Applicability of the doubly labelled water method to the rhinoceros auklet, *Cerorhinca monocerata*

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Summary

The doubly labelled water (DLW) method is an isotope-based technique that is used to measure the metabolic rates of free-living animals. We validated the DLW method for measuring metabolic rates in five rhinoceros auklets (*Cerorhinca monocerata*) compared with simultaneous measurements using the respirometric method. We calculated the CO_2 production rate of four auklets (mean initial body mass: 552 g±36 s.d.) injected with DLW, using the one- and two-pool models. The metabolic rate during the 24-h measurements in a respirometric chamber for resting auklets averaged 16.30±1.66 kJ h^{-1} (n=4). The metabolic rates determined using the one- and two-pool models in the DLW method for the same period as the respirometric measurement averaged 16.61±2.13 kJ h^{-1} (n=4) and 16.16±2.10 kJ h^{-1} (n=4), respectively. The mean absolute percent error between the DLW and respirometric methods was 8.04% using the one-pool model and was slightly better than that with the two-pool model. The differences in value between the DLW and respirometric methods are probably due to oxygen isotope turnover, which eliminated only 10–14% of the initial enrichment excess.

Key words: Doubly labelled water method, Respirometric method, Validation, Rhinoceros auklet

Introduction

Measurement of energy expenditure and how it is allocated to specific activities are central to understanding animal energetics, which are related to the physiological, behavioural and evolutionary ecology of organisms (McNamara and Houston, 1996; Cuthill and Houston, 1997). Energy expenditure and allocation patterns are associated with various aspects of animal locomotion, life history and food requirements (McNeill Alexander and Goldspink, 1977; Trivers, 1985; Nagy, 1987). To gain a deeper understanding of energy use in wild animals, a great deal of time and effort has gone into developing methods to quantify energy expenditure in animals in both laboratory and field studies (Speakman, 1997).

In the laboratory, the most commonly used technique for studying the energetics of animals is to measure the oxygen consumption rate (V_{O_2}) in open-flow respirometric systems (Hill, 1972; Withers, 1977; Koteja, 1996), as the energy expenditure estimated from the V_{O_2} is generally accurate to about 0.5% (Gessaman and Nagy, 1988). However, it is not practical to apply this method to measuring the energy expenditure of free-living animals, as the subjects must be confined in a small chamber.

The doubly labelled water (DLW) method is one of the least-invasive field methods, and has been used for the last half century. This method allows estimation of the energy expended by subjects as they go about their normal activities (Lifson and McClintock, 1966; Speakman, 1997; Butler et al., 2004), and has been used to measure the field metabolic rate (FMR) of many free-living animals, especially flying and diving seabirds (Nagy et al., 1999; Shaffer, 2011). Theoretically, when water labelled with stable isotopes of oxygen and hydrogen (i.e., deuterium) (^{18}O and ^{2}H) is injected into a subject, the isotopes are eliminated gradually, mainly as CO_2 and H_2O. Since ^{2}H leaves as H_2O, while ^{18}O leaves as CO_2 and H_2O, it is possible to estimate the CO_2 production rate (rCO_2), which is an indicator of the metabolic rate, from the difference in the elimination rate of each isotope. The DLW method involves several assumptions when estimating metabolic rates (Lifson and McClintock, 1966; Speakman, 1997). As the assumptions depend on the physiological and physical characteristics of the species under investigation, the accuracy of the DLW method likely varies among species. Therefore, it is important to conduct precise validation studies in various species.

Although many studies have measured the FMR in seabird families using the DLW method (summarised by Ellis and Gabrielsen, 2002; Shaffer, 2011), to our knowledge, only one validation study of the DLW method for adult seabirds has been published to date (Gales, 1989); the difference between the food intake and DLW methods was small (+1.75%, range −5.2% to +12.5%) in the little blue penguin, *Eudyptula minor* (Gales, 1989). However, there have been no previous validations of the DLW method by comparison with the respirometric method in adult seabirds. Therefore, it is still necessary to examine the validity of the DLW method for estimating the metabolic rates of seabird species, which have different body sizes, life histories and physiological/biochemical adaptations.

Given the lack of knowledge regarding the application of the DLW method to seabirds, we compared the energy expenditure of the rhinoceros auklet, *Cerorhinca monocerata*, measured using the open flow respirometric method with that obtained simultaneously using the DLW method.

