Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant

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Summary

1. Time and energy are key currencies in animal ecology, and judicious management of these is a primary focus for natural selection. At present, however, there are only two main methods for estimation of rate of energy expenditure in the field, heart rate and doubly labelled water, both of which have been used with success; but both also have their limitations.

2. The deployment of data loggers that measure acceleration is emerging as a powerful tool for quantifying the behaviour of free-living animals. Given that animal movement requires the use of energy, the accelerometry technique potentially has application in the quantification of rate of energy expenditure during activity.

3. In the present study, we test the hypothesis that acceleration can serve as a proxy for rate of energy expenditure in free-living animals. We measured rate of energy expenditure as rates of O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) in great cormorants (*Phalacrocorax carbo*) at rest and during pedestrian exercise. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were then related to overall dynamic body acceleration (ODBA) measured with an externally attached three-axis accelerometer.

4. Both $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were significantly positively associated with ODBA in great cormorants. This suggests that accelerometric measurements of ODBA can be used to estimate $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ and, with some additional assumptions regarding metabolic substrate use and the energy equivalence of O_2 and CO_2, that ODBA can be used to estimate the activity specific rate of energy expenditure of free-living cormorants.

5. To verify that the approach identifies expected trends in $\dot{V}_{O_2}$ from situations with variable power requirements, we measured ODBA in free-living imperial cormorants (*Phalacrocorax atriceps*) during foraging trips. We compared ODBA during return and outward foraging flights, when birds are expected to be laden and not laden with captured fish, respectively. We also examined changes in ODBA during the descent phase of diving, when power requirements are predicted to decrease with depth due to changes in buoyancy associated with compression of plumage and respiratory air.

6. In free-living imperial cormorants, ODBA, and hence estimated $\dot{V}_{O_2}$, was higher during the return flight of a foraging bout, and decreased with depth during the descent phase of a dive, supporting the use of accelerometry for the determination of activity-specific rate of energy expenditure.

Key-words: heart rate, doubly-labelled water, dynamic acceleration, energy expenditure, oxygen consumption

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Introduction

Rate of energy expenditure is a cornerstone to understanding animal ecology. All animals expend energy and judicious expenditure, mediated via movement of body parts (e.g. in locomotion; Alexander 2003) or physiological/behavioural traits (e.g. hibernation/torpor; Geiser 2004) is critical in survival and thus is a primary focus for natural selection (Brown *et al.* 2004). Comprehensive investigations into the behavioural ecology of animals therefore necessitate that energy turnover and, in particular, the allocation of energy to specific activities, be measured (McNamara & Houston 1996). Currently, however, there are only two major methods for estimating rate of energy expenditure (metabolic rate) in the field, the doubly labelled water method, which provides an estimate of total production of carbon dioxide over the experimental period, and the heart-rate method which, when appropriately calibrated, provides an estimate of rate of oxygen consumption over relatively short as well as over long time periods.

Although both these methods have been used with varying degrees of success, both have their limitations; the doubly labelled water method generally cannot resolve the energetic costs of specific activities while the heart rate method normally involves surgical implantation (and removal) of loggers, with all the operational complications that this involves (see Butler *et al.* 2004 for detailed discussion). Here, we propose a third method for estimating activity-specific metabolic rates in the field using accelerometry. Accelerometers are tiny, inexpensive transducers that can be integrated into loggers for deployment on animals (Ropert-Coudert & Wilson 2005). Such units can be attached to animals externally in a matter of seconds or minutes using conventional tag-attaching techniques (Yoda *et al.* 2001; Watanabe *et al.* 2005). As such, these loggers are accessible, useful tools for a suite of biologists. Internal implantation of these loggers is also a possibility, at least on avian species, and although a more complex method of deployment, circumvents the potential impact on the subject animal of external deployment (Ropert-Coudert *et al.* 2000).

The overall rate of energy expenditure of an adult animal at any one time can be allocated primarily to four bodily functions; basal metabolic rate (BMR) (Frappell & Butler 2004; McKechnie & Wolf 2004; McKechnie, Freckleton & Jetz 2006), temperature-dependent energetic expenditure (Beamish 1990), specific dynamic action (SDA) (e.g. Hawkins *et al.* 1997) and movement (e.g. Alexander 2003). The magnitude of these elements is relatively well understood (White & Seymour 2005) and values for BMR and SDA can be ascribed fairly accurately for free-living animals as a result of indirect calorimetric studies undertaken in the laboratory (Rosen & Trites 1997). In addition, temperature-dependent rate of energy expenditure can be alluded to if the environmental conditions to which the animals are exposed are known (McNab 2002).

Animal activity is defined largely in terms of movement, and muscular contraction, which requires the expenditure of energy, is responsible for this movement (King, Loiselle & Kohl 2004). Hence, accurate quantification of movement should correlate with the energy expended to produce it. Animal movement is typified by variable acceleration, and measurement of acceleration is becoming established as a reliable method of quantifying activity patterns of animals in the field (e.g. Yoda *et al.* 2001). Thus, we postulate that accurate measurement of acceleration of an animal in all three spatial dimensions should provide a good estimate of their rate of energy expenditure while moving. Preliminary work using simple accelerometers on humans, working in just one or two dimensions, has shown that the degree of acceleration does indeed correlate with rate of energy expenditure assessed by rate of oxygen consumption or carbon dioxide production (Campbell, Crocker & McKenzie 2002; Hoos *et al.* 2003; Fruin & Rankin 2004; Kumahara *et al.* 2004). Thus, the accelerometry technique has the potential to provide information about how animals partition their use of both time and energy.

In the present paper, we demonstrate the utility of accelerometric estimation of rate of energy expenditure using electronic devices attached to great cormorants (*Phalacrocorax carbo*), which measured their body acceleration while they were engaged in different activities on land in the laboratory. Using standard respirometry techniques (Withers 2001), we were able to investigate the extent to which body acceleration correlates with the rate of oxygen consumption ($\dot{V}_{O_2}$, an indirect measure of metabolic rate that can be converted to rate of energy expenditure) during activity in this species. We also examined how body acceleration varies as a function of activity in free-living imperial cormorants (*P. atriceps*) to verify that the approach identifies expected trends in $\dot{V}_{O_2}$ from situations with variable power requirements; specifically, $\dot{V}_{O_2}$ related to swimming as a function of depth (Lovvorn & Jones 1991; Lovvorn, Jones & Blake 1991; Wilson *et al.* 1992; Watanuki *et al.* 2003; Tremblay, Cook & Cherel 2005) and the extent to which extra mass carried by the bird in the form of prey affects $\dot{V}_{O_2}$ during flight (Videler *et al.* 1988). The limitations and strengths of the methodology are also discussed.

