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A comparison of methods to evaluate energy expenditure of incubating wandering albatrosses

Scott A. Shaffer
University of California - Santa Cruz, scott.shaffer@sjsu.edu

D P. Costa
University of California - Santa Cruz

H Weimerskirch
Centre d'Etudes Biologiques de Chizé

Comparison of Methods for Evaluating Energy Expenditure of Incubating Wandering Albatrosses

Scott A. Shaffer[1,*]
Daniel P. Costa[1]
Henri Weimerskirch[2]
[1]Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, California 95064;
[2]Centre d'Etudes Biologiques de Chizé, Centre National de la Recherche Scientifique, 79360 Villiers en Bois, France

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ABSTRACT

Measurements of incubation energetics can vary depending on the method used to measure metabolism of an incubating bird. Therefore, we evaluated the energy expenditure of six male and four female wandering albatrosses (*Diomedea exulans* Linnaeus) using doubly labeled water (DLW), the rate of mass loss, and estimates of metabolic water production derived from water influx rate (WIR). Incubation metabolic rates (IMR) determined with DLW (169 ± 21 kJ kg^{-1} d^{-1} SD) were significantly lower than estimates derived from mass loss (277 ± 46 kJ kg^{-1} d^{-1} SD) and WIR (males = 289 ± 60 kJ kg^{-1} d^{-1} vs. females = 400 ± 69 kJ kg^{-1} d^{-1} SD). Estimates of IMR from mass loss and WIR were similar to IMR (305 ± 39 kJ kg^{-1} d^{-1} SD) determined by respirometry in a previous study, and IMR from DLW was similar to estimates based on heart rate (HR; 147 ± 26 kJ kg^{-1} d^{-1} SD) determined in another study. Applying the different measurements of IMR to construct an energy budget, we estimate that a breeding pair of wandering albatrosses spends 124–234 MJ to incubate the egg for 78 d. Finally, IMRs determined with DLW and HR were similar to estimated basal metabolic rates derived from six different allometric equations, suggesting that heat production from adult maintenance metabolism is sufficient to incubate the egg.

Introduction

The cost of incubation has been measured using a variety of methods including (1) respirometry (Gessaman and Findell 1979; Vleck 1981; Grant and Whittow 1983; Brown and Adams 1984; Ricklefs et al. 1986; Gabrielsen et al. 1991), (2) mass loss (Prince et al. 1981; Croxall 1982; Croxall and Ricketts 1983), (3) heart rate (HR; Bevan et al. 1995; J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript), and (4) doubly labeled water (DLW; Flint and Nagy 1984; Ricklefs et al. 1986; Obst et al. 1987; Pettit et al. 1988; Williams 1993). However, these methods are not directly comparable because of differences in measurement interval, measurement technique, precision of the method, and validity of assumptions required (e.g., fraction of substrates metabolized in the mass loss method). Therefore, it is difficult to discern whether differences in the cost of incubation are real or are attributed to the methodology used.

Despite the difference in methodologies, only a few studies have evaluated the cost of incubation by comparing multiple methods to measure metabolism among different species (Grant 1984) or within a single species (Brown and Adams 1984; Ricklefs et al. 1986; Obst et al. 1987; Pettit et al. 1988). These studies indicate that incubation costs range from 0.82 × resting metabolic rate for Bonin petrels (*Pterodroma hypoleuca*) measured with respirometry (Grant and Whittow 1983) to 2.2 × basal metabolic rate (BMR) for Wilson's storm petrels (*Oceanites oceanicus*) measured with DLW and compared to BMR measured with respirometry (Obst et al. 1987). In addition, Obst et al. (1987) determined that measurements of incubation metabolic rate (IMR) determined by mass loss were 43% higher than that determined with DLW. Thus, further examination of the differences in methodologies to measure incubation costs is warranted, especially if incubation costs are used to model energy budgets of breeding birds.

The objective of this study was to compare methodologies used to measure incubation costs by measuring metabolism of incubating wandering albatrosses (*Diomedea exulans*) with (1) DLW, (2) the rate of mass loss, and (3) from estimates of metabolic water production (MWP) derived from water influx rate (WIR). IMRs determined with these three methods were then compared to previous measurements obtained using open-flow respirometry (Brown and Adams 1984) and HR (J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript). Furthermore, we examined how the variations in IMR, determined with different methods, affect the outcome of energy budget models. Also, given that there is considerable variation in the cost of incubation in relation to BMR, we compared IMR of wandering albatrosses to measured (Brown and Adams 1984) and pre-

* Corresponding author; e-mail: shaffer@biology.ucsc.edu.

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dicted BMR (based on several allometric equations) to determine whether adults expend extra energy to incubate their eggs.

