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Author(s): I. L. Boyd, A. J. Woakes, P. J. Butler, R. W. Davis and T. M. Williams

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Validation of heart rate and doubly labelled water as measures of metabolic rate during swimming in California Sea Lions

I. L. BOYD,* A. J. WOAKES,† P. J. BUTLER,† R. W. DAVIS‡ and T. M. WILLIAMS§

*British Antarctic Survey, Natural Environment Research Council, Madingley Road, Cambridge CB3 0ET, UK,
†School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK,
‡Department of Marine Biology, Texas A&M University, PO Box 1657, Galveston, TX 77553, USA and
§Naval Oceans Systems Center, Hawaii Laboratory, PO Box 997, Code 511, Kailua, HI 96734, USA

Summary

1. The measurement of energy expenditures in free-ranging animals is essential if we are to understand fully the interaction between a species and its environment. This study examined the validity of heart rate (f_H) and doubly labelled water (DLW) as measures of field metabolic rate (FMR) in California Sea Lions (*Zalophus californianus*).

2. Oxygen consumption and CO_2 production were measured over 24 h by direct respirometry in six juvenile sea lions. The respirometer consisted of a hood over a flume in which the sea lions were exercised to various levels for 15 min periods throughout each experiment. The exercise regime produced a mean metabolic rate which was 2·3 times the predicted basal metabolic rate (BMR) with mean maxima of 6·27 times the predicted BMR.

3. Simultaneously with direct respirometry, mean CO_2 production was estimated using DLW and O_2 consumption was estimated using f_H, which had previously been calibrated against O_2 consumption.

4. The mean ± SD O_2 consumptions from direct respirometry, f_H and DLW were 11·80 ± 2·40, 11·95 ± 2·17 and 15·01 ± 3·77 ml min^{-1} kg^{-1} respectively. Paired Student's *t*-tests showed no significant difference between O_2 consumption by direct respirometry and the estimates from DLW and f_H. DLW measurements ranged from –10% to +86% of the direct respirometry measurements (mean +36·4%) and f_H measurements ranged from –28% to +23% of the direct respirometry measurements (mean +2·7%).

5. The range of estimated metabolic rates from f_H was largely owing to individual differences in the slopes of the linear relationship between f_H and O_2 consumption. The range of metabolic rates from DLW could be partly attributed to the short duration of the experiments (24–25 h) but this was shown not to be the cause of the tendency to overestimate metabolic rate from DLW. It was concluded that both DLW and f_H are valid methods for measuring FMR in California Sea Lions although it is possible that FMR could be overestimated when using DLW.

Key-words: Energetics, respiration, *Zalophus*

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Introduction

The development of new methods of measuring energy expenditures in animals has allowed the study of animal energetics to move from the laboratory into the field. In particular, the doubly labelled water (DLW; see Appendix 1 for a list of abbreviations) method has proved to be a powerful technique for measuring field metabolic rate (FMR) and has been applied to a wide range of species including birds, reptiles and mammals (Nagy 1980; Speakman & Racey 1988; Bryant & Tatner 1991). However, apart from human studies and one on ruminants (Fancy *et al.* 1986), no validations of DLW have been carried out in large mammals (>5 kg), few DLW studies have been published on large mammals in the field and

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most of these have been on pinnipeds (Costa & Gentry 1986; Costa & Trillmich 1988; Costa, Croxall & Duck 1989; Costa, Antonelis & DeLong 1991; Reilly & Fedak 1991). These studies have shown that, in general, the mean FMR exceeds basal metabolic rate (BMR; estimated from Kleiber 1961) by more than six times and Speakman (1993) has suggested that the DLW method may have overestimated FMR in these studies. Moreover, validation studies of DLW in mammals are not generally carried out on individuals exercising at levels normally found in the field.

The DLW method relies on the principle that water containing labelled hydrogen (D or ^{3}H) is lost from the body water pool of individuals at a slower rate than water containing labelled oxygen (^{18}O). The influx of water from, for example, metabolism and ingestion will deplete both hydrogen and oxygen isotopes of water equally but, because the oxygen component of water is in equilibrium with CO_2 in the blood through the action of carbonic anhydrase, additional labelled oxygen is lost from the body water pool through expired CO_2 (Nagy 1989). The production of CO_2 is proportional to metabolic rate (expressed in ml O_2 min^{-1} kg^{-1}) multiplied by the respiratory exchange ratio (R_E) which varies with the metabolic substrate being used (Schmidt-Nielsen 1975). The technique involves several assumptions which must be upheld for it to operate satisfactorily (Lifson & McClintock 1966; Nagy 1980; Costa 1987; Speakman 1987; 1993; Prentice 1990).

An alternative method of determining metabolic rate is the use of heart rate (Butler 1993). This method uses the Fick equation which shows that the flow of oxygen to the tissues ($\dot{V}O_2$) = tissue oxygen extraction × cardiac stroke volume × heart rate (f_H). Therefore f_H is independent of R_E but it assumes that cardiac stroke volume and the oxygen extraction by the tissues remain constant or that both change in a systematic fashion. In marine mammals the relationship is potentially complicated by the large variation in heart rate which occurs during diving (Thompson & Fedak 1993). However, averaged over whole dive cycles (submergence plus recovery period at the surface), these animals may be in a steady state (Butler 1993) and Fedak (1986) demonstrated a linear relationship between f_H and $\dot{V}O_2$ in a grey seal during swimming when f_H and $\dot{V}O_2$ were averaged over complete dive cycles.

