food availability; (C) I correlated energy expenditure against fitness (survival and reproduction); and (D) I conducted a meta-analysis to place my results within the broader life history continuum (Table 1.2). I measured the energy expenditure of chick-rearing murres across eight years, incubating murres across four years and murre chicks in two years. Across all eight years, I recorded two accurate indicators of feeding conditions: 14-day old chick mass and change in adult energy reserves between incubation and chick-rearing (Gaston & Hipfner 2006).

FIELD METHODS

I analyzed measurements of the energy expenditure of murres at the Coats Island colony (62°35’N, 082°45’W) in Nunavut, Canada, using doubly-labelled water in 1987

(N = 4), 1988 (N = 5), 2006 (N = 23) and 2009 (N = 16) and using time budgets in 2004 (N = 24), 2005 (N = 27), 2006 (N = 98), 2007 (N = 37) and 2008 (N = 23). Chicks were 3-10 d old when adults were sampled, as provisioning rates were constant across that chick age range (Elliott *et al.* 2009). I also analyzed measurements of energy expenditure for chicks using doubly-labelled water in 1987 (N = 2) and 1988 (N = 2) and for incubating adults using doubly-labelled water in 1988 (N = 4), 2006 (N = 15) and 2009 (N = 4) and time budgets in 2005 (N = 15) and 2006 (N = 16). In addition, I recorded chick body mass at three-day intervals across all four years and report mass for 14-day old chicks (Gaston & Hipfner 2006). Loss of adult body mass between incubation and chick-rearing is also a good indicator of feeding conditions (Gaston & Hipfner 2006), and I recorded adult mass loss for all eight years.

DOUBLY-LABELLED WATER

Murres were injected intramuscularly (1987-1988) or in the brood patch (2006, 2009) with doubly-labelled water (2006, 2009: 0.5 or 1.0 mL of 50% H_2O^{18} and 25% D_2O; 1987-1988: 1.91 mL/kg of 15% H_2O^{18} and 0.16 mL/kg of 99% D_2O). The optimal method for measuring equilibrium isotopic values (total body water) in murres is the plateau method at 90 min using the O^{18} equilibrium value (Jacobs *et al.* 2012). In 2006 and 2009, I obtained 100 µL equilibrium blood samples from the tarsal vein 90 min after injection. I released the birds after injection and was able to recapture all but three birds for the equilibrium blood sample. In those three cases, I estimated the isotopic concentrations from the equation between isotopic concentration and body mass (R^2>0.92). Holding a bird for an hour causes the bird to rest/preen on the water for several hours, reducing daily energy expenditure (Schultner *et al.* 2010), and I avoided

that issue by releasing the bird during equilibration; the birds immediately returned to their breeding site and incubated/brooded during the 90 min equilibration period. During the 1987-88 experiments, birds were held for 120 min while the partner was with the chick and obtained equilibrium blood samples at that time. Birds were recaptured a second blood sample from the brachial vein 24-48 h later and, for some birds, also 72 h later.

Samples were timed as close to multiples of 24 h as possible to avoid circadian effects (Speakman & Racey, 1988), and final samples averaged 23 ± 27 min longer than the ideal 24 h multiple. Based on the composition of prey items delivered to chicks, I used a respiratory quotient of 0.81 to estimate daily energy expenditure. As longer sampling periods reduce the error due to day-to-day variance in daily energy expenditure (Speakman & Racey, 1984; Berteaux *et al.* 1986), I calculated daily energy expenditure over the extended time period for individuals that were recaptured more than once for "final" blood samples. Body mass in chick-rearing murres is relatively constant (range = ±8% of average body mass across my study) and any variation occurs largely via metabolically inert lipids (Elliott *et al.* 2008; Jacobs *et al.* 2011). Nonetheless, I included body mass as a covariate.

Capillary tubes that contained the blood samples were distilled (Nagy 1983) to produce CO_2 and H_2 (Speakman *et al.* 1990 for CO_2; Speakman & Krol 2005). The isotope ratios ^{18}O:^{16}O and ^{2}H:^{1}H were analysed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and IsochromμG, Manchester, UK). Samples were run alongside three lab standards for each isotope (calibrated to International standards) to correct delta values to ppm. All samples were run blind to the identity of the bird and

converted to values of daily energy expenditure using a single pool model (Speakman 1993). I assumed a fixed 25% evaporative water flux (equation 7.17: Speakman 1997) which minimises error over a range of conditions (Visser & Schekkerman, 1999; van Trigt *et al.* 2002). Estimates for energy expenditure based on doubly-labelled water in auks are accurate within 2-18% relative to respirometry values from the same individual and within 3% relative to the average respirometry value across the group (Shirai *et al.* 2012). Using different equations for calculating daily energy expenditure results in different absolute values for energy expenditure (Shaffer 2011), but within my dataset those differences had no effect on the hypotheses I tested because values from different equations were highly correlated ($R^2 > 0.95$ for all correlations) and I only examined relative differences.

TIME BUDGET ANALYSES

For those years when I did not measure daily energy expenditure using doubly-labelled water, I estimated daily energy expenditure from time budgets. Specifically, I completed a multiple regression of daily energy expenditure against time spent flying, diving, resting on the water and resting on land. The slope of energy expenditure against time in each activity provided activity-specific metabolic rates. I used activity-specific metabolic rates derived from the same data set that explained 72% of the variation in daily energy expenditure (I used those values reported in Table 1 of Elliott *et al.* 2013).

HANDICAPPING

In 2006 and 2009, I equipped all individuals (including handicapped birds) with time-depth-temperature recorders (TDRs, 3-5 g, Lotek Wireless, Canada) that allowed me to determine time spent flying, diving, resting on water and resting on land (Elliott *et al.*

2009). The small TDRs were attached to the leg and had no measurable impact on behaviour (Elliott, Davoren & Gaston 2007; see also Ropert-Coudert *et al.* 2009). In 1999, 2009 and 2010 handicaps were attached using Tesa tape (1999: activity logger 28 g or 2.8% of murre body mass, 5.6 cm^2 or 6.2% of murre cross-sectional area; 2009 and 2010: accelerometer M190-D2GT, Little Leonardo, Japan, 17 g or 1.7% of body mass, 1.8 cm^2 or 2.0% of cross-sectional area) to the lower back of murres. In 2009, I measured energy expenditure over 48 h using doubly-labelled water for 16 individuals with and without handicaps. I sampled the same individuals twice (24 and 48 h); I measured energy expenditure during 24 h with the handicap and 24 h without the handicap for each individual. As order of handicapping (first or second 24 hour period) had no effect on the magnitude of the effect of the handicaps on time budgets or behaviour (Elliott *et al.* 2007) and energy expenditure during the 24 hours following injection was similar to that during the following 24 hours for the same unhandicapped individual (2041 ± 584 kJ/d vs. 2073 ± 601 kJ/d), I concluded that attachment order had no effect.

SURVIVAL AND REPRODUCTIVE FITNESS: ADULT SURVIVAL, MASS LOSS AND ENERGY DELIVERED TO OFFSPRING

My project is part of a long-term study (1981-2011) and all birds were marked within my project with a unique metal leg band. I only examined birds equipped at my main study site where the band numbers of all breeding individuals were read during the years of study. I supplemented survival estimates for handicapped birds in 2009 with measurements from identical protocols in 2010 (N = 24) and from activity loggers attached in 1999. I also examined survival in relation to non-handicapped energy expenditure in 2004-2009 (I have no information from the 1980s, when experiments

occurred away from the main study area). I examined survival in the following year (i.e. 2000 for birds handicapped in 1999). As part of the ongoing monitoring program, I also resighted >100 unequipped birds in each year. I resighted upwards of 80% of unequipped birds, except in 2011 when polar bear (*Ursus maritimus*) disturbance reduced resighting probabilities (Gaston & Elliott 2012), suggesting that my measurements of apparent survival were robust.

I used energy delivered to the offspring as a proxy for reproductive fitness, as energy delivered to the offspring is closely linked to offspring energy reserves at fledging and post-fledging reproductive success (see model in Elliott, Crump & Gaston 2010). Virtually all offspring survive to fledging so fledging success is not a useful parameter for gauging reproductive fitness at my study site. I recorded the type and length of each food item delivered to offspring by parental murres in three 24-h feeding watches per year. Using mass-length relationships and energy densities derived from prey collected at my study site, I converted feeding watch data into energy delivered per day (Elliott *et al.* 2008; Elliott & Gaston 2008). I measured body mass before and after handicapping. I compared the energy delivered per hour and mass loss by handicapped individuals to that of individuals without handicaps.

ROLE OF A DIGESTIVE BOTTLENECK

I examined the possibility that digestive constraints create an energy ceiling because murres are unable to assimilate energy faster than a particular rate set by the digestive properties of the gut. Such ideas have received much attention over the past three decades (Levey & Grajal 1991; Temeles 1989; Dykstra & Karasov 1993; Kersten & Visser 1996; McWhorter & del Rio 2000; Hansen 2003). For instance, because in some cases it takes

longer to assimilate energy than to consume it, the time budgets of several animals involves short bouts of foraging followed by necessary, longer bouts of digestion (Diamond *et al.* 1986; Guillemette 1998; Heath *et al.* 2010). Specifically, diving animals may follow dive (foraging) bouts with lengthy intra-bout periods at the surface directed towards digestion (Guillemette 1998; Heath *et al.* 2010). The core body temperature declines during diving for many diving birds, including murres (Niizuma *et al.* 2008). Due to cold body temperatures, the efficiency of digestive enzymes and muscle action may be low during diving. Thus, digestion may occur most easily by partitioning time budgets into dive bouts and surface bouts. Also, the heat increment of feeding may aid thermoregulation when sitting at the surface, although that may be equally true of diving to depth (Lovvorn 2007; Kaseloo & Lovvorn 2008; Enstipp *et al.* 2008).

I assumed that murres had roughly 75% digestive efficiency when foraging on fish (long-term digestive efficiency for either sprat or whiting was close to 75% in Hilton *et al.* 2000). Thus, to expend 1327 kJ/d when spending 8067 s submerged during incubation (Elliott *et al.* 2008) would mean that, on average, murres obtain 0.219 kJ per second submerged—or a 10-g fish (4.5 kJ/g; Elliott & Gaston 2008) every ~205 s. To then achieve 2036 kJ/d during chick-rearing (2715 kJ intake assuming 75% assimilation efficiency) and capture an additional 140 kJ/d for the chick (average energy delivery rate to the chick; Elliott *et al.* 2010) would require 13015 s submerged (at 0.219 kJ gained per second submerged). In contrast, average time submerged per day during chick-rearing is 13340 ± 1549 s (Elliott *et al.* 2008). Thus, my calculations appear robust.

The time spent submerged per dive bout (for dive bouts >1 dive; single dive bouts were assumed to be associated with searching) was 976 s, allowing for consumption of

214 kJ of energy (~47 g of fish). Assuming a stomach evacuation rate of 5 kJ/min (for stomach contents of 47 g), and that the last 214 kJ are assimilated at the colony, then it would take 8.3 h to assimilate all of the fish (Hilton *et al.* 2000; Hansen 2003). In contrast, murres in my study averaged 7.0 ± 1.2 h at the surface. Presumably, some assimilation also occurs during diving, albeit at a slower rate than at the surface due to reduced digestive efficiency and gut vasoconstriction at colder temperatures.

My calculations suggest that murres have very little spare time available to digest food; virtually all surface time is spent digesting food obtained during diving. Thus, digestive constraints are one potential mechanism underlying a potential energy ceiling.

META-ANALYSIS AND STATISTICAL ANALYSES

To provide a taxonomic context for my results, I searched the literature for studies that added loads, clipped wings or attached transmitters and reported both changes in chick-provisioning rates (or chick growth rates), and adult body mass or daily energy expenditure relative to controls. Because there were relatively few studies that reported daily energy expenditure, I simply collated and compared the effect size (the difference in average values divided by root mean square of their standard deviations) of energy expenditure among the different studies. For the larger sample of studies that reported adult body mass and chick provisioning/growth rates in birds, I report values as residuals after accounting for degree of handicapping (percent wing area reduction for wing clipping, percent mass increase for added load and percent cross-sectional area increase for back-mounted devices). By examining the residuals, I examined the component of variation in adult body mass or chick growth rates that was independent of the degree of handicapping (presumably, a large handicap would have a greater impact than a small

impact, regardless of other factors). The residuals also accounted for the differences among handicaps; for instance, if wing clipping had a stronger impact on a particular parameter than adding weights, then the slope of the regression between the parameter and the degree of handicapping for wing-clipping would be greater than the slope for adding weights. Of course, there are differences in handicap shape and species locomotory mode, among other possibilities, that would likely play a role in explaining even more of the variance.

Brood value for interspecific studies was calculated following Bókony *et al.* (2009) as:

Log [(Clutch size*Adult mortality rate)/(Clutch size*Broods per year)]

I used PHYLIP (http://evolution.genetics.washington.edu/phylip.html) and the Tree of Life (http://www.tolweb.org) to calculate phylogenetically-independent contrasts. To compare my own individual murre values, I calculated brood value for individual murres as the most-probable number of subsequent clutches an individual is likely to produce for its age based on age-specific survival at my colony (AJG, unpubl. data).

I used R 2.10.1 for all statistical analyses, with an α=0.05 and report all values as means ± SD. I used a general linear model including all interactions to describe daily energy expenditure during chick-rearing with sex, ambient temperature, body mass, body mass change, time to recapture and chick age as independent variables. I also show, on Fig. 1, the results of a one-way ANOVA during chick-rearing and incubation. Results were similar for both all daily energy expenditure and the results of only doubly-labelled water measurements (one-way ANOVA, only doubly-labelled water measurements: $F_{4,65}$ = 0.5, P = 0.74, all daily energy expenditure measurements: $F_{7,243}$ = 1.25, P = 0.28).

Research was approved by the Protocol Management and Review Committee of the University of Manitoba and National Wildlife Research Centre.

Results

INTER-YEAR VARIATION

For those individuals where daily energy expenditure was measured in multiple years, energy expenditure in one year ("years") was strongly linked with energy expenditure in another year (year 1 = 2060 ± 335 kJ/d; year 2 = 2073 ± 411 kJ/d; Fig. 2.1b). Likewise, energy expenditure measured via doubly-labelled water over 48 hr ("days") was correlated with energy expenditure in the subsequent 48 hr (period 1 = 2048 ± 609 kJ/d; period 2 = 2080 ± 592 kJ/d; Fig. 2.1b). Across all 8 years, energy expenditure did not vary with sex, ambient temperature, body mass, body mass change, time to recapture, or chick age (all $p > 0.2$). In particular, energy expenditure did not vary among years (Fig. 2.1c). A power analysis demonstrated that I had an 87% probability of detecting a significant difference at $\alpha = 0.05$, given my sample size and an effect size of 10%. Adult body mass ($F_{7, 122} = 3.81$, $p < 0.001$), 14-day chick mass ($F_{7,275} = 8.37$, $p < 0.001$) and incubation daily energy expenditure (average = 1327 ± 400 kJ/d; Fig. 2.1c) also varied among years. The effect sizes were 68% for adult body mass, 72% for chick growth rates and 52% for incubation energy expenditure. Chick energy expenditure was 289 ± 129 kJ/d pooled across both years.

HANDICAPPING

Overall energy expenditure did not differ significantly between handicapped and non-handicapped birds (pairwise difference = 172 kJ/d ± 572 kJ/d; pairwise $t_{15} = 1.20$, $p = 0.25$, Table 2.2). Birds equipped with accelerometers reduced time spent flying

(handicapped: 11.6 ± 6.2% of time away from the colony; non-handicapped: 18.5 ± 7.5% of time at-sea; pairwise t_{15} = -3.43, p = 0.004), time spent diving (handicapped: 26.2 ± 8.1% of time at-sea; non-handicapped: 30.0 ± 6.0% of time at-sea; pairwise t_{15} = -2.89, p = 0.01) and provisioning rates (pooled 2009 and 2010: pairwise t_{35} = 2.12, p = 0.04) while increasing time resting on the surface (handicapped: 62.3 ± 11.3% of time at-sea; non-handicapped: 51.5 ± 9.2% of time at-sea; pairwise t_{15} = 3.42, p = 0.004). Body mass loss did not differ between groups (pooled 2009-10: pairwise t_{35} = 1.17, p = 0.25). In 2009, daily energy expenditure of handicapped birds increased with age and became more variable (Fig. 1a), but was independent of age in non-handicapped birds (t_{15} = 1.05, p = 0.31, R^2 = 0.07). Likewise, investment in the chick (provisioning rate) increased with age of the adult (brood value; Fig. 2.1), but mass loss declined with murre age (brood value; t_{15} = -2.07, p = 0.04). Whereas, murres averaged 7.0 ± 1.2 h per day at the water surface, I estimated that 8.3 h per day were required to assimilate all energy obtained.

FITNESS CONSEQUENCES

Birds not resighted the following season had lower daily energy expenditure than those resighted in all six years where I resighted individuals in the subsequent year, and that relationship was significant for the pooled data set (resighted: 2151 ± 1109 kJ/d; not resighted: 1777 ± 624 kJ/d; t_{228} = 3.91, P <0.001). Handicapped birds were as likely to be resighted as controls in 1999-2000 (14/15 or 93% handicapped vs. 54/63 or 86% controls), 2009-2010 (15/19 or 79% handicapped vs. 49/56 or 88% controls), and 2010-2011 (12/21 or 57% handicapped vs 29/47 or 62% controls). Energy delivered to the offspring (kJ per day) was not correlated with daily energy expenditure for those birds I observed simultaneously (t_{101} = 1.55, P = 0.12, R^2=0.04).

META-ANALYSIS

Across 25 handicapping studies, investment in self (body mass) decreased with increasing investment in the chick (provisioning rate or chick growth rate, Fig. 2.2a; phylogenetic contrasts: $t_{25} = 3.45$, $p = 0.002$). Chick growth/provisioning rate tended to increase with brood value (Fig. 2.2b), whereas investment in adult's energy stores was independent of brood value across species ($t_{25} = -0.17$, $p = 0.87$; phylogenetic contrasts: $t_{25} = -0.12$, $p = 0.91$).

Discussion

Chick-rearing murres—animals with exceptionally high activity costs—experienced an energy ceiling regardless of energy availability. The ceiling was mediated by behavioural adjustment; when handicapped, flight/dive costs increased substantially but daily energy expenditure remained markedly constant because the animals reduced time spent flying/diving to maintain the same level of energy expenditure. As older birds were able to overcome the energy ceiling over short time-scales by using up their energy reserves, I suggest that the ceiling occurs at the level of intake rather than expenditure. A meta-analysis of data from 25 studies examining the response of breeding animals to handicapping showed that although there was strong evidence of an energy ceiling—a reduction in either self-investment or investment in reproduction—the ceiling was largely independent of the slow-fast life history continuum. Thus, although many breeding animals are apparently limited by an intrinsic energy ceiling, and adjust the time spent in behaviours directed towards self-feeding or offspring nourishment to remain within that ceiling, life history did not appear to play a role in explaining differences in the behaviours that were adjusted.

DAILY ENERGY EXPENDITURE WAS INDIVIDUALLY REPEATABLE (HYPOTHESIS ONE)

Repeatability in daily energy expenditure was higher over short (days) than long time scales (years), which is not surprising given the high individual repeatability in foraging location, diet, flight times and dive depths at my location, with no effect on fitness (Woo *et al.* 2008; Elliott, Woo & Gaston 2010). An individual's physiology may be geared towards a particular level of energy expenditure. When pushed beyond that level there can be consequences for survival and social relationships (e.g. break down of pair bonds), as shown by reduced survival associated with long-term handicapping (Wolf & Schmid-Hempel 1989; Daan, Deerenberg & Djikstra 1996; Paredes *et al.* 2005). My handicapping study was too short to observe such effects. Individual murre's physiology may be geared towards a particular level of energy expenditure as murres adjust the size of energy-related organs (heart and muscle) separately from overall body mass (Jacobs *et al.* 2011). Tradeoffs between body mass (flight costs), fasting endurance (brooding shifts), thermoregulation (insulation), oxidative stress and digestion mediated via body composition may all influence murres' ability to use energy (Drent & Daan 1980; Hammond & Diamond 1997; Speakman & Król 2010).

THE PRESENCE OF AN ENERGY CEILING REGARDLESS OF ENERGY AVAILABILITY (HYPOTHESES TWO AND THREE)

Across treatments and environmental conditions, average energy expenditure during chick-rearing was remarkably constant despite ample statistical power to detect a difference (87% chance of detecting a 10% difference, whereas incubation energy expenditure, chick body mass and adult body mass all showed >50% difference, Fig. 1c).

Chick-rearing murres appeared to operate near a fixed energy ceiling (fixed investment strategy; Kitaysky *et al.* 2000; Tinbergen & Verhulst 2000; Green *et al.* 2009). Indeed, it is remarkable how precisely murres regulated energy expenditure to a given level; handicapped birds decreased time spent flying on average by 40%, which was the amount needed to maintain no net change in estimated energy expenditure. Had flight time been reduced by 30% or 50%, at the same activity-specific costs, I would have been able to detect a statistically significant difference. In contrast, chick growth rates, incubation daily energy expenditure, ambient temperature and adult mass all varied substantially among years, implying that conditions were quite variable. Similarly, in years when adult mass was low at my study site, chick growth rates were also low—again suggesting an energy ceiling that prevented adults from expending more energy to maintain chick growth rates (Gaston & Hipfner 2006). My results are typical (Table 1.2) and many animals may have used similar behavioural mechanisms—alteration of activity budgets—for remaining within their energy ceiling (cf. Ropert-Coudert *et al.* 2007).

Animals are unable to maintain high levels of energy expenditure during poor-food years (Fig. 3). At high levels of energy availability relative to need (incubating birds, supplementally-fed animals, animals with experimentally-reduced broods), animals can down-regulate energy expenditure (Fig. 2.3). At my study site, chick-rearing murres (Table 2.1) apparently exist within some anticipated intermediate level of energy availability relative to need, such that energy expenditure cannot be upregulated (Figs. 2.1c, 2.3). For many breeding animals, energy expenditure can be down-regulated based on energy availability, but cannot be up-regulated for animals working near their energy limit (Fig. 2.3, Table 2.3). For instance, energy availability can be variable at levels

below an intrinsic ceiling where resources are highly variable, leading to the potential for a mis-match between need (brood size) and energy availability (Thomas *et al.* 2000; Kitaysky *et al.* 2000; Tinbergen & Dietz 1994). Pacific kittiwakes (*Rissa tridactyla*) have a small brood size, presumably optimized for low energy availability, and when energy availability increases, because brood size is established a month previous (via clutch size), they have no need to up-regulate energy expenditure as demand is still low (Kitaysky *et al.* 2000). Energy availability may in some conditions provide a boundary for energy expenditure, but animals normally operate at a lower level defined by intrinsic factors linked to their physiology (Fig. 2.3). Nonetheless, energy ceilings vary among individuals (Fig. 2.1b,c) and over evolutionary time-scales (among species or between colonies); energy expenditure often differs among colonies with differing food availability because animals can predict such differences and are adapted to their local level of energy availability (Kitaysky *et al.* 2000; Ballance *et al.* 2009). For instance, individuals can adjust basal metabolism to reduce energy expenditure during periods of high energy demand (Bech *et al.* 2002), yet individuals are unable to make adjustments over shorter time-scales (Table 2.2). I suggest that physiological limitations best explain these observations: while animals can likely adapt individually-variable physiological, morphological or thermal characteristics to correspond with energy availability over evolutionary time-scales, they are unable to do so over short time-scales.

MECHANISMS UNDERLYING AN ENERGY CEILING

Many animals appear to be at an energy ceiling, unable to increase energy expenditure even at the cost of their offspring or their own condition. That ceiling appears to be associated with a physiological constraint rather than energy availability or fitness trade-

offs. One potential physiological constraint is time required to digest food (Kleiber 1975; Kenward & Sibly 1977; Hammond & Diamond 1997). Like many diving birds, murres interrupt foraging (diving) bouts to spend hours resting on the surface between diving bouts. In seabirds feeding on difficult-to-digest prey, much of that surface time is spent digesting prey and digestion rates determine time allocated to dive bouts relative to resting (Guillemette 1998; Heath *et al.* 2010). Digestive constraints also appear to play a role in murres as (1) older individuals used their own reserves to expend more energy even when handicapped, suggesting the constraint occurs at the level of energy intake rather than expenditure, and (2) virtually all surface resting time in murres is required to digest food leaving no additional time available to digest any additional food obtained (Hansen 2003). Digestive constraints, however, do not account for all physiological constraints on energy expenditure. As murres spent less time foraging for their offspring, handicapped birds spent more time resting on the surface than non-handicapped birds despite expending the same amount of energy. If energy expenditure in handicapped birds were limited by time available for digestion, then presumably they could use some of the excess time obtaining and digesting more energy instead of simply resting. I believe that various metabolic constraints evolve in tandem to the same level of energy expenditure. The removal of one constraint does not alter energy expenditure as expenditure is still limited by other constraints, such as thermal constraints, muscle properties and oxidative stress (e.g. Speakman & Król 2010; Beaulieu *et al.* 2011; Selman *et al.* 2012).

NO FITNESS TRADE-OFF BETWEEN ENERGY EXPENDITURE AND SURVIVAL, BUT INCREASED INVESTMENT BY INDIVIDUALS WITH HIGHER BROOD VALUE (HYPOTHESIS FOUR)

High energy expenditure during peak demand (chick-rearing) did not reduce apparent survival (see also Jackson, Trayhurn & Speakman 2001; Welcker *et al.* 2009, 2010). Rather, birds with high energy expenditure had *higher* apparent survival, which I interpret as meaning that birds that were near death were incapable of high levels of energy expenditure (cf. Manini *et al.* 2006). In contrast, those individuals with experimentally-increased energy expenditure often have lower apparent survival (Daan, Deerenberg & Djikstra 1996; Paredes *et al.* 2005; Jacobs *et al.* 2013; my deployment period—24 h—was apparently too short to detect such an effect). While I did not find a fitness cost associated with high daily energy expenditure, I also did not find a fitness benefit, as chick post-fledging survival (as approximated by energy delivery rates to the offspring), was not correlated with daily energy expenditure. Thus, although individual birds at my study site specialize on different foraging strategies (long flights, short flights, deep dives, etc.) with different energy costs (Woo *et al.* 2008, Elliott *et al.* 2009, 2010), no particular strategy provided a better payoff in terms of higher return to the chick.

Despite the constant average level of energy expenditure there was considerable individual variation (Welcker *et al.* 2009, 2010, Green *et al.* 2009; Fig. 2.1b,c). In contrast to the results of the meta-analysis (Fig. 2.2b), some of the individual variation in my study was related to life-history trade-offs. Older individuals, that were less likely to produce many more offspring, were more willing to invest in current broods by having higher chick-provisioning rates and expending larger amounts of energy to compensate

for being handicapped. Similarly, when murres were handicapped with larger handicaps, three out of nine young birds (<10 years old) abandoned the offspring, whereas none of ten old birds (≥10 years old) abandoned (Elliott *et al.* 2007; KHE, unpubl. data). Perhaps old murres maintained the secretion of hormones associated with parental behaviour, such as prolactin, throughout the stress of handicapping (Angelier *et al.* 2007).

For breeding animals, an alteration in activity budgets as an adjustment to increased activity-specific metabolic rates entails a cost for either investment in the individual or its offspring, and those adjustments were evident in my meta-analysis. Individuals either reduced their own body mass (at least over the short-term) or reduced their offspring body mass (Fig. 2.2b). Where a particular species lies upon the trade-off between investment in one's offspring and investment in one's self may be dictated by life history, with individuals from long-lived species maximizing their lifetime reproductive success by favouring their own condition over that of their current offspring (Saether, Andersen & Pedersen 1993; Mauck & Grubb 1995). I found little support for that idea when comparing across 25 studies, including the subset of studies used to justify those claims (Fig. 2b). With a larger selection of both long- and short-lived birds than the two petrels and four passerines reported by Mauck & Grubb (1995), handicapped long-lived birds were not more likely to reduce chick growth rates than handicapped short-lived birds.

IMPLICATIONS FOR MURRE L IFE HISTORY

The existence of an intrinsic limitation on energy expenditure explains the unique "intermediate" life history strategy of murres (Ydenberg 1989; Houston *et al.* 1996; Hansen 2003). Most auks either remain at the breeding site until fledging at near adult size or leave the breeding site soon after hatching, without ever being fed by the parent.

Along with pigeons (Crome 1975), three species of semi-precocial auks are apparently unique among birds in adopting an intermediate strategy of leaving their breeding site at only ~20% of adult body mass and prior to being able to fly. The presence of an energy ceiling in murres explains why parent murres are unable to continue provisioning at the higher rate required by larger offspring regardless of energy availability and why their offspring continue their development at sea, closer to potential food sources.

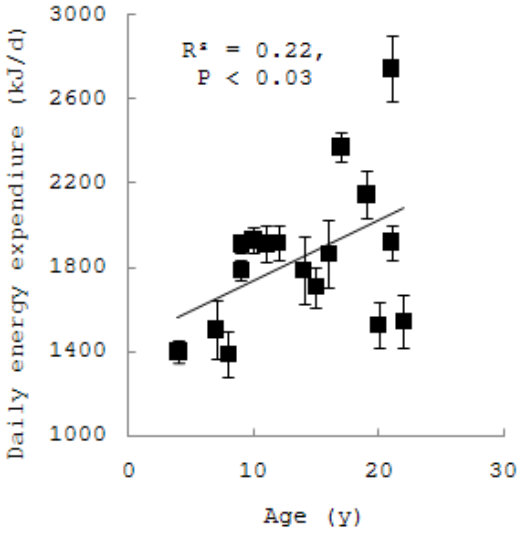
IMPLICATIONS FOR STUDIES OF DEVICE EFFECTS

Accelerometers that are regularly attached to birds of similar or smaller size than murres (Watanuki *et al.* 2006; Sato *et al.* 2007) had a measurable impact on behaviour. Likewise, there was a strong indication of reduced investment in either chick or adult condition following attachment of devices designed to mimic typical bio-logging devices (Fig. 2.2a). Most studies of device effects on daily energy expenditure were unable to measure an effect (Table 2.1), presumably because individuals adjusted their behaviour to remain within their energy ceiling. Increasing body drag by attaching a device augments locomotory costs to a much greater degree than predicted by simple biomechanical modelling, as flow disruption can be much greater than that imposed by a proportional increase in cross-sectional area for a similarly-shaped object (Pennycuick *et al.* 2010). Increasing mass without increasing drag has a smaller effect than predicted by biomechanical modelling (Kvist *et al.* 2001; Nudds & Bryant 2002; Schmidt-Wellenburg, Engel & Visser 2008). I suggest that device-effect studies focus on activity budgets or activity-specific costs rather than on daily energy expenditure and that researchers measure both adult and chick effects simultaneously given the potential trade-off between

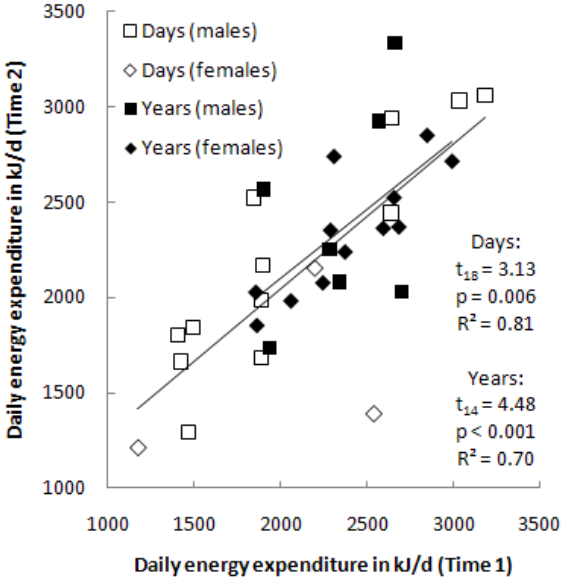
those metrics. Effects are likely particularly pronounced on birds with high wing-loadings that must move underwater (Vandenabeale *et al.* 2012; Elliott *et al.* 2012).

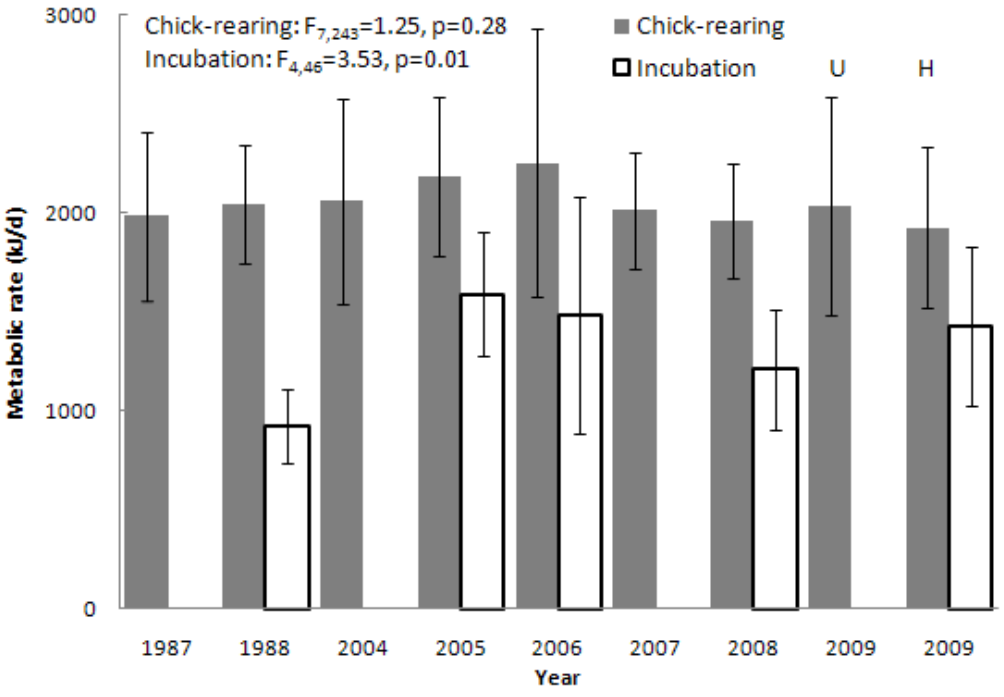
Figure 2.1. (a) Daily energy expenditure from doubly-labelled water experiment for handicapped birds changes with age in chick-rearing thick-billed murres. Uncertainty in doubly labelled water measurements were calculated using a jack-knife approach. Calculations were made using the mean at each point and systematically omitting individual data points. Multiplying the standard deviation of the resulting confidence interval by 1.96 created the estimated uncertainty. (b) Daily energy expenditure is repeatable across time. Lessells & Boag (1987) repeatabilities are shown. (c) Average ± SD energy expenditure during reproduction across eight years (U = non-handicapped, H = handicapped for 2009).

(a)



(b)

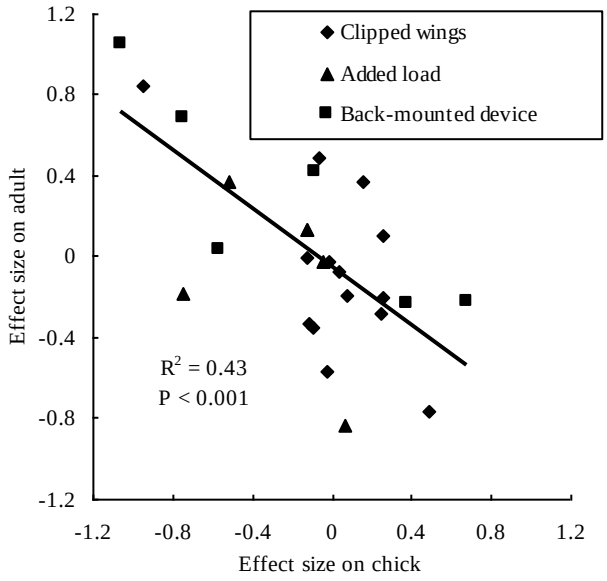




(c)

Figure 2.2. (a) Meta-analysis of studies (N = 25) that handicapped birds (by clipping wings, adding load or mounting a device on the back) and reported both effects on the adult (residual body mass change) and the chick (residual chick growth rate or provisioning rate) relative to controls. (b) Effect size on chick increased with brood value across the same studies as in (a) and for individuals in my study. Effect size = (Average for handicapped – Average for control)/(Pooled standard deviation).

(a)



(b)

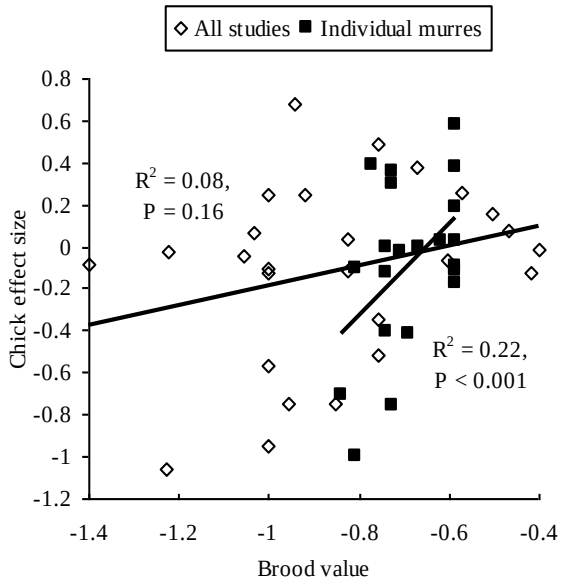


Figure 2.3. A theoretical model describing how daily energy expenditure may change depending on energy availability relative to need, illustrating why animals with both low and high energy availability may have low daily energy expenditure.