Materials and Methods

Study site and field work

Our study was conducted on Teuri Island (44°25′N, 141°19′E), Hokkaido, Japan, from 21 June to 3 July 2010. All experiments followed a protocol approved by the Institutional Animal Care and Use Committee of Nagoya University. Ten rhinoceros auklets were captured at night to measure their metabolic rate using both the DLW and respirometric methods and to determine their natural background isotope abundance.

The birds were held in darkened boxes and transported to the laboratory on Teuri Island. After carefully elevating the abdominal skin to avoid injection into the air sacs, five auklets were injected intraperitoneally with DLW containing 10.3 atom-percent ^{18}O (Taiyo Nippon Sanso, Shinagawa City, Tokyo, Japan), 4.0 atom-percent ^{2}H (Isotech, Miamisburg, OH, USA) and 0.9% NaCl. To quantify the injected dose, the syringe was weighed before and after injection on an electrical balance (Mettler-Toledo, Columbus, OH, USA) to the nearest 0.1 mg. After the injection, the bird was placed in a plastic box for 1 h to allow the injected dose to equilibrate (Degen et al., 1981; Williams and Nagy, 1984; Król and Speakman, 1999). Then, 1 mL of blood was taken from the brachial or tarsus vein of the bird (initial sample), the initial body mass was measured to the nearest 5 g using a spring balance (Pesola, Baar, Switzerland), and the bird was placed in a metabolic chamber (see below). To reduce the error caused by circadian metabolic rhythm, measurement period was adopted as 24 h (Speakman and Racey, 1988). Twenty-four hours after DLW injection, the bird was removed from the chamber, weighed immediately (final body mass), and 1 mL of blood was sampled from the brachial or tarsus vein (final sample). The bill depth and head length of each auklet were measured using Vernier callipers (±0.05 mm) to determine their sex from the discriminant score (Niizuma et al., 1999).

Each blood sample was put into a heparinised tube (Nipro Neo-Tube, NT-HE 1000; Nipro, Osaka City, Osaka, Japan) and centrifuged immediately (12 min, 3000 rpm). The serum was then transferred to a 0.5-mL plastic screw-cap vial with O-rings (AGC Techno Glass, Funabashi City, Chiba, Japan) and frozen at −25°C until isotope ratio analysis.

Isotope ratio analysis

The ^{2}H and ^{18}O isotope concentrations of the serum and DLW dose samples were analyzed according to the procedure of Shirai et al. using isotope ratio mass spectrometry (IRMS; Hydra 20-20, Sercon, Crewe, UK) (Shirai et al., 2012; Perks et al., 2000; Yamada et al., 2009). To estimate the enrichment of the dose sample, 0.00207 g of the dose sample was diluted with 0.99059 g of distilled water before analysis (Speakman, 1997). The serum samples were also diluted six-fold with distilled water by measuring with an electrical balance (Mettler-Toledo) to the nearest 0.01 mg. The enrichment of distilled water was measured using IRMS, as with the diluted serum and dose samples.

The distilled water, diluted serum and dose samples were put into cylindrical tubes and analysed using the water equilibration method (Horita et al., 1989). Water standards (IA-R501, $\delta^{2}H$=+578.53‰; IA-R502, $\delta^{2}H$=+1113.56‰; IA-R503, $\delta^{18}O$=+107.29‰; IA-R504, $\delta^{18}O$=+267.67‰; and IA-R505, $\delta^{2}H$=−66.9‰, $\delta^{18}O$=−9.45‰, relative to Vienna Standard Mean Ocean Water (VSMOW); Iso-Analytical, Crewe, UK) were used to establish calibration curves for normalising the values. Calibration curves were run after every 12 samples. First, for ^{18}O analysis, the cylindrical tubes were filled with CO_2 gas and kept at 25°C for a minimum of 8 h to allow the exchange of ^{18}O between the sample and CO_2 gas. Then, the exchanged CO_2 gas was analysed using IRMS. After analysing the ^{18}O, the cylindrical tubes were put into mini-vials with a Pt catalyst for ^{2}H analysis (Herd et al., 2000). The cylindrical tubes were filled with hydrogen gas and kept at 25°C for at least 36 h to allow exchange between the ^{2}H sample and hydrogen gas. Then, the exchanged hydrogen gas was analysed using IRMS. Each sample was analyzed in duplicate.