The advantages and disadvantages of each of these methods ultimately depend on a combination of their utility and accuracy. In a study on Barnacle Geese (*Branta leucopsis*), Nolet *et al.* (1992) concluded that heart rate was a good measure of metabolic rate as long as data from groups of individuals were used and that the relationship between f_H and $\dot{V}O_2$ had been determined over the full range of f_H. They also showed a good concordance between heart rate and measurement of metabolic rate from DLW. The DLW method relies on recapturing the individual within a set time interval after the start of an experiment, whereas the use of f_H has no such restriction, particularly if data logging systems are used (Woakes, Butler & Bevan 1994). Moreover, f_H may be used to provide information about the energy costs of particular behaviours whereas DLW can only provide an average measure of FMR over the time period of the experiment. When both these methods are used together, it should be possible to estimate the relative costs of specific behaviours within the period of a DLW experiment. Given the potential advantages of using f_H in addition to DLW and of measuring f_H during DLW experiments, the aims of this study were to (1) validate the use of f_H and DLW in exercising California Sea Lions (*Zalophus californianus*, Esson) by comparing the estimates with those obtained by direct measurements of $\dot{V}O_2$ and (2) compare the accuracy of f_H and DLW as measures of metabolism during prolonged periods of swimming. The ultimate aim was to validate both f_H and DLW for measuring field metabolic rate in otariid pinnipeds.

Materials and methods

Six juvenile California Sea Lions (three female and three male, all approximately 1 year old) were used in the study. Two additional sea lions were also tested in the respirometer but they did not adapt well to the experimental apparatus and were released back into the wild before the experiments began. All the animals had been kept at Sea World, San Diego, for 1–3 months after being beached along the coast of California and were transferred under licence (Permit no. 742 under the US Marine Mammal Protection Act) to the Physiological Research Laboratory at Scripps Institution of Oceanography up to 30 days in advance of the experiments. The sea lions were normally kept in outdoor seawater tanks with a suitable haul-out area and fed twice daily on a diet of herring and mackerel [respiratory quotient (RQ) = 0·77, based on standard fat, protein and carbohydrate values for these fish; Pitcher & Hart 1982] supplemented with vitamins. The sea lions were trained to swim under a respirometry hood in a flume and to breathe within the hood. Individuals that were rejected as unsuitable for the experiment were those which failed to learn to exhale in the respirometry hood. As the sea lions could swim faster than the maximum water speed (1·4 m s^{-1}) in the flume, the work load was increased by adding weights to a line and pulley attached to the back of the animal (see Williams, Kooyman & Croll 1991).

In addition to measurement of O_2 consumption and CO_2 production by direct respirometry, CO_2 production was estimated using DLW and O_2 consumption was estimated using f_H. Calibration of heart rate with metabolic rate for the individuals used in these experiments has been reported separately (Butler *et al.* 1992). Each sea lion was fasted for 18–24 h before an

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experiment and was then kept in the flume respirometer for 24–25 h. During this time, individuals were exercised at different water velocities and with different weights on the pulley line. These exercise periods lasted 15 min and were then generally followed by periods of 15 min rest or swimming at low speed. Occasionally, rest periods were extended to 30 min. Depending on the individual seal, 43–81 periods of exercise were performed during the experiment. The water temperature was within the range 19–22 °C.

RESPIROMETRY

An open-circuit system for measuring O_2 consumption and CO_2 production was used. The respirometer was a hood which was the only place within the flume that a sea lion could surface to breathe. Air was drawn through the respirometer hood (volume ≈ 150 l) at a rate of 75–85 l min^{-1}, measured using a calibrated flow metre accurate to 1% on the in-flow line, by a vacuum pump. This flow rate kept the fraction of O_2 in the respirometer above 19·8% and the fraction of CO_2 in the respirometer below 1%. O_2 and CO_2 analysers continuously sampled gas in the exhaust line from the hood. Moisture was removed from the sampled air with Drierite before gas entered the O_2 and CO_2 analysers, and CO_2 was removed with Baralyme before entering the O_2 analyser. The output from the O_2 and CO_2 analysers was sampled every second by a computer, together with the temperature of the ambient air using a thermister. Mean $\dot{V}O_2$ and $\dot{V}CO_2$, adjusted for atmospheric pressure at the beginning and in the middle of each experiment and for relative humidity each hour, were recorded each minute. Atmospheric pressure varied by <1 millibar during any experiment.

The system was calibrated for O_2 with and without water flowing in the flume before and after each experiment using the N_2 dilution technique (Fedak, Rome & Seeherman 1981). For the CO_2 calibration, 10·103% CO_2, calibrated using the Scholander 0·5 cm^3 gas analyser (Scholander 1947), in N_2 flowing at 1–3 l min^{-1} was mixed with the total flow entering the hood which gave a mean concentration of CO_2 in the exhaust gas within the range observed when a sea lion was present in the respirometer. The theoretical fraction of both O_2 and CO_2 expected in the exhaust gases during calibrations was calculated using the equations in Davis, Williams & Kooyman (1985) and these showed the accuracy of the system was within 0·5% for both gases when there was no flow of water in the flume. These calibrations also permitted detection of leaks in the respirometry system. Absorption of CO_2 by the water increased with increased water speed in the flume but there was no evidence of a change in the accuracy of O_2 measurements with increased speed. The system was also calibrated against dry ambient air (20·94% O_2, 0·03% CO_2) each hour during experiments while animals were in the respirometer. These calibrations took place while the seal was at rest and when metabolic rate was stable. Each calibration lasted ≈5 min. When a calibration was performed metabolic rate at the beginning of each calibration was assumed also to apply during calibrations. The 95% response time of the respirometry system was 2–3 min. All gas measurements were converted to STPD.

