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RESEARCH ARTICLE

Correlations of metabolic rate and body acceleration in three species of coastal sharks under contrasting temperature regimes

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ABSTRACT

The ability to produce estimates of the metabolic rate of free-ranging animals is fundamental to the study of their ecology. However, measuring the energy expenditure of animals in the field has proved difficult, especially for aquatic taxa. Accelerometry presents a means of translating metabolic rates measured in the laboratory to individuals studied in the field, pending appropriate laboratory calibrations. Such calibrations have only been performed on a few fish species to date, and only one where the effects of temperature were accounted for. Here, we present calibrations between activity, measured as overall dynamic body acceleration (ODBA), and metabolic rate, measured through respirometry, for nurse sharks (*Ginglymostoma cirratum*), lemon sharks (*Negaprion brevirostris*) and blacktip sharks (*Carcharhinus limbatus*). Calibrations were made at a range of volitional swimming speeds and experimental temperatures. Linear mixed models were used to determine a predictive equation for metabolic rate based on measured ODBA values, with the optimal model using ODBA in combination with activity state and temperature to predict metabolic rate in lemon and nurse sharks, and ODBA and temperature to predict metabolic rate in blacktip sharks. This study lays the groundwork for calculating the metabolic rate of these species in the wild using acceleration data.

KEY WORDS: Respirometry, Acceleration data logger, Elasmobranch, Field metabolic rate, Bioenergetics

INTRODUCTION

The life history and ecology of animals are inextricably linked to the methods and efficiency with which they use and obtain energy, and thus the ability to estimate the metabolic rate of free-ranging animals is fundamental to understanding their ecology (McNamara and Houston, 1996; Brown et al., 2004). The proportion of energy dedicated to particular behaviours and tasks determines the success and fitness of individuals, and by extension populations (Tolkamp et al., 2002; Metcalfe et al., 2016). Therefore, quantifying the metabolic rate of animals in the field can provide insight into how energetic demands drive behavioural decisions and ecological interactions (McNamara and Houston, 1996; Sims, 2003). This is especially important for marine predators, which play a significant role in ecosystem dynamics through top-down control and behaviourally mediated effects on prey species (Dill et al., 2003; Heithaus et al., 2008). Foraging needs are directly driven by energy requirements and metabolic rate (Williams et al., 2004), and thus understanding the energy expenditure of free-ranging predators is crucial to quantifying predator–prey dynamics and determining the energetic impacts that these animals have on their ecosystems. This has become increasingly important as populations of marine predators continue to decline worldwide (Dulvy et al., 2014; Barreto et al., 2016), threatening the stability of these ecosystems (Ferretti et al., 2010).

A number of methods have proved successful in measuring the metabolic rate of a variety of taxa in the laboratory, most notably doubly labelled water (DLW) and respirometry. Translating these measurements to free-ranging animals in the field, however, remains difficult, particularly in aquatic environments. DLW has been successful in estimating field metabolic rates (FMRs) in air-breathing species, but this method cannot be applied to fully aquatic, water-breathing animals because of the high flux of water across the gills (Speakman, 1997; Butler et al., 2004). Using respiratory frequency to estimate metabolic rates of air-breathing marine taxa such as cetaceans has also been successful (e.g. Roos et al., 2016), but again this method is difficult to apply to water-breathing taxa. Instead, respirometry has become the standard in measuring metabolic rate in fishes (Carlson et al., 2004). However, metabolic rates measured under controlled laboratory conditions are not always directly applicable to field environments (Lowe and Goldman, 2001), and respiration generally cannot be directly measured under natural conditions in the field, particularly in water-breathing species, as it requires the animal to be in an enclosed space. As a result, using respirometry to estimate FMR requires respiration to be correlated with other parameters that can be measured in the field. The most common of these methods is heart rate telemetry, which uses the correlation between heart rate (f_H) and oxygen consumption ($\dot{M}_{O_2}$), and has been used to estimate FMR in both terrestrial and aquatic taxa. However, this method can result in large errors, as heart rate can be affected by a variety of factors such as stress level, body size, environmental variables and individual variation, which can alter the f_H–$\dot{M}_{O_2}$ relationship (Butler et al., 2004; Green, 2011; Halsey et al., 2011a). These errors appear particularly prevalent in fish species (Thorarensen et al., 1996; Carlson et al., 2004). Although a few studies have produced strong correlations between heart rate and metabolic rate in fish (Clark et al., 2010), overall the application of the heart rate method for measuring energy expenditure in fish has been limited.

Using body acceleration as a proxy for metabolic rate has shown great potential as a method of estimating energy expenditure in the

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List of symbols and abbreviations