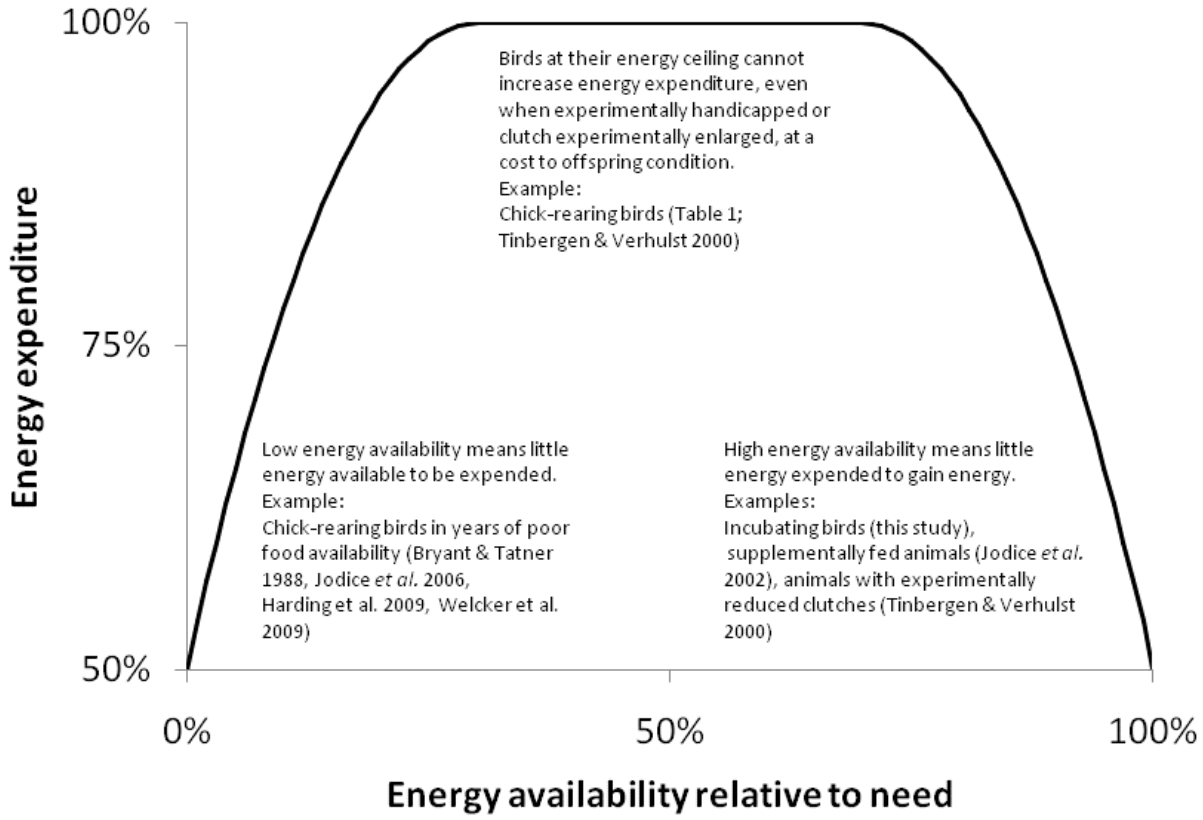


Table 2.1. None of the studies that examined daily energy expenditure with and without handicapping showed statistical significance at the sequential Bonferroni-corrected α-value of 0.004. The overall effect size across all 13 studies is -0.01 (SE= 0.33, z-test P = 0.51). In contrast, 5 of 6 studies that examined locomotory costs for similar-sized handicaps revealed statistical significance at the sequential Bonferroni-corrected α-value of 0.017.

Species (L = Lab study; **Non-flyers in bold**)	Handicap (as a percentage of body mass)	Energy expenditure ± SD		P	Source
		Handicapped (N)	Non-handicapped (N)		
Daily energy expenditure (kJ/d)					
Thick-billed murre[1]	Accelerometer (1.7%)	1926 ± 405 (16)	2036 ± 552 (16)	0.25[3]	My study (2009 only)
White-tailed tropicbird[1]	Radio transmitter (2.2%)	1693 ± 803 (5)	778 ± 244 (5)	0.04	Pennycuick *et al.* 1990
Brant (L)	Dummy radio (2.9%)	550 ± 137 (4)	594 ± 103 (4)	0.62	Sedinger, White & Hauser 1990
Northern bobwhite (L)	Radio transmitter (3.1%)	157 ± 23 (5)	150 ± 6 (5)	0.77	Hernández *et al.* 2004
Common tern[1]	Radio transmitter (6.2%)	368 ± 58 (6)	343 ± 37 (7)	0.45	Klaassen, Becker & Wagener 1992
Zebra finch (L)	Backpacks (27%)	48.1 ± 7.9 (9)	53.4 ± 6.6 (7)	0.34	Nudds & Bryant 2002
House wren[1]	Wings clipped by 4.2%	43.0 ± 3.1 (16)	40.5 ± 2.6 (16)	0.18	Tieleman *et al.* 2008
Pied flycatcher[1]	Removed two primaries	64.2 ± 5.4 (9)	61.2 ± 5 (13)	0.37	Moreno *et al.* 1999
African penguin[1]	Swim speed logger (0.6%)	2007 ± 405 (5)	2433 ± 796 (10)	0.29	Nagy, Siegfried & Wilson 1984[4]
Adélie penguin[1]	Dummy (0.8%)	5323 ± 1115 (4)	5790 ± 1115 (4)	0.71	Culik and Wilson 1992
Little blue penguin[1]	Activity logger (5.5%)	1349 ± 112 (6)	1671 ± 130 (4)	0.04	Gales *et al.* 1990
Takahe[2]	Radio transmitter (1.9%)	1274 ± 42 (6)	1174 ± 42 (6)	0.03[3]	Godfrey, Bryant & Williams 2003
Meadow vole (L)	Radio transmitter (7.9%)	92 ± 16 (10)	91 ± 17 (10)	0.45	Berteaux *et al.* 1996b
Locomotory (flying or swimming) costs (kJ/h) associated with attaching a device that primarily increased body drag rather than load					
Thick-billed murre[1]	Accelerometer (1.7%)	631 ± 111 (16)	505 ± 114 (16)	0.0005	My study (flight)
Rock pigeon	Dummy (2.5-5%)	292 ± 45 (8)	158 ± 22 (8)	<0.0001[3]	Gessaman & Nagy 1988
Rose-colored starling (L)[6]	Box + antennae (2.6%)	0.225 ± 0.064 (3)[5]	0.116 ± 0.040 (3)[5]	<0.0001	Pennycuick *et al.* 2012
Adélie penguin (L)[6]	Dummy (0.8%)	435 ± 30 (5)	334 ± 7 (4)	0.0003	Culik and Wilson 1991
Atlantic cod (L)[6]	Dummy (3.1%)	146 ± 14 (7)	127 ± 5 (7)	0.01	Steinhausen *et al.* 2006
European eel (L)[6]	Dummy (1.6%)	27.4 ± 8.6 (9)	25.4 ± 6.9 (9)	0.60	Methling *et al.* 2011

[1]Measured while rearing offspring; general linear model output including all four activities (see Methods) had $R^2 = 0.77$

[2]Flightless runner; change in energy costs thought to reflect changes in thermoregulation

[3]Pairwise comparisons of the same individuals with and without handicaps

[4]Recalculated from values presented in their Tables 1 and 2 during the period away from the colony

[5]Drag coefficient; I only reported on experiments with live animals as coefficients on frozen specimens are unrealistic (Pennycuick *et al.* 2012)

[6]Studies used respirometry (all other studies used doubly-labelled water)

Table 2.2. Predictions derived from the five hypotheses tested in my study.

Hypothesis	Prediction	Method	Supported?
1. Repeatability	Energy expenditure for a given individual would be repeatable across time	Measure energy expenditure for the same individual within and across years	Yes
2. Energy availability[1]	Energy expenditure would vary across environmental conditions	Measure energy expenditure across years and handicapping treatments.	No
3. Ceiling	Energy expenditure would not vary across environmental conditions or be associated with fitness	Same as for (2) and (4).	Yes
4. Trade-offs[1]	(i) Energy expenditure would be associated with fitness, such as offspring condition (birds that fed their offspring more often would be more active) and adult survival (less energy available for self-maintenance). (ii) Regardless of what limits energy expenditure, increased investment in energy for adult energy stores (self-feeding) when environmental conditions deteriorate would mean decreased energy available to feed offspring. However, older birds and short-lived bird species, both with lower future reproductive potential, would expend more energy feeding offspring when environmental conditions deteriorate because their current brood value is higher.	(i) Correlate reproductive success and survival with energy expenditure. (ii) Measure adult mass loss (an indication of self-feeding), chick growth rate and energy expenditure with and without handicaps.	Partially (i) No (ii) Murres: yes; Meta-analysis: no

[1]Meta-analysis used to examine whether results held across broader taxonomic groups.

Chapter Three. Thyroid hormones correlate with resting metabolic rate, not daily energy expenditure, in two charadriiform seabirds

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My contribution: I conducted all the field work (with field assistants, and with logistical help from T. Gaston and S. Hatch) and laboratory work (with laboratory assistants, except for the doubly-labelled water analyses, which occurred in J. Speakman's lab), completed all statistical analyses and wrote the paper (with comments from all authors). V. Palace provided instruction and instrumentation for the thyroid hormone analyses. J. Welcker aided in the development of ideas.

Summary

1. Thyroid hormones affect *in vitro* metabolic intensity, increase basal metabolic rate in the lab, and are sometimes correlated with basal and/or resting metabolic rate in a field environment.
2. Given the difficulty of measuring metabolic rate in the field—and the likelihood that long-term measurement of metabolic rate in the field jeopardizes other measurements—I examined the possibility that circulating thyroid hormone levels were correlated with resting metabolic rate in two free-ranging bird species with high levels of energy expenditure (the black-legged kittiwake, *Rissa tridactyla* and thick-billed murre, *Uria lomvia*). Because basal metabolic rate and daily energy expenditure are purported to be linked, I also tested for a correlation between thyroid hormones and daily energy expenditure. I examined the relationships between free and bound levels of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) with daily energy expenditure (DEE) and with 4-hr long measurements of post-absorptive and thermoneutral resting metabolism (resting metabolic rate; RMR).
3. RMR but not DEE increased with T3 in both species; both metabolic rates were independent of T4. T3 and T4 were not correlated with one another. DEE correlated with body mass in kittiwakes but not in murres, presumably owing to the larger coefficient of variation in body mass during chick-rearing for the more sexually dimorphic kittiwakes.
4. I suggest T3 provides a good proxy for resting metabolism, but DEE does not, in these seabird species.

Introduction

As pleiotropic hormones, thyroxine (T4) and triiodothyronine (T3) are involved in the regulation of a multitude of physiological traits, but they are best known for their role in the regulation of tissue oxygen consumption and thermogenesis *in vitro* (Bobek *et al.* 1977; McNabb 2000; Hulbert 2000). T4 and T3 are also well known for their *in vivo* association with basal metabolic rate in homeotherms, at least in the laboratory (Nicol *et al.* 2000; Silvestri *et al.* 2005; Johnstone *et al.* 2005; Kim 2008). By increasing cellular metabolism, T3 can both increase basal metabolic rate and promote thermoregulation. Nonetheless, regulatory heat, the extra heat produced in response to cool temperatures (thermoregulation), is primarily under nervous control via shivering (McNabb 2007). It is obligatory heat, the additional heat increment above essential life processes necessary to maintain thermoneutrality and the primary constituent of basal metabolic rate, which is controlled by T3 in homeotherms (McNabb 2007).

Information on the role of thyroid hormones in the regulation of basal metabolic rate (BMR) in wild homeotherms, however, is limited and equivocal (Burger & Denver 2002; Chastel *et al.* 2003; Vezina *et al.* 2009; Li *et al.* 2010). For instance, administration of T4 can increase basal metabolic rate and/or daily energy expenditure (al-Adsani *et al.* 1997; Banta & Holcombe 2002; Johannsen *et al.* 2012), and it is often unclear whether there is a direct effect of T4 or whether T4 is activated via deiodination (McNabb 2000; Johannsen *et al.* 2012). Furthermore, whereas T3 is the biologically active molecule in mammals, T3 and T4 have similar physiological potency in some birds, even though the affinity of the thyroid receptor for T3 is almost identical between birds and mammals (McNabb 2007). The ratio of T3:T4 is sometimes used as a diagnostic for thyroid-related

illnesses, as homeostasis in the presence of thyroid illness can distort that ratio (Mortoglou & Candiloros 2004). More recently, the T3:T4 ratio has been used as an indicator of toxic contamination in wild animals because some thyroid hormone mimics out-compete T3 at the binding site of the carrier molecules (Verreault *et al.* 2004; Brar *et al.* 2010).

Basal metabolic rate is the minimal energy cost for a non-breeding, post-absorptive, resting and thermoneutral adult animal. Daily energy expenditure (DEE) is the average daily energy cost. Both BMR and DEE vary within species (Bech *et al.* 1999; Fyhn *et al.* 2001; Speakman *et al.* 2003). One potential cause of variation in BMR (or resting metabolic rate, RMR, when one or more of the assumptions for BMR are violated) is that some individuals obtain a large amount of energy regularly and therefore have large and metabolically-active organs, such as the gastro-intestinal tract, heart and skeletal muscles (Hammond & Diamond 1997; Speakman *et al.* 2003). Those costly organs are required to sustain the high DEE required to obtain and assimilate that energy. Other individuals obtain less energy and therefore have smaller and less active organs. Variation in BMR can thus reflect the variation in energy expenditure required for maintaining internal organs at a resting state, and as a consequence, one might anticipate a correlation between BMR and DEE. Such correlations have been reported by some authors (Nilsson 2002; Tieleman *et al.* 2008; Careau *et al.* 2012), but not others (Meerlo *et al.* 1997; Fynh *et al.* 2001; Speakman *et al.* 2003). If high metabolic intensity is associated with high daily energy expenditure, high basal metabolic rate and high thyroid hormone levels, I might anticipate a relationship between those variables. Given the role of T3 and T4 or of the T3/T4 ratio in regulating resting metabolism, and the putative energetic link between

maintenance metabolism and DEE, I predicted that these thyroid hormones may also be involved in regulating DEE. If that were the case, then circulating levels of T3 and T4 should correlate with both maintenance metabolism and DEE. In support, a previous study used thyroid hormones as indicators of DEE in a charadriiform bird (Duriez *et al.* 2004).

I examined the strength of correlations between post-absorptive, thermoneutral and unstressed RMR, DEE and thyroid hormone levels in free-living, breeding thick-billed murres (*Uria lomvia*) and black-legged kittiwakes (*Rissa tridactyla*). I treat unstressed RMR as a surrogate for BMR. Both kittiwakes and murres are piscivorous seabirds of the order Charadriiformes that are characterized by high levels of energy expenditure relative to other seabirds (Shaffer 2010). Kittiwakes are sexually dimorphic (males are ~10% larger than females), whereas murres are largely monomorphic (males are <5% larger than females) with seasonal variation in size obscuring sex-specific trends. Kittiwakes are small (~400 g) gulls that spend most of their time at sea flying and foraging at the surface while murres are large (~1000 g) auks that forage by diving to depths of up to 150 m. I examined RMR during incubation because RMR likely peaks at that time, at least for kittiwakes (Bech *et al.* 2002). I examined DEE during chick-rearing because time spent flying, and thus DEE, likely peaks during chick-rearing, at least for murres (Croll 1990). I considered both bound and unbound hormone levels of T4 and T3 to examine three predictions: (1) RMR and DEE would both correlate with thyroid hormone levels, (2) that relationships would be stronger with free than with total thyroid hormone levels, and, (3) RMR and DEE would be correlated across individuals within each species.

Materials and methods

FIELD METHODS

I studied basal metabolic rate in adult kittiwakes from 15 to 30 June 2010 (N = 32) at Middleton Island, Alaska, and murres from 13 to 24 July 2011 (N = 53) at Coats Island, Nunavut. All birds were in the latter half of incubation (eggs 15-25 d old). I studied daily energy expenditure of some of the same individual kittiwakes from 4 to 20 July 2010 (N = 24) when kittiwakes had chicks between 10 and 20 days old. Likewise, I also studied the same individual murres from 25 July to 10 August 2009 when murres had chicks between 5 and 15 days old (N = 22; same observations as Elliott *et al.* 2013). At my Arctic study sites, air temperatures were usually moderate (10-20°C), the night was very brief and birds generally foraged actively throughout both day and night. Birds were captured at the nest with a noose pole (murres) or a hook (kittiwakes). Only one adult per nest was captured and I did not correct body mass for linear size as such corrections do not improve estimation of lean or lipid mass in either murres or kittiwakes (Jacobs *et al.* 2012). Murres were sexed using sex-specific PCR primers applied to a drop of blood on filter paper (Elliott *et al.* 2010a) whereas kittiwakes were sexed behaviourally by position during copulation. Both parents share incubation and chick-rearing duties, at least during the period of time covered by the current studies. Research was approved by the Protocol Management and Review Committee of the University of Manitoba under protocol F11-020.

RESTING METABOLIC RATE

I used open-flow respirometry to measure metabolic rate of incubating birds in a respirometry chamber. Birds were captured between 0600-2200 hrs local time (0430-

2030 solar time) as they were switching off from their incubation shift (~10 hr for kittiwakes and ~12 hr for murres, Gill *et al.* 2002, Elliott *et al.* 2010). The ambient temperature (T_a) during the measurements was within the birds' thermoneutral zone (range = 15.1 – 18.2°C; higher temperatures appeared to result in discomfort and increased metabolic rate; see Gabrielsen *et al.* 1988 for thermoneutral zone).

Immediately after capture, birds were weighed using a spring balance (Pesola, Switzerland) and transported to a small building and placed in a FoxBox II® (Sable Systems, Las Vegas, Nevada) respirometer for the measurement of RMR. I used magnesium perchlorate to dry outside air, which was pumped through a 15 L respiratory chamber with a flow rate of ~1.0 L/min (kittiwakes) or ~2.0 L/min (murres) using the pump and flow meter built into the FoxBox II respirometer. The effluent air was then passed through a FoxBox II® carbon dioxide analyzer. Carbon dioxide was then removed from the air using soda lime and the effluent was passed through a FoxBox II oxygen analyzer. I recorded effluent air concentrations every 15 seconds. To reduce drift, the oxygen analyzer was encased in Styrofoam. Both the oxygen and carbon dioxide analyzers were calibrated at the start and end of each field season using pure nitrogen and 30% oxygen-70% nitrogen stock gas. I measured baseline gas levels (scrubbed of water, and, for the oxygen analyzer, of carbon dioxide) for one hour before and after each measurement and calibrated the baselines to 20.6% oxygen and 0.04% carbon dioxide at the start of each measurement. Calibrating the baseline to 20.6%, instead of the traditional 20.95%, should have little effect on my calculations as those calculations use only the difference in percent oxygen between steady-state and ambient air, and both values would be similarly biased downward. I calculated RMR as the lowest 10-minute

running average of instantaneous oxygen consumption using formula 3A in Withers (2001), after accounting for washout delay, although as birds were in steady state, the effect of correcting for instantaneous oxygen consumption had less than 1% effect on oxygen and carbon dioxide measurements. RMR (W) was calculated from the value of oxygen consumption rate using a conversion factor of 20.1 J ml O_2^{-1}. Because carbon dioxide production rate can be less sensitive to drift than oxygen consumption rate, I repeated all analyses using carbon dioxide production rate.

DAILY ENERGY EXPENDITURE

I used the doubly-labelled water method to measure daily energy expenditure (DEE) during chick-rearing. Upon capture, I weighed the birds and injected them intra-peritoneally with 0.5mL doubly-labelled water (64.0 atom percent excess ^{18}O and 36.2 atom percent excess ^{2}H). Birds were then released and recaptured after 90 min (murres) or kept in a cloth bag for 60 min (kittiwakes), at which time I obtained an initial blood sample to allow measurement of equilibrium isotopic concentrations (Speakman 1997). Murres that were released immediately returned to their breeding site and were inactive (brooding or incubating) until recaptured. Background blood samples were taken from 8 unlabeled adult kittiwakes and 9 unlabelled adult murres to determine the mean background level of isotopes. I recaptured, reweighed and resampled all injected individuals after an average of 48.0 h (SD = 2.4 h, range = 46-55 h). All samples were flame-sealed into capillary tubes until analysis at the University of Aberdeen via isotope ratio mass spectrometry (see Speakman & Krol 2005). Water for analysis of ^{2}H and ^{18}O was obtained by vacuum distilling blood samples into glass Pasteur pipettes. ^{2}H enrichment was determined from hydrogen gas derived from the distilled water by online

chromium reduction. ^{18}O enrichment was analyzed by equilibration of distilled water with CO_2-gas of known oxygen isotopic enrichment using the small-sample equilibration technique (Speakman & Krol 2005). Isotope ratios were then determined by gas source isotope mass spectrometry (IRMS) with isotopically characterized gases of H_2 and CO_2 in the reference channels. Enrichment of the injectate was estimated by a dilution series with tap water and mass spectrometric analysis of 5 subsamples of each solution (Speakman & Krol 2005).

Initial body water was determined using the plateau method from the ^{18}O dilution space, which agrees well with directly measured values for murres and other animals (Speakman 1997; Jacobs *et al.* 2012). I assumed percent body water did not change over the course of my measurements. I calculated energy expenditure using a single pool model corrected for fractionation effects assuming a fixed evaporative water loss of 25% (equation 7.17, Speakman 1997). Based on direct examination of adult diet, the rate of CO_2-production was converted into estimates of DEE (W) using a caloric equivalent of 27.1 J mL CO_2^{-1} (murres: 81% protein, 14% fat, 5% carbohydrate) and 27.4 J mL CO_2^{-1} (kittiwakes: 80% protein, 15% fat, 5% carbohydrate). The doubly-labelled water method accurately estimates daily energy expenditure at the individual level (uncertainty generally <10%) for charadriifom birds and other animals (Visser & Schekkerman 1999; Van Trigt *et al.* 2002; Shirai *et al.* 2012). Furthermore, values derived from a number of alternative equations are all highly correlated ($R^2 > 0.95$), and as it is the relative (rather than absolute) value of DEE that is relevant to my results, the use of alternative equations would have little impact on my conclusions.

THYROID ASSAYS

Upon both capture and immediately after RMR measurements (murres) or only immediately after RMR measurements (kittiwakes), birds were blood sampled (1 mL from the alar vein into a heparnized syringe using a 25G needle). Likewise, upon capture and immediately after DEE measurements (murres) or only immediately after DEE measurements (kittiwakes), birds were blood sampled in a similar manner. I obtained only one blood sample from each kittiwake because of their small size. Blood samples were stored on ice for <4 h, centrifuged at 2000*g* for 10 minutes, the plasma was removed and stored at –20°C for the remainder of the field season (1-2 months) and then shipped to the University of Manitoba on dry ice and stored at –80°C until analysis. I determined both total and free T3 and T4 concentrations in duplicate by radioimmunoassay using a commercially available kit on unextracted plasma with a slight modification (MP Biomedical kits 06B258710, 06B257214, 06B254216, 06B254029). The modification required longer incubation times for equilibration from the suggested 1-2.5h to 6h (JW, KHE, unpubl. data). Sample hormone concentrations in blood from 10 murres and 10 kittiwakes with a 6h incubation time were highly correlated with concentrations from the same samples with the recommended, shorter incubation period ($R^2 > 0.9$ for all hormones). Blood samples were also taken from 10 kittiwakes and 10 murres prior to placement in the FoxBox respirometer and following measurement of RMR. Strong positive correlations between hormone levels before and after measurement of RMR (total T3: $R^2 = 0.89$, $t_9 = 3.69$, $P = 0.005$; total T4: $R^2 = 0.84$, $t_9 = 3.25$, $P = 0.01$), suggest that hormone levels post-chamber were representative of circulating levels and not affected by stress associated with capture. Intra-assay variability was 4.5 ± 1.1% (total

T3), 4.9 ± 0.9% (free T3), 3.9 ± 0.6% (total T4) and 4.8 ± 1.5% (free T4), inter-assay variability was 5.5% (total T3), 6.1% (free T3), 5.9% (total T4) and 7.1% (free T4) and all assays were parallel with the standard curve following serial dilution (ANCOVA: $P > 0.2$).

DATA ANALYSIS

I used simple, least-squares regression to examine the relationship between total and free T3 and T4 and between those hormones and RMR and DEE. I examined thyroid hormone levels and body mass simultaneously to eliminate the possibility that a correlation between energy expenditure and thyroid hormones was due to the correlation between thyroid hormones and body mass associated with thermoregulation. I also constructed a general linear model for both RMR and DEE with both free and total T3, free and total T4, sex, body mass, time of day (circularly transformed using the cosine of time since midnight; RMR only), ambient temperature and deviations from a 24-hour sampling interval (DEE only) as independent variables. To account for the effect of body mass, I also present data as residual of energy expenditure on body mass. I included ambient temperature as a covariate to test whether I was operating within the birds' thermoneutral zone. All values are shown as averages ± SD. All statistical analyses were performed using R.2.4.1.

Results

Body mass and RMR were roughly double in murres compared to kittiwakes, whereas DEE was slightly less than three times as high (Table 3.1). Free hormone levels, but not total hormone levels were higher in murres implying lower levels of the binding proteins (Table 3.1). The respiratory exchange ratio was slightly higher in kittiwakes than murres

(Table 3.1). As is the case in most studies of any duration, RMR was generally recorded during the last half of measurements (average = 3.1 ± 0.4 h after start of measurements).

RMR during incubation was not correlated with DEE during chick-rearing in kittiwakes (t_{23} = -1.09, P = 0.30, R^2 = 0.10) or murres (t_{21} = 0.51, P = 0.67, R^2 = 0.03). Total T3 and free T3 were correlated with each other in incubating kittiwakes (t_{36} = 6.06, P < 0.0001, R^2 = 0.59) and murres (t_{52} = 9.13, P < 0.0001, R^2 = 0.62). Total T4 and free T4 were correlated with each other in incubating kittiwakes (t_{36} = 3.79, P = 0.0006, R^2 = 0.39) and murres (t_{51}= 2.45, P = 0.02, R^2 = 0.16). Total T3 was not correlated with total T4 in murres (t_{51} = 0.11, P = 0.91, R^2 = 0.00) or in kittiwakes (t_{36} = -0.65, P = 0.52, R^2 = 0.02). Free T3 was not correlated with free T4 in murres (t_{51} = 1.48, P = 0.14, R^2 = 0.04) or kittiwakes (t_{36} = 0.44, P = 0.67, R^2 = 0.01). The ratio of free T3 to total T3 was correlated with the ratio of free T4 to total T4 in incubating kittiwakes (t_{36} = 2.16, P = 0.04, R^2 = 0.24) and murres (t_{41} = 2.97, P = 0.005, R^2 = 0.22).

When considered simultaneously within a general linear model, RMR in kittiwakes increased with total T3 (t_{36} = 4.24, P < 0.001) and body mass (t_{36} = 3.32, P = 0.002), but was independent of free T4 (t_{36} = -0.62, P = 0.54), total T4 (t_{36} = 0.27, P = 0.79), ambient temperature within the thermoneutral zone (t_{36} = -0.07, P = 0.95), sex (t_{36} = 1.14, P = 0.21), time of day (t_{36} = 1.22, P = 0.19) and body mass (t_{36} = 1.88, P = 0.07). Using carbon dioxide production, rather than oxygen consumption, to calculate RMR did not alter the significance of any of those parameters. In contrast, DEE in kittiwakes increased with body mass (t_{17} = 4.20, P = 0.002), but was independent of free T3 (t_{17} = -0.58, P = 0.57), free T4 (t_{17} = -1.88, P = 0.09), total T3 (t_{17} = -0.12, P = 0.90), total T4 (t_{17} = -1.52, P = 0.16), sex (t_{17} = 0.84, P = 0.42) and ambient temperature (t_{17} = 0.11, P = 0.93).

Similarly, univariate correlations showed that RMR in kittiwakes was most closely linked to T3, whereas DEE was most closely linked to body mass (Table 3.2, Figs. 1,2).

When considered simultaneously within a general linear model, RMR in murres increased with total T3 (t_{50} = 4.39, P < 0.0001) and body mass (t_{50} = 16.29, P < 0.0001) but was independent of free T4 (t_{50} = -0.13, P = 0.90), total T4 (t_{50} = 0.53, P = 0.60), time of day (t_{50} = 1.01, P = 0.32), sex (t_{50} = 0.69, P = 0.52) and ambient temperature within the thermoneutral zone (t_{50} = -0.17, P = 0.90). Using carbon dioxide production, rather than oxygen consumption, to calculate RMR did not alter the significance of any of those parameters. In contrast, DEE in murres was independent of free T3 (t_{15} = 0.58, P = 0.57), free T4 (t_{15} = -1.88, P = 0.09), total T3 (t_{15} = -0.12, P = 0.90), total T4 (t_{15} = -1.52, P = 0.16), ambient temperature (t_{15} = 0.11, P = 0.93), sex (t_{15} = 0.56, P = 0.58) and body mass (t_{15} = 0.71, P = 0.52). Similarly, univariate correlations showed that RMR in murres was most closely linked to T3, whereas DEE was independent of all seven variables (Table 3.2, Figs. 3.1,3.2).

Discussion

T3 predicted mass-corrected RMR (Fig. 3.1), suggesting that T3 can be a useful indicator of RMR in some species of wild birds, especially in conjunction with body mass. Nonetheless, I caution that T3 probably proves useful only at a single location at a single time of year as both thyroid hormones and RMR likely vary nonlinearly with environment (Burger & Denver 2002). My results challenge assertions, developed primarily from studies of non-charadriiform birds, that T4 is as biologically active as T3 in birds (McNabb 2007). Comparisons of the likely thyroid receptor structure based on DNA sequences may help predict variation in receptor affinity to T3 and T4 among bird

species, although thyroid receptors in birds are similar to mammals and do have higher affinity for T3 (Weirich & McNabb 1984; Bellabarba *et al.* 1988; McNabb 2007). In contrast, the absence of a relationship between T3 and DEE suggests that T3 is not an effective proxy for DEE as previously illustrated for a lizard (*Sceloporus undulatus*; Joos & Johnalder 1990) and humans (Starling *et al.* 1998). T3, however, may be a good indicator of DEE for inactive animals where BMR, or at least RMR, may constitute a substantial proportion of DEE (Toubro *et al.* 1996) or where thermoregulatory costs are high (Duriez *et al.* 2004). However, T3 is not a good indicator of DEE in humans (Starling *et al.* 1998), which would be a prime example of a species where RMR may constitute a substantial proportion of DEE. DEE did correlate with body mass (rather than T3), but only for kittiwakes, the species with greater sexual dimorphism, and consequently more extensive individual variation in body mass (Bech *et al.* 2002; Elliott *et al.* 2008; Jacobs *et al.* 2011).

The percent bound T3 was similar to the percent bound T4 suggesting that both hormones are similarly affected by carrying capacity, namely concentration of the binding proteins, albumin and transthyretin in circulation (Hulbert 2000). Birds lack specific T4-binding proteins and rely primarily on the less-specific transthyretin (17-32%) and albumin (66-75%) carriers for transport (McNabb 2007). Due to the absence of an effect of T4 on DEE or RMR, I suggest that T4 acts mainly as a reserve for rapid upregulation of T3 titres via cellular deiodinases when a rapid increase in basal metabolic rate, or other processes regulated by T3, is required. Possible exceptions are where T3:T4 ratios are influenced by exogenous thyroid mimics, such as polychlorinated biphenyls (McNabb 2000). Interestingly, my correlations were higher between RMR and total T3

than free T3, further supporting the idea that metabolic rate is primarily regulated via increased deiodination (production of T3) than via changes in levels of binding proteins, such as transthyretin.

My measurements of RMR were similar to those made by other authors, including those that attempted to measure BMR. My value of 6.51 W for resting murres weighing 980 g during incubation compares favorably with the 6.9 W reported for post-absorptive, non-breeding murres weighing 803 g (Croll & McLaren 1993), 7.0 W for post-absorptive murres weighing 1025 g (Hawkins *et al.* 2005) and 5.1 W for post-absorptive, breeding murres weighing 819 g (Gabrielsen 1988). My value of 3.49 W for resting kittiwakes weighing 432 g during incubation also compares favorably with the 3.34 W reported for kittiwakes weighing 365 g (Gabrielsen 1988) and 3.31 W for "basal" metabolism in kittiwakes weighing 345 g during incubation (Bech *et al.* 1999).

Birds were captured as they were relieved by their partner at the nest site and kept for four hours. Assuming 10 h (kittiwakes) and 12 h (murres) incubation shifts, the birds were likely post-absorptive during measurements of RMR, as gut retention rates in charadriiform birds are <6 h (Hilton *et al.* 2001) and murres, at least, seldom have anything in their guts when captured at the colony. For birds in which measured pre- (<3 minutes from capture) and post-measurement hormone levels were analyzed, there was no significant difference in corticosterone levels for either species (for kittiwakes, those birds were excluded from the current analysis, as the remaining kittiwakes were not sampled pre-measurement). As my birds were likely post-absorptive, I found no evidence for daily rhythms in RMR for my Arctic-nesting birds (see Results) and as my birds were unstressed, RMR is likely a close approximation of BMR.

Daily energy expenditure (DEE) at the time of peak energy demands (chick-rearing), and RMR at a time when birds spend a substantial proportion of their time resting (incubation) were not correlated within individual breeding kittiwakes and murres. RMR was correlated with T3, and T3 measured at the same time as the DEE measurements was, if anything, slightly inversely correlated with DEE. The absence of a relationship between DEE and T3 therefore provides additional support for the absence of a positive relationship between RMR and DEE—although DEE may be correlated with the component of RMR that is independent of T3. DEE increases between incubation and chick-rearing for both kittiwakes and murres, as birds increase flying time two-fold between those periods, and there is an increase in the size of some metabolically active organs between these two breeding stages (Bech *et al.* 2002; Elliott *et al.* 2008; Jacobs *et al.* 2011). However, some metabolically intensive organs decrease in size between incubation and chick-rearing (Bech *et al.* 2002; Elliott *et al.* 2008) and BMR decreases between those two time periods, at least for kittiwakes (Bech *et al.* 2002). One potential mechanism underlying individual variation and seasonal trends in RMR could be that RMR is primarily determined by body temperature and metabolic intensity rather than by organ size, at least over the range of organ size variation apparent in nature outside of long-distance migration and similar extreme events (Rønning *et al.* 2008). Although BMR and DEE are sometimes correlated in interspecific comparisons (Daan *et al.* 1990), my results provide support for the conclusion that RMR/BMR and DEE are not directly linked at the level of the individual within a species (Meerlo *et al.* 1997; Speakman *et al.* 2003). Further study, directly examining the correlation between RMR and DEE within

the same life-history stages, is required to conclusively demonstrate the absence of a correlation.

Table 3.1. Average (±SD) values for metabolic rate and thyroid hormone levels for black-legged kittiwakes and thick-billed murres.

	Kittiwake	Murre
Incubation body mass (g)	432 ± 38	980 ± 78
Chick-rearing body mass (g)	420 ± 41	998 ± 51
Resting metabolic rate (W)	3.49 ± 0.56	6.51 ± 0.81
Respiratory exchange ratio	0.74 ± 0.03	0.71 ± 0.04
Daily energy expenditure (kJ/d)	788 ± 127	2036 ± 552
Free T3 (pg/mL)	14.9 ± 5.3	21.1 ± 13.3
Total T3 (ng/dL)	396 ± 173	360 ± 314
Free T4 (ng/dL)	1.24 ± 0.61	1.53 ± 0.89
Total T4 (μg/dL)	2.79 ± 0.85	2.41 ± 0.91

Table 3.2. Correlation coefficients for relationships between thyroid hormones and either DEE or post-absorptive, unstressed RMR. Correlations that are statistically significant ($P < 0.05$) are shown in bold.

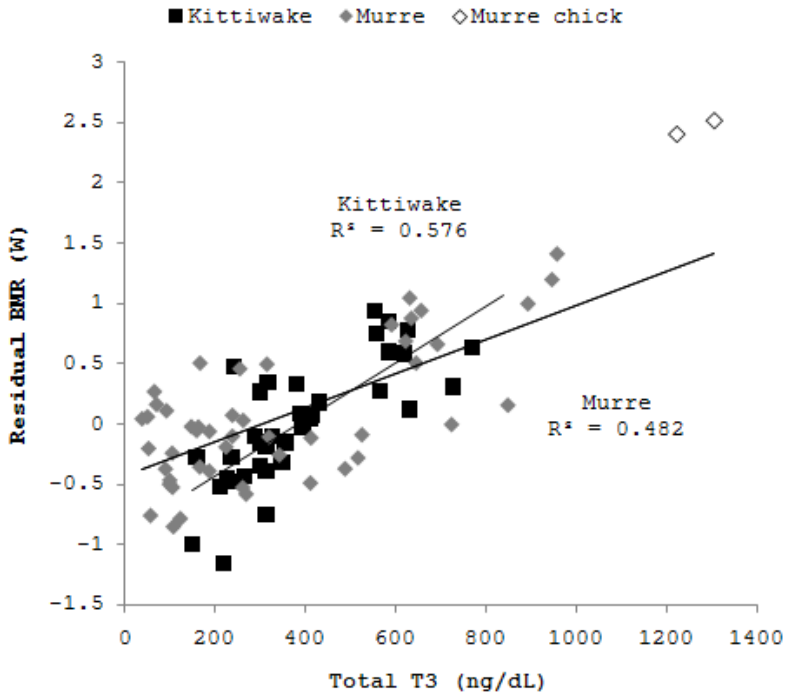
	Free T3	Total T3	Free T4	Total T4	Body mass
Kittiwakes					
RMR	**0.591**	**0.729**	-0.270	0.054	**0.458**
DEE	0.007	-0.247	0.270	-0.001	**0.618**
Murres					
RMR	**0.453**	**0.491**	0.015	0.145	**0.733**
DEE	-0.157	-0.261	0.204	0.102	0.027

Table 3.3. Correlation coefficients for relationships between total T3 and RMR or BMR across different species of birds.

Species	R	Reference
Black-legged kittiwake *Rissa tridactyla*	0.73	My study
House sparrow *Passer domesticus*	0.62	Chastel *et al.* 2003
Little bunting *Emberiza pusilla*	0.78	Liu *et al.* 2006; Zheng *et al.* 2013
Red knot *Calidris canutus*	0.28	Vezina *et al.* 2009
Thick-billed murre *Uria lomvia*	0.49	My study

Figure 3.1. (a) Residuals of post-absorptive, unstressed RMR on body mass during incubation increase with total T3 for both thick-billed murres (grey symbols) and black-legged kittiwakes (black symbols). Values for two murre chicks (open symbols) are shown but not included in the regression. (b) Residuals of daily energy expenditure during chick-rearing on body mass are not related to total T3 for thick-billed murres and black-legged kittiwakes.