DOUBLY LABELLED WATER

After obtaining an initial blood sample from the rear flipper to obtain the background enrichment of isotopes, each sea lion was injected (i.m. *latissimus dorsi*) with a measured dose (±0·001 g) of water enriched for $H_2{}^{18}O$ (4–5·5 g, 90·4% enrichment) and ^2H_2O (5·4–6·8 g, 99·9% enrichment). Complete equilibration of ^3H_2O with body water occurs 1–2 h after injection in sea lions and fur seals (Costa 1987). In this experiment, 3–4 h was allowed to elapse to permit equilibration of the oxygen and hydrogen isotopes with the body water pool. A second blood sample was obtained 3–4 h after initial injection and immediately before each sea lion was placed in the flume and respirometer. Individuals were also weighed to the nearest 0·2 kg at this time. A final blood sample was obtained immediately after each sea lion was removed from the flume and respirometer at the end of each experiment. Blood samples were placed in sealed tubes, centrifuged within 4 h of sampling and then the plasma was flame sealed in glass capillary tubes for storage prior to analysis. Plasma samples were analysed at Centrum voor Isotopen Onderzoek, Gröningen, the Netherlands [see Speakman *et al.* (1990) for calibration of data from this laboratory] and followed the methods described by Masman (1986). Isotopic enrichments were determined in triplicate on a SIRA-9 mass spectrometer. Initial enrichments varied from 102·05 to 155·05 ppm excess for ^{18}O and 1693·4 to 2332·8 ppm excess for ^3H. The mean ± SEM coefficients of variation of the ppm excess for all samples analysed were 1·49 ± 0·27 and 0·77 ± 0·10 for ^{18}O and ^2H respectively. $\dot{V}CO_2$ from DLW measurements can be calculated from several different standard equations available in the literature (Lifson & McClintock 1966; Coward *et al.* 1985; Schoeller *et al.* 1986; Speakman 1993; Speakman, Nair & Goran 1993). Although several of these have been developed for humans, most have been applied to other species in the past, including pinnipeds (e.g. Costa & Gentry 1986), so we calculated $\dot{V}CO_2$ using each equation to examine the consequences of these calculations for the final result. $\dot{V}O_2$ was estimated by dividing the estimate of $\dot{V}O_2$ by the mean respiratory exchange ratio obtained from direct respirometry.

HEART RATE

At least 24 h before the beginning of an experiment, a

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data logger for recording heart rate information (Woakes *et al.* 1994) was placed within a sealed metal box and attached with epoxy glue to the hair on the back of each sea lion. Two electrocardiogram (ECG) electrodes (self-adhesive human stress electrodes; NDM, Dayton Ohio, USA) were placed on shaved, degreased skin in the mid-line of the back at the levels of the pectoral and pelvic girdles, and attached to the data logger. These electrode positions gave good clean ECG patterns even in active animals. Plastic covers were placed over the electrodes and attached to the surrounding fur with epoxy glue. The data logger averaged and stored f_H every 30 s throughout the experiment. Owing to failure of one heart rate recorder during experiments, simultaneous measurements of f_H and DLW were only available for five of the six sea lions. An additional experiment, without DLW, was subsequently carried out on the sea lion which had a faulty heart rate logger (Seal 1640). $\dot{V}O_2$ was estimated from f_H by calculating the expected $\dot{V}O_2$ separately for each of the six calibration runs presented by Butler *et al.* (1992). As the same individuals were used in the calibrations as in the validation experiments presented here, estimates of $\dot{V}O_2$ from f_H for individuals were obtained only from the other five individuals in the study, i.e. we did not use the calibration data for individuals to estimate $\dot{V}O_2$ for that individual; only calibration data from the other individuals were used. Heart rate was calculated as the mean for the period of the experiment and, when substituted in each calibration equation, this gave five independent measurements of $\dot{V}O_2$, similar to those which would be obtained for heart rate measurements in an uncalibrated wild sea lion, and the mean of these five estimates was used as the estimate of $\dot{V}O_2$ from f_H.

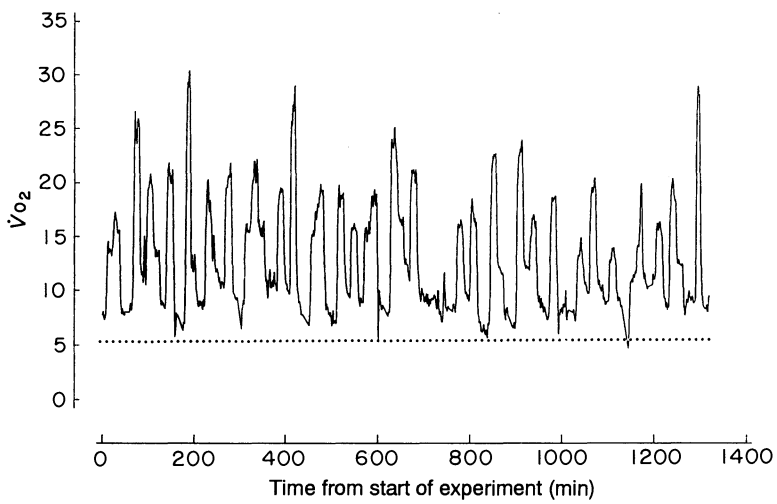

Fig. 1. Variation in O_2 consumption ($\dot{V}O_2$, ml min^{-1} kg^{-1}) throughout an experiment on Sea Lion 1655. The dotted line shows the predicted basal metabolic rate (Kleiber 1961).

