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Development of a Novel Physiology Tag to Measure Oxygen Consumption in Free-Ranging Seabirds

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Executive summary

Ambitious renewable energy targets in many countries has led to the proposed installation of several thousand wind turbines throughout coastal areas of Europe. These are often located on offshore sandbanks, overlapping with important habitats for many seabird species. Potential displacement to avoid offshore wind farms may have energetic consequences for seabird individuals. However, at present there is a paucity of data linking energetic changes associated with displacement, in part, due to a lack of suitable technology for measuring energy expenditure in free-ranging seabirds. A method that is used widely in human research to provide real-time measurements of energy use is near-infrared spectroscopy (NIRS). Here, we describe the initial development and deployment of a NIRS logger to measure muscle oxygen saturation (SmO2) in European shags (*Phalacrocorax aristotelis*). The NIRS logger was adapted from an existing wearable NIRS system for humans and was deployed on two free-ranging shags on the Isle of May, Scotland during the summer of 2019. Upon retrieval, both loggers were in good condition and did not suffer water ingress or damage during the deployment. However, only one logger recorded data (02h 16m 30s) and, although the NIRS system did collect biologically relevant SmO2 measurements, the duration of viable data was relatively short (~15 mins). Nevertheless, this study demonstrates the first use of a non-invasive NIRS sensor to measure energy expenditure in free ranging seabirds and provides the first non-invasive muscle oxygen measurements in a free-ranging wild animal. It therefore provides the first successful steps in the development of a new technology for measuring high resolution changes in energy expenditure in free-ranging seabirds.

Introduction

Ambitious renewable energy targets have been developed by many countries to mitigate potential impacts of climate change (Jay 2010; Toke 2011). This has led to the proposed installation of several thousand wind turbines throughout coastal areas of Europe. In Scotland, offshore renewables have the potential to make a significant contribution to the Scottish Government's target of being Net Zero Carbon by 2045.

Proposed wind farms are often located on offshore sandbanks, which are also important habitats for many seabird species (Wanless, Harris & Greenstreet 1998). This has led to concerns about potential impacts on seabirds, including injury or mortality as a result of collisions with turbine blades (for review see: Drewitt & Langston 2006), displacement to less favourable habitats and/or increased flight costs due to a change in trajectories to avoid the wind farm (Searle *et al.* 2014; Searle *et al.* 2018). For example, Krijgsveld *et al.* (2011) quantified barrier effects of the 'Noordzeewind' wind farm near Egmond aan Zee for northern gannets and derived a macroavoidance rate of 0.64. Similarly, Welcker and Nehls (2016) found significant displacement of 5 species of seabird with 75-92% lower abundance inside the German offshore wind farm 'alpha ventus' compared to outside the wind farm.

Displacement and/or changes in flight trajectories to avoid offshore wind farms may have energetic consequences for individuals. However, at present there is a paucity of empirical data linking energetic changes associated with displacement and barrier effects and the survival and reproduction of seabirds (Searle *et al.* 2018). This has led to attempts to model the potential energetic consequences of avoiding offshore wind farms by a range of seabird species. For example, Masden *et al.* (2010) developed an energetic model of flight based on species-specific morphometrics, and typical foraging distances and trips per day, and investigated the effects of increased travel distances as a result of avoiding a simulated wind farm. Their predictions suggested that for European shags (*Phalacrocorax aristotelis*), great cormorants (*Phalacrocorax carbo*), common guillemots (*Uria aalge*), and Atlantic puffins (*Fratercula arctica*), daily energetic expenditure could increase by up to 170% (Masden *et al.* 2010).

However, uncertainties about the true energetic consequences of displacement remain due to, in part, a lack of suitable technology for measuring energy expenditure at an appropriately high resolution in free-ranging seabirds. Doubly-labelled water techniques have been used previously to measure the field metabolic rates of seabirds (e.g. Gabrielsen, Mehlum & Nagy 1987; Weimerskirch *et al.* 2003) but this only provides single measurements over days or weeks. Alternatively, heart

rate measurements, based on the physiological relationship between heart rate (fH) and oxygen consumption rate ($\dot{V}O_2$), can provide high resolution estimates of energy expenditure in free-living animals; however, this generally requires species-specific calibration in controlled laboratory conditions (Butler *et al.* 2004).

Overall dynamic body acceleration (ODBA) is used increasingly as a calibrated proxy for energy expenditure in free-ranging animals (e.g. Hicks *et al.* 2017). Energetic costs of movement constitute the majority of energy expended by individual animals (Karasov 2015); in theory therefore, body acceleration, which can be measured using animal-borne accelerometer tags (Johnson & Tyack 2003), should correlate with energy expenditure and provide an index of $\dot{V}O_2$ (Wilson *et al.* 2006; Gleiss, Wilson & Shepard 2011; Elliott *et al.* 2013). However, the efficacy of ODBA as a measure of energy expenditure in diving species is currently open to debate given the equivocal results from marine mammal and seabird studies in captivity (Fahlman *et al.* 2008; Hindle *et al.* 2010; Halsey *et al.* 2011). For example, Fahlman *et al.* (2008) reported a significant relationship between VO_2 and ODBA for diving Steller sea lions (*Eumetopias jubatus*). However, re-analyses of these data by Halsey *et al.* (2011) suggests that the relationship between ODBA and $\dot{V}O_2$ is relatively poor for Steller sea lions, particularly when compared to results from terrestrial species. Halsey *et al.* (2011) present further data showing that ODBA is also a poor predictor of $\dot{V}O_2$ in double-crested cormorants (*Phalacrocorax auratus*); they conclude that, while ODBA shows promise for estimating energy expenditure in many terrestrial and fully aquatic species, it may well be that other methods of measuring field metabolic rate will prove more suitable for at least some diving species (Halsey *et al.* 2011).

An alternative method that is used widely in human research to provide real-time measurements of blood oxygenation is near-infrared spectroscopy (NIRS) (e.g. Mancini *et al.* 1994). NIRS uses infrared light from LEDs shone onto the skin, which passes through the underlying tissue and is scattered back to a receiver located next to the LEDs. As oxygenated [HbO_2] and deoxygenated haemoglobin [HHb] have different absorption spectra, spectral analysis of the refracted light determines the relative concentrations of these chromophores. Real time oxygen consumption can therefore be measured by comparing rate of change in [HbO_2] and [HHb]; this information can be used to effectively calculate oxygen consumption within the underlying tissue.

