Estimating the rate of oxygen consumption during submersion from the heart rate of diving animals

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Submitted 2 October 2006; accepted in final form 3 January 2007


How animals manage their oxygen stores during diving and other breath-hold activities has been a topic of debate among physiologists for decades. Specifically, while the behavior of free-ranging diving animals suggests that metabolism during submersion must be primarily aerobic in nature, no studies have been able to determine their rate of oxygen consumption during submersion (V\(_{O2D}\)) and hence prove that this is the case. In the present study, we combine two previously used techniques and develop a new model to estimate \(V_{O2D}\) accurately and plausibly in a free-ranging animal and apply it to data for macaroni penguins (Eudyptes chrysolophus) as an example. For macaroni penguins at least, \(V_{O2D}\) can be predicted by measuring heart rate during the dive cycle and the subsequent surface interval duration. Including maximum depth of the dive improves the accuracy of these predictions. This suggests that energetically demanding locomotion events within the dive combine with the differing buoyancy and locomotion costs associated with traveling to depth to influence its cost in terms of oxygen use. This will in turn effect the duration of the dive and the duration of the subsequent recovery period. In the present study, \(V_{O2D}\) ranged from 4 to 28 ml min\(^{-1}\) kg\(^{-1}\), indicating that, at least as far as aerobic metabolism was concerned, macaroni penguins were often hypometabolic, with rates of oxygen consumption usually lower than that while resting in air (10.3 ml min\(^{-1}\) kg\(^{-1}\)).

FREE-RANGING, DIVING ANIMALS frequently engage in bouts of repeated and prolonged foraging dives. For decades, physiologists have attempted to understand how these animals manage their oxygen stores to achieve such behavior (for a review, see Ref. 10). Despite behavioral evidence indicating the contrary, the majority of these studies have tended to conclude that a large proportion of dives exceed the calculated aerobic dive limit (cADL) of the species in question (e.g., Ref. 30). cADL is defined as the volume of usable oxygen stores divided by the rate of oxygen consumption during submergence (\(V_{O2D}\)). Under this definition, when a dive reaches the cADL, all usable oxygen has been depleted. However, the brain and central nervous system will always require some oxygen to maintain functionality; so if all oxygen is exhausted, then the animal will die. Therefore, to state that a dive may exceed the cADL merely indicates that the estimate of cADL is incorrect. This is not to say, however, that all natural diving utilizes only aerobic metabolism (11, 12). Kooyman et al. (38) demonstrated that diving animals will initiate the accumulation of lactate above resting levels well before all available oxygen has been consumed. The dive duration (\(t_d\)) at which this accumulation of lactate occurs is the aerobic dive limit (37, 38), now often termed the diving lactate threshold (DLT; 10). In the case of the Weddell seal (Leptonychotes weddellii), the DLT was \(-20–25\) min and the maximum \(t_d\) recorded (probably close to cADL) were approximately two to three times this. However, only \(-3%\) of observed dives had durations in excess of the DLT, and these were usually followed by extended periods at the surface, presumably to recycle accumulated lactate. Clearly cADL and DLT represent vastly different thresholds in the physiology of diving, and the two quantities should not be confused [see Butler (7) for a thorough discussion of this topic]. Furthermore, that the scope for energy expenditure is variable between dives, as a result of activities undertaken within the dive (e.g., Ref. 54), also indicates that to consider both cADL and DLT as single, one-dimensional, inflexible thresholds is an oversimplification of a complex system.

As indicated above, the majority of behavioral studies investigating the time budgets of diving animals suggest that surface interval (\(t_s\)) durations are not long enough to allow for recovery from anaerobic metabolism i.e., that dives are generally almost completely aerobic (49) and thus do not exceed the DLT, let alone the cADL. This implies then that, when determining cADL, either the calculation of usable oxygen stores and/or the estimate of \(V_{O2D}\) are incorrect. There is surely a limit to the inaccuracy in estimates of total body oxygen stores (35). Certainly, it seems unlikely that total body oxygen stores could be two or three times greater than current estimates, as would be necessary for all diving to be within the cADL (40). This indicates that most of the inaccuracy lies in the estimation of \(V_{O2D}\). As a result, a more accurate method to estimate \(V_{O2D}\) is required, if we are to develop a solid working knowledge of the energetic costs of diving from which we can investigate physiological adaptations more robustly.

Progress with this work has been limited since it is not possible to measure \(V_{O2D}\) directly (14). Several experimental techniques have been employed to estimate \(V_{O2D}\), but the results of these studies vary substantially. Respirometry has been used to calculate \(V_{O2}\), while animals are at the surface between dives from man-made holes in the ice of the Antarctic (13), and/or while they swim or rest in a static water channel or flume (e.g., Ref. 17). A recent new approach utilizes body

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movement as a calibrated proxy for \( V_O^2 \) (55). A first step in measuring \( V_O^2D \) has been made by measuring the decline in the partial pressure of oxygen (\( P_O^2 \)) in the air sacs during dives of emperor penguins (\( A. forsteri \); 34). In free-ranging animals, isotopic techniques, including doubly labeled water, have often been used to estimate \( V_O^2 \) during complete foraging trips and to equate this (or a proportion of this) to \( V_O^2D \) (e.g., Ref. 15). Heart rate (\( f_H \)) has been recorded during free-ranging diving in a variety of species, including elephant seals (\( M. leucomelaena \); 31), Antarctic fur seals (\( A. gazella \); 5), and South-Georgian shags (\( P. georgianus \); 2). If a relationship has been established between \( f_H \) and \( V_O^2 \) for a given species, then it is possible to estimate the \( V_O^2 \) of a complete dive cycle \( [n] \) and the subsequent \( s \) from the mean \( f_H \) of that dive cycle (e.g., Refs. 20 and 24). Unfortunately, however, \( f_H \) measured while the animal is submerged or on the surface cannot be used to estimate \( V_O^2 \) directly, either while submerged or during the subsequent surface interval, as studies have shown that during diving the relationship between \( f_H \) and \( V_O^2 \) is not consistent (4, 21).