STATISTICAL METHODS

Differences between means, and between means and individual values, were examined with Student's paired *t*-tests. In cases where multiple comparisons were made using paired *t*-tests and where significant differences were found, allowance was made for the possibility of detecting significant differences by chance. Variations around mean values were expressed as the coefficient of variation [CV, (SD/mean)×100]. Predicted BMR was calculated from the equation provided by Kleiber (1961) and was used to correct for variations in body mass when comparing the degree of elevation of metabolic rate owing to exercise across individuals and species.

The experiments were shorter than is ideal for experiments involving DLW and, potentially, this could have introduced variability in the DLW measurements of metabolic rate. In ideal circumstances it would have been most appropriate to maintain seals in the respirometer for at least one half-life (4–6 days) of the isotopes in the body water pool (Nagy 1980). The duration of the experiments was limited because 24 h was close to the maximum duration over which the heart rate electrode pads would remain in place (we were not permitted to implant the electrodes subcutaneously in these individuals as would normally be done in the wild) and it was also close to the maximum time over which the sea lions remained calm within the respirometer. Potential variation in the fractional turnover rates of isotopes owing to errors in the measurement of isotope enrichments was assessed by calculating the fractional turnover rate of each combination of replicate measurements of isotope enrichment (minus background enrichments) determined for each sea lion at the beginning and the end of the experiments. For three replicate measurements of both isotopes in each blood sample, this yielded 81 (3^4) separate measurements of the fractional turnover rate and the total hydrogen and oxygen dilution spaces. Each of these yielded a different measurement of $\dot{V}CO_2$. This method is similar to that used by Speakman & Racey (1987).

Results

O_2 consumption measured by direct respirometry showed marked variation throughout each experiment with maxima corresponding to periods of exercise and minima corresponding to periods of rest (Fig. 1). Measurements of O_2 consumption were more reliable than those of CO_2 production because a proportion of the CO_2 was absorbed by the water in the flume and this proportion tended to increase with the speed of the flume. Therefore, CO_2 production measured by direct respirometry (Table 1) will be underestimated. However, mean ± SE R_E (0·76 ± 0·05) was close to the RQ of the diet (0·77) and was also closer to the expected RQ for proteins than to that of fats (Schmidt-

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Table 1. Mean $\dot{V}O_2$, $\dot{V}CO_2$ and R_E measured by direct respirometry during the whole of each experimental period. In all California Sea Lions excluding one, both $\dot{V}O_2$ consumption and CO_2 production were estimated simultaneously using f_H and DLW respectively

Tag no.	Serial no.*	Body mass (kg)	Duration (h)	Direct respirometry (mean ± SD) $\dot{V}O_2$ (ml min^{-1} kg^{-1})	$\dot{V}CO_2$ (ml min^{-1} kg^{-1})	R_E	Multiple of BMR†
1639	1	33·7	24·75	10·64 ± 4·44	8·07 ± 3·10	0·78 ± 0·17	2·28
1640(1)‡	2	33·1	24·70	11·79 ± 5·96	8·05 ± 3·86	0·70 ± 0·11	2·51
1640(2)§	2	33·0	10·30	15·74 ± 7·88	11·23 ± 5·23	0·73 ± 0·12	3·34
1647	3	29·5	24·88	10·88 ± 5·13	8·17 ± 3·59	0·77 ± 0·13	2·25
1653	6	38·1	24·45	12·05 ± 5·03	8·56 ± 3·39	0·72 ± 0·12	2·66
1654	4	32·8	24·50	8·64 ± 4·15	7·06 ± 2·94	0·85 ± 0·16	1·83
1655	5	25·7	24·38	12·86 ± 5·06	9·40 ± 3·42	0·74 ± 0·09	2·57
Mean ± SEM		32·3 ± 1·6	22·6 ± 2·2	11·80 ± 0·90	8·65 ± 0·54	0·76 ± 0·02	2·49 ± 0·19

* Serial number provided by Butler *et al.* 1992.
† Kleiber (1961).
‡ Experiment without f_H measurement of $\dot{V}O_2$ consumption.
§ Experiment without DLW measurement of CO_2 production.

Nielsen 1975; Table 1). The exercise regime resulted in a mean ± SE metabolic rate of 11·80 ± 2·40 ml O_2 min^{-1} kg^{-1} which was, on average, 2·3 (1·8–2·6) times the predicted (Table 1). The mean maximum metabolic rate achieved was 29·75 (26·21–35·77) ml O_2 min^{-1} kg^{-1} or 6·3 (5·4–7·6) times the predicted BMR, although these maxima were of short duration (1–2 min).

The mean ± SD $\dot{V}O_2$ estimated from f_H was 11·95 ± 2·17 ml min^{-1} kg^{-1} which was 2·52 ± 0·4 times the predicted BMR (Table 2). This was not significantly greater than $\dot{V}O_2$ measured by direct respirometry (paired $t = 0·139$, df = 5, $P > 0·05$) and, on average, represented a 2·70% (range –27·7 to +23·6%, SD = 19·58%) overestimate of O_2 consumption.