AICc	corrected Akaike's information criterion
BL	body length
COV	coefficient of variability
DLW	doubly labelled water
DO	dissolved oxygen
f_H	heart rate
FMR	field metabolic rate
$\dot{M}_{O_2}$	oxygen consumption
ODBA	overall dynamic body acceleration
RMR	routine metabolic rate
SDA	specific dynamic action
SMR	standard metabolic rate
TL	total length

field. This technique is based on the principle that animal movement, which can be measured through multi-dimensional acceleration, results directly from muscle contraction, which is catalysed by adenosine triphosphate (ATP) hydrolysis and thus requires oxygen (Wilson et al., 2006; Gleiss et al., 2011). Past accelerometry studies have investigated the relationship between animal movement and metabolic rate, most commonly measured through respirometry, and have indicated strong correlations between the two in a variety of animal taxa, including birds (Wilson et al., 2006; Green et al., 2009; Halsey et al., 2009a,b, 2011b; Elliott et al., 2012), amphibians (Halsey and White, 2010), marine turtles (Enstipp et al., 2011; Halsey et al., 2011c), mammals (Fahlman et al., 2008; Halsey et al., 2009a), marine invertebrates (Payne et al., 2011; Lyons et al., 2013; Robson et al., 2012) and fish (Clark et al., 2010; Gleiss et al., 2010; Yasuda et al., 2012; Wilson et al., 2013a,b; Wright et al., 2014; Mori et al., 2015). These studies have shown great promise in the applicability of accelerometry as a proxy for metabolic rate, maintaining strong correlations across diverse taxa and through a variety of behaviours, with low error compared with other techniques (Halsey et al., 2009a). Accelerometers are also relatively easy to deploy on free-ranging animals, requiring external attachment rather than the surgical implantation required by heart rate telemetry. Therefore, this technique offers an excellent opportunity to translate laboratory measurements of metabolic rate to free-ranging animals, particularly during activity.

Before accelerometer data can be used to estimate FMR, laboratory calibrations correlating movement, typically represented by overall dynamic body acceleration (ODBA), with oxygen consumption need to be established. These laboratory calibrations have been conducted for relatively few species of fish, including red sea bream (*Pagrus major*; Yasuda et al., 2012), Japanese sea bass (*Lateolabrax japonicas*; Mori et al., 2015), European sea bass (*Dicentrarchus labrax*; Wright et al., 2014), sockeye salmon (*Oncorhynchus nerka*; Clark et al., 2010; Wilson et al., 2013a,b) and scalloped hammerhead sharks (*Sphyrna lewini*; Gleiss et al., 2010). As temperature is widely regarded as the most important environmental factor driving metabolic rate of ectotherms (Gillooly et al., 2001; Kingsolver, 2009; Angilletta et al., 2002), it is imperative that temperature is accounted for in these calibrations. Despite this importance, the effects of temperature on the ODBA–$\dot{M}_{O_2}$ relationship have only been examined in one of these fish species, the European sea bass (Wright et al., 2014). The effects of temperature on ODBA–$\dot{M}_{O_2}$ relationships have also been directly examined in a few other ectotherm species, including green turtles (*Chelonia mydas*; Enstipp et al., 2011), American lobsters (*Homarus americanus*; Lyons et al., 2013) and king scallops (*Pecten maximus*; Robson et al., 2016). The lack of laboratory calibrations of ODBA and metabolic rate that include temperature is largely due to the considerable logistical difficulties and expense associated with performing accurate calibrations at divergent temperatures, including holding animals captive for long time periods and having access to a respirometry system with reliable temperature control. These issues are particularly pertinent for larger and more active species, including many marine predators, which are difficult to hold in captivity and require large respirometry facilities. Regardless of these challenges, determining how temperature affects the ODBA–$\dot{M}_{O_2}$ relationship is an essential component to translating laboratory calibrations to estimates of FMR, which will almost certainly include variable temperatures.

In this study, we tested whether ODBA is able to accurately predict oxygen consumption at a range of experimental temperatures in three species of coastal sharks, nurse sharks [*Ginglymostoma cirratum* (Bonaterre 1788)], lemon sharks [*Negaprion brevirostris* (Poey 1868)] and blacktip sharks [*Carcharhinus limbatus* (Müller and Henle 1839)]. These species present a range of energetic strategies, with nurse sharks representing an inactive, benthic species, blacktip sharks representing an active, ram-ventilating species, and lemon sharks characterizing an intermediate activity level. Calibrations were performed in a static respirometry system and included a wide range of volitional swimming activity. These laboratory calibrations of the relationship between ODBA, $\dot{M}_{O_2}$ and temperature will allow for FMR to be extrapolated from acceleration data collected from free-ranging sharks.

MATERIALS AND METHODS

Capture and maintenance

Respirometry experiments were conducted on juvenile nurse, lemon and blacktip sharks. Nurse sharks (N=8, 53–132 cm total length, TL) were captured via rod and reel from the Florida Keys, USA. Lemon sharks (N=27, 69–100 cm TL) were captured with cast nets from Cape Canaveral, FL, USA and the Florida Keys. Blacktip sharks (N=8, 53–64 cm TL) were captured with rod and reel from Terra Ceia Bay, FL, USA. All animals were transported to Mote Marine Laboratory in Sarasota, FL, USA, and held in a 150,000 l indoor, recirculating tank for the duration of experiments. Sharks were fed a diet of herring, squid and shrimp to satiation every other day, but were fasted prior to the beginning of trials to achieve a post-absorptive state. Nurse sharks were fasted for at least 72 h prior to trials, and lemon and blacktip sharks were fasted for at least 48 h prior to trials. All sharks were kept on a constant 12 h light:dark cycle. This work was approved by the Mote Marine Laboratory Institutional Animal Care and Use Committee (approval no. 09-09-NW1).

Respirometry trials were run for two temperature groups representing the low (~20°C) and high (~30°C) ends of the temperature range these species are likely to experience in the wild. Sharks were acclimated to trial temperatures in the holding tank for at least 1 week prior to experimentation. Trials with lemon and blacktip sharks were all run within 2 months of initial capture. Nurse shark trials were run with individuals that had been maintained in captivity for at least 1 year.