(a)



(b)

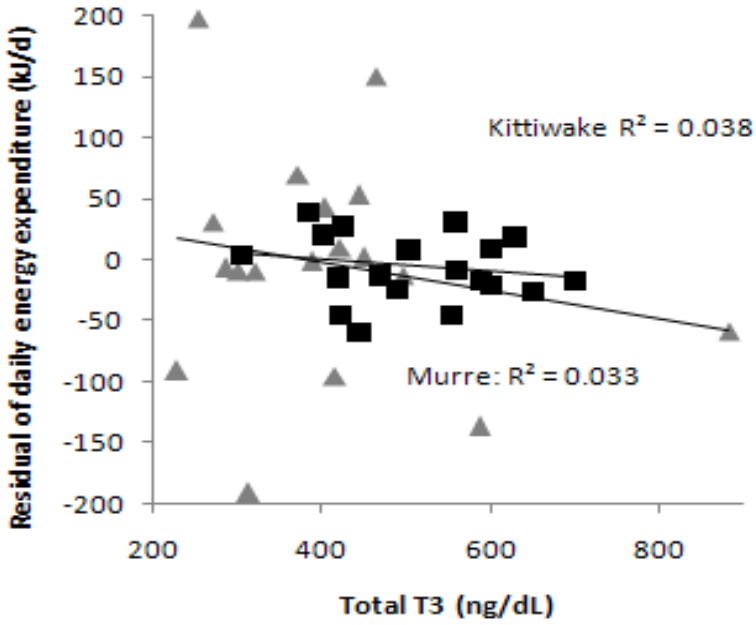
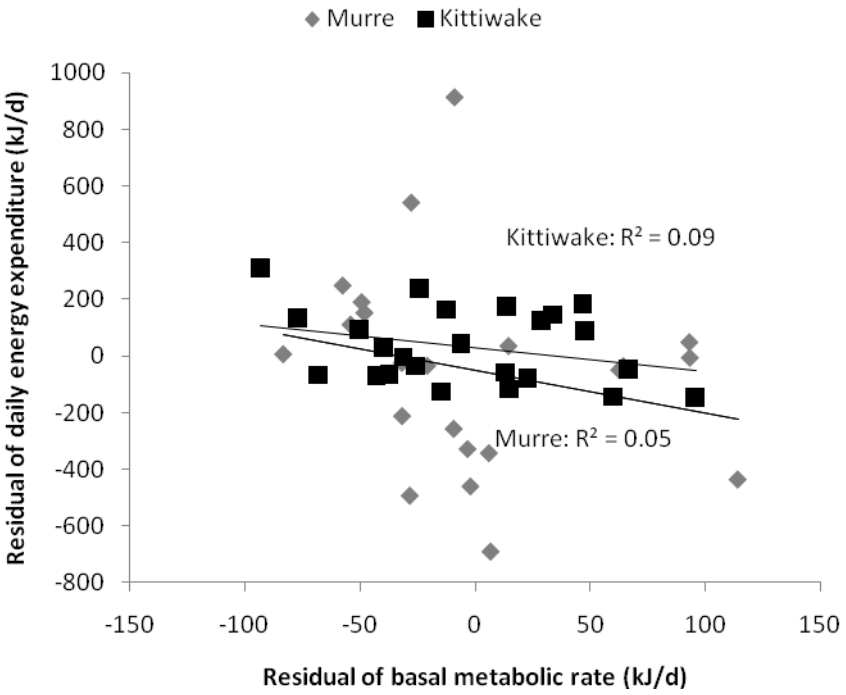


Figure 3.2. Residuals of post-absorptive, unstressed RMR on body mass during incubation are not correlated with residual of DEE on mass during chick-rearing.



Chapter Four. Ageing gracefully: physiology but not behaviour declines with age in diving birds

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My contribution: I conducted all the field work (with field assistants, and with logistical help from T. Gaston; except for the 2004 and 2010 deployments) and laboratory work (with laboratory assistants), completed all statistical analyses and wrote the paper (with comments from all the authors). Y. Ropert-Coudert supplied loggers and helped design the experiment.

Summary

1. Senescence-related mortality is higher in long-lived animals than short-lived animals, yet many long-lived birds show few signs of physiological aging. Likewise, there are few reports of physiological senescence in long-lived diving homeotherms that frequently encounter hypoxic conditions.
2. To examine aging within a long-lived diving homeotherm, I studied blood oxygen stores (N = 93), resting metabolism (N = 43) and foraging behaviour (N = 310) of known-age thick-billed murres (*Uria lomvia*). Because murres dive exceptionally deep for their size and have a very high metabolism, I expected that very old murres would show signs of physiological senescence. I paid particular attention to resting metabolism as I argue that these maintenance costs reflect those experienced during deep dives.
3. Blood oxygen stores (hematocrit), resting metabolic rate and thyroid hormone levels all declined with age in birds 3-30 years of age. In birds measured longitudinally three years apart, thyroid hormone levels and hematocrit were both lower, suggesting progressive changes within individuals rather than selective disappearance of particular individuals. I found no effect of age on dive depth, dive duration, descent rate or dive efficiency or wing beat frequency during flight.
4. Declining metabolism with age in long-lived murres with high-energy costs contrasts with findings for long-lived birds with low energy costs that do not show a decline in resting metabolic rate with age. Presumably, birds studied to date with low energy costs either (i) already have minimal energy costs, (ii) experience less advantage owing to strategic reductions in energy expenditure, or (iii) experience fewer costs associated with high energy expenditure.

5. Physiological changes occurred in tandem with advancing age in murres, but offset each other such that there was no detectable decline in behavioural performance.

Introduction

As many diving animals are both long-lived and regularly encounter hypoxic environments that can cause tissue damage, they may be particularly susceptible to senescence (Hindle *et al.* 2009a; Beaulieu *et al.* 2011; Le Vaillant *et al.* 2013). Actuarial senescence, the increasing rate of mortality with age, is more important in long-lived than short-lived wild animals as a higher proportion of mortality is attributable to senescence in the former relative to the latter (80% vs. <10%, respectively; Ricklefs 2008; 2010; Turbill & Ruf 2010). Furthermore, breath-hold diving creates a hypoxic environment for tissues, and the regular hypoxic exercise associated with diving should result in chronic hypoxia (Hindle *et al.* 2009a,b; 2010). Specifically, the use of alternative metabolic pathways (glycolysis) and lactate build-up should result in the increased production of free oxygen radicals by mitochondrial complex I during glycolysis and/or the suboptimal performance of antioxidant enzymes due to changes in pH (Hulbert 2006; Hindle *et al.* 2009a,b). Furthermore, repeated ischemia-reperfusion at the surface may lead to potentially harmful damage to tissues (Hoerter *et al.* 2004; Hindle *et al.* 2009a,b; Beaulieu *et al.* 2011). Thus, diving animals may be particularly susceptible to senescence associated with oxidative damage. Although breath-hold divers are expected to be good models for measuring senescence in the wild because of their potential for tissue damage, few studies have examined variation in dive performance with age. Those studies that have examined variation in dive performance with age emphasized behavioural aspects

rather than physiological aspects, and focused largely on patterns at the start of life (Zimmer *et al.* 2011; Le Vaillant *et al.* 2012).

Metabolism, measured in aerobic organisms as the rate of oxygen utilization, constitutes an important constraint on dive duration, and so variation in metabolism with age is likely to have an impact on dive performance. Maintenance or resting metabolism may be particularly important because during deep dives—those dives where lactate production may lead to the greatest changes in pH and that are most likely to cause tissue damage—animals enter a state of hypometabolism where metabolic rate is reduced to near or below resting levels (Niizuma *et al.* 2007; Meir *et al.* 2008; Ponganis, Meir & Williams 2010). Although no study has examined variation in resting metabolism with age in a diving organism, several studies documented a decline in resting metabolic rate with age (Blackmer *et al.* 2005; Moe *et al.* 2007). I use the term resting metabolic rate, rather than basal metabolic rate, as many studies reporting basal metabolic rate violate the strictest definition of basal metabolic rate and in the context of a relationship with aging the difference is likely relatively unimportant. Indeed, most homeotherms show a declining trend in metabolism with age although patterns vary even within a taxonomic group, such as rodents (Table 4.1). Researchers adopting a longitudinal approach have demonstrated that declines in metabolism with advancing age occur within individuals rather than through the selective mortality of individuals with a high resting metabolic rate (Moe *et al.* 2009; Broggi *et al.* 2010). Changes in whole-body metabolism may reflect changes in body composition, such as changes in the volume of metabolically intense tissue, or they may represent reductions in average tissue metabolic intensity. Hypothyroidism—a decline in thyroid hormone levels—is a typical consequence of

human aging, especially for females (e.g. Spaulding 1987; Djordjevic *et al.* 1990; but see Piers *et al.* 1998). As thyroid hormone levels are associated with resting metabolic intensity, measurement of thyroid hormones alongside metabolism provides the possibility of separating changes in resting metabolism due to changes in body composition from changes due to metabolic intensity.

Dive performance will also depend on oxygen stores. Whereas most oxygen stores reside in the muscles of larger animals, blood oxygen stores are more important for small animals, where >50% of oxygen can be stored as haemoglobin and <10% as myoglobin (McIntyre, Campbell & MacArthur 2002; Elliott *et al.* 2010b). Hematocrit (the ratio of packed red blood cell volume to total blood volume) is strongly correlated with hemoglobin content (Elliott *et al.* 2008; Elliott *et al.* 2010b) and is therefore a good indicator of oxygen stores available to small divers. As hematocrit is often positively associated with aerobic scope (the ratio of maximum energy output to basal energy output; see Bishop 1999), and aerobic scope declines with age in mammals (Stones & Kozma, 1985; Goldberg, Dengel & Hagberg 1996; Chappell, Rezende & Hammond 2003), it is reasonable to assume that hematocrit would also decrease with age in diving animals.

If there is one animal that may be pushed to the limit when expending energy, then, it would be a breath-hold diving bird that experiences both high metabolism and the demands of diving (Hindle *et al.* 2009a,b; Beaulieu *et al.* 2011; Le Vaillant *et al.* 2012). To examine how diving behaviour, metabolism and oxygen stores change with age in long-lived animals, I studied the thick-billed murres (*Uria lomvia*), a deep-diving bird with exceptionally high-energy costs, that regularly exceeds its aerobic dive limit (or dive

lactate threshold; Croll *et al.* 1992; Elliott *et al.* 2008a; Elliott *et al.* 2008b; 2013). I hypothesized that its ecology would cause high levels of senescence and predicted reduced (i) dive performance, (ii) resting metabolic rate, (iii) thyroid hormone levels and (iv) hematocrit with age.

Materials and methods

I studied dive behaviour (2004-11; chicks 3-15 d post-hatch: N = 147 males, 163 females; incubation: 52 males, 34 females) and flight behaviour (2009-11; all chick-rearing; see sample sizes in following section) of breeding thick-billed murres, at Coats Island, Nunavut (62°57'N, 82°00'W). I also studied oxygen stores, resting metabolism and thyroid hormone levels during the latter half of incubation (eggs 15-25 d) from 10-20 July 2008, 1-20 July 2009 and 13-24 July 2011. Flight costs for murres are the highest activity-specific costs, as a multiple of basal metabolic rate, of any homeotherm measured to date (Shaffer 2011; Elliott *et al.* 2013). Murres also dive much deeper and for longer periods of time than equivalent-sized penguins, apparently regularly exceeding their calculated aerobic dive limit (Croll *et al.* 1992; Watanuki & Burger 1999; Elliott *et al.* 2008b; 2010b). Because of their exceptional lifestyles in terms of both diving performance and overall energy expenditure, murres might be particularly susceptible to metabolism-related senescence. While previous research examining age-related changes in metabolism among long-lived seabirds have focused on procellariiforms (Blackmer *et al.* 2005; Moe *et al.* 2007) with low daily energy expenditure (Shaffer 2011), studies of long-lived wild birds have detected few signs of declining immunity (Lecomte *et al.* 2010; Apanius & Nisbet 2006), reproduction (Nisbet, Apanius & Friar 2002; Coulson & Fairweather 2003) or metabolism (Galbraith *et al.* 1999; Blackmer *et al.* 2005; Moe *et al.*

2007) with age that are characteristic symptoms of aging in mammals and short-lived birds (Cichon, Sendecka & Gustafsson 2003; Saino *et al.* 2003; Moe *et al.* 2009; Palacios *et al.* 2007; Cote *et al.* 2010; Holmes & Ottinger 2003; these ideas are reviewed by Nisbet 2001; Ricklefs 2010; Holmes & Martin 2009).

At my Arctic study sites, air temperatures were usually moderate (10-20°C), the night was brief (2-4 h depending on date) and birds generally foraged actively throughout the day, including the brief night. Birds were captured at the nest with a noose pole. Both parents share incubation and chick-rearing duties, at least during the period of time covered by the current studies. Only one adult per nest was captured. I determined the sex of murres using sex-specific PCR primers (Elliott *et al.* 2010a). I did not correct body mass for linear size, as such corrections do not improve estimation of lean or lipid mass in murres (Jacobs *et al.* 2012). All procedures were approved under the guidelines of the Canadian Council on Animal Care (University of Manitoba F11-020).

I worked with murres 3-30 years old, which should provide an ample age range over which to detect signs of senescence, as the oldest wild large auks recorded by the North American bird banding lab (www.pwrc.usgs.gov/bbl/longevity/Longevity_main.cfm) were 33 (Atlantic puffin, *Fratercula arctica*) and 28 years old (rhinoceros auklet, *Cerorhinca monocerata*; razorbill, *Alca torda*; and both species of murres, *U. lomvia* and *U. aalge*) and the UK lab (http://blx1.bto.org/ring/countyrec/results2011/longevity.htm) were 41 (razorbill—one exceptionally old bird recorded), 35 (Atlantic puffin) and 32 years old (common murre). Furthermore, declines in survival and reproduction were observed in common murres in Britain apparently in their mid-20s (Crespin *et al.* 2006; Lewis *et al.* 2007). I also provide hematocrit and metabolism data from chicks (0 years

old) for comparative purposes, but I do not include chicks in statistical analyses as metabolism in chicks is presumably impacted by growth.

DIVING AND FLYING BEHAVIOUR

I attached Lotek (Canada) LTD1100 (5 g; 3 s continuous recording; 2004-2007), LAT1400 (5 g; 15 s continuous recording, 1 s continuous recording when depth > 5 m; 2008) and LAT1500 (3 g; 15 s continuous recording, 1 s continuous recording when depth > 3 m; 2008-2011) time-depth recorders to the legs of parental murres and extracted dive behaviour (frequency, depth and duration) from the pressure log and time budgets (time spent flying, resting on water and resting at the colony) from the temperature log (Elliott *et al.* 2008a; 2009). The leg-mounted recorders do not measurably impact murre behaviour (Elliott *et al.* 2007). I also attached Little Leonardo (Japan) UME-D2GT accelerometers (17 g; 1 s continuous recording of depth, 32 Hz continuous recording of acceleration in surge and heave directions; see Watanuki *et al.* 2003) in 2010-11 (N = 66) and CATTRAQ (Catnip Technologies, USA) GPS loggers (25 g; 2 min continuous recording, 13 s continuous recording when speed about 4 m/s) in 2010-11 (N = 38) to the back of parental murres using Tesa tape. I recorded body mass before and after each deployment.

At Coats Island, males forage primarily at night and females during the day (Elliott *et al.* 2010b). Average dive depth is shallower for males because they feed at night when visibility is limited. At a given time of day, there is no apparent difference in dive depth between males and females, except in the late afternoon (Fig. 4.1), where females dive deeper than males, probably because males are pursuing risk-averse prey types (amphipods) through the water column at that time (Elliott *et al.* 2010b). To exclude any

effect of time of day and sex on dive depth, I included only measurements between 06h00 and 16h00. Foraging behaviour occurs along three major axes in murres at my study site (Elliott *et al.* 2008b): dive depth (prey depth), dive shape (prey habitat, benthic vs. pelagic) and effort (prey energy quality); all foraging variables are correlated with one of those three axes. I therefore recorded dive depth (and duration), dive shape (time allocation at depth index as described in Elliott *et al.* 2008b) and several metrics of effort: descent rate as a residual on dive depth, surface pause duration as a residual on dive depth and dive efficiency (time spent at the bottom divided by total time in the dive cycle, Croll *et al.* 1992).

I used the Fast Fourier Transform in Igor Pro (WaveMetrics, Lake Oswego, USA) to determine wingbeat frequency during flight. I used both the x and y components, excluding the first and last 15 s of flight (to avoid changes in wingbeat frequency associated with take-off and landing; Sato *et al.* 2007). I considered the frequency with the strongest maximum in the frequency domain to reflect the wingbeat frequency, and averaged that value across the x and y components after accounting for the effect of hourly wind speed as measured at the Environment Canada weather station at Coral Harbour (Sato *et al.* 2007). I considered only the first flight leaving the colony, as mass may be higher during subsequent flights if the bird has fed or is returning with a fish. I used the GPS logger to measure ground speed. The speed distribution was strongly bimodal with a minimum (<0.1%) of measurements at 5 m/s and I considered a flight to occur when there were six consecutive measurements >5 m/s.

OXYGEN STORES

I used hematocrit as a measure of oxygen stores in murres. Hematocrit is closely correlated with hemoglobin content in murres (Elliott *et al.* 2008a). Muscle oxygen stores are low in murres due to the conflicting demands of flight (Croll *et al.* 1992; Elliott *et al.* 2010b) and respiratory stores may be adjusted to dive depth (Elliott, Gaston & Davoren 2007) and are therefore lower in most dives than the maximal values assumed in most calculations of oxygen store partitioning (e.g. Croll *et al.* 1992; Elliott *et al.* 2010b). Blood oxygen stores are therefore a major source for energy production for diving murres (52% of overall oxygen stores compared with 3% for muscle oxygen stores; Elliott *et al.* 2010b). I measured hematocrit in 2008 (N = 62) and 2011 (N = 31, same birds used for respirometry), including, in 2011, 20 individuals that were also sampled in 2008.

For 10 birds, I also measured plasma volume using Evans blue dye (Croll *et al.* 1992). I injected the birds with Evans blue dye (0.2 mL of 2.5 g/ L) in the brachial vein and obtained a blood sample from the opposite wing 15 min later (Croll *et al.* 1992). I centrifuged the samples for 15 min at 10 000 *g* to remove turbidity. I measured absorbance at 615 and 415 nm using a Biotek plate reader (Fisher Scientific, Ottawa, Canada) and determined plasma volume using pooled plasma diluted with a known concentration of Evans blue dye. The correlation coefficient between dilution and the difference in absorbance for the standard curve was R = 0.99.

RESTING METABOLIC RATE

I used open-flow respirometry with a FoxBox II® (Sable Systems, Las Vegas, USA) system to measure metabolic rate of incubating birds in a respirometry chamber. Birds were in their thermoneutral zone (~15 °C) and there was no diel rhythm in metabolic rate

during my measurements (0430-2030 solar time) at my Arctic study site. Birds were likely post-absorptive during the final measurement periods as they were held for four hours and captured as they were switching off from their incubation shift (~12 hr for murres, Elliott *et al.* 2010). Resting metabolic rate increased with body mass (Elliott *et al.* 2013) and I used the residual of resting metabolic rate on body mass for all analyses. Further details of resting metabolic rate measurements are presented elsewhere (Elliott *et al.* 2013).

THYROID ASSAYS

Thyroid hormones provide an alternative index of basal metabolic rate. Upon capture, I obtained 1 mL of blood from the alar vein of the same birds used for hematocrit measurements. Blood samples were stored on ice for <4 h, centrifuged at 2000 *g*, the plasma was removed and stored at –20°C for the remainder of the field season (1-2 months) and then shipped to the University of Manitoba, Canada, on dry ice and stored at –80°C until analysis. I determined both total and free T3 and T4 concentrations in duplicate by radioimmunoassay using a commercially available kit on unextracted plasma (MP Biomedical kits 06B258710, 06B257214, 06B254216, 06B254029). I also measured thyroid hormone levels in the same murres sampled for hematocrit in 2008 (N = 46) and re-sampled in 2011 (N = 20). Thyroid hormone levels correlated with resting metabolic rate in murres (Chapter Three; see also Welcker *et al.* 2013). More details of the thyroid hormone measurements are presented elsewhere as the current measurements occurred on the same assays as those reported therein (Chapter Three).

Results

DIVE BEHAVIOUR

There was no relationship between age and a variety of diving parameters (Fig. 4.2; Table 4.2). Those relationships were also non-significant for incubating birds (all $P > 0.15$). Likewise, for those chick-rearing birds resampled 4-8 years later, dive depth, residual surface interval on dive depth, time allocation at depth index and descent rate index were no different (paired t-test, all $P > 0.20$). Wingbeat frequency decreased with tail wind strength ($t_{54} = -8.91$, $P < 0.0001$) but was independent of age. Flight speed increased with tail wind strength ($t_{54} = 3.51$, $P < 0.0001$) but was independent of age.

OXYGEN STORES

Hematocrit declined with age in murres (2008: $t_{60} = -3.70$, $P = 0.0005$, excluding chicks; 2011: $t_{29} = -4.04$, $P = 0.0004$, Fig. 4.3). In birds resampled three years later, hematocrit had also declined ($t_{19} = -3.42$, $P = 0.003$). Likewise, plasma volume showed a declining trend with age, but that trend was not statistically significant ($t_9 = -1.20$, $P = 0.26$), and therefore blood oxygen stores declined with age ($t_9 = -3.32$, $P = 0.009$).

RESTING METABOLIC RATE AND THYROID HORMONE LEVELS

Resting metabolic rate decreased with age ($t_{42} = -2.75$, $P = 0.009$, excluding chicks; Fig. 4.4a). Hematocrit was correlated with resting metabolic rate ($t_{31} = 4.31$, $P < 0.001$, $R^2 = 0.61$). Total plasma T3 ($t_{42} = -2.71$, $P = 0.01$, excluding chicks; Fig. 4.4b), but not total T4 ($t_{42} = -0.31$, $P = 0.76$), declined with age. In birds resampled three years later, total T3 also declined ($t_{19} = -2.55$, $P = 0.02$), but T4 did not ($t_{19} = -0.25$, $P = 0.81$). The ratio of free to bound T3 increased weakly with age ($t_{46} = 2.44$, $P = 0.02$, $R^2 = 0.12$), implying a reduction in capacity with age.

Discussion

Blood oxygen stores, resting metabolism and thyroid hormone levels declined with age, supporting three of my four predictions about aging in murres. In contrast, I observed no change in dive or flight performance. Under the assumptions that blood oxygen stores are indicative of total oxygen stores and that maintenance costs (resting metabolic rate) make up a substantial proportion of dive costs during deep dives, a decline in oxygen stores may be balanced by a decline in oxygen utilization rate:

$$dive\ duration \propto \frac{Oxygen\ stores}{Oxygen\ utilization\ rate};$$

as both the numerator and denominator decrease in tandem, there is no effect on dive duration. Apparently, physiological changes do occur with age in this long-lived species, but have no detectable effect on behavioural performance.

Resting metabolic rate also declined with age (Fig. 4.4). One possible explanation is the selective disappearance of birds that expend more energy (Blackmer *et al.* 2005; Moe *et al.* 2007; 2009). However, as I also observed declines in thyroid hormone levels with age within the longitudinal component of my study, I believe that my results represent a decline in metabolism within individuals. Four observations made elsewhere support a connection between elevated metabolism and reductions in metabolism with age: (1) in contrast to my results, resting metabolic rate was independent of age in procellariform seabirds with low daily energy expenditures (Blackmer *et al.* 2005; Moe *et al.* 2007); (2) there was a decline in metabolic rate with age in great tits (*Parus major*) at a site with high winter metabolic rate, but not at a site with low winter metabolic rate (Broggi *et al.* 2010); (3) the decline in metabolic rate with age (effect size in Table 4.1) was higher for

short-lived animals with high mass-specific metabolic rates than for long-lived animals with low mass-specific metabolic rates (see also Shoyama *et al.* 2011); and, (4) in different mutants of round worms (*Caenorhabditis elegans*), lifespan explains 91% of the variation in the rate of decline in metabolism with age (Shoyama *et al.* 2009). Although resting metabolic rate declined with age, I do not believe that aerobic scope declined with age as (1) time spent flying, which is the main determinant of daily energy expenditure in murres (Elliott *et al.* 2013), did not vary with age suggesting that daily energy expenditure does not vary with age; (2) resting metabolic rate declined with age, which would tend to offset any change in maximum aerobic performance; and, (3) wingbeat frequency and flight speed, which would represent performance near maximum output did not vary with age.

Reductions in metabolism with age can be viewed as strategic restraint on the part of individuals that are likely to encounter energy-related senescence or non-adaptive deterioration associated with an energetically expensive lifestyle. Those declines occurred roughly linearly with age in murres (Fig. 4.4), as is the case in adult humans, implying that mitochondrial respiratory enzymes also likely decline linearly with age (Trounce, Byrne & Marzuki 1989; Yen *et al.* 1989; Short *et al.* 2005, and references in Table 4.1). In contrast, metabolism in invertebrates declines exponentially with age (Shoyama *et al.* 2009). Murre ageing patterns therefore best fit a model of constant decay, whereby the number of mitochondria being produced is being constantly outweighed by a fixed amount by the number of mitochondria destroyed throughout an individual's lifespan (Shoyama *et al.* 2009).

As thyroid hormone levels, like resting metabolic rate, declined with age, reduction in metabolism with age apparently reflects reduced metabolic intensity rather than a change solely in body composition. Hypothyroidism is a typical consequence of human aging (e.g. Spaulding 1987; Djordjevic *et al.* 1990; but see Piers *et al.* 1998). Presumably, as is the case in laboratory studies (Hunt *et al.* 2006), declines in basal metabolic rate with age are associated with declines in mitochondrial energy production. Further study using magnetic resonance could elucidate whether an increase in the proportion of metabolically-inert tissues, such as lipids (~9% the metabolic rate of protein, Scott *et al.* 1996), also play a role in declining basal metabolic rate with age. Furthermore, plasma capacity declines with age in mammals and similarly the concentration of thyroid binding proteins, primarily transthyretin in birds, appeared to decline with age in murres as the ratio of free to bound T3 increased weakly with age (e.g. Spaulding, 1987; Wiener *et al.* 1991). In humans, a decline in thyroid hormone levels can occur either through a decline in thyroid-stimulating hormone (TSH) with downstream effects on thyroid hormone levels or a decline in thyroid hormone synthesis with no change in TSH, possibly due to changes in the morphology of the thyroid follicular cells (e.g. Spaulding, 1987; Wiener *et al.* 1991). Similar adjustments in thyroid status apparently occur in aging long-lived murres, although I have no information on whether the declines originate at the level of the brain, thyroid or periphery.

Hematocrit declined with age (Fig. 4.3), possibly reflecting declining bone marrow cell replication rates with age. As hematocrit is often used as an index of body condition (e.g. Donnelly & Sullivan, 1998; Murphy, 2010), one interpretation of my results would be that older birds are in poorer body condition. Alternatively, hematocrit is known to

correlate with metabolic rate in many animals because substantial oxygen carrying abilities are required to sustain high oxygen consumption rates (Carpenter 1975; Palomeque & Planas 1978)—although the two can clearly be de-coupled as in the case of young murres with low hematocrit and high metabolism (Fig. 4.3). If hematocrit directly responds to metabolism, then declining hematocrit with age may be a direct consequence of the declining resting metabolism with age that I measured. Finally, lower hematocrit equates to lower blood viscosity (Williams 2011), which in turn could reduce the susceptibility of old birds to heart attacks or other cardiovascular disease. Regardless, hematocrit is strongly correlated with blood hemoglobin concentration and blood haemoglobin stores represent ~50% of oxygen in diving auks (Croll *et al.* 1992; Elliott *et al.* 2010b), and thus declining hematocrit with age implies reduced oxygen availability during the dive.

Behavioural performance was maintained into old age as no changes in any component of flight or dive behaviour were detected over the age range of birds tested. Similarly, old common terns (*Sterna hirundo*) showed no decline in daily activity levels or reproduction (Galbraith *et al.* 1999). In contrast, old male, but not female, albatrosses show behavioural deterioration with the oldest males gaining less mass per time spent at sea and foraging at different locations than young males (Catry *et al.* 2006; Lecomte *et al.* 2010). Apparently, murres are able to adjust physiological changes such that there is no net effect on behaviour, at least at my study site where foraging conditions are relatively good (Gaston *et al.* 2005; Gaston *et al.* 2009). Murres may have no option to adjust behaviour, as biomechanical constraints are too tight to allow much flexibility during flying and diving (Elliott & Gaston 2005; Thaxter *et al.* 2010).

My results provide circumstantial evidence in support of the "rate of living" theory of aging. If energy expenditure causes senescence, young birds, that are unlikely to live long enough to experience senescence, may be selected to expend maximal levels of energy to maximize abilities to establish breeding sites and maximize chick growth rates, whereas old birds may be selected to reduce energy expenditure, and their rate of senescence, as they are more likely to die of senescence-related causes. Alternatively, if high levels of energy expenditure eventually lead to the degradation of the thyroid hormone, circulatory or respiratory systems, or other systems associated with energy utilization, then reduced resting metabolic rate with age may be symptomatic of physiological decay ("senescence"). Of course, my study is purely correlational and it is possible that declines in resting metabolism with age are coincidental and not associated with actuarial senescence. Further studies examining the exact system (e.g. changes in TSH, follicle cell morphology, thyroid hormone carrying capacity, or body composition) associated with declining resting metabolism are necessary to disentangle these possibilities, alongside studies examining why wild birds die and the pathology of reduced metabolic rate.

Figure 4.1. Average dive depth (± SE) relative to time of day (GMT-5.5 or CDT-0.5) for 56 male and 48 female thick-billed murres. Average dive depth was calculated for each one-hour bin for each individual, and then averaged across individuals.

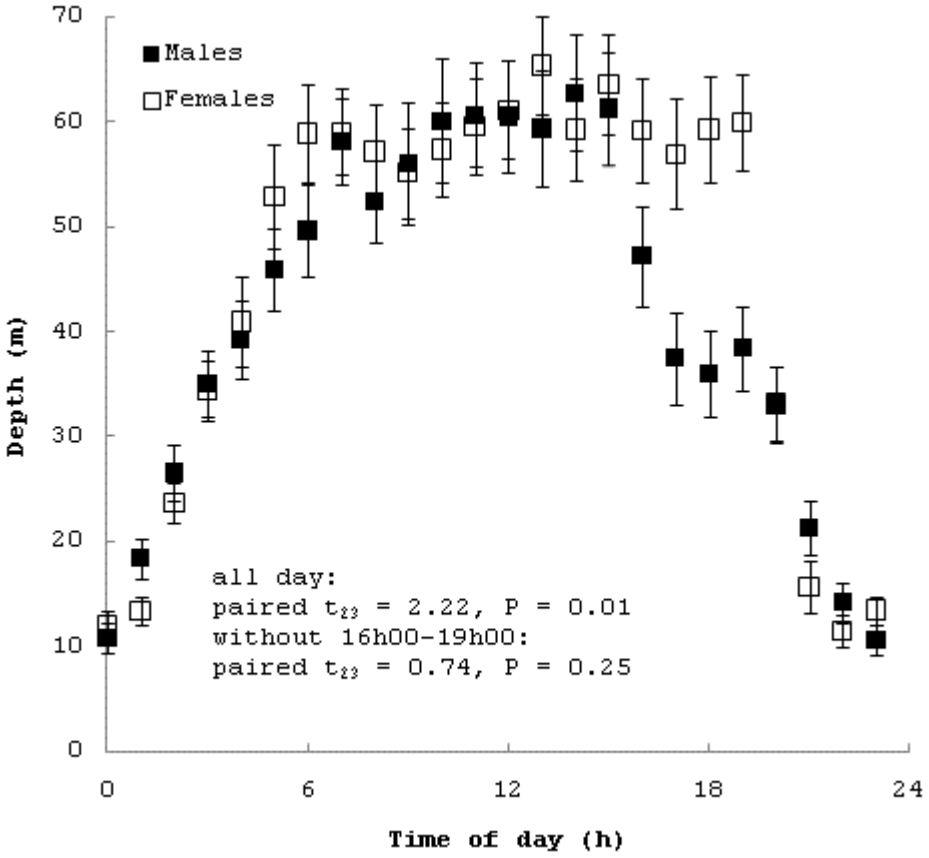


Figure 4.2. Relationship between age and (a) dive depth (06h00-16h00), (b) descent rate (after accounting for depth), (c) dive shape (time allocation at depth index) and (d) surface interval (after accounting for dive depth) in chick-rearing thick-billed murres.

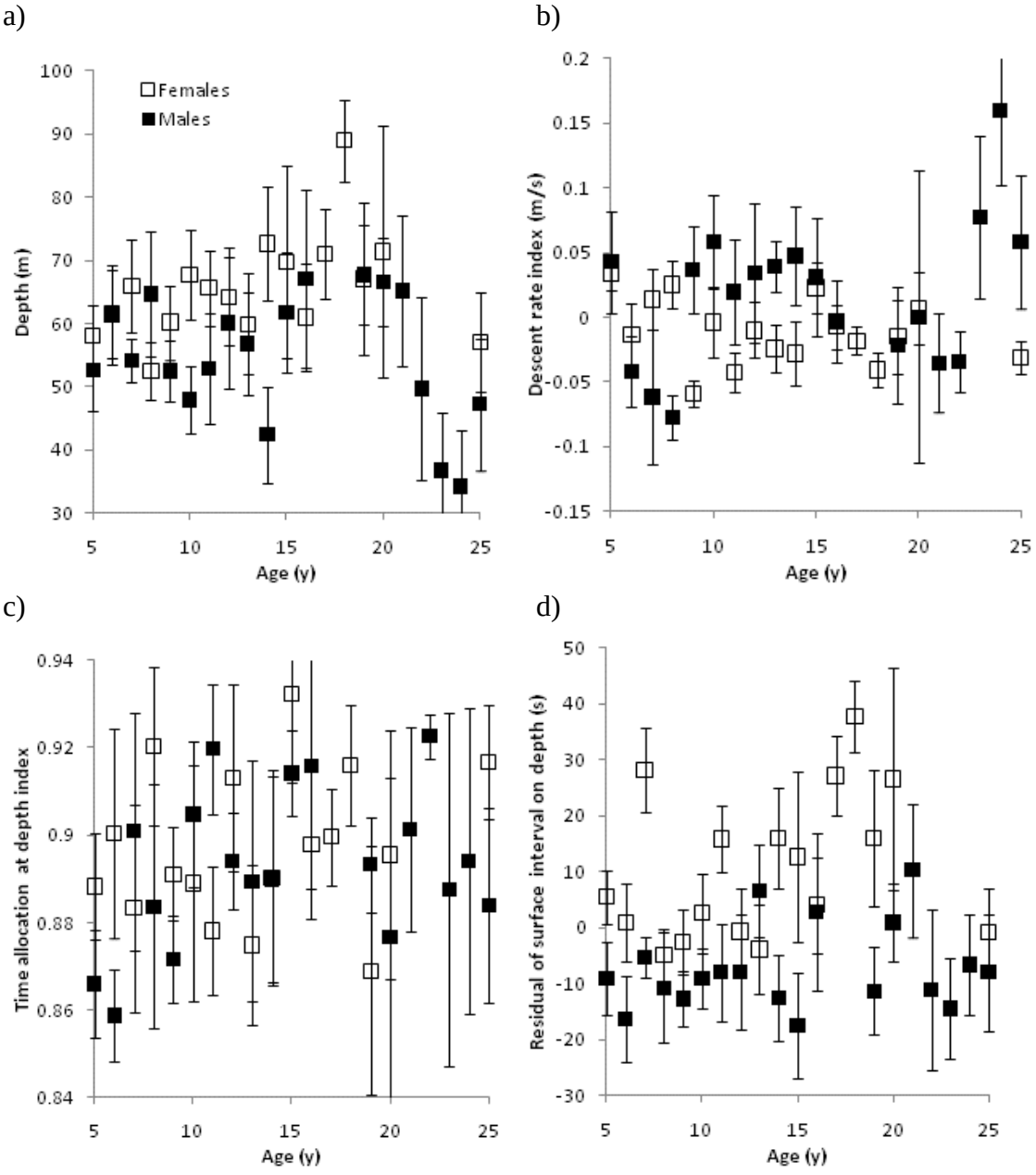


Figure 4.3. Hematocrit declined with age in wild thick-billed murres.

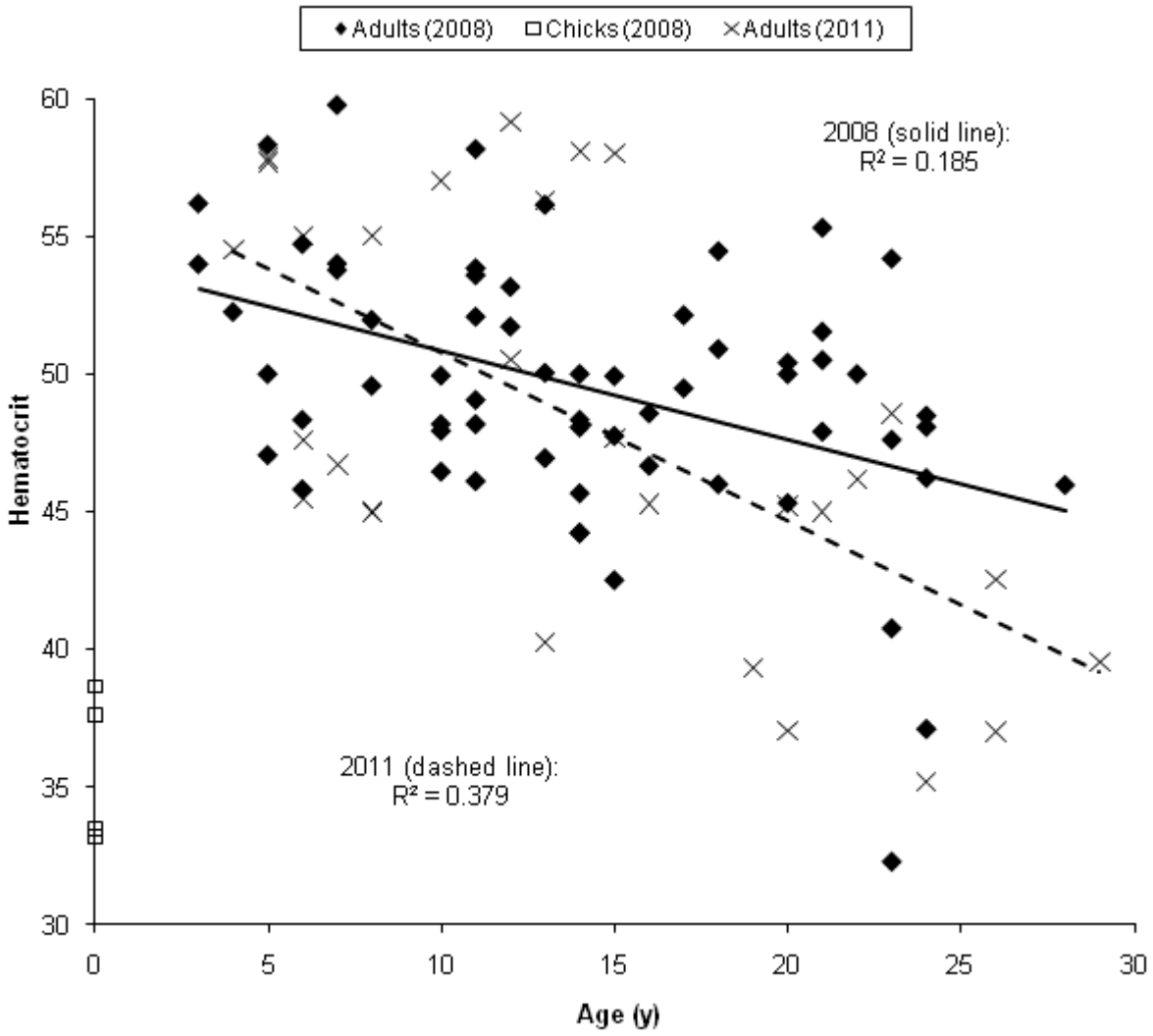
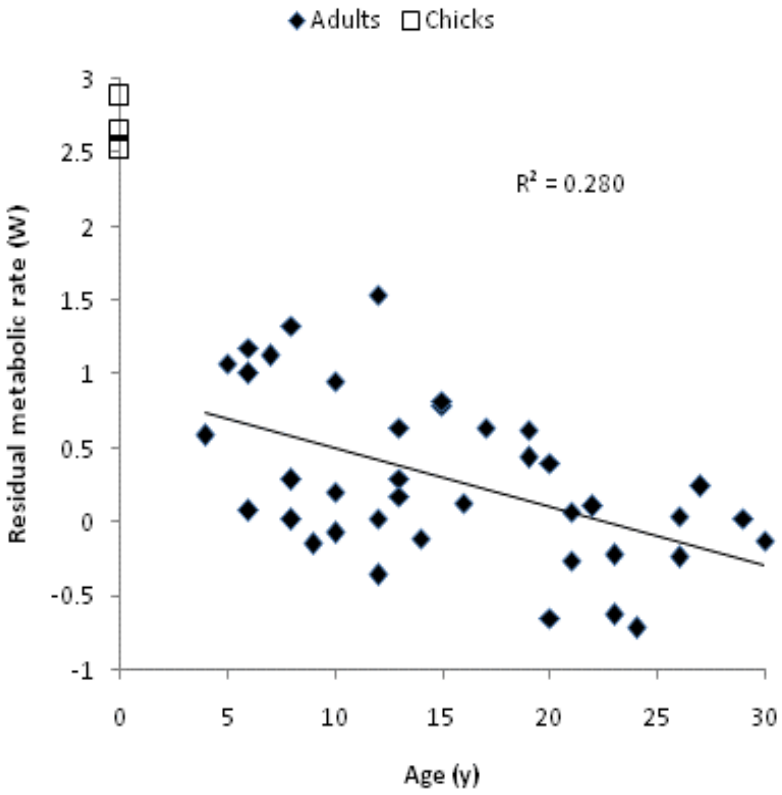


Figure 4.4 (a) Post-absorptive, resting metabolic rate (residual on body mass) and (b) triiodothyronine (T3) in wild thick-billed murres declined with age.

