Assessing Cetacean Energetic Reserves; A review of Approaches and New Developments

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Abstract

The ability accurately gauge the energetic reserves of free-roaming cetaceans, is of elevated importance in conservation biology as this factor has direct implications for individual fitness, survival and reproductive success. In a rapidly changing environment, monitoring individual and population energetic reserves can also offer vital insight into ecosystem health and species resilience to accelerated habitat change. The intrinsic complications of studying cetaceans, however, render direct measurement of energetic reserves impractical in these species and currently there is an absence of standardized methodologies applicable to both dead and live animals. Energetic reserves are mainly stored in the blubber of cetaceans in form of lipids and traditionally, methodological approaches for assessment have targeted either blubber measurements, body composition or, conversely, whole body morphometry. New developments and the increasing cost-efficiency of drone technology and transcriptome sequencing continue to offer new avenues for robust assessment. This paper reviews the current state of the field, the capacity and limitations of traditional approaches and evaluates new approaches for their potential to provide a standardised approach to energetic reserve assessment across life-categories and species.

Introduction

The importance of effective wildlife health assessment in diverse areas of conservation biology is widely recognized (Ryser-Degiorgis, 2013). The challenge has never been greater than in the current era of accelerated climate change, habitat degradation and biodiversity loss. In marine mammals, individual health has been summarised as being the result of complex interactions between immune status, body condition, pathogen and toxicant exposure (Burek et al., 2008) as well as the genetic and environmental conditions that interact with these factors. A compromise of any one of these can lead adverse health outcomes.

In marine mammals, the role of body condition, or more specifically "energetic reserves" has been extensively studied and found to be an important determinant of individual fitness (Peig and Green, 2010). Particularly female energetic reserves appear to carry significant implications for reproductive success (IWC, 2001; Lockyer, 1986; Williams et al., 2013). Long gestation and lactation periods come at a high energetic cost which is entirely assumed by females (Miller and Hall, 2012). It has been observed that females right whale *(Eubalaena australis),* forego pregnancy (Seyboth et al., 2016) in years of low food availability, similar reduced reproductive output has been observed in killer whales *(Orcinus orca)* (Ward et al., 2009) and fin whales *(Balaenoptera phyalus)* (Williams et al., 2013) in response to prey abundance reduction. This correlation with energy reserves is extended to offspring viability and survival. Early-term abortion as well as adverse outcomes for fetal growth, neonatal mass, weaning mass and calf body condition are just some of the end-points studied which have been correlated with maternal energy reserves (Atkinson and Ramsay, 1995; Christiansen et al., 2016; Christiansen et al., 2014; Knowlton et al., 1994).

Body size in many large cetaceans further appears to carry evolutionary advantages in terms of predator avoidance, male competitive behaviors (Christiansen et al., 2016) and in the evolution of the capital breeding strategy, where larger energy stores are necessary to support a negative energy budget associated with extended off-spring dependence periods. Unsurprisingly, individual energy reserves have also been suggested as important predictors of cetacean behavioral patterns. For example, in poorer energetic reserves may be forced to increase their foraging effort to compensate, which in turn may lead to them taking greater risks, for example through greater exposure to predators (Miller and Hall, 2012), or utilizing sub-optimal food sources.

Aside from the evidenced "top-down" implications of individual energy reserves on diverse areas of individual fitness and population fecundity; animal energy reserves may also serve as a marker for the evaluation of habitat quality, or any changes hereto (Speakman, 2001). In this context marine mammals have been advocated as "sentinels" of ocean health (Bengtson Nash et al., Submitted; Bossart, 2011). This ability is of elevated significance in light of broad scale rapid change occurring to cetacean environment globally (Bossart, 2011).

Despite the demonstrated links between cetaceans energetic reserves and individual fitness, population and ecosystem health, there are currently no non-lethal, standardized approaches for routine evaluation of this health parameter. As such there exists a need for robust, minimally invasive measures that can be applied to both dead and live animals, and is appropriate for a broad application on large numbers of individuals, such as that required in long-term monitoring programs.

The following is a review of the diverse methodologies applied to date for the purpose of energy reserves assessment in cetaceans, as well an introduction to novel methodologies in the area. The capacity and limitations of available approaches are discussed in the context of their potential to fill the technical research need for a robust, standardised approach to energetic reserve assessment across life-categories and species.

TRADITIONAL APPROACHES

The most commonly applied measures, have their origins in commercial whaling and can be roughly separated into three categories based upon their focus of evaluation, namely blubber, body composition or body morphometry measures respectively.