Methods

LABORATORY WORK

Great cormorants (*P. carbo*) of approximately 4–6 weeks of age were collected under permit from nests at Rutland Water Nature Reserve, UK, during April 2004 and 2005. They were transported immediately to the School of Biosciences at The University of Birmingham, UK, where they were housed for 4 months in an indoor facility that included water baths. They were maintained on a hand-delivered diet of defrosted sprats. Each bird received a daily vitamin supplement (fish eater tablets,

Mazuri Zoo Foods, Essex, UK) concealed within the sprat. When approximately 5–6 months old, the birds were transferred to a 130 m² outdoor aviary that included a 50-cm-deep pond. Birds continued to be maintained on a diet of sprat with a daily vitamin supplement in the outdoor aviaries. During November 2005, five cormorants (three from the 2004 cohort, two from 2005) were fitted with loggers (largest dimensions $65 \times 36 \times 22$ mm, mass 35 g), which recorded triaxial acceleration ($0–6$ g) at 32 Hz with 22-bit resolution in a 128 Mb RA memory. The three axes for the acceleration transducers were calibrated by rotating the units through all combinations of pitch and roll ($0–360°$ for both rotations) so that output from the transducers in millivolts could be converted into g. Devices were attached to feathers on the lower back using TESA tape (Wilson *et al*. 1997) and care was taken to attach the units in identical positions on all individuals. Birds [mean mass $1·98 \pm 0·07$ (SE) kg] were placed in a respirometer chamber and walked on a treadmill while measurements of rates of oxygen consumption ($\dot{V}_{O_2}$, ml min^{-1}) and carbon dioxide production ($\dot{V}_{CO_2}$, ml min^{-1}) were recorded using standard positive pressure open-flow respirometry (Withers 2001).

During the course of the experiment, the birds also engaged in activities such as preening and wing-flapping. All the activities of the birds while in the respirometer chamber were filmed. The respirometer chamber comprised a 210 L clear acrylic box, into which room air was pumped (Rietschle Thomas 2688CHI44, Hants, UK). The flow rate through the chamber was 55 L min^{-1}, measured with an Aalborg $0–100$ L min^{-1} mass flow controller. Good mixing within the chamber was assured by the inclusion of three 12×12 cm fans, and incurrent air temperature was measured by means of a negative temperature coefficient thermistor calibrated with a Grant GR150 precision water bath. Relative humidity of incurrent air was measured with an electronic hygrometer, and water vapour was mathematically scrubbed to provide a flow rate corrected to standard temperature pressure dry (STPD). A subsample of the chamber air was drawn off and passed through a column of indicating Drierite™ (Hammond Drierite Co, Xenia, OH, USA) and an ML206 O_2 and CO_2 analyser (ADInstruments, Bella Vista, NSW, Australia) calibrated with custom gas mixtures provided by a Wösthoff gas mixing pump (type 2M301/a-F, Bochum, Germany). The voltage outputs of the gas analyser and thermistor were recorded at a sampling frequency of 4 Hz by a PowerLab ML750 A/D converter (ADInstruments) and Chart software (ADInstruments). The hygrometer reading was noted and recorded periodically. The O_2 signal was conditioned with a 2 Hz low-pass filter and 9-point Bartlett weighted signal averaging. The CO_2 signal was conditioned with a 5 Hz low pass filter and 9-point Bartlett weighted signal averaging. Partial pressures of ambient O_2 and CO_2 were recorded for 3 min in every 20 min, and the baseline check was automated with a solenoid valve (SMC model EVT307, Radio Spares, Corby, UK) controlled by the ML750 and Chart

software. N_2 dilution tests (Fedak, Rome & Seeherman 1981) were used to check for any leaks within the system; the system was accurate to within $\pm 2\%$.

Each bird was exercised at up to seven different speeds ($0·29–0·54$ m s^{-1}) presented in a random order. This speed range includes the lowest speed available on the treadmill and the highest speed the birds would maintain, although not all birds would walk at the higher speeds. Each speed was maintained for a sufficient length of time to allow for gas equilibration (at least 10 min), and $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated as 2–5-min averages of the steady-state values observed once the system was in equilibrium. Data were excluded if birds showed evidence of fatigue and were unable to maintain station within the respirometer for a sufficient length of time to allow for gas equilibration. Birds were allowed one or two rest periods during the exercise protocol, and were rested until $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were stable. Resting $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ during these periods (speed = 0 m s^{-1}) were calculated as the average over a 2–5-min period when $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were stable and the system was in equilibrium. The birds were often active during these 'resting' periods, for example exploring the box or preening, and each resting period was included in the calibration regressions. During the final resting period at the completion of the exercise protocol, the respirometer chamber was made as dark as possible to induce a deep rest in the bird and the lowest mean equilibrium value of $\dot{V}_{O_2}$ over a period of 2–5 min was recorded.

FIELDWORK

Fieldwork was conducted on 15 imperial cormorants *P. atriceps* breeding at Punta León (43°04′ S, 64°2′ W), Chubut, Argentina during November and December 2005. Birds were equipped with devices in a manner identical to the cormorants in Birmingham except that the devices were of two different types; a device identical to that used in Birmingham set to record at 32 Hz (used on three individuals), and another device (mass 40 g) set to record at 9 Hz and recording 13 channels of data with a resolution of 22 bits into a 512 Mb memory (used on 12 individuals). Only triaxial acceleration, recorded in a manner identical to that of the logger used in Birmingham, and depth are relevant here. The three axes for the acceleration transducers were calibrated from both logger types by rotating the units through all combinations of pitch and roll ($0–360°$ for both rotations) so that output from the transducers in millivolts could be converted into g.

All cormorants fitted with devices were brooding small chicks. The cormorants were caught using a specially designed crook, which was used to remove them slowly from the nest. The fitting procedure took less than 5 min after which the birds were immediately returned to the nest where they continued with brooding. Equipped cormorants were observed at a distance through binoculars whenever possible and detailed notes made of their behaviours for comparison with

the accelerometry traces. The birds were allowed to forage for a single trip before the devices were retrieved.

DERIVATION OF OVERALL DYNAMIC BODY ACCELERATION (ODBA)

Downloaded acceleration data from the three axes were converted from mV into *g* using the calibrations of transducer output vs. transducer angle with respect to gravity (see above) and the three signals were individually smoothed using running means over 1 s. Then for each channel, the specific values for the smoothed data for any particular time interval were subtracted from the corresponding unsmoothed data for that time interval to produce a value for *g* resulting primarily from the dynamic acceleration (the static acceleration resulting from body angle with respect to gravity having been removed). Derived values were then converted into absolute positive units and the resultant values from all three channels then added to each other to give an overall value for the triaxial dynamic acceleration experienced by the birds. These values were then used in regressions of overall dynamic body acceleration (ODBA) vs. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ for the cormorants studied in the laboratory.

STATISTICAL ANALYSIS

Parameter estimates for the relationship between ODBA and $\dot{V}_{O_2}$, and ODBA and $\dot{V}_{CO_2}$, were made using repeated-measures analysis of covariance (ANCOVA); α was set at 0·05.