Calculations in the DLW method

The plateau and intercept methods was used to determine the isotope dilution spaces for ^{2}H (N_d, mol) and ^{18}O (N_o, mol), and to estimate total body water, respectively (Speakman, 1997). N_d and N_o were calculated using the general equations:

$$N_d = \frac{H_{inj} \times (H_i - H_d)}{H_b - H_i}$$

$$N_o = \frac{O_{inj} \times (O_i - O_d)}{O_b - O_i}$$

where H_{inj} and O_{inj} represent the respective DLW dose (^{2}H or ^{18}O, mol), H_d and O_d represent the isotope concentrations (^{2}H or ^{18}O, ppm) in the DLW dose, and H_b, H_i, O_b and O_i represent the isotope concentrations (^{2}H or ^{18}O, ppm) of the background and initial samples, respectively. For each bird, the dilution space ratio ($R_{dilspace}$, dimensionless) was calculated by dividing the total body water value obtained from ^{2}H dilution by the value obtained from ^{18}O dilution (Speakman, 1997). When the initial isotope enrichment of the body water (H_d or O_d, ppm) was calculated by the intercept method, we used the following equation (Król and Speakman, 1999):

$$H_d = anti\ln(\ln(H_{init} - H_b) + k_d) + H_b$$

$$O_d = anti\ln(\ln(O_{init} - O_b) + k_o) + O_b$$

where O_{init} and H_{init} are the initial isotope enrichments of body water pool (^{2}H or ^{18}O, ppm); k_d and k_o are the isotope turnover rate between the initial and final samples (h^{-1}, see below). The turnover rates for ^{2}H and ^{18}O (k_d and k_o, respectively, day^{-1}) were determined using the two-sample technique and calculated as follows:

$$k_d = \frac{\ln(H_i - H_b) - \ln(H_f - H_b)}{t}$$

$$k_o = \frac{\ln(O_i - O_b) - \ln(O_f - O_b)}{t}$$

where H_f and O_f represent the respective isotope concentrations (^{2}H or ^{18}O, ppm) of the final samples and t represents the time interval between the initial and final samples (days) (Lifson and McClintock, 1966; Speakman, 1997). Ideally, background isotope levels should be determined for each animal before injection with labelled water (Speakman and Racey, 1987). However, this increases both the handling time and disturbance to the animal. Tatner determined that use of background levels from uninjected birds did not affect the estimated metabolic rate (Tatner, 1990). As in other seabird studies (e.g., Adams et al., 1986; Birt-Friesen et al., 1989; Hodum et al., 1998; Visser et al., 2000), we determined the natural background isotope abundances in five uninjected adult auklets. The background isotope level averaged 1994.55 ppm (range 1993.89–1995.12 ppm) for ^{18}O and 148.15 ppm (range 145.86–149.90 ppm) for ^{2}H. We used these mean background levels to calculate the CO_2 production rate (rCO_2, mL day^{-1}).

There are two main models for calculating the rCO_2, i.e., the one- and two-pool models. Compared with the oxygen isotope, the injected hydrogen isotope exchanges reversibly with hydrogen on the exposed amino groups of proteins (Culebras and Moore, 1977; Matthews and Gilker, 1995). The estimated body water pool based on the hydrogen isotope dilution is 3–4% greater than the oxygen space because of the reversible exchange (Schoeller et al., 1986; Speakman et al., 1993). There are two ways to address this problem: 1) to ignore the discrepancy and use the oxygen dilution space as a true estimate of the body water pool (one-pool model) and 2) to modify the equation so that each turnover is expressed relative to its own dilution space (two-pool model). For the rhinoceros auklet, rCO_2 was computed using both the one- and two-pool models of Speakman to evaluate the applicability of the rCO_2 estimations (Speakman, 1997). The equations are as follows:

Speakman's one-pool method (Speakman, 1997) (equation 7.17):

$$rCO_2 = \frac{N}{2.078}(k_o - k_d) - 0.0062 \times k_d \times N$$

Speakman's two-pool method (Speakman, 1997) (equation 7.43):

$$rCO_2 = \frac{N}{2.078}(k_o - R_{dilspace} \times k_d) - 0.0062 \times N \times R_{dilspace} \times k_d$$

