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Measurement of individual and population energetics of marine mammals

Sara J. Iverson, Carol E. Sparling, Terrie M. Williams, Shelley L.C. Lang, and W. Don Bowen

8.1 Introduction

The overriding currency of all animal life is energy. Animals have evolved strategies of energy acquisition and use, but these strategies also experience tradeoffs between energy allocated to maintenance, activities, growth, and reproduction and are central to our understanding of life histories and fitness. Thus 'energetics'—the study of the metabolic requirements, energy use, and output of animals—underpins many areas of physiology, ecology, evolutionary, and population biology, and even ecosystem dynamics.

Marine mammals pose many challenges for the study, interpretation, and comparison of individual and population energetics. For instance, the ability to study captive animals is often restricted to a few of the smaller marine mammal species. Although opportunities in the wild may be greater, there remain serious limits to our abilities to study species in remote locations (e.g. polar bears, *Ursus maritimus*, in the high arctic), in unstable habitats (e.g. ice-associated pinnipeds in the Bering and Chukchi Seas), or due to endangered status (e.g. monk seals, *Monachus* spp., southern sea otters, *Enhydra lutris nereis*, and vaquita, *Phocoena sinus*). As a group, cetaceans pose further difficulties because of their limited accessibility and large size. Adaptive insulation (blubber) is important in temperature management (Iverson 2009a) and the presence of this comparatively inert tissue can add complexity to the issue of defining the metabolically relevant body mass in marine mammals. Conversely, the sea otter (which does not have blubber) and some fur seals are small species with little internal insulation that rely mainly on thick pelage and the strategy of using heat generated from continual activity, and specific dynamic action (SDA) from frequent feeding, to offset thermoregulatory costs (Costa and Kooyman 1984; Mostman-Liwanag *et al.* 2009).

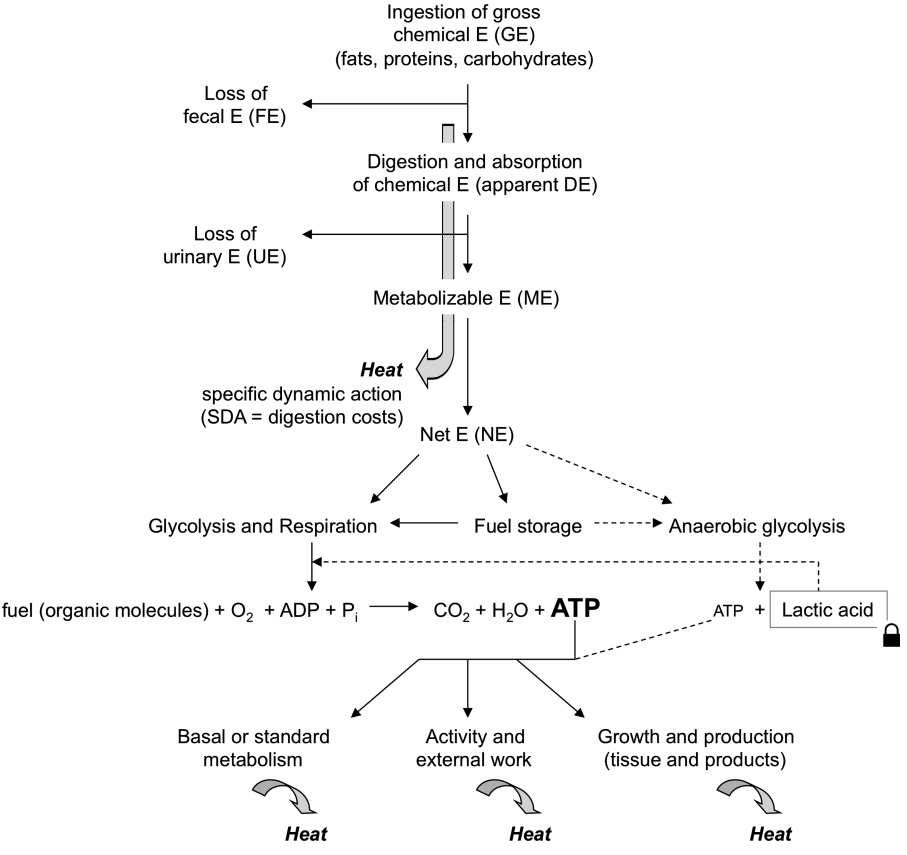

Fig. 8.1 Pathways of energy (E) acquisition and use in animals, illustrating the components that are generally measurable and form the basis for existing methods used for determining aspects of animal energetics. SDA occurs as a summed consequence of digestion and absorption processes, including synthesis of nitrogenous wastes to be exported in urine. Thus, although SDA occurs along the whole process, because its term is subtracted from ME to obtain NE, it is depicted as arising after ME. Digestible E (DE) is referred to as apparent DE because faeces contain both metabolic and undigested nitrogen. Ultimate E use can be divided into three major types of physiological work—biosynthesis, membrane transport, and external mechanical work—but are here divided into the three general categories of summed costs which are most pertinent to evaluating whole animal energetic budgets. 'Respiration' refers to the entire mitochondrial oxidative catabolic pathway, which includes the Krebs' citric acid cycle, electron transport chain, and oxidative phosphorylation, and results in the complete oxidation of fuels. The amount of adenosine triphosphate (ATP) produced from oxidative (aerobic) metabolism is about 19-fold greater than that produced during anaerobic glycolysis alone, and is thus represented by the larger font size under respiration. During anaerobic glycolysis,

Measurement and interpretation of 'maintenance metabolism' (i.e. BMR, SMR, see Box 8.1) also remains problematic for marine mammals. The rigorous conditions required for determining BMR are quite difficult to achieve for living marine mammals (including how 'resting' is even defined, as marine mammals can rest while completely submerged at depth and breath-holding, while floating at the surface intermittently breathing, and while lying on solid substrate in air). Overall, interpretation of BMR is complicated by how we define body mass, by the reliance of many species on extended periods of fasting, by characteristic apnoea and bradycardia when submerged, and by the fact that metabolic rate (MR) while actively diving may actually be lower than MR measured under criteria specified for BMR (e.g. Sparling and Fedak 2004). Boyd (2002a) rejected the idea that BMR, as classically defined for terrestrial mammals, can be realistically quantified in marine mammals. Additionally, the extent to which marine mammals use oxidative versus anaerobic metabolism (Fig. 8.1), and manage lactate production, especially under conditions of pushing physiological limits while diving (e.g. Kooyman *et al.* 1980), also warrant further study.

8.1.1 Definitions

All animals must exchange energy with their environment. The process begins in animals with energy input through the ingestion of food (fats, proteins, and carbohydrates), followed by digestion (chemical and enzymatic degradation of foodstuffs) and cellular absorption of the smaller energy-containing organic molecules produced, such as fatty acids and glycerol, amino acids, nucleotides, and monosaccharides. At several early points following ingestion, energy is lost from the animal—some directly to the faeces (undigested material) and some, after absorption, to the urine (e.g. waste metabolites) (see Fig. 8.1). The remainder of the absorbed energy-containing molecules comprises metabolizable energy (ME). However, the processing costs of both digestion and absorption result in some additional energy being expended and lost as heat (SDA, see Fig. 8.1). The remaining net energy (NE) is what the animal has to use for all physiological processes. Some of this NE is stored directly into tissues as fuel reserves, primarily as fats and proteins, with limited storage of carbohydrate as glycogen. The remainder undergoes catabolism, which comprises the cellular processes that harvest chemical free energy. During catabolism some energy is lost as heat to the surroundings, while the rest is used primarily to generate and store ATP, the

Fig. 8.1 (continued)
ATP is regenerated in the absence of O_2 through the reduction of pyruvate to lactic acid (lactate), which not only contains a large potential E source 'locked' in the incompletely oxidized compound, but requires sufficient O_2 to get rid of it (that is, metabolize it via the respiration pathway) and, until such time, the animal incurs an 'O_2 debt' during which it may be debilitated from further activity.

main energy-transferring molecule in the cell. The newly generated ATP then powers cellular work (mechanical, transport, chemical, and synthetic) by coupling exergonic reactions to endergonic reactions. Stored fuels can later be mobilized for catabolism during periods when nutrients are not available from the digestive tract. Once again, this cellular work produces heat, which is lost to the surroundings. In general, the most prevalent and efficient pathway used in all these ATP-driven catabolic processes is oxidative cellular respiration, in which cells consume oxygen (O_2), and produce carbon dioxide (CO_2) as a direct consequence of metabolism (see Fig. 8.1). The critical shuttle mechanism is:

$$\mathrm{ADP} + \mathrm{P_i} \quad \rightleftarrows \quad \mathrm{ATP}$$

whereby energy from foodstuff bonds is used to drive the reaction to the right. When the reaction goes to the left, ADP is produced and energy is released for physiological work (P_i, inorganic phosphate).