Blubber Measures

Blubber tissue is comprised mainly of white adipose tissue in a matrix of collagen fibers, blood vessels, fibroblasts and immune cells (Ahima and Flier, 2000). Blubber, is a critical component of mammalian adaptation to the aquatic environment (Koopman et al., 2002) and serves a multitude of physiological functions including; body hydrodynamics, water balance, buoyancy, thermal insulation, defines body shape and streamlines and acts as a biological spring, reducing necessary energy for locomotion (Fish, 2000; Koopman et al., 2002; Ryg et al., 1988). One of its primary function, however, is the storage of energy in the form of lipids (Ackman et al., 1975b; Iverson, 2002 ; Lockyer, 1987). Whilst some visceral lipid storage occurs, the blubber tissue is significantly influenced by energetic condition, as the stored lipids constitute the excess of energy after covering the energetic needs for metabolism, maintenance and growth (Parry, 1949). This storage provides an integrated indication of foraging effort and foraging success (Miller and Hall, 2012), providing a good measure of the energy balance (Miller et al., 2011). Maintaining adequate body condition and thus blubber reserves is of critical importance, both for the energy-related blubber functions and its various physiological roles. Blubber has therefore presented the focus of the majority of methods applied for the evaluation of energetic reserves in cetaceans.

*Blubber Mass***:**

Due to intense energy storage in the blubber tissue, it is expected that blubber mass may be used as an accurate proxy for the estimation of total lipid stores. Direct measure of blubber mass has its origin in commercial whaling, where the whale was flensed and the blubber where weighted directly by weight scale, giving a direct measure of the total lipid stores. Nevertheless, dealing with a cetacean carcass is not easy task. First, the blubber is cut off in long strips called “blanket piece” and then broken down to more manageable proportions. As the mass increases, it also increases the difficulty of measuring. In cetaceans, blubber mass can constitute 15%–55% of total body mass (Aguilar et al., 1999; Lockyer, 1976). The technique is restricted to handling of carcasses, however, data obtained in this manner from commercially or incidentally caught or stranded animals has been used to provide baseline physiological information. For example Read (1990) estimated the body condition of 220 harbor porpoises *(Phocoena phocoena)*, killed incidentally in commercial fishing operation, using direct measurement of blubber mass regressing it against body size, finding variation in body condition among reproductive classes. Similar study were conducted in franciscanas *(Pontoporia blainvillei)*, where blubber weight measurements were found to correlate with age classes (Caon et al., 2007). Today, the direct blubber mass approach is still used in whaling operations such as the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA). This program used “Fat weight” (blubber weight + visceral fat) of harvested Antarctic minke whales *(Balaenoptera bonaerensis)*, as a body condition indicator, showing an significant decrease in blubber weight during 2 decades (Konishi et al., 2008).

Blubber Thickness:

i. Direct Measurement

Measurement of blubber thickness at various body locations, also has its origin in commercial whaling and like blubber mass, is bases on an inferred relationship between blubber volume and overall energy reserves (Hanks, 1981; Schulte-Hostedde et al., 2005). Blubber thickness, and all the indices derived from it, are the most commonly applied measures used to mirror energy reserves. The direct, physical measure of blubber thickness has been shown to provide an accurate reflection of energy reserves (Lockyer, 1987; Víkingsson, 1995). For example a study in fin whale and sei whale *(Balaenoptera borealis)*, found pregnant and resting females has the thickest blubber when compared to juvenile females and adult males. Juvenile males were found to have the thinnest blubber of all age and gender categories (Lockyer et al., 1985). A study in minke whale *(Balaenoptera acutorostrata)*, found a seasonal variation in the blubber thickness (Niæss et al., 1998). In harbor porpoises, differences in blubber thickness among the reproductive groups were found (Koopman, 1998). Both blubber thickness measure, single-site and multiple site has been used in different studies depending of the study design. When a single-site is used, it needs to be taken at the site were the blubber is most variable (Lockyer et al., 1985). Konishi (2006) reported that a single-site, medial lateral blubber thicknesses measurement in Antarctic minke whale, highly correlated with total blubber mass, thus it was a suitable body condition indicator. Nevertheless, multiple site measures has been advocated for a better representation, as it is well known that blubber thickness is not homogenous across the body surface (Lockyer et al., 1984).