Mathematical or modeling approaches have also been developed to ascertain \( V_O^2D \). Recent work has included the effects of buoyancy and metabolic rate while resting (e.g., Ref. 30). Woakes and Butler (57) developed a model to calculate mean \( V_O^2D \) at the mean \( t_D \) and \( t_S \) of tufted ducks (\( A. fuligula \)) diving freely in a laboratory tank. The concept used was based on a technique first applied by Scholander (48), which obtained an estimate of \( V_O^2D \) by partitioning the oxygen uptake between dives into the amount of oxygen consumed during the surface interval and the amount consumed during the preceding dive. While instructive, this approach could only provide an estimate of \( V_O^2D \) for “average” dives. With a greater experimental data set and improved mathematical model, Halsey et al. (27) refined this approach. They controlled for the effects of \( t_S \) by plotting changes in oxygen uptake after 10 s (sufficient time to replenish the oxygen stores of tufted ducks) against \( t_D \). Then, by assuming that \( V_O^2 \) while at the surface and while descending and ascending to the foraging site (a tray at constant depth) were fixed quantities regardless of \( t_D \), the slopes of these regressions represented \( V_O^2D \) during the foraging part of the dive (i.e., the part of the dive varying with \( t_D \)). This represented the first direct and tangible estimate of \( V_O^2D \) during diving. Due to a relatively small sample size, Woakes and Butler (57) and even Halsey et al. (27) were obliged to assume that \( V_O^2 \) at the surface was independent of \( t_S \) duration, thus regressing only \( t_D \) against oxygen uptake at fixed durations of \( t_S \).

In contrast to these laboratory studies, recordings of heart rate during free-ranging diving are able to include a far greater number and variety of dives, allowing some of the limitations and assumptions of previous models to be eliminated. If \( f_H \) over the dive cycle is used to estimate \( V_O^2 \), then it is possible to develop the models of Scholander (48), Woakes and Butler (57), and Halsey et al. (27) and not only quantify the rate of oxygen consumption during the dive cycle, but investigate how variability in different elements of the dive cycle (e.g., diving depth, \( t_D \), \( t_S \)) might influence it. This development of the original model by Scholander (48) apportions total heart beats recorded over the diving cycle (\( \Sigma H_{DC} \)) to the time spent either submerged or on the surface. In the present study, we use an example data set of over 500,000 dives by macaroni penguins (\( E. chrysolophus \)) for which heart rate has been recorded, to illustrate how statistical modeling can take advantage of such a large number of dives, and demonstrate how \( V_O^2D \) can be predicted from factors as straightforward as the \( t_S \) and dive depth. A key finding is that the duration of a surface interval often reflects the occurrence of energy-costly activities during the previous dive, not simply the duration of that dive.

We suggest that our estimates of \( V_O^2D \) in macaroni penguins are the most direct, plausible, and meaningful obtained to date for a free-ranging animal. This approach could have applications in other studies where it is of interest to look at rate of oxygen consumption at a fine scale, beyond that which can be achieved using respirometry or other methods that measure oxygen uptake, which is only equivalent to oxygen consumption when an animal is in steady state. Such applications could include studies of apnea (1, 39) or short periods of exercise (e.g., Ref. 33).

**MATERIALS AND METHODS**

**Data Collection**

The approach in the present study is based on the reanalysis of data collected on the diving behavior and energetics of macaroni penguins (22, 23). Briefly, the penguins were implanted with data loggers that recorded heart rate every 10 s and dive depth every 2 s. Data were analyzed from the winter migration of 18 penguins (9 male, 9 female) during 2002, since this period represented the most complete set of data for a single phase of the annual cycle (23). Data from both sexes were pooled, since, while the diving behavior and capacity varies between male and female macaroni penguins (23), there is no difference between the sexes in the relationship between \( f_H \) and \( V_O^2 \) (26).

Several diving species intersperse foraging dives with shallow nonforaging dives, thought to have a role in the recovery from oxygen debt (e.g., Ref. 50). This behavior is not shown by macaroni penguins so, as in previous studies (23), only foraging dives (maximum depth > 10 m) were used in the analysis. Since \( f_H \) was recorded less frequently than dive depth, dives with a \( t_D \) or \( t_S \) of <10 s (which would have included the majority of dives to <10 m in any case) were ignored. Dives with a \( t_S \) > 600 s were also ignored, as these were assumed to represent interbout periods rather than surface intervals for recovery from the previous dive and preparation for the next. In total, 514,106 dives were analyzed.

Conversions of \( f_H \) to mass specific rate of oxygen consumption (\( sV_O^2 \); \( ml\cdot min^{-1}\cdot kg^{-1} \)) were made using equation 14 from Green et al. (26). Conversion to \( sV_O^2 \) is appropriate for this species, as they have a mass exponent of one (25). As with all studies of this type, using \( f_H \) as a proxy for \( sV_O^2 \) assumes that the oxygen pulse (cardiac stroke volume multiplied by oxygen extraction by the tissues) varies with \( f_H \) in a predictable way under all physiological circumstances (6). It is not possible to use the heart rate method to predict \( V_O^2 \) accurately for individual animals (6). Therefore, in the present study, all models were constructed and calculations computed using \( f_H \) and \( \Sigma H_{DC} \) for each individual animal. Resulting mean values of \( f_H \) and \( \Sigma H_{DC} \) for all animals were then converted to estimates of \( V_O^2 \), as described above. The standard error of the estimate (SEE) for estimates of \( sV_O^2 \) was calculated following the procedures established by Green et al. (25).