As the experimental design involved the participation of each bird in only a single respirometry and accelerometry session it was not possible to include body mass as a fixed factor in the analysis, because bird identity and body mass were perfectly confounded. However, body mass is potentially correlated with both ODBA and $\dot{V}_{O_2}$, and might therefore be a useful additional parameter in a model used to predict $\dot{V}_{O_2}$ from ODBA. It was therefore important to check whether there was a significant effect of body mass on $\dot{V}_{O_2}$, or if there was a significant interaction between body mass and ODBA. Thus, to circumvent the problem of a single mass value for each bird, one ODBA and $\dot{V}_{O_2}$ pair were selected randomly for each bird, and the effect of body mass and ODBA on $\dot{V}_{O_2}$ were examined first with a full factorial analysis of variance (ANOVA) with body mass and ODBA as fixed factors and, if the interaction term was not significant, with an ANCOVA with body mass as a covariate. Because at least five ODBA and $\dot{V}_{O_2}$ pairs were available for each bird this procedure was repeated five times, and α was set at a Bonferroni corrected level of 0·01. No ODBA and $\dot{V}_{O_2}$ pair was included in more than one analysis, and care was taken to ensure that each subset contained a wide range of ODBA values (i.e. each subset of five data pairs included at least two pairs measured during walking, and two measured during non-walking periods).

During laboratory sessions, care was taken to ensure

that a range of $\dot{V}_{O_2}$ values was obtained both during periods of walking and periods of non-walking, to try to ensure that the range of $\dot{V}_{O_2}$ for walking and non-walking overlapped. However, initial data analysis showed that ODBA values for walking and non-walking did not overlap, and visual inspection suggested that the walking and non-walking periods may be best described by separate relationships. While techniques for fitting two-part regressions exist (e.g. Chappell 1989; Nickerson, Facey & Grossman 1989; Yeager & Ultsch 1989), these methods nevertheless require a subjective judgement about which of the single-phase and two-phase fits is most appropriate. In the present case it could reasonably be hypothesized, a priori, that a single-phase regression is appropriate because ODBA integrates all mechanical work performed by the animal, or that a two-phase regression is appropriate because the location of the logger in close association with the trunk will emphasize locomotory movements of the body, and be less sensitive to movements of the extremities (e.g. the head and neck during preening). Thus, in order to compare the single-phase and two-phase fits objectively, and determine which is most appropriate, we adopted Burnham & Anderson's (2001) approach for model comparison and calculated Akaike's information criterion (AIC) as a measure of model fit for both the single-phase and two-phase models. AIC was calculated as $-2\times$ the log-likelihood of the model, plus $2\times$ the number of estimable parameters (Burnham & Anderson 2001). This addition penalizes the number of parameters in a model, ensuring that the best model is not necessarily the one with the most parameters. The best model was the one with the lowest AIC. The probability that a model was the best of the two tested was measured by its Akaike weight (Burnham & Anderson 2001), the relative likelihood of a model compared to the other.

Results

LABORATORY WORK

The acceleration patterns of the five cormorants walking at different speeds on the treadmill showed characteristic double peaks in all axes, with the form of the acceleration trace changing systematically with speed (Fig. 1). Mean overall dynamic body acceleration (ODBA) correlated with both $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (Fig. 2) in all birds with no significant differences between individuals. The overall best fit for the relationship between ODBA and rate of oxygen consumption was:

$$\dot{V}_{O_2} = 92\cdot3 \text{ODBA} + 52\cdot1 \quad (r^2 = 0\cdot81, F = 136\cdot5,$$
$$P < 0\cdot0001) \text{ (Fig. 2a)}$$

The equivalent relationship for rate of carbon dioxide production was:

$$\dot{V}_{CO_2} = 84\cdot9 \text{ ODBA} + 31\cdot3 \quad (r^2 = 0\cdot81, F = 140\cdot6,$$
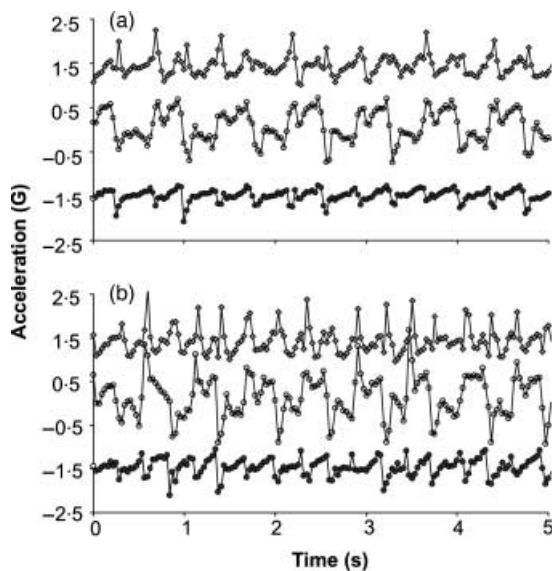$$P < 0\cdot0001) \text{ (Fig. 2b)}$$

Fig. 1. Changes in overall acceleration recorded by triaxial accelerometers recording at 32 Hz during walking at different speeds of (a) 0·36 m/s and (b) 0·54 m s^{-1} by a great cormorant. Note that offsets of 1 *g* have been applied to the top and bottom channels (negative and positive additions, respectively) so as to separate the traces from the three transducers and make them clearly distinguishable from each other.

Fig. 2. Relationship between overall dynamic body acceleration and (a) oxygen consumption and (b) carbon dioxide production for five great cormorants resting and walking at different speeds on a treadmill.

Mean respiratory exchange ratio (RER, $\dot{V}_{CO_2}/\dot{V}_{O_2}$) was 0·73 ± 0·02 (SEM).

A single-phase regression was found to provide the best description of the relationship between ODBA and $\dot{V}_{O_2}$, given the data (AIC = 45·5 and 47·2 for single-phase and two-phase regressions, respectively). Comparison of Akaike weights (w_i) showed that the single-phase regression ($w_i = 0·71$) was 2·4× more likely to be the best fit than the two-phase regression ($w_i = 0·29$).

For the five subsets of data, ANOVA revealed no significant interaction between body mass and ODBA ($F = 0·88$–375·3, $P = 0·52$–0·03), and ANCOVA revealed no significant effect of body mass ($F = 0·016$–19·1, $P = 0·91$–0·05). However, as n was only five in each case, ODBA was a significant predictor of $\dot{V}_{O_2}$ in only two of the five subsets ($F = 203·0$, 146·9, 42·6, 7·63, 3·59; $P = 0·005$, 0·007, 0·02, 0·11, 0·20). Nevertheless, these results indicate that the lack of inclusion of body mass as a fixed factor in the analyses has not produced less valid prediction relationships.

