

Is basal metabolic rate influenced by age in a long-lived seabird, the snow petrel?

Børge Moe^{1,*}, Frédéric Angelier², Claus Bech¹ and Olivier Chastel²

¹Department of Biology, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway and

²Centre d'Étude Biologiques de Chizé (CEBC), Centre National de la Recherche Scientifique, 79360 Villiers en Bois, France

*Author for correspondence at present address: Norwegian Institute for Nature Research (NINA), Division of Arctic Ecology, NO-9296 Tromsø, Norway (e-mail: borge.moe@nina.no)

Accepted 26 July 2007

Summary

Ageing is associated with a decline in basal metabolic rate (BMR) in many species, including humans. The evolutionary and physiological causes underlying the relationship between age and BMR are poorly understood. Studies of procellariiform seabirds may provide valuable insight because they have a longer maximum lifespan than expected from their body size and rates of energy metabolism. Such studies are rare, however, because there are few populations with a high proportion of individuals of known age.

We performed a cross-sectional study of energy metabolism in relation to age in a long-lived seabird, the snow petrel *Pagodroma nivea*. In an Antarctic population that has been subject to a long-term research program,

including annual banding of chicks since 1963, we measured BMR of individuals aged between 8 and 39 years. We show that the BMR of the snow petrel does not decrease with increasing age. BMR seems to be sustained at a fixed level throughout the investigated age-span.

We review this result in light of the disposable soma theory of ageing, and we discuss whether species-specific relationships between age and basal metabolic rate can be related to differences in maximum lifespan.

Key words: ageing, basal metabolic rate, body condition, disposable soma theory, diurnal rhythm, long-lived seabirds, oxidative stress hypothesis, *Pagodroma nivea*, senescence.

Introduction

Ageing (or senescence) is a decline in physiological functioning with age accompanied by a decrease in reproductive performance and an increase in mortality rate (Rose, 1991; Holmes and Austad, 1995). The 'rate of living' theory, which arose nearly a century ago (Rubner, 1908; Pearl, 1922), advocated that the degree of ageing and life expectancy was determined by the rate of energy metabolism. This idea was based on the observation that many species with low mass-specific metabolic rates had a relatively long lifespan.

The 'free radical' theory (Harman, 1956), now often referred to as the 'oxidative stress' hypothesis, provided a mechanistic explanation for the rate of living hypothesis. Free radicals and oxidants, termed reactive oxygen species (ROS), are produced as by-products of oxidative phosphorylation (oxidative ATP production) in the mitochondria (Cadenas and Davies, 2000). ROS are unstable and highly reactive molecules, and they cause damage to DNA, proteins and lipids. The potential for damage depends on the ROS production and the counteracting effects of antioxidant defence mechanisms, and this is known as oxidative stress (e.g. Beckman and Ames, 1998). The consequent damage to cells and associated organs undergoing oxidative stress is believed to be cumulative and thus to underlie the process of ageing.

Inter-specific comparisons made across and within classes

show strong relationships between body size and energy metabolism, as well as between body size and lifespan. However, such comparisons also reveal large variation in lifespan between animals with similar metabolic rates (Speakman et al., 2002; Speakman et al., 2003; Speakman, 2005). This strongly suggests that the relationship between energy metabolism and ageing differs among different groups of animals.

Birds deserve special attention because they have a longer maximum lifespan and fewer signs of ageing than expected from their body size and rates of energy metabolism (Holmes and Ottinger, 2003). Long-lived seabirds especially have low rates of extrinsic mortality (e.g. predation, contagious disease, starvation, weather-related stress). Evolutionary theory predicts that species with low rates of extrinsic mortality should benefit from evolving mechanisms that prevent age-related cellular damage. On the other hand, species with high rates of extrinsic mortality should not benefit from evolving special adaptations for combating age-related cellular damage, because they usually die for other reasons than senescence. Species with different rates of extrinsic mortality, and different lifespans, are expected to have different optimal investment in somatic maintenance and repair. This view is known as the 'disposable soma theory of ageing' (Kirkwood and Rose, 1991; Kirkwood and Austad, 2000). Mechanisms that prevent age-related cellular damage are expected to have a genetic basis (Kirkwood, 2002), and ageing

is thought to result from accumulation of cellular damage as a direct consequence of evolved limitations in the genetic settings of maintenance and repair functions.

Energy metabolism is a potential predictor for lifespan, but physiological mechanisms underlying energy metabolism may also be subjected to age-related effects themselves. It is a well-known phenomenon in humans that the basal metabolic rate (BMR) declines with age (Benedek et al., 1995; Ryan et al., 1996; Piers et al., 1998; Hunter et al., 2001). For simplicity, we do not distinguish between BMR and resting metabolic rate when referring to published studies, because the functional difference between the two is probably not relevant when addressing questions about age-related effects. Decline in BMR with age has also been reported in rats (Greenberg, 1999; Even et al., 2001; Miyasaka et al., 2003) and dogs (Speakman et al., 2003). However, age-related decline in metabolic rate does not seem to be universal (O'Connor et al., 2002; Promislow and Haselkorn, 2002; Sukhotin et al., 2002; Chappell et al., 2003).