ii. Deep-core Biopsy

Direct measurement of blubber thickness is carried out by cutting through the skin and blubber down to the muscle and measuring the full depth of the blubber tissue with a ruler. In its traditional application, therefore it can only be applied to stranded and harvested animals. Approximation of blubber thickness obtained through deep-core biopsy systems, have, however been developed and applied for measurement in free-ranging individuals (Cornick et al., 2016; Reeb and Best, 2006; Waugh et al., 2014). Deep biopsies have been successfully used in specific studies aimed at e.g. integument and postnatal ecdysis in right whale (Reeb and Best, 2006; Reeb et al., 2007; Reeb et al., 2005) and vertical distribution of lipids, fatty acids and organochlorine contaminants in humpback whales (*Megaptera novaeangliae*) (Waugh et al., 2014). However, its potential as a routine monitoring technique is limited due to the higher logistical effort involved in sampling, the greater level of invasiveness, as well as uncertainties relating to whether a full depth biopsy has been obtained. Whilst substantial blubber tissue is obtained with such systems, unless the biopsy captures some of the underlying muscle sheath, uncertainty remains regarding its penetration depth through the blubber (Geraci and Bruce-Allen, 1987). In addition, the loss of tension in the collagen matrix of the tissue, once the biopsy is taken or the blubber tissue is cut, cause tissue expansion, leading to a small but measurable increment in thickness (Aguilar et al., 2007). There are no data regarding the short and long term effects on the health of the individuals (Cornick et al., 2016), associated with this technique. However, Geraci and Bruce-Allen (1987) reported an important difference in wound healing time between bottlenose dolphins *(Tursiops truncatus)* and belugas (*Delphinapterus leucas*). While bottlenose dolphins presented a healing time of 7 days, belugas took between 30 and 40 days to heal. Concluding, that the main factor associated with healing time is the thickness of the epidermis. Uncertainty therefore remains regarding the long term health effects to targeted animals.

iii. Ultrasound measurement

Ultrasound is an alternative way to measure blubber thickness that have showed to correlated with direct measurement (Cartee et al., 1995; Zeng et al., 2015). It relies on the concept of sound travelling at different speeds through tissues of different density (Curran and Asher, 1974). This method has been more widely used in pinnipeds (Gales and Burton, 1987; Noren et al., 2015), for its easy application when the animals are on land. In cetaceans, it has been used in both stranded and free-roaming animals. In stranded animals, it has the advantage of reducing the loss of lipids and tissue tension that occurs during the necropsies. Additionally, it provides fast and valuable information on the distribution and structure of fat, enabling e.g. the distinguishment of different blubber layers that can be presented according to the species (Zeng et al., 2015). Also, for improved accuracy, as advocated with direct measures, measurements can easily been taken at different parts of the body. However it has been found to be more reliable on fresh carcasses, due to the normal decomposition changes in the body.

In captive or free-ranging cetaceans that can be temporarily restrained, the technique similarly performs well. For example Cornick et al. (2016), reported a mean measurement bias of 0.20 cm between ultrasound and rule data, in belugas. The method has been successfully applied to captive harbour porpoises (Kastelein et al., 1995), bottlenose dolphins (Cartee et al., 1995) and gray whale *(Eschrichtius robustus)* (Curran and Asher, 1974). The pre-requisite of restraint during measurement however limits its application to small cetaceans and similarly places limitations on the number of animals that can feasibly be assessed. Whilst measurements have been made on larger free-roaming cetaceans from a boat , with great precision (Miller et al., 2011). This method requires further standardization of the measure with both sampling position on the body and length of the animal, estimated by measurement from stereo video images of the animal or aerial photogrammetry. Further, operation of ultrasound equipment were reported as difficult to operate under boat-based conditions (Moore et al., 2001), plus the extra effort of video images or aerial photogrammetry renders this technique impractical for broad scale inclusion in routine monitoring programs.

Blubber Lipid Content:

Changes in nutritional status not only affects blubber thickness, but also blubber composition particularly its lipid content (Aguilar and Borrell, 1990). Therefore, blubber lipid content or lipid percentage, has become a common technique applied to evaluate energetic reserves of cetaceans

(Ryan et al., 2013). The base assumption of the technique is that the lipid content of blubber is representative of the area where the sample was taken (Ryan et al., 2013). Although applied in both, dead and free-roaming individuals, results have shown to be more accurate from samples derived from necropsies (Krahn et al., 2004; McKinney et al., 2014; Ryan et al., 2013), likely due to available sample size relative to surface area, and hence proportional lipid loss from the sampled tissue. Remote biopsies present well documented sampling effect leading to a loss of lipid (Krahn et al., 2007; Krahn et al., 2004; Ryan et al., 2013), and higher error when handling small sample amounts. Sample handling and approach contributes to the discrepancy observed in the lipid-percent values obtained from biopsy versus necropsy samples in killer whales, beluga whales (Krahn et al., 2004) and fin whales (Ryan et al., 2013). All of the above study findings indicated that blubber lipid content was not representative of directly harvested blubber tissue. As such, extra care must be taken in interpreting the data in quantitative analysis of lipid.