Accelerometry

During trials, sharks were equipped with Cefas G6A+ acceleration data loggers (Cefas Inc., Lowestoft, UK), which recorded triaxial acceleration at 25 Hz, depth and temperature. Tags were attached to the first dorsal fin of the sharks at two points using monofilament

Journal of Experimental Biology (2017) 220, 397-407 doi:10.1242/jeb.146993

(Fig. 1) at least 18 h before the start of a trial. As sharks tagged in the field would need to be tracked acoustically to retrieve the data loggers, the loggers used in captive trials were paired with a mock acoustic tag (model V9, Vemco Inc., Bedford, NS, Canada) in order to maintain the same weight and drag as tags used in field studies (see Fig. 1). The paired tag package measured 37×36×15 mm and weighed 23 g in air, representing 0.2–2.2% of the body mass of the study animals. The frontal cross-sectional area of the tag was 4.3 cm^2, equal to between approximately 2% and 10% of the cross-sectional area of the study animals based on girth measurements, assuming a round cross-section for the animals.

Respirometry

Trials were conducted in a circular, closed respirometer constructed from a modified fibreglass holding tank with a diameter of 2.45 m, as described in Whitney et al. (2016). Briefly, the respirometer was sealed using a lid constructed from a PVC frame with plastic sheeting stretched across it, and dissolved oxygen (DO) and temperature were measured using a handheld DO meter (model Pro Plus, Yellow Springs Instruments, Yellow Springs, OH, USA). To ensure even water mixing in the respirometer and create water flow past the DO meter for accurate measurements, a pump shuttled water from the outside to the centre of the tank past the DO probe. The pump and DO meter were enclosed in a circular cage made of PVC and rigid plastic mesh during lemon and nurse shark trials to protect the instruments from the animals and encourage the sharks to swim in full circles around the outer edge of the tank (Fig. 1). This mesh cage was not used during trials with blacktip sharks because it appeared to induce stress in the animals. Using a circular tank may increase overall costs of swimming compared with straight-line swimming because of the increased energy required for turning (Hughes and Kelly, 1996; Wilson et al., 2013a,b). However, this factor should be accounted for by the accelerometers, as the increased costs of turning are associated with the increased body movement required for turning.

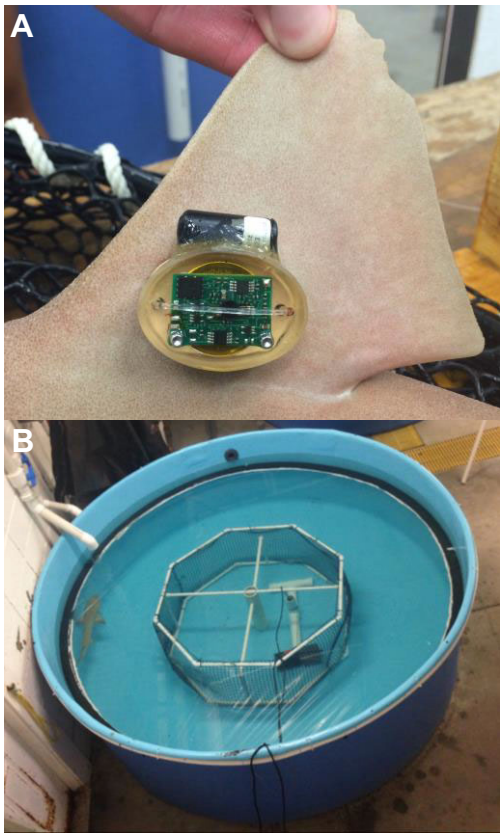

Fig. 1. Tag and experimental set-up. (A) An accelerometer joined to an acoustic transmitter, attached to the dorsal fin of a juvenile nurse shark. (B) Static respirometry set-up, featuring a pump system that passes water past the dissolved oxygen (DO) meter through a T-shaped pipe for accurate DO measurements without creating water flow in the respirometer.

Lemon and nurse sharks were placed into the respirometry system at least 12 h prior to the start of trials to allow them to acclimate to the system overnight. Blacktip sharks appeared to fatigue after extended periods in the smaller tank system, and were acclimated to the respirometer for 1 h prior to the start of trials rather than overnight. After the acclimation period, the respirometer tank was isolated from its flow-through system and sealed with the lid. The tank was surrounded by a curtain to limit extraneous disturbances, and the trials were monitored remotely using a live digital video feed. DO and water temperature were recorded every 5 min, and shark behaviour was monitored constantly throughout the trials. Swimming speed was calculated in body lengths (BL) s^{-1} by measuring the time a shark took to complete a full lap of the respirometer 3 times every 5 min during periods of consistent swimming activity. Trials began with the DO near 100% saturation, and were run until DO reached 80% saturation.

To assess background respiration, a blank respirometer (without an animal) was measured for 4 h during each group of trials.

Data analysis

Intervals of the trials where sharks displayed consistent swimming or resting behaviour for at least 20 min were used for analysis. Mass-specific oxygen consumption rate ($\dot{M}_{O_2}$, mg O_2 kg^{-1} h^{-1}) was calculated for each of these analysis intervals using Eqn 1:

$$\dot{M}_{O_2} = \frac{(S-b)60V}{M}, \quad (1)$$

where S represents the slope of the oxygen degradation curve in minutes, b is the slope of the background respiration curve, V is the volume of the respirometer in litres and M is the mass of the shark in kg. The volume of the shark (<10 l) was considered to be negligible relative to the respirometer volume (2494 l), representing an error of <0.5%, and was thus not incorporated into our model.

