

RESEARCH ARTICLE

Counting calories in cormorants: dynamic body acceleration predicts daily energy expenditure measured in pelagic cormorants

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ABSTRACT

The integral of the dynamic component of acceleration over time has been proposed as a measure of energy expenditure in wild animals. We tested that idea by attaching accelerometers to the tails of free-ranging pelagic cormorants (*Phalacrocorax pelagicus*) and simultaneously estimating energy expenditure using doubly labelled water. Two different formulations of dynamic body acceleration, [vectorial and overall DBA (VeDBA and ODBA)], correlated with mass-specific energy expenditure (both $R^2=0.91$). VeDBA models combining and separately parameterizing flying, diving, activity on land and surface swimming were consistently considered more parsimonious than time budget models and showed less variability in model fit. Additionally, we observed evidence for the presence of hypometabolic processes (i.e. reduced heart rate and body temperature; shunting of blood away from non-essential organs) that suppressed metabolism in cormorants while diving, which was the most metabolically important activity. We concluded that a combination of VeDBA and physiological processes accurately measured energy expenditure for cormorants.

KEY WORDS: Doubly labelled water, Accelerometry, Field metabolic rate

INTRODUCTION

Energy expenditure is an important currency in animal ecology, but is difficult to measure in the wild. The doubly labelled water (DLW) method provides only a single, time-averaged estimate whereas heart-rate methods need to account for cardiovascular adjustments independent of energy expenditure (Speakman, 1997; Butler et al., 2004; Green et al., 2009; Green, 2011). The invention of miniature accelerometers has led to the development of accelerometry as a novel technique for estimating energy expenditure that accurately predicts oxygen consumption for humans (Plasqui and Westerterp, 2007; Halsey et al., 2008; Leenders et al., 2006), captive or semi-captive animals (Williams et al., 2004; Fahlman et al., 2008; Wilson et al., 2006; Halsey et al., 2009; Enstipp et al., 2011), domesticated animals (Green et al., 2009; Miwa et al., 2015), animals in the wild (Payne et al.,

2011; Elliott et al., 2013a; Bishop et al., 2015) and even relatively sedentary animals (Lyons et al., 2013).

Accelerometry is based on the principle that energy expended over a time period is equivalent to the mechanical work done, W . W is defined to be the integral of force, F , applied in the direction of travel, integrated over the distance travelled, x :

$$W = \int F dx. \quad (1)$$

For a body moving at a nearly constant instantaneous speed, such as an automobile, F involves no change in acceleration and is merely the force equal to the counteracting forces (i.e. friction, drag, buoyancy, gravity). However, animal locomotion usually involves rapid accelerations and decelerations during each gait cycle such that there is a net force unequal to the counteracting forces (Gleiss et al., 2011). A basic assumption in accelerometry is that most energy expenditure is associated with that net force. According to Newton's second law, net force, F_{net} , is proportional to the product of mass, m , and acceleration, a , such that (Eqn 1) can be simplified to:

$$W \approx \int F_{\text{net}} dx = mv \int a dt = mv\text{DBA}. \quad (2)$$

For a given constant mass and velocity, v , mechanical work is then approximated by the integral of acceleration over time and is termed dynamic body acceleration, DBA (see also eqn 7 in Gleiss et al., 2011). In practice, DBA is calculated after removing the static component (gravity) unassociated with work (Wilson et al., 2006). Both sides of Eqn 2 have the dimensions of mechanical work, $\text{kg m}^2 \text{s}^{-2}$, as in this formulation DBA consists of the integral of acceleration over several muscle cycles (e.g. wing beats during flight, flipper beats during swimming or steps during running) and has the dimension m s^{-1} . Alternatively, the mass-specific mechanical work completed (mass-specific energy expenditure) for an animal travelling at a constant average velocity is proportional to DBA:

$$\frac{W}{m} \propto \text{DBA}. \quad (3)$$

Although accelerometry has been used to quantify the fine-scale energy use of animals in the wild (Williams et al., 2014; Bishop et al., 2015), the method has seldom been validated in the wild (but see Fahlman et al., 2008; Elliott et al., 2013a; Duriez et al., 2014; Bishop et al., 2015). The technique assumes not only that an accurate measure of mechanical work can be derived from acceleration, but also that energy expenditure in animals is primarily associated with mechanical power. However, mechanical power in homeotherms is only $\sim 20\%$ of total metabolic energy costs, and oxygen consumption rates can sometimes only correlate weakly with mechanical power because

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List of symbols and abbreviations

AIC	Akaike's information criterion
AICc	Akaike's information criterion with finite sample size correction
A_n	measured acceleration in direction n
A_n	static component to acceleration
D	duration
DBA	dynamic body acceleration
DLW	doubly labelled water
DMR	diving metabolic rate
EE	energy expenditure
F	force
FMR	flying metabolic rate
F_{net}	net force
m	mass
ODBA	overall dynamic body acceleration
RMR	resting metabolic rate
SMR	surface swimming metabolic rate
T	time
v	velocity
VeDBA	vectorial dynamic body acceleration
W	mechanical work
x	distance travelled
α	intercept

of variation in muscle efficiency, body heat production, digestion and other factors (Ward et al., 2001; Rey et al., 2015). For instance, energy lost as a result of thermoregulation, digestion and basal metabolism are not accounted for by accelerometry. However, accelerometry has proven accurate in spite of this, and indeed activity may be an important component of thermoregulation and 'resting' metabolism (Green et al., 2009).