FIELDWORK

As in the laboratory birds, the various cormorant activities could be identified readily by inspection of the trace of triaxial accelerometry (Fig. 3). Examples include flight, nest building, walking (cf. Fig. 1), washing at sea and diving as well as respiratory frequency, which was particularly apparent in resting birds. ODBA during resting on land had a mean value of 0·092 (SD 0·03, $n = 12$) but varied substantially according to activity and even as a function of the extent of the activity. Thus, for example, ODBA decreased with increasing
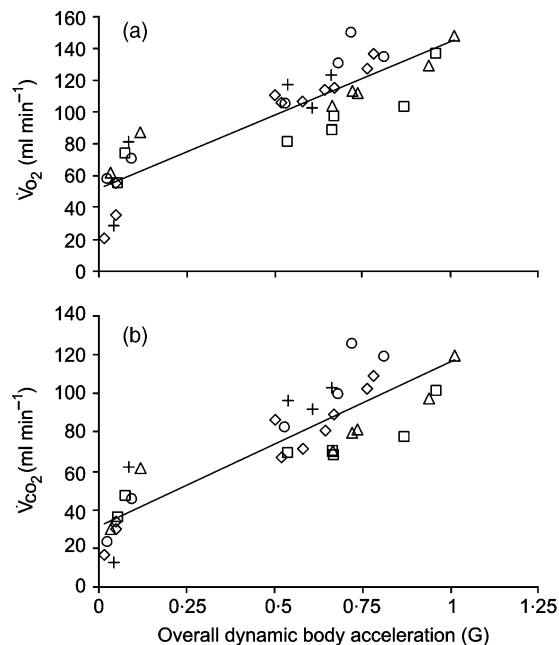
depth during the descent phase of dives to any specific depth (e.g. Figure 4a). This was due primarily to the birds being subject to decreasing upthrust (Fig. 4b), as plumage and respiratory air volume decreased with depth (Wilson *et al.* 1992) and as rate of descent did not vary during the course of such dives ($P > 0·05$). ODBA also varied for flight, being systematically lower in birds setting out to forage ($x = 0·826$, SD 0·087, $n = 12$; $t = 3·17$, $P < 0·01$) than in birds returning from foraging ($x = 0·944$, SD 0·095, $n = 12$), presumably laden with food and probably also with water (Ribak, Weihs & Arad 2005) (Fig. 5).

Discussion

The significant positive relationship between rate of oxygen consumption ($\dot{V}_{O_2}$) and overall dynamic body acceleration (ODBA) (Fig. 2) indicates that the accelerometry technique is a potentially useful method for determination of activity-specific $\dot{V}_{O_2}$ in cormorants. Accurate determination of activity-specific $\dot{V}_{O_2}$ is critical because, during activity, the costs of movement may outweigh those of any of the other functions by a factor of more than 10 (Darveau *et al.* 2002; Weibel *et al.* 2004) and currently accounts for most of the uncertainty in the compilation of energy budgets in free-living animals. The variance that we observe in our relationship between $\dot{V}_{O_2}$ and ODBA (Fig. 2) will be due, in part, to energy-expending processes of the body that do not relate to movement (see below) but will also be due to methodological errors. A number of factors contribute to variance in the ODBA–metabolic power relationship, discussed below.
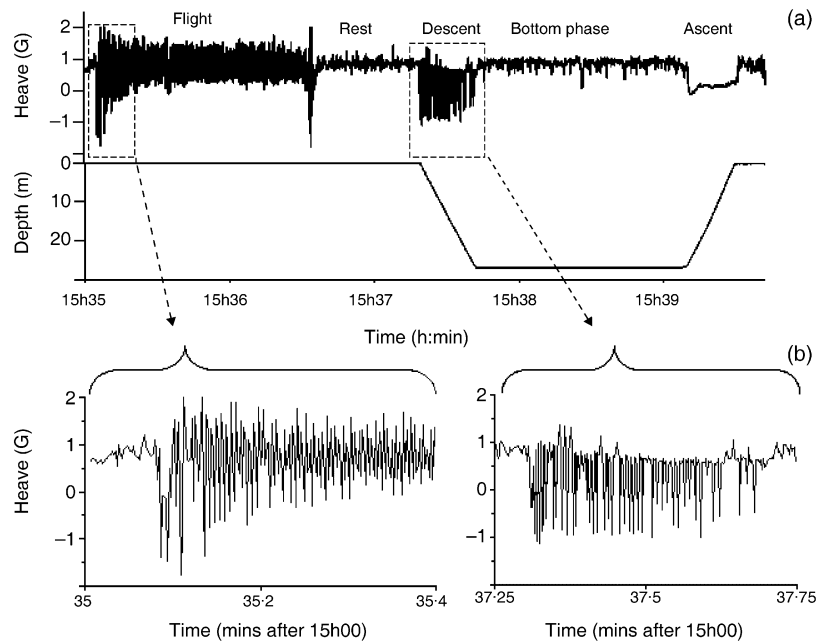
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*Journal of Animal
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1081–1090

Fig. 3. Example of the triaxial acceleration signal logged from a male imperial cormorant during a single foraging trip. (a) Changes in the heave axis during take-off from the water surface, a short period of flight followed by a rest at the water surface and then a dive. (b) The heave acceleration during the take-off and during the descent of the dive. Note that during flight and the descent of the water column, wing beats and foot kicks, respectively, are discernable as peaks. The take-off is characterized by greater heave amplitudes and the descent of the water column during dives has both higher amplitudes and higher frequencies closer to the surface where upthrust is greatest (cf. Fig. 4).
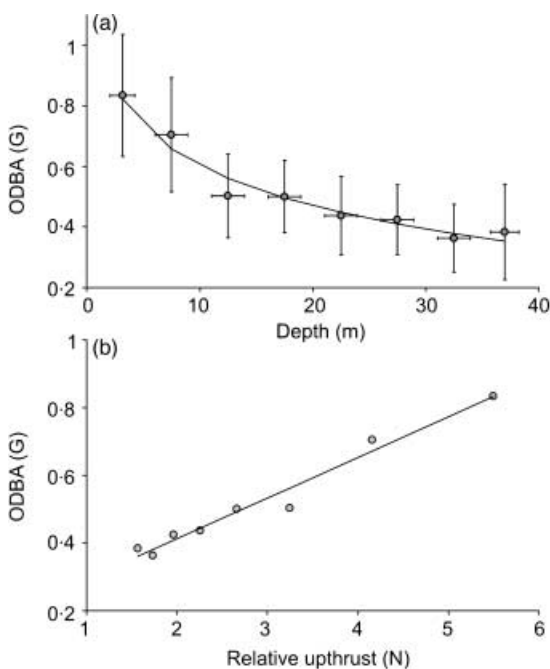

Fig. 5. Overall body dynamic acceleration for 12 imperial cormorants during flight when leaving the colony to forage and when returning with food after foraging to provision their brood. The difference between outward and return ODBA for flight is significant ($t = 3.17$, $P < 0.01$).