**Calculation**

**Structure of models.** All calculations were based on the principle that the total volume of oxygen consumed during a dive cycle (\( \Sigma V_O^2_{DC} \)) is a function of \( t_D \), \( t_S \), \( V_O^2 \), and the rate of oxygen consumption during the subsequent surface interval (\( V_O^2S \)). This is summarized by Eq. 1.
OXYGEN CONSUMPTION DURING SUBMERSION

\[ \Sigma_{O2,DC} = (V_{O2,D} \times t_d) + (V_{O2,S} \times t_s) \]  

An important point to note is that \( V_{O2,S} \) represents the rate at which oxygen is consumed by the tissues during the surface interval. This is not equal to total oxygen uptake during the surface interval, which has to be sufficient to match oxygen used during both the previous dive and the subsequent surface interval and will, therefore, be equal to \( \Sigma_{O2,DC} \). As explained above, a calibration relationship between \( f_{d0} \) and \( V_{O2} \) can be used to estimate mean \( V_{O2} \) of a complete dive cycle from the mean \( f_{d0} \) of that dive cycle. Therefore, the same relationship can also be used to estimate the \( \Sigma_{O2,DC} \) of a dive cycle from the \( \Sigma_{O2,DC} \) for that dive cycle. Thus, Eq. 1 can be converted to Eq. 2.

\[ \Sigma_{O2,DC} = (f_{d0} \times t_d) + (f_{s0} \times t_s) \]  

Model 1

While the physiological state of a diving animal changes unpredictably during the course of a dive as they adjust their circulation and balance the demands of foraging, locomotion, and oxygen conservation (e.g., Ref. 3), it is reasonable to suppose that changes are much more predictable during the subsequent surface interval. If so, then at any given value of \( t_s \), we assume that within an individual animal, \( f_{s0} \) is a constant. In other words, for all surface intervals of, for example, 30 s, \( f_{s0} \) will always be similar. Therefore, at a given \( t_s \), \( f_{s0} \times t_s \) will also be a constant (\( \alpha \)). Similarly, if a surface interval is a period of recovery from the previous dive, then it seems reasonable to expect that during dives with a given surface interval the rate of energy expenditure (represented by \( f_{d0} \)) during the previous dive will also be a constant (\( \beta \)). While many studies show a close association between \( t_d \) and \( t_s \), there will still be sufficient variability in \( t_s \) within a given category of \( t_s \) so that Eq. 2 can be rearranged to give Eq. 3.

\[ \Sigma_{O2,DC} = (t_d \times \beta) + \alpha \]  

Model 2

Certain studies, both in the laboratory and in the field, indicate that mean \( f_{d0} \) of dive cycles is lower during longer dives (3, 24). It is possible that \( f_{d0} \) also decreases as \( t_d \) increases. If this is correct, then, rather than assume \( f_{d0} \) is constant within a category of \( t_d \), it is replaced with a simple linear function of \( t_d \) (Eq. 4). Incorporating this relationship into the model should improve the estimates of \( f_{d0} \) and \( f_{s0} \). As before, at a given \( t_s \), \( f_{s0} \times t_s \) is assumed to be a constant (\( \alpha \)), therefore, substituting \( f_{d0} \) in Eq. 2 with Eq. 4 gives us Eq. 5.

\[ f_{d0} = (t_d \times \varphi) + \beta \]  

\[ \Sigma_{O2,DC} = (t_d \times \varphi) + (t_d \times \beta) + \alpha \]
Any further validation of the model within the data set risks forming a circular argument. While a more robust test of the models would be to input data from other diving bouts, in this case, this would entail using data from another season. The seasonal variability in diving capacity shown by macaroni penguins (22, 23) means that a test of the models with data from another part of their annual cycle is not necessarily valid. Therefore, the models were tested on the data set used to derive them, in order at least to determine which model gave the most accurate estimates of $\sum_{tD}$ and, within that, $f_{HD}$ and $f_{HDC}$. To limit the circularity of the validation, the data were organized in a different fashion from that for the derivation of the models. For each animal, all dives were initially classified into bins by $tD$ (4 s). Within each 4-s bin of $tD$, dives were then classified into 4-s bins of $tS$ and then mean depD was calculated for each bin for each animal. A grand mean of depD for all animals was then calculated for each bin of $tS$. For comparison, the same approach was also applied to $\sum_{tDC}$ calculated from the upper 95% confidence limits.*

RESULTS

Table 1 shows the summary statistics for the regressions of each model within bins of $tS$, within each penguin. Mean $r^2$ was significantly different between all four models (ANOVA with Tukey’s post hoc testing, $P < 0.001$ in each case). Both the intercept and slope were significant in nearly every case in model 1. However, the mean $r^2$ of model 1 regressions was the lowest of the four. All three multiple regressions fitted the data very well, although the coefficients of the slopes were statistically significant, most frequently, and had higher partial correlation coefficients for model 3. Furthermore, model 3 had the highest mean $r^2$. Certainly, adding all of the variables into one model (model 4) did not appear to produce the most descriptive model.