Material and Methods

Wandering albatrosses were studied during the early incubation period in January–February 1999 on Possession Island, Crozet Archipelago, southwestern Indian Ocean (46°S, 52°E). The weather during this period was cool (9.8° ± 3.8°C SD), with moderate to heavy rain and relatively high humidity (90.8% ± 9.3% SD), and persistent west-southwesterly winds (10.0 ± 8.2 km h^{-1} SD; maximum 117 km h^{-1}).

The metabolic rates of 10 incubating wandering albatrosses (six males and four females) were determined (1) using the DLW method (Lifson and McClintock 1966; Nagy 1980, 1983; Speakman 1997) and (2) the mass loss method (Croxall 1982) and (3) from estimates of MWP derived from WIR. The sex of each bird was determined by plumage characteristics (Weimerskirch et al. 1989), and prior reproductive histories and ages were determined from banding records (Weimerskirch and Jouventin 1987). The mean age of male albatrosses was 22.8 ± 5.7 yr SD and the mean age of female albatrosses was 18.3 ± 11.6 yr SD. All but one bird had at least two previous breeding attempts.

Nests were monitored daily for changeovers between partners, and study birds that had initiated an incubation bout within 12–24 h of their return from sea were chosen. A bird was removed from its nest and its egg covered to reduce heat loss. A cloth hood was placed over the bird's head and 3−4 mL of blood was sampled from a vein on the tarsus for background determination of oxygen-18 and tritium. Each bird was then given an intraperitoneal injection of 8–10 mL of sterile water containing 10 atom % oxygen-18, 2.15 MBq mL^{-1} of tritiated water, and 0.9% NaCl. Mass of the injected volume was determined by weighing the syringe before and after injection on a portable electronic balance (±0.01 g; Ohaus 200, Pine Brook, N.J.). Following the injection, birds were weighed to the nearest 50 g with a Salter spring balance (Salter Weightronix, West Bromwich, U.K.) and then returned to the nest to allow isotopes to equilibrate with total body water (Degen et al. 1981). A second blood sample of 4–6 mL was collected from a tarsal vein 150–180 min postinjection, although 100 min was determined to be sufficient for complete isotope mixing with body water (Fig. 1). At the end of the measurement interval, a final 4–6 mL of blood was collected, and birds were reweighed. Wandering albatrosses never leave their nests unattended because there is a high risk of egg predation from aerial scavengers. Therefore, we were confident that incubating birds would not engage in activities away from the nest when observers were absent from the colony.

All blood samples were collected with a syringe, transferred to a vacutainer (B-D brand with no additives, Beckton-Dickinson, Franklin Lakes, N.J.), and stored at 5°–8°C before centrifugation. Serum was transferred to 2-mL plastic screw cap vials (with silicon O-rings; Sarstedt, Newton, N.C.) and frozen at −5° C until analyses were performed in April 1999. The specific activity of tritiated body water was determined in triplicate by liquid scintillation spectrometry (Beckman LS 6500, Beckman Coulter, Fullerton, Calif.) of 90 μL of serum water in 10 mL of Ecolite^{+} scintillation cocktail (ICN Pharmaceuticals, Costa Mesa, Calif.). Water was obtained by distilling 100-μL aliquots of serum, following methods described in Ortiz et al. (1978). Specific activity of oxygen-18 water was determined by mass ratio spectrometry of water distilled from blood serum (Metabolic Solutions, Nashua, N.H.).

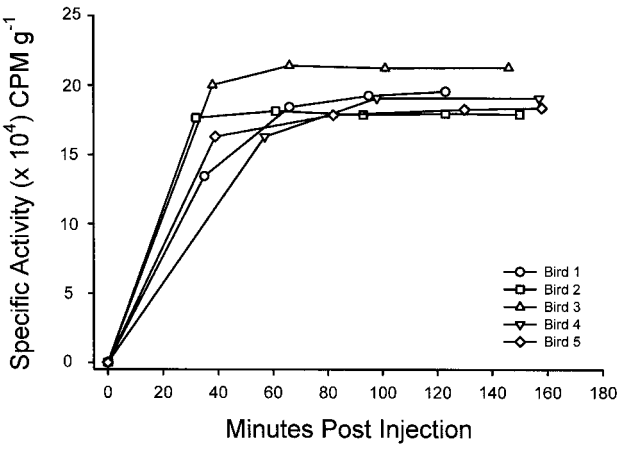

Figure 1. Specific activity of tritium in blood samples of five adult wandering albatrosses, in counts per minute per gram (i.e., CPM g^{-1}), plotted against time after injection of isotope. Background samples were collected immediately before isotope injection (intraperitoneally). Equilibration time of tritium within total body water occurred within 100 min postinjection.