Fig. 4. (a) Overall dynamic body acceleration of imperial cormorants as a function of depth during the descent phase of 27 dives terminating at a maximum depth of 40–42 m. Points show means for data within 5 m categories (bars SD). (b) Mean ODBA regressed against the relative upthrust for that depth, with upthrust calculated assuming a body plumage air of 170 mL kg^{-1} (Gremillet *et al.* 2005), respiratory air of 160 mL kg^{-1} (ref. in Wilson *et al.* 1992), body mass of 2·2 kg (Quintana unpublished data) and a bird body density that is, otherwise, neutrally buoyant in seawater (Wilson *et al.* 1992).
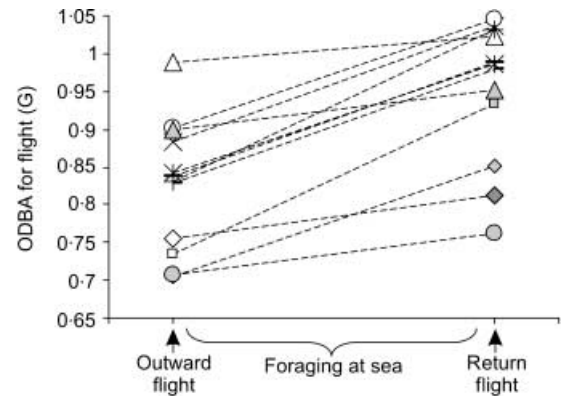
ESTIMATION OF RATE OF ENERGY EXPENDITURE FROM $\dot{V}_{O_2}$

The present study is concerned with demonstration of the utility of accelerometric estimation of rate of energy expenditure, rather than the ecological consequences of energy use. Thus, we have chosen to relate ODBA to $\dot{V}_{O_2}$, which was measured rather than metabolic rate (in W), which requires conversion from O_2 consumption to energy use and incorporates additional assumptions. The exact relationship between $\dot{V}_{O_2}$ and metabolic power

1087

*Activity-specific
metabolic rate in
free-living animals*

depends on the metabolic substrate being utilized. When pure carbohydrate is metabolized and the respiratory quotient (RQ, the ratio of CO_2 formed to O_2 used at the cellular level) is 1, 1 mL O_2 s^{-1} = 21·1 W, while for pure fat metabolism, i.e. when RQ is 0·71, 1 mL O_2 s^{-1} = 19·6 W (Butler & Bishop 2000). Under steady-state conditions, such as those employed in the present study, RQ is equivalent to the respiratory exchange ratio (RER, $\dot{V}_{CO_2}/\dot{V}_{O_2}$), which is the exchange ratio at the lungs. However, these energy equivalences have been validated for only a few species, and there is scope for error when converting from $\dot{V}_{O_2}$ to rate of energy expenditure (Walsberg & Hoffman 2005). Furthermore, RER and RQ may be lower than 0·71 in fasting birds (Nolet *et al.* 1992; Chaui-Berlinck & Bicudo 1995; Hawkins *et al.* 2000; Walsberg & Hoffman 2005), but energy equivalences of O_2 are available only for the range 0·71–1, and it is not clear how data should be treated in these cases (see Walsberg & Hoffman 2005 for further discussion).

THE ACCURACY OF THE ACCELEROMETER SYSTEM

The use of acceleration to measure movement is logical because muscular contraction resulting in movement produces acceleration (or deceleration) in a corresponding moving part (usually a limb). Thus, theoretically, if accelerometers were put onto all moving parts of the body, it should be possible to quantify all movement and, because muscular contraction requires the consumption of oxygen, estimate the oxygen uptake of the muscles associated with each of the moving parts. However, because the moving parts of the body are all connected with the trunk, any movement should produce a corresponding, albeit dampened, movement in this area. In general, the more substantial the movement (i.e. a greater rate of change of position) in the extremities, the greater will be the movement of the trunk, even if the body is not subject to some overall translocation. Thus, single accelerometers to monitor all elements of animal movement should be placed on the trunk where movement in any of the extremities can be perceived. Placement of the accelerometer system and the exact adherence to the feathers is likely to be critical, and slight variations of such are likely to have produced some of the interindividual variance that we observed in measured acceleration as a function of speed in our captive cormorants.

Although the actual overall patterns in acceleration that the body experiences over the three spatial dimensions will be complex and dependent on the various types of muscle movement, triaxial accelerometers mounted on the body trunk should allow accurate quantification of this (Pfau, Witte & Wilson 2005). For this reason, accelerometry in the three axes is likely to give the best correlation with $\dot{V}_{O_2}$ which may, at least in part, explain our high correlation coefficients compared to those from other studies using mono- or biaxial transducers (Campbell *et al.* 2002; Hoos *et al.* 2003).

Indeed, the coefficient of determination for the ODBA–$\dot{V}_{O_2}$ relationship reported here ($r^2 = 0.81$, Fig. 2a) is comparable to that reported for the f_H–$\dot{V}_{O_2}$ relationships of king and macaroni penguins ($r^2 = 0.73$–0.79; Froget *et al.* 2004; Green *et al.* 2005). Ultimately, however, the extent to which mono-, bi- or triaxial transducers are appropriate to define the energy expended by movement will depend on the animals and the movements and, in an initial phase at least, this will require examination on a species-by-species basis.

WHAT MIGHT AFFECT THE ODBA–$\dot{V}_{O_2}$ RELATIONSHIP GENERICALLY?

Despite the potential that accelerometry might have to serve as a proxy for $\dot{V}_{O_2}$, and hence rate of energy expenditure during activity, there are a number of situations which, if they are not simulated during the calibration procedure, could lead to errors. These include the effect that elastic ligaments and tendons (Bennett & Taylor 1995), different animal gaits (Rubenson *et al.* 2004) and resonant frequencies might have on the ODBA–$\dot{V}_{O_2}$ relationship (Dutto *et al.* 2004). We note that substantial elasticity in tendons during animal movement will result in a correspondingly large acceleration signal, much of which actually represents energy that can be re-used by the animal in question (Alexander 1988). In addition, it is not intuitively obvious how the rate of energy expenditure of terrestrial animals moving up slopes of different inclines might relate to ODBA (Gottschall & Kram 2005).

Thus, it is important that when the accelerometry method is to be used to estimate $\dot{V}_{O_2}$ in the field, the calibration procedure incorporates all the factors that may reasonably affect the relationship in the field. Similar complexities have been identified, but have not been a hindrance, with the heart rate method. For example, the f_H–$\dot{V}_{O_2}$ relationship differs between flight and treadmill exercise in geese (Ward *et al.* 2002). In reptiles such as lizards, feeding, body temperature, heating and cooling, as well as level of activity, affect the relationship between heart rate and rate of oxygen consumption and have to be incorporated into the final model (Clark, Butler & Frappell 2006). It is also important that the accuracy of the method is determined by performing suitable validation experiments and that, if at all possible, the standard error of the estimate is determined so that mean estimates can be statistically compared (see Green *et al.* 2001).