NIRS has been used successfully to measure haemoglobin (Hb) O_2 saturation levels in the locomotory muscle of emperor penguins (*Aptenodytes forsteri*) (Williams, Meir & Ponganis 2011). This previous study developed a NIRS instrument which was

attached to feathers on the back of the birds, with the sensor head implanted into the locomotor muscle to make measurements of Hb saturation (Williams, Meir & Ponganis 2011). This provided confirmation that NIRS can be used to accurately measure Hb saturation levels in muscles of birds as well as providing important insights into the dive response in penguins; however, the approach was invasive, requiring full anaesthesia to implant sensors under the skin with sutures to muscles (Williams, Meir & Ponganis 2011). More recently, research has shown that an animal-borne NIRS sensor can be used to measure $[HbO_2]$ and $[HHb]$ in freely diving, captive harbour seals (*Phoca vitulina*) without the need for sensor implantation (McKnight *et al.* 2019). With appropriate development, this has the potential to provide a non-invasive means of measuring muscle $\dot{V}O_2$ in free-ranging birds.

Here, we describe the initial development of an animal-borne NIRS sensor and recording logger to measure muscle O_2 saturation (SmO_2) in free-ranging birds. We then report on the results of deployments of the logger on free-ranging European shags to determine the efficiency of the loggers, and to estimate activity-specific (e.g. diving, flying, resting) energy expenditure in shags. We then discuss the implications of the results of the development, deployment and future perspectives for the technology.

Methods

NIRS tag

To measure the oxygen consumption and heart rate of free-ranging European shags, an animal-borne archival NIRS sensor was developed from an existing wearable dual-wavelength continuous wave NIRS system for humans (Moxy Monitor, Hutchison, Minnesota, USA). The Moxy Monitor is an autonomous data logger that consists of a light emitting optode (LEO) and two receiver photodiodes (RPD), and a microprocessor recording system. These were housed in a plastic case which is relatively small (61 x 44 x 21 mm) and weighs approximately 48 g in air (Figure 1). Muscle O_2 saturation (SmO_2) measurements were made at sampling rates up to 2 Hz and it can store approximately 10,800 measurements onboard the device. Importantly for applications on free ranging animals, the Moxy Monitor is designed for use in human exercise physiology, so the measurements are relatively insensitive to potential motion artefacts if contact with the skin surface can be maintained.

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Figure 1: A Moxy-3 NIRS sensor that was the basis of the development of the seabird NIRS logger to measure heart rate and muscle O_2 saturation. Note three optical windows on the top of the sensor which house the light emitting optode and the two receiver photodiodes.

To ensure that the sensor was suitable for deployment on free-ranging birds, a series of hardware modifications were carried out; specifically, NIRS sensors were appropriately adapted to make skin contact between the feathers, and data logging technology were effectively miniaturised and ruggedized, and adapted to ensure attachment to the bird was safe and secure.

To waterproof the sensor head, the LEO and RPDs, and printed circuit boards (PCBs) of the sensor head were first removed from the plastic case. The LEO and RPDs were then removed from the PCBs and fitted with short extension leads (approximately 50 mm in length) (Figure 2). To ensure that the whole system was waterproof, the PCBs were covered with a thin coating of spectrally transparent epoxy (EPO-TEK 301, Epoxy Technology, Billerica, MA, United States). To ensure that epoxy encapsulated the electronics but did not cover the optical window on the optodes, the sensor head was cradled in a custom-built silicone mould. This allowed the internal components to be waterproofed by epoxy but ensured the external surface and LEDs remained exposed.

For the purposes of the study, it was assumed that bird Hb and myoglobin have the same optical properties as human ones given the previous application of NIRS in other bird species (Williams, Meir & Ponganis 2011; Williams & Ponganis 2013).
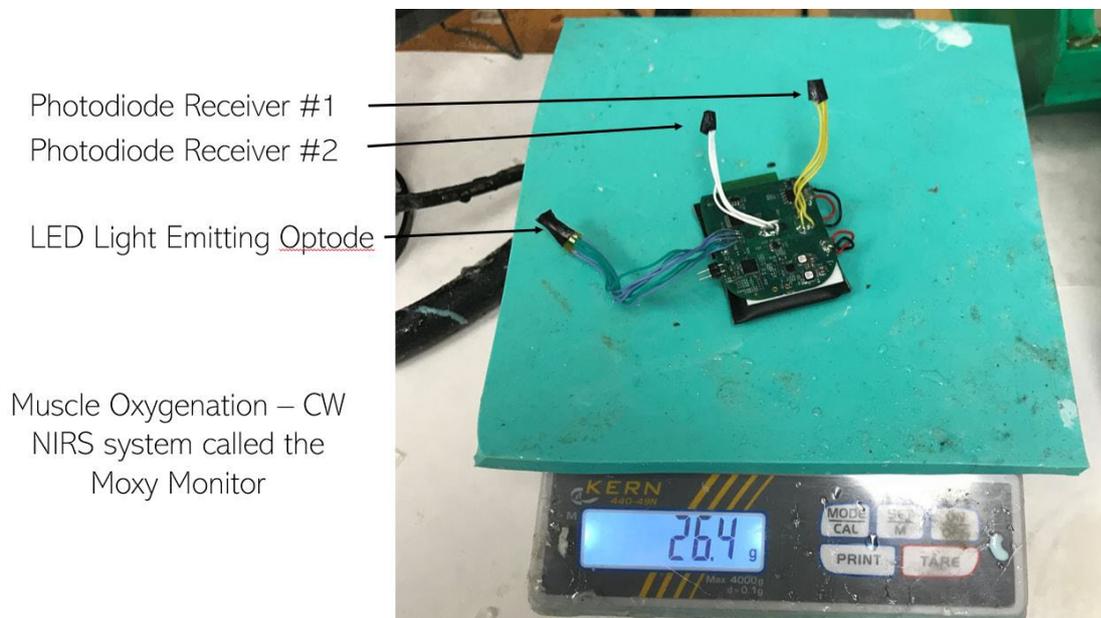

Figure 2: Photograph of modified Moxy-3 NIRS sensor showing the light emitting optode and photodiode receivers, connecting to the PCB using the short extension leads; the overall weight (in air) of the system (excluding attachment Tesa™ tape) was 26.4 g.