The characteristics of this exchange of energy between an animal and its environment, outlined in Fig. 8.1, form the basis for the different ways in which animal energetics are measured, both directly and indirectly. In principle, measurement of any of the components illustrated can provide insight into some aspect of an animal's metabolism and energy budget. The MR of an animal (Box 8.1) is defined as the rate at which it converts chemical energy to heat and external work. Thus, quantification of an animal's heat production is a direct measurement of MR. Since most external work is usually also degraded to heat, heat remains the most direct measurement of MR, accomplished using calorimetry with an animal enclosed in an insulated chamber. Although feasible for smaller animals (as discussed below), such measurements are progressively more difficult for large species including most pinnipeds and cetaceans. Therefore, it is rarely used. Rather, indirect methods of

Box 8.1 Acronyms and definitions of terms used in energetics studies

(See also Fig. 8.1 for further terms)

Allometry: the systematic change in MR (or body proportions) with increasing body size, usually expressed as $Y = Y_o M^b$, where Y is a process rate (e.g. BMR, MMR), Y_o is a normalization constant, M is body mass, and b is a scaling exponent.

AS (aerobic scope for activity): measure of an individual's upper metabolic limits, defined as the relative increase in an animal's MR between rest and maximal MR under the same conditions: $\dot{V}O_{2max}/\dot{V}O_{2rest}$ (i.e. $\dot{V}O_{2max}$ being MMR, $\dot{V}O_{2rest}$ being SMR or RMR).

BMR (basal metabolic rate): the rate at which energy is used by an organism at complete rest (i.e. 'maintenance', assumed to be the lowest stable level MR of an individual). In order to appropriately compare MR across animals, Kleiber (1975) put forth the comprehensive criteria that the animal must be at rest but awake, within its TNZ, stress-free, post-absorptive, mature (not growing), and not reproducing.

EE (energy expenditure): the rate of energy consumption per unit time.
FMR (field metabolic rate): the rate of metabolism that includes the total cost of all activities of a free-living animal.
MMR (maximal metabolic rate): the rate of metabolism at maximal activity and muscle work. May involve both aerobic and anaerobic biochemical pathways.
MR (metabolic rate): the rate of energy consumption by an organism, which is the rate of its conversion of chemical energy to heat and external work. This is measured in calories (cal) as units of heat (the energy required to raise 1 g of water from 14.5 °C to 15.5 °C) or in joules (J) as units of work (the work done to move 1 kg through 1 m), and expressed per unit time as a rate ($1\ J = 1\ kg \cdot m^2 \cdot s^{-2}$). Joules are now the standard international (SI) unit of energy measure, but can be readily interconverted with calories: 1 cal = 4.184 J.
RMR (resting metabolic rate): the MR when at rest, but otherwise not strictly meeting all criteria for Kleiber's BMR (e.g. when an animal is still digesting food).
RQ (respiratory quotient): the ratio of the volume of CO_2 produced to the volume of O_2 consumed during aerobic catabolism. The range of respiratory quotients for animals in energy balance varies with the substrate being metabolized. Fat and carbohydrate catabolism have an RQ of 0.71 and 1.00, respectively. Protein has an RQ of 0.8–0.9, depending on the amino acids being catabolized. RQ (measured at cells) is sometimes called the respiratory exchange ratio (RER, measured at respiratory organs).
SDA (specific dynamic action): the increase in metabolic rate as a result of digestion and absorption processes. The magnitude and duration of SDA varies with the size and composition of the meal. (Also called the heat increment of feeding, HIF.)
SMR (standard metabolic rate): synonymous with BMR, but SMR refers to measurement at a standard temperature.
TNZ (thermal neutral zone): the range of external temperatures at which an animal's requirements for metabolic heat production are at minimum. Above this zone energy must be expended to keep cool, and below more metabolic energy must be used for heat production.
$\dot{V}O_{2max}$**:** maximum rate of O_2 consumption of an individual; a measure of aerobic capacity.

measuring metabolism, although in principle accompanied by greater uncertainties or errors, in reality provide better information on how an animal uses energy under natural or varying conditions. Measurements of O_2 consumption, CO_2 production, water (H_2O) turnover, food energy intake, nutrient and energy balance, fuel storage and subsequent mobilization, tissue growth, synthetic products (e.g. fetus, milk), as well as lactate production (see Fig. 8.1), can all provide valuable insights into animal energetics (see also definitions in Box 8.1).

8.2 Measurement of metabolism

This section details some of the methods available for the estimation of MR of marine mammals.

8.2.1 Direct calorimetry

Quantification of energy released as heat over a given period is the most direct method of measuring MR. Direct calorimetry is performed by placing an animal in a sealed insulated chamber or calorimeter and measuring the heat given up to the circulating air and to water flowing through the chamber walls (e.g. McLean and Tobin 1987; Walsberg and Hoffman 2005). In practice, direct calorimetry is cumbersome and calorimeters are expensive and difficult to maintain. Hence, direct calorimetry is rarely used, especially for large animals, and has not been used in marine mammals. Furthermore, it is impossible to measure the energetic cost of specific activities of wild animals in their natural environment using direct calorimetry. An alternative to direct calorimetry is the minimum heat loss method, which uses physical measurements of heat transfer across the blubber and body surface to infer total heat loss, but it is based on assumptions that are likely inappropriate (reviewed in Boyd 2002a).

8.2.2 Respirometry (measurement of gas exchange)

MRs of animals can be estimated indirectly from the measurement of other variables related to energy utilization, such as O_2 consumption and energy balance (e.g. see Fig. 8.1). 'Indirect calorimetry', although in principle representing any indirect method, most often refers to the measurement of rates of gas exchange (where $\dot{V}O_2$ = O_2 consumption, $\dot{V}CO_2$= CO_2 production) and the translation of these quantities into a heat or work equivalent. Because steady-state metabolism consumes O_2 and produces CO_2 in known amounts (see Fig. 8.1) depending on the type(s) of food molecules being catabolized (i.e. the RQ, Box 8.1), their measurement can be used as an indirect assessment of MR. Respirometry refers to the measurement of these gases. Most relevant for use in marine mammals is open-flow (or open-circuit) respirometry, where air is pumped through a metabolic chamber at a rate that constantly replenishes the O_2 depleted by the animal while removing the CO_2 and water vapour produced by the animal (e.g. Renouf and Gales 1994; Boily and Lavigne 1995; Sparling *et al.* 2006) (Fig. 8.2 and Box 8.2).

There have been several innovative approaches to adopting this technique for use on marine mammals, which take advantage of the fact that they perform many activities underwater yet must return to the surface to breathe. This constraint has enabled researchers to quantify the metabolic costs of swimming and diving in both captive and free-ranging animals. Thus, open-flow respirometry has been applied to sea otters diving in a simulated environment (Yeates *et al.* 2007), otters and pinnipeds swimming in flumes (Davis *et al.* 1985; Fedak *et al.* 1988; T.M. Williams 1989), and exercising cetaceans (Worthy *et al.* 1987; T.M. Williams *et al.* 1993). Examples for free-ranging marine mammals include an isolated ice-hole model developed in the Antarctic for the study of Weddell seals (*Leptonychotes weddellii*, Kooyman *et al.* 1973; T.M. Williams *et al.* 2004), an open-ocean Steller sea lion