Accelerometer data were analysed using Igor Pro (Wavemetrics, Lake Oswego, OR, USA). Static acceleration (indicating animal body position) was separated from dynamic acceleration (indicating animal movement) using a 3 s box smoother (Shepard et al., 2008), which was sufficient to remove tailbeat noise from static acceleration traces. ODBA was calculated as the sum of the absolute value of the three dynamic acceleration axes. A mean ODBA value was calculated for each $\dot{M}_{O_2}$ analysis interval during respirometry trials, to provide paired ODBA–$\dot{M}_{O_2}$ points for model analyses.

A species-specific mean standard metabolic rate (SMR), the metabolic rate at rest, was calculated for lemon and nurse sharks at each experimental temperature by averaging metabolic rates during all resting intervals for that species and temperature grouping. Because blacktip sharks are a ram-ventilating species, SMR was not directly calculated, but was estimated using the intercept of the ODBA–$\dot{M}_{O_2}$ relationship. Routine metabolic rate (RMR), the metabolic rate during volitional activity, was calculated for each species and temperature grouping as the mean metabolic rate of all periods where the study animals showed consistent swimming

Journal of Experimental Biology (2017) 220, 397-407 doi:10.1242/jeb.146993

activity. Rest periods for lemon and nurse sharks were not included in RMR calculations.

Predictive modelling

Quantitative analyses were performed in R (http://www.R-project.org/), using the lme4 (Bates et al., 2015) and MuMIn (http://CRAN.R-project.org/package=MuMIn) packages. Linear mixed models were constructed to describe the relationship between ODBA and oxygen consumption for each species, with ODBA, activity state (active or inactive) and temperature group as predictor variables, and individual included as a random effect. Because lemon sharks were collected from two different locations, capture location was included as a predictor variable in the lemon shark model. Models were compared using the small-sample corrected Akaike's information criterion (AICc), residuals, log likelihood and R^2 of the models. Normality of the residuals of the optimal models was tested using an Anderson–Darling test (Wright et al., 2014).

Because of the difficulties and expense inherent in running respirometry trials with large, active animals, it was only possible to conduct trials at two experimental temperatures. As a result, temperature group was included as a factor in the model, rather than as a continuous variable, because with two temperature treatments, the model would assume a linear correlation between temperature and metabolic rate. However, previous work has decidedly shown that metabolic rate scales exponentially with temperature in ectotherms, including fish, according to a Q_{10} relationship (Clarke and Johnston, 1999; Gillooly et al., 2001). Therefore, we calculated Q_{10} values both for the SMR of each species and for the intercept of the predictive model in each species to provide a method of scaling these values exponentially with temperature in the absence of more detailed experimental data. A Q_{10} of the RMR was also calculated for blacktip sharks, as they do not exhibit a SMR. This RMR Q_{10} was calculated using only RMR data from the two temperature groupings that overlapped in ODBA levels to ensure that the comparison was made between metabolic rates from similar activity levels, as volitional activity tends to increase with temperature (Halsey et al., 2015). All Q_{10} values were calculated according to the Van't Hoff equation (Eqn 2):

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}, \quad (2)$$

where R_1 is the metabolic rate at temperature T_1, and R_2 is the metabolic rate at temperature T_2.

Model validation

To validate our models, we used a jack-knife approach to estimate the prediction error of the optimal model (e.g. Halsey et al., 2009a, 2011c; Halsey and White, 2010; Enstipp et al., 2011). In this technique, each individual was excluded from the analysis in turn and a new predictive equation established using the remaining individuals. The new predictive equation was applied to ODBA data from the excluded individual to produce predicted $\dot{M}_{O_2}$ values for each analysis interval, which were compared against the measured $\dot{M}_{O_2}$ values. The standard error of the estimate (s.e.e.) was calculated for each species using these predicted values, and used to assess the coefficient of variability (COV), as s.e.e.×100/measured value (Green, 2011). The algebraic per cent error was also calculated, as [(estimated $\dot{M}_{O_2}$ – observed $\dot{M}_{O_2}$)×100]/observed $\dot{M}_{O_2}$. These calculations were made for each species as a whole and for each individual. Paired-sample *t*-tests were used to determine whether there were significant differences in mean measured $\dot{M}_{O_2}$ and predicted $\dot{M}_{O_2}$ for any species or individual.

RESULTS

Oxygen consumption and ODBA were simultaneously measured for all three shark species at temperatures near 20 and 30°C. Because of difficulties in keeping blacktip sharks in captivity for extended periods, calibration experiments were only possible with one individual at temperatures near 20°C. This resulted in insufficient data to construct a full ODBA–$\dot{M}_{O_2}$ calibration for blacktip sharks near 20°C, although the pair of trials conducted on this individual allowed for a preliminary ODBA–$\dot{M}_{O_2}$ calibration at that temperature. Although swimming activity was volitional, all three species presented a wide range of activity levels during trials, with ODBA during active periods ranging from 0.07 to 0.24 g. Swimming speeds ranged from 0.23 to 0.45 BL s^{-1} in nurse sharks, 0.42 to 0.77 BL s^{-1} in lemon sharks and 0.58 to 0.84 BL s^{-1} in blacktip sharks. Individual animals contributed an average of 9±6 paired ODBA–$\dot{M}_{O_2}$ points to the calibration analyses. Metabolic rates were highest in blacktip sharks, at 362 mg O_2 kg^{-1} h^{-1} at 29.3°C, with a RMR approximately 49% higher than in lemon sharks (243 mg O_2 kg^{-1} h^{-1}) and 162% higher than in nurse sharks (138 mg O_2 kg^{-1} h^{-1}) at similar temperatures (Table 1). Some of the nurse shark metabolic rate data were previously published in Whitney et al. (2016).