In the present study we used the snow petrel *Pagodroma nivea* Forster as a model species for examining age effects on physiological performance. Snow petrels, like other high-latitude seabirds, exhibit a rather active lifestyle with relatively high metabolic rates compared to other non-passerine bird species (Weathers et al., 2000; Hodum and Weathers, 2003). *P. nivea* is a medium-sized petrel, and, like other procellariiform seabirds, is a long-lived species. The oldest individual in our study population was ringed as adult in 1966 and is still alive. It must be at least 46 years old, given that the minimum age at first breeding is 6 years. Mean life expectancy of snow petrels is ~30 years, given that the annual adult survival is 0.95 and mean age at first breeding is 10 years (Jenouvrier et al., 2005) (C. Barbraud, personal communication). Thus, the maximum recorded lifespan of snow petrels is about 3 times the predicted maximum lifespan for birds with the same body mass (Lindstedt and Calder, 1976). Our study population has been subject to a long-term ringing and monitoring program (see Materials and methods), which provided us with access to individuals of known age. Hence, this population was very well suited for our study: a cross-sectional study on the relationship between BMR and age. In addition, the long-term study of this particular population includes information about the average breeding success and the proportion of breeders at the year of hatching (Jenouvrier et al., 2005). Breeding success and proportion of breeders vary substantially between years, and this variation is partly explained by physical environmental variability (Jenouvrier et al., 2005). We also think that this variation reflects biotic environmental variability (including food availability). We therefore used breeding success and proportion of breeders at the year of hatching as proxies for overall environmental conditions during early development (rearing conditions) of the adults of known age.

Environmental conditions may affect early development and subsequent adult phenotype (Gebhardt-Henrich and Richner, 1998). If early environmental conditions induce different adult metabolic phenotypes, as was shown for a passerine species (Verhulst et al., 2006), it is important for two reasons to test for this. First, such an effect may influence an entire cohort's BMR, which needs to be controlled for in any statistical analysis of age-affected BMR. Secondly, data on early developmental

conditions and subsequent adult metabolic rate are scarce and totally lacking for wild populations.

In the present study, we measured BMR, a key parameter of energy metabolism, as an indicator of physiological functioning. The aim of our study was to investigate whether physiological functioning was negatively affected by age in a long-lived seabird, the snow petrel, as could be expected if accumulated oxidative stress has caused cellular damage. On the other hand, if long-lived seabirds have evolved adaptations for preventing age-related oxidative damage up to old age, one would expect either that physiological functioning was not negatively affected by age, or there would be a delayed onset of such negative effects.

Materials and methods

Study population and age determination

The study was carried out on Ile des Pétrels, Pointe Géologie Archipelago, Terre Adélie, Antarctica (66°40'S, 140°01'E) during the austral summer of 2004/05 (December–January). The population of snow petrels *Pagodroma nivea* Forster on this island has been subject to a long-term ringing and monitoring programme (Chastel et al., 1993; Barbraud and Weimerskirch, 2001). Chicks have been ringed every year since 1963, and a substantial proportion of the current population consists of individuals of known age, because ringed chicks have returned to the colony to breed as adults later in life. Data on the average breeding success and the proportion of breeders (i.e. number of breeding pairs relative to the number of non-breeding pairs present in the colony) in the year of hatching (Jenouvrier et al., 2005) were used in this study as proxies for the overall environmental conditions in the year of hatching (i.e. environmental conditions during early development) of the adult snow petrels of known age. The present study was located outside the sub-colonies used for demographic studies to avoid any disturbance to the ongoing long-term demographic studies (Jenouvrier et al., 2005).

Metabolic measurements

Rates of O₂ consumption were measured in adult snow petrels resting in the dark in thermoneutral conditions by open-flow respirometry (Withers, 1977) using a parallel two-chamber system. Outside air was dried using silica gel and pumped through two ~10-litre metabolic chambers with flow rates between 0.9 and 1.3 l min⁻¹, depending on the size of the birds. The flow rates entering each metabolic chamber were regulated with calibrated mass flow controllers (Bronkhorst Hi-Tec, type F-201C-FAB-22-V, Rurlo, Holland), and the flow rates were chosen to obtain O₂ concentrations above 20% in the chambers. Excurrent air was again dried, before a fraction of the air was directed to the O₂ analyser (Servomex type 244A, Crowborough, East Sussex, UK). An automatic valve system located very close to the oxygen analyser switched between excurrent air from the two chambers every 45 min. Hence, the oxygen analyser alternated between measuring the O₂ concentration from chamber 1 and chamber 2. The O₂ analyser was calibrated with dry atmospheric air (20.95%) and pure stock nitrogen. Any changes in the O₂ concentration readings in dry atmospheric air post-experiment from those taken pre-experiment were controlled for by assuming a linear drift. Measurements of the O₂ concentration in excurrent air were stored, along with the

measurements of ambient temperatures in the metabolic chambers, flow rates (1 min^{-1}) and valve position (1 or 2) on a data logger (type Squirrel, Grant, Cambridge, UK), at 30 s intervals. The respirometry system was housed in a heated laboratory, and we managed to maintain a relatively stable room temperature, which ensured thermoneutral conditions inside the metabolic chambers. Ambient temperature at the time of BMR measurement (range $13\text{--}19^\circ\text{C}$) was within the thermoneutral zone of the snow petrels (Weathers et al., 2000).

The metabolic measurements were performed on non-breeding individuals and failed breeders only, mainly to avoid disturbance to breeding birds, but also to avoid some confounding factors. These include temporal variation in BMR that is known for breeding birds (Bech et al., 2002) and the belief that non-breeders would carry reduced food loads and thus more likely to be postabsorptive during the BMR determinations. The measurements lasted for ~ 10 h, to ensure that birds could become rested and postabsorptive during this period. Consequently, we assumed that heat increment of feeding was unlikely to significantly influence our BMR measurements, although the petrels may metabolise small amounts of stomach oils (Weathers et al., 2000).

We performed measurements during two periods of the day. 'Day' measurements started (on average) at 11:30 h and ended at 20:50 h, while 'night' measurements started (on average) at 22:10 h and ended at 09:00 h. High-latitude seabirds usually lack a diurnal rhythm in energy metabolism (Bryant and Furness, 1995), but snow petrels tend to be more active during the night than the day (Bretagnolle, 1988). Therefore, we tested for a potential diurnal rhythm in BMR in order to control for it in the statistical analyses of BMR variation (see Statistics).

