

Ageing gracefully: physiology but not behaviour declines with age in a diving seabird

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Summary

1. A higher proportion of long-lived animals die from senescence than short-lived animals, yet many long-lived homeotherms show few signs of physiological ageing in the wild. This may, however, differ in long-lived diving homeotherms that frequently encounter hypoxic conditions and have very high metabolic rates.
2. To examine ageing within a long-lived diving homeotherm, we studied resting metabolism and thyroid hormones ($N = 43$), blood oxygen stores ($N = 93$) and foraging behaviour ($N = 230$) of thick-billed murres (*Uria lomvia*). Because murres dive exceptionally deep for their size and have a very high metabolism, we expected that ageing murres would show signs of physiological senescence. We paid particular attention to resting metabolism as we argue that these maintenance costs reflect those experienced during deep dives.
3. Blood oxygen stores (haematocrit), resting metabolic rate and thyroid hormone levels all declined significantly with age in incubating murres 3–30 years of age. In birds measured longitudinally 3 years apart, thyroid hormone levels and haematocrit were both significantly lower, suggesting progressive changes within individuals rather than selective disappearance of individuals with high metabolic rates. Within our longitudinal data set, we found no effect of age on dive depth, dive shape or behavioural aerobic dive limit.
4. A meta-analysis of changes in resting metabolism with age across 15 animal species demonstrated that such declines are pervasive across most of the kingdom. The rate of decline was highest in species with high energy expenditure supporting a linkage between metabolism and senescence.
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.

Key-words: basal metabolic rate, behavioural senescence, cost of hypoxia, diving, physiological senescence, rate of living theory, resting metabolic rate, thick-billed murre

Introduction

Long-lived diving animals represent valuable models to examine senescence in the wild. Firstly, 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 (Ricklefs 2008, 2010; Turbill & Ruf 2010). Whereas relatively few individuals in short-lived species will live long enough to be senescent in the wild, a larger proportion of individuals in long-lived

species will be senescent. Perhaps because of stronger selection for postponed physiological senescence (defined as declining physiological capacity with age) in long-lived birds, studies of long-lived wild birds have detected few signs of declining immunity (Apanius & Nisbet 2006; Lecomte *et al.* 2010), 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 routinely accompany physiological ageing in mammals and short-lived birds (Cichon, Sendecka & Gustafsson 2003; Holmes & Ottinger 2003; Saino *et al.* 2003; Moe *et al.* 2009; Palacios *et al.* 2007; Cote *et al.* 2010; these ideas are reviewed by Nisbet 2001; Ricklefs 2008, 2010; Holmes &

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Martin 2009). Secondly, breath-hold diving results in: (i) a hypoxic internal environment with repeated ischaemia-reperfusion at the surface, which can potentially damage tissues, and (ii) the use of alternative metabolic pathways (glycolysis) that causes an increased production of free oxygen radicals by mitochondrial complex I during glycolysis and suboptimal performance of antioxidant enzymes due to changes in pH associated with lactate build-up (Hoerter *et al.* 2004; Ramirez, Folkow & Blix 2007; Hulbert 2008; Hindle *et al.* 2009a,b; Hindle, Mellish & Horning 2011; Beaulieu *et al.* 2011). Thus, diving animals may be particularly susceptible to oxidative damage and associated senescence. Nonetheless, those studies that have examined variation in dive performance with age in birds emphasized behavioural aspects rather than physiological aspects and focused on patterns at the start of life (Zimmer *et al.* 2011; Le Vaillant *et al.* 2012; Pelletier *et al.* 2014).

Dive performance in breath-hold divers is a function of both oxygen stores and rate of oxygen utilization. Therefore, aerobic metabolism – the rate of oxygen utilization – constitutes an important constraint on dive duration, and variation in metabolism with age is likely to impact dive performance. Maintenance or resting metabolism may be particularly important because during deep dives – those dives where lactate production may lead to 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 resting levels (Niizuma *et al.* 2007; Meir *et al.* 2008; Ponganis, Meir & Williams 2010; Elliott *et al.* 2013b). Although no study has examined variation in resting metabolism with age in a diving organism, several studies documented variation in resting metabolic rate with age in non-diving animals (Table 1; we 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, but these violations are unlikely to impact the relationship with age). Most homeotherms show a declining trend in resting metabolism with age although patterns vary even within a taxonomic group, such as rodents (Table 1). Declines in metabolism with advancing age occur within individuals rather than through the selective mortality of individuals with high resting metabolic rates (Moe *et al.* 2009; Broggi *et al.* 2010). Such changes in whole-body metabolism may reflect changes in (i) body mass or composition, particularly the volume of metabolically intense tissue, or (ii) average tissue metabolic intensity. As hypothyroidism – a decline in thyroid hormone levels triggering reduced cellular metabolic intensity – is a typical consequence of human ageing, especially for females (e.g. Spaulding 1987; Djordjević *et al.* 1990; but see Piers *et al.* 1998), option (ii) appears to play a role in humans. Measurement of thyroid hormones alongside metabolism provides the possibility of separating options (i) and (ii) in other animals.