Although alternative accelerometer-derived metrics have been formulated (e.g. Spivey and Bishop, 2013), DBA remains the most commonly reported technique for converting accelerometer profiles into energy expenditure. Apart from variation in efficiency at which mechanical work is converted into metabolic energy expenditure and variation in the importance of inertial (constant velocity) work not incorporated within DBA, estimates of DBA from accelerometers can also lead to erroneous estimates of energy expenditure because of device orientation as only acceleration in the direction of motion provides useful work (Gleiss et al., 2011). Those issues explain why the relationship between DBA and metabolism is different between flying and walking (Laich et al., 2011; Elliott et al., 2013a). Indeed, several studies have failed to find a relationship between DBA and energy expenditure, either when body mass variation may have confounded relationships (Guillemette and Butler, 2012; Dalton et al., 2014; Volpov et al., 2015; M. Kristiansen, Can energy expenditure of free-ranging kittiwakes be estimated by body acceleration? MSc thesis, University of Tromsø, 2014) or when cardiovascular adjustments during diving overwhelmed any relationship with mechanical work (Halsey et al., 2011; Meir et al., 2013). It is therefore important to validate DBA in free-living animals, especially across different locomotory modes, temperatures and other vagaries present in the wild environment.

There remains debate over the proper approach to take in formulating a useable measure of DBA. One of the earliest measures, termed overall dynamic body acceleration (ODBA), was calculated as the summation of acceleration in three orthogonal spatial axes (Wilson et al., 2006). However, Gleiss et al. (2011) raised concerns about ODBA, arguing non-independence in the

axes of motion and advocating instead for the use of vectorial dynamic body acceleration (VeDBA). In comparing these two interpretations of DBA, Qasem et al. (2012) found little difference between the VeDBA and ODBA. However, this study was self-acknowledged as being limited in scope, given its investigation into only a single activity (treadmill walking) in humans and a small number of captive animal species. The authors went on to suggest that disparity between these two formulations may ultimately be dependent upon the type of motion recorded, the type of animal and gait. Therefore, a logical next step is to investigate VeDBA and ODBA in wild organisms that exhibit a range of locomotory modes.

Cormorants provide an interesting model for testing both the validity of DBA in the wild and the alternate formulations of DBA because they use four different locomotory modes (movement on land, flying, surface swimming and diving), and DBA has been extensively validated for cormorants in the laboratory (Wilson et al., 2006; Laich et al., 2011; but see Halsey et al., 2011). Daily energy expenditure has seldom been reported in the order Suliformes (Shaffer, 2011; referred to there as 'Pelecaniformes' from which Suliformes has recently been split), only once in cormorants (Keller and Visser, 1999), and never in a small cormorant species, presumably because many cormorants are very sensitive to disturbance. The two-sample DLW method requires holding large animals for over an hour, and that lengthy period of restraint can cause abandonment of offspring or altered behaviour (Schultner et al., 2010; Hinsley et al., 2011). Obtaining direct estimations of daily energy expenditure would be useful for validating estimates from bioenergetic models used to quantify the impact of cormorants on fisheries (Ridgway, 2010). Such values would complement estimations of metabolism obtained from cormorants in dive tanks using respirometry (e.g. Schmid et al., 1995; Enstipp et al., 2005, 2006, 2007) or in the wild using heart rate (Bevan et al., 1997; Grémillet et al., 2005). Cormorants spend a large proportion of their active time diving (Kotzerka et al., 2011), and diving could be an important component of daily energy expenditure. As cormorants have wettable plumage, they are less buoyant than most seabirds, but are still positively buoyant at the surface and work harder to descend than to ascend (Cook et al., 2008, 2010).

Pelagic cormorants (*Phalacrocorax pelagicus* Pallas 1811) are the most widely distributed and abundant cormorant in the North Pacific, yet because they are sensitive to disturbance and nest in small, loose colonies on steep cliffs, information on many aspects of their biology is limited. Apart from early reports from observations at the surface (Hobson and Sealy, 1985; Cooper, 1986) and a recent paper at our study site (Kotzerka et al., 2011), the underwater foraging behaviour is largely undescribed and daily energy expenditure has not been documented. To provide additional insight into the foraging behaviour of this species, we attached accelerometers for 24–96 h to wild pelagic cormorants while simultaneously estimating daily energy expenditure using DLW. We used a single-sample approach with only 15 min handling time to reduce disturbance to the bird. Our primary objective was to determine whether DBA predicted daily energy expenditure in a foot-propelled diving bird, the pelagic cormorant, that also walks and flies. Secondary objectives were to estimate the daily energy expenditure of small, wild cormorants for the first time, and to compare ODBA and VeDBA formulations in the wild.

MATERIALS AND METHODS

Our observations were made at the Middleton Island 'tower' colony (59°26' N, 146°20' W), Alaska, USA, which is situated at the edge of the continental shelf in the northern Gulf of Alaska. Several

factors affect cormorant populations on the island. The 1963 earthquake moved the entire island up by several metres and the western cliffs that once fell directly into the ocean are now several hundred metres from the shore, and have eroded over time into a gentler slope. Cormorants are a major component of eagle pellets on the island in summer (T. van Nus, personal communication) and eagle population recovery (Middleton: from zero pairs in 1984 to five pairs in 2012, with many more subadults) has impacted cormorant populations throughout the Pacific Northwest, but especially at Middleton, where they are more accessible because of more gently sloping sites (Elliott et al., 2011). During the 1977–2008 warm-water regime, capelin (*Mallotus villosus*) largely disappeared from the diet, with negative impacts on many seabird species (Hatch, 2013). Decreased site quality, increased predation and reduced food availability have reduced the cormorant population on the island from a high of ~7000 pairs in the late 1970s to 535 active nests in 2012, which still places Middleton Island as one of the largest pelagic cormorant colonies in the world. Pairs were accessible in the tower colony, to which artificial wooden ledges have been added for research, viewable from inside the building through sliding panes of one-way mirror glass (Gill and Hatch, 2002). Cormorant productivity on the tower was average in 2012, a cold-water year (46 fledglings from the 85 tower sites, but only 16 nestlings present mid-chick-rearing from the entire rest of the island because of high predation), and so daily energy expenditure likely reflected typical foraging conditions.