(a)



(b)

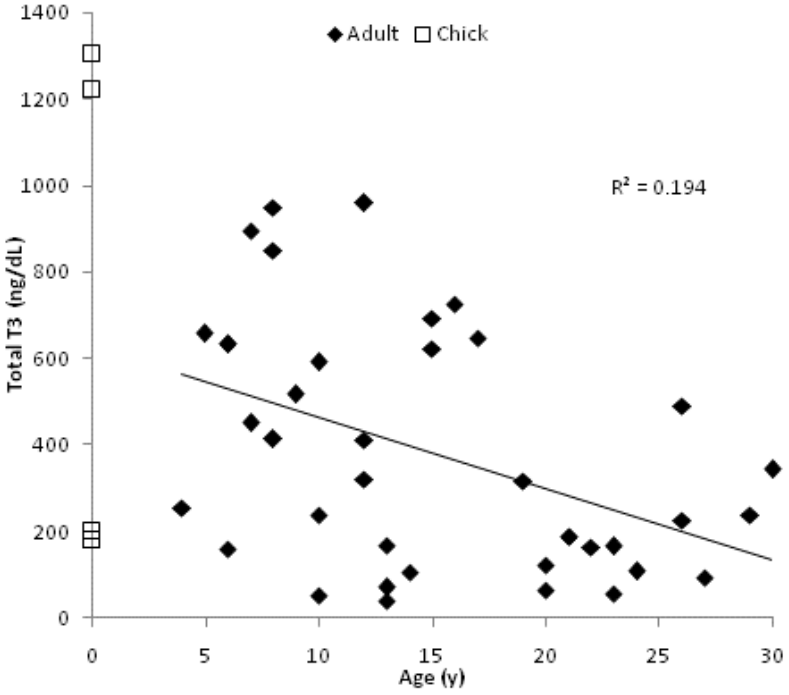


Table 4.1. Representative studies that have examined changes in resting or basal metabolism with age in animals. Statistics are presented after accounting for sex, mass and other factors, if such factors were accounted for and found significant in the study. If the authors did not present a particular statistic, I calculated the statistic from data presented in the paper. Otherwise, I present the data as "NR" (not reported). Excluding invertebrates, where changes in metabolism with age may be confounded by indeterminate growth, and averaging all values for a single species, effect size (β) was -2.13 ± 0.58 (z-test, $P < 0.0001$). Studies on species that included individuals ≥20 years of age ("long-lived species") tended to have a higher effect size than those that did not ("short-lived species"; one-tailed $t_8 = 2.08$, $P = 0.07$). The table is meant to be representative and is not an exhaustive summary of the medical literature. Studies in bold reported what they referred to as basal metabolic rate; remaining studies reported what they referred to as resting metabolic rate. W = study occurred on wild animals.

Species	N	R	B	P	Age (y)	Reference
Human *Homo sapiens*	54	NR	-1.6[3]	<0.001	25-81	Benedek *et al.* 1995
	43	NR	-1.1[3]	0.005	18-69	Ryan *et al.* 1996
	62	NR	-0.4[3]	0.006	18-77	Piers *et al.* 1998
	58	-0.57	-0.5	<0.01	23-77	Hunter *et al.* 2001
Snow petrel *Pagodroma nivea* (W)	38	-0.14	-1.0	0.30	8-39	Moe *et al.* 2007
Thick-billed murre *Uria lomvia* (W)	43	-0.51	-3.6	<0.001	3-30	My study
Leach's storm-petrel *Oceanodrama leucorhoa* (W)	94	NR	-1.5	0.14	6-28[1]	Blackmer *et al.* 2005
Naked mole rat *Heterocephalus glaber*	24	NR	0.7	0.50	1-20	O'Connor *et al.* 2002
Dog *Canis lupus*	105	-0.32	-5.1	<0.001	1-12	Speakman *et al.* 2003
Great tit *Parus major* (W)	694	NR	-5.0	<0.001	1-7	Bouwhuis *et al.* 2011
	35	NR	-3.8	0.007	1-7	Broggi *et al.* 2010
	13	NR	0.7	0.49	1-4	Broggi *et al.* 2010
Zebra finch *Taeniopygia guttata*	25	-0.37	-2.5	<0.01	1-5	Moe *et al.* 2009
Deer mouse *Peromyscus maniculatus* (male)	211	0.01	0.1	0.92	0-5	Chappell *et al.* 2003
Deer mouse (female)		-0.12	-1.2	0.23		
Rat *Rattus norvegicus*	12	NR	-2.4[2]	<0.001	1-2	Even *et al.* 2001[3]
Fruit fly *Drosophila* sp.	NR	NR	NR	NR[4]	0-<1	Promislow and Haselkorn, 2002
Mussel *Mytilus edulis*	175	NR	-0.5	0.64[5]	2-10	Sukhotin *et al.* 2002
Flatworm *Schmidtea polychroa*	28	NR	NR	0.82	0-3	Mouton *et al.* 2011
Roundworm *Caenorhabditis elegans*	100	-0.88[6]	-5.8	<0.001	0-<1	Shoyama *et al.* 2009
Milkweed bug *Oncopeltus fasciatus*	60	-0.86	-5.5[7]	<0.001	0-<1	McArthur and Sohal, 1982

[1]Reported as "breeding age" of 1-23 years; Leach's storm-petrels begin breeding around age 5 (Blackmer *et al.* 2005) and I added five years to each age.

[2]Effect size calculated from t-test comparing young adults with old individuals; the authors do not present a regression against age.
[3]Miyasaka *et al.* (2003) also show declining metabolism in rats between 12-44 weeks, but their sample may be biased in the context of senescence due to growth effects in young rats. Greenberg (1999) also shows a decline in rat organ basal metabolic rate between 6 months and 18 months, but does not present statistical analyses or the data in a fashion simple to reanalyze for my table.
[4]Report that metabolism showed no significant relationship with age, except in one species (*D. melanogaster*), where metabolism increased with age ($P < 0.00014$).
[5]The authors report a significance of 0.02 on an ANOVA with each year class as a categorical variable, as three year classes have significantly lower metabolic rates. I reanalyzed the presented data in their Fig. 2b with age as a continuous variable (linear regression) to be compatible with the other studies in my table.
[6]Statistics averaged across all eight strains. See also Shoyama *et al.* (2007) and Braeckman *et al.* (2002).
[7]We calculated statistics separately for all three temperature regimes, starting at the age when mortality began (20 days for 30°C and 18°C, 30 days for 25°C) and then averaged statistics across all three groups or, for P-value, included group as a covariate.

Table 4.2. Relationship between age and dive behaviours for 310 wild, known-age thick-billed murres. The coefficients of determination are calculated across individuals (not averaged by age class, as in Fig. 2) and on best-fit quadratic relationships to provide the possibility of either linear or non-linear changes with age. In each case, sex was also included as a covariate.

Metric	t_{308}	P	R^2
Dive depth	0.40	0.69	<0.01
Dive duration	0.45	0.65	<0.01
Residual surface pause on depth	0.34	0.73	<0.01
Residual descent rate on depth	0.52	0.50	<0.01
Time diving per day	0.21	0.83	<0.01
Dive efficiency	-0.52	0.61	<0.01

Chapter Five. Patterns of aging in two long-lived charadriiform birds: partitioning of energy into different physiological systems changes with age within an overall energy ceiling

Kyle H. Elliott, Lorraine Chivers, John R. Speakman, Yan Ropert-Coudert, Anthony J. Gaston, Scott A. Hatch, W. Gary Anderson, and James F. Hare

My contribution: I conducted all the field work (with field assistants, and for the GPS loggers on kittiwakes in collaboration with L. Chivers, and with logistical support from T. Gaston and S. Hatch), except for work partly included in retrospective survival, reproductive and foraging analyses, and laboratory work (with laboratory assistants; except for the doubly-labelled water analyses, which were completed at J. Speakman's lab), completed all statistical analyses and wrote the paper (with comments from G. Anderson and J. Hare). Y. Ropert-Coudert supplied loggers and helped design some of the experiments.

Summary

1. A substantial proportion of mortality in long-lived wild animals is believed to be associated with senescence. However, several studies have failed to detect physiological senescence in long-lived wild animals, especially birds. Most studies to date have examined only a single parameter or parameters associated with only a single physiological system.
2. To examine physiological and behavioural senescence in two species of long-lived birds, I assessed 29 physiological traits and seven behavioural traits alongside five demographic parameters in two species of long-lived wild charadriiform seabirds (thick-billed murres *Uria lomvia* and black-legged kittiwakes *Rissa tridactyla*). For many of the parameters, I obtained values three years later from the same individual, demonstrating that patterns occurred longitudinally within individuals and were thus not attributable to the selective appearance or disappearance of individuals.
3. Mortality increased with age, whereas reproductive success increased with age and then declined with age in the oldest individuals. Although lay date became earlier with age, until old age when the trend reversed, there was no relationship between age and egg size, clutch size or either circulating or LHRH-induced levels of testosterone and estradiol. There was also no change in diet, foraging locations, chick provisioning/growth rate or time budgets with age, and consequently daily energy expenditure did not change with age. In contrast, several measures of resting metabolic rate or intensity declined with age and levels of superoxide dismutase, an antioxidant, in the blood increased with age.

Taken together, these traits suggest an increase in defense against oxidative stress as the animal ages.

4. Cell-mediated immune function and/or the inflammatory response declined with age, whereas antibody-mediated immune function did not change with age, suggesting that individuals switched from innate to memory-based defenses as they aged. Cholesterol levels increased while hematocrit decreased with age.
5. My data demonstrate that although all individuals faced the same overall energy ceiling, birds from different age classes directed that energy towards different physiological components (resting metabolism, antioxidants, immunity). Interestingly, I found changes in physiology with age in the absence of changes in behaviour. I interpret those findings in the light of the rate of living theory of aging.

Introduction

Actuarial senescence, the increasing rate of mortality with age, is more important in long-lived animals than short-lived animals as a higher proportion of mortality is attributable to senescence in the former relative to the latter (80% vs <10%, respectively; Ricklefs 2008; 2010; Turbill & Ruf 2010). Although most long-lived animals may not die directly from cancer or neural disease, progressive deterioration with age appears to cause older animals to be more susceptible to mortality, whether as a result of predation, disease or other extrinsic or intrinsic causes. As many long-lived wild animals apparently live long enough to experience senescence, it should be easier to detect physiological and behavioural senescence in long-lived than in short-lived wild animals. In contrast, long-lived wild birds show few signs of declining immunity (Lecomte *et al.* 2010; Apanius &

Nisbet 2006), reproduction (Nisbet, Apanius & Friar 2002; Coulson & Fairweather, 2003) or metabolism (Galbraith *et al.* 1999; Blackmer *et al.* 2005; Moe *et al.* 2007) with age that are characteristic symptoms of aging in mammals and short-lived birds (Moe *et al.* 2009; Palacios *et al.* 2007; Holmes & Ottinger 2003; these ideas are reviewed by Nisbet 2001; Ricklefs 2010; Holmes & Martin 2009). Given the apparent importance of senescence in the mortality patterns of long-lived animals, how are long-lived birds able to maintain their physiological health into old age? One possibility is that avian brains are particularly expensive to maintain and that cognitive processes that allow birds to interact efficiently with elements of their environment (e.g. prey, predators, conspecifics) deteriorate prior to other physiological systems (Driscoll *et al.* 2003; Pitnick, Jones & Wilkinson 2006). That idea is supported by reduced behavioural, but not physiological, performance in older albatrosses (Catry *et al.* 2006; Lecomte *et al.* 2010).

Why do animals senesce? Given the enormous number of potential causes of death, I currently have little understanding of which physiological system first undergoes senescence. Presumably, many physiological and behavioural traits are inextricably linked, and examining only a single marker can lead to misidentification of the primary determinant of aging (Lecomte *et al.* 2010). There is therefore a crucial need to examine many different biomarkers (both behavioural and physiological) over the full natural lifespan in wild animals.

Studies of physiological or behavioural aging in long-lived animals are usually cross-sectional (Galbraith *et al.* 1999; Nisbet *et al.* 2002; Blackmer *et al.* 2005; Apanius & Nisbet 2006; Moe *et al.* 2007; Lecomte *et al.* 2010) because to follow an individual that lives ~30 years over its entire lifespan would require a researcher's entire active career.

Furthermore, many of the current techniques for measuring physiology or behaviour of wild animals were unavailable or impractical in a field environment 30 years ago. Nonetheless, because particular traits may be associated with reduced survival or delayed recruitment, the selective appearance or disapperance of individuals with that trait may cause age-related patterns in the absence of senescence within a particular individual (Forslund & Pärt 1995; van de Pol and Verhulst 2006). Therefore, there is a growing need for longitudinal studies of aging within the same individuals (Nussey *et al.* 2008; Moe *et al.* 2009; Broggi *et al.* 2011; Riechert, Chastel & Becker 2012) , even for long-lived animals.

Organ systems associated with metabolism may be of particular interest, especially for long-lived birds. For a given body mass, birds live longer than their mammalian counterparts despite having a higher metabolism (Hulbert 2007). The classic "rate of living" theory claims that metabolism determines life expectancy so that organisms with a higher metabolism will experience a reduced life span (Rubner 1908; Pearl 1922). In particular, the free radical theory of aging postulates that reactive oxygen species are a byproduct of metabolism that cause cellular dysfunction resulting in cumulative oxidative stress that ultimately causes organ malfunction and death (Harman 1956). Under these scenarios, it could be expected that long-lived birds would be particularly susceptible to mortality associated with high metabolism and oxidative stress. As reviewed in Chapter Three, many animals (and especially those with high metabolism) show a reduction in resting metabolism with age. However, fewer studies have examined daily or maximal energy expenditure, which may be more important in the context of oxidative stress. One study found no change in daily energy expenditure with age in common terns (*Sterna*

hirundo; Galbraith *et al.* 1999). As many chick-rearing birds are believed to be working at their maximal possible level, and constrained by internal physiology (Chapter Two; Welcker *et al.* 2010), changes in levels of daily energy expenditure may be accomplished by varying physiology with age.

The effect of oxidative stress on aging is affected by the balance between the production of reactive oxygen species (ROS) and antioxidant or damage repair enzymes that remove or mitigate ROS (Monaghan 2009). There are many steps within that system that can be influenced. For instance, fewer ROS are generated per oxygen molecule, ROS production is localized to mitochondrial complex I and peroxides are generated at a slower rate in birds compared to laboratory rodents (Ku & Sohal 1993; Lopez-Torres *et al.* 1993; Barja *et al.* 1994; Herrero & Barja 1998; Barja & Herrero 1998; Pamplona *et al.* 2005). In contrast, antioxidant enzyme levels are sometimes higher and sometimes lower in birds compared to lab rodents (Ku & Sohal 1993; Lopez-Torres *et al.* 1993; Barja *et al.* 1994). In general, however, avian cells have better resistance to oxidative stress than mammalian cells (Ogburn *et al.* 1999, 2001; Pamplona *et al.* 2005) although older birds have higher levels of 8-oxo-dG in captive passerines and lower levels of 8-OH-dG in wild species (both biomarkers of DNA oxidation) than young birds (Liu 2004). Clearly, better understanding is needed of how oxidative stress and antioxidant levels change with age in wild birds.

The circulatory system plays a critical role in metabolism as it delivers oxygen to the tissues. Thus, it could be expected that changes in metabolism with age may be mirrored by changes in oxygen delivery capability, for instance via variation in the quantity of red blood cells (either by changes in hematocrit or changes in blood volume). Furthermore,

the absence of reduction in condition prior to mortality in birds instigated Ricklefs (2008) to suggest that senescence in birds may be associated with cardiovascular failure. Specifically, high levels of metabolism may impose oxidative stress or wear-and-tear in the heart or related organs. Cardiovascular disease plays an important role in mammalian senescence and heart failure has been documented for a number of bird species, especially long-lived parrots (Juan-Sallés *et al.* 2011). If cardiovascular disease plays an important role in the senescence of wild animals, then one would expect that biomarkers for cardiovascular disease, such as cholesterol, would increase with age in wild animals.

Changes in basal metabolism with age may be partially accomplished by altered investment in the immune system. Immunosenescence—declining immune function with age—is a pervasive consequence of mammalian aging (Grubeck-Loebenstein & Wick 2002, Effros 2003, Miller *et al.* 2005). Although the avian immune system is similar in its general features to that of mammals (Lavoie 2005), and some simple metrics are known to sometimes decline with age in passerine birds (Saino *et al.* 2003; Cicohon *et al.* 2003; Palacios *et al.* 2007, 2010), few studies have examined immunosenescence in long-lived birds. Those studies that have examined immunosenescence in long-lived birds have generally not found a difference; bacteria-killing ability of frozen blood (representing enzymatic capacity; Lecomte *et al.* 2010), haptoglobin concentration (Lecomte *et al.* 2010) and circulating levels of antibodies (Apanius and Nisbet 2003, 2006; Lecomte *et al.* 2010) did not vary with age. In contrast, the inflammatory response to phytahemagglutinin (a novel antigen) did decline with age in two long-lived birds (Haussman *et al.* 2005). As might be expected, in tree swallows (*Tachycineta bicolor*) some aspects of the immune system declined with age while others did not (Palacios *et al.*

2007, 2011). For instance, whereas two earlier studies had shown a decline in the concentration of antibodies produced in response to a novel antigen (sheep red blood cells or Newcastle disease vaccine; Saino *et al.* 2003; Cicohon *et al.* 2003), Palacios *et al.* (2007) found no change in antibody response to sheep red blood cells with age. There is therefore a need to examine several different components of the immune system simultaneously to determine what systems are most sensitive to aging.

Rather than representing variation in investment in one's self via immunity, variation in metabolism may represent variation in investment in offspring. For instance, clutch size and sex steroid levels decline with age in captive short-lived birds but not wild long-lived birds (Nisbet *et al.* 2002; Holmes & Martin 2009). In particular, reduced reproductive success with age in long-lived birds is only associated with terminal illness; once the last year of life is removed from analysis, there is no decline in reproductive success with age (Coulson & Fairweather, 2003; Reed *et al.* 2008). Thus, whereas reductions in reproductive success with age, such as menopause, are a universal mammalian trait (Cohen *et al.* 2006), that does not appear to be the case for birds.

Foraging behaviour can impact both reproduction and self-investment, depending on whether individuals shuttle energy intake towards their offspring or themselves. Recently, foraging ecology has been discussed as a particularly important component of senescence in long-lived animals, as old male (but not female) albatrosses are less successful than middle-aged albatrosses at sea (Catry *et al.* 2006; Lecomte *et al.* 2010). Lecomte *et al.* (2010) posit that reduced foraging abilities underly senescence in wild animals, possibly through reduced muscle/metabolic abilities affecting flight abilities or reduced cognitive abilities influencing spatial memory. However, in their case a regime shift has occurred

such that there is now greater productivity north of their study colony than south of the colony. Old birds continue foraging on the formerly productive region south of the colony, possibly because spatial memory for newly learned locations declines with age, whereas spatial memory for locations learned long ago does not change with age, as is the case in humans (Rosenbaum *et al.* 2012). Thus, "senescence" may not represent a decline in abilities with age but merely an inability of old birds to alter their foraging routines (Lecomte *et al.* 2010). That study highlights the importance of understanding environmental changes occurring within the lifetime of the individuals being studied.

To understand the patterns underlying senescence in long-lived birds, I examined the largest array of physiological and behavioural metrics measured to date within individual charadriiform birds of known age. First, I documented changes in mortality and reproductive success relative to age. In contrast to the procellariform seabirds examined to date, charadriiform seabirds have very high energy costs (Shaffer 2011; Elliott *et al.* 2013). I therefore examined several aspects of their physiology assocoiated with metabolism, including resting metabolic rate, daily energy expenditure, antioxidant levels and hematocrit. I measured behaviours that may relate to metabolism, such as activity budgets and chick-provisioning/growth rates. I also examined measures of foraging success, including body mass, cholesterol, corticosterone and glucose, and several measures of immunity and reproductive investment. Finally, because both populations have recently undergone a major switch in diet (murres: mid-90s; Gaston *et al.* 2003; kittiwakes: 2008; Hatch *et al.* 2013), I examined whether old birds showed different foraging patterns or diet compared with young birds. I was interested in documenting the patterns that mirrored the changes in mortality or reproductive success with age.

Methods

I studied black-legged kittiwakes at Middleton Island, Alaska and thick-billed murres at Coats Island, Nunavut. Both species were studied during mid-chick-rearing: chick-rearing murres had chicks 3-10 d old whereas chick-rearing kittiwakes had chicks 10-20 d old. I examined many different metrics of aging, and modified several among field seasons based on the results of the previous field season. I captured murres using a noose pole and kittiwakes using a wire fashioned into a hook. I did not correct body mass for linear size as such corrections do not improve estimation of lean or lipid mass in either murres or kittiwakes (Jacobs *et al.* 2012). For instance, in my kittiwake data set, body mass alone explains 92% of the variation in the residual of body mass on the first principal component of head-bill, tarsus, culmen and wing length and 93% of the variation in the residual of body mass on head-bill, and consequently size-corrected mass explains little of the variation beyond mass alone. Murres were sexed using sex-specific PCR primers applied to a drop of blood on filter paper (Elliott *et al.* 2010a) whereas kittiwakes were sexed behaviourally by position during copulation. Both parents share incubation and chick-rearing duties, at least during the period of time covered by the current studies. Research was approved by the Protocol Management and Review Committee of the University of Manitoba under protocol F11-020.

The average age of kittiwakes banded as chicks and rebanded as adults over the course of the study (given full colour bands signifying their first recapture since fledging) was 5.05 y (SD = 1.47 y) for females and 5.35 y (SD = 1.83 y) for males; there was no difference between sexes (t_{105} = 0.54, P = 0.62). Due to the paucity of old birds, I therefore added five years to the age of any adult bird when first banded, and, if that bird

was older than 15, I included it in the study and estimated its age as the age of first banding plus five. Thus, an unbanded bird that was first caught 11 years previous to a given measurement would be considered 16 and included in my study. As only one bird (out of 107) was caught at two years of age (none younger) and only one at nine years of age (none older), the bird was likely to be within the age range 14-19. In contrast, the average age of first breeding ("recruitment") was 7.27 y (SD = 1.79 y) for females and 6.96 y (SD = 1.47 y) for males; most birds bred for the first time roughly two years after being banded.

Individual quality or heterogeneity is a major confounding problem when testing for the effect of senescence (Nisbet 2001; van de Pol & Verhulst 2006; Nussey *et al.* 2008). I overcame that problem in two ways. First, for many parameters I used a longitudinal approach that involved measuring the same individual several years later. Second, I averaged across age-classes (three year bins) to reduce the effect of individual variation. As some of my techniques are inherently variable (e.g. accuracy of the doubly-labelled water technique is ~10-20% on individuals compared with ~3% on groups; Speakman 1997, Shirai *et al.* 2012), averaging also reduced variability inherent in the methods employed. Nonetheless, as the variability itself is also of interest (Williams 2008), we included other factors in the overall model for many analyses, which were conducted on the entire (non-averaged) dataset.

Many parameters were expected to either increase or decrease with age. For those parameters, I included only a linear age term in analyses. For other parameters, such as foraging performance or parental abilities, where low (or high values) were expected at both the start and end of life, we included a quadratic term in analyses (age^2). Although

the relationships are unlikely to be functionally quadratic, the quadratic term may be sufficient to provide a simple description of patterns (e.g. Reid *et al.* 2003).

ENERGY EXPENDITURE

Daily energy expenditure peaks during chick-rearing for murres and kittiwakes, whereas resting metabolic rate peaks during incubation (Bech *et al.* 2002). I therefore used doubly-labelled water to measure daily energy expenditure in chick-rearing murres (chicks 3-15 d old; N = 42) and kittiwakes (chicks 10-20 d old; N = 41) and open-flow respirometry to measure post-absorptive resting metabolic rate in incubating (egg age: 15-25 d) murres (N = 43) and kittiwakes (N = 41). Methods are described in more detail in Chapters Three and Four; my values include some of the values reported in those chapters. To account for the potential confounding effect of body mass, I report all values as residuals on body mass. I also measured oxygen carrying capacity during incubation.

Daily energy expenditure: doubly-labelled water. The optimal method for measuring equilibrium isotopic values in murres is the plateau method at 90-120 min using the ^{18}O equilibrium value (Jacobs *et al.* 2012). I injected birds in the brood patch with doubly-labelled water and obtained equilibrium blood samples at 60 min (kittiwakes) or 90 min (murres). I recaptured the birds and obtained a second blood sample from the brachial vein 24-72 h later. Samples were timed as close to multiples of 24h as possible to avoid circadian effects (Speakman & Racey, 1988). As longer sampling periods reduce the error due to day-to-day variance in daily energy expenditure (Speakman & Racey, 1984), I calculated daily energy expenditure over the extended time period for individuals that were recaptured more than once for second blood samples. All samples were run blind to the identity of the bird and converted to values of daily energy expenditure using a single

pool model with a fixed 25% evaporative water flux (equation 7.17: Speakman 1997) and a respiratory quotient of 0.81. Using this equation, estimates for energy expenditure based on doubly-labelled water in charadriiform seabirds are accurate to within 2-18% relative to respirometry values from the same individual and within 3% relative to the average respirometry value across the group (Shirai *et al.* 2012). Using different equations for calculating daily energy expenditure results in different absolute values for energy expenditure (Shaffer 2011), but within my dataset those differences had no effect on the hypotheses I tested because I examined only relative differences and I used the same equation for all analyses.

Daily energy expenditure: time budgets. Because I had robust activity-specific metabolic rates for murres, I augmented my sample size with daily energy expenditure estimated from time budgets (N = 205). To convert time budgets into daily energy expenditure, I used activity-specific metabolic rates that explained 72% of the variation in daily energy expenditure (see "Time budget models" Elliott *et al.* 2013b,c).

Resting metabolic rate. I used open-flow respirometry with a FoxBox II® (Sable Systems, Las Vegas, USA) system to measure the resting metabolic rate of incubating birds in a respirometry chamber. Birds were in their thermoneutral zone (15°C) and there was no diel rhythm in metabolic rate during my measurements (0430-2030 solar time) at my Arctic study site. Birds were post-absorptive as they were held for four hours and captured as they were switching off from their incubation shift (~12 hr for murres, Elliott *et al.* 2010; ~10 hr for kittiwakes; Gill *et al.* 2002). I also measured body temperature of resting, incubating birds in June 2013 with a Fisher Scientific traceable thermometer (model 14-648-45) placed 1 cm into the cloaca. Birds that flew off before being captured

had higher body temperature (41.6 ± 0.2 °C (SE)) than those caught before they flew off (40.8 ± 0.2 °C, t_{42} = 2.34, P = 0.02); those caught while sleeping had a lower body temperature (40.5 ± 0.2 °C), but the difference was not statistically significantly different from those caught before they flew off (t_{38} = 0.80, P = 0.43). Consequently, I only used data from those that had not flown off and were not caught while sleeping.

Oxygen carrying capacity. I used measured hematocrit by collecting blood in capillary tubes and spinning the tubes at 2000 g for 10 min. Hematocrit is closely correlated with hemoglobin concentration in murres (Elliott *et al.* 2008a). I measured hematocrit in separate individual murres in 2008 (N = 62) and 2011 (N = 31), and, in 2011, 20 individuals that were also sampled in 2008. I also measured hematocrit in kittiwakes in 2010 (N = 105) and 2012 (N = 32; same birds as in 2010). I also measured plasma volume using Evans blue dye (Croll *et al.* 1992; N = 10 murres, N = 32 kittiwakes). I injected the birds with Evans blue dye (0.2 mL of 2.5 g/ L) in the brachial vein and obtained a blood sample from the opposite wing 15 min later (Croll *et al.* 1992). I centrifuged the samples for 15 min at 10 000 g to remove turbidity. I measured absorbance at 615 and 415 nm using a Biotek plate reader and determined plasma volume using pooled plasma for each species diluted with a known concentration of Evans blue dye. The correlation coefficient between dilution and the difference in absorbance for the standard curve was R = 0.99.

HORMONE AND ANTIOXIDANT ASSAYS

Within 3 min of capture, I obtained 1 mL of blood from the alar vein. Blood samples were stored on ice for <4 h, centrifuged at 2000*g* for 10 min, the plasma was removed and stored at –20°C for the remainder of the field season (1-2 months) and then shipped

to the University of Manitoba, Canada, on dry ice and stored at –80°C until analysis. I measured thyroid hormone levels during incubation (egg age: 15-25 d) as an alternative index of resting metabolic rate (Chapter Three; Elliott *et al.* 2013a; Welcker *et al.* 2013), corticosterone (baseline) levels during chick-rearing (12-17 d old) as an index of stress and reproductive hormones (testosterone and estradiol) during pre-laying (15-20 May 2012; average 8 d pre-laying) as an index of reproductive readiness. To examine antioxidant capacity, I measured both total antioxidant capacity (primarily micromolecules) and superoxide dismutase concentration (as a measure of enzymatic antioxidant capacity). Antioxidant capacity was measured on the same plasma samples collected for baseline corticosterone analyses. All samples were measured in duplicate.

Thyroid hormones. I determined both total and free T3 and T4 concentrations in duplicate by radioimmunoassay using a commercially available kit on unextracted plasma (MP Biomedical kits 06B258710, 06B257214, 06B254216, 06B254029). I also measured thyroid hormone levels in the same murres sampled for hematocrit in 2008 (N = 46) and re-sampled in 2011 (N = 20). Thyroid hormone levels correlated with resting metabolic rate in two charadriiform birds (Chapter Three; Welcker *et al.* 2013). More details of the thyroid hormone measurements are presented elsewhere as the current measurements occurred on the same assays as those reported therein (Chapter Three).

Corticosterone. I used a radioimmunoassay (RIA) to measure corticosterone levels in duplicate using 25 μL of plasma extracted into 1.2 mL of ethanol and then dried down with a sample concentrator (Savant, Fisher Scientific) before being reconstituted in 100 μL of radioimmunoassay buffer. For a subset of samples, there was no difference between ethanol-extracted plasma corticosterone levels and those extracted using

dichloromethane. Extracted samples were reconstituted in 100 μL of RIA buffer (0.1 M phosphate buffer, 0.9 % NaCl, and 0.5 % bovine serum albumin). A standard curve was created by adding known concentrations (0.01 to 50 ng ml^{-1}) of corticosterone standard (Steraloids) into RIA buffer. To each of the test tubes, I then added 100 μl of tritiated (5000 disintegrations per minute) corticosterone (GE Healthcare, 78.1 MBq mol^{-1}, Code TRK406) and 100 μl of sheep serum anti-corticosterone (diluted 1:50000; Antibodies-online, Code ABIN343319). After 1 h incubation at room temperature, samples were incubated at 4°C for a minimum of 18 h. The reaction was stopped through the addition of 100 μl of dextran-coated charcoal to each assay tube. After 15 min on ice, assay tubes were centrifuged for 30 min at 4°C (2500 × g), and the supernatant was decanted into 7 ml scintillation vials. Finally, 4 ml of scintillation fluid (Ultima Gold, Perkin Elmer) was added to each assay tube, and tubes were counted on a liquid scintillation counter (LS6500, Beckman Coulter) for 5 min. All samples were counted in duplicate, and corticosterone concentration of each sample was interpolated from the standard curve that was counted in triplicate. Inter-assay and intra-assay coefficients of variation for corticosterone were 7.3 and 5.2%, respectively, samples demonstrated good parallelism (ANCOVA, murres: $F_{4,22} = 1.24$, $p = 0.32$; kittiwakes: $F_{4,22} = 0.99$, $p = 0.45$). The minimum detection level was 0.025 ng ml^{-1}.

Corticosterone-binding globulin. I measured corticosterone-binding globulin (CBG) levels on baseline plasma samples according to established protocols (Breuner and Orchinik 2002; Love *et al.* 2004). I stripped endogenous corticosterone from plasma by incubating 5 μL of plasma in 495 μL of dextran-coated Norit charcoal (10 g/L) for 30 min at room temperature prior to centrifuging for 10 min at 3000*g*. I determined total

binding capacity (in triplicate; 50 µL of stripped plasma, 50 µL buffer, 50 µL of tritiated corticosterone; 1:300 dilution) and non-specific binding (in duplicate; 50 µL of stripped plasma, 50 µL 1 µg/mL corticosterone, 50 µL of tritiated corticosterone) for each individual. Following 2 h incubation, I used a Brandel harvester to separate the free and bound fractions using glass fiber filters (Whatman GF/B) pre-soaked for 1 h in 25 nM Tris buffer with 0.3% polyethyleneimine. I suspended filters in scintillation fluid for 24 h before measuring radioactivity. Intra-assay variability was 3.2% and inter-assay variability was 9.6%. I estimated concentration of free corticosterone (H_{free}) as:

$$H_{free} = 0.5\left[H_{total} - B_{max} - K_d \pm \sqrt{(B_{max} - H_{total} + K_d)^2 + 4H_{total}K_d}\right]$$

where K_d = 1.20 for kittiwakes and 1.18 for murres, B_{max} is the concentration of CBG molecules and H_{total} is the concentration of total corticosterone (Barsano and Baumann 1989).

Estradiol and testosterone. I used the same protocol as corticosterone to measure estradiol and testosterone. However, estradiol was concentrated 8-fold (200 µL of plasma into 25 µL buffer) and testosterone was concentrated 2-fold (50 µL of plasma into 25 µL buffer) following ethanol extraction. To each of the test tubes, I added 100 µl of tritiated (5000 disintegrations per minute) testosterone or estradiol (Perkins Elmer, Waltham MA, 78.1 MBq mol^{-1}, Code NET517250UC or NET370250UC) and 100 µl of goat serum anti-testosterone or anti-estradiol (diluted 1:50000; testosterone: Fitzgerald, Acton MA, 20-TR05T, estradiol: Antibodies-online, Code ABIN289331). Along with a standard curve, each assay was incubated at room temperature for 1 h before being left refrigerated overnight. After stopping the reaction with dextran-coated charcoal (as described for the corticosterone radioimmunoassay), the samples were counted on the scintillation counter.

The inter-assay and intra-assay coefficients of variation were 4.2 and 5.1% (testosterone) and 7.2 and 9.3% (estradiol), respectively.

Superoxide dismutase concentration. Superoxide dismutases are a family of metalloenzymes that catalyze the dismutation of superoxide free radicals into oxygen and hydrogen peroxide. I measured levels of superoxide dismutase in red blood cells using the Cayman Chemical (Ann Arbor MI) superoxide dismutase kit (item 706002). I lysed 25 μL of red blood cells by adding 100 μL of ice-cold milliQ water, centrifuged at 10 000g for 10 minutes, and then diluted the supernatant 1:100 with sample buffer. The intra-assay coefficient of variation was 6.4%.

Total antioxidant capacity. I measured total antioxidant capacity of the samples collected for baseline corticosterone assays using the Cayman Chemical (Ann Arbor MI) antioxidant assay kit (item 709001). Total antioxidant capacity is measured relative to Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which is a water-soluble analog of vitamin E. Antioxidants in the plasma include superoxide dismutase, other enzymes, α-tocopherol, carotenoids, glutathione and uric acid. The cooperation of different antioxidants provides greater defense against oxidative stress than any single compound. Thus, the overall antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual components. The assay measures the ability of plasma to inhibit the oxidation of 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] by metmyoglobin. I diluted 5 μL of plasma in 100 μL of sample buffer prior to assaying. The intra-assay variation was 2.6%. I then measured uric acid concentration via the Cayman Chemical uric acid kit (item 700320). I also diluted 5 μL of plasma in 100 μL of sample buffer prior to assaying. The intra-assay coefficient of

variation was 3.8%. I considered true antioxidant capacity to be total antioxidant capacity – antioxidant capacity of uric acid (as circulating levels of uric acid were unlikely to reflect adaptation for antioxidant defence).

IMMUNOCOMPETENCY

The immune system is complex and there are many different components to a "healthy" immune system. To examine immunosenescence in seabirds, I therefore examined several aspects of the immune system. All measurements were begun on chick-rearing birds sampled for corticosterone. Second measurements for phytohemagglutinin and Newscastle disease vaccine response occurred 24 h and 17 d later, respectively; sample sizes for those groups were reduced because not all birds were recaught.

Phytohemagglutinin. I injected birds in the right patagium (the skin between the caprometatarsus and the ulna/radius) with 0.2 mg of phytohemagglutinin (Sigma-Aldrich, St Louis MO, mixed in 0.05 mL of PBS 0.9% NaCl). The thickness of the wing web was measured to the nearest 0.01 mm using a digital micrometer (model 293; Mitutoyo Inc., Tokyo, Japan) before injection and exactly 48 h later. The difference in wing web thickness before and after injection was taken as an index of the swelling response.

Newcastle disease vaccine. For kittiwakes only, I injected birds with TRIPLEVAC (Merck Animal Health, Whitehouse Station NJ), a live Newcastle disease vaccine (B1 strain), according to the instructions with the vaccine. Newcastle disease is a contagious bird disease associated with a family of viruses occurring in both wild and domestic birds (and transmissible to humans) that causes respiratory and nervous symptoms, depending on the strain (Cichon *et al.* 2003). I treated Newcastle disease as a novel antigen and examined the responsiveness of the birds' immune system to the novel antigen. I verified

that each individual had not previously encountered the vaccine by obtaining and testing a blood sample for each individual pre-vaccination. The vaccine was reconstituted daily with water. Birds were injected during chick rearing (chicks 10-20 d old), and a baseline blood sample was taken prior to injection. I then obtained a second blood sample 17 d later. I used 100 μL of plasma to measure the circulating level of anti-NDV antibodies pre-injection and after 17 d using a kit (product code NDVC-2P, ID-VET, Montepellier, France). Intra-assay variability averaged 13.2%.