Dive performance also depends on oxygen stores. Blood oxygen stores are more important than muscle oxygen stores for small animals, such as shrews, moles and auks

(>50% of oxygen is stored as haemoglobin and <10% as myoglobin; McIntyre, Campbell & MacArthur 2002; Elliott *et al.* 2010b). Haematocrit (the ratio of packed red blood cell volume to total blood volume) is strongly correlated with haemoglobin content (Velguth, Payton & Hoover 2010; Elliott *et al.* 2010b) and is therefore a good indicator of oxygen stores available to small divers. As haematocrit is often positively associated with aerobic scope (maximum sustained output ÷ resting metabolic rate) and aerobic scope declines with age in some homeotherms (Stones & Kozma 1985; Goldberg, Dengel & Hagberg 1996; Bishop 1999; Chappell, Rezende & Hammond 2003), haematocrit may also decrease with age in diving homeotherms. Nonetheless, both blood and muscle oxygen stores did not decline with age in Weddell seals (*Leptonychotes weddellii*), and consequently, the aerobic dive limit did not vary with age (Hindle, Mellish & Horning 2011).

To examine how diving behaviour, metabolism and oxygen stores change with age in long-lived animals, we studied the thick-billed murre (*Uria lomvia*). Flight costs for murrets are the highest, as a multiple of basal metabolic rate, of any homeotherm measured to date (Shaffer 2011; Elliott *et al.* 2013b). Murrets also dive much deeper and for longer periods of time than equivalent-sized penguins, apparently regularly exceeding their aerobic dive limit (or 'dive lactate threshold'; Croll *et al.* 1992; Watanuki & Burger 1999; Elliott *et al.* 2008b, 2010b). We hypothesized that the murrets' ecology and high metabolism would cause high levels of senescence and predicted reduced (i) resting metabolic rate, (ii) thyroid hormone levels, (iii) haematocrit and (iv) dive performance with age. We examined both physiological ('healthspan') and behavioural senescence under the assumption that physiological senescence would precede behavioural senescence.

Materials and methods

We studied thick-billed murrets at Coats Island, Nunavut (62°57'N, 82°00'W), where birds breeding at 3–30 years old provided an ample age range over which to detect signs of senescence, as the oldest wild large auks recorded by the North American bird-banding laboratory (www.pwrc.usgs.gov/bbl/longevity/Longevity_main.cfm) were 33 (Atlantic puffin, *Fraterecula arctica*) and 28 years old (rhinoceros auklet, *Cerorhinca monocerata*; razorbill, *Alca torda*; and both species of murrets) and by the European bird-banding data base (http://www.euring.org/data_and_codes/longevity-voous.htm) were 42 (razorbill and common murre, *Uria aalge*), 40 (Atlantic puffin) and 27 years old (thick-billed murre). Furthermore, declines in survival and reproduction for murrets in their mid-20s were observed both in our population and in common murrets in Britain (Crespin *et al.* 2006; Lewis *et al.* 2006; Reed *et al.* 2008; Elliott 2013).

We studied dive behaviour during the chick-rearing period 3–15 day post-hatch (Table 2). We also studied oxygen stores, resting metabolism and thyroid hormone levels during the latter half of incubation (eggs 15–25 day) from 10 to 20 July 2008, 1 to 20 July 2009 and 13 to 24 July 2011. At our sub-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 night, so, there was no clear period of inactivity. Birds were captured at the nest with a

Table 1. Summary of studies that have examined changes in resting or basal metabolism with age in animals. The table is meant to be representative of studies examining variation in resting metabolism with age and is not an exhaustive summary of the medical literature. 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, we calculated the statistic from data presented in the paper. Excluding invertebrates, where changes in metabolism with age may be confounded by indeterminate growth, and averaging all values for a single species, average effect size (β , slope divided by standard error on the slope) was -2.13 ± 0.58 (z-test, $P < 0.0001$)

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

*NR = Not reported and not calculable from data presented in paper.

[†]Effect size calculated from *t*-test comparing young adults with old individuals; the authors do not present a regression against age.

[‡]Study occurred on wild animals.

[§]Study reported what they referred to as basal metabolic rate; remaining studies reported 'resting metabolic rate'.

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

**Miyasaka *et al.* (2003) also show declining metabolism in rats between 12 and 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 and 18 months, but does not present statistical analyses or the data in a fashion simple to reanalyse for our table.