We used males exclusively in behavioural analyses to reduce variation in body mass as a confounding variable when correlating daily energy expenditure with DBA (larger individuals have higher energy expenditure, but, because of Newton's second law, lower acceleration for a given force). Furthermore, when we initially captured three females, two abandoned and one did not return for several days. Males showed no abandonment and appeared to be bolder and less stressed following capture. We identified males by their larger bill size. All birds in the study were marked with a steel band and a laminated plastic band numbered for individual identification.

All work adheres to The Association for the Study of Animal Behaviour/Animal Behaviour Society Guidelines for the Use of Animals in Research. Methods were approved by the University of Manitoba in accordance with the guidelines of the Canadian Council on Animal Care (protocol no. F11-020). Devices were ~1% of body weight, which is below the recommended limit of 3%, yet likely the handling still had an impact. We used non-breeding individuals for 3 h restraints to minimize impact on reproductive success. We used a single-sample method (handling time <15 min) to minimize handling stress. We used tail-mounted accelerometers because birds acted agitated and preened back-mounted accelerometers.

Accelerometry

We caught 23 chick-rearing pelagic cormorants on their nests with a metal hook through a slit underneath a one-way window installed at each nest in the tower. All cormorants were wrapped tightly in a towel and measured on an Ohaus electronic scale (± 1 g). Handling time for cormorants was always less than 15 min, during which time we attached CEFAS G6A accelerometers (18 g; sampling interval=1 s for pressure; sampling interval=25 Hz per axis for triaxial acceleration; grooves sanded in for attachment) to the four central tail retrices using two cable ties. We recaptured the birds after 24–96 h. One of the accelerometers fell off before retrieval and for five of the deployments acceleration was accidentally recorded at 1 Hz when the bird was not diving. Consequently, our sample size

was 17 birds. We used the pressure log to determine time spent underwater and the acceleration logs to determine when the bird was flying, on land or swimming at the water surface (Laich et al., 2008). ODBA was calculated as:

$$\text{ODBA} = |A_x - \bar{A}_x| + |A_y - \bar{A}_y| + |A_z - \bar{A}_z|, \quad (4)$$

and VeDBA was calculated as:

$$\text{VeDBA} = \sqrt{(A_x - \bar{A}_x)^2 + (A_y - \bar{A}_y)^2 + (A_z - \bar{A}_z)^2}, \quad (5)$$

where A_n is the measured acceleration in direction n and \bar{A}_n is the 2-s running mean representing the static component to acceleration. All accelerometer data are archived (Dryad Digital Repository doi:10.5061/dryad.23td2; see Elliott et al., 2013b).

Doubly labelled water

The two-sample DLW technique requires holding the cormorant for 3 h to allow the injectate to equilibrate with body water, and, thus, to quantify dilution space of the isotope. Given that extensive restraint alters birds' behaviour, and consequently daily energy expenditure (Schultner et al., 2010; Hinsley et al., 2011), we attempted a modified two-sample technique. To reduce disturbance and associated possibility of abandonment with a costly accelerometer attached, we injected the DLW intravenously and our second sample was taken only 12 min after injection to reduce the impact of extended restraint on behaviour. In a second set of birds that were not equipped with accelerometers, samples were taken after both 12 min and 3 h so that the equilibrium blood sample could be estimated from the 12 min blood sample. However, the single-sample method based on body mass was more accurate than the modified two-sample approach, and we therefore used the single-sample approach. Even with our modified method, the single female we attempted proved very difficult to recapture (recaptured only after isotopes had diluted to near-background levels), and we only included males in the present study.

We estimated daily energy expenditure using a single-sample approach after we were unsuccessful with our modified two-sample approach. Given the strong correlation between body mass and isotope dilution (Fig. 1), we are confident that the single-sample approach provided accurate values. At the time of first capture, we

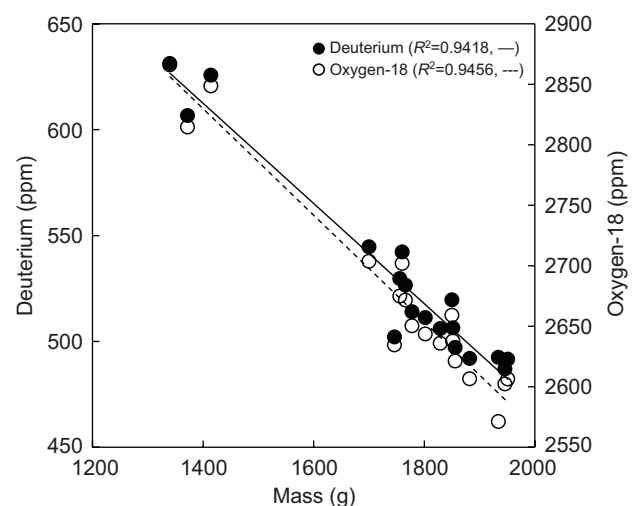


Fig. 1. Oxygen-18 and deuterium were inversely correlated with body mass following injection with doubly labelled water for pelagic cormorants at Middleton Island in 2012.

injected intravenously 1.2 ml of DLW (50% H₂¹⁸O and 25% D₂O; see Elliott et al., 2013b for details). We injected 40 birds. For 17 birds, we obtained a blood sample from the brachial vein of the opposing wing at 12 min and 3 h. For 23 birds, we obtained blood samples only at 12 min. We only attached accelerometers to those 23 birds sampled after 12 min because the shorter sampling time was believed to reduce stress on the bird. We used the other 17 birds to determine a relationship between dilution space at 12 min and dilution space at 3 h. However, the relationships between isotope values at 12 min and 3 h were poor ($R^2=0.27-0.29$; see Elliott et al., 2013b). In contrast, body mass predicted isotope values well (Fig. 1). We consequently used a single-sample method to calculate equilibrium ('initial') isotope values using the plateau method at 3 h for the 17 birds used in the accelerometry study, as detailed in Elliott et al. (2013b).