Initial total body water was calculated using the initial dilution space of oxygen-18. Final body water content was calculated as the initial fractional water content times the final body mass. Carbon dioxide production was calculated using Equation (2) in Nagy (1980), and water flux was determined using Equations (4) and (6) in Nagy and Costa (1980). These equations assume that total body water volume changes linearly through time (Nagy 1980; Nagy and Costa 1980). Carbon dioxide production was converted to units of energy expenditure in kilojoules (kJ) using a conversion factor of 1 L CO_2 = 25.2 kJ (Adams et al. 1986). This conversion factor was based on the chemical composition of a squid and fish diet consumed by albatrosses (Clarke and Prince 1980; Croxall and Prince 1980).

Because incubating wandering albatrosses do not leave their nest to eat or drink, water influx from metabolism should be equivalent to MWP and thus provide a measure of metabolic rate. Therefore, metabolic rates were estimated from the rate of

water flux, assuming that 0.026 mL H_2O is liberated per kilojoules of fat or protein catabolized (Schmidt-Nielsen 1990). Finally, energy expenditure was determined by the rate of mass loss, assuming the composition of mass lost (50.8% fat, 35.7% water, and 13.5% protein) was similar to that determined for great-winged petrels, *Pterodroma macroptera* (Groscolas et al. 1991). Thus, energy expenditure in kilojoules per day was calculated using an energy equivalent of 22.4 kJ g^{-1} ([0.508 × 39.4 kJ g^{-1} fat] + [0.135 × 17.99 kJ g^{-1} protein]; equivalency data from Groscolas et al. 1991; protein data from Whittow 1986) for mass lost in grams per day over the incubation fast.

Statistical analyses were performed using SYSTAT 9.0 (Wilkinson 1996) with a significance level of $P < 0.05$ for *t*-tests (two tailed), correlation analyses, and general linear models (ANOVA and ANCOVA). Unless stated otherwise, all data are presented as means ± 1 SD.

Results

Body Size and Mass Loss

Male wandering albatrosses were 17.6% heavier than females at the start of their incubation bouts (Table 1; $t = -2.59$, df = 8, $P = 0.032$). However, there were no significant sex differences in the means of total body mass loss (−0.71 ± 0.17 kg), mass loss per day (−119 ± 26 g d^{-1}), percentage mass change per day (−1.23% ± 0.20% d^{-1}), or initial total body water (48.9% ± 4.7%). In addition, initial body mass significantly correlated with mass loss per day (Fig. 2; $r = 0.699$, $F = 7.7$, df = 1,8, $P = 0.024$) but not with total mass loss ($r = 0.488$, $F = 2.0$, df = 1,8, $P = 0.194$).

Energy Expenditure and Water Flux

The cost of incubation (kJ d^{-1}) determined with DLW was 26% higher in male wandering albatrosses compared to females (Table 1; $t = -2.36$, df = 8, $P = 0.046$), but mass-specific energy expenditure (169 ± 21 kJ kg^{-1} d^{-1}) was not significantly different between the sexes. IMR determined by mass loss exhibited no sex difference in either absolute energy expenditure (2,676 ± 575 kJ d^{-1}) or mass-specific energy expenditure (277 ± 46 kJ kg^{-1} d^{-1}). In contrast, incubation costs determined by WIR exhibited no significant sex difference in absolute energy expenditure (3,172 ± 729 kJ d^{-1}), but mass-specific costs (males = 289 ± 60 kJ kg^{-1} d^{-1} vs. females = 400 ± 69 kJ kg^{-1} d^{-1}) were significantly different between the sexes ($t = 2.36$, df = 7, $P = 0.041$). Mass-specific WIR was significantly different between the sexes (males = 8 ± 2 mL H_2O kg^{-1} d^{-1} vs. females = 10 ± 2 mL H_2O kg^{-1} d^{-1}; $t = 2.50$, df = 7, $P = 0.041$), whereas absolute WIR (mL H_2O d^{-1}) exhibited no sex difference (Table 1).

