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The use of body mass loss to estimate metabolic rate in birds☆

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ABSTRACT

During starvation, energy production occurs at the expense of body reserve utilisation which results in body mass loss. Knowing the role of the fuels involved in this body mass loss, along with their energy density, can allow an energy equivalent of mass loss to be calculated. Therefore, it is possible to determine daily energy expenditure (DEE) if two body mass loss measurements at an interval of a few days are obtained. The technique can be cheap, minimally stressful for the animals involved, and the data relatively simple to gather. Here we review the use of body mass loss to estimate DEE in birds through critiquing the strengths and weaknesses of the technique, and detail the methodology and considerations that must be adhered to for accurate measures of DEE to be obtained. Owing to the biology of the species, the use of the technique has been used predominantly in Antarctic seabirds, particularly penguins and albatrosses. We demonstrate how reliable the technique can be in predicting DEE in a non-Antarctic species, common eiders (*Somateria mollissima*), the female of which undergoes a fasting period during incubation. We conclude that using daily body mass loss to estimate DEE can be a useful and effective approach provided that (1) the substrate being consumed during mass loss is known, (2) the kinetics of body mass loss are understood for the species in question and (3) only species that enter a full phase II of a fast (where substrate catabolism reaches a steady state) and are not feeding for a period of time are appropriate for this method.

1. Introduction and background

Many species of birds have periods in the annual cycle when energy use exceeds energy acquisition. In response to this energy deficit, a loss in body mass is typically observed. Such periods can include moult, migration and the incubation period. Characteristically, when food is scarce or events preclude the ability to forage, animals will rely predominantly on endogenous stores of fat that are generally deposited during the weeks preceding an anticipated change in food availability (Blem, 1976; Biebach, 1996; Walsberg, 1988; Witter and Cuthill, 1993; Dawson et al., 2000; Portugal et al., 2007). As fat is the most energy dense substrate in the body, stored as triglycerides in deposits of white adipose tissue, it is these fat stores that are catabolised during periods of energy deficit and typically account for the majority of mass loss (see Blem, 1990, for full review).

For certain birds this energy deficit can be extreme, and this typically occurs for species which are unable to forage at all as a consequence of the particular event taking place at that time. For example, incubating and moulting penguins (Sphenisciformes) are entirely restricted to land and are therefore unable to forage. Penguins are restricted to land during moult because they lack feathers and fat for insulation when in the water, and during incubation because of the distance to the sea from safe breeding and moult sites (e.g. Croxall, 1982; Cherel and Groscolas, 1999; Green et al., 2005). Similar restrictions are faced by other moulting and incubating seabirds such as the albatrosses (Diomedeidae), shearwaters and petrels (Procellariiformes) (e.g. Cherel and Groscolas, 1999). For those interested in studying the energetics of events such as incubation and moult in birds, it can be possible to utilise this fasting period to determine the daily energy expenditure (DEE) of the fasting animal, through the recording of daily body mass loss (DBML). Here, we review the use of the DBML technique to estimate DEE. We present details of the methodology, compare the technique to other approaches available for measuring DEE in birds, and present a worked example of how to apply the technique.

During starvation, energy production occurs at the expense of body reserve utilisation which results in body mass loss (e.g. Cherel et al., 1994). When birds are experiencing a long-term fast, the significant body fuels which are catabolised are lipids and proteins (Blem, 1976; Blem, 1990). Knowing the role of these fuels in body mass loss, along with their corresponding energy content, can allow

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Abbreviations: BMR, basal metabolic rate; DBML, daily body mass loss; DEE, daily energy expenditure; DHR, daily heart rate; DMR, daily metabolic rate; DLW, doubly-labelled water; f_H, heart rate; HRDLs, heart-rate data loggers; IMR, incubation metabolic rate; M_b, body mass; MR, metabolic rate; RHR, resting heart rate; RPY, respirometry; TOBEC, total body electrical conductivity; $\dot{V}O_2$, rate of oxygen consumption.

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an energy equivalent to be calculated (Barre, 1975; Pinshow et al., 1976; Prince et al., 1981; Croxall and Ricketts, 1982). Body mass loss during a prolonged fast is best described as an exponential function of time, $W_t = W_0 e^{-kt}$, where W_0 is the mass at the start of the fast, W_t the mass t days later, and k is a constant, which here is proportional to the birds mass loss per day (e.g. Croxall, 1982). Therefore, it is possible to determine DEE during this period if at least two body mass loss measurements at an interval of a known number of days are obtained. For example, Shaffer et al. (2001) weighed adult incubating wandering albatrosses (*Diomedea exulans*) at the onset of the incubation period, and again 21 days later at the end of the incubation shift, thus allowing them to calculate a mean DBML of 112 g for the duration of egg incubation and its associated fast. Use of the DBML technique to estimate DEE has been used predominantly in Antarctic and sub-Antarctic seabirds, particularly penguins and albatrosses (Fig. 1, see also Croxall, 1982). This is a result of the particular life-cycle and life history of these groups, as when on land, these species are fasting completely for periods ranging from days to months (e.g. Groscolas, 1988). It is also partly due to the behavioural nature of these species, in that they are tolerable to regular weighing and general human activity around the nest and/or moult site. However, any species that undergoes a fast of sufficient duration can be a possible candidate for the DBML technique, providing that body mass and measurement time intervals are accurately recorded, and the activity (e.g. moult) and individual sex and status are known.