ESTIMATING $\dot{V}_{O_2}$, AND HENCE RATE OF ENERGY EXPENDITURE, OF FREE-LIVING CORMORANTS USING ODBA

The value of ODBA in helping to determine the cost of various activities of free-living animals can be demonstrated by examining the data on the behaviour of imperial cormorants in Patagonia to highlight certain trends. As pointed out by Yoda *et al.* (2001) and Watanabe

et al. (2005), accelerometers are generally useful for determining different categories of animal behaviour (Ropert-Coudert *et al.* 2004) and this is certainly apparent in our results. Beyond this, however, authors have also been able to allude to the biomechanics of locomotion, for example examining the effect of air-mediated buoyancy on stroke frequency during the descent and ascent in diving mammals and birds (Sato *et al.* 2003; Watanuki *et al.* 2005). The effects of depth are apparent in that stroke frequency tends to decrease with increasing depth during the descent of birds due to compression of the respiratory system and hence a reduction in buoyancy (Wilson *et al.* 1992), while ascent is largely passive (Watanuki *et al.* 2003; Kato *et al.* 2006). The reverse is generally true of marine mammals, which are negatively buoyant below about 50 m depth (Sato *et al.* 2003; Williams *et al.* 2000). While it seems clear that the costs of swimming at depth in both mammals and birds will be indicated by limb stroke frequency, the inability of systems to integrate stroke amplitude (cf. Wilson & Liebsch 2003) means that $\dot{V}_{O_2}$ while swimming at depth either has to be determined directly using respirometry techniques, usually under laboratory-type conditions (Williams *et al.* 2004; Enstipp, Gremillet & Jones 2006), or indirectly using the heart rate method on free-ranging animals in the field (Froget *et al.* 2004; Green *et al.* 2003), although for both these methods the values obtained integrate costs for the whole dive/surface period. Alternatively, $\dot{V}_{O_2}$ during dives can be estimated based on mechanical models (Lovvorn, Croll & Liggins 1999), or estimated indirectly using a multiple regression approach (Woakes & Butler 1983; Halsey, Butler & Woakes 2005).

Our ODBA data from imperial cormorants clearly show a decreasing ODBA with increasing depth when constant rates of descent are maintained. Indeed, calculation of the upthrust experienced by these birds shows that 97% of the variance in ODBA is explained by air volumes, which is to be expected if ODBA relates to the $\dot{V}_{O_2}$ involved in the descent. An ODBA of 0·17 *g* is predicted for birds descending at a vertical rate of 1·17 m s^{-1} (rate of descent did not change with depth in dives to 40 m), with an increase of 0·12 *g* for every *N* upthrust experienced (Fig. 4b). ODBA decreased, on average, by 54% during descent from 3·2 to 37·0 m; thus, if we assume that the relationship between ODBA and $\dot{V}_{O_2}$ is similar for walking great cormorants (Fig. 2) and swimming imperial cormorants, this represents a 32% decrease in $\dot{V}_{O_2}$. Similarly, the use of ODBA as a measure of energy expended can be expanded to other power-variable activities such as flight. Our results show, for example, that the ODBA for cormorants flying while laden with food for their chicks is 14% higher than when flying unladen (cf. Hambly, Harper & Speakman 2004). Again, assuming that our great cormorant relationship between ODBA and $\dot{V}_{O_2}$ can be used on imperial cormorants, $\dot{V}_{O_2}$ when laden is estimated to be 8·5% higher than when unladen. It is not known if the ODBA–$\dot{V}_{O_2}$ relationship determined in the present study is relevant to a flying bird, but the difference in ODBA between empty and laden birds suggests that the technique does show promise. Although experiments have been conducted to examine the energetic consequences of loading terrestrial animals (e.g. Irschick *et al.* 2003), this is rare in flying animals (Gessaman & Nagy 1988; Videler *et al.* 1988; see also Butler *et al.* 1998; who documented a decrease in heart rate during the autumn migratory flight of barnacle geese as they lost body mass). There are obvious difficulties in determining the energetic cost of flight, even though quantification of the energetic costs of transporting extra mass by flying species is critical in understanding a variety of behavioural strategies (Lindstrom & Alerstam 1992).

THE UTILITY OF ODBA FOR STUDIES OF THE ENERGETICS OF FREE-LIVING ANIMALS

Given the limitations and difficulties involved in measuring rate of energy expenditure of free-living animals (Butler *et al.* 2004), measurement of triaxial dynamic acceleration appears to have great potential for deriving the energy expenditure involved in locomotion. Furthermore, it may also be possible to derive estimates of energy expenditure during other types of movement, such as respiration, and to combine accelerometry with measurements of other variables such as depth (e.g. Fig. 3a), body temperature and heart rate. Although the present study has highlighted a large number of areas where the methodology may be subject to error, the overall goodness-of-fit of ODBA with $\dot{V}_{O_2}$ from our laboratory data (Fig. 2) coupled with clear, explicable trends from free-living birds obliged to work under conditions of varying mechanical power (Figs 4 and 5) indicate that the concept is fairly robust. This, together with the ability of accelerometry to identify behaviours (Watanabe *et al.* 2005), should ultimately allow researchers to determine how free-living animals partition their time and energy into particular behavioural strategies.

References

Alexander, R.M. (1988) *Elastic Mechanisms in Animal Movement*. Cambridge University Press, Cambridge.

Alexander, R.M. (2003) *Principles of Animal Locomotion.* Princeton University Press, Princeton.

Beamish, F.W.H. (1990) Swimming metabolism and temperature in juvenile walleye, *Stizostedion-vitreum-vitreum. Environmental Biology of Fishes*, **27**, 309–314.

Bennett, M.B. & Taylor, G.C. (1995) Scaling of elastic strain-energy in kangaroos and the benefits of being big. *Nature*, **378**, 56–59.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Burnham, K.P. & Anderson, D.R. (2001) Kullback–Leibler information as a basis for strong inference in ecological studies. *Wildlife Research*, **28**, 111–119.

Butler, P.J. & Bishop, C.M. (2000) Flight. *Sturkie's Avian Physiology*, 5th edn (ed. G.C. Whittow), pp. 391–435. Academic Press, San Diego, CA.

Butler, P.J., Green, J.A., Boyd, I.L. & Speakman, J.R. (2004) Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods. *Functional Ecology*, **18**, 168–183.

Butler, P.J., Woakes, A.J. & Bishop, C.M. (1998) Behaviour and physiology of Svalbard barnacle geese *Branta leucopsis* during their autumn migration. *Journal of Avian Biology*, **29**, 536–545.

Campbell, K.L., Crocker, P.R.E. & McKenzie, D.C. (2002) Field evaluation of energy expenditure in women using Tritrac accelerometers. *Medicine and Science in Sports and Exercise*, **34**, 1667–1674.