Oxygen consumption rates were calculated using formula 1d in Withers (Withers, 1977), assuming a constant RQ of 0.73, and corrected for wash-out delays in the system using the method given by Niimi (Niimi, 1978). In this way, we obtained the instantaneous O_2 consumption rates. The first 2 min of every 45 min interval were excluded from the analyses to ensure that no air from the previous chamber remained in the oxygen analyser at the time of measurement. Basal metabolic rate (BMR) was defined as the lowest 20 min running average value during the 10 h sampling period. The length of the interval (20 min running average) was chosen after we had plotted the minimum values of the metabolic rate (MR), calculated in five randomly selected experimental runs using intervals that varied from 5 to 60 min. This plot showed that the chosen interval was outside the part of the curve where MR could be underestimated, i.e. above the part of the curve where MR decreases rapidly with decreasing length of the intervals (see Meerlo et al., 1997). Body masses of the birds were weighed, to the nearest 1 g, before and immediately after each experiment. A linear decrease in body mass during the experiment was assumed when calculating the body mass at the time that BMR was obtained.

Blood sampling, molecular sexing and hormone assay

The birds were bled from the alar vein with a 1 ml heparinised syringe immediately after the metabolic measurement (i.e. they were bled in the morning or in the evening). Blood samples were centrifuged and plasma and red cells were separated and stored at -20°C until plasma was assayed. The red blood cells were

used for the molecular sexing. We used a molecular method adapted from Fridolfsson and Ellegren (Fridolfsson and Ellegren, 1999), which is described in further detail elsewhere (Weimerskirch et al., 2005).

Because snow petrels were held in the respirometer for a prolonged period of time (10 h), we measured plasma levels of total corticosterone at the end of the BMR measurement to see if birds were experiencing a stressful situation. Plasma concentrations of corticosterone were determined by radioimmunoassay at the CEBC as previously described (Lormée et al., 2003). All samples were run in one assay (intra-assay variation: 7.8%, $N=5$ duplicates). Blood samples that were collected within 3 min (*sensu* 'baseline level') (Lormée et al., 2003; Romero and Reed, 2005) were considered to reflect the potential stress experienced by the birds into the respirometer and not the stress of being removed and handled outside the respirometer. Corticosterone levels (average: 6.0 ng ml^{-1} , $N=7$) were not significantly different to those measured within 3 min in the field (average: 5.4 ng ml^{-1} , $N=45$; t -test, $t=0.5$, $\text{d.f.}=50$, $P=0.6$) (Angelier et al., 2007a). The majority of the BMR values were obtained during the last 1–3 h of the metabolic measurement. These corticosterone levels, therefore, indicate that we have obtained a measure of BMR with very little error due to stress.

Data handling and statistical analyses

A total of 67 snow petrels, either non-breeders or failed breeders from 50 different nests, were subjected to BMR measurements. BMR data of both pair members from the 17 nests where both were measured were considered as independent data, because BMR of the pair members were not significantly correlated (BMR residuals: $r=0.12$, $P=0.65$; BMR: $r=0.39$, $P=0.12$; where 'BMR residuals' refers to residuals from regression of BMR on body mass and 'BMR' refers to whole animal BMR not corrected for mass). The total sample of 67 snow petrels included 38 individuals of known age.

As male and female snow petrels differ greatly in body size (Barbraud and Jouventin, 1998), indices of body condition were calculated separately for each sex. Body condition was calculated as the residual from a reduced major axis regression of body mass against structural body size (Green, 2001). To obtain one variable for structural body size, a factor score (PC1) was extracted with a principal component analysis including the length of the skull (head + bill), culmen, tarsus, wing and the tip bill depth for males and females separately. The PC1 for males explained 80% and the PC1 for females explained 82% of the variation in these traits, and all traits correlated positively to the PC1 ($r>0.85$).

We used a general linear model (GLM) with type III sum of squares to perform analyses of covariance (ANCOVA) and variance (ANOVA). All variables were inspected graphically to ensure linearity, and correlation tests were used prior to analyses to ensure that there was no collinearity among the explanatory variables. All statistical tests were performed with SPSS version 14.0 (2005).

To test for any cyclic diurnal pattern in BMR, we transformed the time when BMR was obtained from hours to radians ($00:00 \text{ h}=0\pi$; $12:00 \text{ h}=\pi$; $24:00 \text{ h}=2\pi$), and included the term 'cosine(time of BMR)' as a covariate in the GLM analysis. To

test whether BMR differed between the two measurement periods, we included the term “Measurement (‘Day’ vs ‘Night’)” in the GLM analysis, where ‘Day’ and ‘Night’ refer to measurements that were started during the day (at 11:30 h) and during the night (at 22:10 h), respectively.

Results

Sources of variation in BMR

BMR and body mass had a significant allometric relationship, with BMR scaling to body mass by the power of 0.68 (Table 1, Fig. 1). In addition to body mass, body condition was also positively related to BMR (Table 1). Thus, individuals with a higher body condition for a given body mass had a slightly higher BMR. There was a weak ($r^2=0.06$), but significant, effect of the term cosine(time of BMR) on the variation in BMR (Table 1, Fig. 2). This shows that BMR of the snow petrels followed a cyclic diurnal pattern, with predicted BMR being lowest at 12:00 h and highest at 24:00 h, indicating that the most active phase in energy metabolism is around midnight. Average BMR did not differ between the two measurement periods ‘day’ and ‘night’ (Table 1), because BMR was generally obtained in the morning and in the evening during the ‘night’ and ‘day’ measurements, respectively (see Fig. 2). Furthermore, sex or the interaction between sex and body mass and between sex and body condition did not explain a significant amount of the variation in BMR (Table 1).