ODBA–$\dot{M}_{O_2}$ correlations and model selection

Metabolic rate was positively correlated with temperature, changing the intercept of the ODBA–$\dot{M}_{O_2}$ relationships in all species. However, the slopes of the ODBA–$\dot{M}_{O_2}$ relationships did not significantly change with temperature in any species (ANCOVA interaction effects $P>0.05$). The SMR Q_{10} was 2.99 for nurse sharks and 2.96 for lemon sharks, and the RMR Q_{10} was 2.67 for blacktip sharks.

ODBA scaled linearly with oxygen consumption in all species and temperature groupings (Fig. 2). The inclusion of the individual as a random effect greatly improved model fit in all predictive models (ΔAICc>35). Variability was due to inter-individual differences in the intercept, i.e. the SMR. Capture location was not a significant predictor of $\dot{M}_{O_2}$ for lemon sharks ($P>0.05$), and thus was removed from the model.

Table 1. Standard and routine metabolic rate (SMR and RMR) for the three shark species

Species	Temp. group (°C)	*N*	Mass range (kg)	No. of trials	SMR range	Mean SMR	RMR range	Mean RMR	SMR/RMR Q_{10}
Nurse	23.9	8	5.5–11.12	35	21–44	34.8±6	67–112	95±11	2.99
	29.3	8	7.8–12.4	53	55–90	62.9±8	111–166	138±15	
Lemon	20.6	20	2.07–3.46	32	38–108	64.1±16	100–219	152±30	2.96
	29.5	16	1.74–2.95	29	116–220	168.5±23	172–308	243±32	
Blacktip	21.6	1	3.2	2	–	–	126–182	161±19	2.67
	29.4	7	1.03–1.47	7	–	246*	282–448	362±39	

Metabolic rate is given as mass-specific oxygen consumption rate ($\dot{M}_{O_2}$), in mg O_2 kg^{-1} h^{-1}; range and mean±s.d. values are given.
*The SMR reported for blacktip sharks was extrapolated from the intercept of the ODBA–$\dot{M}_{O_2}$ relationship.

Journal of Experimental Biology (2017) 220, 397-407 doi:10.1242/jeb.146993

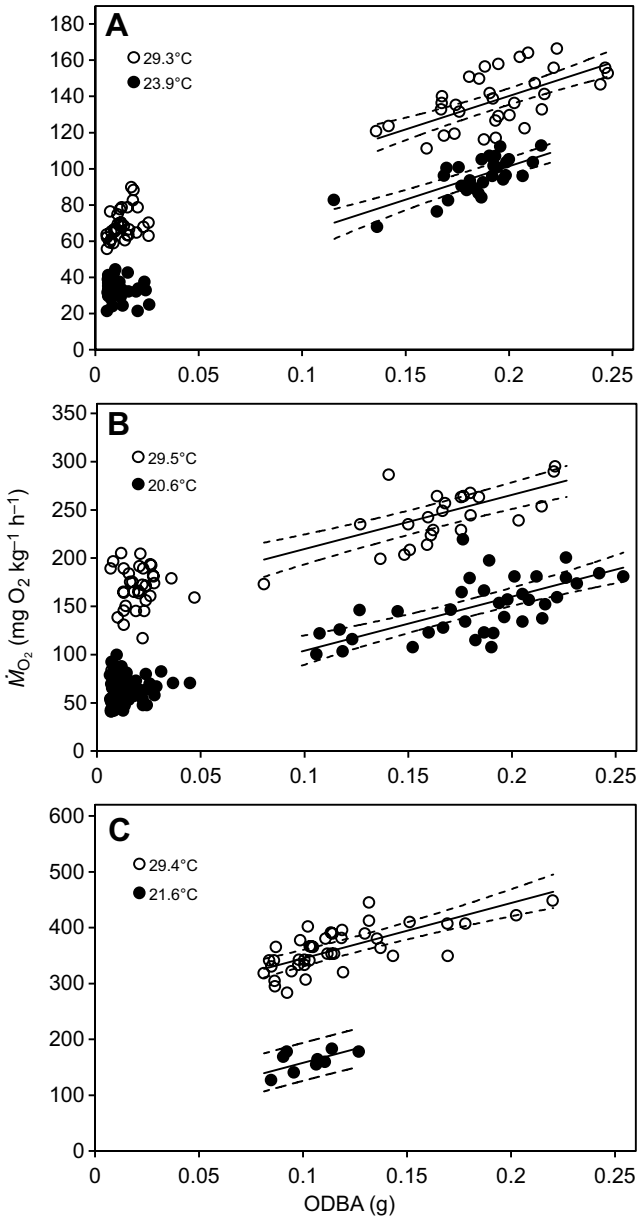

Fig. 2. Overall dynamic body acceleration–oxygen consumption (ODBA–$\dot{M}_{O_2}$) relationships for sharks at the two calibration temperatures. (A) Nurse sharks, (B) lemon sharks and (C) blacktip sharks. Trendlines show relationships described by the fixed effects of the best-fit models for each species, with the formulas given in Table 3. Dashed lines show 95% confidence intervals. The cooler water blacktip data are based on a single shark and do not represent a complete ODBA–$\dot{M}_{O_2}$ calibration at that temperature.