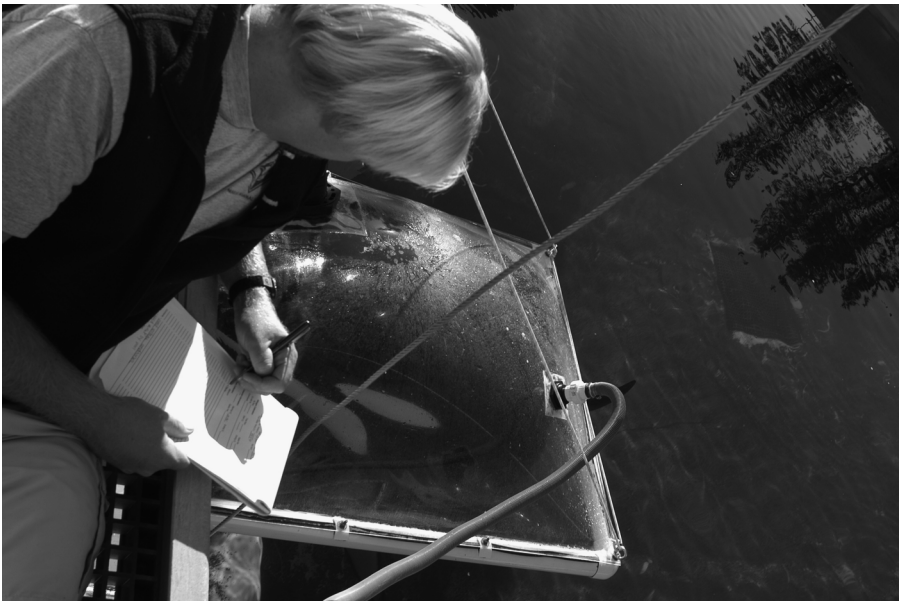

Fig. 8.2 Killer whale (*Orcinus orca*) resting in a metabolic hood for open-flow respirometry. Note that the water surface provides an airtight seal with the hood, thus creating a respirometer for the animal. Hoses connect to a vacuum pump that provides air into the hood at 500 L per minute and samples to the gas analysers. For this and other species studied, the air flow rate must be high enough that the animal is never in danger of being hypoxic, yet low enough so that the amount of O_2 consumed ($\dot{V}O_2$) by the animal can be detected with precision, ideally between 20 and 21% O_2. Expired air from this open-circuit respirometer is continuously sampled by gas analysers for determination of the fractional concentration of O_2 and CO_2, which is recorded with a laptop computer. Calculation of the rates of $\dot{V}O_2$ or $\dot{V}CO_2$ is reliant on knowledge of flow rates into and out of the chamber or hood, plus the fractional concentrations of the gas mixtures in and out of the system. The exact equation used also depends on where the flow meter is relative to the chamber and on whether or not water vapour and CO_2 are present or removed from the air stream prior to analysis (see Withers *et al.* (1977) for a general discussion, and Fedak *et al.* (1981) for details regarding marine mammal respirometry). Energy expenditure (EE) can be calculated from measured $\dot{V}O_2$ using the energy equivalent of O_2. The simplest method is to assign a mean energy equivalent for O_2 of 4.83 kcal per litre of O_2. However, this value will vary with the substrate being oxidized (from 4.73 at an RQ of 0.71 to 5.04 at an RQ of 1). In the absence of a measured RQ, one can assume an RQ based on diet. The equation of Weir (1949) allows the calculation of EE if both O_2 and CO_2 are measured: EE (kcal/day) $= \dot{V}O_2(3.9) + \dot{V}CO_2(1.1)$. (Photo courtesy of Terrie M. Williams.)

(*Eumetopias jubatus*) laboratory (Hastie *et al.* 2007), and a combined respirometry/simulated foraging approach (Sparling *et al.* 2007).

The size of some marine mammals, especially the larger odontocetes and mysticete whales, prevents the use of many of the open-flow respirometry methods described in the preceding section. Instead, expired gases may be collected as the whale breathes into a non-permeable bag or balloon. This has been done successfully for trained killer whales (Kriete 1995), as well as grey whales (*Eschrichtius robustus*) in captivity (Sumich 2001) and in open water (Sumich 1983). A cautionary note when attempting this method concerns the assurance of normal breathing patterns by the subject. Forced exhalations due to obstruction of the blowhole or individual animal responses can result in elevated end-tidal gas levels, which lead to an overestimation of MR (T.M. Williams, personal observation).

Despite the difficulties, there are several advantages for using respirometry methods for energy assessments. Most importantly, this technique allows the detailed quantification of energy costs related to specific activities. Thus, the effects of temperature regulation, exercise, digestion, age, body size, at-sea behaviours, moulting, pregnancy, and lactation among others can be evaluated. In addition, if both O_2 consumption and CO_2 production are measured, then the RQ (see Box 8.1) can be calculated, which provides information about the fuel being metabolized. If only O_2 consumption is measured (common in marine mammal studies) then information on the fuel substrate used is needed to translate $\dot{V}O_2$ into actual EE (i.e. RQ in Box 8.1 and Fig. 8.2 legend). Furthermore, some calculation techniques are relatively insensitive to variations in RQ (Fedak 1986) in cases where only $\dot{V}O_2$ is known.

The disadvantages of respirometry are that the measurement of gas exchange can be used in few situations, and results must be carefully interpreted in light of the experimental design. In general, the behavioural repertoire of captive marine mammals relative to their wild counterparts cannot be reproduced; this is especially apparent for costs related to interactions between animals. Perhaps, most important for marine mammal energetics is the difficulty of measuring a true diving MR. Open-flow respirometry can only measure gas exchange while the animal is at the water surface. Partitioning the submergence and surface MR post dive is complicated at best. During the surface interval, MRs will include the O_2 consumed for surface resting as well as for recovery from the previous dive and for replenishing O_2 stores for subsequent dives. Different analytical approaches have been used to solve this problem. The simplest is where the $\dot{V}O_2$ taken up during a surface period is assumed to have been consumed over the entire previous dive plus the surface recovery period; this is expressed as diving MR, but it does not attempt to partition between O_2 consumption while submerged versus at the surface (Castellini *et al.* 1992; Reed *et al.* 1994; Sparling and Fedak 2004). Another approach is to first measure the RMR (generally determined at the onset of the trial) and then calculate the diving O_2 consumption from the post-dive O_2 uptake minus the volume of O_2 equivalent to the RMR (Hurley and Costa 2001; T.M. Williams *et al.* 2004a; Hastie *et al.* 2007). If the

latter approach is used, it is important that the resting $\dot{V}O_2$ during the post-dive recovery period is equivalent to the pre-dive resting $\dot{V}O_2$. Also, there is the problem of delayed metabolic responses and recovery in diving marine mammals. Sparling *et al.* (2007) provided evidence supporting the hypothesis that diving seals can defer the metabolic costs of digestion until after periods of active foraging, and thereby maximize the O_2 available for extending the duration of dives. This highlights the importance of the timescale over which measurements are made. If diving animals are deferring the metabolic costs of certain processes then it is crucial that any study attempting to quantify energetic costs does so over an appropriate period of time .

8.2.3 Doubly labelled water (DLW) and isotope dilution

Doubly labelled water (DLW)

Gas exchange can also be measured indirectly using radio- or stable-isotopes of hydrogen and oxygen. Thorough accounts of the theory, history, techniques and advantages and disadvantages of the DLW technique have been published (Speakman 1997; Butler *et al.* 2004). This technique involves quantitatively dosing weighed animals with either deuterium- or tritium-labelled water (^2H_2O or ^3H_2O, respectively) and isotopic O_2 (^{18}O), and then sampling the body water (usually blood, but also urine or saliva) after complete isotope distribution (equilibration) throughout the body water pool. A blood sample may need to be collected before isotope administration to determine background levels, and is absolutely required if the individual has previously been dosed. In principle, the isotope mixture can be administered intramuscularly (IM), intravenously (IV), intraperitoneally (IP), subcutaneously (SC), or orally (gastric intubation). During equilibration the animal must not consume food or water. Equilibration can take about 1–6 h, depending on the species and body size, and this should be confirmed with either serial sampling to confirm a plateau in isotope concentration or waiting for the appropriate established maximum period. The animal is then released and later recaptured once, or in a series of recaptures, to collect a blood (or other) sample for the measurement of subsequent isotope elimination over a specified period (days).

The decision to use ^2H_2O or ^3H_2O will depend largely on the size of the study animal and analytical methods available, and on regulations concerning the use of radioisotopes (^3H_2O). Usually, ^2H_2O is most practical for individuals weighing less than about 130 kg, especially if infrared spectrometry is used for analysis (Oftedal *et al.* 1987b; Oftedal and Iverson 1987; Iverson *et al.* 1993); however, if analysed using isotope-ratio mass spectrometry (e.g. Speakman 1997, 2001; Sparling *et al.* 2006), ^2H_2O has been used in walrus (*Odobenus rosmarus*) weighing up to 1597 kg (Acquarone and Born 2007). ^3H_2O is used in relatively small quantities and analysed using liquid scintillation spectrometry (e.g. Ortiz *et al.* 1978; Reilly and Fedak 1990). Samples are analysed for ^{18}O using isotope-ratio mass spectrometry (e.g. Speakman 1997, 2001; Sparling *et al.* 2006).