The term cosine(time of BMR), of the general form $\cos(x)$, predicts a function with the lowest values at π (12:00 h) and the highest values at 0π and 2π (00:00 h and 24:00 h) (see Fig. 2). We tested this prediction with a non linear regression, and tested whether the estimate of ‘B’, in the term $\cos(x+B)$, was significantly different from zero in the following equation:

$$\log_{10}\text{BMR} = A \times \cos(x+B) + C \times \log_{10}(\text{body mass}) + D \times \text{body condition} + E. \quad (1)$$

The estimate of ‘B’ (-0.444 , 2 s.e.m.=0.85) was not significantly different from zero, and, consequently, we

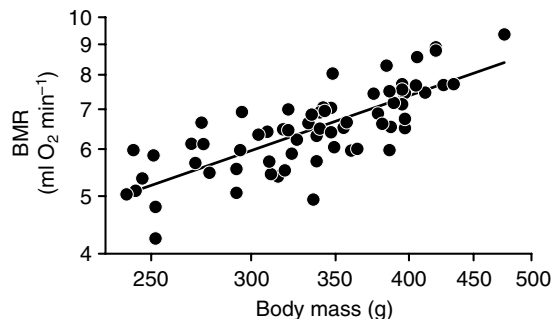


Fig. 1. BMR ($\text{ml O}_2 \text{ min}^{-1}$) in relation to body mass (g) ($N=67$). The axes are log-scaled, and the regression line represents a significant relationship ($P<0.0005$). The regression line is not adjusted for other effects. $\log\text{BMR}=(0.74\pm 0.07) \times \log(\text{body mass}) - (1.06\pm 0.19)$, $r^2=0.61$.

regarded $\cos(x)$, instead of $\cos(x+B)$, as the most parsimonious term. Hence, we did not find support for using a model that assumed the lowest values to occur at a significantly different time than at 12:00 h (π).

No age-related effects on BMR or body condition

BMR was measured in 38 individuals of known age (range 8–39 years). Age was not a significant predictor of the variation in BMR ($F_{1,34}=1.1$, $P=0.3$, Table 2, Fig. 3), and neither were two proxies of environmental conditions at birth, i.e. average breeding success and proportion of breeders at the year of hatching of the adult snow petrels ($F<0.8$, $P>0.38$, Table 2). Body mass, body condition and the term cosine(time of BMR) were included in this analysis, because they were found to be significant predictors of BMR in the analysis from Table 1. However, the term cosine(time of BMR) was not significant in this analysis, and it was therefore not included in the final model that included age (Table 2).

Age did not significantly explain any of the variation in body condition ($F_{1,36}=2.2$, $P=0.15$, Table 3). Furthermore, sex did not

Table 1. Basal metabolic rate (BMR) of snow petrels in relation to different explanatory variables

	d.f.	F	P	r^2	Estimate (s.e.m.)
Final model					
Body mass	1,61	85.6	<0.0005	0.58	+0.68 (0.074)
Body condition	1,61	5.9	0.02	0.09	+0.00052 (0.0002)
Cosine(time of BMR)	1,61	4.0	0.05	0.06	+0.021 (0.011)
Rejected terms					
Sex (Females vs Males)	1,60	1.2	0.27	0.02	-0.012 (0.011)
Measurement (‘Day’ vs ‘Night’) ^c	1,59	0.7	0.40	0.01	-0.009 (0.011)
Sex \times Body mass	1,58	0.7	0.42	0.01	
Sex \times Body condition	1,57	0.0	0.93	0.00	

The model is obtained with a GLM^a, and non-significant variables are stepwise excluded one by one. Values of rejected terms are those before they were excluded from the model. $N=65^b$.

^aThe GLM was performed with \log_{10} -transformed BMR as dependent variable and with \log_{10} -transformed body mass (g), body condition (see Materials and methods) and cosine of the time (in rad, see Materials and methods) when BMR was obtained as covariates, and with measurement (‘day’ vs ‘night’ measurements) and sex as factors.

^bWe had BMR data on 67 snow petrels, but two individuals had missing data on structural body size, which was used to calculate body condition.

^c‘Day’ and ‘Night’ refer to measurements that were started during the day (at 11:30 h) and during the night (at 22:10 h), respectively.

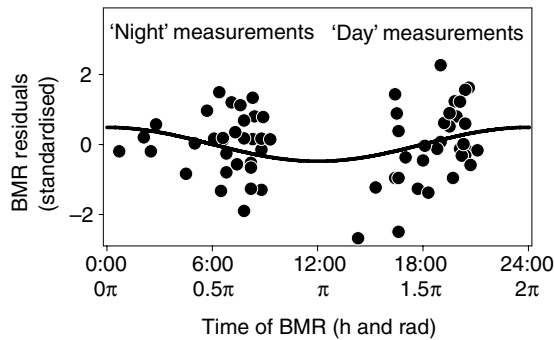


Fig. 2. Standardised BMR residuals in relation to the time when the BMR value was obtained for individual snow petrels ($N=65$). Time is expressed in h (0:00–24:00) and rad (0π – 2π). The regression line represents a significant effect of the term cosine (time of BMR) on BMR ($P=0.05$) and indicates a cyclic diurnal rhythm in BMR with the lowest predicted values at 12:00 h and the highest predicted values at 00:00 h. ‘Night’ and ‘day’ measurements refer to BMR measurements that were started during the night (at 22:10 h) and the day (at 11:30 h), respectively. The BMR residuals were obtained from the model in Table 1 (BMR controlled for body mass and body condition). Standardised residuals are divided by an estimate of their standard deviation (s.d.), and have mean=0 and s.d.=1.

explain any significant amount of the variation in body condition ($F_{1,35}=0.2$, $P=0.65$, Table 3), and neither did average breeding success and proportion of breeders at the year of hatching of the adult snow petrels ($F<0.1$, $P>0.77$, Table 3). BMR and body condition thus appear to be at rather fixed levels irrespective of age, at least in the investigated part of the lifespan of the adult snow petrels.