In all three species, the model with the lowest AICc included temperature group and ODBA as predictive variables, with individual included as a random effect. However, while this model may best describe $\dot{M}_{O_2}$ in the laboratory, our goal was to create a model that will provide the most accurate estimates of FMR when applied to field data. It is likely that resting ODBA in the field will be highly variable as a result of extraneous water movement from waves or currents (Whitney et al., 2010). Therefore, a model incorporating a binary activity state (active/resting) would lead to more accurate FMR estimates during rest periods because the elevated resting ODBA due to water movement can be separated from ODBA due to shark movement. Because of this, we adopted a model for nurse and lemon sharks that includes activity state, and an interaction between ODBA and activity state. This model had a ΔAICc <2, a higher R^2 and log likelihood, and smaller mean residual than the top model in both species, and thus does not represent a substantial drop in model fit. In this activity state model, ODBA did not significantly describe $\dot{M}_{O_2}$ during resting periods for nurse sharks or lemon sharks, with the slopes of ODBA–$\dot{M}_{O_2}$ relationships during inactivity not significantly different than zero. This suggests that using a mean SMR determined by temperature to estimate $\dot{M}_{O_2}$ during resting periods would have predictive power similar to that of using the correlation with ODBA. Therefore, for lemon and nurse sharks, we used a mean SMR to estimate $\dot{M}_{O_2}$ during inactivity, and a linear correlation with ODBA to estimate $\dot{M}_{O_2}$ during activity, where both the SMR and intercept of the active ODBA–$\dot{M}_{O_2}$ equation scale exponentially with temperature according to their respective Q_{10} values. The intercept of the ODBA–$\dot{M}_{O_2}$ relationship in blacktip sharks also scales exponentially with temperature. Because blacktip sharks are constantly active, activity state was not included in models for this species.

The linear relationships in these optimal models showed strong correlations between ODBA and $\dot{M}_{O_2}$ during activity, with R^2>0.91 (Table 2), and Anderson–Darling tests provided no evidence that the residuals of the optimal models deviated from normality (P>0.05). The intercepts of the active ODBA–$\dot{M}_{O_2}$ relationships were not equal to the measured SMR for nurse or lemon sharks (Tables 1 and 3), and therefore scale with a different Q_{10} value. The Q_{10} of the active ODBA–$\dot{M}_{O_2}$ intercept was 5.11 for nurse sharks, 3.20 for lemon sharks and 6.27 for blacktip sharks (Table 3).

Using the linear ODBA–$\dot{M}_{O_2}$ relationship, $\dot{M}_{O_2}$ during active periods ($\dot{M}_{O_2,A}$) can be predicted using Eqn 3 in all three species:

$$\dot{M}_{O_2,A} = a(\text{ODBA}) + b, \quad (3)$$

where a is the slope of the ODBA–$\dot{M}_{O_2}$ relationship during active periods, which is species specific, but independent of environmental influences, and b is the intercept of the relationship, which varies with environmental factors. In our case, this environmental variable was temperature, which scales this intercept according to Eqn 4:

$$b = b_c \cdot S^{\frac{T_b - T_c}{10}}, \quad (4)$$

where b is the intercept calculated at temperature T_b, b_c is the intercept of the ODBA–$\dot{M}_{O_2}$ relationship assessed during calibration at temperature T_c, and S is the scaling factor determined for the species, i.e. the Q_{10} value. $\dot{M}_{O_2,R}$, the $\dot{M}_{O_2}$ during resting periods, or SMR, also scales with temperature according to Eqn 4, where b is the estimated SMR at temperature T_b, b_c is the measured SMR at temperature T_c, and S is the SMR Q_{10}.

Model validation

The optimal model was used in the jack-knife validation exercise to compare predicted $\dot{M}_{O_2}$ against measured $\dot{M}_{O_2}$. The predicted values were plotted against the measured $\dot{M}_{O_2}$ values (Fig. 3), with the slopes of these relationships significantly different from 1 in lemon and nurse sharks (P<0.05), tending to slightly underestimate $\dot{M}_{O_2}$ at high activity levels. The algebraic error of the $\dot{M}_{O_2}$ predictions was below 2% in all three species. The overall species COV was 12.3% for nurse sharks, 14.9% for lemon sharks and 7.7% for blacktip sharks (Table 3). The COV was lower in nurse and lemon sharks when only active data were considered, at 8.7% and 9.8%, respectively. These errors were higher when applied to individuals rather than the species

Journal of Experimental Biology (2017) 220, 397-407 doi:10.1242/jeb.146993

Table 2. Model selection criteria for predictive models of $\dot{M}_{O_2}$ in all three shark species