Deployment on free-ranging birds

To determine the efficiency of the NIRS loggers, and to estimate activity-specific (e.g. diving, flying, resting) energy expenditure in free-ranging seabirds, the NIRS loggers were deployed on European shags breeding on the Isle of May, Scotland (56°11′N, 2°33′W) during the summer of 2019; this was carried out as part of a long-term life history and behavioural study of this species (Daunt *et al.* 2007; Bogdanova *et al.* 2014). European shags are medium-sized diving seabirds that feed largely benthically on small fish (Watanuki *et al.* 2008). During chick rearing, they typically make 1-4 foraging trips a day (Wanless & Harris 1992; Sato *et al.* 2008). Two adult male shags (aged 7 and 10 years) were caught at their nests using a standard British Trust for Ornithology (BTO) catching method (a pole with a crook at the end) and were fitted with loggers. The LEO and RPDs were affixed to the down feathers using a small amount of Loctite® 422 Instant Adhesive and were mounted in a line laterally (approximately 15 mm apart) across the back of the shag. This aimed to ensure that the LEO and RPDs maintained contact with the skin and maximised the likelihood of measuring SmO_2 in the latissimus dorsi wing muscles. The logger was

attached immediately caudal to the LEO and RPD locations to the lower back using Tesa™ tape (Figure 3).

In combination with the NIRS loggers, a Technosmart Axy-Trek Marine tags (Technosmart Europe srl, Rome, Italy) combining GPS, tri-axial accelerometry and time depth recorder (TDR) (weight 25 g, dimensions 53x23x10 mm) was deployed on each of the birds. This was attached to the lower back immediately to the rear of the NIRS logger using Tesa™ tape (Figure 3). The raw data recorded acceleration in three axes (x - surge, y - sway, z - heave) and pressure at 25Hz (i.e. 25 records per second). GPS locations were recorded at a nominal sampling frequency of 0.05Hz (i.e. one record every 20 seconds). In total the combined weight of the two tags (in air) for the deployment was 51.4 g. The deployment of the dual tag system (NIRS and GPS/accelerometer/TDR) was similar in scope to the attachment of other more commonly used tags such as GPS, and the time taken between capture and release of the birds was approximately 25 minutes

All procedures involving shags were approved by the University of St Andrews School of Biology Ethics Committee (Reference number SEC19014) and were carried out under a British Trust for Ornithology Schedule 1 Ringing and Special Methods Licence (C / 4671; Ref 19/39), and a Scottish Natural Heritage National Nature Reserve Licence (RP 19_1).

Figure 3: Photograph of the accelerometer and NIRS tags deployed on a European shag on the Isle of May. The tags were mounted dorsally and were affixed to the feathers using Tesa™ tape; the NIRS tag was mounted forward of the accelerometer tag.

Classification of behaviour

To determine whether the NIRS measurements were robust to activity type in the shags, and to provide activity-specific estimates of SmO$_2$, accelerometry and time depth data from the Technosmart tags were used to classify the behaviour of the two birds. The classification was performed using the semi-automated approach described below where 1) behaviour was classified into activity states using a series of predetermined metrics and thresholds; 2) the results were sense-checked by experts in shag behaviour; and 3) the metrics and thresholds were refined based on their feedback.

Pressure data

We initially converted "pressure" (recorded in millibars) into "depth" (in metres) using Equation 1.

Equation 1

$$depth = 10 * (pressure - atm)/atm$$

where *atm* is standard atmospheric pressure (1013.25mb).

However, air pressure can vary markedly over time as a result of changes in weather conditions; we corrected for this by smoothing and zero-correcting the resulting depth values following the approach used in the '*diveMove*' package (Luque 2007) in the software 'R' (R Core Team 2017). Smoothing involved taking a running median over blocks of 3 records; zero-correction involved taking a running 2% quantile over blocks of 30 minutes (45,000 records) and deducting this from the depth values. The rationale behind the second step was that (a) within any 30 minute period at least 2% of the time will be spent not diving, (b) for this 2% of time the depth must be zero and (c) any variations in air pressure within a 30 minute are likely to be negligible.

Accelerometer data

We first separated each accelerometer axis into "static" and "dynamic" components using a moving window of two seconds. The two-second moving average of each component forms the "static" component, and the difference between these averages and the raw data values forms the "dynamic" component (Collins *et al.* 2015). From the static acceleration values for the three axes we calculated static pitch using Equation 2.

Equation 2

$$\text{static pitch} = \tan^{-1}\left(\frac{x}{\sqrt{y^2 + x^2}}\right)$$

Two sets of metrics relating to dynamic acceleration were then calculated as: 1) the standard deviation, $\text{RSD}_{a,60}$, of dynamic acceleration over a 60-second moving window for each axis $a = x, y, z$ (Collins *et al.* 2015); and 2) the percentage of variation (RSQ_a) in 10-second range that is explained by the 2-second range, for each of the three axes (Equation 3)

Equation 3

$$\text{RSQ}_a = 100 * \frac{\left(u_{a,10} - u_{a,2}\right)^2 + \left(l_{a,10} - l_{a,2}\right)^2}{\left(u_{a,10} - l_{a,10}\right)^2}$$

where $(l_{a,2}, u_{a,2})$ and $(l_{a,10}, u_{a,10})$ denote the ranges over 2-second and 10-second moving windows respectively for each axis. The resulting percentage, for each axis, is then smoothed over a 30-second moving window.

The former metric (RSD_a) provides a measure of variability in each axis, and the latter (RSQ_a) provides a measure of predictability (i.e. the extent to which variation in time arises from the repetition of a pattern).

Classification of foraging

We first identified periods of foraging behaviour using the depth values we derived from the pressure records, using the approach of Sato, Mori and Yoda (2001). We assumed that all records for which depth is deeper than 1m correspond to dives (i.e. foraging). However, foraging bouts also include gaps between the dives (Wanless *et al.* 1993). To assess which gaps are associated with foraging, and which are not, we looked at the distribution of the gaps between dives, in terms of (a) the gap in time and (b) the difference in depth (in metres) between the maximum dive depths. For each of these distributions, for each bird, we then calculated the point of inflection in a double exponential model (Sato, Mori & Yoda 2001). For any pair of dives we then assumed that the period between them is a "pause" (i.e. part of a foraging bout) if the gaps in both time and depth between them are lower than the points of inflection. We assumed that the period between dives is not a pause (i.e. is non-foraging behaviour) if the gaps in either time or depth are higher than the corresponding points of inflection.