A vital element of using DBML to estimate DEE is a detailed knowledge of the energy content of the material lost, i.e., the body fuels that comprise the body mass loss (Croxall, 1982; Groscolas, 1988; Groscolas et al., 2010). Historically, in penguins for example, the body mass loss has been estimated to comprise *c.* 95% fat and 5% protein (Stonehouse, 1967; Johnson and West, 1973; Le Maho and Declitte, 1974). However, subsequent studies that directly measured body composition through destructive sampling in moulting penguins estimate that body mass loss comprises *c.* 38%–55% fat and *c.* 6%–9% protein, the remainder being water (Groscolas and Clement, 1976; Williams et al., 1977; Cooper, 1978). Such differences result in estimates of DEE ranging from less than measured basal metabolic rate (BMR) to double. This demonstrates the fundamental difficulty in using data on body mass loss in fasting birds to calculate energy costs; the lack of information on the exact composition of the material lost. As Croxall (1982) summarised, “the estimates of energy expenditure are only as good as the estimates of the substrates consumed”.

Further complications for the estimate of substrates consumed during DBML are that the substrate loss ratio is not constant throughout the fasting process (Cherel and Le Maho, 1985; Groscolas, 1988; Robin et al., 1988; Groscolas, 1990; Cherel and Groscolas, 1999). In penguins, for example, the decrease in body mass is not steady throughout a fast (Fig. 2). This can potentially provide a source of error for determining DBML if mass is measured only a few times. The mass loss for a fasting penguin can be divided into three main, distinct phases (Fig. 2, for full review, see Cherel and Le Maho, 1985; Cherel et al., 1988; Groscolas, 1990): a short initial period (phase I) of high but decreasing DBML, a long period (phase II) of steady and low DBML and finally (phase III) a period of high increasing DBML. The variation in DBML during different phases of a fast has consequences for determining DEE because the proportion of lipids and proteins being catabolised differs in between phases (Groscolas, 1988). Energy equivalent of lipids is about 2.4 times greater than that of protein, and

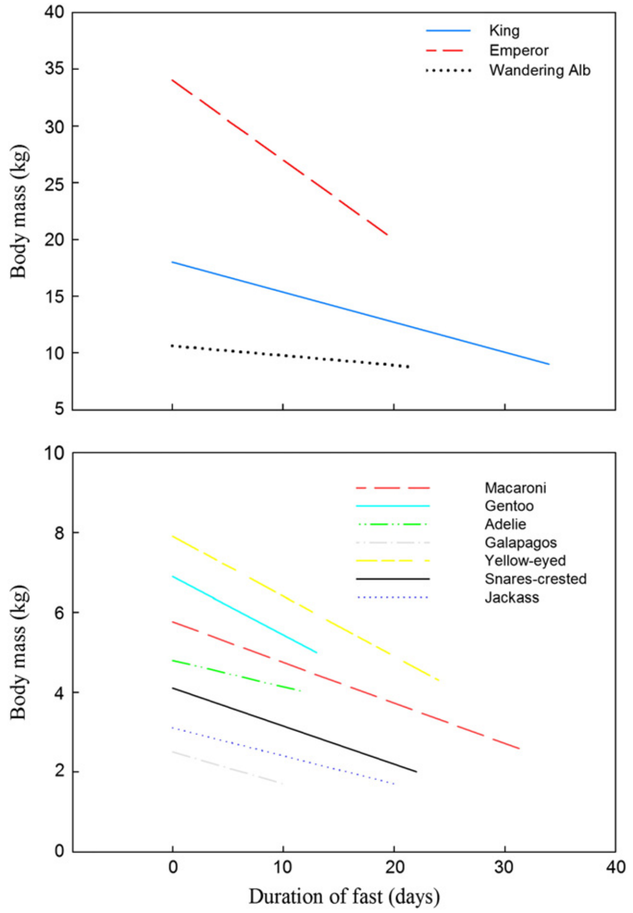

Fig. 1. Body mass loss through fasting during moult and incubation for various species of penguin (top and bottom panel) and wandering albatrosses (top panel).
Data are adapted from Croxall (1982) and references therein.