Isotope concentrations measured at equilibration allow calculation of the body water pool size (see Section 8.3.2). EE is inferred from estimated CO_2 production, which is calculated from the differential elimination of the hydrogen and oxygen isotopes (i.e. labels) from the body water pool (see Box 8.2). The oxygen isotope is eliminated from the body by continuous flux through the body of both water and expired CO_2, but the hydrogen isotope is only eliminated by water flux. The difference between the two elimination rates is correlated with CO_2 production (Lifson *et al.* 1955). Multiplying the difference in the gradients of the exponential declines in isotope enrichments over time by the size of the body water pool gives a quantitative estimate of CO_2 production. There are many complexities involved in correcting for differential distribution spaces of the labels and fractionation during elimination, involving a number of alternative calculation methods (Speakman 1997). Sparling *et al.* (2008) found a good correspondence between DLW-derived estimates of daily EE and those measured using continuous open-flow respirometry, although unusually high MRs have been observed in some studies (e.g. Boyd *et al.* 1995a). Several equations are available for calculating the rates of CO_2 production in DLW studies, varying between one- and two-pool models (separate calculations for hydrogen body pool and oxygen body pool) and differing in the treatment of fractionation effects. Sparling *et al.* (2007) described these different approaches and concluded that two-pool models, which use the measured dilution space ratio and include a correction for fractionation effects, are most appropriate for marine mammals. Speakman's model (1997; eqn 7.43 therein) was judged to be the most appropriate for future marine mammal studies (see Box 8.2).

The principal advantage of DLW is that it provides a measure of FMR, integrating the costs of all activities over the measurement period. This is the most ecologically relevant measure of metabolism. Although expensive, the costs of isotopes, in particular ^{18}O, have declined and developments in mass spectrometer technology have enabled labelling at lower dosages. Thus, DLW studies of marine mammal MR are now feasible for more species, but larger doses of isotope are still required for the same level of precision and accuracy in larger animals. Methods are not well-suited to free-ranging cetaceans or sirenians due to the general capture and restraint requirements. Additionally, field studies can be logistically difficult, with the need to recapture the same animal within a relatively short, specified time window (e.g. about 7–10 days). Although this period is long compared to that needed for techniques such as respirometry, there remain limits to the ecological relevance of individual measures of EE made using DLW. However, useful estimates of FMR can be gained for many species if field studies are carefully designed and executed at several times of the year. An important issue that is often overlooked in DLW studies is that of generating measures of uncertainty in estimates of FMR. Large random errors can occur when using DLW to measure metabolism in individuals, so it is usually important to use grouped data when describing MR using DLW. A Monte Carlo simulation and bootstrapping approach incorporating uncertainty in input parameters (e.g. analytical variability, measurement error in isotope injection volume)

Box 8.2 Calculations of gas exchange in studies of metabolism

Respirometry

There are numerous configurations of open-flow respirometry systems and the basic equations for calculating $\dot{V}O_2$ and CO_2 vary depending on which is used. Generally in marine mammal studies, flow rate is measured downstream of the chamber and a subsample of the flow is dried (using a chemical drying agent) before going to the gas analysers—the equations below assume this is the case.

- If CO_2 is not measured and is absorbed after flow measurement but prior to O_2 measurement, RQ has to be assumed and $\dot{V}O_2$ can be calculated as follows:

$$\dot{V}O_2 = (FiO_2 - FeO_2) \cdot F/(1 - FiO_2 + RQ \cdot (FiO_2 - FeO_2))$$

- If CO_2 is measured:

$$\dot{V}O_2 = F \cdot ((FiO_2 - FeO_2) + FiO_2 \cdot (FiCO_2 - FeCO_2))/(1 - FiO_2)$$

where Fi = the fractional concentration of O_2 or CO_2 in incurrent air, Fe = the fractional concentration in excurrent air, and F = the rate of flow through chamber.

The above equations require the regular calibration of flow meters and gas analysers. However, the following is a simple formula for use where extreme accuracy is not required, RQ is assumed, gas is dried, and CO_2 is absorbed before the gas analyser.

$$\dot{V}O_2 = (0.2094\dot{V}N_2/0.8)(\Delta C/\Delta C^*)$$

This involves using the nitrogen (N_2) calibration technique of Fedak *et al.* (1981) where ΔC and ΔC^* refer to the deflection of the O_2 analyser during measurement and calibration, respectively, and $\dot{V}N_2$ is the volume (or flow rate) of N_2 used in the calibration. This has the advantage of eliminating the need to calibrate the O_2 analyser or measure the flow past the animal, thus producing increased accuracy. This is also particularly useful for situations of high ambient humidity as it is relatively insensitive to errors due to water vapour.

Doubly labelled water (DLW)

Sparling *et al.* (2008) suggested that the most appropriate calculation model for marine mammals was the double-pool model from Speakman (1997):

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

where: rCO_2 = rate of CO_2 production; $k_{o,d}$ = rate of turnover of O_2 and deuterium

(^2H_2O) isotopes; $R_{dilspace}$ = ratio of the two dilution spaces N_d/N_o; N = body water pool calculated as $N_o + (N_d/R_{dilspace})/2$.

There are also equivalent equations when tritium (^3H_2O) is used as a label (see Speakman 1997). Tritium has the advantage of being easy to measure with great accuracy and precision by detecting the beta particles it emits. However, this radioactivity also means that there are safety implications, and hence require very strict safety procedures for its use and disposal. A further disadvantage is the absence of background levels of tritium—covariation in drifts in the background levels of ^{18}O and deuterium tend to cancel each other out—this covariation cannot be used to minimize error when using tritium.

has been used in previous studies using DLW to generate confidence limits for estimates of EE (Boyd *et al.* 1995a; Speakman 1995; Sparling *et al.* 2008).

Singly labelled water (SLW)

Hydrogen isotopes of water (^2H_2O or ^3H_2O) alone, can also be used to estimate MR, EE, and food intake and at a substantially lower cost for both label and analyses. Quantitatively dosing an animal and sampling (again, usually blood) after equilibration allows measurement of the dilution space and total body water pool, and from this total body energy can be estimated (see Sections 8.3.1 and 8.3.2). A second dose and equilibration after a specified period allows estimation of change in total body energy over the interval between doses. If the animal is fasting throughout this interval, which occurs for routine periods in many marine mammals, this change equates directly to total EE. In addition, serial sampling of the body pool after equilibration allows measurement of water flux over time, which can be used to estimate EE and energy/food intake, given knowledge about the food type consumed and calculation of metabolic water production (MWP, see Section 8.5.2, Box 8.3 for an estimation of milk intake; for food intake see the adaptation of this equation in Bowen *et al.* (2001) and Muelbert *et al.* (2003) which accounts for the digestible energy (DE) of prey).

8.2.4 Proxies for assessing energy expenditure (EE)—heart rate (f_H) and stroking rate (f_S)

The cryptic behaviours of most wild marine mammals make energetic assessments by respirometry and DLW methods challenging. New tagging technologies coupled with knowledge concerning the correlation between many physiological parameters and MR enable the use of proxies for free-ranging marine mammals. Depending on the species and question to be evaluated, f_H provides such a useful proxy. Under steady-state conditions, there is a good linear relationship between f_H and MR in most vertebrates studied to date (Butler *et al.* 2004). This is based on the relationship (Fick 1870) between $\dot{V}O_2$ and f_H:

$$\dot{V}O_2 = (C_aO_2 - C_vO_2) \cdot V_S \cdot f_H$$

where V_S is the stroke volume of the heart and $C_aO_2 - C_VO_2$ is the difference in O_2 concentration between arterial and mixed venous blood. Although there are large variations in f_H due to diving activity, Fedak (1986) demonstrated a linear relationship between $\dot{V}O_2$ and f_H in a grey seal (*Halichoerus grypus*) if values were averaged over complete dive cycles. Similar results have been reported for Californian sea lions (*Zalophus californianus*), harbour seals (*Phoca vitulina*), and bottlenose dolphins (*Tursiops truncatus*) (T.M. Williams *et al.* 1991, 1993; Butler *et al.* 1992; Boyd *et al.* 1995a).

The general configuration of an f_H recording system consists of electrodes that detect the heart rate signal connected to a logger which stores the signal for later processing. The logger may be directly attached to the electrodes or the signal may be relayed via radiotelemetry to an externally mounted logging device. The f_H signal has been measured in marine mammals using the R-wave detector (most commonly used), the Holter monitor, and the digital electrocardiogram recorder (see Ponganis 2007 for a detailed review). Off-the-shelf tags (tested on free-ranging marine mammals) are currently manufactured by Wildlife Computers (Redmond WA) and UFI Technologies (Morro Bay, CA), using surface-mounted or implantable electrodes. Implantable electrodes require surgery and have been known to induce substantial inflammatory responses in some species (Green *et al.* 2009). Artefacts due to muscle-generated electrical impulse must be avoided; the ability to assure this will dictate the selection of an R-wave detector versus a digital electrocardiogram recorder. In the latter case, skeletal muscle artefacts can be screened and corrected. Surface attachments will only last a finite amount of time and it is also important to recognize that surface electrode attachments have a finite life, with the subsequent decay of f_H signals leading to a falsely assumed decrease in f_H.