Table 1: Age, study period, initial mass, mass loss, initial total body water, incubation metabolic rate, energy expenditure, and water influx of incubating adult wandering albatrosses in 1999

Bird	Age (yr)	Study Period (d)	Initial Mass (kg)	Total Mass Loss (kg)	Daily Mass Loss (g d^{-1})	TBW_i (%)	IMR	EE (kJ d^{-1}) DLW	ML	WIR	Water Influx (mL d^{-1})
Females:											
48	>9	5.98	8.55	−.60	−100	53.5	.27	1,342	2,252	…	10[a]
A11	>34	6.25	9.10	−.75	−120	46.2	.32	1,689	2,693	2,923	76
M9	20	7.09	9.15	−.95	−134	49.1	.23	1,212	3,007	4,101	107
M16	10	5.99	9.35	−.55	−92	42.3	.26	1,405	2,061	3,560	93
Mean	18.3	6.33	9.04	−.71	−112	47.8	.27	1,412	2,503	3,528	92
SD	11.6	.52	.34	.18	19	4.7	.04	201	428	590	15
Males:											
J	>32	6.75	9.45	−.75	−111	56.9	.29	1,575	2,494	2,171	56
F	16	6.18	9.60	−.50	−81	53.4	.34	1,900	1,816	2,373	62
A9	22	4.18	10.00	−.50	−120	49.8	.29	1,686	2,685	3,765	98
A41	24	4.73	10.90	−.70	−148	48.5	.25	1,576	3,322	2,589	67
A	25	6.24	11.35	−.75	−120	46.1	.25	1,666	2,698	2,989	78
M15	18	6.01	12.50	−1.00	−166	43.2	.31	2,235	3,734	4,075	106
Mean	22.8	5.68	10.63	−.70	−124	49.7	.29	1,773	2,791	2,994	78
SD	5.7	1.00	1.18	.19	30	4.9	.03	256	668	773	20

Note. TBW_i = initial total body water determined from the dilution of oxygen-18 in total body water; IMR = incubation metabolic rate in milliliters of CO_2 per gram per hour, calculated from Equation (2) in Nagy (1980); EE = energy expenditure measured by doubly labeled water (DLW), mass loss (ML), and water influx rate (WIR).

[a] This value was an outlier and was not included in the statistical analyses.

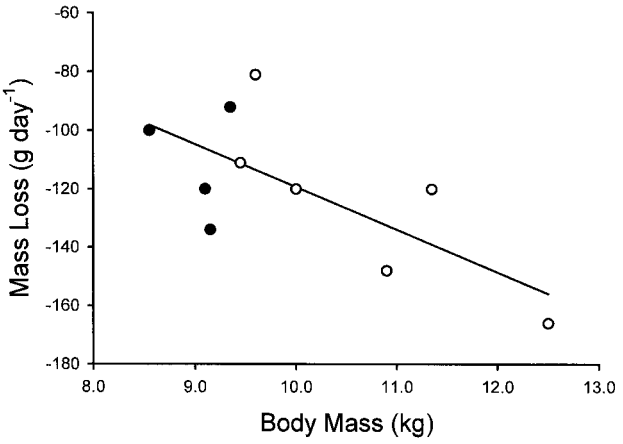

Figure 2. The change in mass per day as a function of initial body mass of incubating wandering albatross adults ($r = 0.699$, $F = 7.7$, df = 1, 8, $P = 0.024$). The open circles represent males; the filled circles represent females.

Comparison of Incubation Costs between Methods

The cost of incubation determined with all three methods was significantly different between the methods used to estimate energy expenditure (Fig. 3; ANOVA, $F = 24.8$, df = 3, 25, $P < 0.001$). IMR determined with DLW was significantly lower and less variable than all other methods ($P < 0.001$, Tukey's HSD multiple comparison). Energy expenditure determined by mass loss was significantly lower than the IMR of females determined by WIR ($P = 0.001$, Tukey's HSD multiple comparison) but similar to IMR of males determined using WIR ($P = 0.923$, Tukey's HSD multiple comparison). Both DLW and the mass loss method produced lower estimates of IMR than that previously reported by Brown and Adams (1984) using open-flow respirometry (Fig. 3; 305 ± 39 kJ kg^{-1} d^{-1}). IMR determined with DLW was also similar to estimates of energy expenditure determined by HR (Fig. 3; 147 ± 26 kJ kg^{-1} d^{-1}; J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript).

Discussion

Comparison of Methods to Measure Incubation Energetics

The results of this study showed that there were considerable differences in metabolic rates of incubating birds when using different methods to measure metabolism of the same individuals. Of all three methods (i.e., DLW, mass loss, and WIR), DLW produced the lowest and least variable measure of IMR (Fig. 3). This may be due to the fact that DLW produces a direct measure of CO_2 production (Lifson and McClintock 1966), whereas the mass loss method and WIR infer CO_2 production from estimates of energy expenditure. Estimates of energy expenditure based on mass loss are entirely dependent on knowing or assuming the fractional composition of metabolic substrates utilized in the fasting bird (Grant 1984). At present, we are unaware of any study that has measured the compositional changes in body stores of fasting albatrosses; therefore, we assumed body mass loss was equivalent to 22.4 kJ g^{-1}, which was similar to the energy equivalent of mass loss for great-winged petrels (mean body mass, ~680 g; Groscolas et al. 1991). An energy equivalent of 22.4 kJ g^{-1} yields an IMR based on mass loss that is 63% higher than IMR determined with DLW. Since fat contributes the greatest total proportion of mass loss and has the highest energy content, we can determine the sensitivity of our assumption by adjusting the fractional contribution of fat when estimating IMR from mass loss. A change of 10% in fat contribution results in a 33% (−10%) to 90% (+10%) overestimate of IMR compared to that determined with DLW. Hence, relatively minor changes in fractional composition of metabolic substrates can vary metabolic rate substantially.