Classification of other behaviours

Non-foraging records were further classified into behavioural states, using metrics derived from the accelerometer data. We first classified any non-foraging records for which the static pitch exceeds 45 degrees as being "on land", on the basis that this body angle (associated with standing) can only be maintained when birds are on land. For the remaining (non-foraging, non-land) records we classified records that have both high variation ($\text{RSD}_z > 0.45$) and high predictability ($\text{RSQ}_z > 95$) in heave (the z-axis) as "flying", as these are characteristic of the steady flapping flight performed by shags (Sato *et al.* 2008). The thresholds (of 0.45 and 95%) were selected visually. Finally, we flagged records with extremely low variability ($\min(\text{RSD}_x, \text{RSD}_y, \text{RSD}_z) < 0.02$) as being "suspect", and classified all remaining records as "resting at sea".

Removal of short behavioural bouts

The next stage was to remove implausibly short behavioural bouts. We did this in two steps:

a)   for each 10-second period we classified the behaviour within the period to be the behaviour that occurs most frequently, unless the period contained some records that were classed as "foraging", in which case we defined the entire period as "foraging".

b)   whenever a behavioural bout had a duration of less than x seconds, where $x \leq 30$, we looked at the bouts immediately prior to and subsequent to this: if both of these had the same behaviour, and both had a duration longer than x seconds, then we reclassified the current behavioural bout as having the same behaviour as the prior and subsequent bouts.

Removal of suspect records

Finally, for each tag, we removed all records after the time at which the first behavioural bout classed as "suspect" occurred. We did this because exploratory data analyses suggested that the "suspect" records related to the period after the tag had been removed from the bird (the exact time of retrieval was not recorded in the field).

Sense-checking

The final classification was evaluated visually, by plotting the behaviour classification in relation to the variation in depth and accelerometry metrics over time. It was also evaluated against the GPS data, which were not used in the construction of the classification, and therefore provide a check on the plausibility of the results.

VO$_2$ estimation using NIRS

The Moxy Monitor data loggers provides continuous measurements of local muscle oxygen saturation (SmO$_2$) as a percentage and total haemoglobin (THb) using NIRS which can be used to measure localised oxygen consumption (VO$_2$) usage during exercise. Here, VO$_2$ was estimated using previous SmO$_2$ calibrations of VO$_2$ from the Moxy Monitor system (Crum *et al.* 2017). The data on SmO$_2$ (%) and VO$_2$ (ml.min.kg^{-1}) presented in this previous study (Crum *et al.* 2017) were analyzed within Generalized Linear Models (Gamma error family) using a logit link function (Dobson 1990), and the resultant model parameters were used to convert SmO$_2$ to VO$_2$; these are presented as mean VO$_2$ values over a rolling 10 second window across the dataset.