The size of the body water pool was 52·1 ± 2·1% of body mass and the ^2H body water pool was 1·046 ± 0·006 times the size of the ^{18}O water pool (Table 3). O_2 consumption calculated from DLW depended upon which of the equations in the current literature was used to calculate CO_2 production (Table 4). Overall, the equations provided by Speakman *et al.* (1993, equations R1 and R2) gave values which were closest to those measured using direct respirometry (Table 4) but these were still, on average, 36·4% (range –10·2% to +86·0%, SEM = 15·4) greater than the values obtained from direct respirometry. At the opposite extreme, metabolic rate derived from the equation of Lifson & McClintock (1966) was 46·3% greater than expected from direct

Table 2. Mean heart rate (f_H) and O_2 consumption, calculated from f_H using calibration curves of O_2 consumption against f_H for six individual California Sea Lions (Butler *et al.* 1992)

Tag no.	Mean f_H ± SD (beats min^{-1})	$\dot{V}O_2/f_H$ (ml O_2 beat^{-1} kg^{-1})*	$\dot{V}O_2$ from f_H (ml min^{-1} kg^{-1})†	Mean $\dot{V}O_2$ from f_H ± SD (ml min^{-1} kg^{-1})‡	Multiple of BMR§	% deviation from expected¶
1639	94·00 ± 21·68	0·11	10·41	10·37 ± 1·84	2·21	–2·5
1640(2)	100·54 ± 23·25	0·16	15·26	11·38 ± 1·26	2·42	–27·7
1647	93·45 ± 20·69	0·12	11·31	9·91 ± 1·68	2·05	–9·8
1653	109·43 ± 18·55	0·11	12·60	14·46 ± 2·48	3·19	16·7
1654	94·95 ± 22·25	0·09	8·96	10·68 ± 1·67	2·27	23·6
1655	111·27 ± 20·23	0·12	13·62	14·90 ± 2·68	2·97	15·9
Mean ± SEM	100·61 ± 3·25	0·11 ± 0·01	12·0 ± 0·9	11·95 ± 0·89	2·52 ± 0·19	2·7 ± 8·0

* $\dot{V}O_2$ measured by direct respirometry (Table 1).
† Estimated from the regression for the individual in which f_H was measured.
‡ Estimated from five regressions of $\dot{V}O_2$ on f_H calibrated for each Sea Lion (Butler *et al.* 1992) and excluding the Sea Lion in which f_H was measured. The regressions equations were:

1639: $\dot{V}O_2 = 0·201 f_H - 8·486$ ($r^2 = 0·908$)
1640: $\dot{V}O_2 = 0·342 f_H - 19·122$ ($r^2 = 0·879$)
1647: $\dot{V}O_2 = 0·279 f_H - 14·656$ ($r^2 = 0·931$)
1653: $\dot{V}O_2 = 0·242 f_H - 13·770$ ($r^2 = 0·672$)
1654: $\dot{V}O_2 = 0·198 f_H - 9·662$ ($r^2 = 0·818$)
1655: $\dot{V}O_2 = 0·250 f_H - 14·161$ ($r^2 = 0·921$)

§ Kleiber (1961).
¶ Deviation of the mean estimate of $\dot{V}O_2$ measured from f_H relative to the estimate derived from direct respirometry.

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respirometry. Using the Speakman (1993) equation, the mean $\dot{V}O_2$ was 15·01 ± 3·77ml min^{-1} kg^{-1}. The difference between the measurements of O_2 consumption from direct respirometry and DLW approached significance (paired $t = 2·50$, df = 5, $P = 0·054$). There was no significant difference between the O_2 consumption estimated using f_H and that estimated using DLW (paired $t = 1·08$, df = 5, $P > 0·4$).

The relatively short duration of the experiments (24·5 h; Table 1) meant that the depletion of both the ^2H and ^{18}O isotopes of water was only 9·0% (range 7·7–11·3%) and 13·8% (range 12·1–17·5%) respectively. This meant that small errors in the measurements of the initial and final isotope enrichments in the blood would have relatively large effects on the fractional turnover rates of the hydrogen and oxygen water pools. To assess this potential error, the fractional turnover rate was calculated for each potential pairing of initial and final isotope enrichments using the triplicate determinations for each. Using the equation of Speakman *et al.* (1993, equation R2), this provided a distribution of potential $\dot{V}O_2$ measurements for each individual which is illustrated in Table 5 as the mean ± 95% confidence intervals for the estimate of $\dot{V}O_2$ from DLW. Despite a coefficient of variation around the measured enrichments of <2%, this resulted in a mean coefficient of variation of 35 ± 13% around the O_2 consumption values (Table 5). Moreover, the range of potential estimates of metabolic rate from DLW, accounting for variation owing to the accuracy with which fractional turnover rates could be estimated in these experiments (Table 5), shows that in four of the six seals, the actual metabolic rate from direct respirometry (Table 1) was significantly less than the metabolic rate from DLW. In the case of sea lion 1654 there were two clear groupings of $\dot{V}O_2$ values resulting from errors in the measurement of initial and final isotope enrichments; one with 12·6 ± 1·8 ml min^{-1} kg^{-1} and another with 25·1 ± 1·8 ml min^{-1} kg^{-1}. This was caused by a single isotope determination which did not conform to the overall pattern. The lower grouping of $\dot{V}O_2$ values was clearly the more accurate in this case. In the case of this individual, the $\dot{V}O_2$ for DLW (Table 5) overestimated actual $\dot{V}O_2$ by 86%. For illustration, exclusion of the higher grouping of $\dot{V}O_2$ estimates reduced this to 48% and reduced the mean difference from expected to 33·5 ± 10·3%.