So far, lipid content has been the most widely used proxy of body condition, for stranded and free-roaming animals. It has been applied in mysticetes, namely fin whales (Ackman et al., 1975b; Aguilar and Borrell, 1990; Lockyer, 1987), sei whales (Ackman et al., 1975b) and humpbacked whales (Ackman et al., 1975b) as well as odontocetes, namely bottlenose dolphin (Samuel and Worthy, 2004), franciscanas (Caon et al., 2007), sperm whales *(Physeter macrocephalus)* (Evans et al., 2003), striped dolphins *(Stenella coeruleoalba)* (Gómez-Campos et al., 2011), harbour porpoises (Koopman et al., 1996; Koopman et al., 2002).

Access to historical whaling data has enabled application of a “lipid content” measure, namely whaling oil yields. Most recently, Braithwaite et al. (2015), used this measure to draw links between the body condition of humpback whales, as assumed from annual oil yields, and krill densities in the corresponding Antarctic feeding ground of the population .

Blubber Trunk Lipid Mass (BTLM):

Although not a widely used index, blubber trunk lipid mass can be considered a proxy for blubber mass. It is derived by considering the total amount of lipid stored in the trunk blubber mass and it is calculated as BTL (Kg) = %lipids in blubber x blubber weight. Results presented by Gómez-Campos et al. (2011) in striped dolphin, exceed the accuracy of lipid content assessment alone. The limitations of measures only applicable to dead animals, however, similarly apply to this approach.

The above outlined measures blubber measures share common limitations. Some studies have found a weak correlation between blubber thickness and nutritional conditions (Caon et al., 2007; Evans et al., 2003; Gómez-Campos et al., 2011; Read, 1990), which can be attributed to the fact that lipid content of blubber can vary independently of blubber thickness (Ackman et al., 1975a). Blubber plays an important role in energy storage, however also plays a role in other important physiological functions which, may present a threshold on the amount of lipid that can be lost from the tissue without jeopardizing its ancillary physiological functions (Gómez-Campos et al., 2011; Noren et al., 2015; Waugh et al., 2012). On the other hand, measurements of blubber thickens does not take account of visceral fat deposits, that seems to act as a secondary energy storage in the body cavity (Lockyer et al., 1985), it is suggested, that these deposits might be more mobile than those in blubber (Niæss et al., 1998), being the last in and the first out in the lipid dynamics. Another important factor is that energy contribution given by proteins, carbohydrates and intracellular lipids, are not taken into account with this measurement (Aguilar et al., 2007). These arguments among others have clear implications for blubber based assessments.

Body Composition

This analysis divides the body mass, into components according with its physical properties. Pace and Rathbun (Pace and Rathbun, 1945) determined that the composition of fat-free body mass (FFM) in mature mammals has a relatively constant proportion of water (~73%) and protein (~20%). Although, this proportion remains valid, it has been found that the precise values for the relationship between total body water (TBW) and other body component is species-specific (Iverson et al., 2010). So, first this relation must be established for the species. Once the values of this relation are known, from TBW content of an animal, can be calculated the FFM and protein content; and subtracting the FFM to the total body mass, it is possible to know the total body fat (TBF).

Isotope dilution:

The most used method to determine the TBW, is though hydrogen isotope (^2H2O or ^3H2O) dilution. Both of the hydrogen atoms of H2O, are completely exchangeable with isotopic water labelled with either deuterium (D or ^2H) or tritium (^3H). The method consists on giving the animal a mass specific dose of the isotope, then subsequent blood samples are obtained at specific intervals to develop a dilution curve. Allowing to calculate the isotopic dilution space, what in turn provides an estimate of TBW (Castellini and Mellish, 2015). Although, apparently the results are quite accurate, the challenges involved are quite significant. Specifically, the high cost of the isotopes, the need to handle the animals for the purpose of weighing and obtaining blood. These limit application to captive animals. Since the logistics of the technique complicates its implementation, the majority of the studies have been carried out in pinnipeds and very few on cetaceans, common dolphins *(Delphinus delphis)* (Hui, 1981), Pacific white-sided dolphin *(Lagenorhynchus obliquidens)*, Pacific bottlenose dolphin *(Tursiops gilli)* and pilot whale *(Globicephala scammoni)* (Telfer et al., 1970). Most of the studies with research questions focusing in specific physiological functions such as osmosis and water consumption and flux.