Other studies have also noted sizeable discrepancies in IMR measured with DLW and mass loss (Wilson's storm petrels [Obst et al. 1987] and Laysan albatrosses, *Phoebastria immutabilis* [Pettit et al. 1988]). In addition, Grant and Whittow (1983) determined that Laysan albatrosses primarily catabolized

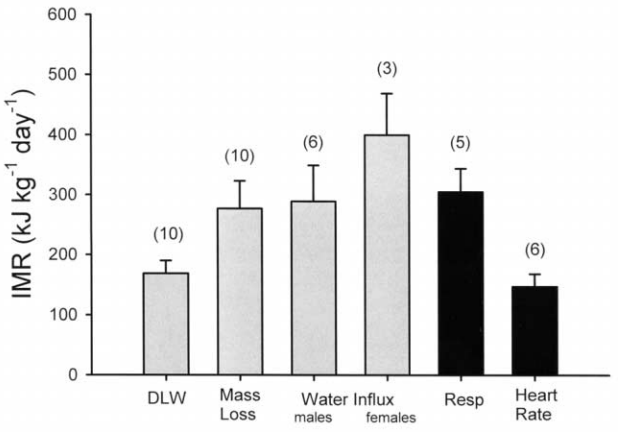

Figure 3. Incubation metabolic rate (*IMR*) of adult wandering albatrosses. IMR (*gray columns*) was determined simultaneously on the same individuals using measurements obtained from (1) doubly labeled water (*DLW*), (2) the rate of mass loss, and (3) the rate of water influx for each sex (sexes were significantly different, $t = 2.36$, df = 7, $P = 0.041$). For comparison, IMR (*black columns*) of wandering albatrosses measured using open-flow respirometry (Brown and Adams 1984) and heart rate (J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript) are presented but were not included in the statistical analyses. Error bars are standard deviations, and samples sizes are given in parentheses. IMR measured with DLW was significantly different from that determined with mass loss and water influx ($P < 0.001$; Tukey's HSD multiple comparison).

fat during incubation. If we assume that body mass loss of wandering albatrosses was 100% fat, IMR would be equivalent to 4,698 kJ d^{-1}, which is 2.9 times higher than IMR measured with DLW and nearly equivalent to the daily energy expenditure of foraging wandering albatrosses (Shaffer et al. 2001).

Previous research has shown that the rate of body mass loss and its fractional composition (i.e., proportion of fat, protein, and water) change during prolonged fasting periods in birds with the highest rate of mass loss occurring in the initial stages of the fast (Le Maho et al. 1976; Cherel et al. 1988). This occurs because metabolism has not yet reached a steady state while birds continue to empty their gastrointestinal tracts and excrete high proportions of nitrogenous waste (Cherel et al. 1988). Thus, our assumption that mass loss is equivalent to 22.4 kJ g^{-1} over the entire fasting period could be in error, especially if we consider that our measurements were initially collected 12–24 h after birds had returned from sea. This would have included the period when the rate of mass loss was highest. Indeed, the rate of mass loss measured in this study (−1.23% body mass d^{-1} over ~6 d) was higher than that reported for wandering albatrosses nesting on South Georgia (−0.9% body mass d^{-1} over ~20 d; Croxall and Ricketts 1983). However, the difference in the rates of mass loss still would not account for the discrepancy between IMRs measured with DLW and mass loss because both methods were measured simultaneously on the same individuals. Furthermore, mass loss did not correlate with energy expenditure. It is conceivable that reliable estimates of IMR could be obtained from mass loss if measurements are collected during the period of steady mass loss (Groscolas et al. 1991); however, the kinetics of body mass loss in fasting albatrosses has yet to be adequately studied.