We used a value of 0.85 for the respiratory quotient. All DLW calculations occurred in the Speakman laboratory blind to the accelerometry measurements that were calculated by an IgorPro script. As we were interested in activity-specific metabolic rates (and had measured activity independently), we assumed that activity-specific metabolic rates did not vary significantly with time of day (i.e. diel variation in energy expenditure was related primarily to diel variation in activity) and did not adjust values that were collected away from 24-h cycles. All DLW data are openly archived (Dryad Digital Repository doi:10.5061/dryad.23td2; see Elliott et al., 2013b).

Given that daily energy expenditure has only been reported once previously for cormorants, and never previously for a small cormorant, we report the actual daily energy expenditure value. It might be expected that cormorants raising two chicks would have higher daily energy expenditure than those raising one chick, and so we use a *t*-test to compare values between cormorants with two chicks and cormorants with one chick.

Statistical analyses

We performed simple linear regressions to compare how total VeDBA and total ODBA compared against a null model of duration of deployment in predicting DLW-estimated energy expenditure. However, as each activity (resting on land, surface swimming, diving and flying) is likely to have different costs and different DBA–metabolic rate relationships, we estimated energy expenditure using a multiple regression. As we were concerned about the overparameterization of our models because of a high ratio of parameters to sample size in some models, we used an information theoretic approach. Specifically, we compared the effectiveness of different time budget and DBA models using an Akaike's information criterion (AIC) approach, which penalizes models that increase the number of parameters without improvement in fit. Additionally, a finite sample size correction, a technique which additionally penalizes parameters, was applied to AIC scores because of the small sample size ($n=17$).

First, we considered time budget models separately parameterizing the aforementioned activities:

$$EE = \text{DMR} \cdot T_d + \text{FMR} \cdot T_f + \text{SMR}_s \cdot T_s + \text{RMR}_l \cdot T_l + \alpha, \quad (6)$$

where EE is the total DLW-estimated energy expenditure during the sampling period, DMR is diving metabolic rate, FMR is flying metabolic rate, SMR_s is surface swimming metabolic rate on the water, RMR_l is resting metabolic rate on land, T_d is time spent diving, T_f is time spent flying, T_s is time spent surface swimming, T_l is time spent on land and α is the intercept. The metabolic rates for

each activity were determined using a multiple linear regression to provide DMR, FMR, SMR and RMR. The values were therefore averages within each activity and each includes relatively energy-intensive periods. For example, preening and walking at the colony would be included in RMR_l, and active prey chasing in DMR.

Second, we considered models where energy expenditure was proportional to our formulations of DBA, with a different calibration coefficient for each activity:

$$EE = a \cdot \text{DBA}_d + b \cdot \text{DBA}_f + c \cdot \text{DBA}_s + d \cdot \text{DBA}_l + \alpha, \quad (7)$$

where DBA_{*n*} is total DBA (the integral of DBA over all time spent in each activity *n*) in each activity: diving, flying, surface swimming and activity on land. In time budget as well as VeDBA models, we also considered models in which activities were combined in place of separate parameterization.

Beyond mechanical costs and activity specific metabolic rates, there are physiological processes that must be considered when predicting energy expenditure. Oxygen consumption in penguins declines exponentially with dive duration (Stockard et al., 2005) as birds enter a state of hypometabolism where heart rate and peripheral body temperature decrease and blood is shunted past non-vital organs. The same exponential model improves estimation of dive costs in auks (Elliott et al., 2013b). Several authors have observed that activity does not predict energy consumption during diving by homeotherms (Halsey et al., 2011; Meir et al., 2013); one possible explanation for this is that activity costs are overwhelmed by hypometabolism. Evidence for such hypometabolism has been observed in other cormorant species (Bevan et al., 1997). Changes in heart rate and body temperature reduce oxygen consumption during the dive. Therefore, we compared VeDBA models against those that included a term accounting for non-linear hypometabolism during the dive in place of DBA_d:

$$EE = a \cdot \sum (1 - e^{0.79D}) + b \cdot \text{DBA}_f + c \cdot \text{DBA}_s + d \cdot \text{DBA}_l + \alpha, \quad (8)$$

where the summation is taken over all dives of duration *D*. The exponential form of the hypometabolism is derived from the shape of oxygen utilization in penguins (fig. 7 in Stockard et al., 2005). The same model provided a good fit for murre (Elliott et al., 2013b). The exponential model has been altered from the exponent of 1.23 in the murre model (Elliott et al., 2013b) to a value of 0.79 based on cormorant average dive duration being roughly 64% of that recorded in the murre study. For theoretical reasons, DBA should correlate with mass-specific energy expenditure, and we show all results on a mass-specific basis.