Natural antibody levels. I measured circulating levels of natural antibodies, which are genetically-coded inherent immunoglobulin molecules that are part of the innate immune system (no exposure to antigen necessary). Natural antibodies and complement (the enzyme cascade that causes cell lysis) were measured using a hemolysis-hemagglutination assay by serially-diulting 25 μL of plasma with Dulbecos PBS (Sigma-Aldrich, St Louis MO) in the presence of rabbit red blood cells in Alsevers (Hemostat, Dixon CA). Assays were run in duplicate, with chicken plasma run as positive controls on each plate. I followed exactly the protocol described by: http://ccoon.myweb.usf.edu/ecoimmunology.org/nAbs.html (accessed 8 August 2013). I scored both lysis and agglutination.

Haptoglobin. Haptoglobin and related proteins (such as ovotransferrin) are acute phase proteins that bind iron—particularly, haemoglobin—that is released during infection from the lysis of erythrocytes. The haemoglobin-protein complex is then removed from circulation, preventing parasites from obtaining nutrition by consuming haemoglobin. Haptoglobin is therefore a good indicator of acute infection. I measured the iron-binding capacity of plasma by adding 10 μL of plasma to 96 well plates and the reagents precisely

as described in the Phase Range haptoglobin assay kit instructions (Tri-Delta, Maynooth, Ireland, item TP-801). Prior to the addition of reagent 2 (at zero minutes) and 8 minutes after the addition of reagent 2, I read the output at 630 nm on a Biotek plate reader. I calculated haptoglobin concentration using the standard curve based on the difference in absorbance between 0 minutes and 8 minutes, to account for turbidity in the plasma samples. I used 10 μL to reduce pipetting errors; intra-assay variability was 2.7%. I found 8 minutes to represent the peak in absorbance within my samples. The standard curves had $R > 0.999$. Values were log-transformed prior to analyses to obtain normality.

BEHAVIOUR

I examined several different behaviours simultaneously. Those behaviours included at-sea foraging behaviour from time budgets and behaviour at the colony (allopreening and aggression).

Time budgets. I attached Lotek 1100 time-depth-temperature recorders (product LTD_1100, Lotek Wireless, St John's NL) to 160 murres (2004-09) and 61 kittiwakes (2010). I used the temperature log to determine when birds were at the colony, resting on the water or flying (Elliott *et al.* 2008; Chivers *et al.* 2012). For murres, I also used pressure to record time spent diving.

Allopreening and aggression. I videotaped 45 pairs of murres and 63 pairs of kittiwakes for 24 h a day for 10 d during incubation and 5 d during chick-rearing in 2009 (murres) and 2010 (kittiwakes). I based my measurement of allopreening and fighting on Kober and Gaston (2003). I recorded time spent allopreening, and whether allopreening was directed towards its partner or a neighbour (I excluded allopreening not directed towards its partner or neighbour, which was <1% of all time spent allopreening). I

recorded time spent in aggression, whether fighting was directed towards its partner, a neighbour or another bird, and recorded aggression in terms of increased severity: beak-pointing; jabbing without contact; jabbing with contact; and grasping.

SURVIVAL AND BODY MASS

I recorded body mass of each bird during capture using a Pesola scale. I recorded the presence and absence of individual birds on study plots, based on unique colour band combinations (kittiwakes) or unique band numbers, read via telescope (murres). I recorded survival intensively over the winters 2008-09 (murres only), 2009-10, 2010-11 and 2011-12 (kittiwakes only), as survival was relatively constant over those winters. The data was entered into program MARK, along with the age of each individual and the year.

Mortality curves in animals either follow a Gompertz distribution (exponentially-increasing mortality with time) or a Weibull distribution (mortality increases with a power function of time; Ricklefs 2008, 2010). Human mortality follows a Gompertz distribution, whereas mortality in some animals follows a Weibull distribution (Ricklefs 2008, 2010; Finch 2012). I used Akaike's information criterion, which penalizes models that are needlessly complex, to compare among three different possible functions (exponential or Gompertz, power or Weibull, or linear).

REPRODUCTIVE SUCCESS

Reproductive failure can occur at several stages, including copulation, egg production, incubation, chick-rearing and fledging. I therefore examined various metrics of reproductive success associated with these different stages.

Egg size. For kittiwakes (it is problematic measuring egg size in murres and assigning parentage because they nest in groups rather than distinct nests and because the eggs

easily fall off the cliff during measurement), I measured the length, breadth and weight of all eggs produced, and recorded the order in which each of up to three eggs was produced.

Hatching and fledging success. As part of ongoing monitoring projects, breeding sites were observed each day (either from a blind for murres or through one-way glass for kittiwakes), and outcome (number of eggs, number of chicks) was recorded. I assumed that any murre that reached 14 d fledged successfully and any kittiwake that reached 40 d fledged successfully.

Chick growth and energy delivery rates. I weighed kittiwake chicks every five days from birth (day 0 to day 40). Because of the difficulty of assigning a murre chick to its parent during weighing (because most murres nest in groups rather than distinct nests), and because murres deliver readily visible prey to their offspring, I examined energy delivery rates of parental murres, rather than chick growth rates—which had the additional advantage of assigning a parameter to a particular parent rather than mating pair, where each member of the pair is usually of different age. Energy delivery rate is correlated with chick growth rate (Jacobs *et al.* 2013). In each year, at least three continuous (24 or 48 h) observational sessions of breeding sites were carried out from a blind situated within 5 m of the birds. I did not conduct feeding watches when it was too dark to see deliveries (roughly 01:00-02:00 in late July; 23:00-0:400 in mid August) because chicks are rarely fed at this time (Elliott *et al.* 2008). During these observation sessions, prey items delivered to chicks were identified whenever possible. Size was estimated assuming that the length of the white streak on the bill is 5 cm (Gaston *et al.* 2003; Hipfner *et al.* 2006). Prey energy content was calculated from species-specific

energy densities and mass-length regressions developed at Coats Island (Elliott and Gaston 2008).

Prey composition and stable isotope analysis. For murres, I also examined prey composition delivered to offspring during the feeding watches. Stable isotope analysis provided a continuous variable for examining changes in prey composition relative to age, and a possibility of looking at diet in kittiwakes (where prey items are not visible). As diet has shifted from Arctic to temperate fish species at Coats (Gaston *et al.* 2003), and I had more old birds in later years, I restricted my analyses of prey composition to 2005-11 (after the switch) to avoid confounding year effects with age effects. To determine whether trophic level or foraging locations changed with age in murres, I collected plasma (half life = one week) and red blood cells (half life = one month) blood samples from chick-rearing murres during 2003, 2006, 2007, 2008 and 2009 (murres) and 2012 (kittiwakes). Adult blood samples were collected in unheparinized cryovials, kept warm and immediately centrifuged. All samples were kept frozen until preparation in the laboratory, where they were freeze-dried, encapsulated in tin and sent to the University of Winnipeg Stable Isotope Laboratory for analysis of $\delta^{13}C$ and $\delta^{15}N$ isotopes.

Foraging locations. I attached CATTRAQ GPS loggers (14 g for kittiwakes, 30 g for murres due to resin needed to seal loggers to prevent becoming water-logged at depth) to the backs of 22 known-age murres in 2010-11 and 86 known-age kittiwakes in 2013. For murres, I set the loggers to record every 5 min when the bird was moving at <10 km/h and 10 s when the bird was moving >10 km/h. Because kittiwakes are more variable in their flight speed, I set the loggers to record every 30 s for kittiwakes. I then mapped the location of one foraging trip per bird.

Results

SURVIVAL AND BODY MASS

Mortality increased as a linear function of age in kittiwakes (t_{19} = 4.67, P = 0.0003) and an exponential function in murres (t_{21} = 2.61, P = 0.02, Fig. 5.1). A linear function slightly outperformed a power or exponential function in kittiwakes (ΔAIC_{power} = 0.85; $\Delta AIC_{exponential}$ = 1.61) while an exponential function slightly outperformed a power or linear function in murres (ΔAIC_{power} = 0.32; $\Delta AIC_{exponential}$ = 2.44) In a general linear mixed model that considered body mass longitudinally across several years, body mass varied with sex (kittiwake: t_{2357} = 9.89, P < 0.0001; murre: t_{542} = 1.01, P = 0.22), date (kittiwake: t_{2357} = -8.45, P < 0.0001; murre: t_{542} = -7.22, P < 0.0001), individual (kittiwake: t_{2357} = 5.66, P < 0.0001; murre: t_{542} = 10.31, P < 0.0001), age (kittiwake: t_{2357} = -6.01, P < 0.0001; murre: t_{542} = 6.54, P < 0.0001), and age*date (kittiwake: t_{2357} = -24.1, P < 0.0001; murre: t_{542} = -14.2, P < 0.0001) but not year (kittiwake: t_{2357} = 0.85, P = 0.33; murre: t_{542} = 1.34, P = 0.18). In general, body mass increased with age during incubation for murres and decreased with age during chick-rearing for kittiwakes (Fig. 5.2).

IMMUNOCOMPETENCY

There was no effect of sex or date on lysis (kittiwake; date: t_{81} = -1.17, P = 0.25; sex: t_{81} = 0.39, P = 0.70; murre; date: t_{81} = -0.17, P = 0.86; sex: t_{64} = -1.09, P = 0.28), agglutination (kittiwake; date: t_{81} = -0.02, P = 0.64; sex: t_{81} = 1.59, P = 0.12; murre; date: t_{64} = -0.11, P = 0.66; sex: t_{64} = -1.88, P = 0.07), haptoglobin (kittiwake; date: t_{81} = -0.26, P = 0.79; sex: t_{81} = -0.15, P = 0.89; murre; date: t_{64} = -0.01, P = 0.96; sex: t_{64} = 2.45, P = 0.16), response to Newcastle disease vaccine (kittiwake; ate: t_{59} = -1.06, P = 0.30; sex: t_{59}

= 0.96, P = 0.34) and phytohemagglutinin (kittiwake; sex: t_{26} =-1.84, P = 0.08; murre; sex: t_{29} =0.65, P = 0.52). I therefore pooled all samples. Within the pooled samples, three metrics of the cell-mediated and/or inflammatory response declined with age (Fig. 5.3). In contrast, two measures of the antibody-mediated response did not vary linearly with age, although response to Newcastle disease vaccine was impaired in both young and old kittiwakes. Lysis in murres (t_{24} = -2.55, P = 0.02), but not kittiwakes (t_{16} = 0.45, P = 0.66), was lower within the same individual sampled three years later. Haptoglobin (kittiwake: t_{16} = -0.30, P = 0.77, murre: t_{24} = -0.49, P = 0.63) and agglutination (kittiwake: t_{16} = 0.71, P = 0.49, murre: t_{24} = 0.16, P = 0.88) were no different within the same individuals sampled three years later.

ENERGY EXPENDITURE AND BLOOD PARAMETERS

Post-absorptive resting metabolic rate increased with body mass (kittiwake: t_{32} = 2.29, P = 0.03; murre: t_{38} = 4.04 P = 0.0003), decreased with age (kittiwake: t_{32} = -3.25, P = 0.003; murre: t_{38} = -3.14, P = 0.003, Fig. 5.4) and was independent of sex (kittiwake: t_{32} = 0.89, P = 0.38; murre: t_{38} = 0.16, P = 0.87) and date (kittiwake: t_{32} = -0.49, P = 0.63; murre: t_{38} = -0.15, P = 0.88). Similarly, body temperature in kittiwakes decreased with age (t_{38} = -3.84, P = 0.0005, Fig. 5.4) and body mass (t_{38} = -2.55, P = 0.02), but was independent of date (t_{38} = -1.55, P = 0.13) and sex (t_{38} = 0.83, P = 0.41). Thyroid hormone levels tended to decline with age in both species, with the exception of total T4 (kittiwakes: total T3: t_{31} = -4.09, P = 0.0003, free T3: t_{31} = -3.14, P = 0.003, total T4: t_{31} = -0.81, P = 0.43, free T4: t_{31} = -2.06, P = 0.05; murres: total T3: t_{45} = -2.32, P = 0.02, free T3: t_{45} = -2.48, P = 0.02, total T4: t_{45} = -1.53, P = 0.13, free T4: t_{45} = -2.11, P = 0.04), but was independent of date (kittiwakes: total T3: t_{31} = 0.39, P = 0.70, free T3: t_{31} = -1.11, P

= 0.28, total T4: t_{31} = -0.13, P = 0.90, free T4: t_{31} = 0.28, P = 0.78; murres: total T3: t_{45} = -0.88, P = 0.39, free T3: t_{45} = -0.34, P = 0.74, total T4: t_{45} = 2.36, P = 0.02, free T4: t_{45} = 0.56, P = 0.58), sex (kittiwakes: total T3: t_{31} = 1.15, P = 0.27, free T3: t_{31} = 0.30, P = 0.76, total T4: t_{31} = 0.45, P = 0.65, free T4: t_{31} = 0.15, P = 0.87; murres: total T3: t_{45} = 0.74, P = 0.46, free T3: t_{45} = 0.05, P = 0.96, total T4: t_{45} = 0.10, P = 0.92, free T4: t_{45} = -1.36, P = 0.18) and body mass (kittiwakes: total T3: t_{31} = 0.51, P = 0.61, free T3: t_{31} = -0.51, P = 0.61, total T4: t_{31} = 1.14, P = 0.27, free T4: t_{31} = -0.84, P = 0.41; murres: total T3: t_{45} = 0.58, P = 0.57, free T3: t_{45} = 1.58, P = 0.12, total T4: t_{45} = 1.76, P = 0.08, free T4: t_{45} = 0.34, P = 0.74); the ratio of free to bound T3 (an index of blood carrying capacity) also tended to increase with age (kittiwake: t_{31} = 1.81, P = 0.08; murre: t_{45} = 3.36, P = 0.002), but was independent of body mass (kittiwake: t_{31} = -1.77, P = 0.09; murre: t_{45} = -0.63, P = 0.53), date (kittiwake: t_{31} = 0.37, P = 0.71; murre: t_{45} = 1.70, P = 0.10) and sex (kittiwake: t_{31} = 0.39, P = 0.70; murre: t_{45} = -0.99, P = 0.33). In birds resampled three years later, total T3 also declined (murres: t_{19} = -2.55, P = 0.02; kittiwakes: t_{16} = -2.37, P = 0.03).

Plasma volume in kittiwakes increased with body mass (t_{39} = 4.43, P = 0.00007, Fig. 5) but not age (t_{39} = 0.49, P = 0.63) or sex (t_{39} = 0.99, P = 0.33). Cholesterol in kittiwakes increased with age (t_{80} = 2.04, P = 0.04; Fig. 5) but not body mass (t_{80} = 1.04, P = 0.24), time of day (t_{80} = -0.38, P = 0.71) or sex (t_{80} = 0.69, P = 0.50). Hematocrit decreased with age (murre: t_{85} = -4.59, P < 0.00001; kittiwake: t_{97} = -2.05, P = 0.04, Fig. 5.5) and tended to be higher for males (murre: t_{85} = 2.02, P = 0.04; kittiwake: t_{97} = 1.42, P = 0.16) but did not vary with body mass (murre: t_{85} = 0.08, P = 0.94; kittiwake: t_{97} = 0.16, P = 0.87) or date (murre: t_{85} = 0.54, P = 0.59; kittiwake: t_{97} = 0.10, P = 0.92). In birds resampled three

years later, hematocrit also declined or tended to decline (murre: $t_{19} = -3.42$, $P = 0.003$; kittiwake: $t_{16} = -1.99$, $P = 0.05$). Daily energy expenditure (residuals after accounting for body mass) did not vary with age, sex, date or body mass, or longitudinally across the same individual (all $P > 0.05$, Fig. 5.6). Superoxide dismutase levels in kittiwakes increased with age ($t_{72} = 3.18$, $P = 0.002$) and decreased with body mass ($t_{72} = -2.02$, $P = 0.05$, Fig. 7), but were independent of date ($t_{72} = -0.10$, $P = 0.92$) and sex ($t_{72} = 1.01$, $P = 0.32$). Uric acid levels, total antioxidant levels and total antioxidants – uric acid equivalents were independent of age, sex, date and body mass (all $P > 0.05$).

BEHAVIOUR

Aggression (residual on date and sex) decreased with age (kittiwakes: $t_{105} = -3.48$, $P = 0.002$; murres: $t_{91} = -5.11$, $P < 0.0001$, Fig. 5.8). Conversely, allopreening (residual on date and sex) did not vary with age (kittiwakes: $t_{105} = 0.47$, $P = 0.64$; murres: $t_{91} = 0.47$, $P = 0.64$, Fig. 8). Time spent flying, at the colony and foraging did not vary with age, sex, date or body mass, or longitudinally across the same individual (all $P > 0.05$, Fig. 5.6)

REPRODUCTION

Free baseline corticosterone levels declined with age (kittiwakes: $t_{86} = -2.99$, $P = 0.01$; murres: $t_{61} = -2.37$, $P = 0.03$, Fig. 5.9), with age^2 also being significant for murres ($t_{61} = 2.13$, $P = 0.04$). Free baseline corticostereone declined for the same individuals measured three years later (kittiwakes: $t_{19} = -2.43$, $P = 0.02$; murres: $t_{35} = -2.88$, $P = 0.005$). Egg size ($t_{1802} = 0.86$, $P = 0.39$) and estriadiol levels ($t_{39} = 0.17$, $P = 0.86$) were independent of female kittiwake age after accounting for relative lay date. Testosterone levels decreased with age in male kittiwakes after accounting for relative lay date ($t_{48} = -3.10$, $P = 0.01$). After accounting longitudinally for the effect of the individual, lay date (relative to the

median for that year) decreased with age (kittiwakes: $t_{1802} = -2.99$, $P = 0.001$; murres: $t_{842} = -3.35$, $P = 0.0007$, Fig. 5.11). After accounting longitudinally for the effect of the individual, fledging success increased with age (kittiwakes: $t_{1802} = 5.01$, $P < 0.0001$; murres: $t_{842} = 7.52$, $P < 0.0001$, Fig. 5.11), with age^2 also being significant (kittiwakes: $t_{1802} = -5.67$, $P < 0.0001$; murres: $t_{842} = -2.09$, $P = 0.03$, Fig. 5.11).

DIET AND FORAGING LOCATIONS

In murres, percent of invertebrates ($t_{32} = 1.82$, $P = 0.08$), capelin ($t_{32} = 0.01$, $P = 0.99$), Arctic cod ($t_{32} = 0.59$, $P = 0.55$) and benthic fish ($t_{32} = 0.10$, $P = 0.92$) was similar between birds at least 20 years of age (recruited to the colony prior to the switch from cod to capelin) and those known to be less than 10 years old (recruited after the switch from cod to capelin) during 2004-2011. Furthermore, when age and year were inserted into a linear regression for percent of Arctic cod and capelin in the diet across the entire study (1993-2011), year had a strong effect (Arctic cod: $t_{470} = -7.17$, $P < 0.0001$; capelin: $t_{470} = 4.89$, $P < 0.0001$) but age did not (Arctic cod: $t_{470} = 0.77$, $P = 0.44$; capelin: $t_{470} = 1.35$, $P = 0.18$). Between 2004 and 2011, specialization did not increase with age ($t_{187} = -1.77$, $P = 0.08$, $R^2 = 0.02$) or known age ($t_{60} = -1.95$, $P = 0.06$, $R^2 = 0.06$). Total prey energy content per day (chick-provisioning rate) did not change longitudinally with age in murres ($t_{243} = 1.88$, $P = 0.06$, $R^2 = 0.01$), after accounting for individual ($t_{243} = 5.88$, $P < 0.0001$), year ($t_{243} = 3.21$, $P = 0.002$) and chick age ($t_{243} = 2.16$, $P = 0.04$). Chick growth rate likewise did not change with age ($t_{895} = 0.60$, $P = 0.55$) in kittiwakes after accounting for chick age ($t_{895} = 29.0$, $P < 0.0001$) and individual ($t_{895} = 7.41$, $P < 0.0001$). There was no relationship between age and $\delta^{13}C$ or $\delta^{15}N$, either for plasma (murres: $\delta^{13}C$: $t_{66} = 0.33$, $P = 0.74$, $R^2 = 0.00$; $\delta^{15}N$: $t_{66} = 0.35$, $P = 0.73$, $R^2 = 0.00$; kittiwakes: $\delta^{13}C$: t_{51}

= 0.80, P = 0.43, R^2 = 0.00; $\delta^{15}N$: t_{51} = 0.42, P = 0.68, R^2 = 0.00) or for red blood cells (murres: $\delta^{13}C$: t_{27} = -0.26, P = 0.79, R^2 = 0.00; $\delta^{15}N$: t_{27} = 0.10, P = 0.92, R^2 = 0.00; kittiwakes: $\delta^{13}C$: t_{51} = 0.55, P = 0.59, R^2 = 0.00; $\delta^{15}N$: t_{27} = 0.18, P = 0.86, R^2 = 0.00). Likewise, there was no relationship between age and latitude (murre: t_{27} = 0.27, P = 0.80; kittiwake: t_{86} = 0.27, P = 0.80) or longitude (murre: t_{27} = 0.44, P = 0.66; kittiwake: t_{86} = 0.80, P = 0.43) during foraging (Fig. 5.10).

Discussion

I examined 36 behavioural and physiological traits in two species wild charadriiform seabirds. To the best of my knowledge, this is the most comprehensive examination of multiple metrics of aging in a wild animal. In contrast to procellariform seabirds with a low metabolism (Catry *et al.* 2006; Lecomte *et al.* 2010), charadriiform seabirds showed physiological but not behavioural changes with age. In general, time budgets, foraging behaviour, diet, aggression and allopreening did not vary with age (except that very young birds appeared to be more aggressive). In contrast, several immune, metabolic, antioxidant, cardiovascular and endocrine parameters varied with age. Furthermore, I attempted to document for at least one variable within each parameter group that such changes occur (or do not occur) longitudinally within individuals, demonstrating that the trends are not due to selective appearance or disappearance of individuals.

DEMOGRAPHY

Both kittiwakes and murres showed declines in survival and reproductive success associated with senescence. Specifically, I observed reductions in survival (increased mortality) with advancing age in both kittiwakes and murres. Reduced survival with age has been inferred previously for common murres (Crespin *et al.* 2006). In contrast, two

studies in the Atlantic recorded no evidence for reduced mortality with advancing age in kittiwakes (Coulson, 2010; Steiner *et al.* 2010). Survival is lower in Atlantic kittiwakes, and perhaps extrinsic mortality factors are more important for Atlantic kittiwakes, obscuring the effect of senescence. Reproductive success increased with age between at the start of life, before levelling off, and finally declined at the end of life. A similar pattern at the start of life (increasing reproductive success with age) has been shown previously for thick-billed murres (de Forest & Gaston, 1996), common murres (Crespin *et al.* 2006) and other seabirds (Ratcliffe *et al.* 1998; Barbraud & Weimerskirch, 2005; Nevoux *et al.* 2007). Reproductive senescence has also been demonstrated in many other seabirds (Barbraud and Weimerskirch 2005, Nevoux *et al.* 2007). For kittiwakes, most of the variation in reproductive success with age was associated with hatching success; there was little variation associated with clutch size or fledging success. Presumably, if a pair is successfully able to hatch an egg, the individuals have the ability to successfully fledge the chick. The same would also be true for murres where there is no variation in clutch size and where fledging success is almost always 100% (de Forest & Gaston 1996).

ENERGY EXPENDITURE

Daily energy expenditure did not vary with age, although some subcomponents varied. Thus, as shown in Chapter Two, individuals appeared to work at their energy ceiling, and that ceiling did not vary with age. Despite the absence of variation in daily energy expenditure, resting metabolic rate declined with age. Presumably, either any variation in resting metabolic rate was obscured or counteracted by variation in activity costs (due to wind speed, temperature or other components that I did not measure).

Galbraith *et al.* (1999) also report the absence of a relationship between daily energy expenditure and age in breeding common terns. In their case, middle-aged male terns delivered more food than old or young terns, so they considered middle-aged terns to be more energy efficient than old or young terns. With a much larger sample size, I found no variation in chick-provisioning rates, and I could therefore find no support for the idea that middle-aged birds were more efficient. Rather, I believe that by the time birds are old enough to successfully raise an offspring, they are experienced enough at finding food in the vicinity of the colony that there is no discernible reduction in foraging performance in young birds. Similarly, birds seemed to continue to invest in muscle performance and spatial memory during old age such that there was no reduction in energy efficiency during foraging (cf. Hindle *et al.* 2009a; Rosenbaum *et al.* 2012).

Several measures of resting metabolic rate declined with age, as would be expected for two species with high metabolic rates (as discussed in detail in Chapter Four). As body temperature and T3 declined with age, I argue that the decline in resting metabolic rate represents a decline in metabolic intensity rather than a change in different organ sizes. An absence of variation in digestive/metabolic organ size is likewise supported by the absence of variation in daily energy expenditure with age, which I argue in Chapter Two may be set by digestive physiology. In the laboratory, declines in metabolic rate with age in mammals are associated with declines in mitochondrial energy production (Hunt *et al.* 2006) and that may also apply to birds. Likewise, hypothyroidism is often a biomarker for aging in mammals (Spaulding 1987).

In conjunction with a decline in resting metabolic rate with age, levels of superoxide dismutase in the red blood cells of kittiwakes increased with age. Levels of total

antioxidants in the plasma did not vary with age, but those levels are likely primarily influenced by micromolecules (uric acid, carotenoids, vitamins) whose abundance may have little association with the prevention of oxidative stress (Monaghan 2009). For instance, uric acid is the end result of protein catabolism and may represent the fasting state irrespective of oxidative status. The decline in metabolic rate coupled with an increase in enzymatic defenses suggest that for charadriiform birds with inherently high metabolic rate, that older birds reduce metabolism and increase defenses to prevent oxidative stress. In young birds, unlikely to live to the age at which such issues become significant, there is no reason to invest in antioxidant defenses or reduce metabolism. In older birds approaching ages where senescence is important, it may become more important to invest in those defenses.

Hematocrit declined with age while cholesterol increased, with no change in plasma (or blood) volume. The decline in hematocrit with age was more pronounced in murres than kittiwakes, and may be associated with the influence of diving (Chapter Four). In contrast, Nisbet *et al.* (2013) found no effect of age on hematocrit in common terns. Hematocrit is often used as an index of condition (e.g. Donnelly & Sullivan, 1998; Sánchez-Guzmán *et al.* 2004; Murphy, 2010; Nisbet *et al.* 2013), and one interpretation would be that older birds are in poorer body condition. Alternatively, hematocrit is known to correlate with metabolic rate in many animals because substantial oxygen carrying abilities are required to sustain high oxygen consumption rates (Carpenter 1975; Palomeque & Planas, 1978) and the declining hematocrit with age may be a direct consequence of declining resting metabolism with age. If birds are attempting to avoid cardiovascular disease, which may increase with age as indicated by the high levels of

cholesterol in old birds, they may reduce blood pressure, viscosity and heart rate. Interestingly, cholesterol has been used as an index of body condition (Alonso-Alvarez *et al.* 2002; Sánchez-Guzmán *et al.* 2004), suggesting that old birds are in better condition, in contrast with the index provided by hematocrit.

IMMUNOCOMPETENCY

The vertebrate immune system consists of both innate (or non-specific) immunity and acquired (or adaptive) immunity (Roitt *et al.* 1998; Palacios *et al.* 2012). Each component has both cellular and humoral subcomponents. I examined both components, and all measures of cellular immunity (4 out of 4 measures), but few measures of humoral immunity (1 out of 3 measures) declined with age. Haptoglobin levels, a measure of the inflammatory response, also declined with age. As haptoglobin is an iron-binding protein and hematocrit also declined with age, declines in haptoglobin may be associated with reduced hematocrit in old animals. Furthermore, the decline in response to Newcastle vaccine, the one antibody-mediated response that declined with age, was almost entirely due to a decline in the oldest age class (Fig. 3e). In general, older birds, which were more likely to have already encountered a particular pathogen, invested less in innate immunity and relied more on memory-based immunity (Holmes & Martin 2009). Consequently, the decline in resting metabolic rate with age may partly represent a decline in energy directed towards the cellular immune response.

Haussman *et al.* (2005) also documented a decline in cellular immune response with age across three species as measured by the swelling response to phytahemagglutinin while Palacios *et al.* (2007, 2012) documented a decline with age in B-cell proliferation to both phytahemagglutinin and conA. I also found a decline in the ability of plasma to

lyse foreign particles (rabbit red blood cells) with age and in haptoglobin titres, unlike Palacios *et al.* (2007, 2012) and Lecomte *et al.* (2010). Thus, my data support the idea that the cellular immune response and the associated inflammatory response decline with age in birds. I found no linkage between natural antibody levels and age in both species. Likewise, Apanius & Nisbet (2003, 2006), Palacios *et al.* (2007, 2012) and Lecomte *et al.* (2012) all found that natural antibody levels did not decline with age in birds. I found a weak decline in the humoral response to Newcastle disease vaccine in kittiwakes; Palacios *et al.* (2007, 2012) found no relationship between humoral response to sheep red blood cells and age in passerine birds; in contrast, both Saino *et al.* (2003) and Cichon *et al.* (2003) found a decline with age in passerine birds in the antibody response to Newcastle disease vaccine and sheep red blood cells, respectively. Taken together, my results support the idea that the cellular immune response, and its associated inflammatory response decline with age in birds, but that the humoral response is largely maintained into old age. The parallelism between my cell-mediated/inflammatory responses relative to age and my antibody-mediated responses relative to age also support the idea that the immune system can be compartmentalized into different components in wild birds (Matson *et al.* 2006; Mendes *et al.* 2006; Palacios *et al.* 2012).

REPRODUCTIVE AXIS AND FORAGING ECOLOGY

Whereas reduced investment in reproduction during old age is characteristic of all mammals (Cohen 2004), and is typically found in short-lived birds (Ottinger *et al.* 1995; Preston *et al.* 2011; Duval 2012), the reproductive axis is maintained into old age in long-lived terns (Ottinger *et al.* 1995). Specifically, Coulson & Fairweather (2003) posited that long-lived kittiwakes show no reduced investment in offspring as they age. Rather, any

declines in reproductive success can be associated with terminal illness. In my dataset, the youngest birds had higher luteinizing hormone releasing hormone (LHRH)-induced testosterone (and fought more often), but I interpret that as being due to young birds attempting to find and maintain a site rather than any effect of senescence *per se*. Rather, the absence of further changes in testosterone, or any change in estradiol or egg volume with female age, supports the idea that individuals continued investing in reproduction into old age.

In contrast with long-lived albatrosses (Catry et al. 2006; Lecomte et al. 2010), many different metrics of foraging ability showed no variation with age, including diet, location, activity budgets and chick growth/provisioning rates. In particular, despite a high degree of individual specialization (Woo et al. 2008), both species appear to be flexible enough to adapt to their changing prey base. Thus, even those individuals that originally specialized on cod eventually switched to capelin at Coats (Elliott et al. 2009b); apparently an old murre can learn new tricks. I believe that most of the reduction in reproductive success is associated with learning to successfully prevent the egg from falling off the cliff (i.e. coordination with the partner) rather than with foraging success directly (de Forest & Gaston 1996); presumably, by the time a bird is capable of raising a chick it is already a successful forager.

Fig. 5.1. Over-winter mortality increased with age. Among the examined functional relationships, the best-fit function for kittiwakes (open triangles) was linear and for murres (closed squares) was exponential.

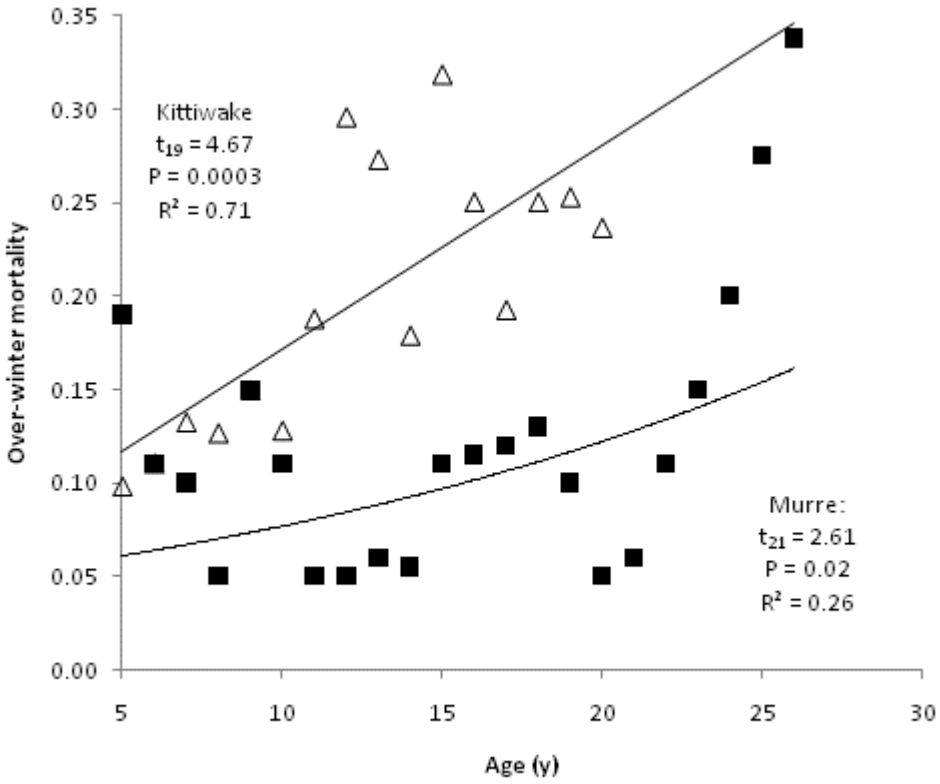


Fig. 5.2. Body mass during (a) incubation increased with age in thick-billed murres (closed squares) but not black-legged kittiwakes (open triangles), whereas during (b) chick-rearing body mass decreased with age in black-legged kittiwakes but not thick-billed murres.

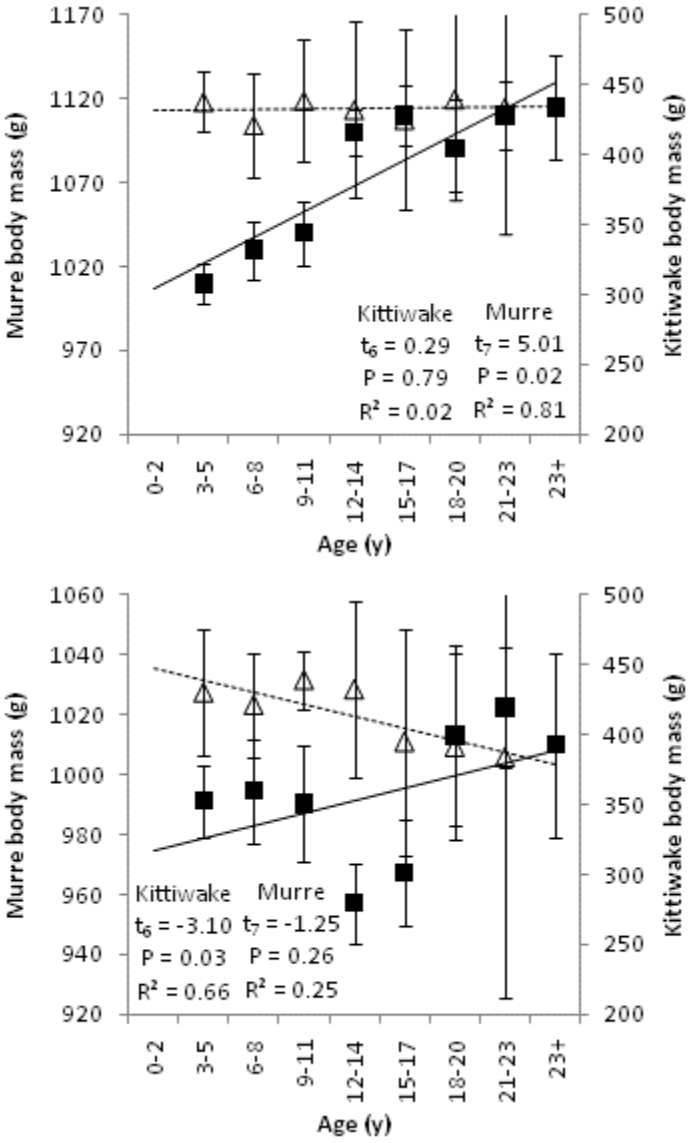


Fig. 5.3. Several measures of the inflammatory, cell-mediated and/or lytic immune system declined with age in chick-rearing murres (solid symbols and line) and kittiwakes (open symbols and broken line), including (a) circulating haptoglobin concentration, (b) change in patagium thickness (swelling) in response to injection with phytohemagglutinin and (c) lysis score, a measure of the plasma's ability to lyse rabbit red blood cells. (d) The agglutination score (agglutination – lysis), a measure of the plasma's ability to agglutinate rabbit red blood cells (a measure of innate or natural antibody levels) did not vary with age. (e) Response to Newcastle disease vaccine (percent vaccine molecules bound 17 d after injection – percent bound prior to injection) varied quadratically with age in chick-rearing kittiwakes. Age 0-2 class were chicks and therefore not breeding.