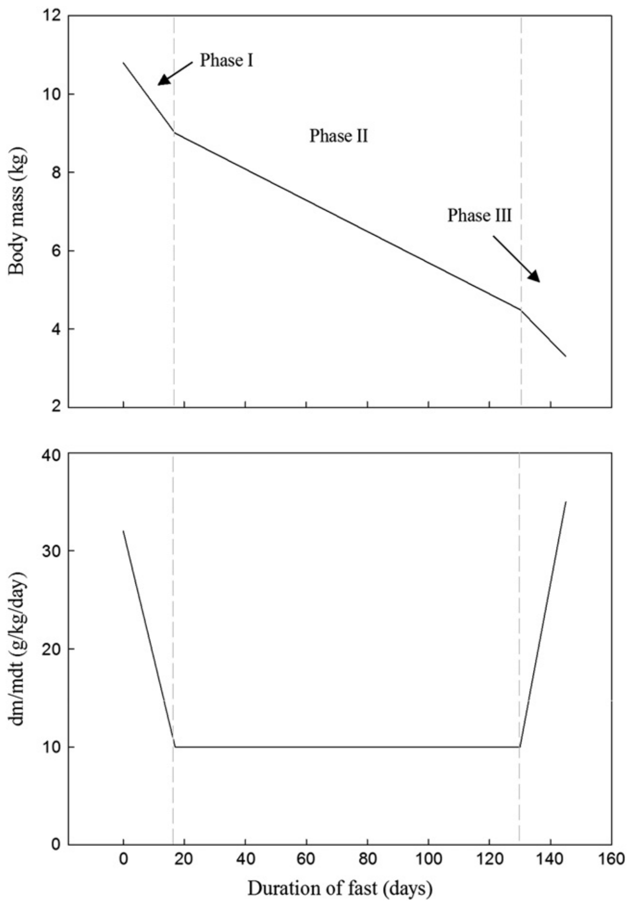

Fig. 2. Changes in body mass (top panel) and specific daily body mass loss (bottom panel), through the course of winter fasting in a king penguin chick.
Figure adapted from Cherel and Le Maho (1985) and Groscolas (1988).

protein utilisation is associated with water loss (hydration of tissues containing proteins being about seven times more than that of adipose tissue) (Groscolas, 1988; Cherel and Groscolas, 1999). As a consequence, the body mass loss associated with a given energy production from fuel oxidation is roughly eight times less from lipids than from proteins. Thus, relatively small changes in substrate use manifest as large effects on estimated metabolic rate (MR) (Shaffer et al., 2001). For example, in wandering albatrosses, a change of 10% in the contribution of fat to the substrate being consumed results in a 33% (−10%) to 90% (+10%) overestimate of DEE during incubation when compared to other techniques. Therefore, as proposed by Groscolas (1988), only phase II should be used for estimating DEE from DBML (i.e. only when the bird is in deep fast), and MR has reached a steady state and substrate consumption is relatively stable. Failure to do so will result in overestimation of MR. In phase I, it is likely that emptying of the gastrointestinal tract will cause false estimations of DEE, as DBML is then not entirely a result of lipid or protein catabolism. Therefore, this technique should be restricted to birds that undergo only long fasting shift lengths (Stovey, 1982; Groscolas, 1988). Application of the technique then requires detailed knowledge of the kinetics of body mass loss and body composition during fasting of each species being studied, but data on body composition changes during a fast can be difficult to collect.

2. How can you measure the fractional composition of the metabolic substrate being catabolised?

It is apparent that using DBML to measure DEE has its strengths. It is relatively cost-effective and straightforward to obtain values of body mass, particularly from those Antarctic species which are confiding and do not get stressed by handling. In principle, the DBML approach is non-invasive and does not always require any legislation or licensing to utilise the technique (some countries, however, will require a license to weigh birds, particularly those of conservation concern). However, the benefits of this technique are only applicable if, the fractional composition of metabolic substrate utilised during fasting is known. In practice, the need to know the metabolic substrate being utilised often prevents the DBML technique from being cheap and non-invasive. Measuring as opposed to predicting the composition of the metabolic substrate being utilised can be expensive, invasive, and again have potential problems with measurement errors. Here, we briefly describe some of the most frequently used non-destructive techniques for investigating body composition in birds that may be used in conjunction with the DBML approach for estimating DEE.

Total body electrical conductivity (TOBEC) is an indirect method of assessing body composition that relies on the principle that the electrical conductivity of an organism is proportional to its fat-free mass (e.g. Wirsing et al., 2002; Hyanakova and Szebestova, 2010). The output signal from TOBEC will give an estimate of lean body mass that can be converted into fat mass (Walsberg, 1988) by subtracting the lean mass from the total body mass (Scott et al., 2001). However, the chambers that the animals are placed in are small, and are not suitable for species such as penguins or albatrosses. A number of factors can influence TOBEC readings also and consequently its accuracy (Scott et al., 1996). With the TOBEC instrument, the most uniform section of the magnetic field occurs in the centre of the sampling area (Walsberg, 1988; Scott et al., 2001) so it is vital to position the animal within the coil in a manner that is easy to repeat. Because positioning of the subject is so important when using TOBEC, each time a reading is taken, the animal must remain still, and in the same position within the chamber for all recordings. With birds, various methods have been devised to achieve the desired 'stillness' that is required to gain accurate readings from TOBEC, including rubber bands (Skagen et al., 1993), Velcro jackets (Scott et al., 1991) and nylon mesh stockings (Walsberg, 1988). Anaesthesia has been used during TOBEC measurements (Witter and Goldsmith, 1997; Gillooly and Bayliss, 1999), to ensure the animal is positioned uniformly in the chamber, to reduce movement and possibly to reduce stress on the animal (Scott et al., 2001). However, anaesthesia is not necessarily suitable for use in conjunction with TOBEC in endotherms, as the associated drop in body temperature alters the TOBEC reading (Tobin and Finegood, 1995).

Ultrasound works on the principle that a high frequency sound emitted from a source will travel through a medium until it hits a reflector, and then return as an echo to the source. Time differences between sound emission and the returning echo, and the intensity of the echo may be used to obtain information about distance and about the properties of the reflector (Starck et al., 2001). Ultrasound is a relatively simple method for body composition analysis (Baldassarre et al., 1980). All that is required is a detailed knowledge of the anatomy of the studied animal and experience at interpreting the images (Starck and Burann, 1998). When considering non-invasive techniques of body composition, ultrasound is particularly useful because it allows morphometric measurements of structures *in situ*, and requires only manual restraint of the animal, so in comparison to TOBEC, it is a relatively non-stressful method. For birds, however, the dense layer of feathers represents an obstacle to the use of ultrasonography for body composition analysis. Such problems can be overcome by using a multipurpose ultrasound gel to part the feathers and expose a small area of skin without causing any long term damage to the feathers or the bird (Farhat and Chavez, 2001).