Results

Behavioural activity states

The initial separation of foraging bouts from non-foraging behaviours and the final classification of all behaviours obtained from the GPS-accelerometer-TDR tags are shown in Figures 4 and 5, respectively. Over the 45.5 hrs of deployment (across both birds), behaviour was classified into four categories. The majority (83%) of time was spent on land, 11% was spent foraging, 4% resting at sea and the remaining 2% flying. This matches the results from previous research on shag daily time activity budgets (Wanless et al. 2005: 89% on land, 6% foraging, 2% resting at sea, 3% flying in 2001; 81% on land, 12% foraging, 2% resting at sea, 5% flying in 2002) and suggests that there was no major disruption in the birds' behaviour as a result of our logger deployments.
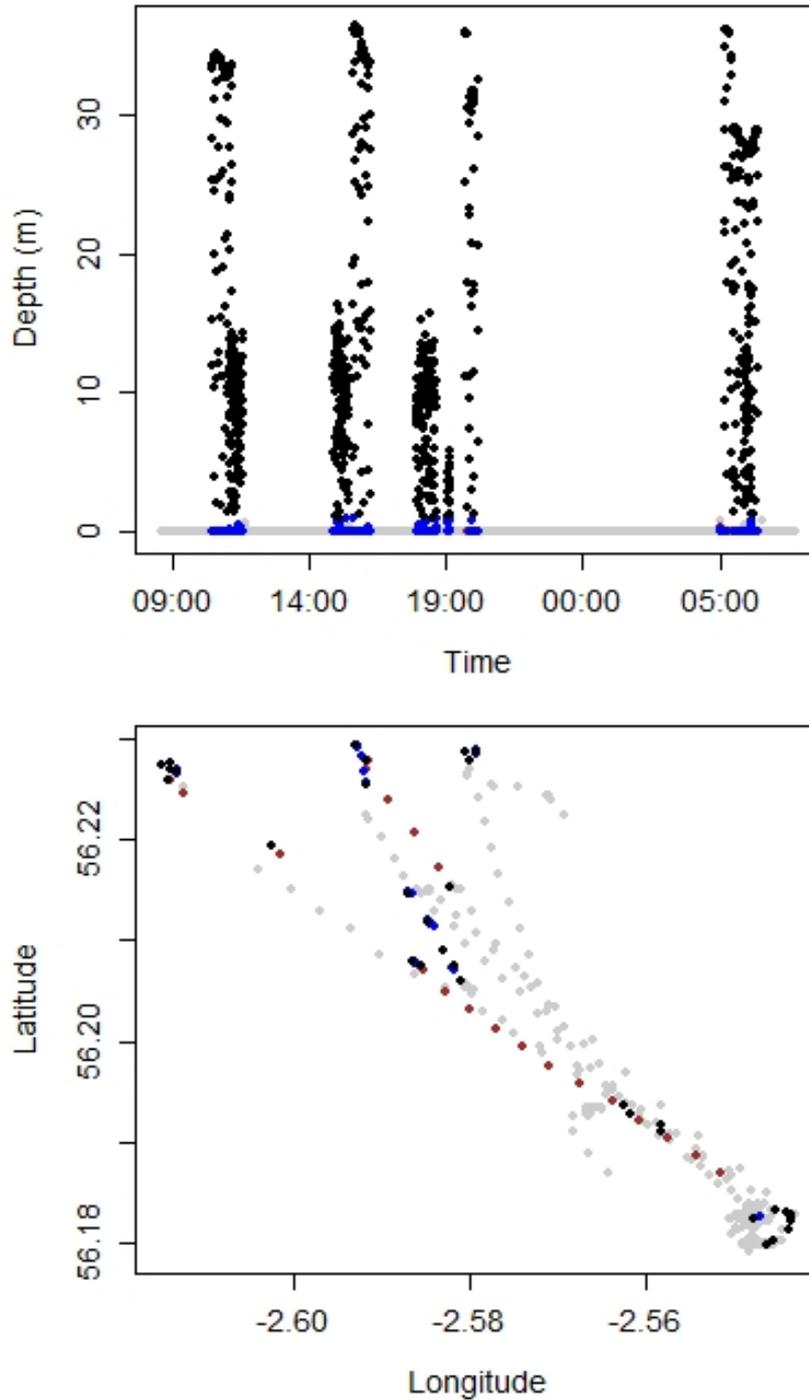
Figure 4: Plots over time (top) and space (bottom) of the records (for both individuals) that are classified as "dives" (shown in black) and "pauses within foraging bouts": for the latter these can either be defined using thresholds for gaps in both depth and time ("TD", shown in blue) or just using thresholds for gaps in time ("TO", shown in red). Note that all records that are classed as pauses using "TD" will also, by definition, be classed as pauses using "TO". Records that are classed as non-foraging using either approach are shown in grey.
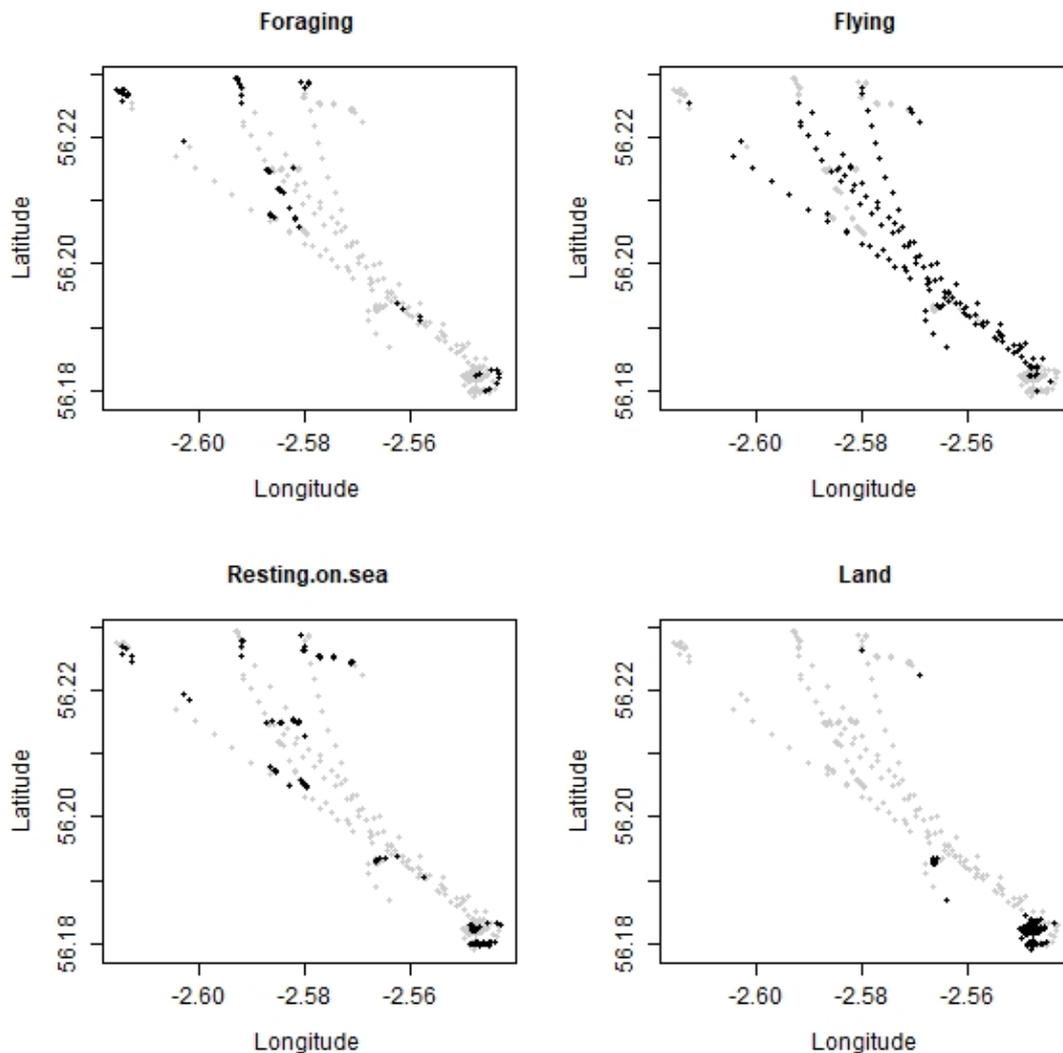
Figure 5: Recorded locations, from GPS tracking data associated with records that are assigned to each of the four behaviours in turn (black) and to the remaining behaviours (grey). Data from both individuals are used in constructing these graphs.

NIRS measurements

Of the two NIRS systems deployed, both were in good condition when they were retrieved and did not suffer water ingress or damage during the deployment. This included periods of flight and dives to depths greater than 30 m. However, SmO_2 data were only successfully retrieved from one device; failure to retrieve data from the second device was a result of the system erroneously switching into a programming mode following the recharge of the battery after tag recovery. Once in programming mode, data could not be downloaded from the device. The system that successfully operated recorded a total of over two hours (02h 16m 30s) of NIRS data. Whilst the system had been programmed to sample at 2Hz, a software error resulted in a sample frequency of 0.5Hz during the deployment; unfortunately, as a sample rate of at least 2Hz is required to identify the cardiac waveform, this

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precluded heart rate measurements during the deployment. However, the system did provide continuous 0.5Hz measures of SmO_2 (Figure 6).