^{††}Report that metabolism showed no significant relationship with age, except in one species (*Drosophila melanogaster*), where metabolism increased with age ($P < 0.00014$).

^{‡‡}Statistics averaged across all eight strains. See also Braeckman *et al.* (2002)

^{§§}We calculated statistics separately for all three temperature regimes, starting at the age when mortality began (20 days for 30 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.

^{¶¶}The authors report a significance of 0.02 on an ANOVA with each year class as a categorical variable, as 3 year classes have significantly lower metabolic rates. We reanalysed the presented data in their Fig. 2b with age as a continuous variable (linear regression) to be compatible with the other studies in our table.

noose pole. We determined the sex of murres genetically, behaviourally or because an individual was paired with a mate of known sex (Elliott, Crump & Gaston 2010a). We did not correct body mass for size, as such corrections do not substantially 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 protocol # F11-020).

OXYGEN STORES

The oxygen store is the oxygen available to the diving animal. Blood oxygen stores are a major source of energy production for diving murres (52% of overall oxygen stores compared with 3% for muscle oxygen stores; Elliott *et al.* 2010b). Furthermore,

respiratory stores may be adjusted to dive depth 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). We therefore used blood oxygen stores – principally haematocrit, which is closely correlated with haemoglobin content in murres – as a measure of oxygen availability during the dive. Upon capture, we obtained 1 mL of blood from the alar vein and centrifuged those samples for 10 min at 9600 *g* (Accuspin micro17, ThermoFisher, Burlington, Canada). We measured haematocrit on incubating birds (adults 3–29 year) in 2008 ($N = 62$) and 2011 ($N = 31$, same birds used for respirometry), and, in 2011, an additional 20 individuals that were also sampled in 2008.

Blood oxygen stores will depend on both haematocrit and blood volume. Therefore, for 10 birds, we also measured plasma volume using Evans blue dye (Croll *et al.* 1992). We injected the birds with Evans blue dye (0.2 mL of 2.5 $g L^{-1}$) in the brachial

Table 2. Summary of variables measured in the current study, with details on devices

Variable	Year	Sample size*	Device	Weight (g)	Recording interval	How attached
Dive behaviour	2004–2007	I: 74 (5); C: 124 (50)	Lotek-LTD1100	5	3 s	Leg
Dive behaviour	2008	C: 23 (3)	Lotek-LAT1400	5	1 s if depth >5 m	Leg
Dive behaviour	2009–2011	I: 11; C: 25 (24)	Lotek-LAT1500	3	1 s if depth >3 m	Leg
Dive behaviour	2009–2011	I: 24; C: 118 (36)	Little Leonardo-UME-D2GT	16	1 s	Back
Dive behaviour	2013	C: (12)	CNRS-LUL	2.5	1 s	Leg
Dive behaviour	All	I: 109 (5); C: 230 (127)				
Haematocrit	2008, 2011	I: 93 (20)				
Plasma volume	2008	I: 10				
Resting metabolic rate	2011	I: 43				
Thyroid hormones	2009, 2011	I: 46 (20)				

C, chick-rearing; I, incubating.

*Total number of individuals; number of individuals sampled in multiple years in parentheses. Sample sizes are not equal to total sample size because of repeated sampling of individuals with different devices.

vein and obtained a blood sample from the opposite wing 15 min later (Croll *et al.* 1992). We centrifuged the samples for 15 min at 10000 *g* to remove turbidity. We measured absorbance at 615 and 415 nm using a Biotek plate reader (Fisher Scientific, Ottawa, ON, 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 $R = 0.99$. We assumed

$$\text{Blood O}_2 = 38.1 * 0.96 * 1.2 * (0.95 * 0.3 + 0.7 * 0.7)$$

$$* \text{Hematocrit} * \frac{\text{Plasma Volume}}{1 - \text{Hematocrit}}$$

$$= 0.34 * \text{Hematocrit} * \frac{\text{Plasma Volume}}{1 - \text{Hematocrit}}$$

based on the assumptions that (i) 95% of arterial blood (30% of blood volume) and 70% of venous blood (70% of blood volume) is saturated, (ii) the oxygen-binding capacity of the blood is 1.2 mL g^{-1} , (iii) 96% of blood oxygen is used in the dive, and (iv) haemoglobin content (g per 100 mL) = $38.1 * \text{Haematocrit}$, where haematocrit is a proportion (Croll *et al.* 1992; Elliott *et al.* 2008a, 2010b).