The most commonly used tool for measuring body compositional changes non-destructively, particularly in conjunction with the DBML technique, is the isotope dilution method (Speakman, 2001). Fat contains substantially less water than lean tissue, so increases in fat are associated with a decrease in water content as a percentage of body mass (see Speakman et al., 2001 for full review). Unfortunately, this technique is frequently applied without an appropriate calibration against total body water in the organism, and frequently no estimation of equilibration time for the isotope is gained (but see also Crum et al., 1985; Degen et al., 1986, 1991; Mata et al., 2006 for examples of validation and calibration). This omission is important because the labelled isotope must equilibrate fully with the water pool in order for the size of the pool to be estimated accurately (see Figs. 1, 3; Shaffer et al., 2001). Failing to allow appropriate time for equilibration leads to underestimation of the body water pool, and overestimation of fat. Using isotopes can also be costly, especially for larger animals, and can also cause disturbance to the animals through repeated administration of the isotope and subsequent blood sampling. It has been shown also that use of the isotope dilution

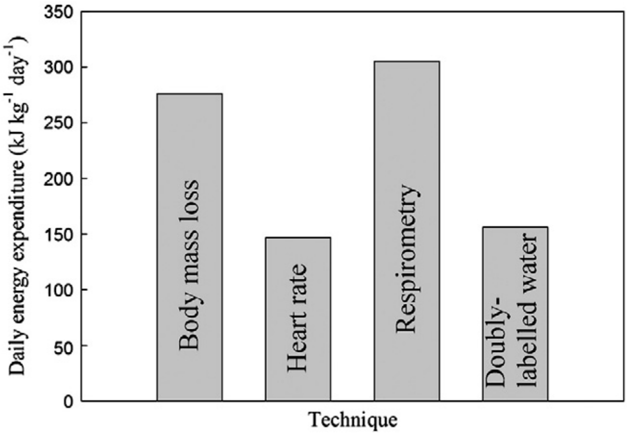

Fig. 3. Comparison of methods for evaluating energy expenditure of wandering albatrosses taken from Shaffer et al. (2001). Estimates of energy expenditure during incubation obtained from body mass loss were significantly higher from those obtained via doubly-labelled water and the heart rate technique.

technique typically overestimates total body water by an average of 3–4% (Crum et al., 1985; Degen et al., 1986, 1991; Speakman et al., 2001; Mata et al., 2006; Eichorn and Visser, 2008) which would result in body composition estimate error, particularly for fat content.

The final method for measuring the fractional substrate catabolised during mass loss is destructive sampling. This method requires sampling of individual birds at various stages in moult, incubation etc., and using traditional 'gold-standard' techniques for quantifying body composition (e.g. Baduini et al., 2001). These methods include fat extraction via Soxhlet apparatus and Kjeldahl method for determining protein (Reynolds and Kunz, 2001). The use of destructive sampling can raise ethical questions about the study and also prevents repeated measures sampling from the same individual. However, destructive sampling does provide an accurate measure of body composition. The technique could best be employed through the validation and calibration of a non-destructive technique against the gold-standard, on a small sub-sample of birds sampled at a range of fasting stages. Therefore, accurate measures of body composition, and in particular, body composition changes, could be obtained from the same individuals throughout a fast period, and in particular the relevant phase of the starvation period (phase II) (see Groscolas, 1988). This would then allow accurate measurements of metabolic substrate catabolism to be achieved, to give the most accurate values of DEE from DBML. Without such information on body composition changes, use of the DBML technique will in most cases lead to spurious measures of DEE.

3. How does the body mass loss technique compare to other techniques that estimate DEE?

Perhaps the most thorough comparison of techniques for studying DEE is that of Shaffer et al. (2001). These authors used a variety of techniques for comparison of incubation MR (IMR) in wandering albatrosses. They compared the DBML, heart rate (f_H), respirometry (RPY) and doubly-labelled water (DLW) techniques. Shaffer et al.'s (2001) analysis showed DLW values to be significantly lower and less variable than other methods. Estimates of IMR for the wandering albatrosses were 276 kJ kg^{-1} day^{-1} (DBML), 147 kJ kg^{-1} day^{-1} (f_H), 305 kJ kg^{-1} day^{-1} (RPY) and 156 kJ kg^{-1} day^{-1} (DLW) respectively (Fig. 3). Shaffer et al. (2001) used DLW as the assumed correct measure, and concluded that DBML significantly overestimated IMR (276 kJ day^{-1} for DBML compared to 156 kJ day^{-1} for DLW). Interestingly, the RPY (see Lighton et al., this issue) measurements reported were 305 kJ day^{-1}. Like Shaffer et al. (2001), other authors have also concluded that RPY measurements are not always accurate, and too disturbing for the animals causing what was it typically concluded to be overestimates of MR. For example, Weimerskirch et al. (2002) observed that being inside a respiratory chamber significantly increased MR and f_H compared to subjects for which f_H was measured in their natural habitat. However, Green et al. (2005) demonstrated that being inside a respiratory chamber actually significantly lowered MR as the birds in the chamber were sheltered from the Antarctic elements (for further discussion, see next section).