A recent study found that body water pool derived ^{18}O using the plateau method has the highest correlation with actual amount of body water pool (Jacobs et al., 2012). Therefore, in our study, N_o and $R_{dilspace}$ determined by the plateau method were used for the calculation of metabolic rates using the one- and two-pool models. To convert units in $mLCO_2\ day^{-1}$ into energy equivalents, we assumed that 1 mL of CO_2=25.11 J (Gessaman and Nagy, 1988). The water efflux (rH_2O, mL day^{-1}) is equal to the sum of the water loss from respiration, skin and excreta, and was computed using the turnover rate of ^{2}H from the equation of Bevan et al. (Bevan et al., 1995) (based on Nagy and Costa, 1980) as follows:

$$rH_2O = \frac{(N_f - N_i) \times \ln[(H_i \times N_i)/(H_f \times N_f)]}{\ln(N_f/N_i) \times t}$$

where N_i represents the initial body water pool and is assumed to be N_o determined by the plateau method, and N_f represents the final body water pool and is inferred from the final body mass, assuming the same percentage of body mass as measured for the initial body water pool.

Respirometric method

The accuracy of the DLW method was evaluated by comparing the estimates of the metabolic rates of adult rhinoceros auklets with the concurrent direct respirometric method for the oxygen consumption rate (V_{O_2}). V_{O_2} was measured using an open-flow respirometric system consisting of a 20-L acrylic metabolic chamber and an oxygen analyser (Xentra 4100; Servomex, Crowborough, UK). The accuracy of the oxygen analyser was better than 0.02% over the entire (0–100%) range of oxygen concentration. The metabolic chamber was submerged in a thermostatic water bath and maintained at 22.3℃±1.5℃ (mean±s.d.), which was assumed to be within the thermoneutral zone of rhinoceros auklets. Based on the equation given by Ellis and Gabrielsen, we assumed their lower critical temperature was 15℃ (Ellis and Gabrielsen, 2002). The chamber temperature (*Tc*) and atmospheric pressure (*Pa*) were recorded using loggers (*Tc*: ±0.7℃, Thermochron Type-SL; KN Laboratories, Ibaraki City, Osaka, Japan; *Pa*: ±1.5 hPa, TR-73U Thermo Recorder; T&D Corp., Matsumoto City, Nagano, Japan) every 1 minute. The flow rate (V_E) of the chamber was fixed at 2.0 L min^{-1} using a mass flow controller (±2%, Type HM1171A; Tokyo Keiso, Minato City, Tokyo, Japan). The effluent air was dried over silica gel and a fraction of the dry effluent air was directed into the oxygen analyser. The oxygen analyser was calibrated using dry outside air (set to 20.946% oxygen) and pure stock nitrogen (set to 0.000% oxygen). The oxygen concentration in the effluent air (F_{EO2}) was read by a computer every minute. V_{O_2} was calculated using formula 3A presented by Withers as follows (Withers, 1977):

$$V_{O_2} = \frac{V_E \times (F_{IO2} - F_{EO2})}{1 - (1 - RQ) \times F_{IO2}}$$

We assumed RQ=0.8, which minimises error in the estimated rate of energy expenditure when RQ is unknown (Koteja, 1996), and that the oxygen concentration of influent air (F_{IO2}) was 20.946%. The body mass of the rhinoceros auklets was assumed to decrease linearly from the initial to the final body mass (see above). In calculating the energy expenditure from V_{O_2}, a conversion coefficient of 20.1 kJ L^{-1} was used (Schmidt-Nielsen, 1997). Each bird's energy expenditure recorded every one minute was converted to the mean metabolic rate per hour to allow comparison with energy expenditures measured using the DLW method. All results are given at standard temperature and pressure for dry gas (STPD).

Data analysis

We used the mean arithmetic percent error and mean absolute percent error to evaluate the accuracy of the DLW method (Gessaman et al., 2004). The absolute percent error is independent of sign, and is consequently unaffected by errors of the opposite sign, which cancel each other in the arithmetic mean. All mean values are presented ±s.d.

The initial enrichments of both ^{18}O and ^{2}H in one auklet (no. 3; see Table 1) were much lower than for the other birds, although this auklet had a body mass similar to the others. This probably indicated a failure of injection caused by the injected DLW dose leaking from the needle puncture or the needle going through the skin of the bird. Therefore, we eliminated the data for the DLW method for this bird.