RESTING METABOLIC RATE

Resting metabolic rate, here defined as the metabolic rate of resting, thermoneutral and post-absorptive individuals, is the rate of energy consumption when an animal is at rest and may be representative of the oxygen consumption rate during diving. Resting metabolic rate is an important metric for physiological senescence as it is a controlled measure of the baseline energy consumption of the organism. We used open-flow respirometry with a FoxBox II® (Sable Systems, Las Vegas, NV, USA) system to measure metabolic rate of incubating birds in a respirometry chamber ($N = 43$ adults aged 4–30 year). Birds were in their thermoneutral zone (Croll & McLaren 1993; all measurements here occurred at 15.1–18.2 °C), and there was no diel rhythm in metabolism during our measurements (0430–2030 solar time) at our Arctic study site. Birds were post-absorptive as they were captured at the end of their incubation shift (*c.* 12 h for murre, Elliott, Crump & Gaston 2010a) and then held for 4 h. Both parents share parental duties, at least during the period of time covered by the current studies. One adult per nest was captured. Resting metabolic rate increased with body mass (Elliott *et al.* 2013a), and we used the residual of resting metabolic rate on body mass for all analyses.

THYROID ASSAYS

Thyroid hormones (triiodothyronine, T3; and thyroxine, T4) provide an indirect index of resting metabolic rate and cellular metabolic intensity (Elliott *et al.* 2013a; Welcker *et al.* 2013). We used thyroid hormones primarily as secondary measures of resting metabolic rate and to separate variation in metabolic intensity from variation in body composition. Upon capture, we obtained 1 mL of blood from the alar vein of the same birds used for haematocrit 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 on dry ice and stored at –80 °C until analysis. We determined total T3 and T4 and free T3 concentrations in duplicate by radioimmunoassay on unextracted plasma (MP Biomedical kits 06B258710, 06B254216, 06B254029; Elliott *et al.* 2013a). We measured thyroid hormone levels in the same incubating murre (adults 4–30 year) sampled for haematocrit in 2008 ($N = 46$) and re-sampled in 2011 ($N = 20$).

DIVING BEHAVIOUR

Murre diving behaviour occurs along three major axes at our study site (Elliott *et al.* 2008b): dive depth (representing prey depth), dive shape (V- or U-shaped, representing pelagic or benthic prey habitat) and effort (representing prey energy quality); all diving variables are correlated with one of those three axes. We therefore recorded dive depth (and duration), dive shape (time allocation at depth index) and surface pause duration as a residual on dive depth as a metric of effort. The latter metric can also be considered an index of the behavioural aerobic dive limit or dive lactate threshold, with surface pauses increasing once oxygen stores are exhausted (Croll *et al.* 1992; Hindle, Mellish & Horning 2011). We attached leg-mounted (Lotek time-depth recorders) and back-mounted time-depth (Little Leonardo) recorders to murre. The larger back-mounted recorders measurably alter murre behaviour (Elliott, Davoren & Gaston 2007; Elliott *et al.* 2008b), and we therefore considered recorder type in our statistical analyses. When we attached Little Leonardo and Lotek time-depth recorders simultaneously to the same individuals, the Little Leonardo recorders overestimated depth consistently relative to the Lotek recorders with no effect on dive duration. We converted all depths recorded by the Little Leonardo recorders using the formula: $\text{Lotek Depth} = 0.897 * (\text{Little Leonardo Depth}) + 0.781$ ($R^2 = 0.998$). At Coats Island, males forage primarily at night and females primarily during the day (Elliott, Crump & Gaston 2010a). Thus,

average dive depth is shallower for males because they feed partially at night when visibility is limited. To reduce the effect of time of day and sex on dive depth, we only included measurements between 06:00 and 16:00 h, when dive depth is independent of time of day. For each individual and dive metric, we calculated the average value, for all dives between 06:00 and 16:00 h and across each deployment ranging from 24 to 96 h in length. We focused our analyses on dives completed during chick rearing, when body mass does not vary with age (Gaston & Hipfner 2006), so as to remove mass as a potential confounding factor. Also, birds dive deeper during chick rearing (Elliott *et al.* 2008a) and therefore are more likely to be near their physiological limit at that time.

STATISTICAL ANALYSES

We used only known-age birds for physiological measurements. We included birds banded as adults, and therefore not of known age, at an intensive study plot (Q Plot), for the measurements of dive behaviours. We added 5 year to the age of all birds banded as adults at Q, as that is the average age at which adults recruit to the colony all new adults were routinely banded each season at Q, and site philopatry is very high for adults (de Forest & Gaston 1996; Elliott 2013). Including birds banded systematically as adults did not create any bias in estimating senescence in the case of a linear decline, nor greatly decreased the power of a test for senescence, in a congeneric *Uria* species (Crespin *et al.* 2006). Because of the potential impact of sex-specific differences in diving behaviour on physiology (Young *et al.* 2013), we included sex as a covariate. We used general linear mixed models implemented in R 3.0.2 (<http://www.r-project.org>) with individual as a random effect and age, year, device (diving only) and sex as fixed effects.