In general, SmO_2 levels remained greater than 80% of normoxic muscle oxygenation during the initial period of deployment (marked by the red polygon in Figure 5). However, from approximately 09:45 onwards there is marked variability in the measurements. Rapid levels of muscle deoxygenation could only result from significant and sustained energetic exertion, which could perhaps be expected from sustained flight or apneic swimming. However, the marked variability in the data is apparent throughout an 'on land' behavioural state (Figure 6) where the bird's body angle is maintained at 45° and it is assumed that activity is relatively low. Considering these data are SmO_2 values from secondary-flight muscles, the pronounced apparent deoxygenation and reoxygenation while the animal is on land suggest that the SmO_2 signal may have been contaminated by LEO/RPD movements, or inadequate contact between LEO/RPD and the bird's skin; it was therefore considered that measurements after 09:45 were inaccurate and did not represent biologically relevant measurements of SmO_2.
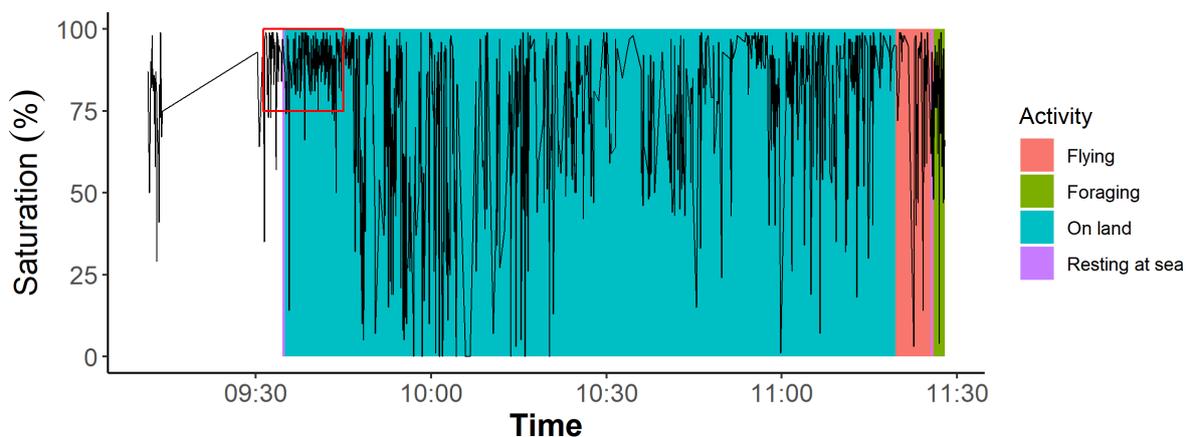

Figure 6: Plot of SmO_2 (%) measurements and behavioural states derived from the accelerometer data over the duration of the deployment on shag number A1.

Using the section of reliable data (denoted by the red box in Figure 6), oxygen consumption (VO_2) was estimated to demonstrate the utility and application of NIRS to measure energetic expenditure in free-ranging birds. SmO_2 measurements during this period ranged from 14.0 to 99.0% with a median of 91.0 (95% CIs = 70.0-99.0%) (Figure 7). Using the GLM based on the data from (Crum *et al.* 2017), whole body VO_2 estimates ranged between 8.9 and 74.6 ml.min.kg^{-1} with a median of 10.9 (95% CIs = 8.9-20.0) ml.min.kg^{-1}. It is important to highlight that, although the duration was relatively short, no NIRS data appear to have been recorded when the bird was in the behavioural state 'Resting at sea'.
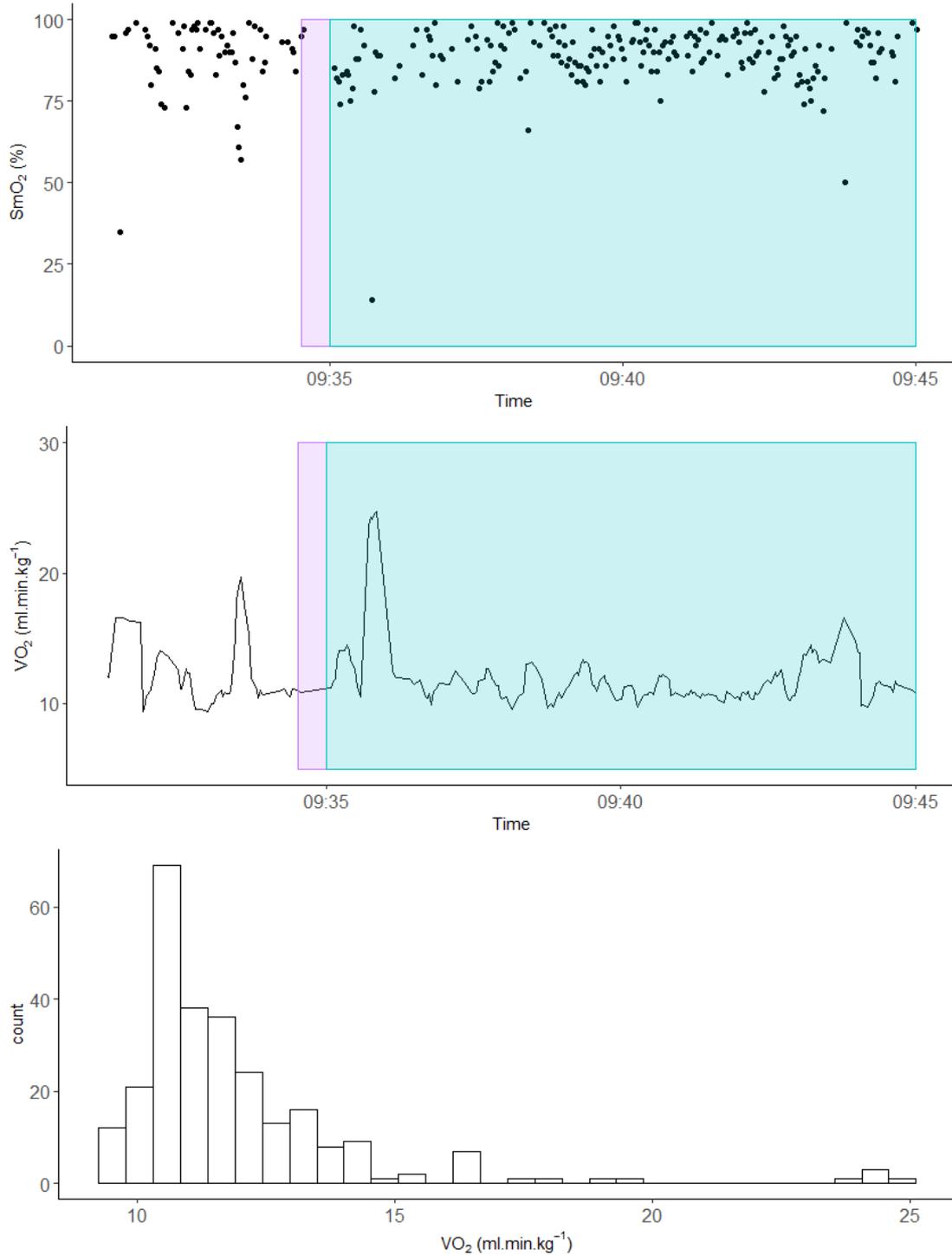
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Figure 7: Plot of the measured SmO_2 (%) across approximately 15 minutes during the deployment (denoted by the red polygon in 6) (upper). Estimated mean whole body VO_2 (ml.min.kg^{-1}) across a rolling 10-second window during this period using the calibrations determined by Crum *et al.* (2017) (middle). Histogram of the estimated whole body VO_2 (ml.min.kg^{-1}) measurements (lower). In the upper and middle panels, the coloured polygons represent the behavioural states derived from the accelerometer data shown in Figure 6.