Discussion

We have shown that there is no detectable decrease in BMR with age in snow petrels ranging from 8–39 years. To our knowledge, there is only one other published study on BMR and

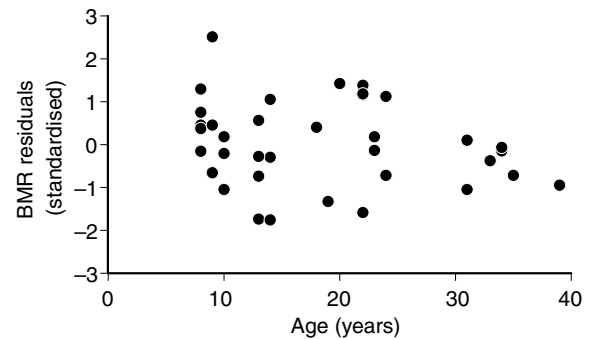


Fig. 3. Standardised BMR residuals in relation to age (years) of snow petrels ($N=38$, range 8–39 years old). The birds are of known age because they were ringed as chicks. The residuals were obtained from the final model in Table 2 (BMR controlled for body mass and body condition). Standardised residuals are divided by an estimate of their standard deviation (s.d.), and have mean=0 and s.d.=1.

age in long-lived seabirds. That study (Blackmer et al., 2005) did not find any significant decrease in BMR with age (ranging from 1–23 years) in Leach’s storm-petrels (*Oceanodroma leucorhoa*). In short-lived bird species, we are only aware of two studies. BMR decreases with age both in captive zebra finches (*Taeniopygia guttata*; B.M., B. Rønning and C.B., unpublished data) and in natural populations of great tits (Broggi et al., 2007). Those studies are longitudinal and cross-sectional, respectively, and show rather strong declines in BMR with age.

Our present study was cross-sectional (i.e. based on only one BMR measurement per individual), and statistical analyses of cross-sectional data are less powerful in detecting significant trends with age compared to longitudinal data (i.e. repeated measurements within the same individuals over a time span). One could argue that a larger sample size could have revealed a

Table 2. Results of a GLM^a analysing basal metabolic rate of adult snow petrels in relation to age, proportion of breeders (PB at hatching) and average breeding success at year of hatching (BS at hatching)

	d.f.	<i>F</i>	<i>P</i>	<i>r</i> ²	Estimate (s.e.m.)
Final model					
Body mass	1,35	27.4	<0.0005	0.44	+0.62 (0.12)
Body condition	1,35	6.6	0.02	0.16	+0.0007 (0.0003)
Rejected terms					
Age	1,34	1.1	0.30	0.03	–0.0007 (0.0007)
BS at hatching	1,33	0.8	0.38	0.02	+0.03 (0.03)
Cosine(time of BMR)	1,32	0.7	0.42	0.02	+0.01 (0.013)
PB at hatching	1,30	0.5	0.49	0.02	–0.05 (0.07)

GLM, general linear model; BMR, basal metabolic rate; PB, proportion of breeders; BS, breeding success.

Body mass, body condition and the term cosine(time of BMR) were also included in the analysis. PB and BS at hatching are used as proxies for the overall environmental conditions during early development of the adults of known age. The birds were of known age because they were ringed as chicks. ($N=38$)^b.

Non-significant variables are stepwise rejected one by one, and values of rejected terms are those before they were excluded from the model.

^aThe GLM was performed with log₁₀-transformed BMR body mass (g). The term ‘cosine(time of BMR)’ refers to the cosine of the time (in rad) when BMR was obtained.

^b $N=37$ when PB at hatching are included in the model.

Table 3. *Body condition^a of adult snow petrels in relation to age, sex, proportion of breeders (PB at hatching) and average breeding success at year of hatching (BS at hatching)*

	d.f.	F	P	r ²	Estimate (s.e.m.)
Age	1,36	2.2	0.15	0.06	+0.63 (0.43)
Sex (Females vs Males) ^c	1,35	0.2	0.65	0.01	+3.7 (8.1)
PB at hatching	1,33	0.1	0.77	0.00	+14.3 (47.3)
BS at hatching	1,32	0.0	0.86	0.00	-4.4 (24.7)

PB and BS at hatching are used as proxies for the overall environmental conditions during early development of the adults of known age. The birds were of known age because they were ringed as chicks. ($N=38$)^b.

All variables were non-significant in this GLM analysis and were stepwise rejected one by one. Values of rejected variables are those before they were excluded from the model.

^aSee Materials and methods for information about how body condition was calculated.

^b $N=37$ when PB at hatching are included in the model.

^cAll the interaction terms with sex were non-significant, and are not shown in this table.

significant trend and revealed a type II error in our analysis of BMR in relation to age (Table 3, Fig. 3). However, the correlation coefficient and the slope were so close to zero that it is unlikely that a larger sample size would have revealed a significant negative relationship. Another problem with cross-sectional data is that we cannot control for selective appearance or disappearance of individuals to/from the population sampled (van de Pol and Verhulst, 2006), which would occur if for example individuals with low or high BMR had different survival rates.

A potential confounding factor in analysing the relationship between age and BMR is a potential cohort effect in BMR due to different environmental conditions during early development in different years. We used breeding success and proportion of breeders at the year of hatching as proxies for environmental conditions during early development, because we expected those parameters to be good descriptors of the overall environmental conditions at the year of hatching (Jenouvrier et al., 2005). However, there was no significant relationship between BMR and average breeding success or proportion of breeders at the year of hatching, and consequently no indications that environmental conditions at year of hatching induced differences in BMR among different cohorts. Individuals reared under different early environmental conditions may attain different adult metabolic phenotypes (Verhulst et al., 2006), but we would need data on food availability and food quality to further test these relationships in the snow petrel.