Typically, the strength of a model is estimated using the coefficient of determination (R^2). However, the DLW method only estimates daily energy expenditure at the level of the individual within $\sim\pm 18\%$ (Speakman, 1997; Butler et al., 2004; Jones et al., 2009; Shaffer, 2011), including in auks (Shirai et al., 2012). To determine how that level of inaccuracy could impact the R^2 value, we ran 1000 simulations of our DLW-estimated daily energy expenditure values. Each simulation varied daily energy expenditure by a randomly generated amount, where the randomly generated amount followed a normal distribution with 95% confidence intervals of $\pm 18\%$. The average R^2 value of the randomly generated data and our actual data was 0.949. That is, a technique that accurately explained all of the variation in daily energy expenditure and that was 100% precise could only be expected to have an R^2 value of 0.949. Virtually all of the

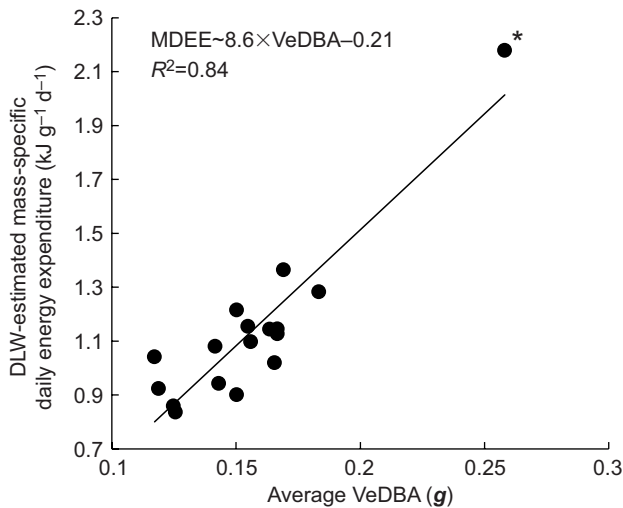


Fig. 2. Average vectorial dynamic body acceleration (VeDBA; g) versus doubly labelled water (DLW)-estimated mass-specific daily energy expenditure (MDEE; $\text{kJ g}^{-1} \text{d}^{-1}$) for pelagic cormorants at Middleton Island in 2012 ($R^2=0.84$, $P<0.0001$). $R^2=0.42$ with removal of outlying point denoted by asterisk.

explainable variance was accounted for, given that our best-fit time budget and VeDBA models had R^2 values of 0.90–0.94. Therefore, we report deviations from measured values as a measure of the goodness-of-fit of our models.

RESULTS

DLW-estimated mass-specific daily energy expenditure was positively correlated with average ODBA ($R^2=0.83$, $t_{15}=8.7$, $P<0.0001$) and average VeDBA ($R^2=0.84$, $t_{15}=8.819$, $P<0.0001$; Fig. 2). Likewise, DLW-estimated mass-specific total energy expenditure was positively correlated with total (integrated over entire deployment period) ODBA ($R^2=0.91$, $t_{15}=12.43$, $P<0.0001$) and total VeDBA ($R^2=0.91$, $t_{15}=12.64$, $P<0.0001$; Fig. 3A). Conversely, DLW-estimated mass-specific total energy expenditure was only weakly correlated with duration of deployment ($R^2=0.42$, $t_{15}=3.542$, $P<0.01$; Fig. 3B), demonstrating that the correlation between total DBA and DLW-estimated mass-specific total energy expenditure was not entirely due to both variables being correlated with deployment duration. This interpretation is supported by the significance of both duration of deployment ($P<0.0001$) and average VeDBA ($P<0.0001$) when concomitantly considered in a multiple linear regression against DLW-estimated mass-specific total energy expenditure.

Given the inherent relationship between duration of deployment and total DBA, we also compared total DBA ($n=15$) and time budget ($n=15$) models that uniquely parameterized four different activities (flying, diving, surface swimming and resting on land) using AIC evaluation (Tables S1, S2). Because of the strong relationship between total ODBA and total VeDBA ($R^2=0.99$, $t_{15}=211.4$, $P<0.0001$), we only considered a single total DBA formulation for AIC analysis. Total ODBA was omitted given its marginally poorer performance and because of the theoretical arguments put forward by Gleiss et al. (2011) and Qasem et al. (2012). The most parsimonious model identified by AIC analysis for predicting DLW-estimated mass-specific total energy expenditure was a total VeDBA model parameterizing surface swimming separate from all other modes of locomotion (Table 1).

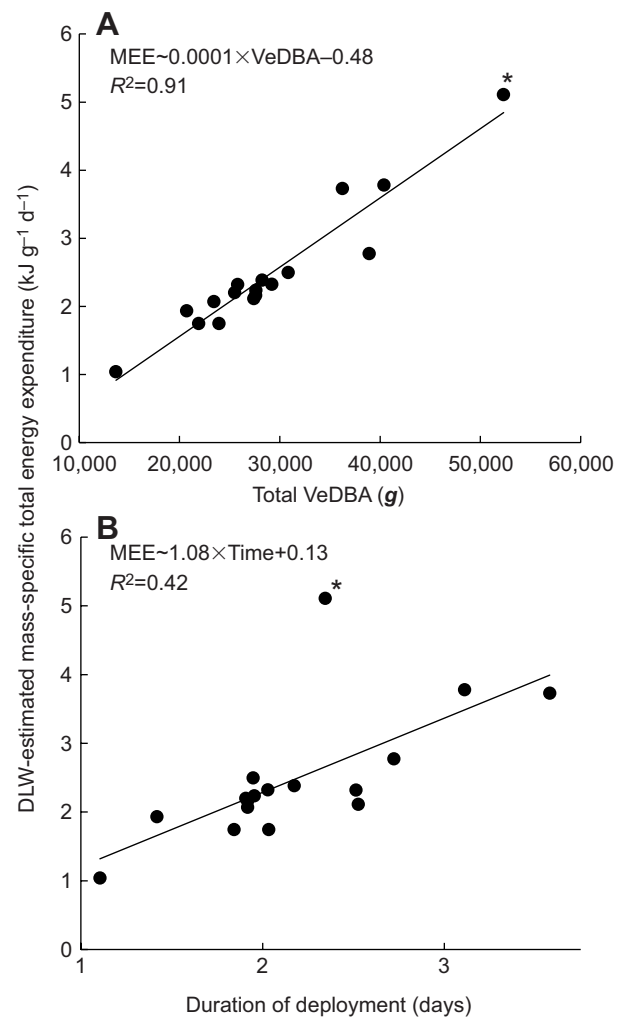


Fig. 3. Comparisons between DLW-estimated mass-specific total energy expenditure (MEE) and either total VeDBA or deployment duration over 24–96 h for pelagic cormorants at Middleton Island in 2012. (A) Total VeDBA (g) versus MEE ($P<0.0001$); (B) deployment duration versus MEE ($P<0.01$). $R^2=0.83$ and 0.79 for A and B, respectively, with removal of non-significant outlying points denoted by asterisks.

