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

Masaki Shirai1,*, Motohiro Ito2, Ken Yoda1 and Yasuaki Niizuma3

1Graduate School of Environmental Studies, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan
2National Institute of Polar Research, 10-3 Midori-cho, Tachikawa, Tokyo 190-8518, Japan
3Faculty of Agriculture, Meijo University, 1-501, Shiogama-ku, Tenpaku-ku, Nagoya 464-8502, Japan

*Author for correspondence (shirai.masaki@h.mbox.nagoya-u.ac.jp)

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 CO2 production rate of four auklets (mean initial body mass: 552±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.

© 2012. Published by The Company of Biologists Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial Share Alike License (http://creativecommons.org/licenses/by-nc-sa/3.0).

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 O2) in open-flow respirometric systems (Hill, 1972; Withers, 1977; Koteja, 1996), as the energy expenditure estimated from the VO2 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) (¹⁸O and ²H) is injected into a subject, the isotopes are eliminated gradually, mainly as CO₂ and H₂O. Since ²H leaves as H₂O, while ¹⁸O leaves as CO₂ and H₂O, it is possible to estimate the CO₂ production rate (rCO₂), 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 18O (Tayo Nippon Sanso, Shinagawa City, Tokyo, Japan), 4.0 atom-percent 2H (Isetech, Miamisburg, OH, USA) and 0.9% NaCl. To quantify the injected dose, the syringe was weighed before and after injection on an electrical balance (Pesola, Baar, Switzerland), and the bird was placed in a metabolic chamber (see below). To reduce the error caused by circadian metabolic rhythm, the measurement period was adopted as 24 h (Speakman and Racey, 1988). Twenty-four hour 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 vein (final sample). The bill depth and head length of each auklet were measured immediately (final body mass), and 1 mL of blood was sampled from the brachial vein (final sample). The bill depth and head length of each auklet were measured immediately (final body mass), and 1 mL of blood was sampled from the brachial vein (final sample). To correct the error caused by circadian metabolic rhythm, measurement period was adopted as 24 h (Speakman and Racey, 1988). Twenty-four hour 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 vein (final sample). The bill depth and head length of each auklet were measured immediately (final body mass), and 1 mL of blood was sampled from the brachial vein (final sample).

Isotope ratio analysis
The 2H and 18O 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., 2008; Yamada et al., 2009). To estimate the enrichment of the dose sample, 0.0028 kg 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 background isotope abundance. For each bird, the dilution space ratio (Rdilspace, dimensionless) was calculated by dividing the total body water value obtained from 2H dilution by the value obtained from 18O dilution (Speakman, 1997). When the initial isotope enrichment of the body water (Hb or Oa, ppm) was calculated by the intercept method, we used the following equation (Krol and Speakman, 1999):

\[ H_b = \text{anti} \ln \left( \frac{\ln(H_{\text{final}} - H_b)}{\ln(H_{\text{initial}} - H_b)} \right) + k_d + H_b \]

\[ O_a = \text{anti} \ln \left( \frac{\ln(O_{\text{final}} - O_a)}{\ln(O_{\text{initial}} - O_a)} \right) + k_d + O_a \]

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

\[ k_d = \frac{\ln(H_{\text{final}} - H_b) - \ln(H_{\text{initial}} - H_b)}{t} \]

\[ k_o = \frac{\ln(O_{\text{final}} - O_a) - \ln(O_{\text{initial}} - O_a)}{t} \]

where \( H_b \) and \( O_a \) represent the respective isotope concentrations (2H or 18O, ppm) of the final samples and \( t \) represents the time interval between the initial and final samples (days). To quantify the oxygen isotope dilution is 3–4% greater than the oxygen 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 18O and 148.15 ppm (range 145.86–149.90 ppm) for 2H. We used these mean background levels to calculate the CO2 production rate (rCO2, mL day\(^{-1}\)).

There are two main models for calculating the rCO2, 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 (Schloer 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, rCO2 was computed using both the one- and two-pool models of Speakman to evaluate the applicability of the rCO2 estimations (Speakman, 1997). The equations are as follows:

Speakman’s one-pool model (Speakman, 1997) (equation 7.17):

\[ r_{\text{CO2}} = \frac{N}{2 \times 0.078} \times (k_d - k_o) - 0.0062 \times k_d \times N \]

Speakman’s two-pool model (Speakman, 1997) (equation 7.43):

\[ r_{\text{CO2}} = \frac{N}{2 \times 0.078} \times (k_d - R_{\text{dilspace}} \times k_o) - 0.0062 \times N \times R_{\text{dilspace}} \times k_d \]

A recent study found that body water pool derived 18O using the plateau method has the highest correlation with actual amount of body water pool (Jacobs et al., 2012). Therefore, in our study, \( N \) and \( R_{\text{dilspace}} \) determined by the plateau method were used for the calculation of metabolic rates using the one- and two-pool models. To convert units in mLCO2 day\(^{-1}\) into energy equivalents, we assumed that 1 mL of CO2 = 25.11 J (Gessaman and Nagy, 1988). The water efflux (rH2O, 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 2H from the equation of Bevan et al. (Bevan et al., 1995) (based on Nagy and Costa, 1980) as follows:

Validation of DLW method 1142
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 (\( VO_2 \)). \( VO_2 \) was measured using an open-flow respirometric system consisting of a 20-L acrylic metabolic chamber and an oxygen analyser (XenAir 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°C ±1.5°C (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°C (Ellis and Gabrielsen, 2002). The chamber temperature (\( T_c \)) and atmospheric pressure (\( P_a \)) were recorded using loggers (\( T_c \): ±0.7°C, Thermochron Type-SL; KN Laboratories, Ibaraki City, Osaka, Japan; \( P_a \): ±1.5 hPa, TR-73U Thermo Recorder; T&D Corp., Matsumoto City, Nagano, Japan) every 1 minute. The flow rate (\( F_i \)) 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 dired 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 (\( F_{EO_2} \)) was read by a computer every minute. \( VO_2 \) was calculated using formula 3A presented by Withers as follows (Withers, 1977):

\[
VO_2 = \frac{V, \times (F_{O_2} - F_{O_2})}{1 - (1 - RQ) \times F_{O_2}}
\]

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 (\( F_{iO_2} \)) 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 \( VO_2 \), a conversion coefficient of 20.1 kJ L\(^{-1}\) day\(^{-1}\) (\( ko \)) was used. The discrepancies between the values measured using the DLW method were +2.14% and 8.04%, respectively, when the one-pool model and two-pool models to compute \( rCO_2 \) indicated that the one-pool model was more appropriate for birds weighing <1 kg (Nolet et al., 1992; Dykstra et al., 1997; Visser and Scheekerman, 1999; Visser et al., 2000; Gessaman et al., 2004). Although our results showed that the one-pool model was 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 \( k/d\) 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., \( k/d\) ratios close to 1.0), decreasing the accuracy of the DLW method (Jones et al., 2009). However, the \( k/d\) 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
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 rCO₂ 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 (Speakman, 1997). We concluded that the DLW method can yield reasonable estimates of CO₂ production and metabolism in rhinoceros auklets under laboratory conditions.

Acknowledgements
We thank Yuuya Suzuki and Megumi Shikata for their assistance in the field, Yutaka Watanuki for his valuable suggestions and help, and Naoki Tomita for help with the isotope analysis. This work was conducted with the support of the Meijo University AGRIOMICS project.

Competing Interests
The authors have no competing interests to declare.

References
Adams, N. J., Brown, C. R. and Nagy, K. A. (1986). Energy expenditure of free-ranging Wandering Albatrosses Diomedea exulans. Physiol. Zool. 59, 583-591.
Bevan, R., Speakman, J. R. and Butler, P. J. (1995). Daily energy expenditure of tufted ducks - a comparison between indirect calorimetry, doubly labelled water and heart rate. Funct. Ecol. 9, 40-47.
Birt-Friesen, V. L., Montevcechi, W. A., Cairns, D. K. and Macko, S. A. (1989). Activity-specific metabolic rates of free-living Northern Gannets and other seabirds. Ecology 70, 357-367.
Butler, P. J., Green, J. A., Boyd, I. L. and Speakman, J. R. (2004). Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods. Funct. Ecol. 18, 168-183.
Culebras, J. M. and Moore, F. D. (1977). Total body water and the exchangeable hydrogen. I. Theoretical calculation of nonaqueous exchangeable hydrogen in man. Am. J. Physiol. 232, R54-R59.

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 |      |      |
| Injectate enrichment | 0.157 | 0.161 |       | 0.158 | 0.158 |      |      |
| Injectate (moles) | 17.61 | 20.49 | 21.63 | 19.29 | 19.75 | 0.067 |      |
| Initial injectate (moles) | 0.1426 | 0.1308 | 0.1210 | 0.1323 | 0.1317 | 0.0089 |      |
| Final injectate (moles) | 17.92 | 21.11 | 22.25 | 19.93 | 20.30 | 1.85 |      |
| Initial enrichment | 39,858.99 | 39,858.99 | (39,858.99) | 39,858.99 | 39,858.99 |      |      |
| ²H (ppm) | 102,693.55 | 102,693.55 | (102,693.55) | 102,693.55 | 102,693.55 |      |      |
| ¹⁸O (ppm) | 102,693.55 | 102,693.55 | (102,693.55) | 102,693.55 | 102,693.55 |      |      |
| Dilution ratio (Rdilspace) | 1.014 | 1.026 | 1.038 | 1.043 | 1.037 | 0.007 |      |
| % TBW (¹⁸O) | 62.42 | 65.86 | 65.65 | 64.36 | 64.57 | 1.58 |      |
| % TBW (²H) | 17.86 | 21.04 | 21.73 | 20.49 | 20.25 | 1.85 |      |
| Final enrichment | 468.55 | 432.71 |        | 413.46 | 446.45 | 440.29 | 23.19 |
| Body water pool – intercept method |       |      |       |      |      |      |      |
| N₀ (mole) | 17.92 | 20.54 | 21.68 | 19.32 | 19.80 | 1.72 |      |
| N₀ (mole) | 17.67 | 21.11 | 22.20 | 19.93 | 20.30 | 1.85 |      |
| % TBW (¹⁸O) | 62.23 | 65.86 | 65.65 | 64.36 | 64.57 | 1.58 |      |
| Dilution ratio (Rdilspace) | 1.014 | 1.026 | 1.026 | 1.031 | 1.025 | 0.008 |      |
| kₒ (day⁻¹) | 0.0726 | 0.0541 | 0.0534 | 0.0458 | 0.0565 | 0.0114 |      |
| kₒ (day⁻¹) | 0.0726 | 0.0541 | 0.0534 | 0.0458 | 0.0565 | 0.0114 |      |
| kₒ (day⁻¹) | 0.0726 | 0.0541 | 0.0534 | 0.0458 | 0.0565 | 0.0114 |      |
| Water efflux (mL day⁻¹) | 33.31 | 27.38 | 42.29 | 33.41 | 34.10 | 6.15 |      |
| Water efflux (mL day⁻¹) | 33.31 | 27.38 | 42.29 | 33.41 | 34.10 | 6.15 |      |
| Water efflux (mL day⁻¹) | 33.31 | 27.38 | 42.29 | 33.41 | 34.10 | 6.15 |      |