Results

The initial body mass and mass change during the metabolic measurements of rhinoceros auklets was 552±36 g (n=4) and 32.7±9.8 g day^{-1} (n=4), respectively (Table 1). Using the DLW method, the one- and two-pool models gave metabolic rates of 16.61±2.13 (n=4) and 16.16±2.10 kJ h^{-1} (n=4), respectively, during the 24-h measurement period (Table 1). The difference between body water pools derived from the plateau and intercept methods was only 0.1–0.3% (Table 1). The dilution space ratio ($R_{dilspace}$) determined by the plateau method ranged between 1.026 and 1.043 (n=4, Table 1), which was within the range found for other taxa (Speakman, 1997). The rates of water efflux varied among the birds with a mean of 34.10±6.15 (range 27.38–42.29) mL H_2O day^{-1} (n=4) (Table 1). The auklets eliminated 11–14% of the initial enrichment excess of the oxygen isotope.

Energy expenditure in the auklets was determined to be 16.30±1.66 kJ h^{-1} (n=4) during the 24-h measurement period when using the respirometric method (Table 1). Using the one-pool model, the mean arithmetic and absolute percent errors in the DLW method were +2.14% and 8.04%, respectively, while the respective values using the two-pool model were −0.62% and 8.35%.

Discussion

In estimating the body water pool, the hydrogen isotope dilution space (N_d) exceeded the oxygen isotope dilution space (N_o) by 3–4% (see Materials and Methods). Therefore, the dilution space ratio ($R_{dilspace}$) should theoretically vary between 1.01 and 1.06 (Culebras and Moore, 1977; Matthews and Gilker, 1995). We confirmed that $R_{dilspace}$ determined by the plateau method ranged between 1.026 and 1.043 in our data, which was within the expected range; therefore, the analytical error in our results was negligible (Speakman, 1997).

The choice between the one- and two-pool models depends on the amount of hydrogen in the subjects, which in practice is related to body size (Speakman, 1987). The one-pool model assumes that there is no subsidiary flux of hydrogen (e.g. fat synthesis) while the two-pool model assumes the magnitude of any subsidiary hydrogen flux relative to the total hydrogen turnover is the same as $R_{dilspace}$ (Speakman, 1987). Since measures of deuterium incorporation in fat synthesis indicate a greater incorporation in small animal (Speakman, 1993), the one-pool model is thought to more appropriate for small birds (<1 kg (Speakman, 1997); <4 kg (Butler et al., 2004)). Three of five studies on birds that evaluated the relative accuracy of using one- and two-pool models to compute rCO_2 indicated that the one-pool model is more appropriate for birds weighing <1 kg (Nolet et al., 1992; Dykstra et al., 1997; Visser and Schekkerman, 1999; Visser et al., 2000; Gessaman et al., 2004). Although our results showed that the one-pool model is more appropriate than the two-pool model for birds weighing <1 kg, there was little difference in the degree of error between the models (Table 1). The "swap-over point" in performance between the one- and two-pool model equations may not be as clear-cut (Speakman, 1997). To investigate the importance of one- or two-pool models, it is necessary to obtain more information and to compare species with different body masses.

The mean arithmetic and absolute percent error of the DLW method for estimating the metabolic rates in rhinoceros auklets were +2.14% and 8.04%, respectively, when the one-pool model was used. The discrepancies between the values measured using the DLW and respirometric methods may be explained by the effects of a high water efflux rate, as previous studies suggested that a high water efflux rate influences the accuracy of the DLW method (Bevan et al., 1995; Jones et al., 2009). The k_d/k_o ratio represents the proportion of the oxygen turnover that is linked to the hydrogen turnover and indicates the magnitude of the water efflux rate (Speakman, 1997). With a higher water efflux rate, the difference in isotope turnover rate of hydrogen and oxygen is small (i.e., k_d/k_o ratios close to 1.0), decreasing the accuracy of the DLW method (Jones et al., 2009). However, the k_d/k_o ratio of the rhinoceros auklets was 0.428±0.067, and the mean water efflux rate of the auklets was 51% below the level (69.9 mL day^{-1}) predicted for captive birds based on the allometric

Table 1. Body masses, background isotope levels, dose details, isotope dilution spaces, turnover rates and ratios, metabolic rates, and water efflux rates for five rhinoceros auklets used in the DLW validation.