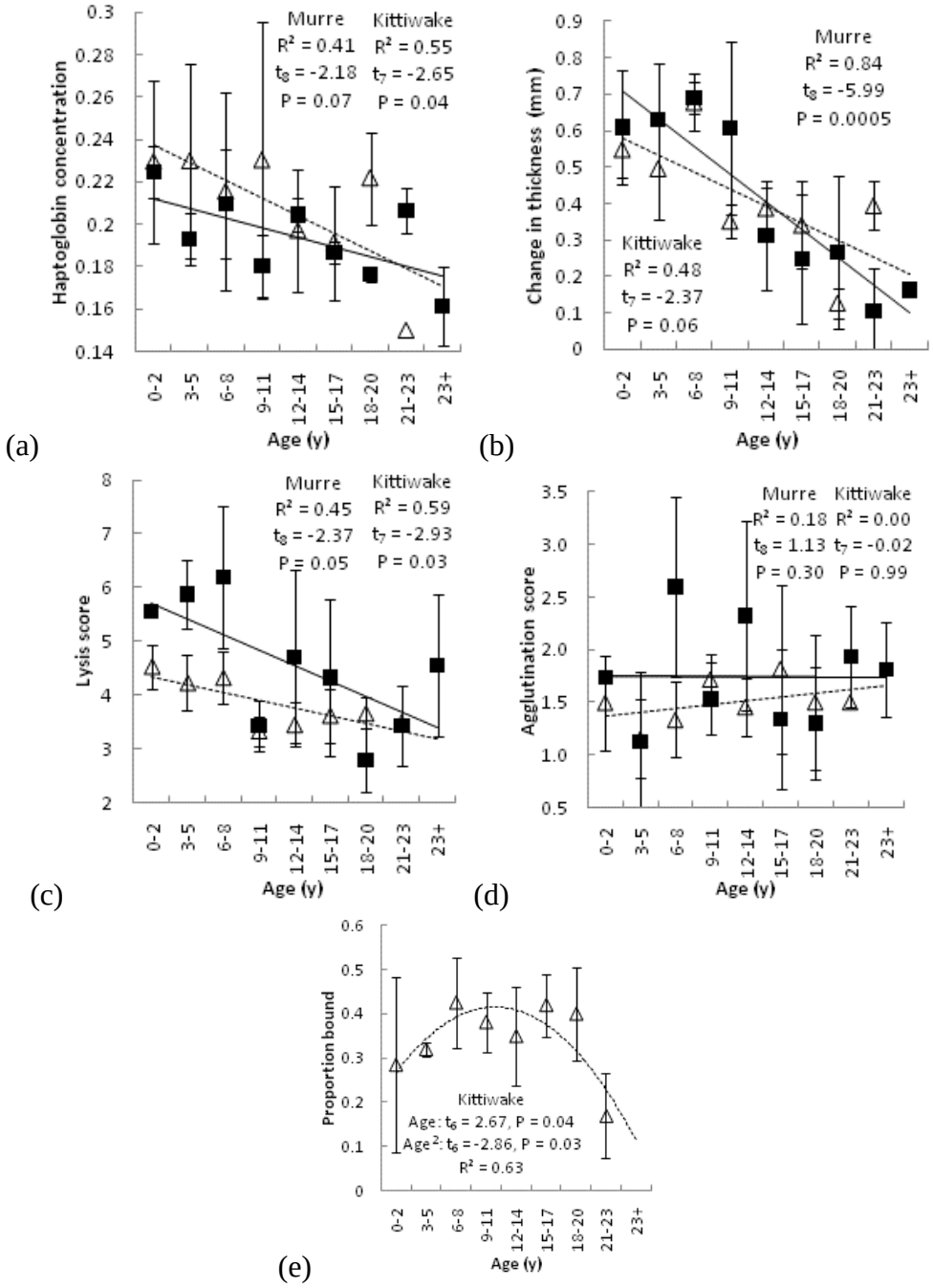
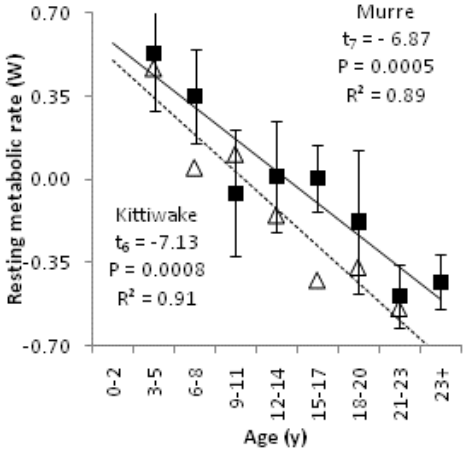
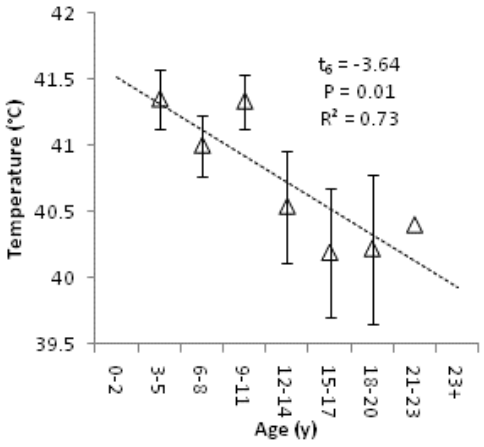


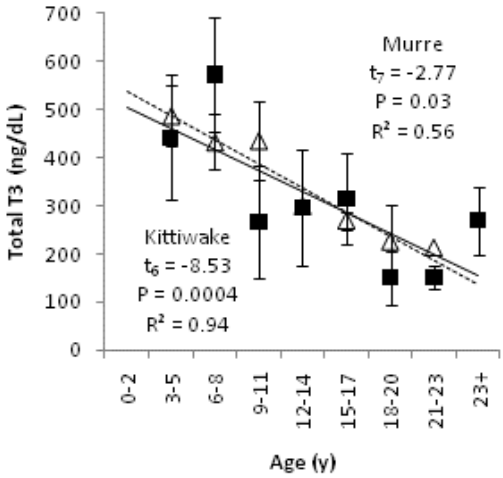
Fig. 5.4. (a) Post-absorptive resting metabolic rate (residual on body mass), (b) body temperature in kittiwakes (open symbols), (c) total T3, (d) free T3, (e) total T4 and (f) free T4 tended to decline with age in incubating murres (closed symbols) and kittiwakes.



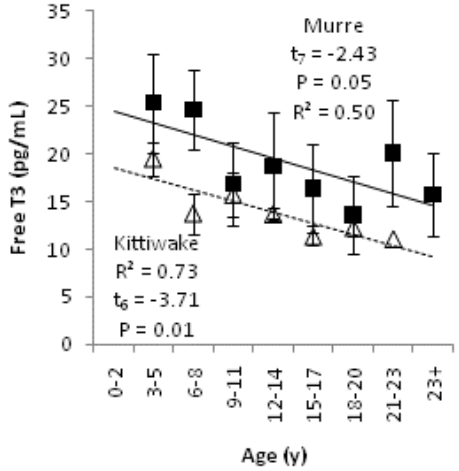
(a)



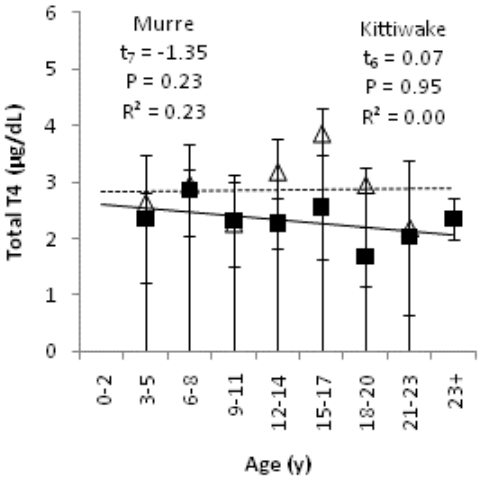
(b)



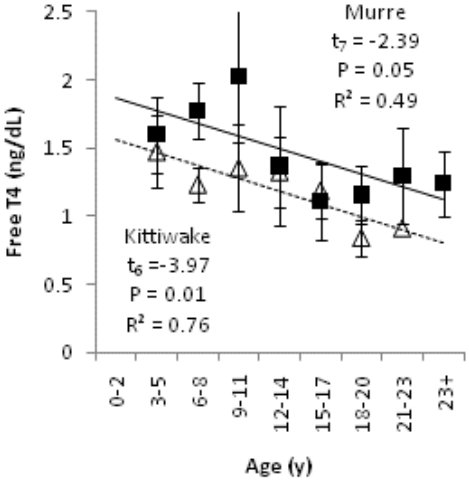
(c)



(d)



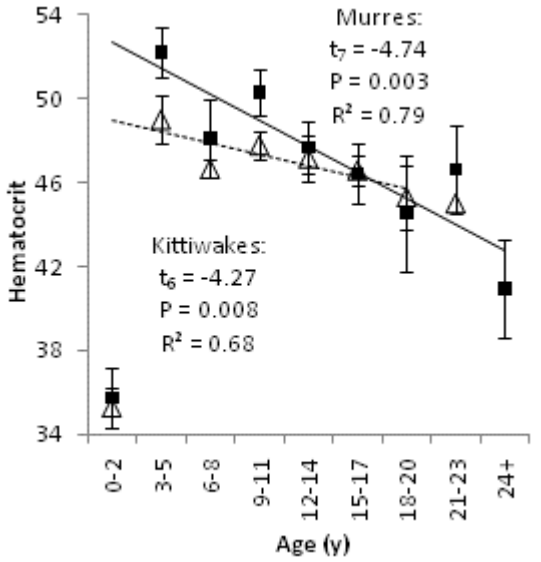
(e)



(f)

Fig. 5.5. (a) Hematocrit declined, (b) plasma volume increased and (c) cholesterol and (d) the ratio of free to bound T3 increased with age in incubating murres (closed symbols) and kittiwakes (open symbols). Chicks (age class 0-2) were not included in regressions.

a)



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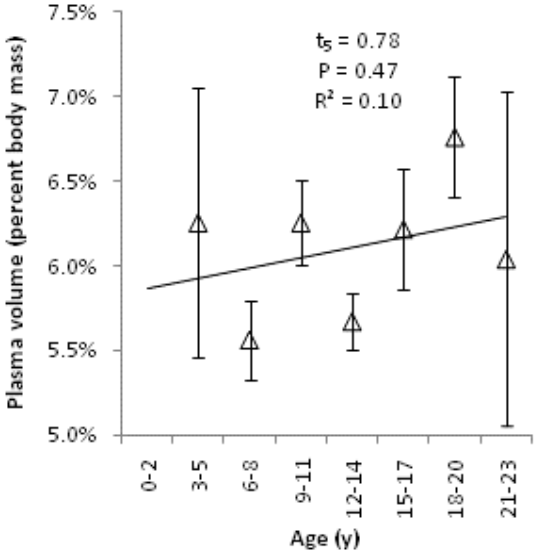

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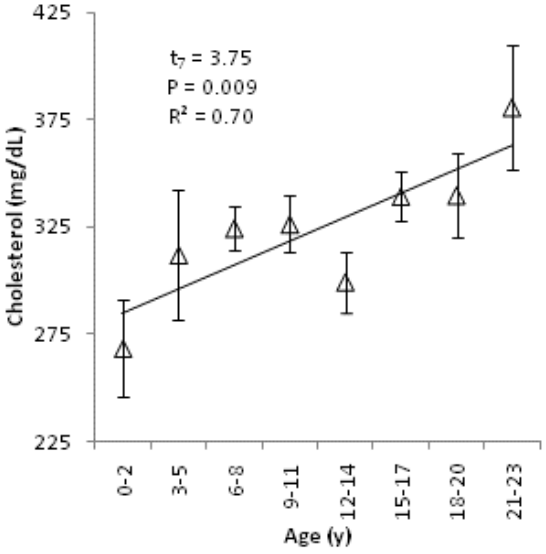

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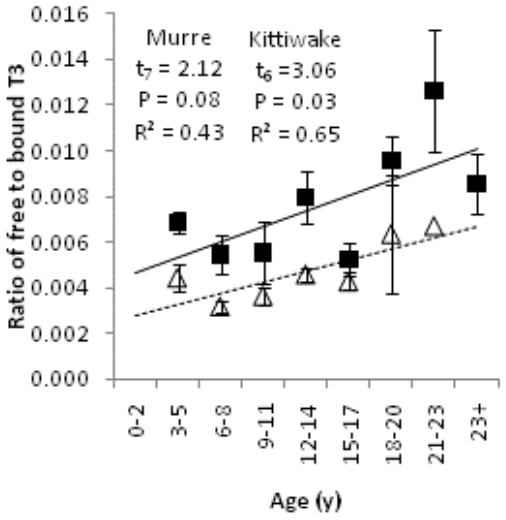
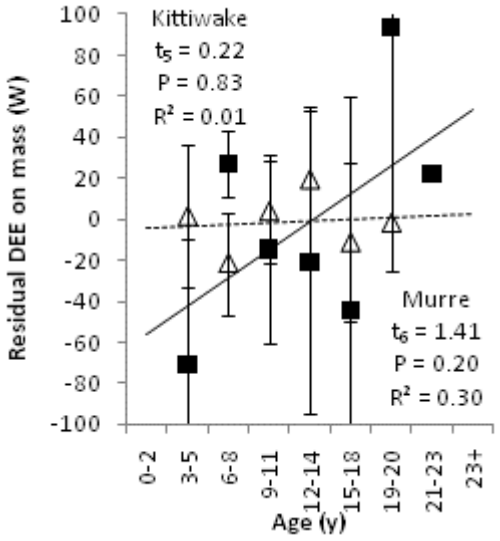


Fig. 5.6. (a) Daily energy expenditure, (b) time spent flying, (c) time spent foraging and (d) time spent at the colony did not vary with age in chick-rearing murres (closed symbols) and kittiwakes (open symbols).

a)



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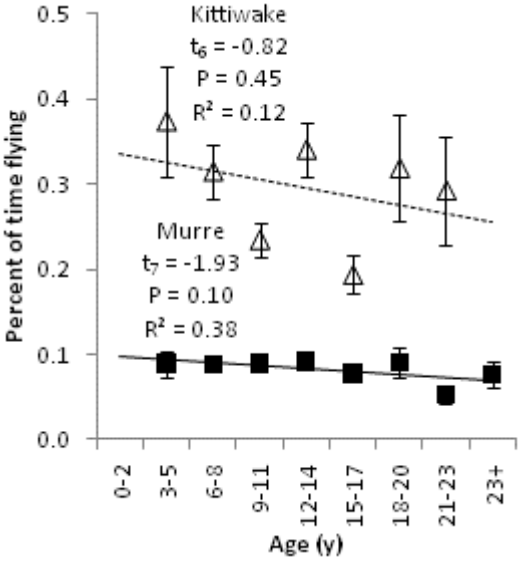

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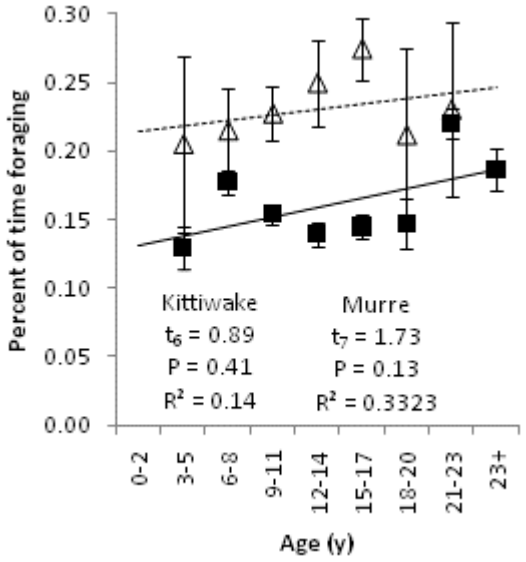

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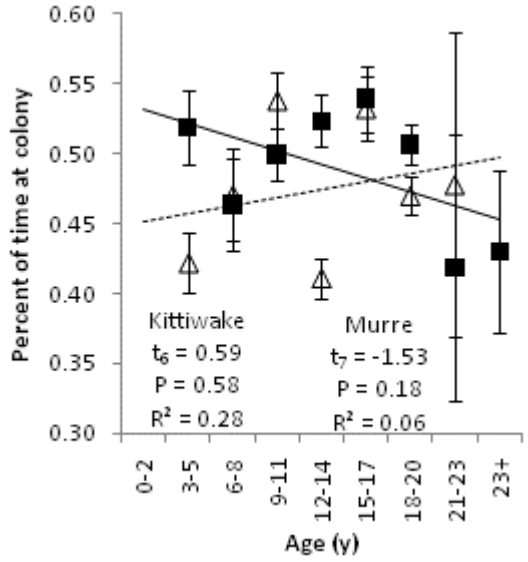
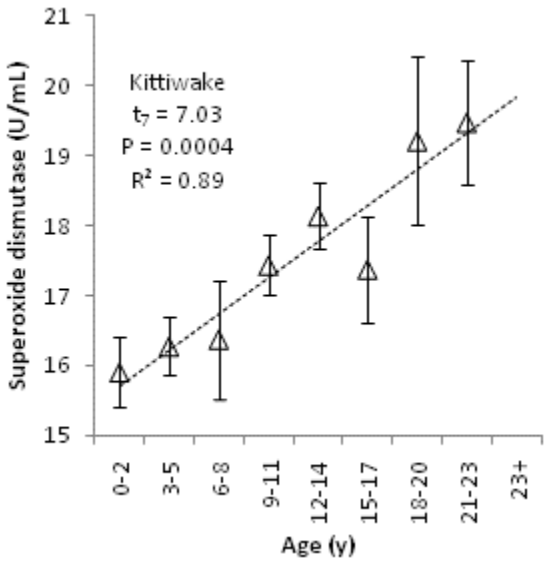
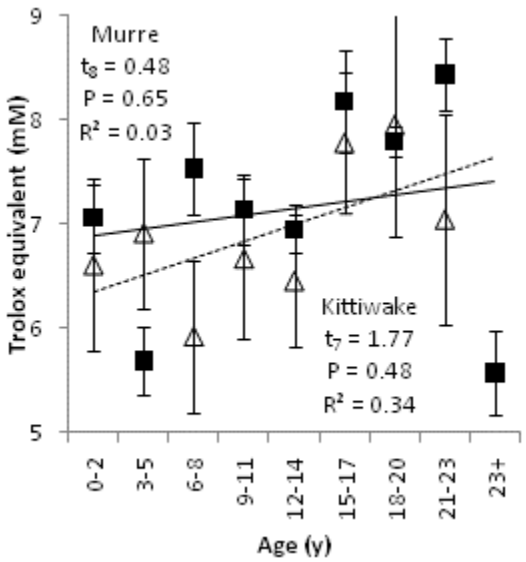


Fig. 5.7. (a) Superoxide increased with age, whereas (b) total antioxidant capacity, (c) uric acid level and (d) total antioxidant capacity – uric acid equivalent did not vary with age in murres (closed symbols) and kittiwakes (open symbols). Chicks (age class 0-2) were not included in regressions. Total antioxidant capacity is measured as trolox equivalent antioxidant capacity, which measures the antioxidant capacity of the plasma as compared to the Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard.

a)



b)



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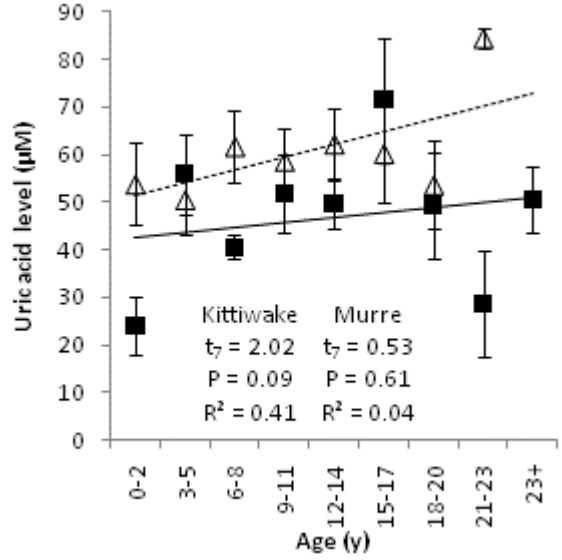

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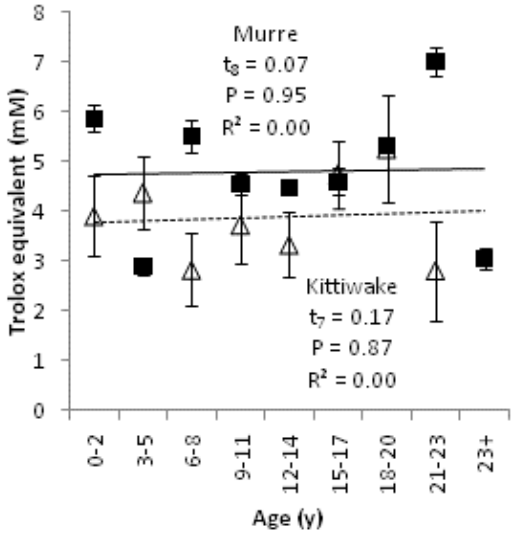
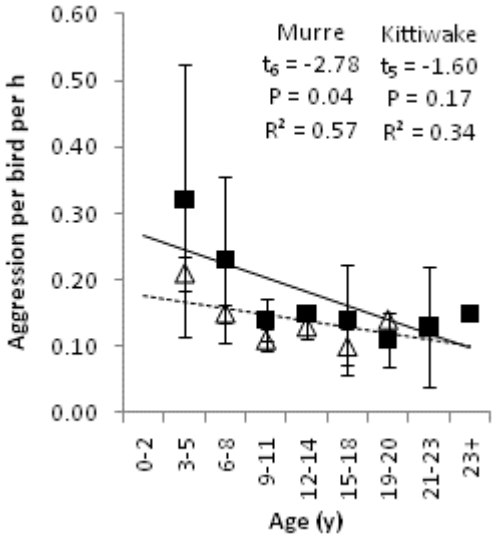


Fig. 5.8. (a) Aggressiveness, (b) allopreening, and (c) chick-provisioning (murres, closed symbols) or chick growth (kittiwakes, open symbols; residual after accounting for age and status—A, B or single) rates were independent of age, except very young birds appeared to show higher aggression in murres.

a)



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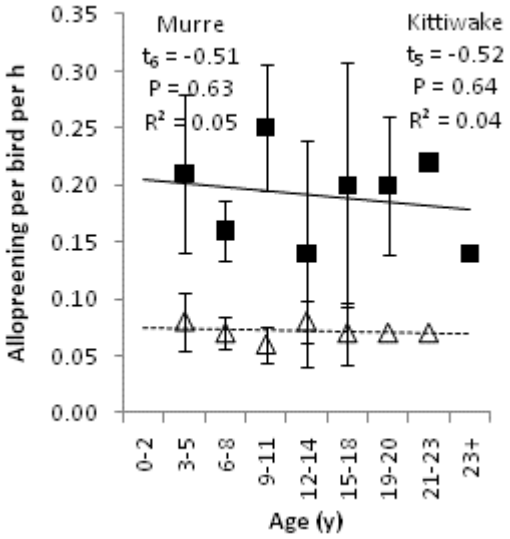

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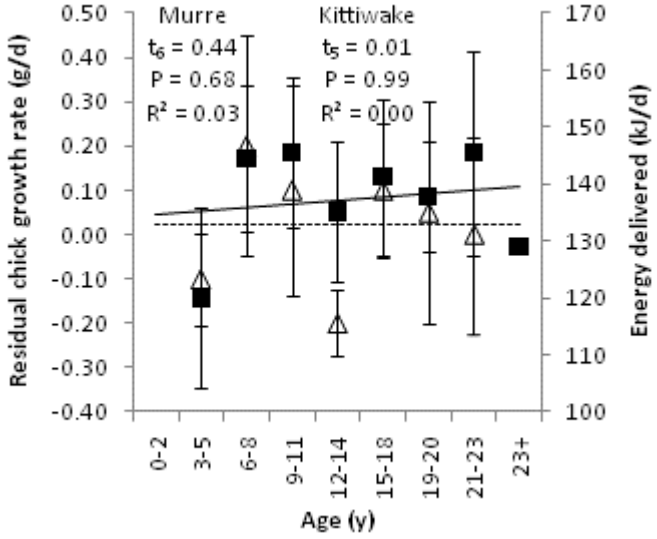
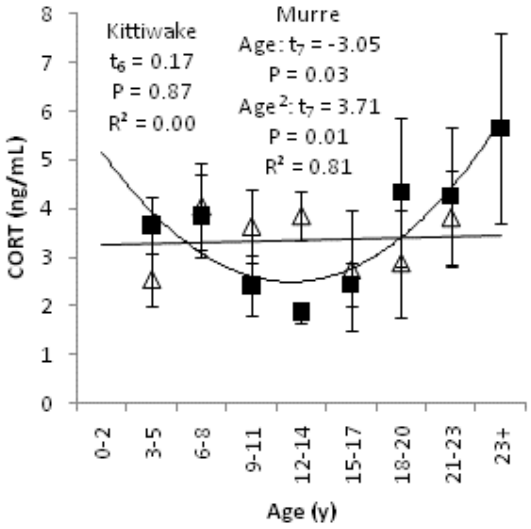


Fig. 5.9. (a) Total corticosterone levels varied with age in murres (closed symbols) but not kittiwakes (open symbols) while (b) corticosterone-binding globulin varied with age in kittiwakes but not murres, such that (c) free corticosterone varied with age in both murres and kittiwakes; (d) glucose levels were independent of age in both species.

a)



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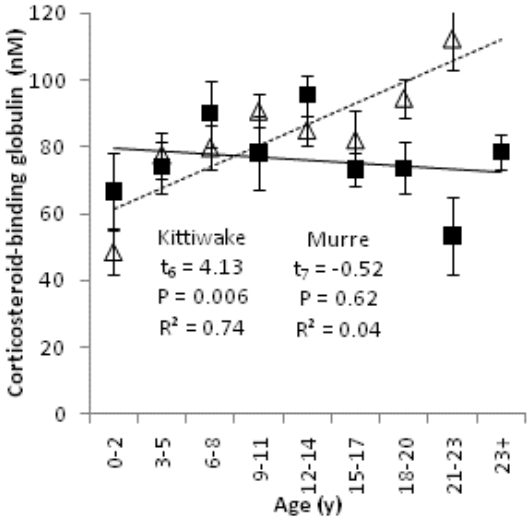

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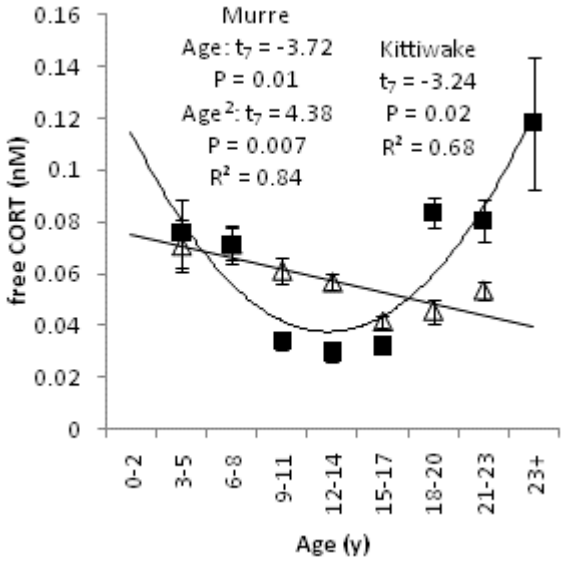

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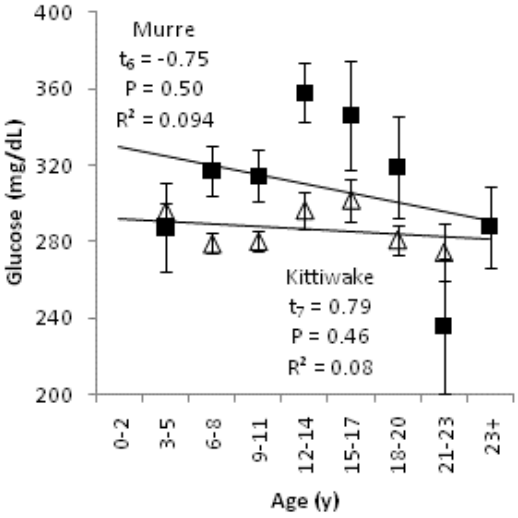
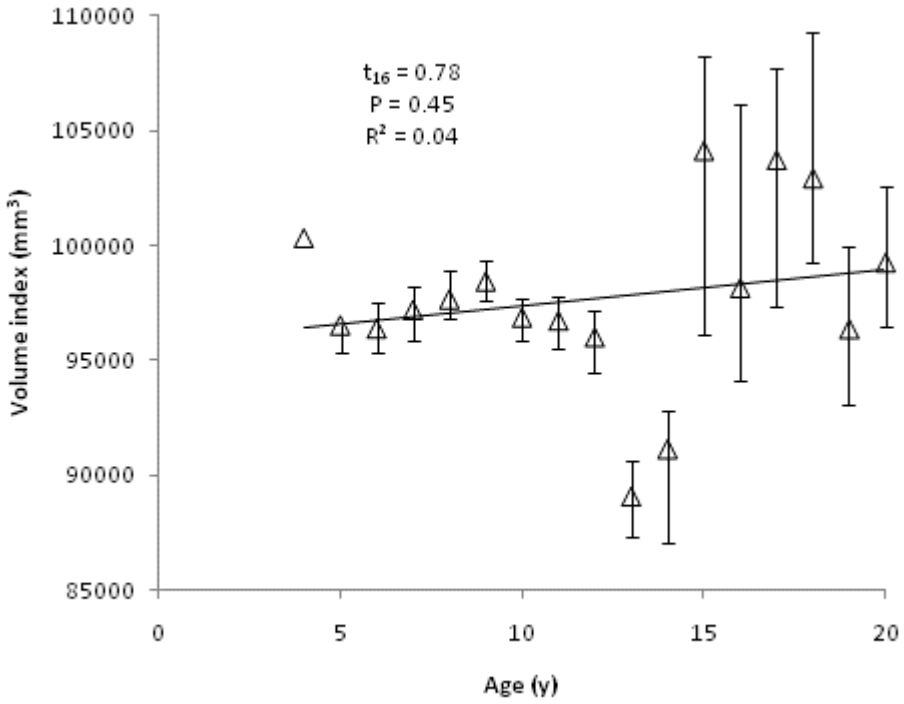


Fig. 5.10. (a) Egg size for females, (b) baseline (filled) and peak (open) estradiol in females, and (c) peak (open) but not baseline (filled) estradiol in males, does not vary with age in kittiwakes.

a)



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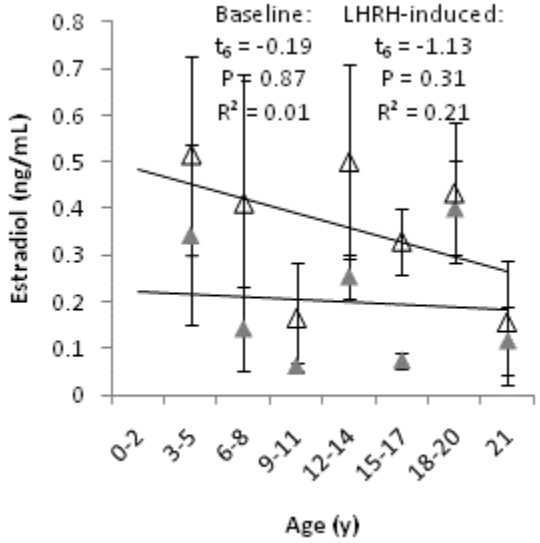

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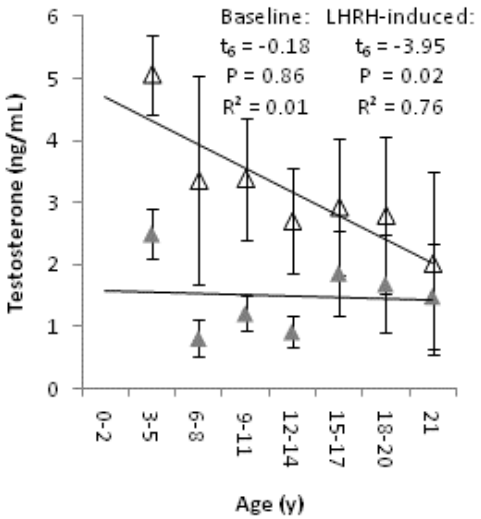
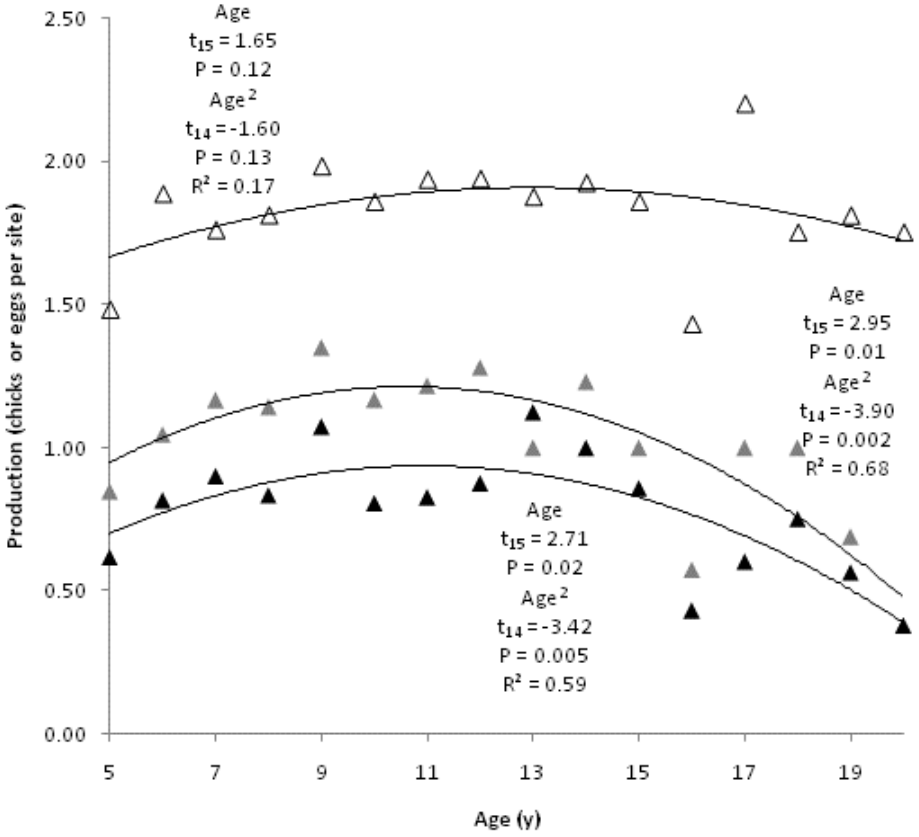


Fig. 5.11. (a) Clutch size (open symbols), hatching success (grey symbols), and (c) fledging success (black symbols) in black-legged kittiwakes; (c) fledging success in thick-billed murres; and (b) lay date in both murres (closed symbols) and kittiwakes (open symbols) varied with age.

a)



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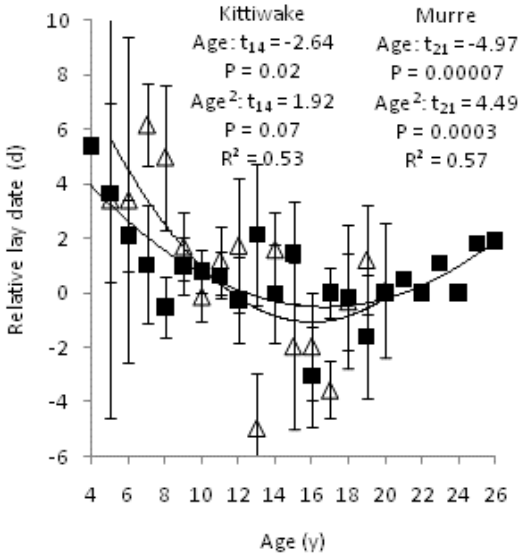

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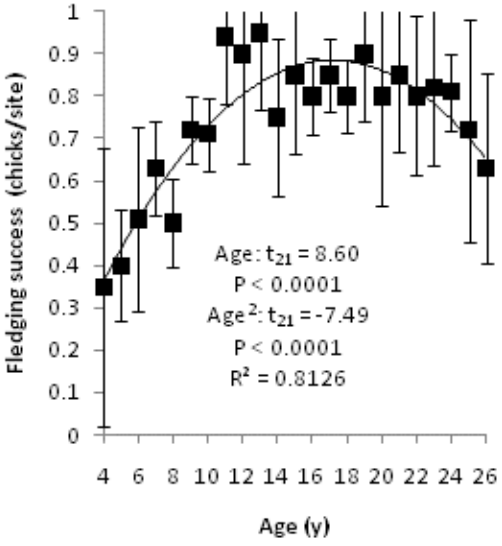
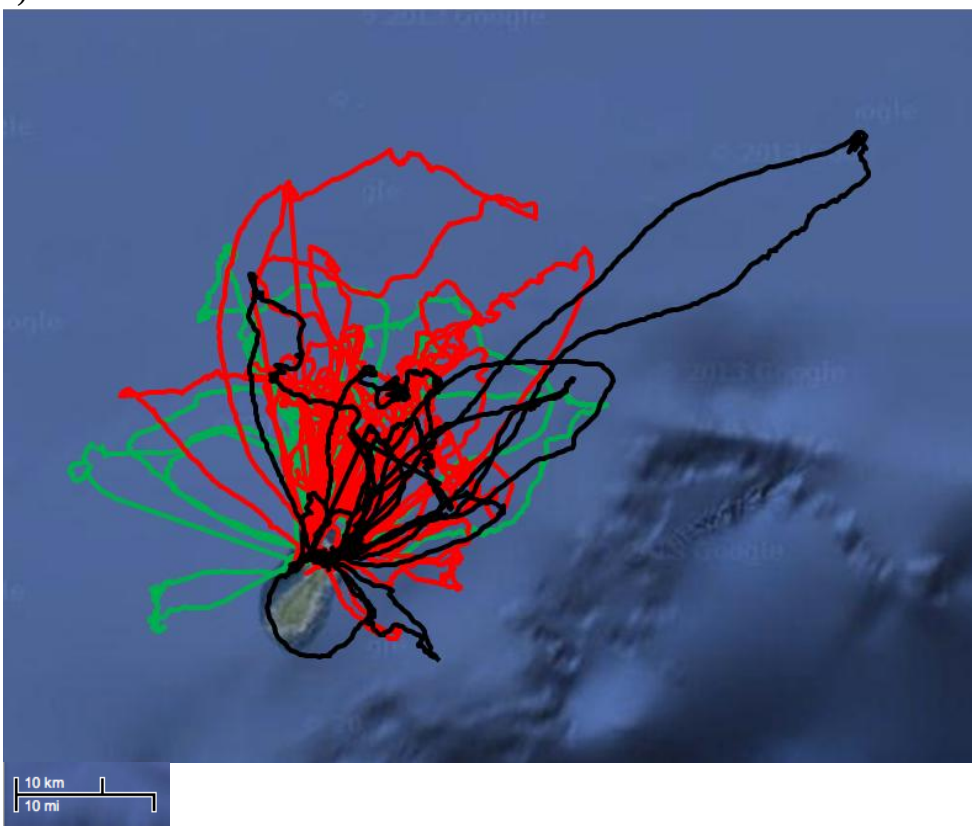


Fig. 5.12. Foraging locations of (a) black-legged kittiwakes (green: 5-9 y; red: 10-15 y; black: 16-23 y) and (b) thick-billed murres (green: 5-9 y; red: 10-19 y; black: 20-26 y).

a)



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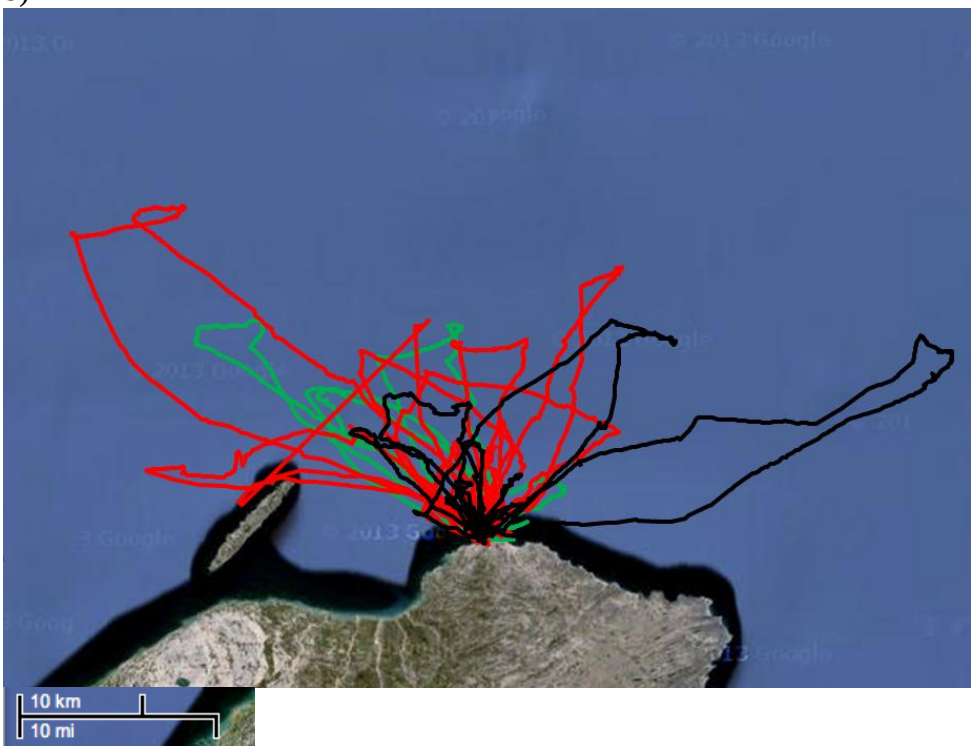

Chapter Six. The prudent parent meets old age: constraint and restraint in senescing seabirds

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My contribution: I conducted all the field work (with field assistants, and with logistical help from T. Gaston and S. Hatch) and laboratory work (with laboratory assistants; the only exception was the 2008 corticosterone analyses, completed by K. O'Reilly), completed all statistical analyses and wrote the paper (with comments from G. Anderson and J. Hare).