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 ¹⁸O using the plateau method was used.

The authors have no competing interests to declare.
Cuthill, I. C. and Houston, A. I. (1997). Managing time and energy. In Behavioural Ecology: An Evolutionary Approach, 4th edition (ed. J. R. Krebs and N. B. Davies), pp. 19-41. Cambridge, MA: Blackwell Science.

Degen, A. A., Pinshow, B., Alkon, P. U. and Arnon, H. (1981). Tritiated water for estimating total body water and water turnover rate in birds. J. Appl. Physiol. 51, 1185-1188.

Dykstra, C. R., Meyer, M. W. and Karasov, W. H. (1997). Validation of the doubly labeled water method in bald eagles (Haliaetus leucocephalus) and a comparison of two equations for the calculation of energy expenditure. Physiol. Zool. 70, 19-26.

Ellis, H. L. and Gabrielsen, G. W. (2002). Energies of free-ranging seabirds. In Biology Of Marine Birds (ed. E. A. Schreiber and J. Burger), pp. 359-407. Boca Raton, FL: CRC Press.

Gales, R. (1989). Validation of the use of tritiated water, doubly labeled water, and 32Na for estimating food, energy, and water intake in little penguins, Eudyptula minor. Physiol. Zool. 62, 147-169.

Gessaman, J. A. and Nagy, K. A. (1988). Energy metabolism: errors in gas-exchange conversion factors. Physiol. Zool. 61, 307-313.

Gessaman, J. A., Newgrain, K. and Green, B. (2004). Validation of the doubly-labeled water (DLW) method for estimating CO2 production and water flux in growing poultry chicks. J. Avian Biol. 35, 71-96.

Herd, S. L., Vaughn, W. H. and Goran, M. I. (2000). Comparison of zinc reduction with platinum reduction for analysis of deuterium-enriched water samples for the doubly labeled water technique. Obes. Res. 8, 302-308.

Hill, R. W. (1972). Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. J. Appl. Physiol. 33, 261-263.

Hodum, P. J., Sydeman, W. J., Visser, G. H. and Weathers, W. W. (1998). Energy expenditure and food requirement of Cassin’s Auklets provisioning nestlings. Condor 100, 546-550.

Horita, J., Ueda, A., Mizukami, K. and Takatori, I. (1987). Automatic 8D and 818O analyses of multi-water samples using H2O and CO2-water equilibration methods with a common equilibration set-up. Appl. Radiat. Isot. 40, 801-805.

Jacobs, S. R., Elliott, K., Guigueno, M. F., Gaston, A. J., Redman, P., Speakman, J. R. and Weber, J. M. (2012). Validation of the use of doubly labeled water for estimating CO2 production and water flux in growing poultry chicks. J. Avian Biol. 35, 71-96.

Koteja, P. (1996). Measuring energy metabolism with open-flow respirometric systems: which design to choose? Funct. Ecol. 10, 675-677.

McNamara, J. M. and Houston, A. I. (1996). State-dependent life histories. Nature 380, 215-221.

McNeil, Alexander, R. and Goldspink, G. (1977). Mechanics And Energetics Of Animal Locomotion. London: Chapman and Hall.

Nagy, K. A. (1987). Field metabolic rate and food requirement scaling in mammals and birds. Ecol. Monogr. 57, 111-128.

Nagy, K. A. and Costa, D. P. (1980). Water flux in animals: analysis of potential errors in the tritiated water method. Am. J. Physiol. 238, R454-R465.

Nagy, K. A. and Peterson, C. C. (1988). Scaling Of Water Flux Rate In Animals. Berkeley: University California Press.