Bioelectrical impedance analysis (BIA):

Bioelectrical impedance analysis is a methodology imported from human use, used to estimate the body composition measuring the opposition of an electric current flow (electrical impedance) through body tissue. Because FFM contains most of the body water and electrolytes conductivity is greater than in fat. The electrical impedance is used to estimate TBW, what in turn, is used to estimate FFM and therefore, by difference with body weight, body fat (Kyle et al., 2004). In live animals any movement of the animal during measurement will significantly affect the repeatability of measurements. Although, the technique is relatively easy and fast, it is less accurate and precise in comparison to others (Iverson et al., 2010), which must take into account.

Body Morphometry

As with blubber mass, whole body mass has also been used as a proxy for body condition and energy reserve estimates. The obvious complications of a direct measure, have led to the use of morphometric parameters such as, the length, or the combination of length and girth, to predict body mass (Boyd et al., 2010b; Cattet et al., 1997). These parameters are based on the assumed linear association between body length and mass. Whilst there have been many proposed variations of morphometric measurements and indices , all make the central assumption of a measurable relationship between body mass and body condition (Jakob et al., 1996; Peig and Green, 2010)

Body Girth:

i. Direct Measure

This measure has been taken routinely in stranded or captured alive animals. By convention it is measured in front of the dorsal fin, where the animal's girth is at maximum (Boyd et al., 2010b). This value has been taken for monitoring body fat condition usually taking part in an index (Lockyer, 1986), or regressed against the standard body length (Gómez-Campos et al., 2011). The value of this measure should be taken with caution as there are several factors that can affect it. In stranded animals the degree of bloating and decomposition affects the measurement (Boyd et al., 2010b). Caon et al. (2007) in franciscanas, and Konishi (2006), in minke whales, found a positive correlation between body girth and blubber weight and body weigh respectively. Lockyer (1986) found significant differences in body girth between reproductive groups, in fin whales, and similar evidence was found by George et al. (2015), in bowhead whales *(Balaena mysticetus)*. By contrast, Gómez-Campos et al. (2011) and Read (1990), found that body girth were poorly correlated with blubber mass, in striped dolphin and harbour porpoise, respectively. This measure has also been applied to animals in captivity or live captured animals. However, in addition to the difficulties that capturing and handling of a wild animal entails, Lockyer et al. (2003), reported that the measures were variable and difficult to take, because even small movements by the animals (*e.g.* breathing) could affect them. The contrasting results between studies and species, together with the difficulty of performing the measurement, and the fact that body girth is affected highly due to the changes in the mass of the protein and carbohydrates located below the blubber (Aguilar et al., 2007), limit the performance and applicability of this measure.

Due to the challenge of the direct measurement of different body parts, in free-roaming and even in stranded individuals, the use of morphometry through photographic images of the individual (photogrammetry) to determine animal size (Best and Rüther, 1992; Cubbage and Calambokidis, 1987; Durban et al., 2015; Koski et al., 2006; Whitehead and Payne, 1981) has become popular. Nevertheless, just a few studies have linked it to body condition. For this approach it is essential that a mechanism for scaling the individual within the picture is derived (Zein, 2013). The different approaches that have successfully applied such scaling are discussed below.

ii. Stereo-photogrammetry

Stereo-photogrammetry consists of measures taken from simultaneous photographs employing two stereo cameras separated in space and placed in known positions relative to the individual (Hall-Martin and Ruther, 1979). This technique allows the measurement of the individual in three dimensions (Cubbage and Calambokidis, 1987). Known morphological dimension of the species is used to determine unknown dimensions, by scaling. This method is cumbersome, requires complex transformations due to relative and absolute orientation of the animals (Zein, 2013), and it can be difficult to implement from small-boat research platforms alongside routine data collection (Durban and Parsons, 2006; Jaquet, 2006). Nevertheless it has been used successfully in hector's dolphins *(Cephalorhynchus hectori)* to measure body length (Bräger and Chong, 1999).

iii. Laser-photogrammetry

This system has two variants, in the first, two lasers project dots separated by a known distance on a part of the animals' body (Durban and Parsons, 2006; Webster et al., 2010). In the other, a laser measures the distance between the camera and the animal (Jaquet, 2006). Both, allowing calculation of the animals' body length.