Species	Model	Mean residual	log Likelihood	AICc	ΔAICc	R^2 (fixed effects only)	R^2 (inc. random effects)
Nurse	$\dot{M}_{O_2}$≈ODBA+Temp	6.99	–465.0	940.0	–	0.95	0.95
	$\dot{M}_{O_2}$≈ODBA*Temp	6.93	–464.4	940.7	0.7	0.95	0.95
	$\dot{M}_{O_2}$≈ODBA*AS+Temp	**6.88**	**–464.0**	**941.9**	**1.9**	**0.95**	**0.96**
	$\dot{M}_{O_2}$≈ODBA+AS+Temp	6.98	–465.0	942.0	2.0	0.95	0.95
	$\dot{M}_{O_2}$≈ODBA*Temp+AS	6.91	–464.2	942.4	2.4	0.95	0.95
	$\dot{M}_{O_2}$≈AS*Temp	8.11	–486.1	984.1	44.1	0.93	0.93
	$\dot{M}_{O_2}$≈AS+Temp	8.33	–488.0	986.1	46.1	0.93	0.93
	$\dot{M}_{O_2}$≈Temp	23.4	–625.5	1259.0	319	0.16	0.46
Lemon	$\dot{M}_{O_2}$≈ODBA+Temp	14.32	–807.3	1624.2	–	0.91	0.92
	$\dot{M}_{O_2}$≈ODBA*Temp	14.22	–806.9	1625.7	1.5	0.91	0.93
	$\dot{M}_{O_2}$≈ODBA*AS+Temp	**14.19**	**–805.9**	**1625.8**	**1.6**	**0.91**	**0.93**
	$\dot{M}_{O_2}$≈ODBA+AS+Temp	14.32	–807.3	1626.6	2.4	0.91	0.92
	$\dot{M}_{O_2}$≈ODBA*Temp+AS	14.11	–805.6	1627.3	3.1	0.92	0.93
	$\dot{M}_{O_2}$≈AS+Temp	16.74	–831.5	1673.0	48.8	0.89	0.90
	$\dot{M}_{O_2}$≈AS*Temp	16.85	–831.1	1674.3	50.1	0.89	0.90
	$\dot{M}_{O_2}$≈Temp	25.54	–932.5	1872.9	248.7	0.53	0.71
Blacktip	**$\dot{M}_{O_2}$≈ODBA+Temp**	**16.32**	**–230.0**	**470.0**	**–**	**0.91**	**0.94**
	$\dot{M}_{O_2}$≈ODBA*Temp	16.18	–230.0	471.9	1.9	0.91	0.94
	$\dot{M}_{O_2}$≈Temp	24.70	–248.1	504.1	34.1	0.83	0.86

The top five models including both temperature (Temp) and ODBA are shown, along with models including only temperature and activity state (AS), and those including only temperature. Individual was included as a random effect in all models. Models in bold were chosen as the optimal model for field estimation. As blacktip sharks are constantly active, activity state was not included as a factor in models for this species. An asterisk indicates where interaction effects between variables were included in the models.

as a whole, with COVs of individuals up to 14.9% in nurse sharks, 25.3% in lemon sharks and 9.7% in blacktip sharks (Table 3). However, there were no significant differences in the mean estimated $\dot{M}_{O_2}$ and measured $\dot{M}_{O_2}$ in any species overall or for any individual (paired-sample *t*-tests $P>0.05$).

DISCUSSION

Despite the importance of quantifying the energy use of marine predators, few studies have been able to undertake this task because of the challenge of estimating metabolic rates of free-swimming animals. Our results showed significant correlations between ODBA and $\dot{M}_{O_2}$ in all three study species, confirming the applicability of acceleration as a proxy for metabolic rate in sharks, and adding further support to the concept of using accelerometry as a method of estimating FMR for fishes. This is also the first study to our knowledge that has explicitly tested the effects of temperature on the slope of the relationship between ODBA and $\dot{M}_{O_2}$ in fish, and one of only two studies to include the effects of temperature in ODBA–$\dot{M}_{O_2}$ calibrations in fish, greatly adding to our understanding of how to best incorporate temperature into calibration models for accurate estimation of FMRs.

Temperature effects in ODBA–$\dot{M}_{O_2}$ calibrations

Temperature was the most important factor in $\dot{M}_{O_2}$ estimation, which is expected as metabolic rate scales exponentially with temperature according to the Q_{10} equation. ODBA was the next most important factor, indicating the predictive power of using fine-scale activity measures above using more generalized activity states. The slope of the ODBA–$\dot{M}_{O_2}$ relationship held constant within the temperature range studied here, which represents the range of temperatures these species are likely to encounter in the field. It is possible that the slope of the relationship may not hold constant near the thermal extremes for a species, where aerobic scope declines, activity is limited and metabolism is more likely to occur anaerobically (Farrell, 2009).

The fact that the slope remained constant within the normal temperature range is not unexpected. The slope of the ODBA–$\dot{M}_{O_2}$ relationship represents the additional amount of energy required to increase activity by a unit of ODBA, akin to the activation energy per unit of activity or movement. As the same amount of muscle contraction is required per unit of increased activity regardless of temperature, this activation energy would also be expected to remain constant, while temperature increases the SMR. A handful of previous studies have examined the relationship between ODBA or swimming speed, $\dot{M}_{O_2}$ and temperature in fish, with similar results, determining that temperature does not significantly affect the slope of the relationship between swimming speed and metabolism in some fish species (Claireaux et al., 2006; Whitney et al., 2016), or between ODBA and metabolism in American lobster (Lyons et al., 2013). However,

Table 3. ODBA–$\dot{M}_{O_2}$ calibration relationships and associated error in the three shark species