In the Shaffer study, f_H and DLW gave estimates of DEE that were not significantly different from each other. Although Shaffer et al. (2001) concluded the value of DEE obtained from RPY experiments were too high, the same technique (RPY) was used to calculate the $\dot{V}_{O_2}/f_H$ relationship, for the f_H value obtained (see Green, this issue, for full review). Shaffer et al. (2001) concluded that DLW and f_H were the most accurate techniques for measuring DEE in the albatrosses, not DBML (or RPY). The values obtained from DLW and f_H in the incubating albatrosses were also similar to those derived from predictive allometric equations for determining basal metabolic rate (see White, this issue), and the authors concluded that incubation for the adult albatrosses did not require additional energy input above maintenance metabolism, mainly because high nest attendance meant no requirement for reheating of eggs. Values of BMR (as defined by Frappell and Butler, 2004), however, are obtained from a bird that is post-absorptive, but not in a state of fasting or starvation. If the recommendation of Groscolas (1988) is upheld, and DBML estimates are only taken from fasting birds in phase II, they would not necessarily be comparable to the BMR of a well fed bird that is simply post-absorptive.

Shaffer et al. (2001) did not know the body mass loss kinetics of wandering albatrosses, and assumed a substrate catabolism during the fasting period that was equivalent to that of a fasting king penguin (*Aptenodytes patagonicus*) (Croxall, 1982). As discussed previously, incorrect assumptions regarding substrate catabolism can lead to a significant measurement error. Further investigation of the Laysan albatross (*Phoebastria immutabilis*), for example, showed that they catabolised fat primarily and very little protein during incubation (Grant and Whittow, 1983; Pettit et al., 1988). If the mass loss during incubation in the wandering albatrosses is assumed to be solely 100% fat, the DEE would then be *c.*4698 kJ day^{-1} derived from the DBML technique as opposed to *c.*2647 kJ g^{-1} from the 50.8% fat and 13.5% protein substrate used (Shaffer et al., 2001). Using primarily fat as the substrate catabolised during the fast yields values of IMR predicted to be the same as foraging for that species. It has been proposed that DBML as a method for measuring DEE would include activities such as social interactions, both sexual and antagonistic, general movements around the nest site and so on, and would, therefore, overestimate IMR. However, this was in comparison to DLW (Shaffer, this issue), which also only gives an average DEE rate for the given period of time between administration of the isotope and subsequent blood sampling, hence presenting the same problem of the value of DEE derived from DBML, as it will include a range of behaviours in addition to incubation. Perhaps the only approach that would adequately identify periods of social interactions and general activity around the nest would be a combination of f_H and accelerometry (see Green, this issue; Halsey et al., this issue). Individual inspection of f_H and tri-axis accelerometry traces could potentially identify periods of general activity and social interactions, and account for them in the calculations of DEE.

In a recent article, Groscolas et al. (2010) studied in more depth the comparisons of DEE between DBML and f_H. They set out guidelines for the use of DBML method which included the confirmation of only using mass loss (and thus its energy equivalent) from the consistent phase II of a fasting animal. Reported values of mass loss during this phase II varied between 55 and 247 g day^{-1} in king penguins, a 4.5 fold range. Consequently, the corresponding range of DEE estimations was 1315 to 5903 kJ day^{-1}. Groscolas et al. (2010) stressed the importance in knowing the energy equivalent of the mass lost, and compared estimates of DEE from DBML and f_H. Like Green et al. (2005) has reported previously (and contrary to Shaffer et al., 2001), Groscolas et al. (2010) found no significant differences in estimated DEE between the two methods. The value of 23.9 kJ day^{-1} used for the conversions of the catabolised metabolic substrate in the king penguins were only 7% different from direct analysis of body composition in two other long-term fasting seabirds (Groscolas, 1988; Groscolas et al., 1991, 2010). This suggests that with the appropriate known information, the DBML method can be a good predictor of DEE. However, during the analytical process, the authors used a linear regression fit (Fig. 1 of that study) to their established relationship between body mass and total energy content (kJ 10^{-3}). Further study of this suggests that the relationship may in fact be non-linear. For example, using a quadratic fit shows the quadratic term to be significant, with a minor increase in r^2. Nevertheless, if the quadratic relationship is used to calculate the energy equivalences of body mass it varies from 15.3 kJ g^{-1} at a body mass of 9 kg, to 36.4 kJ g^{-1} at a body mass of 17 kg. These values are similar to the energy equivalences of protein (17.8 kJ g^{-1}) and lipid (39.3 kJ g^{-1}), respectively. Within the range of body masses used for the calibration relationship in Groscolas et al. (2010) (9.7–12.9 kg), they will be somewhere between overestimating energy expenditure by 28% and underestimating it by 7%. Based on the mean masses of the individuals shown in Table 1 of the paper (*c*. 10 kg), they may overestimate DEE by around 10%, on average. What this demonstrates is the importance of approaching the analysis correctly when using the DBML method to estimate DEE, as small changes in energy conversions can equate to large differences in DEE, particularly for those individuals with large residuals from the line of best fit.