As a test of the four models, each was used to predict $\sum_{tDC}$ for all combinations of $tD$ and $tS$ (4-s bins) from the validation data set. Figure 1 shows predicted $\sum_{tDC}$ as a function of measured $\sum_{tDC}$ for all four models. A weighted regression (APPENDIX A) was used to assess predicted $\sum_{tDC}$ as a function of measured $\sum_{tDC}$. All four regressions were significantly different from the line of equality ($t$-tests, $P < 0.001$, Table 2) but with such large sample sizes ($n = 1,381$ in each case) this is not surprising. To provide further comparisons of the validity of each model, the mean percentage error of predictions made using the four models was also calculated (Table 2). Mean percentage error gives a measure of the accuracy of predictions compared with a measured standard. Mean percentage error was lowest for model 3, although all four models were accurate (<5% in each case). The standard error of the mean percentage error was greatest for model 3, implying that this model was less precise than the others. However, in each case the standard error of the mean was rather low and the resulting confidence intervals of mean percentage error would also have been relatively small. As a result, the conclusion is that the precision for all models was relatively high.

Figure 1 and Table 2 both suggests that all four different models can be used to predict $\sum_{tDC}$ with a relatively high degree of accuracy. However, the aim of the modeling procedure was to derive a technique for the reliable estimation of $\sum_{tD}$. Each model was used to predict $\sum_{tD}$ for each combination of $tD$ and $tS$ in the validation data set. cADL was then determined for each combination by dividing usable oxygen stores for macaroni penguins (58 ml/kg ± 10%) by this value of $\sum_{tD}$. This procedure was also repeated for $\sum_{tDC}$ calculated from measured $\sum_{tDC}$ (Table 3). Only under models 1 and 3 were the vast majority of dives within the cADL. $\sum_{tD}$ predicted using models 2 and 4 barely increased the proportion above estimates made using $\sum_{tDC}$.

In conclusion, models 1 and 3 appear to be superior in predicting both $f_{HD}$ and $\sum_{tDC}$. The addition of an effect of $tD$ on

<table>
<thead>
<tr>
<th>Model</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
<th>$\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%Significant</td>
<td>%Significant</td>
<td>PCC</td>
<td>%Significant</td>
</tr>
<tr>
<td>1</td>
<td>98.4</td>
<td>99.7</td>
<td>0.057±0.003</td>
<td>48.5</td>
</tr>
<tr>
<td>2</td>
<td>74.7</td>
<td>75.5</td>
<td>0.128±0.007</td>
<td>61.5</td>
</tr>
<tr>
<td>3</td>
<td>97.6</td>
<td>86.5</td>
<td>0.063±0.004</td>
<td>59.3</td>
</tr>
<tr>
<td>4</td>
<td>73.6</td>
<td>77.9</td>
<td>0.063±0.004</td>
<td>59.3</td>
</tr>
</tbody>
</table>

Each regression was constructed for each penguin within surface interval duration bins of 4 s. A total of 371 regressions were calculated for each model. Each model had different variables and different numbers of variables (see text for details). In each case, $\alpha$ was the intercept, while variables $\beta$, $\gamma$, and $\delta$ represented different slopes. %Significant is the percentage of the 371 regressions in which the intercept/slope were found to have a significant effect on $\sum_{tDC}$. PCC is the mean ± SE partial correlation coefficient of these variables in the model. The mean $r^2$ ± SE for each of the 371 regressions for each model is also shown.
f_{HD} in models 2 and 4 barely increased the accuracy of prediction (Tables 1 and 2) and produced improbable results in terms of the proportion of dives within the cADL (Table 3). When comparing models 1 and 3 with each other, there is little to choose between them. However, while the accuracies in terms of individual regressions and calculation of cADL are very similar, model 3 adds an extra easily measured variable (depD), which increases the accuracy of prediction of Σ_{HDC} (Tables 1 and 2) and reduces the variability in the percentage of dives within the cADL (Table 3). We suggest that other studies explore all possible models when applying this technique in future, but in the present study we have selected model 3 as the best way to predict both f_{HD} (Eq. 10) and Σ_{HDC} (Eq. 11) in macaroni penguins.

### Table 2. Regression relationships and mean percentage error between measured and predicted total Σ_{HDC}

<table>
<thead>
<tr>
<th>Model</th>
<th>Intercept</th>
<th>Slope</th>
<th>( r^2 )</th>
<th>Mean Percentage Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.0</td>
<td>0.87</td>
<td>98.6</td>
<td>-2.21±0.34</td>
</tr>
<tr>
<td>2</td>
<td>54.3</td>
<td>0.85</td>
<td>97.8</td>
<td>-4.41±0.26</td>
</tr>
<tr>
<td>3</td>
<td>37.2</td>
<td>0.85</td>
<td>98.0</td>
<td>-0.44±0.43</td>
</tr>
<tr>
<td>4</td>
<td>95.4</td>
<td>0.78</td>
<td>91.9</td>
<td>-1.67±0.33</td>
</tr>
</tbody>
</table>

Predictions were made for a range of combinations of dive duration (\( t_d \)) and surface duration (\( t_s \)), using 4 different models. Regressions were weighted to account for different numbers of dives within each combination of \( t_d \) and \( t_s \).