Results

PHYSIOLOGY

Haematocrit declined with age in murres (2008: $t_{60} = -3.70$, $P = 0.0005$; 2011: $t_{29} = -4.04$, $P = 0.0004$, Fig. 1). In birds resampled 3 years later, haematocrit also declined ($t_{19} = -3.42$, $P = 0.003$; Fig. 2). Likewise, plasma volume showed a declining trend with age ($t_9 = -1.20$, $P = 0.26$), and therefore blood oxygen stores declined with age ($t_9 = -3.32$, $P = 0.009$).

Resting metabolic rate decreased with age ($t_{42} = -2.75$, $P = 0.009$; Fig. 3a). Haematocrit increased with resting metabolic rate ($t_{31} = 4.31$, $P < 0.001$, $R^2 = 0.61$). Total T3 ($t_{42} = -2.71$, $P = 0.01$; Fig. 3b), but not total T4 ($t_{42} = -0.31$, $P = 0.76$), declined with age. In birds resampled 3 years later, total T3 also declined ($t_{19} = -2.55$, $P = 0.02$; Fig. 2), but T4 did not ($t_{19} = -0.25$, $P = 0.81$). The ratio of free to bound T3 increased with age ($t_{46} = 2.44$, $P = 0.02$, $R^2 = 0.12$), implying a reduction in carrying capacity of the hormone with age.

BEHAVIOUR

There was no relationship between age and a variety of diving parameters in chick-rearing birds when analysed using a general linear mixed model with individual as a

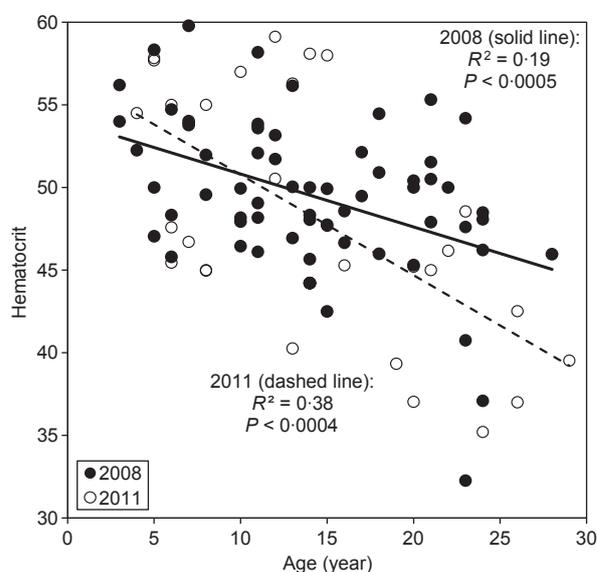


Fig. 1. Haematocrit declined with age in wild thick-billed murres.

random factor (Fig. 4; Table 3). Those relationships were also non-significant with age for incubating birds (all $P > 0.15$).

Discussion

Blood oxygen stores, resting metabolism and thyroid hormone levels declined with age, supporting three of our four predictions about ageing in thick-billed murres. However, we observed no change in dive performance, including the behavioural aerobic dive limit. Under the assumptions that blood oxygen stores are indicative of total oxygen stores and that maintenance costs make up a substantial proportion of dive costs during deep dives (diving metabolic averaged across the entire dive varied from $1.73 \times \text{RMR}$ during 0.5 min dives to $0.5 \times \text{RMR}$ during 5 min dives; Elliott *et al.* 2013b), a decline in oxygen stores may be balanced by a decline in oxygen utilization rate (dive duration \propto oxygen stores/oxygen utilization rate, and as both the numerator and denominator decrease in tandem, there is no effect on dive duration). Similarly, despite changes in muscle histology, there was no change in oxygen stores or dive performance (behavioural aerobic dive limit) with age in long-lived Weddell seals (Hindle *et al.* 2009a; Hindle, Mellish & Horning 2011). Apparently, physiological changes do occur with age in long-lived species, but have no detectable effect on behavioural performance.