Both, stereo-photogrammetry and laser-photogrammetry, only capture a part of the animal's body, therefore allowing calculation of just specific body parts. In general, as the growth of these parts are not isometric with body length (Clarke et al., 1972), additional calculations and calibrations are required. As such, detailed knowledge of the algorithm relationships is crucial for the implementation of this technique. For example Jaquet (2006), calculated the fluke span in sperm whales and related with body length by using the logarithm in base 10 of both the fluke span (in meters) and the total length (in meters) and calculated a regression between log fluke span and log of the total length.

iv. Underwater-videography

In this method a free diver uses a digital video with underwater housing to obtain full body underwater images, and a portable sonar device to measure the distance between the camera and the animal. The images for the analysis must capture the whole body of the individual, the camera axis must be perpendicular, or nearly so, to the whale's body and as close to the body midline as possible. Subsequently, the body length measured from the distance between the tip of the rostrum and of the notch at the center of the tail flukes. This methodology has been applied in humpback whales (Nolan and Liddle, 2000). However, despite the fact that body length is an important parameter in the morphometric evaluation of body condition, length alone does not provide much usable information. In addition, the technique is limited by the accessibility of the individual whale or dolphin to approach by a swimmer and requires the water to be clear enough for good video images.

v. Aerial-photography

With this technique, the photographs are taken from above at a known height, of individuals at the water surface. In general the altitude and the lens focal length is used to scale the image (Miller et al., 2012). It does have the advantage that information about allometric relationship is not necessary, since the entire body of the animal can be measured from the photograph (Miller and Hall, 2012). In addition, this technique variant eliminates the horizontal axis and the parallax errors, associated with photogrammetry, explained below. Perryman and Lynn (2002), associated in grey whales, the nutritional condition with the total length and the width of the whale at its widest point. Miller et al. (2012) used measurements of width along the length of the body to estimate body volume and mass of right whales, width was considered as the diameter calculated from girth measurements based on the assumption that the cross-section of a right whale in the region measured is circular. These are some of the few studies that have linked aerial-photogrammetry with body condition. The high costs of aerial photographs are shown as one of the major drawbacks of this method. However, recently the use of unmanned aerial vehicles (UVA) for this purpose has made this technique much more accessible as discussed in later sections.

Several sources of errors are associated with photogrammetry, either related with the technique in which the images are obtained. For example the horizontal axis and the parallax errors, both allied with the position of the individual in the photo (Webster et al., 2010). Or errors associated with measurements of continuous variables. Where the concern is, whether the measurements are accurate (reflective of the true distance measured), for which a series of calibrations are introduced, and precise (differences between repeated measures of the same feature on the same individual) (Perryman and Lynn, 2002). Although there are ways to reduce these errors, the accumulation of small errors can result in a significant deviation.

The lack of consent of the most appropriated morphometric index, has proven to be one of the weaknesses of the approach, with results varying greatly depending of the index of choice (Peig and Green, 2010). This in turn makes comparison of study results difficult. Additionally, there is no clear consensus on whether this method is sufficiently accurate, even at population level. Notable, many have not even been tested for accuracy, precision, sensitivity, biological significance or the range of circumstances under which they may be valid (Cook et al., 2001). The success of an index is based on how precise and accurate it is. While precision refers to the reproducibility of the results, accuracy is how close the obtained result is to the reality. A simple index with not too many variables, in general is more precise but less accurate than a more complex index, that integrate more related variables, compromising precision. The challenge remains in valance these two factors. For a more accurate index it have been advocate that in addition to morphometry measurements, parameters such as age or reproductive class, the day in the feeding season or the stage of the annual reproductive cycle, year and sex, that play a fundamental role in blubber variation, should be consider in the index (Boyd et al., 2010a; Christiansen et al., 2014). The need to include these parameters is especially evident if one takes into account that body mass can change for different reasons other than change in lipid content (Boyd et al., 2010a). Also that body length and blubber thickness have shown a positive, negative or no variation according with the species (Miller et al., 2011) and that total body length was not found to be significantly related to overall lipid, in some species like sperm whale (Evans et al., 2003). Little is known about the patterns of lipid storage and utilization and nutritive condition along the entire length of the body (Miller et al., 2012).

New Developments and Opportunities

Unmanned aerial vehicles (UAVs):

Taking advantage of technological advances, the combination of UVAs and photogrammetry, has allowed the development of a methodology that combines the benefits of aerial- photography, and minimize one of its major limitations, its high cost. Christiansen et al. (2016), recently developed a body condition index (BCI) that captured the variation across the body of humpback whales. Vertical aerial photographs of individuals swimming at the surface, dorsal side facing up, with a straight body axis and peduncle, were taken from an altitude of 30-50 m. With the purpose of scaling the photographs with the size of the research vessel, additional images were taken from 80-120 m, where both vessel and whale were included. The BCI, proposed by Christiansen, reflects the animal superficial area, excluding the head, fins and tale fluke, since this parts contain relatively less energy reserves. Although it is a promising method, it is important to be aware of its limitations. A decrease in lipid concentration is not necessary visible in the body shape (Christiansen et al., 2013). And as discussed before, the quantification of the errors, associated with measurements of continuous variables (accuracy and precision), is essential to reach robustness in photogrammetry studies. Although there are ways to measure these errors with accuracy, they can make the methodology parsimonious.