Not only did the top time budget model exhibit greater deviation from DLW-estimated values than the top total VeDBA model (± 0.22 versus $\pm 0.19 \text{ kJ g}^{-1}$, respectively; Table S1), but time budget models were also highly sensitive to activity parameterization in comparison to the relatively robust total VeDBA models. This is made apparent by the difference in average absolute deviation between DLW estimates and predictions of mass-specific total energy expenditure made by time budget and identically parameterized total VeDBA models (paired t -test, $P<2.9 \times 10^{-5}$, $t_{14}=-6.07$; Fig. 4). In a secondary AIC analysis, models replacing total $\text{VeDBA}_{\text{diving}}$ with a term meant to represent hypometabolic processes while diving were identified as more parsimonious (Table 2). A single outlying individual spent 2.46 times as much time diving as the group average and had a daily energy expenditure 1.89 times higher than the group average. However, including that individual did not change the order of any of the models presented in Table 1.

Daily energy expenditure was similar between birds with one chick ($2166 \pm 89 \text{ kJ day}^{-1}$) and those with two chicks ($2205 \pm 101 \text{ kJ day}^{-1}$; $t_{21}=0.79$, $P=0.26$).

Table 1. AIC comparison of total vectorial dynamic body acceleration (VeDBA; $n=15$) and time budget ($n=15$) models for explaining doubly labelled water (DLW)-estimated mass-specific total energy expenditure (kJ g^{-1}) in wild pelagic cormorants ($n=17$) during four locomotory modes: flying, diving, surface swimming and movement on land

Model	$\Delta\text{AICc}^{*,\ddagger}$	AICc weight	Deviation
$\text{VeDBA}_s + \text{VeDBA}_{\text{all other modes}}^{\S}$	0.00	0.36	± 0.19
$\text{VeDBA}_{\text{all modes}}$	0.32	0.26	± 0.21
$\text{VeDBA}_{f+s} + \text{VeDBA}_{l+d}$	1.15	0.11	± 0.19
$\text{VeDBA}_{f+d} + \text{VeDBA}_{l+s}$	1.39	0.09	± 0.19
$\text{VeDBA}_f + \text{VeDBA}_l + \text{VeDBA}_s + \text{VeDBA}_d$	6.93	0.00	± 0.18
$T_f + T_l + T_s + T_d$	8.08	0.00	± 0.21
$T_{\text{all modes}}$	31.74	0.00	± 0.21

Average absolute deviation between model predicted and DLW-estimated values are reported as a goodness-of-fit measure. All potential time and total VeDBA models were considered; only those with $\Delta\text{AICc} \leq 2$ are presented in addition to total VeDBA and time budget models in which all locomotory modes are combined and those in which modes are separately parameterized. Subscripts are as follows: d, diving; f, flying; l, movement on land; s, surface swimming.

*A finite sample size correction was applied to all AIC values.

\ddagger An additional parameter was added to each model for intercept estimation.

\S Equation for most parsimonious model was: mass-specific energy expenditure = $(5.6 \times 10^{-5} \pm 2.7 \times 10^{-5}) \text{VeDBA}_{\text{surface}} + (1.1 \times 10^{-4} \pm 9.0 \times 10^{-5}) \text{VeDBA}_{\text{all other modes}} - 0.34 \pm 0.24$.

DISCUSSION

Bioenergetic insights offered by DBA

Average ODBA and average VeDBA were strongly correlated ($R^2=0.83$ and 0.84 , respectively) with DLW-estimated mass-specific daily energy expenditure in free-living pelagic cormorants capable of four locomotory modes (activity on land, flying, foot-propelled diving and surface swimming). Similarly, both total ODBA and total VeDBA were strongly correlated with DLW-estimated mass-specific total energy expenditure. The strength of these relationships between DLW-estimated mass-specific total energy expenditure and both measures of DBA were near identical ($R^2=0.91$). Despite the concerns of Gleiss et al. (2011) regarding a summative formulation of ODBA, we, like Qasem et al. (2012), found that there was little difference in the correlative strength of

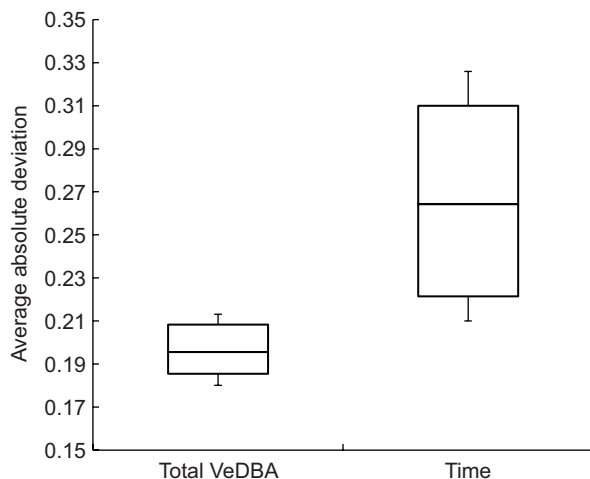


Fig. 4. Average absolute deviation between DLW-estimated mass-specific total energy expenditure (kJ g^{-1}) and predictions made by total VeDBA ($n=15$) and time budget ($n=15$) models. Boxes display mean and interquartile range, whiskers denote maximum and minimum; paired two-tailed t -test, $P < 0.0001$, $t_{14} = -6.07$.