Percentage errors were calculated as \% error = \frac{[\text{predicted} - \text{measured}]}{\text{measured}} \times 100

Figure 2 shows how the different constants associated with model 3 varied as a function of \( t_s \) (constant functions). In all models, \( f_{HD} \) was equal to \( \alpha \) divided by \( t_s \). Weighted polynomial regressions (APPENDIX A) showed that \( f_{HDC} \) varied significantly as a function of \( t_s \) (Fig. 3). Substitution of these constant functions into Eqs. 6 and 7 generated the predictive equations for \( f_{HD} \) (Eq. 10) and \( \Sigma_{HDC} \) (Eq. 11).

\[
f_{HD} = \left(219.7 - 461.6t_s + 451.6t_s^2 - 136.2t_s^3\right)
+ \left[(-1.1 + 8.1t_s - 15.9t_s^2 + 12.3t_s^3 - 3.2t_s^4) \times \text{depD}\right]
\quad (10)
\]

\[
\Sigma_{HDC} = \left[\left(219.7 - 461.6t_s + 451.6t_s^2 - 136.2t_s^3\right) \times \text{t_n}\right]
+ \left[(-1.1 + 8.1t_s - 15.9t_s^2 + 12.3t_s^3 - 3.2t_s^4) \times \text{depD} \times \text{t_n}\right]
+ \left[34.0t_s + 1813.0t_s^2 - 2638.4t_s^3 + 1436.1t_s^4 - 273.5t_s^5\right]
\quad (11)
\]

\( \dot{V}_{O_2D} \) varies considerably as \( t_s \) and depD vary. APPENDIX B contains similar derivations for the other three models. Figure 3 shows a frequency distribution of all the values of \( \dot{V}_{O_2D} \) calculated for the validation data set using Eq. 10 (model 3) and converted to rate of oxygen consumption. During the winter, \( \dot{V}_{O_2D} \) varied from 4 to 28 ml·min⁻¹·kg⁻¹ with a median value of 11.25 ml·min⁻¹·kg⁻¹. The 95% confidence intervals of these predictions, incorporating both the uncertainty in the model development and the error in conversion of...
fH to VO₂ ranged from 1 to 40 ml·min⁻¹·kg⁻¹ but were clustered tightly around the predictions (Fig. 3).

**DISCUSSION**

For over 100 years, physiologists have been striving to discover how animals are able to perform extraordinary feats of diving [see Butler and Jones (9, 10) for reviews of the history of diving physiology studies]. Many of these studies have focused on attempting to estimate VO₂D, despite the obvious challenges of such an objective (14). In the present study, using macaroni penguins as an example, we have developed a model that allows the prediction of VO₂D from fH based on temporal and physical characteristics of the dive (tS and depD). The iterative process of model construction and generation allowed the inclusion of various factors that might feasibly have influenced VO₂D and facilitated the unbiased selection of the most appropriate model. In the example calculation with macaroni penguins, there were still a few dives (1.3% with confidence intervals of 0.3–6.6%) that appeared to exceed the cADL. This can be attributed to variability in the factors within the model and suggests that inclusion of more predictive factors or information about the dives could further improve the accuracy of this technique.

**Factors Influencing Dive Behavior and Energetic Costs**

Perhaps the most interesting aspect of the present study is the factors that proved to be significant and aided in the selection of a model and those which did not. Model 3 gave the best predictions. However, despite being the simplest of all of the models, including only the effects of tS on fH, model 1 also gave excellent predictions of both fH and ΣHDC. While model 3 improved upon this by adding an effect of depth, the most important factor in defining the metabolic cost of a dive appears to be the subsequent tS. To some extent this is a question of cause and effect, as it is unlikely that the rate of metabolism during a particular dive is set by the subsequent tS. Physiologically speaking, Eq. 10 should really be rearranged such that tS is dependent on fH. In other words, the rate at which oxygen is consumed while submerged will have a profound effect on the subsequent tS. Furthermore, the shape of the curve of cumulative oxygen uptake suggests that this relationship is not simply linear (28). That tS and fH are linked is perhaps not surprising, although it is gratifying that the process of model development should reveal this link.

It is also not surprising that depD is an important predictor of VO₂D. Much recent work on diving physiology (with penguins in particular) has revealed that animals often appear to match aspects of their physiology to the intended depth of a dive. King penguins (Aptenodytes patagonicus), Adélie penguins (Pygoscelies adeliae), and Magellanic penguins (Spheniscus

<table>
<thead>
<tr>
<th>Model</th>
<th>%Dives Within cADL</th>
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<tbody>
<tr>
<td>1</td>
<td>97.1 (63.3–100.0)</td>
</tr>
<tr>
<td>2</td>
<td>17.1 (6.0–76.9)</td>
</tr>
<tr>
<td>3</td>
<td>98.7 (89.3–99.9)</td>
</tr>
<tr>
<td>4</td>
<td>10.6 (2.9–67.0)</td>
</tr>
<tr>
<td>VO₂DC</td>
<td>8.9 (3.8–20.4)</td>
</tr>
</tbody>
</table>

Also shown is the %dives that would be within the cADL if it is assumed that VO₂D was equivalent to VO₂ for the complete dive cycle (VO₂DC). 95% Confidence intervals are shown in parentheses.

![Fig. 2](http://ajpregu.physiology.org/)

**Fig. 2.** A: variation in mean (open symbols) heart rate during a surface interval between two dives (fH) as a function of the duration of that surface interval (tS). B and C: variation in the mean (open symbols) of 2 constants (β and ϕ; dimensionless) relating to the response of heart rate during the previous dive as a function of tS. See text for details of derivation of these constants. Also shown are fitted, weighted, polynomial regression curves with 95% confidence intervals (dashed lines) and their equations. All three relationships were significant (P < 0.001, r² = 94.8, 97.5, 89.1, respectively). Data are taken from 18 male and female free-ranging macaroni penguins diving during their winter migration.
magellanicus) modulate their inhaled air volume prior to submersion such that when their intended depth is reached they are neutrally buoyant (46, 56). Magellanic penguins also appear able to predict the rate of oxygen consumption in a forthcoming dive based on its duration and likelihood of prey capture (51). Macaroni penguins actively stroke their flippers during descent but during ascent are able to glide for at least half of the time (45). Mean stroke frequency during descent is independent of dive depth, but during ascent it decreases with increasing dive depth. As a result, in mechanical terms at least, \( \dot{V}O_2D \) will be influenced by depth (30, 53). These data show that penguins make decisions based on complex environmental parameters, suggesting a remarkably efficient use of time during foraging (51). In the case of macaroni penguins, \( \dot{V}O_2D \) and depth interact to define the \( t_s \).