Butler *et al.* (2004) gives a detailed account of the advantages and disadvantages of the use of f_H to estimate MR. The greatest advantage of measuring f_H is that it can potentially provide estimates of EE of free-living animals at varying temporal scales. MR can be estimated for specific types of behaviour (Butler *et al.* 1992), with the potential to examine longer term variation in the FMR of free-living individuals, as demonstrated in penguins (e.g. Green *et al.* 2005).

A difficulty of this method is that the relationship between f_H and MR must be established for each species. Realistically, this can be achieved in the laboratory for only a few marine mammals. Additionally, the $f_H/\dot{V}O_2$ relationship may differ under conditions of digestion (McPhee *et al.* 2003) and extreme bradycardia (Boyd *et al.* 1995a). Studies on birds have shown that the $f_H/\dot{V}O_2$ relationship can vary under differing conditions (e.g. exercise, feeding, thermoregulation) and therefore calibration studies need to be tailored to the likely range of activities undertaken and conditions experienced by free-ranging animals (Green *et al.* 2006). As with many other field methods like DLW, high levels of individual variation have been demonstrated and need to be recognized when interpreting the data (Butler *et al.* 1992; Boyd *et al.* 1995a; McPhee *et al.* 2003).

A comparatively new method for determining the energetic demands of free-ranging marine mammals involves the use of instrumentation to determine f_S. Like heart beats, marine mammals expend energy for each individual swimming stroke taken. With knowledge about the RMR of an animal, the cost of individual swimming strokes, and the number of strokes taken during specific activities, it is possible to calculate the EE for discrete activities. Animal-borne cameras (Davis *et al.* 1999, 2001; Bowen *et al.* 2002) and 3-axis accelerometer microprocessor tags (T.M. Williams *et al.* 2004a; R.P. Wilson, *et al.* 2006) have been used for recording individual strokes. Although a powerful method for detailing the energetic cost of discrete activities including the energetic impact of living in different habitats, the f_S method requires knowledge about the RMR and swimming metabolism of each species. Fortunately, with increased use of this method, stroke-cost libraries for marine mammals are being developed which will provide a database for converting f_S data into an energetic demand (e.g. see T.M. Williams *et al.* 2008 for a comparison of locomotor costs in marine and terrestrial mammals).

8.2.5 Proxies for assessing energy expenditure (EE)—allometry

The logistics of measuring the metabolism of marine mammals, especially under free-ranging conditions, have instigated the use of allometry to predict both short-term and long-term EE. A wide variety of predictive equations are available, but they are only as good as the data comprising them (see Table 9.1 in Boyd 2002a for a compilation of marine mammal metabolic data). Of particular concern is the extrapolation of allometric regressions to include exceptionally large marine mammals for which few data are available. Regardless, the use of allometry can provide a starting point for predicting EE. The main advantage of using allometry is that it is relatively simple and relies only on a measure (or estimate) of body mass. Estimates of metabolism using allometry may be the closest one can get to measuring the MR of the largest cetaceans.

The allometric relationship between body mass and MR is one of the most frequently used energetic relationships. Among marine mammals:

$$\text{BMR} = 1.93\ \text{mass}^{0.87}$$

in phocid seals resting submerged (Lavigne *et al.* 1986) and

$$\text{FMR} = 30.43\ \text{mass}^{0.524}$$

in pinnipeds and cetaceans (Boyd 2002a), where MR is in watts and mass is in kg. Although some debate exists as to the exact value of the exponent for mammals

MR is generally thought to be proportional to body mass raised to ¾ power. The link between allometric predictions of BMR and estimates of FMR is generally a single multiplier. A general rule of thumb is that the RMR of marine mammals averages 2 times the predictions for terrestrial mammals based on Kleiber (1975), and that FMR is 3–4 times BMR (*see* Costa and Williams, 1999; Boyd 2002a). Exceptions do occur and include comparatively low resting MR for manatees, and comparatively low FMR for deep-diving phocid seals.

The simplicity of using allometry to predict MR can be misleading, and may be complicated in the case of marine mammals. The effects of varying amounts of metabolically inert blubber, especially for species that undergo large seasonal changes in body composition, and uncertainties over the true scaling exponent (which may differ between marine and terrestrial species) will affect the applicability of generalized allometric regressions for individual species. Consequently, allometry may be more applicable to the estimation of MR at the group or population level than amongst individuals.

8.3 Estimating body composition

Body composition analysis divides body mass into components on the basis of differing physical properties. The energy content and nature of the major constituents of an animal's body (lipid, protein, and carbohydrate) can be used to estimate the quantity of stored energy and the nutritional status of an individual, but it can also be used to understand energy gain and expenditure, as well as nutrient utilization during fasting. Body mass is divided into fat and fat-free mass (FFM, sometimes referred to as lean body mass). However, FFM is heterogeneous, including protein, water, and bone mineral. A number of approaches are available to estimate the body composition of marine mammals, and the approach used depends largely on the size and type of animal and on the nature of the question being investigated.

8.3.1 Carcass analysis and the relationship between chemical constituents

The most direct method of estimating the energy content of an individual is by means of bomb calorimetry of the whole carcass or, more frequently, of subsamples of the homogenized carcass (e.g. Blaxter 1989; Estes *et al.* 1998). Given that stored carbohydrate is negligible in mammals, direct determination of the fat and protein content of such subsamples (e.g. Oftedal *et al.* 1987b) can also be used to convert to body energy equivalents using standard values for the energy density of fat (39.5 MJ/kg) and protein (23.5 MJ/kg) (Schmidt-Nielsen 1980; Worthy and Lavigne 1983). However, the need to homogenize the entire carcass in a whole-body grinder generally limits this approach to fairly small marine mammal species or juveniles.

Given the large size of marine mammals, dissection is more generally used to prepare the various body components (i.e. carcass, viscera, blubber, and skin) for

analysing separately by either bomb calorimetry or chemical analysis. Care must be taken to reduce evaporation and fluid loss during dissection and to correct for these losses in the final calculations (e.g. Oftedal *et al.* 1989; Reilly and Fedak 1990; Arnould *et al.* 1996). Measurement of the total mass fraction of these body components combined with an estimate of their energy content, has been used to study energy storage and depletion in pinnipeds, cetaceans, and polar bears (e.g. Bryden 1972; Lockyer *et al.* 1985; Bowen *et al.* 1992; Pond *et al.* 1992).

Chemical carcass analysis also provides the standard from which other methods of measuring body composition (see Section 8.3.2) can be validated (e.g. Reilly and Fedak 1990; R. Gales *et al.* 1994b; Arnould *et al.* 1996). That is, from the early empirical studies of Pace and Rathbun (1945), the composition of lean body mass or FFM in mature mammals is assumed to contain a relatively constant proportion of water (~73%) and protein (~20%). Thus, in principle in the two-compartment model, if the total body water (TBW) of an animal is known, from this FFM and thus total body protein (TBP) content can be calculated, with the remainder of the body mass comprising total body fat (TBF). From these values, total body energy (TBE) can be estimated as above. Although this principle remains generally valid, the precise values for the relationship between TBW and other body constituents have been re-examined many times and there is some evidence for species-specific relationships. For instance, Reilly and Fedak (1990) empirically established the relationships between body components and TBW in grey seals and derived the following predictive equations:

$$\%\text{TBF} = 105.1 - 1.47(\%\text{TBW})$$

$$\%\text{TBP} = 0.42(\%\text{TBW}) - 4.75$$

$$\text{TBE (MJ)} = 40.8(\text{body mass, kg}) - 48.5(\text{TBW, kg}) - 0.4$$

These regression equations accounted for 95–99% of the observed variation in gross body components and have been widely used for pinnipeds, but may be best applied to other phocids. Species-specific equations have also been determined for harp seals (*Phoca groenlandica*, R. Gales *et al.* 1994a) and Antarctic fur seals (*Arctocephalus gazella*, Arnould *et al.* 1996). In the absence of species-specific data, application of the most appropriate equation for a given family or age-group is recommended.