4. Applying the technique to species other than Antarctic seabirds

4.1. Introduction

As detailed previously, a substantial bias exists for what bird species the DBML technique has been applied to. Owing to the nature of the technique, it has been largely restricted to moulting and incubating Antarctic seabirds (notably penguins and petrels). Here, we demonstrate that the DBML technique can be used as an effective proxy for DEE in a member of the Anatidae (waterfowl) family in the northern hemisphere which enters a full phase II of a fast.

Common eiders (*Somateria mollissoma*), hereon referred to as eiders, are large (>2 kg) marine ducks which obtain their food exclusively by diving, using a combination of wing and foot propulsion (Heath et al., 2006). Eiders are relying exclusively on body reserves during incubation (Parker and Holm, 1990; Gabrielsen et al., 1991; Bolduc and Guillemette, 2003) and have been studied extensively in the wild because of their unusual incubation, with females incubating the eggs entirely by themselves, only moving from the nest for brief recesses typically at night (Criscuolo et al., 2000; Bolduc and Guillemette, 2003). Prior to the incubation period, the body mass of the females increases by approximately 26% in comparison to that during the winter months (Rigou and Guillemette, 2010). During incubation, females lose approximately 23–36% of body mass (Fig. 4; Criscuolo et al., 2000; Guillemette and Ouellet, 2005; Bolduc and Guillemette, 2003). The aim here is to use the DBML method to estimate DEE during incubation of the eiders, and compare the DEE values to those obtained through simultaneous recordings of f_H (see Green, this issue).

4.2. Materials and methods

4.2.1. Heart rate and conversion to energy expenditure

Heart rate was measured in 20 female eiders in the field using heart-rate data loggers (HRDLs), full details of which, including the implantation procedure, can be found in Guillemette et al. (2004) and Green et al. (2005). Of the twenty instrumented females, 17 returned to the breeding colony one year after logger deployment, and data from 13 were usable for this study. For each bird and for each day of recording the number of heart beats that occurred during the day was summed. Thus, mean daily heart rate (DHR) was calculated as the total number of heart beats during a 24 h period divided by 1440 (the number of min in 24 h) to obtain a value in beats min^{-1}. Resting (minimum) f_H (RHR) was used as a surrogate of maintenance cost. A customized computer program was run to find the five min interval with the lowest average f_H that occurred during the day and use these as RHR (Guillemette et al., 2007).

We used the calibration study of Hawkins et al. (2000, Table 1) to convert f_H data into MR. In that study, the f_H (beats min^{-1}) and $\dot{V}O_2$ (ml kg^{-1} min^{-1}) relationship for six eiders was established using RPY and HRDLs. Hawkins et al. (2000) measured resting $\dot{V}O_2$ for birds fasting in the air and the functional (reduced major axis) relationship was:

$$\dot{V}O_2 = 0.076 f_H + 5.778 \ (n = 6,\ r^2 = 0.54,\ P = 0.039) \qquad (1)$$

$\dot{V}O_2$ was then converted to MR assuming an energy equivalence of oxygen of 20.083 kJ l^{-1}.

The composition of incubating female eiders has been studied destructively, allowing accurate measurements of body composition to be used in calculations of substrate catabolism during body mass loss (Parker and Holm, 1990; Gabrielsen et al., 1991). Body mass loss was converted to DEE assuming that 60.2% of body mass loss of fasting females was composed of lipids and 10.3% of proteins (the rest being water and ash, Parker and Holm, 1990).

5. Results and discussion

Between the 5th and 20th incubation day, mean (±SD) daily body mass loss (DBML) of breeding female eiders was 24.1 ± 7.0 g. DBML did not differ between the first and second halves of the incubation (Paired *t*-test (unilateral), $t = 0.611$, $df = 12$, $P = 0.276$). Mean body mass at the beginning of incubation averaged 2218 ± 140 g (Fig. 4) and was strongly and significantly correlated with body mass loss (body mass (M_b) loss = 0.060 M_b *IncubationStart* − 110.6, $r^2 = 0.89$, $P < 0.001$) indicating that the larger birds lost mass at a higher rate than the small ones. No relationship was found between DHR and body mass loss and between DHR and body mass at the beginning of incubation. Body mass loss (g day^{-1}) was inversely related to the daily time spent diving (body mass loss = − 1.17 M_b *DivingTime* + 27.07, $r^2 = 0.74$, $P < 0.05$) indicating that females losing body mass at a lower rate were potentially feeding. Therefore, we considered below the two groups separately; those that were observed diving during recesses ($n = 7$) and those that were not ($n = 6$).

The critical assumption to convert DBML into energy expenditure is the proportion of lipids and proteins used during starvation. The quantity of water loss estimated is of crucial importance as it influences the proportion of body mass loss that is fuel. From pre-laying to post laying, water would contribute 45% of body mass loss if we take the results of Parker and Holm (1990) for granted. From post-laying to hatching that proportion is now 27% and we used that value in our calculations together with 62.1% and 10.2% for the proportion of fat and protein, respectively. Based on these assumptions, incubating eiders spend 623 ± 198 (SD) kJ day^{-1} (or 321 kJ kg^{-1} day^{-1}). Comparatively, our estimate of DEE using the f_H method gives an MR of 573 kJ day^{-1} (or 326 kJ kg^{-1} day^{-1}).

When splitting our 13 females into two groups, we observed the diving (and presumably feeding) females to lose body mass at a slower

Table 1
Summary of advantages and disadvantages of using the daily body mass loss technique to estimate daily energy expenditure in birds.