Lipophilic Persistent Chemical Burdens as a proxy for lipid dynamics:

Persistent Organic Pollutants (POPs) are synthetic compounds and ubiquitous environmental contaminants. POPs are defined by their persistence, toxicity, propensity to bioaccumulate in organisms and their capacity for long range environmental dispersal. The vast majority of known POPs are lipophilic, accumulating in lipid-rich tissues of organisms with their toxicokinetics being driven by lipid dynamics (Bengtson Nash et al., 2013a; Yordy et al., 2010). Bengtson Nash et al. (2013b), showed that in a distinct breeding stock of southern hemisphere humpback whales, the outer blubber POP burden, increased dramatically between two time-points of the annual migration journey. Animals are expected to lose blubber during the annual migration and associated fast. A concentration effect in POP burdens is expected as the lipid reserved are metabolized but the POPs are not. The authors, however, found a nonlinear relationship between the reduction of the lipid content in blubber and the increase in outer blubber contaminant burdens indicating that lipid loss from the outer blubber layer was insufficient to explain the concentration effect. As mentioned before, there is a threshold under which lipid loss can occur from outer blubber layer, due to the ancillary roles this blubber layer is also involved in. This led the author to deduce that the extra increase in contaminates came from visceral fat deposits and blubber lipid loss from other body locations. Based upon this assumption, Bengtston Nash, developed the POP Concentration Index (CI) approach, to estimating the average population whole-of-body lipid loss or gain between two time points. In the above named study, the authors estimated average population lipid loss by focusing on only the males of a distinct migrating breeding stock. The approach would be equally valid for individuals tracked over time or similarly defined populations. The approach could however not be used to compare two diverse populations with different diets, nor to track populations with a high level of individual exchange with other populations.

Adipocyte metrics:

This approach relies on the fact that adipocyte number remains constant throughout adulthood and fluctuating weight, under normal conditions (Faust et al., 1978). Therefore, a change in energy stored results in a change in the size (volume or area) of the existing adipocytes, rather than a fluctuation in total adipocyte number (Faust et al., 1978). Based on the above information Castrillon et at proposed adipocyte metrics as proxy of energy reserves. The measurements used images from histologically prepared blubber tissue, stained to differentiate the adipocyte cells from the inter-vacuolar space. Adipocyte area is calculated as the adipocyte average area from measurements of individual adipocyte cross-sectional area, through image analysis software. Given the laborious nature of the method, rendering it impractical for high-throughput, routine analysis, an adipocyte index (AI) was developed. AI is defined as the ratio of inter-vacuolar area to adipocyte area within the image. Histological slides were prepared as per adipocyte area, however automated analysis was afforded by imaging software.

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Though, adipocyte metrics seem to perform very well, the limitation are the same limitation as per blubber lipid content. As mentioned before blubber differs in biochemical composition and in function, along its depth. Outer blubber has several functions, in addition to energy storage, which limit the loss of lipids in this layer placing an inherent limitation on the power of both blubber lipid content and adipocyte metrics.

Glide pattern analysis:

The basis for this approach is that because lipids are less dense than other tissue, changes in lipid stores directly involve change in body density (Biuw et al., 2003). Body density can be estimated by analysis of performance during glides, which are periods in which the animal is not actively producing thrust with its swimming organs (Miller and Hall, 2012). There are three different models to estimated body density from glides (Aoki et al., 2011; Biuw et al., 2003; Watanabe et al., 2006), however just one have been applied on cetaceans. The model uses all available glides as measured to fit a three term model of drag, air buoyancy, and tissue buoyancy (Miller et al., 2004). The necessary data information for glides analysis is collected by a high-resolution tag. The great advantage of this method is that it reflects the state of the total body fat store, wherever in the body lipids might be located. However the method is in its development stage. A preliminary study by Miller and Hall (2012), acquired good results in deep diving species, northern bottlenose whales (*Hyperoodon ampullatus*). However in shallow-diving species as humpback whale, the model seems to need adjustments, as apparently it is necessary to take in account diving air volume for each dive, complicating the analysis. The air on the animal density effect is reduced by compression at depth (Biuw et al., 2003), for this reason, it is not an issue in deep dive species. Another variable that can compromise this methodology is that it is unknown how widespread is glide behavior among cetaceans, indispensable behavior in the model (Iverson et al., 2010). Researcher concluded that work remains challenging.