8.3.2 Total body water (TBW) measurement

The most accurate method for non-destructively estimating body composition in marine mammals is based on hydrogen isotope (^2H_2O or ^3H_2O) dilution to determine TBW from the resulting isotope dilution space (Speakman 2001). Other body components and TBE can then be estimated from their empirically derived relationships with TBW (see Section 8.3.1). As with DLW and SLW (see Section 8.2.3), knowing the amount of isotope administered, the isotope concentration at equilibration, and the body mass of the animal allows the dilution space to be calculated, which can then be converted to TBW. That is, while ^2H_2O or

^3H_2O do mix almost entirely with TBW, a small fraction of isotope is lost to rapidly exchangeable hydrogen atoms in organic constituents of the body and thus dilution space will slightly overestimate TBW (reviewed in Bowen and Iverson 1998). A comparison of a number of studies on phocid and otariid pinnipeds, in which both dilution space (using either ^2H_2O or ^3H_2O) and carcass desiccation were measured in the same individuals, revealed a single predictive regression equation which fits all species and can be used to calculate TBW (Bowen and Iverson 1998):

$$\text{TBW} = 0.003 + 0.968 \cdot (\text{dilution space})$$

Using TBW and empirically derived equations (see Section 8.3.1), TBF, TBP, and TBE can then be calculated. A number of detailed descriptions of the field and analytical methods, as well as calculation procedures used in hydrogen isotope dilution studies of body composition, are available in the literature and serve as useful examples of specific procedures (e.g. Reilly and Fedak 1990; Iverson *et al.* 1993; Arnould *et al.* 1996; Speakman 1997, 2001; Mellish *et al.* 1999; Sparling *et al.* 2006; Acquarone and Born 2007; Hall and McConnell 2007).

8.3.3 Ultrasound

Imaging ultrasound is also used to measure blubber depth as an index of body condition. B-mode ultrasound comprises a linear array of transducers that simultaneously scan a plane through the animal; this is viewed as a two-dimensional image on a screen from which measurements of internal structures can be obtained. Using a portable ultrasound scanner, blubber depth is measured at a series of dorsal, lateral, and ventral points along the body of a sedated or restrained animal (e.g. Mellish *et al.* 2004). The blubber depth measurements are combined with measurements of length and girth taken at the same points along the body, and the animal is then modelled as a series of truncated cones from which volume (Vs) of each blubber section can be calculated:

$$V_s = 1/3\pi h\left[(2r_x d_x + r_x d_y + r_y d_x + 2r_y d_y) + \left(d_x^2 + d_x d_y + d_y^2\right)\right]$$

where h is the length of each body cone, $d_{x,y}$ is blubber depth and $r_{x,y}$ is radius of the animals at site x and y (*see* N.J. Gales and Burton 1987; McDonald *et al.* 2008). The total volume of the blubber can be converted to total mass and fat using estimates of blubber density and lipid content; FFM can then be estimated by subtraction (see Section 8.3.1). The method has been validated against carcass analysis and isotopic methods in several species of phocid seals (Slip *et al.* 1992; Worthy *et al.* 1992; N.J. Gales and Burton 1994; Webb *et al* 1998; McDonald *et al* 2008) and has more recently been used in free-ranging bottlenose dolphins (Noren and Wells 2009). To assess reduction in measurements taken, Hall and McConnell (2007) found that a single blubber depth measured at the dorsal midpoint between the

foreflippers was the best predictor (explaining 72.5% of variability) of total body fat in juvenile grey seals.

8.3.4 Bioelectrical impedance analysis (BIA)

Bioelectrical impedance analysis (BIA) was initially developed and validated as a rapid and non-invasive means of estimating TBW and, thus, body composition, in humans (*see* Lukaski 1987). It has since been adapted for use in domestic species and a wide range of free-ranging mammals. Predictive relationships between TBW (estimated by isotope dilution) and impedance measurements have been developed in grey seals (Bowen *et al.* 1999), but results in other pinniped species have been less promising. Tierney *et al.* (2001) found significant, positive relationships between TBW in southern elephant seals and BIA variables, but the level of accuracy was inadequate for BIA to be more useful than the other methods. Similar results were reported in harbour seals (Bowen *et al.* 1998) and in female Antarctic fur seals (Arnould 1995).

BIA is based on the conduction of a known, low-level, alternating electric current through an organism. Conductivity is related to water and electrolyte distribution. Because FFM contains most of the body water and electrolytes, conductivity is greater in fat-free tissues than in fat and, therefore, the impedance of the electrical current is dependent on the body composition of the organism (see Lukaski 1987 for a review of the principles and equations). For each individual species a predictive relationship between TBW (estimated either by carcass analysis or hydrogen isotope dilution) and impedance must be developed. Because electrode configuration and placement on the body can have significant effects on both the magnitude and repeatability of measurements (R. Gales *et al.* 1994b; Farley and Robbins 1994; Arnould 1995) they should be tested to ensure that (1) total body impedance is being measured and (2) that the measurements are reproducible. Complete protocols, including electrode configurations and placements have been described in detail for phocids (Gales *et al.* 1994b), otariids (Arnould 1995), polar bears (Farley and Robbins 1994) and small carnivores (Pitt *et al.* 2006). Any movement of the animal during measurement will significantly affect the repeatability of measurements and, thus, the best results are obtained from anaesthetized individuals (Farley and Robbins 1994; Bowen *et al* 1999; Pitt *et al.* 2006). Although BIA is less accurate and precise than carcass analysis or hydrogen isotope dilution, it is relatively fast and non-invasive and, therefore, may provide a valuable alternative for estimating mean differences in TBW among groups (Bowen *et al.* 1999). Thus, investigators will have to weigh the merit of speed vs. cost and precision for a particular study.

8.3.5 Novel approaches to estimating body composition

Several novel approaches have been used to estimate body composition in marine mammals. One involves examining changes in the diving behaviour of seals instrumented with electronic data loggers. Elephant seals regularly perform dives

during which they spend a large proportion of time drifting passively through the water column (Crocker *et al.* 1996). The rate of drift depends on the buoyancy of the seal, which, in turn, depends on their body composition, with fatter seals drifting more slowly (Irvine *et al.* 2000). Biuw *et al.* (2003) used this observation to examine the theoretical relationships between drift rate and body composition, and carried out a sensitivity analysis to quantify uncertainty caused by varying model parameters. Using data from Argos satellite tags in the model, they were able to estimate the relative lipid content of individual seals to within about $\pm 2\%$ of that estimated by hydrogen isotope dilution, and to estimate changes in body composition by recording changes in the rate of drift during diving in foraging seals. This approach has recently been extended to changes in drift rate estimated from changes in the swimming speed of diving elephant seals recorded by archival electronic tags (Thums *et al.* 2008a). Although drift dives have been recorded in both northern and southern elephant seals and have recently been described in adult male New Zealand fur seals (Page *et al.* 2005b), it is not known how widespread this type of behaviour is among marine mammals and, therefore, how general this approach to estimating body composition might be.

8.4 Energy balance analysis

Measurement of the chemical energy of food entering and leaving an animal's body will provide an indirect estimate of MR, referred to as analysis of energy or material balance. Since not all GE consumed by an animal is available for metabolism, estimates of DE, ME, and NE provide increasingly better representations of what an animal actually has available for physiological work. If an animal is in a stable state (i.e. maintaining its biomass and not shedding products, such as feathers, fur, fetus or milk), then subtracting FE and UE from GE (i.e. ME, Fig. 8.1) will be a measure of its MR in an absorptive state (e.g. if resting, it will be a measure of RMR, Box 8.1). More complicated scenarios are introduced when the animal is not in a steady state, in which case all significant outputs of organic material (including tissue growth) must be measured.

The principle of energy balance analysis is straightforward, but in practice requires controlled feeding studies in captive situations and thus is restricted to the smaller species of marine mammals. In addition, extended acclimation periods of days are required to allow clearance of previous dietary regimes, and feeding study measurements must be taken over periods of at least 2–3 days or more to ensure that average steady-state energy input and output can be measured (e.g. Keiver *et al.* 1984; Lawson *et al.* 1997; Trumble and Castellini 2005). Finally, given that the collection of urine is required for measuring ME (e.g. see Keiver *et al.* 1984; Ronald *et al.* 1984), methods are not well-suited to the study of marine mammals in water and preclude measurement in cetaceans. Hence, even in pinnipeds, most studies have assessed apparent DE only (reviewed in Costa and Williams 1999).

Experimental approaches to energy balance studies and the estimation of DE require the quantitative collection of faeces (uncontaminated by urine). This can be done using either whole faecal collections (requiring collection of all faeces associated with the food consumed) or, more frequently, by feeding an indigestible inert dietary marker and comparing the changes in energy (or a given nutrient) and concentration of the marker in the faeces relative to that in the food consumed (Kleiber 1975). Markers may be naturally occurring in the food or added manually, but should be non-absorbable, non-toxic, have no appreciable bulk, mix thoroughly with digesta, and be accurately analysable. Examples of markers used in marine mammal studies include chromium sesquioxide (Cr_2O_3), naturally occurring manganese (Mn^{2+}), cobalt-ethylenediaminetetraacetic acid (Co-EDTA), silicon tubing pieces, dried kernal corn, unpopped popcorn, and dyed corncob grit (e.g. Keiver *et al.* 1984; Fadely *et al.* 1990; Mårtensson *et al.* 1994; Lawson *et al.* 1997; Rosen and Trites 2000; Trumble and Castellini 2005; Larkin *et al.* 2007). In current practice, the marker is usually fed just at the beginning of the tested diet trial and faecal collection is begun at first appearance of the marker and continued for 24–72 hours (e.g. Keiver *et al.* 1984; Trumble and Castellini 2005). Diet and faeces are collected, homogenized, and analysed for total water, nitrogen, lipid, and energy content by standard procedures and for marker concentration, according to the marker used (see above references for procedural details).