The use of WIR to estimate metabolic rate of fasting animals has been shown to produce comparable results with measurements of metabolism obtained using DLW (Costa and Trillmich 1988). However, in this study, WIR overestimated IMR determined with DLW by 1.7–2.4 times (Fig. 3). This discrepancy could be attributed to errors associated with isotope exchange via evaporative water loss, which can occur when unlabeled water vapor or CO_2 exchanges with labeled water and CO_2 across the respiratory surfaces (Lifson and McClintock 1996; Nagy 1980; Nagy and Costa 1980). Given that ambient air contains less than 0.03% CO_2, it is unlikely that any significant loss of labeled CO_2 occurred in incubating wandering albatrosses. However, the average humidity of the air during our study period was ~90%, so it is possible that water influx was overestimated due to the respiratory exchange with ambient air. Using the mean IMR of male wandering albatrosses determined with DLW (Table 1), we estimate MWP to be about 46 mL H_2O d^{-1} (1,773 kJ d^{-1} × 0.026 mL H_2O kJ^{-1}), which is 70% lower than measured WIR (78 mL H_2O d^{-1}). This means that 32 mL H_2O d^{-1} was unaccounted for in the water influx of these birds. Given that saturated air (90% humidity at 9°C) contains 5.3 mg H_2O L_{air}^{-1} (Weast 1983), it is possible to estimate the error associated with respiratory water exchange. Assuming a respiration quotient of 0.74 for fat and protein catabolism (Weathers et al. 2000) and an oxygen extraction efficiency of 5% (Berger and Hart 1974), we estimate that albatrosses breathe in approximately 2,000 L_{air} d^{-1}. Thus, the exchange of inspired unlabeled water would account for another 11 g H_2O d^{-1} ([2,000 L_{air} d^{-1} × 5.3 mg H_2O L_{air}^{-1}] ÷ 1,000). The remainder (21 mL H_2O d^{-1}) would have to come from an exogenous source because we know that birds do not leave their nests to eat or drink. Therefore, we suggest that birds consumed rainwater that collected on their beaks during the frequent rainstorms that occurred in January and February, 1999. Also, we commonly observed birds snapping at raindrops with their beaks, so rainwater was likely a potential source of water for fasting birds.

Table 2: Time energy budgets of breeding wandering albatrosses during the incubation period

		IMR					FMR		
			Brown and Adams (1984)		This Study		Shaffer et al. (2001)		
	Mass (kg)	Incubation (d)	DEE (kJ d^{-1})	TEE (MJ)	DEE (kJ d^{-1})	TEE (MJ)	Foraging (d)	DEE (kJ d^{-1})	TEE (MJ)
Male	10.63	39	3,242	126	1,773	69	39	4,858	189
Female	9.04	39	2,757	108	1,412	55	39	4,131	161
Total (per pair)				234		124			350
FMR/IMR				1.5		2.8			
IMR + FMR[a]				584		474			

Note. The total incubation period is approximately 78 d, and incubation duties are shared equally by each sex (Tickell 1968; Weimerskirch 1995). Incubation metabolic rate (IMR) determined by Brown and Adams (1984) was 305 kJ kg^{-1} d^{-1}. Field metabolic rate (FMR; 457 kJ kg^{-1} d^{-1}) was measured with doubly labeled water during the incubation period of 1999 at the same breeding colony as birds in this study (Shaffer et al. 2001). DEE = daily energy expenditure; TEE = total energy expenditure.

[a] Total cost for incubation period.

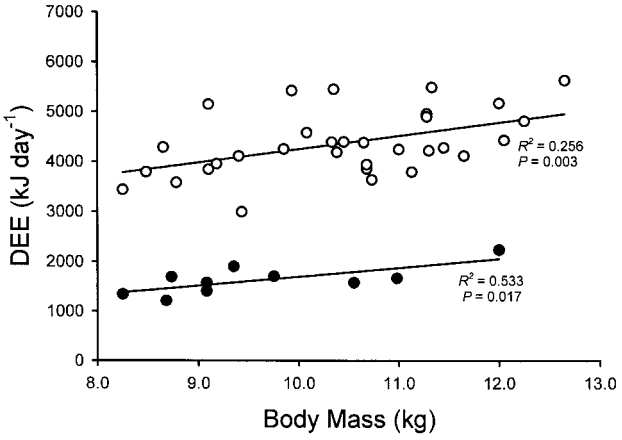

Figure 4. Comparison of daily energy expenditure (*DEE*) as a function of adult body mass for incubating (*closed circles*) and foraging (*open circles*) wandering albatrosses. DEE of incubating and foraging birds was measured using doubly labeled water during the austral summers of 1998 (foraging birds only) and 1999 at the same breeding colony (Shaffer et al. 2001). The slopes of the lines were not significantly different (ANCOVA, $F = 0.432$, df $= 1, 39$, $P = 0.515$), but the difference in cost between incubation and foraging (i.e., intercepts) was about 2.5 times (ANCOVA, $F = 140$, df $= 1, 40$, $P \ll 0.001$).