Rhinoceros auklet	No. 1	No. 2	No. 3	No. 4	No. 5	Mean	s.d.
Sex	Female	Male	Female	Male	Male		
Initial body mass (g)	510	562	(495)	595	541	552	36
Final body mass (g)	481	540	(456)	550	505	519	32
Mass change (g day^{-1})	28.5	21.8	(38.5)	44.6	35.8	32.7	9.8
Measurement period (h)	24.43	24.23	(24.32)	24.20	24.15		
Initial injectate (moles)	0.157	0.161		0.158	0.158		
Injectate enrichment							
^{2}H (ppm)	39,858.99	39,858.99	(39,858.99)	39,858.99	39,858.99		
^{18}O (ppm)	102,693.55	102,693.55	(102,693.55)	102,693.55	102,693.55		
Initial enrichment							
^{2}H (ppm)	493.13	448.68	(298.98)	428.13	460.53	457.62	27.20
^{18}O (ppm)	2881.59	2777.75	(2382.41)	2723.19	2811.28	2798.45	66.26
Final enrichment							
^{2}H (ppm)	468.55	432.71		413.46	446.45	440.29	23.19
^{18}O (ppm)	2761.73	2680.86		2639.53	2709.49	2697.90	51.34
Body water pool – plateau method							
N_d (moles)	17.92	21.11		22.25	19.93	20.30	1.85
N_o (moles)	17.67	20.54		21.68	19.32	19.80	1.72
% TBW (^{18}O)	62.42	65.86		65.65	64.36	64.57	1.58
Dilution ratio ($R_{dilspace}$)	1.026	1.040		1.038	1.043	1.037	0.007
Body water pool – intercept method							
N_d (moles)	17.86	21.06		22.20	19.89	20.25	1.85
N_o (moles)	17.61	20.49		21.63	19.29	19.75	1.72
% TBW (^{18}O)	62.23	65.71		65.51	64.24	64.42	1.60
Dilution ratio ($R_{dilspace}$)	1.014	1.028		1.026	1.031	1.025	0.008
k_d (day^{-1})	0.0726	0.0541		0.0534	0.0458	0.0565	0.0114
k_o (day^{-1})	0.1426	0.1308		0.1210	0.1323	0.1317	0.0089
k_d:k_o	0.509	0.414		0.441	0.346	0.428	0.067
Metabolic rate – one-pool model (kJ h^{-1})	13.76	17.61		16.35	18.72	16.61	2.13
% difference with respirometry	−2.96	2.19		−8.83	18.17	2.14	11.59
Metabolic rate – two-pool model (kJ h^{-1})	13.39	17.10		15.85	18.29	16.16	2.10
% difference with respirometry	−5.60	−0.73		−11.60	15.45	−0.62	11.60
Water efflux rate (mL day^{-1})	33.31	27.38		42.29	33.41	34.10	6.15
Metabolic rate – respirometry (kJ h^{-1})	14.18	17.23	(19.00)	17.93	15.84	16.30	1.66

The data for auklet no. 3 were removed from the mean and s.d. because the DLW dose was likely injected incorrectly. For the calculation of metabolic rate using the DLW method, body water pool derived ^{18}O using the plateau method was used.

equation of Nagy and Peterson (Nagy and Peterson, 1988). This means that rhinoceros auklets have a greater oxygen turnover rate relative to hydrogen, corresponding to an enormous rCO_2 relative to the water efflux rate. This result supports the suggestion that water efflux is unlikely to be an issue for seabirds because the metabolic rates are sufficiently higher than the water efflux rates (Shaffer, 2011). Therefore, the water efflux rates may have little influence on the errors observed in this study.

As an alternative possibility, a previous study suggested that the magnitude of the reduction in blood oxygen isotope levels between injection and sampling likely influences the accuracy of the DLW method (Gales, 1989). The auklets in our experiment eliminated only 11–14% of the initial enrichment excess of the oxygen isotope. This is probably because the subjects were inactive and the experiment was relatively short (24 h). A validation study showed that the duration of measurement with the DLW method (i.e., the amount of oxygen isotope eliminated) significantly influences the absolute percent error of rCO_2, and a relatively long measurement period has greater accuracy (Gessaman et al., 2004). Therefore, low oxygen isotope elimination may lead to the differences between the methods seen in this study.

We found that the one-pool model in the DLW method was more accurate for rhinoceros auklets, and the 8.04% error observed here was within the level of accuracy reported by Speakman (Speakman, 1997). We concluded that the DLW method can yield reasonable estimates of CO_2 production and metabolism in rhinoceros auklets under laboratory conditions.

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Competing Interests

The authors have no competing interests to declare.

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