RESTING METABOLISM DECLINED WITH AGE WITHIN MURRES AND ACROSS ANIMAL SPECIES

Resting metabolic rate declined with age (Fig. 2). Likewise, 16 of 19 studies showed a decline in resting metabolic rate with age and the mean effect size (-2.13 ± 0.58) was strongly significant (Table 1). Thus, a decline in metabolism with age appears to be a general trait conserved

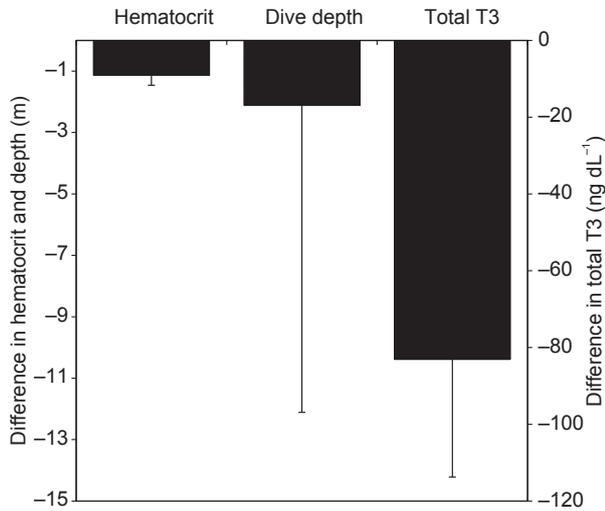


Fig. 2. Longitudinal change in ageing thick-billed murres. Difference in haematocrit, dive depth and total T3 within individual thick-billed murres sampled three (T3, haematocrit) and three to nine (depth) years apart.

across many animal orders. In some cases, the decline was not statistically significant, but given the generality of the declining trend, this may simply represent inadequate sample sizes, especially for old age classes.

The relationship between resting metabolic rate and age varied among species (Table 1), and we hypothesize that a steeper decline in resting metabolic rate with age is associated with higher metabolic demands. Four observations support a connection between increased metabolism and reductions in metabolism with age: (i) in contrast to our results, resting metabolic rate declined more slowly with age in procellariiform seabirds with lower daily energy expenditures (Blackmer *et al.* 2005: Leach's storm-petrel: 141 kJ day⁻¹, 46 g; 71% of the expected value from the 'all seabird' equation of Shaffer 2011; Moe *et al.* 2007: snow petrel: 793 kJ day⁻¹, 245 g; 142% of the expected value; thick-billed murre: 2045 kJ day⁻¹, 1000 g; 153% of the expected value); (ii) 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); (iii) the rate of decline of metabolism with age was inversely proportional to lifespan in roundworms (Shoyama, Shimizu & Suda 2009); and (iv) the decline in metabolic rate with age (effect size in Table 1) was higher for short-lived animals (sampled lifespan <20 year) with high mass-specific metabolic rates than for long-lived animals (≥20 year) with low mass-specific metabolic rates (one-tailed *t*-test comparing effect sizes for the two groups: $t_8 = 2.08$, $P = 0.07$).

As thyroid hormone levels, like resting metabolic rate, declined with age, reduction in metabolism with age reflected reduced metabolic intensity rather than a change solely in body composition. Given that we observed longitudinal declines in thyroid hormone levels, our results likely represent a decline in metabolism with advancing

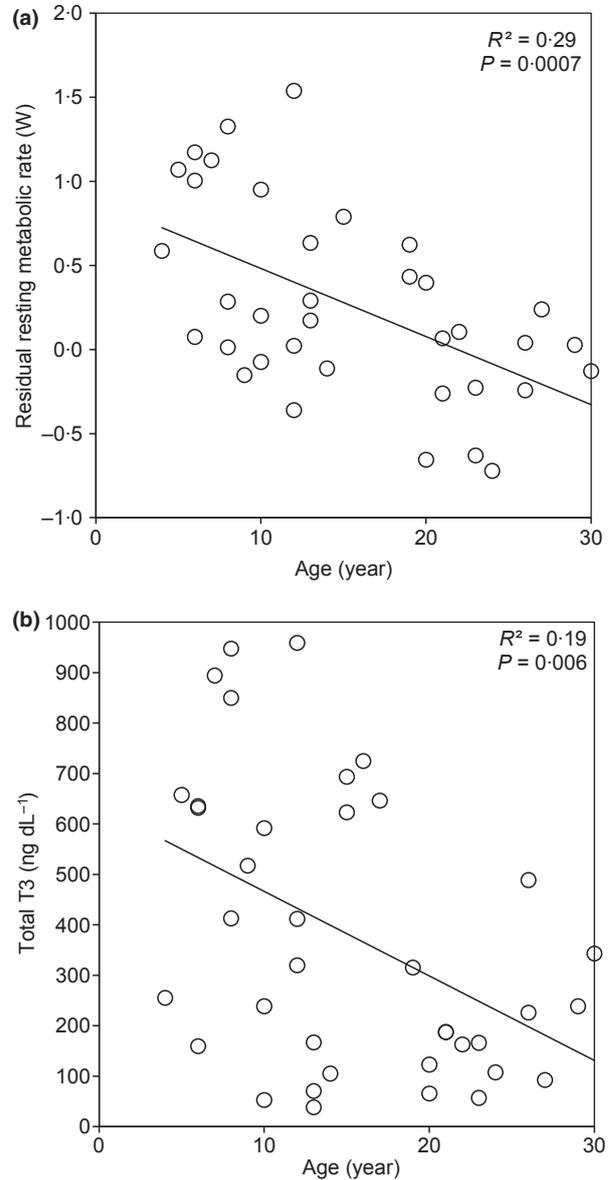


Fig. 3. (a) Post-absorptive, resting metabolic rate (residual on body mass) and (b) triiodothyronine (T3) in wild thick-billed murres declined with age.