Energy and nutrient balance studies provide important insight into understanding animal MR, digestive efficiency, and food and energy requirements. These methods also allow the assessment of differing nutrient and energy availability from different food or prey types. This is because diet quality, quantity, and digestive tract morphology together determine the effectiveness of nutrient and energy extraction from food (e.g. Stevens and Hume 1995). For example, in seals fed different species of fish, or diets of several fish species, digestive efficiency and nutrient/energy extraction differs with diet, meal size, and feeding regime (e.g. Rosen and Trites 2000; Trumble and Castellini 2005). Thus, inferences for evaluating MR from such studies should consider these effects.

8.5 Energetics of lactation

The ability of females to efficiently transfer milk energy to their neonates can have significant consequences for both maternal and offspring fitness. Understanding variation in the patterns of energy transfer both within and among species requires detailed knowledge of both the proximate composition of the milk and the rate of milk production. Substantial individual variation among females in both milk composition and milk output has been noted for a variety of free-ranging mammals (see Lang *et al.* 2009). Thus, when attempting to characterize these components for a species, care should be taken to ensure that a sufficient number of individuals are sampled to provide representative data. In addition, to produce accurate

estimates of milk output and milk energy output of an individual, values for the milk components of that individual should be used rather than the species averages (see Section 8.5.2).

8.5.1 Milk composition

Sample collection

Milk composition can change substantially over the course of lactation (e.g. Oftedal *et al.* 1987a) and attendance bouts (e.g. Costa and Gentry 1986; Georges *et al.* 2001). Consequently, the timing of sampling is an important consideration. Exogenous oxytocin in IM doses of approx. 0.10–0.15 IU kg^{-1} in pinnipeds helps to initiate the milk ejection reflex and facilitates milk collection. Because circulating levels of oxytocin can remain elevated for several hours, a single dose is sufficient to achieve complete evacuation (Mačuhová *et al.* 2004). Repeated doses of oxytocin can alter mammary secretory processes and should be avoided (Oftedal 1984). Milk composition does not appear to change with evacuation of the gland in pinnipeds (Oftedal *et al.* 1987a; Iverson *et al.* 1993), but the same may not be true for cetaceans, polar bears, and otters and thus requires confirmation.

For large and/or generally inaccessible species of marine mammals, obtaining milk samples represents a significant challenge. Although post-mortem sampling may be the only source for some species, it can be very difficult to obtain samples that are representative and uncontaminated (e.g. from blood; see Oftedal 1984, 1997). Manual expression of milk from intact glands immediately post-mortem has been successful in some species (Peaker and Goode 1978; Ponce de Leon 1984; Oftedal *et al.* 1988). However, due to the loss of the milk ejection reflex, complete evacuation of the gland is probably not possible. Milk samples obtained from the stomachs of neonates should never be used to estimate proximate composition, as they will overestimate the water and carbohydrate content and underestimate the fat and protein content of the milk (Oftedal and Iverson 1995).

Samples should be kept frozen until analysed. To avoid exposing collected samples to repeated cycles of freezing and thawing for individual analyses, it is advisable to aliquot fresh samples prior to freezing wherever possible. Thawed samples should be homogenized before analysis.

Methods of analysis

The analysis of marine mammal milk samples follows standard methods for the analysis of dry matter (water), protein, lipid and carbohydrate (reviewed in Oftedal and Iverson 1995). The gross energy (GE) content can be determined directly using bomb calorimetry, or accurately estimated using standard values for the energy density of milk lipid and protein (see Box 8.3). Analyses should be performed independently and in duplicate. Values for individual components should never be calculated by subtraction (e.g. protein = dry matter − [lipid +

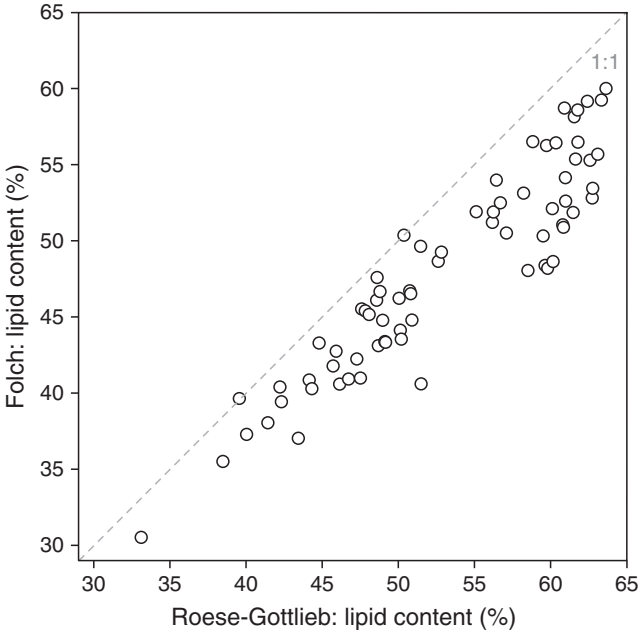

Fig. 8.3 Comparison of milk lipid content values obtained for 71 samples of grey seal milk analysed by the Roese–Gottlieb method (AOAC 2000) and by a modified Folch method (Folch *et al.* 1957; solvent to sample ratio increased from 20:1 to 50:1). Values are averages of duplicate analyses. Differences among duplicates (data not shown) were significantly less for the Roese–Gottlieb (0.14%) compared to the Folch (0.76%; $p < 0.001$, paired *t*-test) method. Estimates of milk lipid content determined by Roese–Gottlieb were significantly higher than those obtained by Folch ($p < 0.001$, paired *t*-test), with the Folch method underestimating milk lipid content by an average of 5.1% and a maximum of 11.6% (S.L.C. Lang and S.J. Iverson, unpublished data).

carbohydrate + ash]), as this will compound errors in measurements. Carbohydrate and non-protein nitrogen are normally found in only trace amounts in the milks of marine mammals. However, because marine mammal milk can be very high in lipids, which contribute most of the GE, lipid analysis warrants special consideration.

The standard and most accurate method for the quantitative determination of milk lipid content is the Roese–Gottlieb method (AOAC 2000). This is a gravimetric method which uses sequential diethyl ether and petroleum ether extractions after pre-treatment of the sample with ammonium hydroxide to disrupt the milk fat globules and break down the hydrophobic casein micelles. Any new method for determining milk fat content should be verified against the Roese–Gottlieb method prior to use. The chloroform- and methanol-based Folch (Folch *et al.* 1957) and Bligh and Dyer (1959) methods have also been used, but these methods are unsuitable for the quantitative determination of total milk lipid content. Direct comparison of the Folch and Roese–Gottlieb methods demonstrates that the values obtained by Folch

are more variable and are underestimated compared with the Roese–Gottlieb method, even when the solvent to sample ratio has been substantially increased (Fig. 8.3). There are similar problems with the Bligh and Dyer method (Iverson *et al.* 2001).

8.5.2 Milk output and milk energy output

Measurement of the rate of output by females (i.e. milk intake, MI, by the neonate) using either DLW or SLW techniques (see Section 8.2.3) has been previously described in detail by Oftedal and Iverson (1987). The daily total water intake (TWI) of the neonate(s) over the period of interest is estimated from the elimination of labelled hydrogen from the body water pool of the neonate (see Section 8.2.3, Box 8.3). MI is then calculated from either a measure of MWP and the water content of the milk (DLW method) or from data on the proximate composition of the milk and the rates of fat and protein deposition of the neonate(s),

Box 8.3 Calculations for estimation of milk energy output

Formula for determining the gross energy (GE) content of milks from proximate composition

L = lipid, CP = crude protein, S = sugar (i.e. carbohydrate):

$$\mathbf{GE}(\text{MJ kg}^{-1}) = \frac{(39.3^{\dagger} \cdot \%\text{L}) + (23.6^{\dagger} \cdot \%\text{CP}) + (16.5^{\ddagger} \cdot \%\text{S})}{100}$$

Alternatively, if non-protein nitrogen (NPN) has been determined (where TP = true protein):

$$\mathbf{GE}(\text{MJ kg}^{-1}) = \frac{(39.3^{\dagger} \cdot \%\text{L}) + (23.6^{\dagger} \cdot \%\text{TP}) + (31.0^{\ddagger} \cdot \%\text{NPN}) + (16.5^{\ddagger} \cdot \%\text{S})}{100}$$

Total water intake (TWI)

In a young, growing animal, changes in hydrogen isotope concentration over time reflect not only water loss and the intake of unlabelled water but also dilution as a result of an increase in the size of the body water pool (Nagy and Costa 1980; Oftedal and Iverson 1987).