Table 3. Estimates of water mass in individual California Seas Lions using $H_2{}^{18}O$ and ^2H_2O

Tag no.	^2H_2O water mass (kg)	$H_2{}^{18}O$ water mass (kg)	Ratio (^2H_2O:$H_2{}^{18}O$)	$H_2{}^{18}O$ mass (% body mass)
1639	18·88	18·13	1·041	53·8
1640	17·55	16·73	1·049	50·5
1647	16·53	15·86	1·042	53·8
1653	20·83	19·97	1·043	52·4
1654	18·45	17·64	1·046	53·8
1655	13·24	12·53	1·057	48·8
Mean ± SEM	17·58 ± 1·05	16·81 ± 1·03	1·046 ± 0·002	52·2 ± 0·9

Discussion

The determinations of O_2 consumption using both f_H and DLW showed variation between individuals which was unrelated to variations observed using direct respirometry. However, in both cases, O_2 consumption was overestimated and, expressed in absolute terms, variability was greater for DLW than for f_H and the average error was also greater for DLW. Although the DLW measurements must be viewed in the context of the short duration of the experiments with the potential errors this could have introduced, these conclusions are broadly similar to other recent validation studies involving f_H and DLW in birds (Nolet *et al.* 1992; Bevan *et al.* 1994).

DLW MEASUREMENTS

Most DLW validation studies of metabolic rate produce good agreement when values are averaged for groups of individuals but there can be considerable ranges of individual errors (Nagy 1989; Roberts 1989; Speakman & Racy 1988; Tatner & Bryant 1989; Nolet *et al.* 1993; Bevan *et al.* 1994). In addition, DLW measurements of metabolic rate tend to overestimate actual metabolic rate, especially in mammals (Speakman & Racey 1988; Speakman 1993). The results of this study conform with these general conclusions from other validation studies. The degree of overestimate appears to be greater in sea lions than in other species of mammals (Speakman & Racey 1988), although this must be considered in the light of the large variability in the accuracy of the DLW estimates of metabolic rate.

Part of the variability in the measurements of $\dot{V}O_2$ from DLW in this study is probably because of the short duration of the experiments and, if we had been able to run the experiments for a longer time, it seems probable that this variation would have been reduced. However, we have used a form of analysis which provides an estimate of the error in metabolic rate from DLW which is attributable to the limits of the experimental design. This showed that potential errors in the estimate of isotope fractional turnover rates cannot account for the apparent overestimate of $\dot{V}O_2$ from DLW in most of the individuals examined. Compared to the results of DLW studies on free-ranging pinnipeds, average metabolic rate in the present study was about one-third of that commonly estimated in

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the field where average metabolic rates of six times BMR have usually been encountered (Costa & Gentry 1986; Costa *et al.* 1989, 1991; Reilly & Fedak 1991). All of the previous studies of pinnipeds have used the Lifson & McClintock (1966) equation for calculating the rate of CO_2 production (Table 4) and, using this equation, our results from DLW significantly overestimated metabolic rate (paired $t=2{\cdot}89$, df $=5$, $P<0{\cdot}03$). Note, however, that as six comparisons were made in Table 4 there was a one in six chance of detecting one significant difference at $P=0{\cdot}03$. Even considering this caveat, the average metabolic rates estimated for pinnipeds in the field may be closer to four times than to six times BMR as supposed previously. This may be because, according to our results, previous studies (Lifson & McClintock 1966) included a 46% overestimate of actual metabolic rate. This is consistent with our observations of the metabolic rates of Sea Lions under different levels of exercise. We had little success in raising metabolic rate to greater than six times BMR in our experimental subjects and we assumed that six times BMR was close to their maximum aerobic metabolic rate. This supports the view that previous studies of FMR in pinnipeds using DLW have overestimated metabolic rate, because it seems unlikely that individuals could have sustained metabolic rates close to maximum for several days, which is the normal duration of experiments examining FMR using DLW.

In post-absorptive individuals the expected R_E would be close to 0·71 because this would be representative of the use of fat as the metabolic fuel. However, changing R_E from the measured value (0·76) to the theoretical value (0·71) only exacerbated the overestimation of $\dot{V}O_2$ from DLW. The R_E in this study was closer to the RQ expected if the Sea Lions were using protein rather than fat as the main metabolic fuel, especially as R_E tends to be reduced in a water flume because of absorbance of CO_2 by the water. The net effect of loss of CO_2 in flume water would be to overestimate $\dot{V}O_2$ from DLW. In a worst case scenario where the real R_E was 1·0 (representative of sea lions using carbohydrate as the metabolic fuel) and the estimated R_E was 0·71 (representing the use of

Table 4. O_2 consumption and CO_2 production, estimated by the DLW method, for individual California Sea Lions using six different equations available in the literature. All values are ml O_2 or CO_2 min^{-1} kg^{-1}