Chappell, R. (1989) Fitting bent lines to data, with applications to allometry. *Journal of Theoretical Biology*, **138**, 235–256.

Chaui-Berlinck, J.G. & Bicudo, J.E.P.W. (1995) Unusual metabolic shifts in fasting hummingbirds. *Auk*, **112**, 774–494.

Clark, T.D., Butler, P.J. & Frappell, P.B. (2006) Factors influencing the prediction of metabolic rate in a reptile. *Functional Ecology*, **20**, 105–113.

Darveau, C.A., Suarez, R.K., Andrews, R.D. & Hochachka, P.W. (2002) Allometric cascade as a unifying principle of body mass effects on metabolism. *Nature*, **417**, 166–170.

Dutto, D.J., Hoyt, D.F., Cogger, E.A. & Wickler, S.J. (2004) Ground reaction forces in horses trotting up an incline and on the level over a range of speeds. *Journal of Experimental Biology*, **207**, 3507–3514.

Enstipp, M.R., Gremillet, D. & Jones, D.R. (2006) The effects of depth, temperature and food ingestion on the foraging energetics of a diving endotherm, the double-crested cormorant *Phalacorax auritus. Journal of Experimental Biology*, **209**, 845–859.

Fedak, M.A., Rome, L. & Seeherman, H.J. (1981) One-step N2-dilution technique for calibrating open-circuit V_{O_2} measuring systems. *Journal of Applied Physiology*, **51**, 772–776.

Frappell, P.B. & Butler, P.J. (2004) Minimal metabolic rate, what it is, its usefulness, and its relationship to the evolution of endothermy: a brief synopsis. *Physiological and Biochemical Zoology*, **77**, 865–868.

Froget, G., Butler, P.J., Woakes, A.J., Fahlman, A., Kuntz, G., Le Maho, Y. & Handrich, Y. (2004) Heart rate and energetics of free-ranging king penguins (*Aptenodytes patagonicus*). *Journal of Experimental Biology*, **207**, 3917–3926.

Fruin, M.L. & Rankin, J.W. (2004) Validity of a multi-sensor armband in estimating rest and exercise energy expenditure. *Medicine and Science in Sports and Exercise*, **36**, 1063–1069.

Geiser, F. (2004) Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual Review of Physiology*, **66**, 239–274.

Gessaman, J.A. & Nagy, K.A. (1988) Transmitter loads affect the flight speed and metabolism of homing pigeons. *Condor*, **90**, 662–668.

Gottschall, J.S. & Kram, R. (2005) Ground reaction forces during downhill and uphill running. *Journal of Biomechanics*, **38**, 445–452.

Green, J.A., Butler, D.J., Woakes, A.J., Boyd, I.L. & Holder, R.L. (2001) Heart rate and rate of oxygen consumption of exercising macaroni penguins. *Journal of Experimental Biology*, **204**, 673–684.

Green, J.A., Butler, R.J., Woakes, A.J. & Boyd, I.L. (2003) Energetics of diving in macaroni penguins. *Journal of Experimental Biology*, **206**, 43–57.

Green, J.A., Woakes, A.J., Boyd, I.L. & Butler, P.J. (2005) Cardiovascular adjustments during locomotion in penguins. *Canadian Journal of Zoology*, **83**, 445–454.

Grémillet, D., Chauvin, C., Wilson, R.P., Le Maho, Y. & Wanless, S. (2005) Unusual feather structure allows partial plumage wettability in diving great cormorants. *Journal of Avian Biology*, **36**, 57–63.

Halsey, L.G., Butler, P.J. & Woakes, A.J. (2005) Breathing hypoxic gas affects the physiology as well as the diving behaviour of tufted ducks. *Physiological and Biochemical Zoology*, **78**, 273–284.

Hambly, C., Harper, E.J. & Speakman, J.R. (2004) The energy cost of loaded flight is substantially lower than expected due to alterations in flight kinematics. *Journal of Experimental Biology*, **207**, 3969–3976.

Hawkins, P.A.J., Butler, P.J., Woakes, A.J. & Gabrielsen, G.W. (1997) Heat increment of feeding in Brunnich's guillemot *Uria lomvia. Journal of Experimental Biology*, **200**, 1757–1763.

Hawkins, P.A.J., Butler, P.J., Woakes, A.J. & Speakman, J.R. (2000) Estimation of the rate of oxygen consumption of the common eider duck (*Somateria mollissima*), with some measurements of heart rate during voluntary dives. *Journal of Experimental Biology*, **203**, 2819–2832.

Hoos, M.B., Plasqui, G., Gerver, W.J.M. & Westerterp, K.R. (2003) Physical activity level measured by doubly labeled water and accelerometry in children. *European Journal of Applied Physiology*, **89**, 624–626.

Irschick, D.J., Vanhooydonck, B., Hertel, A. & Andronescu, A. (2003) Effects of loading and size on maximum power output and gait characteristics in geckos. *Journal of Experimental Biology*, **206**, 3923–3934.

Kato, A., Ropert-Coudert, Y., Gremillet, D. & Cannell, B. (2006) Locomotion and foraging strategy in foot-propelled and wing-propelled shallow-diving seabirds. *Marine Ecology – Progress Series*, **308**, 293–301.

King, A.M., Loiselle, D.S. & Kohl, P. (2004) Force generation for locomotion of vertebrates: skeletal muscle overview. *IEEE Journal of Oceanic Engineering*, **29**, 684–691.

Kumahara, H., Schutz, Y., Ayabe, M., Yoshioka, M., Yoshitake, Y., Shindo, M., Ishii, K. & Tanaka, H. (2004) The use of uniaxial accelerometry for the assessment of physical-activity-related energy expenditure: a validation study against whole-body indirect calorimetry. *British Journal of Nutrition*, **91**, 235–243.

Lindstrom, A. & Alerstam, T. (1992) Optimal fat loads in migrating birds – a test of the time-minimization hypothesis. *American Naturalist*, **140**, 477–491.

Lovvorn, J.R., Croll, D.A. & Liggins, G.A. (1999) Mechanical versus physiological determinants of swimming speeds in diving Brunnich's guillemots. *Journal of Experimental Biology*, **202**, 1741–1752.

Lovvorn, J.R. & Jones, D.R. (1991) Effects of body size, body-fat, and change in pressure with depth on buoyancy and costs of diving in ducks (*Aythya* spp). *Canadian Journal of Zoology–Revue Canadienne de Zoologie*, **69**, 2879–2887.

Lovvorn, J.R., Jones, D.R. & Blake, R.W. (1991) Mechanics of underwater locomotion in diving ducks – drag, buoyancy and acceleration in a size gradient of species. *Journal of Experimental Biology*, **159**, 89–108.

McKechnie, A.E., Freckleton, R.P. & Jetz, W. (2006) Phenotypic plasticity in the scaling of avian basal metabolic rate. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **273**, 931–937.