While our models are unable to include the likelihood of occurrence of prey capture events, our findings may suggest that these events are critical in determining \( \dot{V}O_2D \) and \( t_o \). In other species of free-ranging penguins feeding on euphausids, prey capture events are associated with rapid acceleration and deceleration (44). Rapid acceleration and deceleration during horizontal swimming is energetically expensive and is therefore likely to limit \( t_o \) and distance swum (18). Furthermore, prey pursuit by penguins may involve swimming at speeds higher than the optimal cost of transport, and the power costs of swimming increase with the cube of swim speed (54). As a further complication, the required prey capture speed may vary between prey patches or between different target prey species, all of which will introduce further variability (54). Respirometry studies of Weddell seals suggest that \( \dot{V}O_2D \) is greater during foraging dives than during exploratory dives (40). If prey capture events are unpredictable and energetically expensive, then they are likely to have an important effect in shortening or lengthening dives, and, as a result, the duration of a dive is not necessarily a guide to its energetic cost. In other words, a dive of 1 min duration could vary substantially in \( \dot{V}O_2D \), depending on the type or prey and whether or not energetically expensive prey capture events occur. The duration of the bottom phase of dives and subsequent behavior during ascent (whether an immediate return to the surface or prey searching) vary substantially in macaroni penguins and are dependent on each other (45), suggesting that prey capture rates also vary substantially between individual dives.

While the rejection of models 2 and 4 shows that \( f_{1D} \) is not dependent on \( t_o \) at all, \( t_o \) is of course critical in determining the total energy expenditure of the dive (Eq. 1). There is little reason to assume that the duration of any given event should affect the rate at which energy is expended; in fact, the reverse is more likely. In a resource-limited environment, the rate at which energy is required for a given activity (which, in the case of \( \dot{V}O_2D \), appears to be highly variable and dependent on extrinsic factors) is more likely to limit its duration than vice versa. Hence there is a lack of dependence of \( f_{1D} \) on \( t_o \). Figure 4 illustrates the complex nature of the interactions between \( t_o \), \( t_s \), and depth. Figure 4 shows the tremendous variation in combinations of \( t_o \) and \( t_s \) or depth. While the majority of dives have a \( t_o \)-to-\( t_s \) ratio of \(-5 \), this ratio varies from 0.25 to 18. Clearly, \( t_s \) is influenced by factors other than \( t_o \) alone. This variability in \( t_s \) (i.e., recovery and preparation duration in terms of oxygen restocking, CO2 elimination, and possibly lactate conversion) for a given \( t_o \) presumably represents variability in the level of total oxygen stores before and after a dive (due to the shape of the curve of cumulative oxygen uptake) or energetic costs during the preceding dive, due either to differences in depth or prey capture events (see above) or other energetically costly activities. Similarly, the wide variation in \( t_o \) at a given depth suggests that differing costs within a dive to a given depth may lead to different levels of \( \dot{V}O_2D \), which will in turn affect its duration (\( t_o \)).

Variability in Rate of Oxygen Consumption During Submergence

In the absence of detailed measurements or predictions, many studies have assumed that \( \dot{V}O_2D \) is constant across all

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**Fig. 3.** Frequency distribution (black bars) of rate of oxygen consumption during submersion (\( \dot{V}O_2D \)) estimated from \( t_o \), \( t_s \), and maximum dive depth (depth) using model 3. Also shown are the minimum (grey shadow) and maximum (white shadow) 95% confidence intervals of this distribution.

**Fig. 4.** Frequency plot of combinations of \( t_o \) and subsequent \( t_s \) (A) and \( t_o \) and maximum dive depth (B) recorded from 18 male and female free-ranging macaroni penguins during their winter migration. Shades denote the number of dives observed at each combination in which black = 1,001–10,000, dark grey = 101–1,000, midgrey = 11–100, and light grey = 1–10.
dives and independent of other factors associated with the dive (e.g., Ref. 15). However, in the present study, we show that \( V_{\text{O}2D} \) can have a wide range of values (Fig. 3). Some other studies have also shown this to be the case: for example, postdive respirometry of Weddell seals suggests that \( V_{\text{O}2D} \) is subject to considerable variability (40), which is further supported by theoretical calculations (30). Further evidence in support of this comes from the only two studies that have measured DLT. Both emperor penguins (41), and to a lesser extent Weddell seals (37), show variation in their lactate response at a given \( t_0 \). Figure 2 from Ponganis et al. (41) shows clearly how in one of four penguins (only 22 dives were analyzed) lactate production is substantial during a dive of \( \sim 5.75 \text{ min} \), but in another dive of \( \sim 6.25 \text{ min} \), postdive lactate levels were barely elevated above resting levels. This illustrates that even within an individual animal performing a small number of dives from a fixed location, either oxygen stores or \( V_{\text{O}2D} \) must be subject to substantial variation, and DLT represents rather more than a fixed time threshold beyond which lactate accumulation always occurs, as many authors use it (e.g., Ref. 15).