Table 2. AIC comparison of total VeDBA ($n=15$) models with the addition of a term representing hypometabolic processes while diving (in place of VeDBA_d ; $n=5$) for explaining DLW-estimated mass-specific total energy expenditure (kJ g^{-1}) in wild pelagic cormorants ($n=17$) during four locomotory modes: flying, diving, surface swimming and movement on land

Model	$\Delta\text{AICc}^{*,\ddagger}$	AICc weight	Deviation
$\text{VeDBA}_{f+l} + \text{VeDBA}_s + \sum(1 - e^{-0.79D})^{\S}$	0.00	0.57	± 0.16
$\text{VeDBA}_{\text{all other modes}} + \sum(1 - e^{-0.79D})$	0.74	0.27	± 0.17
$\text{VeDBA}_{f+s} + \text{VeDBA}_l + \sum(1 - e^{-0.79D})$	1.89	0.08	± 0.17
$\text{VeDBA}_s + \text{VeDBA}_{\text{all other modes}}$	3.37	0.02	± 0.19

Average absolute deviation between model predicted and doubly labelled water-estimated values are reported as a goodness-of-fit measure. All potential total VeDBA models were considered; only those with $\Delta\text{AICc} \leq 2$ are presented in addition to the top non-hypometabolism total VeDBA model. Subscripts are as follows: f, flying; l, movement on land; s, surface swimming. D , duration.

*A finite sample size correction was applied to all AIC values.

\ddagger An additional parameter was added to each model for intercept estimation.

\S Equation for most parsimonious model: mass-specific energy expenditure (kJ g^{-1}) = $(1.06 \times 10^{-4} \pm 1.5 \times 10^{-5}) \text{VeDBA}_{f+l} + (5.5 \times 10^{-5} \pm 2.4 \times 10^{-5}) \text{VeDBA}_s + (4.07 \times 10^{-3} \pm 3.77 \times 10^{-4}) \sum(1 - e^{-0.79D}) - 0.22 \pm 0.26$.

these two measures, even after expanding the scope of our investigation to include a wider range of locomotory activities. Our results provide additional support for the use of DBA to estimate energy expenditure of wild animals (Williams et al., 2004; Wilson et al., 2006; Halsey et al., 2009; Duriez et al., 2014; Bishop et al., 2015). Total DBA correlated more strongly with DLW-estimated energy expenditure than DBA averaged over the duration of device deployment. This is unsurprising given that total DBA would be strongly correlated with time of deployment, and therefore total energy expenditure, despite a relatively weak relationship between DLW-estimated mass-specific total energy expenditure and duration of deployment ($R^2=0.42$). Using AIC evaluation, we compared time budget and total VeDBA models while simultaneously evaluating the need for separate activity parameterization.

In comparing total VeDBA and time budget derived models, the inclusion of DBA parameters greatly improved model parsimony over time-based terms (Table 1). The most parsimonious model identified by AIC analysis parameterized surface swimming separately from all other modes of locomotion. The slope for total VeDBA during surface swimming (5.5×10^{-5} , footnote to Table 2) was approximately half that of total VeDBA for all other modes of locomotion (1.06×10^{-4}). Perhaps DBA at the water surface partially represented wave action, explaining why the slope was smaller. Furthermore, given the cold and wet climate of Middleton Island, it is likely that the cormorants undergo activity–thermoregulatory substitution, and so under different environmental conditions, biomechanical activity could be used to offset thermoregulatory energetic costs (Humphries and Careau, 2011). That total $\text{VeDBA}_{\text{flight}}$ was not included as a separately parameterized term in the most parsimonious model for predicting DLW-estimated mass-specific total energy expenditure values (Table 1) was surprising. Previous work has demonstrated that DBA is not directly linked to mechanical flight costs (Spivey and Bishop, 2013), and we suggest the use of the formulae presented by Spivey and Bishop in future analyses of flight costs in wild birds.

Despite these confounding factors, both measures of DBA (ODBA and VeDBA) proved to be accurate predictors of DLW-estimated mass-specific total energy expenditure. Even when distinct activities were not separately parameterized, the strength

of correlation between total ODBA/total VeDBA and DLW-estimated mass-specific total energy expenditure ($R^2=0.91$) is comparable to what has been observed in cormorants for separately considered activities under laboratory conditions (Wilson et al., 2006; Laich et al., 2011). Furthermore, DBA models performed better under AIC analysis when compared with time budget models, supporting the view that accelerometry can be used to predict energy expenditure in the wild. Although the top time budget and total VeDBA models explained similar amounts of variation, the predictive power of total VeDBA models remained relatively high regardless of activity parameterization whereas time budget models were extremely sensitive to the same changes (Table S1). Thus, total VeDBA appeared to be a useful heuristic for comparing energy costs across locomotory modes. This view is supported by the fact that the only activity separately parameterized in the top total VeDBA model (surface swimming) was the activity most subject to confounding environmental vagaries and thus had a smaller calibration coefficient than all other activities (Table 2).