age within individuals rather than the selective disappearance of birds that expend more energy. Hypothyroidism is a typical consequence of human ageing where declines in metabolism with age are associated with declines in mitochondrial energy production (e.g. Spaulding 1987; Djordjević *et al.* 1990; but see Piers *et al.* 1998). Additional study using quantitative magnetic resonance could elucidate whether an increase in the proportion of metabolically inert tissues, such as lipids, also plays a role in declining resting metabolic rate with age. Furthermore, the concentration of thyroid-binding proteins, primarily transthyretin in birds, may have declined with age in murres as the ratio of free to bound T3 increased weakly with age. The concentration of thyroid-binding proteins declines with age in mammals (e.g. Spaulding 1987; Wiener *et al.*

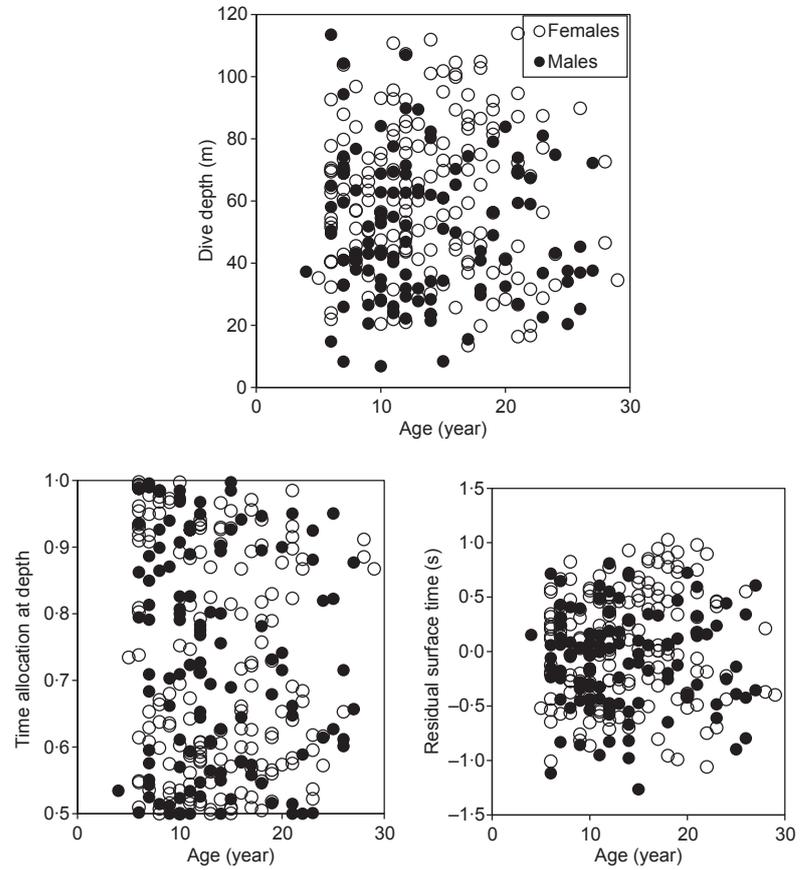


Fig. 4. Relationship between age and dive depth (06:00–16:00 h), dive shape (time allocation at depth index) and surface interval (after accounting for dive depth) in chick-rearing thick-billed murres.

Table 3. Relationship between age and the three axes of dive behaviour for 230 wild, known-age chick-rearing thick-billed murres. Each metric was averaged across all dives recorded between 06:00 and 16:00 h in a given year. Also shown are the relationships between age and thyroid hormones (T3 and T4) and haematocrit. *F*-values generated from general linear mixed models, with individual as a random factor, are shown, with the associated *P*-values in parentheses

Metric	Sex	Age	Device	Year
Dive depth	8.76 (0.004)	2.75 (0.10)	1.19 (0.28)	1.87 (0.07)
Surface pause	0.00 (0.97)	0.07 (0.79)	17.3 (<0.0001)	3.33 (0.002)
Dive shape	11.2 (0.001)	3.69 (0.06)	1.20 (0.28)	2.95 (0.005)
T3	0.06 (0.81)	6.51 (0.01)		0.73 (0.40)
T4	0.09 (0.77)	0.07 (0.80)		0.58 (0.45)
Haematocrit	0.24 (0.63)	11.7 (0.001)		5.29 (0.02)

Values significant at $P < 0.05$ are shown in bold. Residual surface pause on depth.