✓	✗
Comparatively cheap compared to other methods and easy to get equipment	Estimates can be incorrect if metabolic substrate being consumed not known
No stress involved for the animal with minimal handling required	Estimating body composition can be expensive and invasive
Non-invasive in principle with no attachment put on the animal	Measuring body composition non-invasively can introduce errors
No licensing required to gather data (e.g., British Home Office License)	Can only use species which enter a full phase II fast
Minimal technology involved and no specialist knowledge needed	To enable repeated mass measurements, species must be site faithful
Simple to gather data and no specific skills required	Only provides metabolic costs for limited events (e.g., incubation and moult)
Relatively straightforward analysis if substrate consumed is known	Calculates only an average daily energy expenditure over a given duration

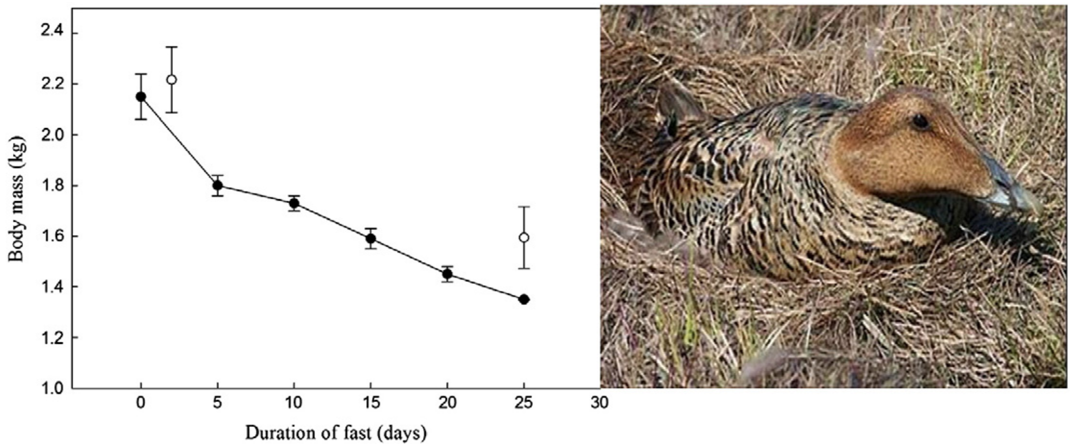

Fig. 4. Mean body mass loss (left panel) in six female common eiders (right photograph) during the 25-day fast associated with the incubation period (closed circles, data adapted from Criscuolo et al., 2002). Open circles are body mass at the start and finish of the incubation process in 13 females taken from the present study (see Methods) breeding at Christiansø Island, Denmark. Error bars represent standard deviation.

rate and spend less energy (based on DBML method, 505 kJ day^{-1}) than their non-diving counterparts (734 kJ day^{-1}), which results in a significant difference ($t = -2.92$, $df = 11$, $P = 0.014$). We cannot statistically test the MR of the two groups estimated from the f_H method (see Green, this issue) although the trend was for the active females to spend more energy (604 kJ day^{-1}) than the non-diving females (537 kJ day^{-1}).

Our estimates of MR of incubating eiders (DBML method = 321 kJ kg^{-1} day^{-1}, f_H method = 326 kJ kg^{-1} day^{-1}) are in general agreement with other studies that have measured the MR of female common eiders while fasting and being inactive in a metabolic chamber. Gabrielsen et al. (1991) have measured the DMR of two wild incubating females using a portable respirometer to be 387 kJ kg^{-1} day^{-1}. Using the same method, these authors also reported the DMR of twelve females caught while in a reproductive stage and brought into the laboratory to be 391 kJ kg^{-1} day^{-1}. Hawkins et al. (2000) measured the metabolic rate of resting common eiders to be 370 kJ kg^{-1} day^{-1}. Gabrielsen et al. (1991) also used the DBML method on incubating females to estimate DMR and found that it was 460 kJ day^{-1} and 24–29% less than the respirometry method. These authors did not report this level of energy expenditure on a mass-specific basis although their estimate stems from the 20th day of incubation (Fig. 1 in Gabrielsen et al., 1991) when females weigh in average 1440 g and giving a mass specific DMR of 319 kJ kg^{-1} day^{-1}.

Our study confirms the conclusion of Gabrielsen et al. (1991) (and possibly also that of Shaffer et al., 2001) that respirometry estimates are higher than DBML estimates. We can exclude the effect of thermoregulation costs as all studies, including ours, were performed above the lower critical temperature of 7 °C measured in that species (Gabrielsen et al., 1991). This is contrary to expectations as the respirometry method uses the most steady and lowest level of gas exchange for any experimental bird whereas the DBML technique integrates all activities and metabolic processes occuring on a 24 h basis. Indeed, although incubating eiders may spend as much as 99.5% of 24 h sitting on the nest (Bolduc and Guillemette, 2003), their behaviour while incubating includes nest maintenance, turning the eggs, standing and watching predators (Guillemette, unpublished observations). We therefore suggest that respirometry is more invasive and more stressful than the DBML method (e.g. Groscolas et al., 2010). Weimerskirch et al. (2002) observed that the minimum f_H of albatrosses while sitting in the metabolic chamber was higher than for individuals incubating on their nest and conclude that it was an effect of handling and the experimental procedure associated with the respirometry method. In support of this, Hawkins et al. (2000) reported a minimum f_H of seven female common eiders while sitting in the metabolic chamber to be 93 beats min^{-1} on average (mean body mass = 1790 g) compared to 74 beats min^{-1} in average for our 13 incubating females (mean body mass = 1760 g). The difference in f_H between a bird in a respirometer and a bird resting in its natural environment is likely to be related to habituation to the experimental set up of the individual birds involved. A wild bird not accustomed to being handled and involved in experimentation is likely to have a more extreme reaction to being inside a respirometry chamber than a captive bird that is trained and habituated to the experimental surroundings.