Equation ref.	Tag no. 1639 $\dot{V}O_2$	1639 $\dot{V}CO_2$	1640(1) $\dot{V}O_2$	1640(1) $\dot{V}CO_2$	1647 $\dot{V}O_2$	1647 $\dot{V}CO_2$	1653 $\dot{V}O_2$	1653 $\dot{V}CO_2$	1654 $\dot{V}O_2$	1654 $\dot{V}CO_2$	1655 $\dot{V}O_2$	1655 $\dot{V}CO_2$	Mean % difference from direct respirometry*
Lifson & McClintock (1966)†	10·38	8·11	15·17	10·68	18·88	14·72	13·52	9·87	17·16	14·76	21·47	15·89	+46·2
Coward *et al.* (1985)‡	9·36	7·32	13·91	9·80	17·92	13·98	12·31	8·89	16·48	14·17	19·81	14·66	+36·3
Schoeller *et al.* (1986)§	9·88	7·72	14·49	10·20	17·98	14·02	12·88	9·40	16·38	14·08	20·59	15·23	+39·6
Speakman *et al.* (1993, eqn R1)¶	9·56	7·47	14·13	9·94	17·63	13·74	12·51	9·13	16·11	13·86	20·14	14·90	+36·4
Speakman *et al.* (1993, eqn R2)**	9·55	7·47	14·12	9·94	17·62	13·74	12·50	9·13	16·11	13·86	20·14	14·90	+36·4
Speakman (1993)††	10·14	7·93	14·73	10·37	18·64	14·54	13·20	9·63	16·95	14·58	20·75	15·36	+43·1

† $rCO_2=\dfrac{N}{2{\cdot}08}(k_o-k_h)-0{\cdot}015k_hN$

where rCO_2 is the rate of CO_2 production (mol h^{-1}), N is the total body water (mol) and k_o and k_h are the fractional turnover rates of the oxygen and hydrogen pools respectively.

‡ $rCO_2=0{\cdot}481\,(N_ok_o-N_hk_h)-0{\cdot}015k_hN_h$

where N_o and N_h are the dilution spaces of ^{18}O and ^{3}H respectively.

§ $rCO_2=\dfrac{N}{2{\cdot}078}(1{\cdot}01k_o-1{\cdot}04k_o)-0{\cdot}0246r_{gf}$ where $N=\left(\dfrac{N_o}{1{\cdot}01}\right)+\left(\dfrac{N_d}{1{\cdot}04}\right)/2$ $r_{gf}=1{\cdot}05N(1{\cdot}01k_o-1{\cdot}04k_h)$

¶ $rCO_2=\dfrac{N}{2{\cdot}196}(1{\cdot}01k_o-1{\cdot}053k_h)$ where $N=\left[\left(\dfrac{N_o}{1{\cdot}01}\right)+\left(\dfrac{N_h}{1{\cdot}0531}\right)\right]/2$

** $rCO_2=\dfrac{N}{2{\cdot}196}(k_o-1{\cdot}0427k_h)$ where $N=\left[N_o+\left(\dfrac{N_d}{1{\cdot}0427}\right)\right]/2$

†† $rCO_2=\dfrac{N_o}{2{\cdot}08}(k_o-Rk_h)-r_{gf}$ where $R=\dfrac{N_h}{N_o}$ $r_{gf}=1{\cdot}05N_o(k_o-k_h)$

* Mean % deviation from the $\dot{V}O_2$ obtained from direct respirometry.

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fat as fuel), this would result in a 41% overestimate of actual $\dot{V}O_2$. Therefore errors in the estimated R_E could make an important contribution to the observed errors in $\dot{V}O_2$ estimated from DLW, but they are unlikely to be as large as described by this example.

The proximate cause of overestimation of metabolic rate from DLW is likely to be that the fractional turnover rate of either the ^2H dilution space is underestimated or that of the ^{18}O dilution space is overestimated. Studies of metabolic rate in fasting pinnipeds that have used ^3H to measure body water have found a close relationship between estimates of metabolic rate from water turnover and estimates based on changes in body composition (Boyd & Duck 1991; Boyd, Arnbom & Fedak 1993), suggesting that errors are most likely to lie with the estimate of the fractional turnover of the ^{18}O dilution space. Speakman & Racey (1988) suggested that an additional irreversible loss of labelled oxygen could occur through its incorporation into urea through the ornithine–arginine cycle. In carnivores such as seals, it might be expected that protein is used as a metabolic substrate and hence there may be a high rate of irreversible oxygen sequestration in urea. Up to 20% of total ^{18}O flux could be accounted for by this route if all energy was obtained from protein metabolism but even a more modest 2–5% loss could result in a 7–21% overestimate of metabolic rate from DLW. This is a potentially important and hitherto largely unexplored source of error in the application of DLW to species which rely on high-protein diets.

Most of the other potential sources of error, including maintenance of constant body water, no resorption of labelled water back into the body, differential loss of isotopes from the body by fractionation, cutaneous exchange of CO_2 (Nagy 1980; Costa 1987) or loss of labelled ^2H through methane production (Midwood *et al.* 1989) or by incorporation of water into fats (Haggerty *et al.* 1991) are probably insignificant, although each could contribute to the observed variance in DLW measurements of metabolism. One assumption, that rates of water and CO_2 flux remain constant, was not upheld by the design of the experiments, although Nagy & Costa (1980) suggested that variations in these rates introduce insignificant errors.

In addition, the equations used to calculate $\dot{V}CO_2$ from DLW assume a constant size of the body water pool. A decline in the size of the body water pool would have occurred during the experiments and this is indicated by the change in mass of 0·5–1 kg between the beginning and end of the experiments. From the measured metabolic rate and the energy density of fat and protein about half of this mass loss would have been owing to the metabolism of storage tissues. Even if the remainder of the mass loss was water, this would have led only to a 1–2% overestimate of metabolic rate from DLW. This is not a significant factor in terms of the total observed variation in metabolic rate from DLW, but it provides a partial explanation for overestimation of metabolic rate using this method.