1991), suggesting a general decline in all aspects associated with thyroid status. In humans, a decline in thyroid hormone levels can occur either through a decline in precursor hormones at the level of the brain or a decline in thyroid hormone synthesis 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 ageing long-lived thick-billed murres, although we have no information on whether the declines originate at the level of the brain, thyroid or periphery.

Resting metabolic rate declined linearly with age in murres (Fig. 3), as is the case in other vertebrates, 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 1). Murre ageing patterns therefore best fit a model of constant decay, whereby the number of mitochondria being produced is constantly outweighed by a fixed amount by the number of mitochondria destroyed throughout an individual's life span (Shoyama, Shimizu & Suda 2009). We suggest that murre cells transcribed fewer mitochondrial enzymes with age in response to lower levels of circulating thyroid hormones. With fewer mitochondria being produced, proportionately fewer mitochondria were destroyed, leading to a net linear reduction in mitochondrial density and metabolic intensity with age.

OXYGEN STORES DECLINED WITH AGE

Haematocrit declined with age (Fig. 1), possibly reflecting declining bone marrow cell replication rates with age. Likewise, haematocrit declined with age in pigeons (*Columba livia*; Prinzing & Misovic 2010). As haematocrit is often used as an index of body condition (e.g. Donnelly & Sullivan 1998; Murphy 2010), one interpretation of our results would be that older birds are in poorer body condition. Alternatively, haematocrit 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, growing animals with both low haematocrit and high metabolism. If haematocrit directly responds to metabolism, then declining haematocrit with age may be a direct consequence of the declining resting metabolism with age that we measured. Although haematocrit declined with age, we do not believe that aerobic scope (maximum sustained output \div resting metabolic rate) declined with age as (i) resting metabolic rate declined with age, which would tend to *increase* aerobic scope; and (ii) daily energy expenditure, which may correlate with maximum sustained output, did not vary or increased with age (Elliott *et al.* 2014b). Regardless, haematocrit is strongly correlated with blood haemoglobin concentration and blood haemoglobin stores are *c.* 50% of total oxygen stores in diving auks (Croll *et al.* 1992; Elliott *et al.* 2010b). Thus, as changes in plasma volume did not offset the declining haematocrit, blood oxygen stores during diving declined with age.

NO EVIDENCE FOR BEHAVIOURAL SENESENCE

Behavioural performance was maintained into old age. We detected no changes in any component of dive behaviour with age. Similarly, old common terns (*Sterna hirundo*) showed no decline in daily activity levels or reproduction (Galbraith *et al.* 1999) and old blue-footed boobies (*Sula nebouxi*) increased reproductive success when injected with lipopolysaccharide to simulate sickness implying maintenance of provisioning behaviour ability (Velando, Drummond & Torres 2006). Le Vaillant *et al.* (2012) also found no change in dive depth or duration with age, but they did not include any very old birds in their study. In contrast, old male, but not female, wandering albatrosses (*Diomedea exulans*) showed behavioural deterioration with the oldest males gaining less mass per time spent at sea and foraging at different locations than young males (Cтры *et al.* 2006; Lecomte *et al.* 2010). Likewise, old female little penguins (*Eudyptula minor*) had longer, but not deeper, dives than middle-aged females (Zimmer *et al.* 2011), although a second study found no difference in dive behaviour or efficiency between young and old penguins at the same study site (Pelletier *et al.* 2014). Apparently, murrelets are able to adjust to physiological changes such that there is no net effect on behaviour, at least at our study site where foraging conditions are relatively good (Gaston *et al.* 2013).

STRATEGIC RESTRAINT OR NON-ADAPTIVE DETERIORATION?

Our results provide circumstantial evidence in support of the 'rate of living' theory of ageing (Pearl 1928). Reductions in metabolism with age can be viewed as strategic restraint on the part of individuals that are likely to encounter energy-related senescence. 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 establish breeding sites and maximize chick growth rates. In contrast, old birds may be selected to reduce those components of energy expenditure not directly associated with reproduction (e.g. RMR) to reduce their rate of senescence, as they are more likely to die of senescence-related causes. Alternatively, if ageing is associated with the non-adaptive degradation of energy-associated tissues, such as the thyroid hormone, circulatory or respiratory systems, then reduced resting metabolic rate with age may simply be symptomatic of physiological senescence. Nonetheless, our 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 brain, 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 metabolism.

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Data accessibility

Data available from the Dryad Digital Repository (Elliott *et al.* 2014a,b; doi:10.5061/dryad.j7737).

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