Nevertheless, the DBML method is not without pitfalls in this instance as suggested by our observations showing that DBML is lower for active and presumably feeding females compared to their non-feeding counterparts. We thus conclude that in addition to the body fuels being consumed, the absence of feeding is also a critical assumption associated with the DBML method. There is, however, a potential problem when examining the validity of an indirect experimental method by reference to other indirect experimental methods, in that the reference method being used for comparison will have errors associated with it also. Historically, $\dot{V}O_2$ measurements via RPY were considered the gold reference standard for measurements of DEE. As discussed previously, RPY can have problems associated with measuring DEE due to the potential effects of stress and confinement. It is highly likely, therefore, that certain approaches will work better with different animals and systems.

6. Summary and future potential

Estimating DEE from DBML, like all techniques, has its advantages and disadvantages (summarised in Table 1). In comparison to other approaches (e.g. HRDLs and f_H, accelerometry), DBML is cheap, relatively straightforward, and involves minimal stress and handling to the animal. In principle, it can be non-invasive and in some jurisdiction may not require licenses or permits (e.g. British Home Office license if working within the United Kingdom). Equipment-specific specialist knowledge is not essential to gather data, and the minimal equipment required to use the technique means the work can be conducted in remote locations where it may not be possible to take large and bulky equipment (e.g. RPY) or perform surgeries (f_H).

However, estimates of DEE can be incorrect if the substrate being consumed during the mass loss period is either not known or estimated incorrectly. The process of obtaining data on body composition can become costly (e.g. TOBEC, ultrasound) and/or invasive (e.g. isotope dilution), and will in itself introduce errors to the analytical process. Perhaps the greatest limitation of the DBML technique is that only species that enter the full phase II of a fast can be used and these species must be accessible and tolerable to being weighed at regular intervals. Moreover, the individuals must be recognisable or individually marked, and be site faithful, either remaining or returning to the same place to allow for repeated mass measurements to be taken, if an individual measure of DEE is required.

When weighing up the advantages and disadvantages in Table 1, it can be easy to determine that the DBML technique is too limited to be of any use; or rather, that the column of disadvantages on the right side (✘) of Table 1 outweigh any of the positives listed in the advantages column on the left (✔). In particular, it is arguable that all the species that it may be possible to utilise the DBML technique on (i.e. enter phase II of a fast for sufficient duration) have already been studied extensively (e.g. Croxall, 1982; Groscolas, 1988). However, this is not necessarily the case and in this review alone we show that the DBML approach is an accurate measure of DEE in incubating female eiders, when compared to f_H estimates and the literature.

There are potentially two key aspects that may open up the DBML technique to a wider use; (1) the use of a population to gain estimates of DEE for a species and/or event rather than individuals, and (2) body composition analysis (either destructive or non-invasive) of a subset of the population at different stages of the fast to determine metabolic substrate use. Using this dual approach there is much potential for the use of the DBML technique. For example, it may be possible to utilise this approach with migrating birds, for instance, waders (Charadriiformes). One population of bar-tailed godwits (*Limosa lapponica*) migrate 11,000 km from the east coast of New Zealand to the west coast of Alaska, non-stop (Gill et al., 2005). It may be possible, therefore, to use mass loss during this migration as a measure of DEE, and birds could be caught prior to departure and then immediately on arrival, and a mean population mass loss ascertained. Alternatively, depending on the ethical standpoint, it would also be possible to destructively collect birds at departure and arrival, thus also allowing detailed body composition analysis to be performed. Studying bird migration would, however, be limited to those species that it is certain are not feeding en route. The DBML technique could also potentially be applied to bird groups such as hummingbirds (Apodiformes) that enter a torpid state for periods of time, and members of the Galliforme group such as the Svalbard ptarmigan (*Lagopus mutus hyperboreus*), which undergo fasting periods due to inclement weather (e.g. Hiebert, 1993; Lindgard et al., 1992). The purpose of this review is not to exhaustively list all possible scenarios in which the DBML technique can be applied, but rather demonstrate the potential the approach has, outside of the penguins, albatrosses, shearwaters and petrels that have already been studied extensively. Finally, reiterating to some degree what Groscolas (1988) stated, the following three key elements must be adhered to when using the DBML technique:

1. The substrate being consumed during the mass loss but be accurately known for that species, and ideally measured rather than estimated.
2. Ideally, regular weighing sessions are required to record body mass loss kinetics and consistency during the fasting process and the associated mass loss.
3. Only species which enter the full phase II of a total fast and do not feed can be used, and data from phase I should not be used for analysis. This phase II period has to be sufficiently long in duration to clearly discriminate between phases of the fast.

If these assumptions and guidelines are strictly adhered to, the potential remains for the DBML technique to be a useful and productive tool in measuring energetics and DEE, both in the field and under laboratory conditions.

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