As the limit of oxygen stores is approached, dives are likely to have a high component of anaerobic metabolism, as in Weddell seals and emperor penguins (Fig. 5). Unfortunately, the present study gives no insight as to what \( t_0 \) and activity might correspond to the DLT for macaroni penguins. It should be noted again that in Weddell seals \(< 3\% \) of dives exceeded the DLT; so for macaroni penguins, the proportion of dives where \( V_{\text{O}2D} \) does not account for all of the metabolism during a dive is also likely to be very low and correspond only to the longest and/or most energetically demanding dives. The ratio of \( t_{\text{DLT}}/t_0 \) (Fig. 4) indicates that only a small percentage of dives by macaroni penguins are likely to exceed their DLT.

### Rate of Oxygen Consumption During Submergence

In the absence of measurements, several authors have speculated on what \( V_{\text{O}2D} \) might be. Some authors have noted that for all dives to be within the cADL, \( V_{\text{O}2D} \) must be lower than that while animals rest in land, i.e., they exhibit (aerobic) hypometabolism. (8). Other studies have used \( \text{VO}_2 \) while animals rest on land as a proxy for \( V_{\text{O}2D} \) (30), which also suggests hypometabolism, since in most species studied to date, \( \text{VO}_2 \) while resting on water is approximately twice that while resting on land. Other studies have used the metabolic cost of swimming in a static water canal but admit that there are limitations with this analysis, which are likely to lead to an overestimation of \( V_{\text{O}2D} \) (16, 18). As mentioned above, in the current study, we have found that \( V_{\text{O}2D} \) is not a fixed value but rather that it varies in association with depth and possibly with the performance of energetically costly activities. Predicted values of \( V_{\text{O}2D} \) ranged from 4 to 28 ml\( \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \) (Fig. 4), although 93% of dives were in the range of 6 to 20 ml\( \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \), and the median was 11.25 ml\( \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \). By way of comparison, mean \( \text{VO}_2 \) was 10.3 ml\( \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \) while macaroni penguins rested on land and 25.6 ml\( \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \) while they rested in water (26). To illustrate the effect of using different proxies for \( V_{\text{O}2D} \), we have recalculated cADL and the proportion of dives within this limit by using a variety of methods (Table 4). This exercise illustrates that for macaroni penguins at least, the present approach is the most plausible estimate of mean \( V_{\text{O}2D} \).

### Table 4. Rate of \( V_{\text{O}2D} \) and the resulting percentage of naturally occurring dives that would be within the cADL

<table>
<thead>
<tr>
<th>Technique</th>
<th>Model 3</th>
<th>( V_{\text{O}2D} )</th>
<th>FMR(^a) (fs method)</th>
<th>FMR(^b) (DLW)</th>
<th>( V_{\text{O}2c} ) (resting in air)</th>
<th>( V_{\text{O}2c} ) (resting on water)</th>
<th>( V_{\text{O}2c} ) (active swimming)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{\text{O}2D} ), ml( \cdot \text{min}^{-1} \cdot \text{kg}^{-1} )</td>
<td>4–28</td>
<td>20–56</td>
<td>32.0±1.5</td>
<td>65.6</td>
<td>10.3±0.5</td>
<td>25.6±2.1</td>
<td>40.3</td>
</tr>
<tr>
<td>%Dives Within cADL</td>
<td>98.7 (93.4–99.7)</td>
<td>8.9 (3.8–20.4)</td>
<td>20.0 (8.5–41.4)</td>
<td>1.2 (0.6–2.4)</td>
<td>100 (100–100)</td>
<td>41.3 (17.1–83.3)</td>
<td>6.9 (3.0–17.1)</td>
</tr>
</tbody>
</table>

Calculations are made for macaroni penguins during their winter migration by using a variety of commonly used proxies. Oxygen stores were assumed to be 58 ml/kg (see text). \(^a\) Data from Green JA, Boyd IL, Wokes AJ, and Butler PJ, unpublished observations. \(^b\) Calculated from mean field metabolic rate (FMR) during premoult extended foraging trip, but reduced by 30% to match observed seasonal decrease in FMR from heart rate (9a) method (data from Ref. 18a). DLW, doubly labeled water. \(^c\) Data from Ref. 26. \(^d\) Calculated as the midpoint of the minimum and maximum values for \( V_{\text{O}2D} \) recorded while animals swam in a static water canal. Where available, means are ± SE or SEE. 95% Confidence intervals for cADL and %cADL (in parentheses) are calculated using means ± SE or SEE. Where error was not available, a nominal 10% of the mean was used.
to date, but in the absence of similar data, resting VO2 in air is the most reasonable substitute.

When compared with resting data, the range of VO2D determined using the present approach suggest that macaroni penguins reduced their metabolic rate below the resting level in water to extend its life (see Ref. 8). This may be possible in part since heat generated by the pectoral muscles during locomotion can be used to substitute for thermoregulatory costs (52). In the present study, we were unable to explore hypometabolism in detail and ascertain whether it might also be associated with observed decreases in abdominal temperature during diving (24). Debate continues on the role of regional heterothermy in energy saving during diving (29, 42, 43, 47). By contrast, recent work on king penguins suggests that such low values of VO2 while resting on water do not represent a hypometabolic state. Unlike fasted birds (as most animals in respirometry studies tend to be), well-fed king penguins had a resting VO2 in water that was not significantly different from that while on land (19). This finding matches data from macaroni penguins which suggest that by the middle of winter, VO2 while inactive at sea (at night, not while foraging) is closer to resting VO2 on land than it is to resting VO2 on water (22).