Discussion

This study demonstrates the first use of a non-invasive NIRS sensor to investigate muscle oxygen saturation in free ranging European shags. Further, the SmO_2 measurements in the current study provide the first non-invasive muscle oxygen measurements in a free-ranging wild animal. Estimates of VO_2 derived from these are similar to the published range of empirical measures of VO_2 in similar seabird species.

The NIRS system we developed was an adapted off-the-shelf muscle oxygenation sensor (Moxy Monitor). This was selected as it is relatively low-cost, is relatively small and lightweight, and is fully autonomous. The Moxy systems were successfully adapted for the marine environment and to ensure it was less than 3% of the instrumented animals' body mass. From a practical perspective, the loggers required a series of technical modifications which, although relatively minor, required specialised engineering facilities. In particular, a skilled electronic engineer was required to remove the LEO and RPDs from the PCB after having been attached via flow soldering, and to reattach these to the extension leads. Further, the application of the waterproof coating and subsequent waterproof tests also required suitable testing facilities, specifically a shallow (0 to 2 bar) pressure-chamber.

The deployment of the dual tag system (NIRS and GPS/accelerometer) on shags was similar in scope to the attachment of other more commonly used tags such as GPS, with the exception of the time taken between capture and release of the birds which was substantially longer (around 25 minutes compared to the typical 10 minutes for the deployment of two loggers). The additional time was required to attach the three NIRS sensors securely to ensure skin contact between the feathers, which was a delicate procedure. The birds behaved normally at release after tag deployment, returning to the nest within a few minutes. The behaviour of the birds over the next *ca*. 24 hours (as recorded by GPS and accelerometers) was markedly different, with one bird foraging more extensively at a further distance from the colony, and one foraging less extensively close to the colony. However, these patterns are within the normal range of behaviours of breeding shags, with time activity budgets comparable to those recorded in past work (Wanless *et al.* 2005) and locations within the typical foraging range of this population during the breeding season (Bogdanova *et al.* 2014). At tag retrieval, the birds both left the nest as the person approached. They returned to the nest after a few minutes and were captured successfully. A small proportion of birds that have been caught previously respond in this way when they first observe someone approaching the nest. However, there are two mitigating circumstances that may explain why we saw this

response in both birds. First, the birds were comparatively late breeders and this subset of the population tends to be more susceptible to disturbance; we had to work on late breeders because of the delay in logger development. Second, they were nesting in locations that were quite distant from the shoreline, and shags are typically more nervous in such circumstances. We had to select birds nesting in these locations because late in the season there is a much smaller pool of birds available for capture. Despite these potential contributory factors, we can't discount the possibility that the short-term adverse effect that we observed was a direct result of this particular deployment. Crucially, however, we have no evidence that these effects were long-term; the birds returned to the nest within a few minutes of release after the loggers had been removed, attended the nest thereafter and fledged their chicks successfully.

In terms of the suitability of the system we developed here for deployment on other species, there are a number of important considerations related to their morphology, behaviour and ecology. The tag weight and shape should be developed taking into account aspects such as body mass, flight type and foraging strategy of the target species (Table 1), to ensure the tags don't add excessive weight and/or substantially increase energetic costs associated with flight and foraging. Particular care should be taken with species such as auks that naturally incur high flight and foraging costs. The overall weight (in air) of the system we used in this project was ~26g; although this would be suitable for deployment on shags and other large species, it is excessive for smaller species of interest such as black-legged kittiwake, common guillemot, razorbill and Atlantic puffin. Following the widely used rule that devices should not exceed 3% of the birds' body weight (Kenward 2001, but see also Vandenabeele et al. 2012), the weight of tags deployed on all species in Table 1 except great black-backed gull and gannet would need to be below 26 g (range 9-25 g; calculation made using the lower weight limit presented). Furthermore, the shape of the device ideally needs to be as streamlined as possible, particularly for diving species, to limit the increased drag (Vandenabeele et al. 2012). Another consideration is the probability of recapturing the birds to retrieve the devices. Although it was possible to attach the NIRS tags and recover them with limited removal of feathers, they did require the attachment of sensors to the down feathers of the birds and this may increase thermal sensitivity. It is, therefore, important to consider the potential consequences of tag deployment in situations where recapture is not successful, and the tag remains attached to the birds for longer than initially intended. Seabird recapture rates are generally high but can vary among and within colonies and years depending on factors such as environmental conditions, locations of nest sites and timing of breeding.

Table 1

Morphological and behavioural traits relevant to the development of NIRS tags for other seabird species of importance to offshore renewables. High wing loading, flapping flight and diving are associated with higher energetic costs.