We show that snow petrels have a cyclic diurnal rhythm in basal energy metabolism. The energy consumption was highest at midnight (00:00 h), and this is in accordance with a behavioural study showing that snow petrels are more active during night (Bretagnolle, 1988). In inter-specific comparisons, the active-phase BMR is reported to be 24% and 31% higher than the rest-phase BMR in non-passerines and passerines, respectively (e.g. Aschoff and Pohl, 1970). The novel aspect of our result is that we find a significant diurnal rhythm in BMR of a high-latitude seabird and show that it follows a cosine function. Energy metabolism of high-latitude seabirds, breeding under continuous daylight, is not expected to differ, or to differ much, between day and night (Gabrielsen et al., 1988; Bryant and Furness, 1995). From the parameter estimates derived from Table 1, we calculated that predicted BMR was 16% higher at 00:00 h compared to at 12:00 h. One should, however, treat this

estimate carefully, because we would need more BMR values around 00:00 h and 12:00 h to obtain a robust estimate of the difference in energy metabolism between noon and midnight (see Fig. 2).

The lack of any significant relationship between BMR and age (Fig. 3) was not confounded by any diurnal variation in BMR. The term cosine(time of BMR) was not significant in the analysis that included age and was not included in that final model (Table 2). Furthermore, the results were also the same if we used the residuals from the final model in Table 1, and analysed the relationship between age and residual BMR [BMR controlled for body mass, body condition and cosine(time of BMR)].

Senescence is a decline in physiological functioning and reproductive performance with age. Our study detected no decline in physiological functioning (BMR) with age (up to 39 years) in the snow petrel. This is consistent with our data on reproductive success for this species (Angelier et al., 2007a), which shows that reproductive performance increases with age from 6 years to 12 years, then stabilises, and does not significantly decline in the oldest petrels of the study. This supports analyses that suggest that birds in natural populations maintain a high level of physical fitness into old age (Ricklefs, 2000). We cannot, however, rule out the possibility that an age-specific decline in physiological functioning may occur in very old individuals, e.g. in individuals older than 39 years. Senescence effects, measured as reduced foraging performance and hormonal changes, have been documented in very old grey-headed albatrosses (*Thalassarche chrysostoma*) (Cady et al., 2006) and black-browed albatrosses (*Thalassarche melanophris*) (Angelier et al., 2007b), respectively.

Our result indicating that snow petrels maintain a high level of physical fitness into old age may support the disposable soma theory (Kirkwood and Rose, 1991; Kirkwood and Austad, 2000). Long-lived seabirds have low extrinsic mortality rates (Lack, 1968) and, from an evolutionary consideration, should benefit from evolving mechanisms that promote a high degree of somatic maintenance and repair which, consequently prevent or delay age-related cellular damage. The pattern that two long-lived bird species do not show age-related decline in BMR (Blackmer et al., 2005) (present study) and two short-lived birds species do show age-related decline in BMR (Broggi et al.,

2007) (B.M., B. Rønning and C.B., unpublished) gives further support to the disposable soma theory. There are two, not mutually exclusive, ways to apply the disposable soma theory to our result. (1) BMR remains stable with age because the somatic maintenance and repair mechanisms have prevented damage on the macromolecules underlying the metabolic pathways in the mitochondria. Hence, the structures in the mitochondria remain intact into old age and BMR can function at the same levels. (2) The sustained BMR with age reflects the energetic costs of having a high degree of somatic maintenance and repair into old age in long-lived species. These two ways represent indirect and direct relationships between somatic maintenance and repair and BMR, respectively. At present, we do not know if it is possible to separate the two.

An example of a long-lived mammalian species with no age-related decline in BMR is the naked mole rat (O'Connor et al., 2002), which is the most long-lived rodent having a maximum lifespan of more than 28 years (Buffenstein, 2005). Other rat species, with a shorter maximum lifespan compared to the naked mole rat, show age-related decline in BMR (Greenberg, 1999; Even et al., 2001; Miyasaka et al., 2003). The decrease in BMR with increasing age in humans (e.g. Piers et al., 1998) contrasts the above mammalian and avian examples, because humans are also very long-lived. We do not have a good explanation for this apparent paradox. It might be that the onset of the decrease is delayed or that the rate of decrease is slower in humans compared to more short-lived mammalian species, but it is difficult to evaluate this from the available literature.

If the relationship between BMR and age is related to species-specific maximum lifespan, one may expect the degree of decrease in BMR with increasing age to be reflected in rates of senescence and in the degree of adaptations for preventing age-related cellular damage. Such adaptations could be associated with (1) better defences against ROS (oxidative protection), (2) less production of ROS or/and (3) better repair of the damage caused by ROS. Membrane properties of cells and mitochondria, such as fatty acyl composition and mitochondrial uncoupling proteins, are suggested to be important both for limiting oxidative damage and for affecting metabolic rate (e.g. Speakman et al., 2004; Criscuolo et al., 2005; Hulbert, 2006; Hulbert et al., in press). At present, however, we can only speculate why different species show different relationships between BMR and age.

The fieldwork was financially and logistically supported by the Institut Paul Emile Victor (IPEV Programme 109). B. Moe was supported by a post-doctoral grant from the Norwegian University of Science and Technology. We thank all workers that have been involved in the long-term study of seabirds in Terre Adélie, G. Bouteloup for assistance in the field, S. Dano, A. Lacroix and C. Trouvé for hormone assays and molecular sexing, and T. Kirkwood, W. A. Buttemer and A. J. Hulbert for providing comments on an earlier version of this manuscript.