Summary

1. Young animals may reduce investment in reproduction owing to high probability of subsequent reproduction (the "restraint" hypothesis). The very oldest animals may also show restraint in reproduction because even a small increase in reproductive investment may lead to death. Alternatively, reproduction may be constrained by senescence (the "constraint" hypothesis). Many studies have observed an increase in reproductive success in long-lived animals with age followed by reduced success at the end of life, but few studies have examined the proximate mechanisms that provide a context for understanding the ultimate causes of these patterns.
2. To address this issue, I examined the stress response (including time to return to the offspring and corticosterone, the principle avian glucocorticoid responsible for directing energy away from reproduction and towards survival following stress) in two species of free-living long-lived seabirds.
3. Several measures of the stress response were highest in both young and very old birds. When birds were challenged exogenously, there was no change in corticosterone levels with age, again implying that they were "choosing" to be restrained.
4. Hormonal cues led to greater investment in adult survival over reproductive output at both the start and end of life consistent with the restraint hypothesis.

Introduction

Life-history theory predicts that iteroparous organisms will reduce investment in current reproduction when costs to future reproductive output outweigh the benefits of current reproduction (Williams 1957; Stearns 1992; Roff 2002). Young animals are expected to invest less energy in reproduction as they have high probability of subsequent reproduction (the "restraint" hypothesis, e.g. Heidinger *et al.* 2006; Desprez *et al.* 2011). Increased investment in current reproduction as the probability of substantial future reproductive output decreases may in part explain why reproductive success usually increases with age (de Forest & Gaston 1996; Newton 1998; Angelier *et al.* 2007; Lewis *et al.* 2006; Coulson 2011). One potential mechanism to account for such patterns is a reluctance to abandon reproduction when faced with stressors such as adverse feeding conditions or risk of predation as birds get older (Heidinger *et al.* 2006, 2010; Wilcoxen *et al.* 2011; Goutte *et al.* 2011).

The oldest animals may show restraint in reproduction because even a small increase in energy expended during reproduction may lead to death (McNamara *et al.* 2009; Heinze & Schrempf 2012). Alternatively, both young and very old animals may lack the ability to maintain high levels of energy consumption (the "constraint" hypothesis), leading to reduced reproductive success (Finkel & Holbrook 2000; Costantini *et al.* 2012). Many studies have observed an increase in reproductive success in long-lived animals with age followed by a reduction at the end of life (de Forest & Gaston 1996; Newton 1998; Angelier *et al.* 2007; Lewis *et al.* 2006; Coulson 2011), but fewer studies have examined the proximate mechanisms, which can provide a context for understanding the ultimate causes of these patterns.

The endocrine stress response provides a mechanism through which the competing demands of life-history trade-offs can be regulated, including those between current versus future reproduction. Glucocorticoids released in response to a stressful event shift energy investment away from reproduction and towards survival (Wingfield & Kitaysky 2002; Satterwaithe *et al.* 2010; Kitaysky *et al.* 2010; Schultner *et al.* 2012). Older breeding individuals may secrete lower levels of glucocorticoid hormones in response to a stressor during breeding (Table 6.1). As glucocorticoid hormones can interact negatively with parental hormones, such as prolactin, thus increasing desertion or reducing other forms of investment in offspring, the net result of reduced glucocorticoid responses can be to maintain investment in offspring in response to a stressor (Angelier *et al.* 2007, 2009; Goutte *et al.* 2010; Kitaysky *et al.* 2010; Lendvai *et al.* 2010; Seltmann *et al.* 2012).

Although reduced levels of glucocorticoid hormones with age could represent restraint via (1) increased investment in offspring when the probability for future reproductive success is low (Heidinger *et al.* 2006), alternative explanations exist. For instance, (2) young individuals may be unlikely to successfully reproduce because they are unable to forage adequately, have inadequate partners, obtain sub-optimal breeding sites or are otherwise inexperienced breeders (de Forest & Gaston 1996; Newton 1998; Angelier *et al.* 2007; Lewis *et al.* 2006; Coulson 2011). In that case, young individuals may show restraint and be more likely to abandon offspring in the face of a stressor because they are unlikely to reproduce successfully in any case, regardless of future reproductive success. Alternatively, senescence or habituation may constrain the stress response. For instance,

(3) older individuals may have habituated to stressors or (4) senescence may cause deterioration in adrenal tissue, both of which may dampen stress responses.

I examined these four hypotheses in two long-lived, cliff-nesting seabirds. I predicted that if (1) were true, then diminished response to a stressful event would be proportional to future reproductive potential and be particularly pronounced (exhibiting the greatest suppression) late in life, when future reproductive prospects are particularly low. If (2) were true, I would anticipate that the rate of diminishment of the stress response would be highest early in life, when reproductive success increases rapidly. I predicted that if (3) or (4) were true, then pre-breeding birds would show similar declines in corticosterone responses as breeding birds. Corticosterone is secreted by the adrenal tissue in response to adrenocorticotropic hormone (ACTH), which is secreted by the pituitary gland in the brain. Increased circulating levels of corticosterone then negatively feedback to the hypothalamus and the pituitary to inhibit further release of ACTH. By first using the corticosterone agonist dexamethasone to suppress natural production of ACTH (and therefore corticosterone) and then exogenously injecting ACTH, I can assess the responsiveness of adrenal tissue. If (4) but not (3) were true, I would observe similar declines in response to stimulation of adrenal tissue during these adrenocorticotropic hormone (ACTH) challenges. To provide a stronger context for examining these relationships, I also examined behavioural adjustments occurring in tandem with a corticosterone response.

Methods

FIELD METHODS

I studied chick-rearing black-legged kittiwakes from 10 to 15 July 2010 (N = 32) and from 12 to 20 July 2012 at Middleton Island, Alaska, and chick-rearing thick-billed murres from 20 to 31 July 2008 (N = 43) and 24 to 30 July 2011 at Coats Island, Nunavut. I also studied pre-breeding (8-22 d pre-lay) kittiwakes at Middleton Island from 5 to 20 May 2012 (I did not study pre-laying murres due to the difficulty of capturing murres before laying). Chick-rearing murres had chicks 3-10 d old whereas chick-rearing kittiwakes had chicks 10-20 d old. I captured murres using a noose pole and kittiwakes using a wire clothes-hanger fashioned into a hook. To avoid reductions in stress response due to habituation, I only used murres from sections of the colony where they had not been handled since banded as a chick ("R" and "Past N" ledges). For kittiwakes, I included years of handling as a covariate. I restrained birds by placing them in cloth bags that tightly fit the body. Only one adult per nest was captured. I did not correct body mass for linear size as such corrections do not improve estimation of lean or lipid mass in either murres or kittiwakes (Jacobs *et al.* 2012). For instance, in my kittiwake data set, body mass alone explains 92% of the variation in the residual of body mass on the first principal component of head-bill, tarsus, culmen and wing length and 93% of the variation in the residual of body mass on head-bill, and consequently size-corrected mass explains little of the variation beyond mass alone. Murres were sexed using sex-specific PCR primers applied to a drop of blood on filter paper (Elliott *et al.* 2010a) whereas kittiwakes were sexed behaviourally by position during copulation. Both parents share incubation and chick-rearing duties, at least during the period of time covered by the

current studies. Research was approved by the Protocol Management and Review Committee of the University of Manitoba under protocol F11-020.

BLOOD SAMPLING

I obtained 1 mL of blood from kittiwakes and 2 mL of blood from murres <3 minutes after capture for baseline hormone levels and 0.5 mL of blood from kittiwakes and 1 mL of blood from murres after 30 minutes of restraint. During ACTH restraint experiments, from both control and ACTH-challenged individuals, I obtained 0.5 mL (kittiwakes) or 1.0 mL (murres) of blood from kittiwakes in <3 minutes, after 30 minutes of restraint and after 60 minutes of restraint. Blood samples were stored on ice for <4 h, centrifuged at 2000*g* for 10 minutes, the plasma was removed and stored at –20°C for the remainder of the field season (<one month) and then shipped to the University of Manitoba on dry ice and stored at –80°C until analysis. I also recorded behaviour during the first 10 minutes of restraint by counting the number of times the bird attempted to escape. I classified birds that never attempted to escape as "calm", those that attempted to escape for 0-2 minutes as "normal" and those that attempted to escape >2 minutes as "not calm". I also recorded the time to return to the colony following restraint.

INJECTIONS

During the chick-rearing experiments, I injected intramuscularly (pectoralis muscle) 45 chick-rearing kittiwakes (42 controls), 40 pre-breeding kittiwakes (44 controls) and 27 chick-rearing murres (44 controls) with 0.2 mL dexamethasone (Sigma D1756: 0.5 mg = kittiwakes; 1 mg = murres) immediately following the baseline blood sample and with 0.2 mL adrenocorticotropic hormone (Sigma A6303 : 45 IU = kittiwakes ; 100 IU = murres) 30 minutes later. I based ACTH dosages on a similar study in another

charadriiform species (Heidinger *et al.* 2010); five murres injected with 200 IU did not have a higher corticosterone response as increased ACTH dosage is more likely to increase the duration of the corticosterone response than its magnitude. I randomly selected every other bird (kittiwakes) or every third bird (murres) as ACTH-challenged birds. Control birds were injected with phosphate-buffered saline at each step. As described earlier, I obtained blood samples 30 minutes after dexamethasone and ACTH injections.

CORTICOSTERONE AND GLUCOSE ASSAYS

I used a radioimmunoassay (RIA) to measure corticosterone levels in duplicate using 25 μL of plasma extracted into 1.2 mL of ethanol (2009-2012) or 25 μL of unextracted plasma (2008; those assays were conducted by K. O'Reilly at the University of Portland). For a subset of samples, there was no difference between ethanol-extracted plasma corticosterone levels and those extracted using dichloromethane. In 2009-2012, extracted samples were reconstituted in 100 μL of RIA buffer (0.1 M phosphate buffer, 0.9 % NaCl [w/v], and 0.5 % bovine serum albumin [w/v]). A standard curve was created by adding known concentrations (0.01 to 50 ng ml^{-1}) of corticosterone standard (Steraloids) into RIA buffer. To each of the test tubes, I then added 100 μl of tritiated (5000 disintegrations per minute) corticosterone (GE Healthcare, 78.1 MBq mol^{-1}, Code TRK406) and 100 μl of sheep serum anti-corticosterone (diluted 1:50000; Antibodies-online, Code ABIN343319). After 1 h incubation at room temperature, samples were incubated at 4°C for a minimum of 18 h. The reaction was stopped through the addition of 100 μl of dextran (0.5% w/v)-coated charcoal (5% w/v) to each assay tube. After 15 min on ice, assay tubes were centrifuged for 30 min at 4°C (2500 × g), and the

supernatant was decanted into 7 ml scintillation vials. Finally, 4 ml of scintillation fluid (Ultima Gold, Perkin Elmer) was added to each assay tube, and tubes were counted on a liquid scintillation counter (LS6500, Beckman Coulter) for 5 min. All samples were counted in duplicate, and corticosterone concentration of each sample was interpolated from the standard curve that was counted in triplicate. Inter-assay and intra-assay coefficients of variation for corticosterone were 7.3 and 5.2%, respectively, samples demonstrated good parallelism (ANCOVA, murres: $F_{4,22} = 1.24$, $p = 0.32$; kittiwakes: $F_{4,22} = 0.99$, $p = 0.45$). The minimum detection level was 0.025 ng ml^{-1}. In 2008, all samples were measured via ^{125}I double antibody radioimmunoassay (MP Biomedical kit 07-120103) directly on unextracted plasma following the kit instructions. The single assay had intra-assay variability of 5.4%.

For kittiwakes, I recorded glucose levels by placing a drop of blood on a test strip and reading the value using a Bayer contour meter (kittiwakes). For murres, I recorded glucose level by adding 5 μL of plasma (5 μL gave a coefficient of variation of 3.1% whereas adding the recommended 2 μL gave a coefficient of variation of 7.2%) to 96-well plates with the reagents from the Wako LabAssay kit (product code: 298-65701) based on the mutarotase method (murres).

CORTICOSTERONE-BINDING GLOBULIN

I measured corticosterone-binding globulin (CBG) levels on baseline plasma samples according to established protocols (Breuner and Orchinik 2002; Love *et al.* 2004). I stripped endogenous corticosterone from plasma by incubating 5 μL of plasma in 495 μL of dextran-coated Norit charcoal (10 g/L) for 30 min at room temperature prior to centrifuging for 10 min at 3000*g*. I determined total binding capacity (in triplicate; 50 μL

of stripped plasma, 50 µL buffer, 50 µL of tritiated corticosterone at 20000 DPM; 1:300 dilution) and non-specific binding (in duplicate; 50 µL of stripped plasma, 50 µL 1 µg/mL corticosterone, 50 µL of tritiated corticosterone) for each individual. Following 2 h incubation, I used a Brandel harvester (Brandel, Gaithersburg, MD) to separate the free and bound fractions using glass fiber filters (Whatman GF/B) pre-soaked for 1 h in 25 nM Tris buffer with 0.3% polyethyleneimine. I suspended filters in scintillation fluid for 24 h before measuring radioactivity. Intra-assay variability was 3.2% and inter-assay variability was 9.6%. I estimated concentration of free corticosterone (H_{free}) as:

$$H_{free} = 0.5\left[H_{total} - B_{max} - K_d \pm \sqrt{(B_{max} - H_{total} + K_d)^2 + 4H_{total}K_d}\right]$$

where K_d = 1.20 for kittiwakes and 1.18 for murres, B_{max} is the concentration of CBG molecules and H_{total} is the concentration of total corticosterone (Barsano and Baumann 1989).

TIME TO RETURN TO THE COLONY (TEMPERATURE LOGGERS)

I attached temperature loggers (Lotek 1100, 5 g or 0.5% of body mass) that recorded time flying and time at the colony (Elliott *et al.* 2008). The loggers were attached to the band using duct tape and total restraint time was <5 min. Using the data from the logger, I recorded time to return to the colony following capture. I also used visual observations to record time to return to the colony following capture and restraint for corticosterone analyses.

METABOLIC RATE

I used the same dataset reported in Chapter Two, including birds of known age. Briefly, I placed post-absorptive, resting birds in a small oxygen chamber and used a Fox Box II to measure oxygen consumption and carbon dioxide production over four hours after

removing water vapour and, for the oxygen analyzer, carbon dioxide. I used equations from Withers (2001) to convert measured percent oxygen into instantaneous oxygen consumption rate, and averaged values over five minute intervals. I then subtracted the lowest 30-minute average instantaneous oxygen consumption rate ("un-stressed resting metabolic rate") from the average instantaneous oxygen consumption rate over the first ten minutes (two intervals). I expressed that value as a residual on body mass.

STATISTICAL ANALYSES

I log-transformed corticosterone levels and time to return to obtain normality. I used general linear models to examine the effect of age, year, time since laying (relative date, which is negative for pre-laying birds), sex, time of day, body mass, sampling time, date and recapture history (kittiwakes only; I avoided murres that had been handled previously as adults) on corticosterone levels. I fit quadratic polynomials to describe the relationship between the parameters documented and age.

I obtained blood samples after 0, 30 and 60 minutes and recorded corticosterone and corticosterone-binding globulin by radioimmunoassay and glucose levels enzymatically. To examine investment in the offspring, I recorded time to return to the offspring following restraint of <5 min (without blood-sampling). To examine the relationship between corticosterone and investment in offspring, I recorded time to return to the offspring following restraint for corticosterone analyses (60 min). As glucose levels are expected to increase in response to corticosterone, similar patterns are expected between glucose and corticosterone. To examine the responsiveness of tissues to corticosterone I examined the residual of glucose levels on corticosterone. A high responsiveness would be associated with a higher level of glucose for a given concentration of corticosterone. I

also recorded the time to return to the colony following restraint. In a separate group of birds where I measured metabolic rate for four hours in an oxygen chamber, I measured metabolic rate during the first 10 minutes of capture (as part of the same experiments reported in Chapter Three).

I log-transformed corticosterone levels and time to return to obtain normality. I used general linear models to examine the effect of age, year, time since laying (relative date, which is negative for pre-laying birds), sex, time of day, body mass, sampling time, date and recapture history (kittiwakes only; I avoided murres that had been handled previously as adults) on corticosterone and glucose levels, and time to return to the colony.

Results

Baseline corticosterone levels declined with body mass in kittiwakes and with relative date in pre-breeding but not chick-rearing kittiwakes (Table 2). Corticosterone concentration at 60 minutes declined with age in chick-rearing murres and kittiwakes but not pre-breeding kittiwakes before increasing again for the oldest age classes (Table 6.2, Fig. 6.1). Corticosterone concentration at 60 minutes in kittiwakes and murres challenged via ACTH was not related to any of the examined parameters (Table 6.2). As corticosterone-binding globulin levels after stress during chick-rearing were independent of age (murres: $t_{55} = 0.56$, P = 0.57; kittiwakes: $t_{77} = 0.89$, P = 0.35), corticosterone patterns were not affected by globulin levels. For instance, free corticosterone at 60 minutes followed a similar quadratic pattern during chick-rearing (Age; murres: $t_{55} = -2.47$, P = 0.01; kittiwakes: $t_{77} = -3.59$, P = 0.0006; Age^2; murres: $t_{55} = 3.61$, P = 0.0007; kittiwakes: $t_{77} = 2.49$, P = 0.01).

Baseline glucose levels declined in concert with baseline corticosterone levels (pre-breeding kittiwakes: $t_{45} = 3.41$, $P = 0.001$; chick-rearing kittiwakes: $t_{82} = 3.28$, $P = 0.002$) and increased with time of day (pre-breeding kittiwakes: $t_{45} = 2.77$, $P = 0.008$; chick-rearing kittiwakes: $t_{82} = 2.99$, $P = 0.004$), but were unaffected by body mass or capture time ($P > 0.05$). The change in glucose levels from baseline to 60 minutes increased with corticosterone levels at 60 minutes (pre-breeding kittiwakes: $t_{41} = 2.56$, $P = 0.01$; chick-rearing kittiwakes: $t_{76} = 2.32$, $P = 0.02$). After accounting for corticosterone levels, glucose declined with age, but was also higher in very old birds (Fig. 6.1).

Birds with lower stress-induced corticosterone were calmer during handling (chick-rearing kittiwakes: $t_{75} = 3.15$, $P = 0.002$; murres: $t_{41} = 2.04$, $P = 0.04$) and took less time to return to their chicks (chick-rearing kittiwakes: $t_{75} = 3.15$, $P = 0.002$; murres: $t_{36} = 5.06$, $P < 0.0001$). Return time declined with age before increasing again for the oldest age classes (Fig. 6.1). Older birds were calmer during handling (kittiwakes: $t_{76} = 1.92$, $P = 0.05$; murres: $t_{41} = 4.21$, $P = 0.0001$) and had a lower oxygen consumption rate during the first 10 minutes of handling relative to their long-term baseline (kittiwakes: $t_{32} = 2.24$, $P = 0.03$; murres: $t_{38} = 2.51$, $P = 0.02$).

Discussion

In support of the restraint hypothesis, the stress response was reduced at both the start and end of life. As would be expected based on a life-history trade-off between current and future reproduction, several measures of the stress response were attenuated at ages when reproductive success was usually high (de Forest & Gaston 1996; Newton 1998; Angelier *et al.* 2007; Lewis *et al.* 2006; Coulson 2011). Specifically, my data matched the predictions of hypothesis (2) and, thus, I conclude that individuals suppress the stress

response when they have a high probability of current successful reproduction. For example, the declines in glucocorticoid response with age were absent in pre-breeding birds and birds challenged with ACTH, and were most obvious at young ages, implying that the attenuated stress response was not due to senescence. As has been the case in many other studies (Table 6.1), the stress response closely tracked reproductive success, which is lower in both young and very old murres and kittiwakes (de Forest & Gaston 1996; Lewis *et al.* 2006; Coulson 2011). In those studies where reproductive success did not decline in old age—either because of selective mortality of "poor quality" individuals or because few exceptionally old birds were included—there was no increase in the stress response at the end of life (Table 1).

Selection based on residual reproductive success is also unlikely on a theoretical basis. A bird with aging parameter 0.1 and extrinsic mortality 0.1, about equivalent to a murre or kittiwake (as predicted from Ricklefs 2010 based on a survival of ~90%), would still have an annual survival probability of 80% at age 25; Coulson (2010) was unable to detect reduced mortality up to age 17—the maximum age he recorded—in kittiwakes. Thus, even very old birds still have high residual reproductive success and selection for any parameter, including selection favouring curtailment of investment based on future prospects, at those ages is likely weak. It is more compelling that natural selection would act to produce a mechanism that effectively acts to reduce responsiveness to a stressor when current reproductive prospects are favourable as opposed to a mechanism reducing responsiveness to stressors when future reproductive prospective are limited years into the future. Presumably, birds are able to assess their own condition, their partner's quality, their site quality and local conditions to determine whether current reproduction is

probable, and convey that message to the hypothalamus, modulating the stress response. The stress response is one mechanism for reducing reproductive effort (or abandoning reproduction) either via behavioural changes or via an effect on other hormones, such as prolactin; seabirds can apparently suppress the stress response when current reproductive value is high.

Multiple measures of the stress response changed with age, including the glucocorticoid response, behaviour during restraint, time to return to the offspring, metabolic rate and glucose production. Assuming that glucose clearance rate is unaffected by age, the observed changes in glucose concentration after accounting for glucocorticoid levels suggest that tissue responsiveness to glucocorticoids (e.g. glucocorticoid receptors) is regulated in tandem with the total adrenal response such that when the stress response is reduced there is less glucose production and release. There was no change in glucocorticoid levels in birds challenged with ACTH suggesting that control of the stress response does not occur via reductions in adrenal tissue with advancing age. Increases in adrenal capacity observed in old primates are thought to be associated with senescence (Sapolski & Altmann 1991; Otte *et al.* 2005) and the absence of a relationship when challenged with ACTH provides additional evidence against the increases at the end of life being associated with senescence in wild birds. In contrast, glucocorticoid response declined with age following ACTH challenge in another charadriiform bird, providing evidence for reduced adrenal capacity (Heidinger *et al.* 2008). One possibility is that because the birds in that study were not first injected with dexamethasone to suppress the stress response prior to ACTH challenge, the response to

the ACTH challenge may have been additive to the existing stress response (Heidinger *et al.* 2008).

My study adds to the growing literature demonstrating a connection between the glucocorticoid stress response and reduced parental behaviours (Lendvai *et al.* 2007; Angelier *et al.* 2009; Ouyang *et al.* 2012; Riechert *et al.* 2012; Thierry *et al.* 2013). For example, older ancient murrelets (*Synthliboramphus antiquus*) were less likely to abandon than younger murrelets when electronic recorders were attached to the birds, presumably reflecting an attenuated stress response with age (Elliott *et al.* 2010). Prolactin, a hormone associated with parental behaviour and secreted directly by the pituitary gland, also declines during stressful events (Chastel *et al.* 2005; Angelier *et al.* 2007; Heidinger *et al.* 2010). Presumably, individuals have a general stress response, controlled at the level of the brain, that affects multiple parameters either via the downstream effect of glucocorticoids on, for example, prolactin, or via reduced prolactin secretion directly at the level of the brain.

One explanation for why the stress response decreases with age is that individuals that are less responsive to stress are favoured by selection. Such an explanation would not account for the increase at very old ages. I also do not believe that habituation to capture and handling explains the decline because I used "naïve" murres and recapture history was not a significant factor in any of the models for kittiwakes. Thus, my study animals were not acclimated to restraint and responded to capture with a general stress response.

Rather, my study provides evidence that the stress response serves as a mechanistic link modulating life-history trade-offs. A major source of reproductive failure for cliff-nesting kittiwakes and murres is the loss of offspring to avian predators or to

dislodgement (de Forest & Gaston 1996; Coulson 2011). Even a few seconds' abandonment dramatically increases the chance of predation by gulls or dislodgement of precariously placed offspring. Thus, birds that remain with their offspring or return quickly following disturbance are more likely to reproduce successfully (Gilchrist *et al.* 1998).

However, birds that do not abandon in the face of risk may experience increased mortality. Murres aged 18-23—ages with low stress response in my dataset—were disproportionately represented among those killed by polar bears (*Ursus maritimus*) and heat/mosquito stress on Coats Island (Gaston & Elliott 2013). In both cases casualties occurred because breeding birds refused to abandon the breeding effort when confronted with lethal threats (Gaston & Elliott 2013).

In summary, I provide evidence for a role of the general stress response in a trade-off between current reproduction and future reproduction [Wingfield & Kitaysky 2002; Boonstra 2004; Satterwaith *et al.* 2010). When the probability of current reproduction is high, individuals show suppressed responses to stress. That stress response favours reproduction over survival in the presence of a variety of stressors, including famine and predation. Future research should focus on the role of prolactin and ACTH in the stress response via direct measurements. I predict that the magnitude of both the prolactin decrease and ACTH increase during a stressor will show a similar curvilinear relationship with age, peaking at middle ages.

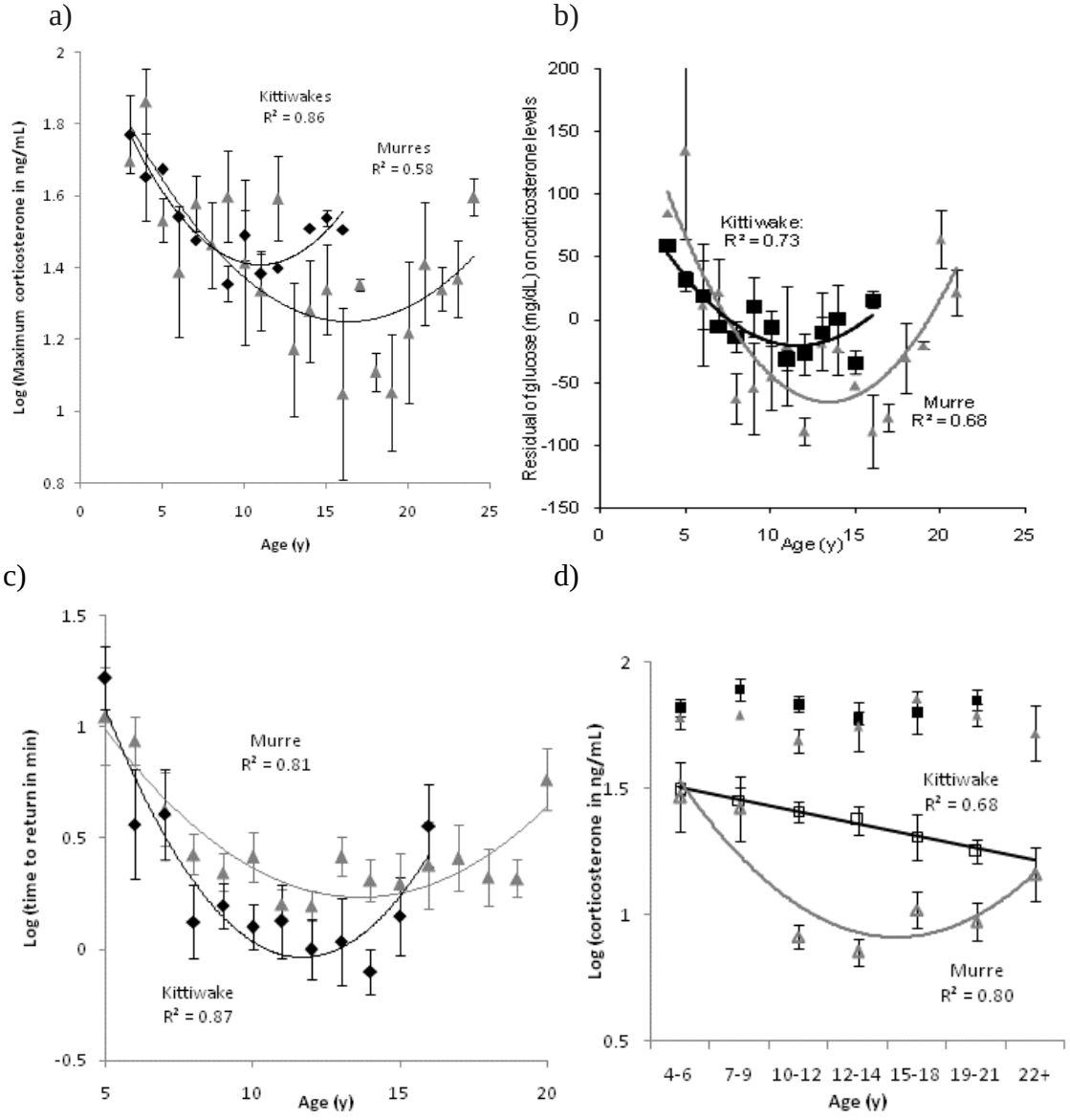
Table 6.1. Comparison of glucocorticoid response to a stressor relative to age with reproductive success relative to age across breeding vertebrates.

Species	**Glucocorticoid response**	**Reproductive success**	**Reference**
Green turtle *Chelonia mydas*	Higher in juvenile	Lower in juvenile	Jessop & Hamann 2005
Human *Homo sapiens*	Increases with age	Declines with age	Reviewed by Otte *et al.* 2005
Dog *Canus lupus*	Increases with age	Not reported	Reull *et al.* 1991
Rat *Rattus norvegicus*	Declines with age	Not reported	Hess *et al.* 1972; Brett *et al.* 1983
Black-legged kittiwake	Lowest in middle age	Highest in middle life	My study; (reproduction: Coulson 2011)
Common tern *Sterna hirundo*	Declines with age	Increases with age	Heidinger *et al.* 2006
Florida scrub-jay *Aphelocoma coerulescens*	Lowest in middle life	Highest in middle life	Wilcoxen *et al.* 2011
Snow petrel *Pagodroma nivea*	Declines with age	Increases with age	Goutte *et al.* 2011 (but see Angelier *et al.* 2007)
Thick-billed murre	Lowest in middle age	Highest in middle life	My study; (reproduction: de Forest & Gaston 1996; Lewis *et al.* 2007)
Wandering albatross *Diomedea exulans*	Lowest in middle life	Highest in middle life	Lecomte 2010

Table 6.2. Results from general linear models including various independent variables for explaining log-transformed baseline and maximum corticosterone (60 minutes after capture) for control (non-injected) and ACTH-injected (dexamethasone at zero minutes, ACTH at 30 minutes—relationships reported for samples collected both at 30 minutes ("DEX") and 60 minutes ("ACTH")) thick-billed murres and black-legged kittiwakes.

	Baseline CORT			Max CORT (Control)			Max CORT (ACTH)			Max CORT (DEX)		
Kittiwakes (pre-breeding)	t	P	β	t	P	β	t	P	β	t	P	β
Body mass	**-3.34**	**0.001**	**-0.42**	1.40	0.18	-0.29	0.94	0.45	-0.13	1.84	0.05	0.35
Relative date	**2.99**	**0.004**	**0.35**	-1.75	0.10	-0.35	-0.88	0.51	-0.14	-0.14	0.82	-0.01
Age	0.44	0.66	0.05	-0.43	0.67	-0.09	0.45	0.66	0.07	-0.69	0.49	0.13
Age^2	0.32	0.84	0.03	0.05	0.95	0.00	0.65	0.54	0.10	0.14	0.88	0.03
Sex	0.78	0.44	0.09	0.32	0.76	0.07	0.15	0.87	0.03	1.02	0.31	0.19
Sampling time	0.52	0.60	0.06	-1.42	0.17	-0.29	-0.48	0.64	-0.08	0.34	0.73	0.06
Hours since sunrise	-0.32	0.75	-0.04	-1.82	0.08	-0.40	**2.18**	**0.04**	**0.39**	1.01	0.31	0.19
Recapture history	0.88	0.38	0.10	0.66	0.51	0.05	0.55	0.71	0.09	0.14	0.88	0.03
Kittiwakes (chicks)												
Body mass	**-2.02**	**0.04**	**-0.22**	1.28	0.21	0.19	0.86	0.40	0.10	1.03	0.30	0.20
Relative date	1.45	0.09	0.19	0.25	0.84	0.05	1.01	0.31	0.12	0.14	0.88	0.03
Age	-1.33	0.11	-0.17	**-3.35**	**0.002**	**-0.55**	0.49	0.63	0.07	**-3.22**	**0.003**	-0.61
Age^2	1.22	0.14	0.15	**2.45**	**0.02**	**0.42**	0.38	0.72	0.04	-0.03	0.98	0.01
Sex	0.54	0.60	0.07	0.29	0.79	0.06	0.85	0.40	0.10	0.18	0.85	0.03
Year	0.17	0.90	0.02	0.25	0.85	0.02						
Sampling time	0.87	0.55	0.11	0.04	0.97	0.01	0.53	0.60	0.08	0.24	0.81	0.05
Hours since sunrise	-1.02	0.15	-0.13	1.16	0.25	0.17	0.55	0.60	0.08	0.68	0.50	0.13
Recapture history	0.03	0.98	0.00	0.41	0.60	0.08	0.81	0.42	0.10	0.22	0.82	0.04
Murres (chicks)												
Body mass	-1.55	0.08	-0.20	0.95	0.35	0.11	-0.02	0.98	0.00	1.41	0.17	0.27
Relative date	0.55	0.61	0.06	-0.34	0.74	-0.04	0.19	0.85	0.03	0.67	0.51	0.13
Age	-1.41	0.09	-0.19	**-2.31**	**0.03**	**-0.34**	1.01	0.32	0.17	**2.42**	**0.02**	**0.46**
Age^2	1.48	0.08	0.20	**3.14**	**0.006**	**0.45**	0.71	0.50	0.14	**2.06**	**0.04**	**0.37**
Year	0.02	0.99	0.00	0.08	0.97	0.00						
Sex	0.22	0.81	0.02	-0.04	0.95	0.00	0.55	0.59	0.09	0.45	0.66	0.09
Sampling time	1.03	0.17	0.12	0.65	0.52	0.08	-0.23	0.82	-0.04	0.13	0.90	0.02
Hours since sunrise	-1.45	0.09	-0.19	-0.44	0.66	-0.06	1.04	0.31	0.17	0.02	0.98	0.00

Figure 6.1. (a) Corticosterone levels after 60 minutes ("maximal"), (b) residual of the change in circulating glucose (mg/dL) between capture and 60 minutes restraint on corticosterone levels after 60 minutes of restraint, (c) average return time to offspring after capture and restraint for <5 min, and (d) corticosterone levels at 30 minutes following injection with dexamethasone (open symbols) and at 60 minutes following injection with ACTH (closed symbols) relative to age in black-legged kittiwakes (black) and thick-billed murres (grey). Values are averages ± SE at each age class; the last age class includes all individuals in that age class or older due to the small sample sizes for old birds ("16" = 16+; age for pooling defined as the age when only one bird is in an age category). All logarithms are base 10.



Chapter Seven. Reduced investment in immune function and hematocrit during chick-rearing by old female, but not male, tree swallows

Kyle H. Elliott, David Bradley, Ryan Norris, David Hussell, W. Gary Anderson and James F. Hare

My contribution: I conducted all the field work (with field assistants, and with logistical help from D. Bradley, R. Norris and D. Hussell) and laboratory work (with laboratory assistants), completed all statistical analyses and wrote the paper (with comments from G. Anderson and J. Hare).

Summary

1. The disposable soma theory suggests that as animals age, they will invest more strongly in the propagation of genes (reproduction) than in the current phenotype (soma) due to the inevitable accumulation of irreparable wear-and-tear on the soma. In contrast, recent theoretical work indicates that an animal may invest less in reproduction as it nears the end of life due to late-life restraint.
2. To examine these ideas in the natural environment, I measured investment in self (antioxidants: superoxide dismutase in the blood and total antioxidant capacity; hematocrit; immune function: response to Newcastle disease vaccine and phytahemagglutinin, natural antibody levels, blood lytic capability and haptoglobin concentration) and offspring (circulating and stress-induced total and free corticosterone, nest visitation rate) in 32 male and 48 female tree swallows (*Tachycineta bicolor*) over two seasons at Long Point, Ontario.
3. Males, but not females, in their first year of life visited the nest less often, had higher baseline and stress-induced corticosterone and lower glucose levels, suggesting reduced foraging abilities. None of these parameters varied after the first year of life for either sex.
4. At the start of chick-rearing, none of the measures of self-investment varied cross-sectionally with age or between birds measured two years apart. However, in females (but not males), the magnitude of the decline in hematocrit and natural antibody levels over the course of chick-rearing increased with age.
5. Tree swallow senescence fits the disposable soma theory of aging: as birds aged, investment in reproduction occurred at the cost of the soma.

Introduction

The disposable soma theory posits that there is a tradeoff in the allocation of energy between reproduction and soma (Hamilton 1966; Kirkwood & Holliday 1979). Because of the inevitable accumulation of wear-and-tear associated with reproductive events, each diminishing the residual reproductive value of the individual, investment in reproduction over soma should increase with age (Kirkwood & Austad 2000). There is now ample evidence in wild populations that senescence (a reduction in survival and/or reproduction with age) occurs in the natural environment (Ricklefs 2008; Jones *et al.* 2008), and that reproduction increases mortality (Daan *et al.* 1996; Paredes *et al.* 2004; Reid *et al.* 2003). For instance, the earlier an individual common murre (*Uria aalge*) chooses to reproduce, the quicker that individual senesces at the end of life (Reed *et al.* 2008). Alternatively, however, recent theoretical work suggested that aging animals may show late life restraint until conditions improve because any additional investment may lead to death (McNamara *et al.* 2009). In that sense, the "rapid senescence" at the end of life, as measured by reduced reproductive capability near death (Reid *et al.* 2003; Reed *et al.* 2008), may represent restraint rather than constraint associated with senescence.