When using DLW, errors in CO_2 production and water flux can result from the physical fractionation of isotopes (Lifson and McClintock 1966; Nagy 1980; Nagy and Costa 1980; Speakman 1997). In this study, we used Equation (2) from Nagy (1980) to calculate CO_2 production from DLW, which accounts for changes in body mass (i.e., water space) rather than correcting for physical fractionation of isotopes. At present, there are no equations that correct for fractionation and changing water space simultaneously when using tritiated water (K. A. Nagy, personal communication). However, we tested whether fractionation caused an error in our estimates of IMR by calculating CO_2 production using Equations (36) of Lifson and McClintock (1966) and Equations (7.18) and (7.44) of Speakman (1997), which correct for physical fractionation. The largest difference in CO_2 production between all four equations was 6.5%, which was not statistically significant (ANOVA, $F = 0.51$, df $= 3, 36$, $P = 0.679$), suggesting that physical fractionation was not a significant source of error in our estimates of IMR from DLW.

Like DLW, open-flow respirometry is a more direct method of measuring oxygen consumption and/or CO_2 production (Speakman 1997). However, an important difference between our method using DLW to measure IMR compared to that of Brown and Adams (1984), which used respirometry, was the duration of the measurement interval. In this study, DLW measurements were carried out over 4–7 d while Brown and Adams (1984) measured IMR with respirometry for only 1–4 h. Therefore, our measurements of IMR would have included periods of sleep while that of Brown and Adams (1984) did not. Sleep is probably an important component of the energy budget of incubating birds because it allows adults to conserve energy during quiescent periods, particularly when incubation bouts extend over several days.

When calibrated with respirometry, HR also provides an indirect measure of metabolism that is sensitive to animal activity, environmental conditions, and stress (J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript). Similar to DLW, WIR, and mass loss, HR can be used to evaluate energy expenditure over long periods. Moreover, HRs of incubating birds produced estimates of energy expenditure (J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript) that were the most comparable to our measurements of IMR using DLW (Fig. 3). The fact that two independent methods produced similar results suggests that previous measurements of IMR (Croxall and Ricketts 1983; Brown and Adams 1984) overestimated the cost of incubation in wandering albatrosses.

Energy Budget of Incubation Period

The cost of incubation is a critical component of reproduction because the rate of development and overall hatching success of the egg(s) depend on the parents' ability to provide the necessary heat for embryonic development (King 1973; Drent 1975). In some species, incubation costs can make up a significant portion of the total energy budget of reproduction, particularly when the duration of the incubation period and/or individual incubation shifts are prolonged (e.g., pelagic seabirds; Whittow 1980, 1983). For wandering albatrosses, the incubation period lasts 78 d (range 75–82 d) and is approximately 22% of the total duration devoted to reproduction (Tickell 1968).

Given that wandering albatrosses have such long incubation periods, variations in the measurements of incubation costs could have a significant impact on estimates of time energy budgets. Previous estimates of the incubation energetics of wandering albatrosses relied on measurements of IMR from open-flow respirometry (Brown and Adams 1984) or mass loss (Croxall and Ricketts 1983). Our data obtained with DLW provides a significantly lower estimate IMR (Fig. 3). Therefore, we modeled the energy budget of a breeding pair of wandering albatrosses using IMR determined with DLW and respirometry to cover the most reasonable range of values. We estimate that the cost of incubating the egg for 78 d ranges from 124–234 MJ, for IMR determined with DLW and respirometry, respectively (Table 2). In addition, Shaffer et al. (2001) measured the field metabolic rates (FMR) of foraging wandering albatrosses using DLW in 1999. Thus, the integrated cost of the entire incubation period (incubation + foraging) is estimated to be 474–584 MJ depending on which value of IMR is used (Table