Species	Body weight (g)	Wing loading	Foraging strategy	Flight type
kittiwake	300-500	low	surface feeding	flapping
guillemot	850-1130	high	pursuit diving	continuous flapping
razorbill	590-730	high	pursuit diving	continuous flapping
puffin	320-480	high	pursuit diving	continuous flapping
herring gull	690-1440	low	surface feeding	flapping
great black-backed gull	1000-2000	low	surface feeding	flapping
gannet	2400-3600	high	plunge diving	partial gliding

Although the NIRS system did collect biologically relevant SmO_2 measurements, the duration of viable data was relatively short (~15 mins). We assume that after this period, the data appear to have been contaminated by LEO/RPD movements, or inadequate contact between LEO/RPD and the bird's skin. A clear avenue for further research is, therefore, the development of a non-invasive mechanical attachment system to maintain positive downward pressure of optics. Nevertheless, the results appear promising and support that NIRS can be a viable energetics tool for avian research. Further, the initial pilot data has proven that NIRS has the capacity to operate successfully on a free-ranging animal and can provide measures of SmO_2 in birds. However, due to the relatively short deployment duration, it is difficult to formally assess the capabilities of the system in a range of activity states as only resting/sedentary states were captured in the usable data.

SmO_2 depletion has been shown to provide an accurate estimate of total body oxygen consumption (Crum *et al.* 2017). This is based on the rationale that during exercise locomotor muscles are the predominant consumer of body oxygen stores. During resting periods, SmO_2 is high (<90%) as the muscles are inactive and baseline metabolic costs are the major contributor to VO_2. During physical exertion, where locomotor muscles are the dominant consumer, the magnitude and rate of SmO_2 depletion correlate and provide an estimate of the rate of total body oxygen consumption (VO_2). Using published values for SmO_2 depletion (as measured with a

Moxy Monitor in humans) to estimate total body oxygen depletion, median baseline (inactive) VO_2 in this study was estimated to be 10.9 (95% CIs = 8.9-20.0) ml.min.kg^{-1} (Figure 5). This is similar to the published range of empirical measures of resting VO_2 in similar seabird species. For example, White, Martin and Butler (2008) measured resting VO_2 rates of 30.9 ± 1.5 ml min^{-1} (which equates to 13.4 ml.min.kg^{-1}) and Hayama and Yamamoto (2011) measured resting VO_2 rates of between 10.5 and 16.9 ml.min.kg^{-1} in great cormorants (*Phalacrocorax carbo*). Similarly, Schmid, Gremillet and Culik (1995) measured VO_2 values of 11.5 ml.min.kg^{-1} (calculated from the allometric relationship $VO_2 = 0.691 M^{0.755}$ where VO_2 is measured in l.h^{-1} and M is mass in kg) in great cormorants standing on land. Unfortunately, a lack of reliable SmO_2 measurements during other behaviours (flight and diving) precluded comparisons of the SmO_2 values in the current study to published empirical estimates of VO_2 during these behaviours'.

From an applied perspective, changes in the behaviour or activity of birds in response to anthropogenic activities or structures may have significant energetic consequences for individuals (Masden *et al.* 2010). However, information on the true energetic consequences of behavioural responses or displacement to seabirds has been limited due, in part, to a lack of suitable technology for measuring energy expenditure in free-ranging seabirds at an appropriately high resolution.

A number of techniques can be used to measure energy expenditure in wild birds but are either at insufficient temporal resolutions (e.g. doubly-labelled water techniques: Gabrielsen, Mehlum & Nagy 1987; Weimerskirch *et al.* 2003) or requires species-specific calibration in controlled laboratory conditions (e.g. heart rate measurements: Butler *et al.* 2004). More recently, the rapid development of high-resolution accelerometers has led to Overall Dynamic Body Acceleration (ODBA) becoming a routinely used proxy for energy use. This provides a useful high-resolution measure; however, a key issue with ODBA estimates of $\dot{V}O_2$ is the lack of any physiological (cardiovascular) input. This has the potential consequence that, during high exertion or diving, reliance on muscle anaerobiosis can make the same biomechanics exponentially more metabolically costly. In other words, if birds were required to operate anaerobically, even briefly, ODBA would fail to capture these energetic costs. For example, it has been shown that biomechanics in isolation is a poor predictor of metabolic acidosis in penguins (Williams, Meir & Ponganis 2011). This is because when muscle switches to anaerobic metabolism there is a significant reduction in the rate of oxygen depletion in the muscle tissue as the oxidative pathway for ATP production is abandoned. However, measuring muscle metabolic dynamics such as those measured in the current study (e.g. SmO_2) provide a direct measure of muscle VO_2, and can detect muscle anaerobiosis. This is, therefore,

robust to anaerobic metabolism and its associated lower rate of recovery (greater metabolic cost), providing a more direct and realistic measure of energetic expenditure in birds. While ODBA is still the most frequently used method of estimating energy expenditure in free-ranging birds, the capacity of NIRS to directly measure tissue-specific oxygenation in real-time, and to capture metabolic changes which ODBA fails to capture, could make NIRS a potentially more reliable and biologically robust method for measuring $\dot{V}O_2$.

Future development

This study confirms that an animal-borne NIRS logger can directly measure energy expenditure in seabirds with a high temporal resolution. Despite the deployment of the system providing only SmO_2 measurements over a relatively short period, there were a number of important, novel and ultimately successful steps achieved here. Ultimately, to ensure that this technology can be utilised practically as a tool for reliably measuring energy expenditure over periods of hours or days, a series of required refinements and developments have arisen from the work thus far.

1) A key area of refinement is the stable attachment of the LEO/RPD to the skin of the birds. In the current study, the LEOs and RPD were independently glued to the down plumage. It seems likely that this provided insufficient contact between the optical hardware and the skin for periods more than several tens of minutes. The development of a mechanical attachment system to maintain positive downward pressure of optics is essential before further deployments. Further, deployments on birds in a more controlled environment (e.g. a captive facility), would be an ideal paradigm to develop attachment mechanisms and to diagnose any potential issues.

2) In its current format the NIRS hardware (Figures 2 and 3) was undesirably configured and housed for free-ranging birds. Both the shape and currently compartmentalised design make the system bulky and resulted in poor fluid-dynamic performance. Effort should be made to create a custom light-weight housing for the components (main PCB and batteries).

3) The NIRS system used in the current study does not provide raw voltage data which would provide a measure of the quality of the NIRS optical signal. Other NIRS systems, provide raw data from which ambient light leak and total photon recovery rate can be interrogated; however, these are larger and significantly more expensive. These two factors provide a direct measure as to whether there is sufficient contact between the skin and optical hardware and would ultimately provide a measure of the reliability of the measured data. Raw HbO_2 and HHb data would also provide more detailed information on

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tissue specific perfusion. While in their current form such systems would appear to be too bulky for deployment on seabirds, development could generate a suitably sized and weighted tag.

Acknowledgements

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