Although there is substantial literature on wild animals showing that part of the "cost of reproduction" is increased senescence (e.g. Reid *et al.* 2003; Paredes *et al.* 2004; Reed *et al.* 2008), few studies examine the underlying mechanism in a natural context, which is necessary to successfully disentangle constraint from restraint. One issue is that most such studies are necessarily cross-sectional, and therefore any changes occurring within individuals may be obscured by variation in individual quality (Nussey *et al.* 2008). It is

therefore necessary to document changes over the course of a single reproductive event, and examine how that might be related to age.

Tree swallows (*Tachycineta bicolor*) are aerial insectivores that are common throughout most of North America. With longevity of ~12 years, tree swallows have exceptional longevity for their size (Hussell 2001). Swallows, including tree swallows, have been used in several studies documenting declining immunity with age (Saino *et al.* 2003; Cichon *et al.* 2003; Haussman *et al.* 2005; Palacios *et al.* 2007, 2010; Vleck *et al.* 2011). In particular, Saino *et al.* (2003) and Cichon *et al.* (2003) both showed that older swallows produced fewer antibodies in response to a novel antigen (sheep red blood cells or Newcastle disease vaccine). Haussman *et al.* (2005) and Palacios *et al.* (2007) demonstrated reduced swelling in response to a phytahemagglutinin challenge in old tree swallows, with Palacios *et al.* (2007) showing reduced T-cell proliferation in response to phytahemagglutinin. Furthermore, when Palacios *et al.* (2010) used lipopolysaccharide to induce an immune response in tree swallows, they found that older birds lost more mass and were more likely to reduce nest visitation rates, which they suggested meant that they had more trouble overcoming the immune challenge.

I used the pre-existing literature on swallow senescence as background for examining senescence in multiple systems simultaneously. Specifically, I respond to the recent appeal that more studies examine multiple traits simultaneously when examining senescence in wild animals (Nussey *et al.* 2008). I examined three measures of self-investment, antioxidant levels, immune function and hematocrit, and three measures of reproductive investment, corticosterone levels (baseline and stress-induced), glucose levels and nest visitation rates in chick-rearing tree swallows. Consistent with the

disposable soma hypothesis, I predicted that as the animals aged, multiple systems would deteriorate. Under the reproductive restraint hypothesis, I also predicted that, old birds would not allow their own systems to deteriorate through a particular reproductive event at a potential cost to their offspring. As an alternative, under the disposable soma hypothesis, I predicted that old birds would allow their own systems to deteriorate to prevent passing the cost onto their offspring.

Methods

I studied tree swallows during the chick-rearing season (1-20 June) of 2011 and 2012 at Long Point, Ontario, Canada. Nests were monitored daily until hatch, and both members of each pair were captured within three days of hatch. After hatch, nests were monitored every three days until day 12, when each nestling was weighed. All blood samples (see volumes and dates below) were obtained from the alar vein. Blood samples were stored on ice for <4 h, centrifuged at 2000*g*, hematocrit recorded, the plasma removed and the plasma stored at –20°C for the remainder of the field season (~two weeks) and then shipped to the University of Manitoba, Canada, on dry ice and stored at –80°C until analysis. In 2011, within 48 h of completed hatch, I obtained a 150 μL sample within 3 minutes of capture and a second 50 μL blood sample after 30 minutes of capture. I then marked one member of each pair with green dye and videotaped the nest for 24 h. I weighed the nestling the night before and the morning after videotaping. In 2012, I obtained a 200 μL blood sample within 3 minutes of capture, injected the bird with Newcastle disease vaccine and phytahemagglutinin and then recaptured the bird 2 d (for phytahemagglutinin) and 12 d (for Newcastle disease vaccine) later. I obtained a second 200 μL blood sample at 12 d. I videotaped nest visitation rates from 800-1100 on

three days (randomly chosen when chicks were between 5 and 10 d old) at each nest. I restricted analyses to birds with four or five nestlings to remove the confounding effect of clutch size.

IMMUNE FUNCTION

The immune system is complex and there are many different components to a "healthy" immune system. To examine immunosenescence in swallows, I therefore examined several aspects of the immune system. All measurements were begun and injections completed within three days of hatch. Both injections occurred at the same time in each bird.

Phytohemagglutinin. I injected birds in the right patagium (the skin between the caprometatarsus and the ulna/radius) with 0.2 mg of phytohemagglutinin (Sigma-Aldrich, St Louis MO, mixed in 0.05 mL of PBS 0.9% NaCl). The thickness of the wing web was measured to the nearest 0.01 mm using a digital micrometer (model 293; Mitutoyo Inc., Tokyo, Japan) before injection and 48 h later. The difference in wing web thickness before and after injection was taken as an index of the swelling response.

Newcastle disease vaccine. I injected birds with TRIPLEVAC (Merck Animal Health, Whitehouse Station NJ), a live Newcastle disease vaccine (B1 strain), according to the instructions with the vaccine. A baseline blood sample was taken prior to injection. I then obtained a second blood sample 12 d later. I used 25 μL of plasma to measure the circulating level of anti-NDV antibodies pre-injection and after 12 d using a kit (product code NDVC-2P, ID-VET, Montepellier, France). Intra-assay variability averaged 13.2%.

Natural antibody levels. I measured circulating levels of natural antibodies, which are genetically-coded inherent immunoglobulin molecules that are part of the innate immune

system (no exposure to antigen necessary). Natural antibodies and complement (the enzyme cascade that causes cell lysis) were measured using a hemolysis-hemagglutination assay by serially-diluting 25 μL of plasma with Dulbecos PBS (Sigma-Aldrich, St Louis MO) in the presence of rabbit red blood cells in Alsevers (Hemostat, Dixon CA). Assays were run in duplicate, with chicken plasma run as positive controls. I followed the protocol: http://ccoon.myweb.usf.edu/ecoimmunology.org/nAbs.html (accessed 8 October 2012). I scored both lysis and agglutination. I measured natural antibody levels at both day 0 and day 12.

Haptoglobin. Haptoglobin and related proteins (such as ovotransferrin) are acute phase proteins that bind iron—particularly, hemoglobin—that is released during infection from the lysis of erythrocytes. The hemoglobin-protein complex is then removed from circulation, preventing parasites from obtaining nutrition by consuming haemoglobin (Horrocks *et al.* 2011). Haptoglobin is therefore a good indicator of acute infection. I measured the iron-binding capacity of plasma by adding 2 μL of plasma to 96 well plates and the reagents precisely as described in the Phase Range Haptoglobin Assay Kit instructions (Tri-Delta, Maynooth, Ireland, item TP-801). Prior to the addition of reagent 2 (at zero minutes) and 8 minutes after the addition of reagent 2, I read the output at 630 nm on a Biotek plate reader. I calculated haptoglobin concentration using the standard curve based on the difference in absorbance between 8 minutes and 0 minutes, to account for turbidity in the plasma samples. I used 10 μL to reduce pipetting errors; intra-assay variability was 2.7% (compared to 7.8% for another set up samples using 5 μL). I found 8 minutes to represent the peak in absorbance within my samples. The standard curves had

R^2 > 0.999. Values were log-transformed prior to analyses to obtain normality. I measured haptoglobin levels at both day 0 and day 12.

HORMONE AND ANTIOXIDANT ASSAYS

Corticosterone. I used a radioimmunoassay (RIA) to measure corticosterone levels in duplicate using 25 μL (2011) or 12.5 μL (2012) of plasma extracted into 1.2 mL (2011) or 0.6 mL (2012) of ethanol. For a subset of samples, there was no difference between ethanol-extracted plasma corticosterone levels and those extracted using dichloromethane, and we therefore used ethanol extraction for all remaining analyses. Extracted samples were reconstituted in 100 μL of RIA buffer (0.1 M phosphate buffer, 0.9 % NaCl [w/v], and 0.5 % bovine serum albumin [w/v]). A standard curve was created by adding known concentrations (0.01 to 50 ng mL^{-1}) of corticosterone standard (Steraloids) into RIA buffer. To each of the test tubes, I then added 100 μL of tritiated (5000 disintegrations per minute) corticosterone (GE Healthcare, 78.1 MBq mol^{-1}, Code TRK406) and 100 μl of sheep serum anti-corticosterone (diluted 1:50000; Antibodies-online, Code ABIN343319). After 1 h incubation at room temperature, samples were incubated at 4°C for a minimum of 18 h. The reaction was stopped through the addition of 100 μL of dextran-coated charcoal (0.25 mL of dextran and 2.5 mL of charcoal into 50 mL of PBS) to each assay tube. After 15 min on ice, assay tubes were centrifuged for 30 min at 4°C (2500 × g), and the supernatant was decanted into 7 mL scintillation vials. Finally, 4 ml of scintillation fluid (Ultima Gold, Perkin Elmer) was added to each assay tube, and tubes were counted on a liquid scintillation counter (LS6500, Beckman Coulter) for 5 min. All samples were counted in duplicate, and corticosterone concentration of each sample was interpolated from the standard curve that was counted in triplicate. Inter-assay and intra-assay

coefficients of variation for corticosterone were 8.4 and 6.1%, respectively, samples demonstrated good parallelism (ANCOVA, $F_{4,22} = 0.81$, $p = 0.53$). The minimum detection level was 0.025 ng ml^{-1}.

Antioxidant capacity. I measured levels of superoxide dismutase in red blood cells using the Cayman Chemical (Ann Arbor MI) superoxide dismutase kit (item 706002). I lysed the 25 μL of red blood cells by adding 100 μL of ice-cold milliQ water, centrifuged at 10 000g for 10 minutes, and then diluted the supernatant 1:100 with sample buffer. The intra-assay coefficient of variation was 7.6%. I measured total antioxidant capacity of the samples collected for baseline corticosterone assays using the Cayman Chemical (Ann Arbor MI) antioxidant assay kit (item 709001). I diluted 2 μL of plasma in 100 μL of sample buffer prior to assaying. The intra-assay variation was 3.1%. I then measured uric acid concentration via the Cayman Chemical uric acid kit (item 700320). I also diluted 2 μL of plasma in 100 μL of sample buffer prior to assaying. The intra-assay coefficient of variation was 4.3%. I considered plasma antioxidant capacity to represent tissue antioxidant capacity, and calculated the true antioxidant capacity to be total antioxidant capacity – antioxidant capacity of uric acid; although uric acid is a major antioxidant in the plasma, it is likely associated with protein catabolism rather than antioxidant protection at the level of the tissue.

Corticosterone-binding globulin. I measured corticosterone-binding globulin (CBG) levels on baseline plasma samples according to established protocols (Breuner and Orchinik 2002; Love *et al.* 2004). I stripped endogenous corticosterone from plasma by incubating 5 μL of plasma in 495 μL of dextran-coated Norit charcoal (1 g/L) for 30 min at room temperature prior to centrifuging for 10 min at 3000*g*. I determined total binding

capacity (in triplicate; 50 µL of stripped plasma, 50 µL buffer, 50 µL of tritiated corticosterone at 20000 dpm; 1:300 dilution) and non-specific binding (in duplicate; 50 µL of stripped plasma, 50 µL 1 µg/mL corticosterone, 50 µL of tritiated corticosterone) for each individual. Following 2 h incubation, I used a Brandel harvester (Brandel, Gaithersburg, MD) to separate the free and bound fractions using glass fiber filters (Whatman GF/B) pre-soaked for 1 h in 25 nM Tris buffer with 0.3% polyethyleneimine. I suspended filters in scintillation fluid for 24 h before measuring radioactivity. Intra-assay variability was 3.2% and inter-assay variability was 9.6%. I estimated concentration of free corticosterone (H_{free}) as:

$$H_{free} = 0.5\left[H_{total} - B_{max} - K_d \pm \sqrt{(B_{max} - H_{total} + K_d)^2 + 4H_{total}K_d}\right]$$

where K_d = 1.05, B_{max} is the concentration of CBG molecules and H_{total} is the concentration of total corticosterone (Barsano and Baumann 1989).

Results

Nest visitation rate by males, but females, increased with age (Table 1). Glucose levels in males also increased with age (Table 1). In contrast, baseline and stress-induced corticosterone decreased with age. However, all those trends were attributable to contributions from birds in the first year of life, and once that year was removed, there was no variation in those parameters with age in either sex. In total, none of 12 different physiological and behavioural parameters varied with age in swallows (Table 1).

Within the 2012 season, hematocrit and natural antibody levels were lower 12 days after the Newcastle disease vaccine challenge (Fig. 7.1). That reduction was associated with age in females, but not males (Fig. 7.1).

Discussion

In support of the disposable soma hypothesis in female tree swallows, throughout a reproductive event that was presumably made more challenging due to vaccination with Newcastle disease vaccine, females continued to visit their offspring at the same rate. That reproduction, however, came at the cost of reduced physical condition in terms of lower hematocrit and immune function. In contrast, Palacios *et al.* (2007) found, at least in one year, a reduction in nest visitation rate (as well as body mass) when old tree swallows were experimentally challenged via simulation of a bacterial infection.

Because of their different levels of investment in reproduction, males and females would be expected to show different senescence patterns under the disposable soma theory. Nonetheless, few studies examine senescence in male birds, in particular (Lecomte *et al.* 2010). In mammals, theoretical models suggest that males should show greater reductions in self-investment with age, and there is data supporting those theories (McElligott et al. 2002). In contrast, I found that female, not male, swallows, showed reduced condition during old age. Apparently, male tree swallows, which may not be fathering their own offspring due to a relatively high level of promiscuity (up to 55% of offspring may be fathered via extra-pair parentage; Conrad *et al.* 2001), were unwilling to sacrifice their own condition during reproduction. Alternatively, males may be less impacted by reproduction as they do not generally contribute to incubation or brooding (Ardia 2005).

One-year-old male (but not female) swallows had lower nest visitation rates, higher corticosterone levels and lower glucose. I interpret this to mean that they were less successful at foraging, as lower blood glucose levels may reflect a longer time period

since the bird last ate. Similarly, high corticosterone levels are often associated with nutritional stress (Angelier *et al.* 2007a,b). Males are larger and feed their offspring less often than females, perhaps implying that they provide larger meals (Ardia 2005). Perhaps it is more difficult to capture the larger loads, meaning that young males were unable to keep up with demand. Ardia (2005) showed that both sexes were equally responsive to changes in demand (via manipulation of clutch size).

Whereas several other studies documented cross-sectional reductions in immune system defenses with age in swallows (Saino *et al.* 2003; Cichon *et al.* 2003; Haussman *et al.* 2005; Palacios *et al.* 2007, 2011), I found no such variation in my study system, even when examining the same characters. Notably, Palacios *et al.* (2007, 2011) found "immunosenescence" in one but not both years of their study. Perhaps immunity is only reduced in old birds during particularly poor years. Alternatively, immunocompetency is associated with individual quality in tree swallows (Ardia 2005) and that may have confounded any trend within individuals.

Hematocrit declined with age over the course of chick-rearing (Fig. 7.1). As hematocrit is often used as an index of body condition (e.g. Donnelly & Sullivan, 1998; Murphy, 2010), one interpretation of my results would be that older females are in poorer body condition at the end of chick-rearing. Rather than investing in bone marrow cell replication rates (and the production of red blood cells), females may have directed more energy towards provisioning offspring. Alternatively, reduced hematocrit may be associated with lower metabolic rate in older swallows late in chick-rearing (we did not measure resting metabolic rate); hematocrit is often positively associated with both aerobic scope and metabolism because high oxygen carrying capacity is needed to sustain

high oxygen consumption rates (Stones & Kozma, 1985; Goldberg, Dengel & Hagberg 1996; Chappell, Rezende & Hammond 2003). However, as most of the energy costs for a swallow would be associated with flight, and neither provisioning rates nor body mass declined with age, we believe that a better explanation is that red blood cell production rates could not keep up with demand and that old females thereby sacrificed condition to produce more offspring. Alternatively, declining hematocrit may be associated with training. Specifically, energy expenditure is often higher during chick-rearing than incubation (e.g. Chapter Two) because adults must fly more to deliver energy to their offspring. In humans, hematocrit declines during training because blood volume increases more rapidly than red blood cell production (e.g. Brun *et al.* 2000). Perhaps the decline in hematocrit during chick-rearing, apparent in both males and old females (Fig. 7.1), represents a switch to higher daily energy expenditure (training), with young females perhaps not increasing daily energy expenditure as much, at a cost to their energy delivery rate.

Further support for the conjecture that old females sacrificed their own somatic tissue for their current reproductive attempt was provided by the decline in natural antibody titre levels during chick-rearing in old, but not young, birds. Given that the birds at that time were responding to a Newcastle disease vaccine challenge, it could be expected that antibody levels would be higher (natural antibody levels and antibody response to a challenge are correlated in tree swallows, Palacios *et al.* 2011). Instead, natural antibody levels were lower in old birds, suggesting that they were directing fewer nutrients towards their immune system and more towards reproduction. In contrast, Palacios *et al.* (2011) showed that when tree swallows were challenged with lipopolysaccharide (to

simulate a bacterial infection), old birds lost more mass and provisioned less. Presumably, the cost of mounting an immune response to a bacterial infection is much greater than to produce antibodies to a vaccine, and the response to the bacterial infection represented an overall greater challenge for older birds.

We found clear sex-specific differences in aging patterns in swallows, with males showing stronger age-related patterns at the start of life (reductions in metrics of foraging success) and females showing stronger age-related patterns later in life (reductions in metrics of investment in somatic tissue with age). Likewise, foraging success declines with age in male, but not female, albatrosses (Catry *et al.* 2006; Lecomte *et al.* 2010). Why do female swallows select a "live fast, die young" senescence pattern while male swallows show restraint early in life and continued investment in the self later in life? As mentioned previously, the explanation may have to do with certainty in parentage. Male tree swallows may be reluctant to invest in offspring that are not their own, as up to 55% of tree swallow offspring may be fathered via extra-pair parentage (Conrad *et al.* 2001). In that sense, my results support the idea that sexual conflict and selection may shape sex-specific aging patterns in vertebrates (Maklakov *et al.* 2009).

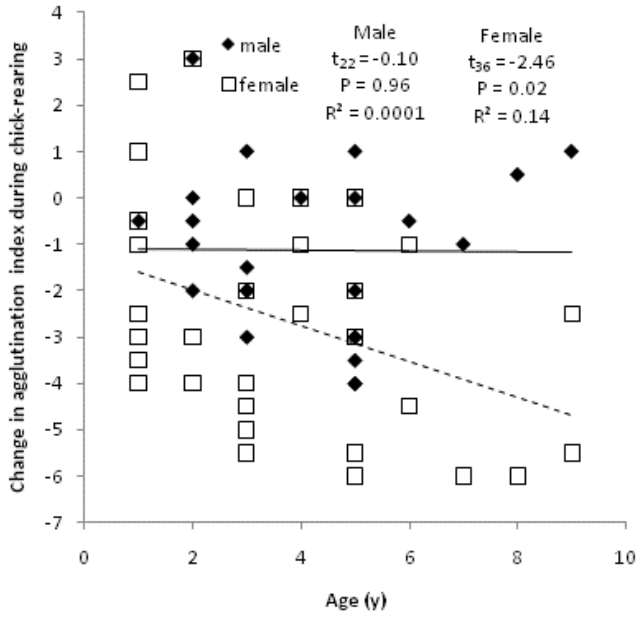
Table 7.1. Statistical relationship between different parameters and age and/or year (t-values, with P-values in parentheses). For parameters not measured in both years, I do not report an effect of year (--).

Parameter	Males (N = 31 in 2011; N = 32 in 2012)		Females (N = 42 in 2011; N = 48 in 2012)	
	Age	Year	Age	Year
Superoxide dismutase	0.94 (0.35)	0.37 (0.71)	0.77 (0.44)	0.81 (0.42)
Total antioxidant[1]	0.39 (0.70)	0.56 (0.58)	0.60 (0.55)	0.98 (0.33)
Agglutination index	-1.63 (0.10)	0.31 (0.75)	-2.00 (0.05)	-1.91 (0.06)
Lytic index	-0.89 (0.38)	0.50 (0.62)	-1.34 (0.19)	-1.53 (0.13)
Haptoglobin	0.22 (0.83)	0.26 (0.79)	0.62 (0.54)	0.11 (0.91
Phytahemagglutinin	0.70 (0.48)	--	0.95 (0.35)	--
Newcastle disease vaccine	-0.87 (0.39)	--	-0.71 (0.48)	--
Hematocrit	0.38 (0.70)	0.08 (0.94)	0.18 (0.85)	0.83 (0.41)
Nest visitation rate	-2.98 (0.004)[2]	0.35 (0.72)	-1.82 (0.07)	0.55 (0.64)
Free baseline corticosterone	-2.91 (0.005) [2]	0.82 (0.22)	0.05 (0.96)	0.23 (0.82)
Free stress corticosterone	-3.50 (0.001) [2]	--	0.39 (0.69)	--
Glucose	2.50 (0.01) [2]	0.77 (0.45)	0.44 (0.66)	0.61 (0.54)

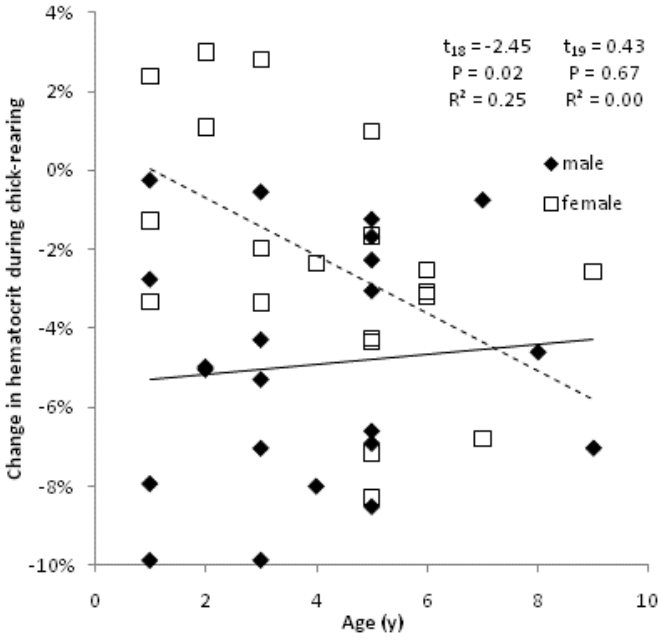
[1]corrected for uric acid

[2]P > 0.05 after excluding first-year birds.

Figure 7.1. The decrease in (a) Agglutination index and (b) hematocrit during chick-rearing was greater for old female (but not male) tree swallows. Dashed line for females, solid line for males.



a)



b)

Chapter Eight. Synthesis

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The current thesis provides the most exhaustive description to date of the physiological and behavioural patterns underlying actuarial and reproductive senescence in four species of bird. Does that solve the aging crisis in Canada? No; but it does provide a more complete comparative perspective of aging in homeotherms. As is the case with most animals with determinate growth (Jones *et al.* 2008), reproductive success and survival both declined with age. In contrast, animals with indeterminate growth, such as some fish or reptiles, may age very differently than animals with determinate growth because of the nonlinear benefits of growing larger, in terms of reduced extrinsic mortality and increased fertility (Ricklefs 2008, 2010). A fish that can increase its reproductive output eight-fold (or more) and virtually eliminate predation by doubling linear length may invest very differently in growth and delayed senescence relative to reproduction compared to a bird that will have a single egg regardless of its age (Vaupel *et al.* 2004; Baudisch & Vaupel 2013).

The most consistent pattern within my dataset was a change in some physiological components with age, but no observed change in behavioural performance. I interpret that to mean that birds invest in maintaining condition until old age. To be able to function adequately during flight, excellent physiological condition may be necessary. For instance, engine failure and body damage occur much less frequently in airplanes than land automobiles (Rausand & Høyland 2004). That is presumably because failure of any component, such as disrepair in the fuselage and/or engine, can cause the "death" of the aircraft. Whereas a rusty automobile can continue to function, or be temporarily stored at the side of the road while awaiting repair, a moving aircraft has fewer options. Likewise, rather than failing due to the gradual deterioration of various organ systems, birds may

fail catastrophically via heart attacks, strokes or other illnesses the symptoms of which appear suddenly prior to death (Ricklefs 2008, 2010). Prior to such catastrophic mortality, condition (i.e. feather structure, muscle and brain performance and other measures of "health") is maintained because (i) variation in any of those components may cause nonlinear increase in turbulence and therefore locomotory costs (which are more important for flying animals due to their higher associated costs) and (ii) condition must be maintained within a narrow range of parameters (relative to running or swimming) for flight to be feasible at all. There is therefore strong selection for avian health to be maintained into old age.

EVOLUTIONARY EXPLANATIONS FOR THE OBSERVED PATTERNS

I began the thesis with several different ultimate explanations for the evolution of aging. Like most studies of aging, my study was purely correlational. It is hard to imagine a manner in which the variable of interest—age—could be manipulated without manipulating a host of other variables at the same time. Thus, it is difficult to provide incontrovertible support for one hypothesis over another—and in many cases several of the hypotheses are generally overlapping. Nonetheless, the patterns I observed fit well within the classical explanations for aging, especially the disposable soma theory. Unlike animals such as desert tortoises, clams and hydras with negative or negligible senescence, I found pronounced declines in survival and reproduction late in life. Notably, in Chapter Five I document continued investment in antioxidant capacity and some components of immune function later in life. Meanwhile, in Chapter Six I document late-life restraint: very old birds directed energy towards themselves rather than their offspring during a

stressor. Thus, as predicted by the disposable soma theory, I found tradeoffs between reproduction and survival, with long-lived birds maintaining investment in the soma well into old age.

Most of the physiological or behavioural systems that I monitored were probably not associated with a single gene in the sense of the simplest statement of the mutation accumulation theory or the antagonistic pleiotropy theory. However, it is easy to envision a gene network regulating many of the physiological and behavioural parameters measured within the current study. As, for the most part, I do not know why birds die in the wild, I did not associate a particular system (or gene network) with increased mortality with age.

The non-programmed theories of aging are predicated on the idea that the force of selection diminishes with age, and that would certainly apply to the birds studied in my dataset; based on my mortality curves (Chapter Five) there would be only nine 30 year-old murres for every hundred 10 year-old murres, and eight 25 year-old kittiwakes for every hundred 5 year-old kittiwakes. Thus, as suggested by mutation accumulation theory, it is possible that random mutations that negatively influence the gene networks associated with immunity, for instance, may be responsible for reduced immune function with age. Alternatively, as suggested by antagonistic pleiotropy theory, it is possible that genes associated with early-life reproduction are linked via pleiotropy with late-life immunosenescence. Without directly examining selection of individual genes or gene networks, it is difficult to refute either theory directly.

Nonetheless, I do not believe that the accumulation of deleterious alleles or pleiotropic effects of positive early-life genes played an important role in the aging patterns

described in the current thesis. Even if few individuals lived into old age, given the importance of survival toward fitness, there would still be some selective advantage weeding out deleterious genes or rearranging early-life genes so that they do not have deleterious late-life effects. Rather, many of the patterns observed in my thesis (e.g. resting metabolic rate, hematocrit, cholesterol, body mass, immunity, superoxide dismutase) showed continual, gradual changes across a broad age range. That trend is more consistent with allocation theory; individuals allocated energy and resources to different physiological and behavioural systems as they aged. Furthermore, in support of the disposable soma hypothesis, individual swallows appeared to invest more heavily in reproduction later in life and less in the preservation of somatic tissue (Chapter Two). Likewise, reduced immunity in aging seabirds could also be found to support the disposable soma hypothesis.

The results presented in the current thesis therefore support both the allocation theory and disposable soma hypothesis. As they aged, individual birds changed how they allocated nutrients and energy, presumably to optimize evolutionary fitness. The allocation patterns fit broadly within the idea that older birds invested more in reproduction at the cost of the soma, although in some cases birds showed late life restraint (Chapter Six), possibly to increase reproductive success in subsequent years.

ISSUES WITH CROSS-SECTIONAL ANALYSES

Although I endeavoured to use a longitudinal approach for several aspects of my study, many aspects are necessarily cross-sectional. Furthermore, it can be argued that 2-4 y is not a particularly useful longitudinal assay for a bird living 20-30 y. Cross-sectional studies are hampered by the potential for cohort effects, and the effect of selective

appearance or disappearance (Forslund & Pärt 1995; Van de Pol & Verhulst 2006; Nussey *et al.* 2008). For instance, if birds with high resting metabolic rate are more likely to die (or to recruit at a younger age), then a decline in resting metabolic rate with age may simply reflect the death at a younger age of those birds with a high resting metabolic rate (or the later recruitment of birds with low resting metabolic rate), rather than the decline of resting metabolic rate with age within an individual.

Individual heterogeneity (the idea that there are "good" and "bad" individuals) is known to be an issue in kittiwakes where there is a strong relationship between reproductive success and survival (Cam *et al.* 2002; but see Steiner *et al.* 2010 who found no relationship using the same dataset). Nonetheless, if these study populations continue to be monitored, it should be possible to determine whether these physiological or behavioural parameters correlate with subsequent survival or with recruitment age.

Steiner *et al.* (2010) argued that rather than having a genetic basis, individual heterogeneity may be associated with early-life effects. Those individuals from cohorts born during good environmental conditions may continue to enjoy the benefit of such conditions many years into the future. Those conditions may be related to environmental conditions in a particular year, habitat quality (Reid *et al.* 2003) or population density (Bonenfant *et al.* 2002). Disentangling all of those effects can be very difficult.

IS AGE THE APPROPRIATE PARAMETER?

I have focused most of my analyses on correlating parameters with age, because age is a relatively accurate and easily documented value. However, it may be that age itself is less important than experience (the number of reproductive events) or the intensity of reproductive events (Kirkwood & Austad 2000; McNamara *et al.* 2009). A year in which

an individual raises two offspring may count more heavily towards aging than a year in which the bird takes a "sabbatical" and does not breed (in murres and kittiwakes, this would usually occur because the bird has been forced from its site by another bird). For old long-lived birds (murres/kittiwakes), "age" is probably very similar to "experience", but that may not be the case for swallows. Documenting such issues is problematic, however, because a bird that does not appear until a later age may have simply bred at a nearby site outside of the current study area.

THE ARCTIC PHOENIX: AGING IN ARCTIC WILDLIFE

Within a given species, or group of species, survival typically declines with increasing latitude (salmon: Koenings *et al.* 1993; thrushes: Ricklefs, 1997; songbirds: Ghalambor & Martin, 2001). The usual explanation for high survival (low "pace of life") in the tropics is that abiotic factors are more important at high latitudes where harsh weather can cause severe mortality than in the benign and stable climate of the tropics. Similarly, in the classical free radical theory of aging, the higher metabolic costs of sustaining a constant internal temperature in the face of harsh Arctic environments are predicted to increase the production of free radicals and oxidative stress, leading to higher rates of mortality among high-latitude individuals.

Despite the prediction that survival should decrease at higher latitudes, many of the longest-lived warm-blooded animals live in the Polar Regions. For example, as mentioned in the Introduction, the longest- lived wild birds (albatrosses, Ricklefs, 2008) and mammals (bowheads/*arviq*, George *et al.* 2011), and the longest-lived animal, the quahog clam *Arctica islandica*, are all found at high latitudes (Strahl *et al.* 2007).

Both physiological and behavioural mechanisms likely underlie the ability of Arctic birds to live extraordinarily long in pulsed-resource systems. Physiological traits linked to body size explain some of the variation: animals tend to be larger near the poles because a low surface area to volume ratio reduces heat loss (Bergmann's rule), and larger animals live longer (Ricklefs, 2008). In addition, some arctic birds have high levels of oxidative defense. For example, when foraging conditions deteriorate, some species (e.g., penguins) show no oxidative damage presumably because of greater oxidative defense (Beaulieu *et al.* 2011). Both albatrosses and terns show no detectable physiological senescence with advancing age (Lecomte *et al.* 2010; Nisbet, 2001). Can Arctic birds such as arctic terns, which migrate up to 80 000 km annually, or thick-billed murres, which have the highest flight costs as a multiple of basal metabolic rate of any bird, sustain high levels of metabolism without any cost to survival?

Recent work on albatrosses suggests a role for behavioural mechanisms (Catry *et al.* 2006; Lecomte *et al.* 2010). Reduced foraging performance is presumably linked to reduced muscle efficiency, but changes in muscle physiology with age have never been examined in Arctic animals. Reduced muscle performance might mean no change in large-scale activity budgets, but reductions in short-scale behaviours (predator avoidance, prey capture).

In contrast, my study indicates that long-lived Arctic birds are facing physiological challenges that result in senescence. In particular, the reductions in basal metabolic rate with age and increases in antioxidant capacity with age (Chapter Five) imply a potential role of metabolism, and potentially oxidative stress, in the senescence of these long-lived arctic animals that are continuously exposed to extreme environments. It would be

interesting to compare defenses against oxidative stress in these birds compared to animals living in more benign environments. Similarly, it would be interesting to know if senescence were particularly measurable during years of particularly harsh environmental conditions, such as heavy-ice years (Gaston et al. 2003).

OLD AGE, ARCTIC ENVIRONMENT AND HIGH TROPHIC LEVEL: A TRIPLE-EDGED SWORD FOR TOXIC CONTAMINATION?

As with other long-lived marine wildlife in the Arctic, seabirds experience "the calamity of so long life" (Rowe, 2008:623). They live in areas with high contamination (Arctic), live long (high bioaccumulation), and feed high in the food web (high biomagnification). Nonetheless, there is the possibility that older birds may acclimate or adjust to contaminant burdens. For example, a recent review based on comparisons between adults and nestlings (Cesh *et al.*, 2010) suggested that the effect of polychlorinated biphenyls and polybrominated diphenyl ethers on thyroid hormones is reduced in old birds. The difference between young and old birds may represent adjustments in the role of the thyroid axis during development. Thyroid hormones in the adult bird are related to metabolism, and old birds may be able to adjust receptivity to hormones so that a constant net effect is achieved. In contrast, thyroid hormones in young birds are associated with growth and may help regulate specific timing of different growth stages. Thus, it may be more difficult to make those adjustments rapidly.

Given the important role of thyroid hormones in regulating aging patterns in wild birds, thyroid disruption may impact senescence. Thyroid hormones clearly play a role in regulating metabolism in these birds (Chapter Three), and show consistent declining patterns with age (Chapters Four and Five). Potentially, thyroid hormone mimics could

bind to thyroid hormone receptors and cause increased resting metabolic rate. During the peak of the chick-rearing period, this could cause greater oxidative stress, greater harm to the soma and therefore accelerate senescence, as suggested in Chapters Four, Five and Seven. To further understand the effect of longevity on contaminant burdens, it would also be important to know whether contamination continues to increase with age, or whether contamination burdens stabilize at equilibrium beyond a certain age (Rowe 2008).

FUTURE DIRECTIONS

Oxidative stress. The variation in resting metabolic rate and superoxide dismutase levels with age (Chapters Four and Five) imply the possibility that oxidative stress is a significant component of aging in murres and kittiwakes. Apart from measuring other antioxidant enzymes (catalase, glutathione-S-transferase) to see if there is a general upregulation in such enzymes with age, it would be useful to measure byproducts of oxidative stress directly. Although reactive oxygen species are too short-lived to measure directly, it is possible to measure lipid peroxidation, protein carbonylation, isoprostanes and DNA damage. I was unable to do so in the context of the current research because they required freezing at -80°C, but that could be possible in future with the use of a nitrogen shipper. Of particular interest would be measuring levels of damage in the muscle tissue itself, as that is where the highest level of mitochondrial respiration occurs. At the same time, it might be possible to measure muscle damage at the tissue level by examining tissue collagen content and integrity (Hindle *et al.* 2009a,b). Small samples (~50 mg) can be obtained from large birds non-lethally.

Hormones. I focused most of my attention on the hypothalamic-pituitary-adrenal axis and in particular the single hormone corticosterone. With development of techniques such as liquid chromatography-mass spectrometry, it is possible to examine multiple steroid hormones simultaneously within a single small (~5 mL) sample. I believe the future will involve measuring multiple hormones (sex steroids, progesterone, aldosterone, thyroid hormones, stress steroids) simultaneously to examine several parameters simultaneously. Aside from measuring multiple hormones simultaneously, in the context of the current thesis, the most important peptide hormone to measure would be prolactin. Prolactin is closely associated with parental behaviour in birds, and variation in that hormone may underlie some of the variation in offspring investment I observed. Further investigation of variation in baseline and stress-induced prolactin with age in murres and kittiwakes is warranted.

Establishing a biomarker for age. Although several physiological measures changed with age—immunity, hematocrit, cholesterol, mass—within my study there is overwhelming individual variation that would make it difficult to use such biomarkers as indicators of age in birds of unknown age. Telomere length and pentosidine have been proposed as potential indicators of age in birds (Haussman *et al.* 2003, 2007; Chaney *et al.* 2003; Fallon *et al.* 2006). Unfortunately, telomere length is only weakly associated with age in murres, at least after excluding chicks from analyses (Young *et al.* 2013) although telomeres are a useful indicator of age in tree swallows (Haussman *et al.* 2003). To date, pentosidine has been primarily examined in skin tissue, such as the webs of aquatic birds (Chaney *et al.* 2003; Fallon *et al.* 2006). Docosahexaenoic acid, a membrane lipid that is particularly resistant to oxidative stress, is another potential

biomarker as it correlates with age in mammals (Hulbert 2006). It would be interesting to measure telomere length, pentosidine concentration and docosahexaenoic acid concentration simultaneously in known-age birds to see which biomarker best predicts age, especially if that trend were similar across different years and locations. Such a biomarker could then be used to age wild birds at locations where long-term marked populations do not exist.

Longitudinal study of inter-individual and intra-individual variation in traits. Although I attempted to monitor longitudinally the same individuals over the course of 2-4 years, it would be fascinating to examine the variation within an individual that occurs over its entire natural lifespan (20-30 years). Such research could demonstrate how changes with age vary with environmental conditions. For instance, senescence in a particular trait may only be measurable during particularly poor years. Of particular interest would be foraging behaviour and endocrinology (Williams 2008). Conditions were generally positive within my study periods, but during a poor year, birds may fly farther to obtain food (Welcker *et al.* 2009). In those cases, flight speed or distance may be particularly associated with age. The downside to such a study is that many initial samples would be required to ensure a sufficient sample size 20 years later due to the disappearance of individuals.

Within my own dataset, I propose to follow up on those measures presented herein by monitoring the reproductive output of those same individuals in the future. If traits such as resting metabolic rate, corticosterone levels or antioxidant levels have fitness consequences—are correlated with subsequent survival or long-term average reproductive success—then they may underlie the variation in quality among individuals.

It would be fascinating to provide a physiological and behavioural underpinning for lifetime reproductive success, and to be able to graph how those physiological and behavioural landscapes impact fitness.

CONCLUSION

It has been a privilege to study the phylogenetic relatives of those same brilliant creatures that Yeats described at Coole. Like Yeats, it is hard not to be impressed when holding a bird that is 33 years old and showing no outward signs of aging; the equivalent would be a human at 90 that shows no difference in behavioural performance to one in the prime of their life. While there are some indications from the cholesterol and hematocrit patterns that heart disease—or other consequences of metabolism—may in fact play a role in senescence in "my" populations, it does at times seem that "their hearts have not grown old".

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