Table 5. Variation in $\dot{V}O_2$ from DLW calculated from combinations of enrichments (corrected for background) in triplicate determinations of blood samples from the California Sea Lions in the study, using the equation provided by Speakman *et al.* (1993, eqn R2). This method of calculation provides an estimate of the error in the $\dot{V}O_2$ from DLW owing to variation in the measurement of isotope enrichment in blood

Tag no.	Mean $\dot{V}O_2 \pm$ SD (ml min^{-1} kg^{-1})	95% confidence limits on mean $\dot{V}O_2$ from DLW	Coefficient of variation	% deviation from expected*
1639	9·55 ± 3·55	8·77–10·33	37·2	–10·2
1640	14·12 ± 6·04	12·78–15·46	42·8	+19·8
1647	17·62 ± 8·49	15·74–19·50	48·2	+61·9
1653	12·50 ± 5·38	11·30–13·70	43·0	+3·7
1654	16·11 ± 7·54	14·43–17·79	46·8	+86·4
1654†	12·61 ± 1·80	12·13–13·09	14·3	+48·7
1655	20·14 ± 4·91	19·04–21·24	24·4	+56·6

* Expected values were those derived from direct respirometry.
† See text for explanation.

HEART RATE MEASUREMENTS

Butler *et al.* (1992) have already demonstrated, using the same group of Sea Lions, that a linear relationship existed between f_H and metabolic rate, and showed that the slope of the relationship was different in each individual. This accounts for the high degree of variation (mean coefficient of variation = 16·1%, calculated from Table 2) in individual estimates of $\dot{V}O_2$ from f_H but is indicative of the variation to be expected in estimates of metabolic rate from f_H of free-ranging individuals. The small difference between metabolic rate obtained from f_H and that obtained by direct respirometry may reflect the slightly different circumstances in which the calibration measurements were made (Butler *et al.* 1992). During these calibrations, metabolic rate and f_H were measured while animals were swimming under steady-state conditions and excluded the potential effects of short-term increases in heart rate owing to stress, rather than to demand for O_2.

The method of calculating $\dot{V}O_2$ from f_H simulated the degree of variation one could expect from the use of f_H to measure metabolic rate in an individual under field conditions. Overall, f_H provided a more precise measure of metabolic rate in exercising Sea Lions, under the conditions in these experiments, than DLW when expressed as a mean from one group of individuals.

COMPARISON BETWEEN f_H AND DLW AS FIELD METHODS

Whether f_H or DLW are used in field studies depends on the questions being addressed. Under the con-

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ditions of our experiment, f_H gave a lower range of variability than DLW. Therefore, under some circumstances, f_H may be superior to DLW for measuring field metabolic rate and these results are in broad agreement with other studies where this type of cross-calibration has been made (Nolet *et al.* 1992; Bevan *et al.* 1994). However, if an overview of average metabolic rate is required from a wide range of individuals then DLW may be the more appropriate technique. Heart rate has the advantage that metabolic rate can be estimated for specific types of behaviour and, unlike DLW, is not constrained by the need to obtain repeat samples of body fluids within a specific time after the beginning of the experiment. Although the heart rate method does rely on a constant relationship between heart rate and oxygen consumption, it does not assume anything about RQ which, as shown by this study, could account for significant errors in metabolic rate estimated from DLW. For this reason alone, it is difficult to extrapolate laboratory-based DLW validation studies on post-absorptive individuals to those in the wild and, when applying the DLW method to wild animals, it is essential to determine the RQ of the diet and possibly to consider the effects of high-protein catabolism on estimated metabolic rate. Although calibration of f_H across individuals can be used to estimate metabolic rate, accuracy can be much improved by calibrating each individual involved in an experiment (Butler *et al.* 1992). As calibration of individuals is not usually possible in the field, this is likely to be the greatest problem associated with the application of f_H to field studies. However, some of these problems may be overcome by applying pooled calibration data from a number of individuals to pooled f_H data from another group of individuals, even if these data were obtained under different conditions from the calibrations (Bevan, Keijer & Butler 1992; Nolet *et al.* 1992). Further studies are also required to determine the relationship between f_H and metabolic rate in species with extreme bradycardia. Despite the apparent overestimate of metabolic rate in most individuals, this study has demonstrated the efficacy of both DLW and heart rate as a means of measuring field metabolic rate in free-ranging sea lions. Grouped means from the heart rate method are likely to provide a close correlation with actual field metabolic rate but those from the DLW method probably represent values towards the upper end of the potential range of field metabolic rates.

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Appendix. List of abbreviations

BMR	basal metabolic rate
DLW	doubly labelled water
f_H	heart rate (beat min^{-1})
FMR	field metabolic rate
k_h	fractional turnover rate of ^2H dilution space (Speakman *et al.* 1993)
k_o	fractional turnover rate of ^{18}O dilution space (Speakman *et al.* 1993)
N	total body water (mol)
N_o	dilution space of ^{18}O
N_h	dilution space of ^2H
rCO_2	rate of CO_2 production (mol h^{-1})
R_E	respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$)
RQ	respiratory quotient
SD	standard deviation of a mean
SEM	standard error of a mean
$\dot{V}CO_2$	CO_2 consumption (ml min^{-1} kg^{-1})
$\dot{V}O_2$	CO_2 consumption (ml min^{-1} kg^{-1})

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