McKechnie, A.E. & Wolf, B.O. (2004) The allometry of avian basal metabolic rate: good predictions need good data. *Physiological and Biochemical Zoology*, **77**, 502–521.

McNab, B.K. (2002) *Physiological Ecology of Vertebrates*. Comstock Cornell, Ithaca, NY.

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

Nickerson, D.M., Facey, D.E. & Grossman, G.D. (1989) Estimating physiological thresholds with continuous 2-phase regression. *Physiological Zoology*, **62**, 866–887.

Nolet, B.A., Butler, P.J., Masman, D. & Woakes, A.J. (1992) Estimation of daily energy-expenditure from heart-rate and doubly labeled water in exercising geese. *Physiological Zoology*, **65**, 1188–1216.

Pfau, T., Witte, T.H. & Wilson, A.M. (2005) A method for deriving displacement data during cyclical movement using an inertial sensor. *Journal of Experimental Biology*, **208**, 2503–2514.

Ribak, G., Weihs, D. & Arad, Z. (2005) Water retention in the plumage of diving great cormorants *Phalacrocorax carbo sinensis*. *Journal of Avian Biology*, **36**, 89–95.

Ropert-Coudert, Y., Bost, C.-A., Handrich, Y., Bevan, R.M., Butler, P.J., Woakes, A.J. & Le Maho, Y. (2000) Impact of externally attached loggers on the diving behaviour of the king penguin. *Physiological and Biochemical Zoology*, **73**, 438–445.

Ropert-Coudert, Y., Gremillet, D., Kato, A., Ryan, P.G., Naito, Y. & Le Maho, Y. (2004) A fine-scale time budget of Cape gannets provides insights into the foraging strategies of coastal seabirds. *Animal Behaviour*, **67**, 985–992.

Ropert-Coudert, Y. & Wilson, R.P. (2005) Trends and perspectives in animal-attached remote sensing. *Frontiers in Ecology and the Environment*, **3**, 437–444.

Rosen, D.A.S. & Trites, A.W. (1997) Heat increment of feeding in Steller sea lions, *Eumetopias jubatus*. *Comparative Biochemistry and Physiology A, Physiology*, **118**, 877–881.

Rubenson, J., Heliams, D.B., Lloyd, D.G. & Fournier, P.A. (2004) Gait selection in the ostrich: mechanical and metabolic characteristics of walking and running with and without an aerial phase. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **271**, 1091–1099.

Sato, K., Mitani, Y., Cameron, M.F., Siniff, D.B. & Naito, Y. (2003) Factors affecting stroking patterns and body angle in diving Weddell seals under natural conditions. *Journal of Experimental Biology*, **206**, 1461–1470.

Tremblay, Y., Cook, T.R. & Cherel, Y. (2005) Time budget and diving behaviour of chick-rearing Crozet shags. *Canadian Journal of Zoology–Revue Canadienne de Zoologie*, **83**, 971–982.

Videler, J.J., Vossebelt, G., Gnodde, M. & Groenewegen, A. (1988) Indoor flight experiments with trained kestrels. 1. Flight strategies in still air with and without added weight. *Journal of Experimental Biology*, **134**, 173–183.

Walsberg, G.E. & Hoffman, T.C.M. (2005) Direct calorimetry reveals large errors in respirometric estimates of energy expenditure. *Journal of Experimental Biology*, **208**, 1035–1043.

Ward, S., Bishop, C.M., Woakes, A.J. & Butler, P.J. (2002) Heart rate and the rate of oxygen consumption of flying and walking barnacle geese (*Branta leucopsis*) and bar-headed geese (*Anser indicus*). *Journal of Experimental Biology*, **205**, 3347–3356.

Watanabe, S., Izawa, M., Kato, A., Ropert-Coudert, Y. & Naito, Y. (2005) A new technique for monitoring the detailed behaviour of terrestrial animals: a case study with the domestic cat. *Applied Animal Behaviour Science*, **94**, 117–131.

Watanuki, Y., Niizuma, Y., Gabrielsen, G.W., Sato, K. & Naito, Y. (2003) Stroke and glide of wing-propelled divers: deep diving seabirds adjust surge frequency to buoyancy change with depth. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **270**, 483–488.

Watanuki, Y., Takahashi, A., Daunt, F., Wanless, S., Harris, M., Sato, K. & Naito, Y. (2005) Regulation of stroke and glide in a foot-propelled avian diver. *Journal of Experimental Biology*, **208**, 2207–2216.

Weibel, E.R., Bacigalupe, L.D., Schmitt, B. & Hoppeler, H. (2004) Allometric scaling of maximal metabolic rate in mammals: muscle aerobic capacity as determinant factor. *Respiratory Physiology and Neurobiology*, **140**, 115–132.

White, C.R. & Seymour, R.S. (2005) Allometric scaling of mammalian metabolism. *Journal of Experimental Biology*, **208**, 1611–1619.

Williams, T.M., Davis, R.W., Fuiman, L.A., Francis, J., Le Boeuf, B.L., Horning, M., Calambokidis, J. & Croll, D.A. (2000) Sink or swim: strategies for cost-efficient diving by marine mammals. *Science*, **288**, 133–136.

Williams, T.M., Fuiman, L.A., Horning, M. & Davis, R.W. (2004) The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. *Journal of Experimental Biology*, **207**, 973–982.

Wilson, R.P., Hustler, K., Ryan, P.G., Burger, A.E. & Noldeke, E.C. (1992) Diving birds in cold water – do Archimedes and Boyle determine energetic costs. *American Naturalist*, **140**, 179–200.

Wilson, R.P. & Liebsch, N. (2003) Up-beat motion in swinging limbs: new insights into assessing movement in free-living aquatic vertebrates. *Marine Biology*, **142**, 537–547.

Wilson, R.P., Putz, K., Peters, G., Culik, B., Scolaro, J.A., Charrassin, J.B. & Ropert-Coudert, Y. (1997) Long-term attachment of transmitting and recording devices to penguins and other seabirds. *Wildlife Society Bulletin*, **25**, 101–106.

Withers, P.C. (2001) Design, calibration and calculation for flow-through respirometry systems. *Australian Journal of Zoology*, **49**, 445–461.

Woakes, A.J. & Butler, P.J. (1983) Swimming and diving in tufted ducks, *Aythya-fuligula*, with particular reference to heart-rate and gas-exchange. *Journal of Experimental Biology*, **107**, 311–329.

Yeager, D.P. & Ultsch, G.R. (1989) Physiological regulation and conformation – a basic program for the determination of critical-points. *Physiological Zoology*, **62**, 888–907.

Yoda, K., Naito, Y., Sato, K., Takahashi, A., Nishikawa, J., Ropert-Coudert, Y., Kurita, M. & Le Maho, Y. (2001) A new technique for monitoring the behaviour of free-ranging Adelie penguins. *Journal of Experimental Biology*, **204**, 685–690.