References

- Angelier, F., Moe, B., Weimerskirch, H. and Chastel, O. (2007a). Age-specific reproductive success in a long-lived bird: do older parents better resist stress? *J. Anim. Ecol.* doi: 10.1111/j.1365-2656.2007.01295.x.
- Angelier, F., Weimerskirch, H., Dano, S. and Chastel, O. (2007b). Age, experience and reproductive performance in a long-lived bird: a hormonal perspective. *Behav. Ecol. Sociobiol.* **61**, 611-621.
- Aschoff, J. and Pohl, H. (1970). Der Ruheumsatz von Vögeln als Funktion der Tageszeit und der Körpergröße. *J. Ornithol.* **111**, 38-47.
- Barbraud, C. and Jouventin, P. (1998). What causes body size variation in the snow petrel? *J. Avian Biol.* **29**, 161-171.
- Barbraud, C. and Weimerskirch, H. (2001). Contrasting effects of the extent of sea-ice on the breeding performance of an Antarctic top predator, the Snow Petrel *Pagodroma nivea*. *J. Avian Biol.* **32**, 297-302.
- Bech, C., Langseth, I., Moe, B., Fyhn, M. and Gabrielsen, G. W. (2002). Energy economy of the arctic-breeding Kittiwake (*Rissa tridactyla*): a review. *Comp. Biochem. Physiol.* **133A**, 765-770.
- Beckman, K. B. and Ames, B. N. (1998). The free radical theory of aging matures. *Physiol. Rev.* **78**, 547-581.
- Benedek, C., Berclaz, P. Y., Jequier, E. and Schutz, Y. (1995). Resting metabolic rate and protein turnover in apparently healthy Gambian men. *Am. J. Physiol.* **268**, E1083-E1088.
- Blackmer, A. L., Mauck, R. A., Ackerman, J. T., Huntington, C. E., Nevitt, G. A. and Williams, J. B. (2005). Exploring individual quality: basal metabolic rate and reproductive performance in storm-petrels. *Behav. Ecol.* **16**, 906-913.
- Bretagnolle, V. (1988). Cycles de présence et rythmes d'activité chez cinq espèces de pétrels antarctiques. *Oiseau Rev. Fr. Ornithol.* **58**, 44-59.
- Broggi, J., Hohtola, E., Koivula, K., Orell, M., Thomson, R. L. and Nilsson, J.-A. (2007). Sources of variation in winter metabolic rate in the great tit *Parus major*. *Funct. Ecol.* **21**, 528-533.
- Bryant, D. M. and Furness, R. W. (1995). Basal metabolic rate of North-Atlantic seabirds. *Ibis* **137**, 219-226.
- Buffenstein, R. (2005). The naked mole-rat: a new long-living model for human aging research. *J. Gerontol. A Biol. Sci. Med. Sci.* **60**, 1369-1377.
- Cadenas, E. and Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic. Biol. Med.* **29**, 222-230.
- Catry, P., Phillips, R. A., Phalan, B. and Croxall, J. P. (2006). Senescence effects in an extremely long-lived bird: the grey-headed albatross *Thalassarche chrysostoma*. *Proc. R. Soc. Lond. B Biol. Sci.* **273**, 1625-1630.
- Chappell, M. A., Rezende, E. L. and Hammond, K. A. (2003). Age and aerobic performance in deer mice. *J. Exp. Biol.* **206**, 1221-1231.
- Chastel, O., Weimerskirch, H. and Jouventin, P. (1993). High annual variability in reproductive success and survival of an Antarctic seabird, the snow petrel *Pagodroma nivea*: a 27-year study. *Oecologia* **94**, 278-285.
- Crisculo, F., Gonzalez-Barroso, M. M., Bouillaud, F., Ricquier, D., Miroux, B. and Sorci, G. (2005). Mitochondrial uncoupling proteins: new perspectives for evolutionary ecologists. *Am. Nat.* **166**, 686-699.
- Even, P. C., Rolland, V., Roseau, S., Bouthegourd, J.-C. and Tomé, D. (2001). Prediction of basal metabolism from organ size in the rat: relationship to strain, feeding, age, and obesity. *Am. J. Physiol.* **280**, R1887-R1896.
- Fridolfsson, A. K. and Ellegren, H. (1999). A simple and universal method for molecular sexing of non-ratite birds. *J. Avian Biol.* **30**, 116-121.
- Gabrielsen, G. W., Mehlum, F. and Karlsen, H. E. (1988). Thermoregulation in four arctic seabirds. *J. Comp. Physiol.* **157**, 703-708.
- Gebhardt-Henrich, S. G. and Richner, H. (1998). Causes of growth variation and its consequences for fitness. In *Avian Growth and Development. Evolution within the Altricial-precocial Spectrum* (ed. J. M. Starck and R. E. Ricklefs), pp. 324-339. New York: Oxford University Press.
- Green, A. J. (2001). Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* **82**, 1473-1483.
- Greenberg, J. A. (1999). Organ metabolic rates and aging: two hypotheses. *Med. Hypotheses* **52**, 15-22.
- Harman, D. (1956). Aging: a theory based on free radical and radiation biology. *J. Gerontol.* **11**, 298-300.
- Hodum, P. J. and Weathers, W. W. (2003). Energetics of nestling growth and parental effort in Antarctic fulmarine petrels. *J. Exp. Biol.* **206**, 2125-2133.
- Holmes, D. J. and Austad, S. N. (1995). The evolution of avian senescence patterns: implications for understanding primary aging processes. *Am. Zool.* **35**, 307-317.
- Holmes, D. J. and Ottinger, M. A. (2003). Birds as long-lived animal models for the study of aging. *Exp. Gerontol.* **38**, 1365-1375.
- Hulbert, A. J. (2006). The links between membrane composition, metabolic rate and lifespan. *Comp. Biochem. Physiol. A*. doi: 10.1016/j.cbpa.2006.05.014.
- Hulbert, A. J., Pamplona, R., Buffenstein, R. and Buttemer, W. A. (in press). Life and death: metabolic rate, membrane composition and life span of animals. *Physiol. Rev.*
- Hunter, G. R., Weinsier, R. L., Gower, B. A. and Wetzstein, C. (2001). Age-related decrease in resting energy expenditure in sedentary white women: effects of regional differences in lean and fat mass. *Am. J. Clin. Nutr.* **73**, 333-337.