In conclusion, we have combined two established techniques (multiple regression calculation of VO2D in the laboratory and studies tend to be), well-fed king penguins had a resting VO2 in water that was not significantly different from that while on land (19). This finding matches data from macaroni penguins which suggest that by the middle of winter, VO2 while inactive at sea (at night, not while foraging) is closer to resting VO2 on land than it is to resting VO2 on water (22).

APPENDIX A

Calculation of Weighted Means

Weighted means of regression coefficients were calculated for each bin of rs since the strengths of the regressions varied between individual penguins. Each regression parameter estimate (E; the intercept and one or more slopes) for each penguin has a standard error (S). The weight (W) of each estimate is calculated as

\[ W = \frac{1}{S^2} \]

The mean estimate (\( \bar{E} \)) is calculated as

\[ \bar{E} = \frac{\sum W_i \times E_i}{\sum W_i} \]

The standard error (S) of this weighted mean is calculated as

\[ S = \frac{1}{\sum W_i} \]

APPENDIX B

Model 1

Total \( \Sigma_{DC} \) was regressed as a function of \( r_s \) within each 4-s bin of \( r_s \) for each penguin. Both \( \alpha \) (equivalent to \( f_{BS} \times r_s \)) and \( \beta \) (equivalent to \( f_{BD} \)) were significant in most of the regressions (Table 1). A weighted mean \( \pm SE \) (APPENDIX B) of \( f_{BD} \) and \( f_{BS} \) was calculated for each 4-s bin of \( r_s \) using the coefficient and its mean \( \pm SE \) for each penguin represented in that bin. The weighting accounted for differences between individual penguins in the variance of the mean value of the dependent variable in each rs bin. Mean \( f_{BS} \) and \( f_{BD} \) were then plotted as a function of \( r_s \) and a weighted polynomial regression (maximum 4th order) was used to ascertain whether there were relationships between \( r_s \) and \( f_{BS} \) or \( f_{BD} \). Both \( f_{BS} \) (3rd order) and \( f_{BD} \) (4th order) varied significantly as polynomial functions of \( r_s \) (Fig. 1).

Substitution of these relationships into Eq. 2 generated our first predictive equation of \( \Sigma_{DC} \) (Eq. 10).

\[ \Sigma_{DC} = [(141.7 + 49.0r_s - 409.4r_s^2 + 476.6r_s^3 - 152.6r_s^4) \times \bar{r_s}] + [163.3r_s + 810.4r_s^2 - 9300r_s^3 + 281.3r_s^4] \]  

MODEL 2

In a similar fashion to model 1, \( \Sigma_{DC} \) was regressed as a function of \( \bar{r_s} \) and \( \bar{r_s}^2 \). Therefore, as well as \( f_{BS} \), coefficients \( \beta \) and \( \varphi \), respectively, were generated for each penguin in each bin of \( r_s \), most of which were statistically significant (Table 1). As before, a weighted mean of \( f_{BS} \), \( \beta \), and \( \varphi \) was calculated for each bin of \( r_s \) and plotted against \( r_s \) (Fig. 2). Weighted polynomial regressions showed that \( f_{BS} \) (3rd order), \( \beta \) (4th order), and \( \varphi \) (4th order) varied significantly as a function of \( r_s \). Substitution of these relationships into Eq. 5 generated our second predictive equation of \( \Sigma_{DC} \) (Eq. 11).

\[ \Sigma_{DC} = [(157.1 - 157.5\bar{r_s} - 797.0r_s^2 + 840.0r_s^3 + 255.1r_s^4) \times \bar{r_s}] + [(2.7 + 67.4r_s - 316.3r_s^2 + 333.0r_s^3 - 102.5r_s^4) \times \bar{r_s}^2] + [386.8r_s - 592.8r_s^2 + 577.0r_s^3 - 175.5r_s^4] \]

MODEL 4

As with models 1–3, \( \Sigma_{DC} \) was regressed as a function of \( \bar{r_s} \), \( \bar{r_s}^2 \), and \( \bar{r_s}^3 \). Therefore, as well as \( f_{BS} \), coefficients \( \beta \), \( \varphi \), and \( \delta \) were generated for each penguin in each bin of \( r_s \), many of which were statistically significant (Table 1). As before, a weighted mean of \( f_{BS} \), \( \beta \), \( \varphi \), and \( \delta \) was calculated for each bin of \( r_s \) and plotted against \( r_s \) (Fig. 4). Weighted polynomial regressions showed that \( f_{BS} \) did not vary significantly as a function of \( r_s \) (Fig. 4). However, the three constants \( \beta \) (2nd order), \( \varphi \) (2nd order), and \( \delta \) (4th order) all did vary significantly as a function of \( r_s \). Substitution of these relationships into Eq. 7 generated our third and final predictive equation of \( \Sigma_{DC} \) (Eq. B2).

\[ \Sigma_{DC} = [(155.9 + 118.6r_s + 155.9r_s^2) \times \bar{r_s}] + [(14.1 - 92.4r_s - 155.9r_s^2 \times \bar{r_s}^3) + [(2.5 - 57.5r_s - 15.9r_s^2) \times \bar{r_s}^4] + [20.5r_s^3 - 3.3r_s^4 \times \bar{r_s} \times \text{dep} \times \text{dep} \times 200.4] \]

ACKNOWLEDGMENTS

The authors thank the editor and anonymous referees for their considerable assistance in improving this manuscript.

GRANTS

This work was originally funded by Natural Environment Research Council (NERC) under their Antarctic Funding Initiative with logistical support provided by the British Antarctic Survey. The authors also thank NERC and La Trobe University for financial support during the writing of this article.
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