Although VeDBA declined during dives because of reduced buoyancy as air volume was compressed and distinct VeDBA profiles associated with dive phases were observed (Cook et al., 2010), dive costs decreased more sharply with depth than implied by biomechanics alone. In other diving homeotherms, activity does not correlate well with energy expenditure (Fahlman et al., 2008; Halsey et al., 2011; Meir et al., 2013; Volpov et al., 2015). We argue that in addition to mechanical activity, dive costs are strongly affected by the suppression of oxygen consumption via reduced heart rate, body temperature, shunting of blood past non-vital organs and other mechanisms, and that physiology, in addition to physics, plays an important role in oxygen consumption during dives. This interpretation is supported by the fact that when physiology and mechanical activity were considered in the AIC model competition, we found that an exponential term meant to account for diving hypometabolism consistently outcompeted a biomechanical activity term. Several authors have calculated diving costs of breath-hold diving homeotherms using biomechanics alone (Lovvorn et al., 2009; Goldbogen et al., 2012; Potvin et al., 2012). Given the importance of physiology, we suggest that such calculations are likely to be inaccurate representations of actual diving costs based on the various attempts to validate such models (Fahlman et al., 2008; Halsey et al., 2011; Meir et al., 2013). Rather, an exponential decrease in metabolism, associated with reduced temperature, heart rate and shunting of blood away from non-essential organs, provided a better fit and was included in all of the most parsimonious models (Table 2).

Total VeDBA models consistently performed well, predicting DLW-estimated mass-specific total energy expenditure within $0.20\pm 1.2\times 10^{-2}$ kJ g⁻¹ on average. This is in contrast to time budget models, which had an average absolute deviation from DLW estimates of $0.26\pm 4.4\times 10^{-2}$ kJ g⁻¹, meaning that, on average, the worst total VeDBA model and the best time budget models were equally deviant from DLW-estimated mass-specific total energy expenditure values (Fig. 4). Although a sample size of $n=17$ increases vulnerability to model overfitting, we do not believe that to be the primary driver of the observed difference between total VeDBA and time budget models. The AIC approach we have taken avoids overfitting by penalizing additional parameters, and the finite sample size correction we have used is specifically designed for handling small sample sizes. If the goodness-of-fit observed in total VeDBA models was an artefact of overfitting, then similarly parameterized time budget models would be expected to perform equally as well. Additionally, if performance by total VeDBA

models was merely a product of overfitting, then goodness-of-fit would correlate with number of model parameters, which is not the case (Table S1). The fact that goodness-of-fit remains high regardless of activity parameterization leads us to conclude that total VeDBA is a more robust metric than activity time budgets for estimating energy expenditure in free-ranging organisms. Although an activity time budget approach can yield energy expenditure estimates that are nearly as accurate as total VeDBA models, this is only after optimal parameterization.

Low daily energy expenditure in pelagic cormorants despite high flight and dive costs

Compared with Suliformes of similar mass, the pelagic cormorant exhibited low daily energy expenditure (Shaffer, 2011), despite relatively high flight and dive costs (Videler, 2005; Elliott et al., 2013b). Flight costs (158 W) averaged from the best-fit model were similar to those reported previously using the same dataset and using only the time budget approach, and are the highest of any bird recorded to date (Elliott et al., 2013b). Given the compromises inherent in moving in three media (land, air and water), it is unsurprising that flight in cormorants and other diving birds are especially high (Lovvorn and Jones, 1994; Videler, 2005; Sato et al., 2007; Elliott et al., 2013b). Wing morphology in diving birds cannot be optimized solely for flight, but must also be altered to reduce drag and buoyancy underwater even for those birds, such as cormorants, that use their feet for locomotion. Wing loading in cormorants ($\sim 100\text{--}150$ N m⁻²) is higher than most other flying, non-pursuit-diving seabirds, including other Suliformes, although not as high as auks or flightless seabirds (Elliott et al., 2013b). Likewise, body mass and aspects of internal physiology in diving birds that also fly is a compromise between what is optimal for diving (e.g. large body, high myoglobin concentration in muscle) and what is optimal for flight (e.g. small body, low myoglobin concentration in muscle to provide more space for mitochondria to power flight; Croll et al., 1992; Elliott et al., 2010). Apart from morphological constraints, flight costs in cormorants may also be high because they have wettable plumage. In the rainy environment of Middleton Island, it may not be possible to completely dry plumage before flight, leading to high flight costs. The high cost of flight likely explains why cormorants cannot soar, unlike most other Suliformes, and why cormorants make only short flying commutes to foraging sites (Watanabe et al., 2011). Interestingly, we observed no difference in DLW-estimated daily energy expenditure between males raising one versus two chicks, despite evidence for such a relationship in other seabirds (Welcker et al., 2015). It is possible that the cormorants were unable to increase offspring provisioning, having reached an energetic ceiling (Tinbergen and Verhulst, 2000). Such an energetic ceiling may be a contributing factor in the low nest survivorship observed in the Middleton Island population and the obligate siblicide present in other sulids (Drummond et al., 1986).

Conclusions

We validated DBA as a technique for estimating energy expenditure in cormorants which were free-ranging over periods of 24–96 h, but not necessarily for very fine-scale behaviours. For finer scale behaviours, it would be interesting to measure heart rate and DBA simultaneously (Bishop et al., 2015). Both ODBA and VeDBA proved equally suitable in estimating energy expenditure. Furthermore, DBA proved a more robust metric of estimating energy expenditure than activity time budgets, which were highly sensitive to activity parameterization. Despite the strong

relationship found between DBA and energy expenditure, we also found moderate evidence for hypometabolic processes while diving. Pelagic cormorants exhibited relatively low daily energy expenditure compared with similarly sized Suliformes, despite higher than expected flight and dive costs. It is likely that these high activity costs influence the foraging behaviour described in the present and previous studies of the Middleton Island population.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

M.R.S. and K.H.E. wrote the paper and conducted data analysis. T.W., S.A.H. and K.H.E. completed the field work. J.R.S. conducted the doubly labelled water analyses and associated calculations.

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Supplementary information

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