- Jenouvrier, S., Barbraud, C. and Weimerskirch, H.** (2005). Long-term contrasted responses to climate of two Antarctic seabird species. *Ecology* **86**, 2889-2903.
- Kirkwood, T. B. L.** (2002). Evolution of ageing. *Mech. Ageing Dev.* **123**, 737-745.
- Kirkwood, T. B. L. and Austad, S. N.** (2000). Why do we age? *Nature* **408**, 233-238.
- Kirkwood, T. B. L. and Rose, M. R.** (1991). Evolution of senescence: late survival sacrificed for reproduction. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **332**, 15-24.
- Lack, D.** (1968). *Ecological Adaptations for Breeding in Birds*. London: Methuen.
- Lindstedt, S. L. and Calder, W. A.** (1976). Body size and longevity in birds. *Condor* **78**, 91-94.
- Lormée, H., Jouventin, P., Trouve, C. and Chastel, O.** (2003). Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies *Sula sula*. *Ibis* **145**, 212-219.
- Meerlo, P., Bolle, L., Visser, G. H., Masman, D. and Daan, S.** (1997). Basal metabolic rate in relation to body composition and daily energy expenditure in the field vole, *Microtus agrestis*. *Physiol. Zool.* **70**, 362-369.
- Miyasaka, K., Ichikawa, M., Kawanami, T., Kanai, S., Ohta, M., Sato, N., Ebisawa, H. and Funakoshi, A.** (2003). Physical activity prevented age-related decline in energy metabolism in genetically obese and diabetic rats, but not in control rats. *Mech. Ageing Dev.* **124**, 183-190.
- Niimi, A. J.** (1978). Lag adjustments between estimated and actual physiological responses conducted in flow-through systems. *J. Fish. Res. Board Can.* **35**, 1265-1269.
- O'Connor, T. P., Lee, A., Jarvis, J. U. M. and Buffenstein, M.** (2002). Prolonged longevity in naked mole-rats: age-related changes in metabolism, body composition and gastrointestinal function. *Comp. Biochem. Physiol.* **133A**, 835-842.
- Pearl, R.** (1922). *The Biology of Death*. Philadelphia: J. B. Lippincott.
- Piers, L. S., Soares, M. J., McCormack, L. M. and O'Dea, K.** (1998). Is there evidence for an age-related reduction in metabolic rate? *J. Appl. Physiol.* **85**, 2196-2204.
- Promislow, D. E. L. and Haselkorn, T. S.** (2002). Age-specific metabolic rates and mortality rates in the genus *Drosophila*. *Ageing Cell* **1**, 66-75.
- Ricklefs, R. E.** (2000). Intrinsic aging-related mortality in birds. *J. Avian Biol.* **31**, 103-111.
- Romero, L. M. and Reed, J. M.** (2005). Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp. Biochem. Physiol.* **140A**, 73-79.
- Rose, M. R.** (1991). *Evolutionary Biology of Aging*. New York: Oxford University Press.
- Rubner, M.** (1908). *Das Problem der Lebensdauer und Seine Beziehung zum Wachstum und Ernährung*. Munich: Oldenberg.
- Ryan, A. S., Nicklas, B. J. and Elahj, D.** (1996). A cross-sectional study on body condition and energy expenditure in women athletes during aging. *Am. J. Physiol.* **271**, E916-E921.
- Speakman, J. R.** (2005). Body size, energy metabolism and lifespan. *J. Exp. Biol.* **208**, 1717-1730.
- Speakman, J. R., Selman, C., McLaren, J. S. and Harper, E. J.** (2002). Living fast, dying when? The link between aging and energetics. *J. Nutr.* **132**, 1583S-1597S.
- Speakman, J. R., van Acker, A. and Harper, E. J.** (2003). Age-related changes in the metabolism and body composition of three dog breeds and their relationship to life expectancy. *Ageing Cell* **2**, 265-275.
- Speakman, J. R., Talbot, D. A., Selman, C., Snart, S., McLaren, J. S., Redman, P., Krol, E., Jackson, D. M., Johnson, M. S. and Brand, M. D.** (2004). Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. *Ageing Cell* **3**, 87-95.
- Sukhotin, A. A., Abele, D. and Portner, H. O.** (2002). Growth, metabolism and lipid peroxidation in *Mytilus edulis*: age and size effects. *Mar. Ecol. Prog. Ser.* **226**, 223-234.
- van de Pol, M. and Verhulst, S.** (2006). Age-dependent traits: a new statistical model to separate within- and between-individual effects. *Am. Nat.* **167**, 766-773.
- Verhulst, S., Holveck, M.-J. and Riebel, K.** (2006). Long-term effects of manipulated natal brood size on metabolic rate in zebra finches. *Biol. Lett.* **2**, 478-480.
- Weathers, W. W., Gerhart, K. L. and Hodum, P. J.** (2000). Thermoregulation in Antarctic fulmarine petrels. *J. Comp. Physiol. B* **170**, 561-572.
- Weimerskirch, H., Lallemand, J. and Martin, J.** (2005). Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. *J. Anim. Ecol.* **74**, 285-289.
- Withers, P. C.** (1977). Measurement of V_{O_2} , V_{CO_2} and evaporative water loss with flow-through mask. *J. Appl. Physiol.* **42**, 120-123.