To account for the changing body water pool size, hydrogen isotope concentrations at time t (H_t) are first corrected (H_t^*) for changing pool size according to the formula:

$$H_t^* = H_t \cdot (N_t/N_i)$$

where N_t is the pool size at time t and N_i is the initial pool size. Note that the correction for changing N requires that N be estimated for each time period, either by a repeated isotope administration or by interpolation from initial and final values for N (e.g. Mellish *et al.* 1999).

The isotope fractional turnover rate (k) is then estimated as the slope of the linear regression of the natural logarithm of H_t^* on time elapsed since isotope administration (see Oftedal *et al.* 1987b).

Total daily water intake (TWI), the sum of water loss (L) and water storage or gain (G), is then calculated as:

$$TWI = L + G = (k \cdot N_{1/2}) + \Delta N$$

where $N_{1/2}$ is the pool size at the mid-point of the study period (assuming a linear change in N with time) and ΔN is the daily change in N. Although changes in N may not be exactly linear, an assumption of linearity will result in relatively small errors unless N changes by more than 40% over the period of study (Nagy and Costa 1980).

Daily milk intake (MI)

The TWI of the suckling neonate(s) includes both the intake of preformed water in milk (MWI) and metabolic water production (MWP) from the catabolism of milk constituents and/or body nutrient stores:

$$TWI = MWI + MWP$$

If neonates have been given DLW (Section 8.2.3), MWP can be estimated from MR and subtracted from TWI. Using the percent water content of the milk, MWI can then be converted to MI.

For SLW (Section 8.2.3), MI is calculated from TWI using the daily fat (F_D) and protein (P_D) deposition rates of the neonate over the sampling period, and data on the percent water (W_M), fat (F_M), protein (P_M), and sugar (S_M) contents of the milk over the corresponding period, and assuming that the oxidation of 1 g of fat, protein, and carbohydrate yields approximately 1.07 g, 0.42 g, and 0.58 g of water, respectively (from Oftedal *et al.* 1987b):

$$MI = 100 \times \frac{TWI + (1.07 \cdot F_D) + (0.42 \cdot P_D)}{W_M + (1.07 \cdot F_M) + (0.42 \cdot P_M) + (0.58 \cdot S_M)}$$

If the values for W_M, F_M, P_M, or S_M change over the period of study, average values should be calculated for these components.

† from Blaxter (1989), ‡ from Perrin (1958).

which allow estimation of MWP (SLW method, see Box 8.3). Daily milk energy intake can then be calculated using the GE content of the milk. Because solid food intake or the drinking of water by the neonate(s) will cause an overestimation of MI, this method is limited to the period that neonates are solely dependent on milk. The optimal frequency and number of recaptures of the neonate(s) for blood sampling over the period of study will depend on factors including suckling pattern and lactation duration, expected rate of water turnover (i.e. MI), patterns of change in the proximate composition of the milk, ease of recapture, and the effects of disturbance on female–offspring interactions (e.g. Oftedal and Iverson 1987; Mellish

et al. 1999; Crocker *et al.* 2001). Milk output/intake cannot be inferred from suckling behaviour, as neither time spent suckling nor the duration of individual suckling bouts accurately reflects milk intake (reviewed in Cameron 1998).

8.6 Population energetics

Up to this point, the focus of this chapter has been on estimating the energy requirements, expenditures, and body composition of individuals. In this section, we extend this to estimating the energy requirements of populations. Population energetics is important in the study of prey consumption, which tends to be an important input into models of ecosystem structure and functioning (e.g. Bundy *et al.* 2009). Population energetics is also important in the assessment of the vulnerability of populations to changes in prey availability and other threats.

The simplest approach to estimating the gross food or energy requirements of a population is to multiply the estimated ration of individuals by the total population size. However, the requirements of individuals will depend on the size, diet, and perhaps reproductive status of individuals and sex. Therefore, in practice, more useful estimates of population consumption are derived from representing both the population and ration as vectors of numbers at each age and the corresponding age- or size-specific rations (Boyd 2002b).

Bioenergetic models typically estimate the gross energy requirements (GER) of a population as the sum of the energy requirements of individuals in the population, according to the following:

$$\mathrm{GER} = \sum_{a=0}^{a=n} n_a(P_a + (A \cdot \mathrm{BMR}_a)/\mathrm{ME})$$

where a = age, n = oldest age, P is the energy used in production (growth), A is a metabolic multiplier to account for activity, BMR (see Box 8.1), and ME (see Fig. 8.1). This recognizes faecal and urinary losses (E_{f+u}) and loss associated with the energy cost of digestion (E_{SDA}). GER can be calculated on any timescale depending on the resolution of the data and the purpose of the model. P is estimated from growth models (usually Gompertz or Richards models fitted to body mass at age data, see Chapter 5). ME is estimated from empirical laboratory studies (e.g. Ronald *et al.* 1984; Rosen and Trites 2000), BMR (in watts) for mammals is estimated using the allometric equation ($3.4 \times$ body mass$^{0.75}$, Kleiber 1975) and the A multiplier is estimated from empirical studies of MR in relation to various types of activity (e.g. Costa *et al.* 1989; Castellini *et al.* 1992; Sparling and Fedak 2004). Again depending on the data and purpose of the model, A can account for the proportion of time in and costs of different activities. For example, Winship and Trites (2003) used the formulation: $A = \mathit{water} \cdot A_{\mathrm{water}} + (1 - \mathit{water}) \cdot A_{\mathrm{land}}$ where *water* is the proportion of time spent in the water, A_{water} is a multiplier of BMR for water, and A_{land} is the multiplier of BMR for land.

Using a multiplier in this way assumes that active MR is a constant multiplier of BMR (i.e. that FMR scales in the same way as BMR).

Where the data will support a more complex representation, the ($A \cdot$ BMR) term may be expressed with a season-specific estimate of FMR (or average daily MR, ADMR) (e.g. Trzcinski *et al.* 2006). GER may also be indexed for differences among sexes and for different periods (e.g. daily compared to seasonal requirements). There are a number of useful examples of this general approach to estimating population GER (Olesiuk 1993; Hammond and Fedak 1994; Mohn and Bowen 1996; Nilssen *et al.* 2000; Winship *et al.* 2001; Boyd 2002b; Trzcinski *et al.* 2006). In each case, the specifics of the models are tailored to the available data. For example, tracking data for grey seals indicate seasonal changes in population distribution (Breed *et al.* 2006). These seasonal differences in distribution can be easily incorporated into estimates of population consumption to better reflect the predation rate on localized prey species (Trzcinski *et al.* 2006).

These models contain three types of errors. The first is measurement error, which is how well we measure input data. The second, process error, is how well parameters of the model fit the data, and the third is model error, the relationships among the processes in the model (Mohn 2009). To date, most models attempt to account for measurement and process error, but little attention has been given to how different models affect estimates and inferences. Uncertainty tends to increase with model complexity. For example, Trzcinski *et al.* (2006) estimated a coefficient of variation of 4–8% associated with an age-structured model of grey seal populations dynamics, but when the energetics were added to the model the coefficient of variation increased to 30%. Modelling with fewer parameters reduces this inaccuracy. However, a more complex model with increased demographic, spatial, and temporal resolution may provide greater insight as to how prey consumption may change as a result of changes in population size and structure and how it may respond to environmental variability.

Measurement error (variability in input parameters) is often incorporated in model estimates of state variables using Monte Carlo techniques to calculate confidence intervals around the predicted values (Mohn and Bowen 1996; Hammill and Stenson 2000; Boyd 2002b; Winship and Trites 2003; Trzcinski *et al.* 2006). All model inputs have an error distribution associated with them based on their empirical standard errors or an estimate of the upper and lower boundaries of plausible values for each variable. Running the model many times by resampling from these distributions provides a distribution of estimates, which can be used to calculate confidence intervals and the coefficient of variation. Parameters with the most amount of uncertainty have wider ranges of possible values and thus contribute more to overall model uncertainty. The implications of uncertainty and bias in the input data on model estimates can be assessed by examining the sensitivity of model predictions to changes in each parameter or group of parameters (Mohn and Bowen 1996; Boyd 2002b).