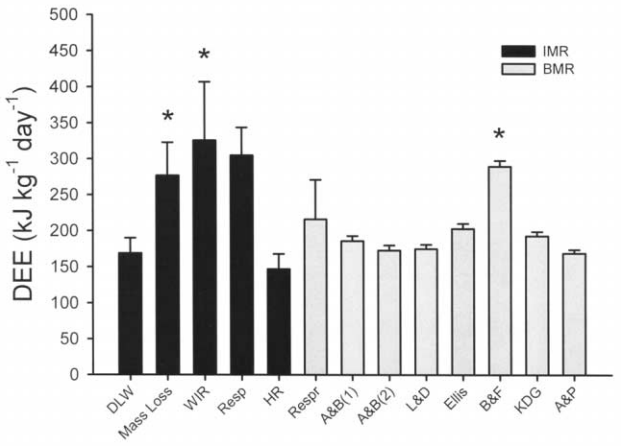

Figure 5. Daily energy expenditure (*DEE*) of incubating wandering albatrosses determined empirically with doubly labeled water (*DLW*), mass loss, water influx rate (*WIR*), respirometry (*Resp*; Brown and Adams 1984), and heart rate (*HR*; J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript). For simplicity of the comparisons, DEE from WIR was combined for both sexes (see Fig. 3). Incubation metabolic rates (*black columns*) were compared to basal metabolic rates (*gray columns*) of wandering albatrosses determined empirically with respirometry (*Respr*; Brown and Adams 1984) and by deriving predictive values using allometric equations and the body masses of all 10 birds in this study. Data for respirometry measurements (incubation and basal metabolic rate) and HR were not included in statistical comparisons because only the means ± SD were presented in the original papers (Brown and Adams 1984; J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript). For all methods (incubation and basal metabolic rates), DEE was statistically different between the means (ANOVA, $F = 37.8$, df $= 9, 89$, $P \ll 0.001$); however, pairwise multiple comparisons showed that three methods were statistically different from all the rest as denoted by the asterisks ($P < 0.001$; Tukey's HSD multiple comparisons). Allometric equations were obtained from the following studies: *A&B* (*1*) and *A&B* (*2*) from Adams and Brown (1984); *L&D* from Lasiewski and Dawson (1967); *Ellis* from Ellis (1984); *B&F* from Bryant and Furness (1995); *KDG* from Kendeigh et al. (1977); and *A&P* (α, rest phase) from Aschoff and Pohl (1970).

2). Moreover, the ratio of FMR to IMR changes from 1.5 (Brown and Adams 1984) to 2.8 (this study; Table 2). The new ratio is similar to that reported for Laysan albatrosses (2.7; Pettit et al. 1988) breeding in the Hawaiian Islands and grey-headed albatrosses breeding on South Georgia (2.3; Costa and Prince 1987). Finally, the difference in cost between incubation and foraging is also consistent across a wide range of body masses in wandering albatross (Fig. 4).

Comparison of Incubation and Basal Metabolism

In a review of seabird energetics, Grant (1984) noted that the cost of incubation varied from 0.82 to 2.2 × BMR for a diverse group of species. The variation largely depended on whether IMR was compared to measured or estimated BMR. Therefore, we compared the IMR of wandering albatrosses determined with DLW, mass loss, WIR, respirometry, and HR to measured BMR of wandering albatrosses (Brown and Adams 1984) and estimated BMR generated from seven different allometric equations. Overall, IMRs determined with DLW and HR (J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript) were similar to six different estimates of BMR and lower than measured BMR (Fig. 5). Considering the potential problems of stress related to the use of respirometry to measure metabolism, we are uncertain about the accuracy of measured BMR presented in Brown and Adams (1984). Furthermore, it is unclear whether birds were measured within their thermal neutral zones, a requirement of BMR. Although measurements were collected under ambient conditions in the field, there were no measurements collected over range of temperatures, thus the thermal neutral zone was not established. Consequently, the metabolic measurements presented in Brown and Adams (1984) may not represent true basal metabolism. Nonetheless, the cost of incubation for wandering albatrosses appears to be comparable to predicted BMR.

The preceding comparison suggests that the cost of incubation for wandering albatrosses is comparable to the cost of basal metabolism. If correct, then the thermal requirements of a bird incubating its egg are no different than that of a bird resting in the colony. This supports King's hypothesis (King 1973), which suggests that heat generated by the metabolism of the adult is sufficient to maintain egg temperature. This may be particularly relevant to pelagic seabirds, like albatrosses, which are large birds (2–10 kg) that lay a single egg weighing 5%–10% of body mass (Tickell 2000). Furthermore, albatrosses never leave the egg unattended so there is no additional cost to rewarm eggs.

In summary, we compared methods of measuring incubation energetics of wandering albatrosses and showed that significant differences exist between the various methods that have been employed. DLW produced the lowest and least variable estimate of energy expenditure, compared to metabolic rates estimated by measuring mass loss, water influx, or respirometry. The cost of incubation estimated by measuring changes in HR (when calibrated with respirometry; J. Weimerskirch, S. A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J. L. Rouanet, unpublished manuscript) also produces similar results to those determined using DLW. Hence, these two methods appear to provide the most accurate estimates of IMR in wandering albatrosses. In addition, the estimates of IMR from DLW and HR were similar to estimates of BMR derived from six different predictive equations, suggesting that wandering albatrosses are able to incubate their eggs without additional energy input above maintenance metabolism.

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