Transcriptome Sequencing:

Improved affordability of genome and transcriptome sequencing offer exciting new possibilities for all areas of cetacean physiology, including health assessment and investigation of metabolic health. Whilst an organism's genome refers to the complete set of genetic material present in a cell or organism, and hereby can be used to investigate differential genetic expression between populations and species, transcriptome sequencing refers to the full set of messenger RNA (mRNA) molecules in an organism, and offer insight into which genes were active (which proteins were being produced) at the time of sampling. Transcriptomics has been successfully used as a predictor of health outcomes in humans (Szabo, 2014) and for the investigation of fasting metabolism in Northen elephant seals *(Mirounga angustirostris)* (Khudyakov et al., 2017). In this study, the blubber transcriptome allowed to identify a large number of genes that were differentially expressed in response to stress. In medical obesity research, the role of white adipose tissue as an endocrine organ has been receiving increasing attention (Trayhurn, 2005). Since adipose tissue produces a range of proteins (Meier and Gressner, 2004), some of which are actively involved in energy balance (Ahima and Osei, 2004), blubber transcriptome could provide valuable insight into adiposity, adipogenesis and lipolysis in this group of mammals with the most highly specialized of mammalian adipose tissue. With this base seems like exploration of transcriptome sequencing from blubber is the next step in the nutritional condition study in cetaceans.

Discussion and Conclusion

The study of cetacean energy reserves is not a simple subject, but one challenged by the inherent difficulties associated with studying large free roaming animals, and the diversity of life-histories of species belonging to this mammalian order. Nonetheless, the powerful insights into diverse areas of individual, population and ecosystem health offered by this single measure, have made quantification desirable and of increasing conservation importance. Numerous methodologies to determine energy reserves, have been proposed over the past century. Despite the fact that most of these methodologies have provided valuable information for the purposes for which they were ascribes, it is important to take into account their respective limitations for routine monitoring and broader comparative purposes.

The inability to restrict free ranging individuals limits the application of many of these measures, for free roaming animals. However, blubber, is one of the most accessible tissues that can be used to acquire robust information about energy reserves in cetaceans. It is relatively easy to access, from both necropsy and biopsy. Nevertheless, it is a complex tissue with diverse and overlapping roles. The fact that blubber differs in biochemical composition and in function along the body and its depth (Lockyer et al., 1984; Strandberg et al., 2008; Zeng et al., 2015), means that its relationship with overall energy stores may asymptote in order to service these ancillary functions. This is particularly the case for measurements obtained from the outer blubber layer which has been shown to be the most stable and the least active into the lipid dynamic (Ackman et al., 1975b; Aguilar and Borrell, 1990; Lockyer et al., 1984), such as those obtained by shallow biopsy.

Few studies have been done based on body composition data. Its high cost and the logistic difficulties, make these methodologies more appropriate for specialized physiological studies rather than with monitoring purposes.

The other set of data come from morphometric analysis. Without doubt, this methodology has improved and will continue to improve with further technological advances. UVAs, has reduced the cost and expedited data collection. However, standardization of the measurement across studies, minimization and quantification of errors, normalization of a body condition index, that ideally take account on the season or the stage of the annual reproductive cycle (Boyd et al., 2010a), require standardizing . A greater difficulty associated with morphometric analysis are the unknowns regarding the changes to nutritional state not necessarily perceivable by body shape (Aguilar and Borrell, 1990; Christiansen et al., 2016).

Great advances are being made continuously in this area of research, however, the need to standardize techniques remains. In line with the International Whaling Commision's call for advances in non-lethal research techniques we have identified the following priority areas for evaluation to facilitate accelerated outcomes in the form of tools to the field.

Identified priorities:

- Evaluation of minimum blubber tissue needed for acceptable handling error in lipid concentration and comparison of findings between biopsied and necropsy collected tissue.
- The priorities for UVAs are, the design and normalization of an appropriated body condition index, which includes the most relevant biological variables related with blubber variation.
- Identification of relevant molecular biomarkers for evaluation of nutritional state.
- Comparative assessment and evaluation of key methods on different species and life states.

The availability of reliable and reproducible diagnostic measures will benefit all areas of cetacean monitoring and conservation. This need has never been greater than in the current era of accelerated climate change, habitat degradation and biodiversity loss.

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