Species	Temp. group	$\dot{M}_{O_2,A}$ formula (mg O_2 kg^{-1} h^{-1})	$\dot{M}_{O_2,A}$ intercept Q_{10}	Algebraic % error	COV (%)			
					Overall	Active	Inactive	Individual range
Nurse	23.9°C	370.7(ODBA)+28.05	5.11	1.47	12.3	8.7	19.7	7.0–14.9
	29.3°C	370.7(ODBA)+65.63						
Lemon	20.6°C	543.0(ODBA)+49.84	3.20	0.78	14.9	9.8	20.1	0.8–25.3
	29.5°C	543.0(ODBA)+155.39						
Blacktip	21.6°C	991.9(ODBA)+58.71	6.27	0.21	7.7	7.7	–	3.2–9.7
	29.4°C	991.9(ODBA)+245.75						

$\dot{M}_{O_2}$ during active periods ($\dot{M}_{O_2,A}$) is estimated based on the linear ODBA–$\dot{M}_{O_2}$ relationship determined by the fixed effects of the linear mixed models, and the associated intercept Q_{10}. The coefficient of variability (COV) is the standard error of the estimate as a percentage of the measured $\dot{M}_{O_2}$.

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 397-407 doi:10.1242/jeb.146993

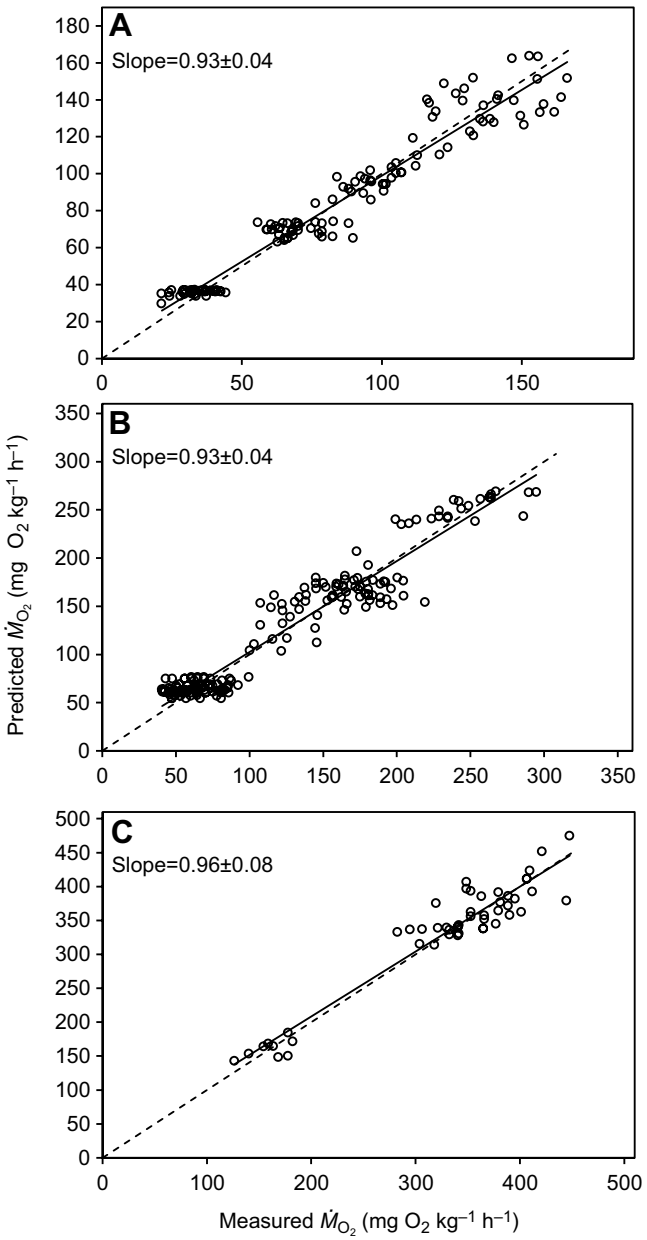

Fig. 3. Error plots showing predicted values of $\dot{M}_{O_2}$ against measured values using the optimal model for each species. (A) Nurse sharks, (B) lemon sharks and (C) blacktip sharks. The slopes of these relationships (solid lines) were significantly different from 1 (dashed line) in nurse and lemon sharks, tending to slightly overestimate low $\dot{M}_{O_2}$ values and underestimate high $\dot{M}_{O_2}$ values. The slopes are given in the plots ±95% confidence intervals.

other studies have found a significant effect of temperature on the relationship between swimming speed and metabolic rate in chub mackerel (*Scomber japonicas*; Dickson et al., 2002) and between body acceleration and metabolism in king scallops (*Pecten maximus*; Robson et al., 2016). If the total value of the activation energy remains constant while the overall metabolic rate increases with temperature, an animal would require a much greater proportion of energy, relative to its metabolic rate, to increase activity at lower temperatures compared with higher temperatures. For example, using the calibration equations determined here, to increase ODBA by 0.1 g (a typical increase between resting and active states), animals would have to increase their metabolic rate by more than 2 times their SMR at temperatures near 20°C, but only by 0.3–0.6 times their SMR at temperatures near 30°C, making activity proportionally more energy efficient at higher temperatures.

Model selection

The model with the lowest AICc in all three species used ODBA and temperature to predict $\dot{M}_{O_2}$ and did not include activity state. However, using a simple linear relationship to predict $\dot{M}_{O_2}$ from field ODBA measurements for both active and inactive periods may incur greater error in the application to field data. As described above, ODBA is not a significant predictor of $\dot{M}_{O_2}$ during resting periods in the laboratory, and there is often substantial water movement in the field due to waves and currents, which can considerably increase resting ODBA values (Whitney et al., 2010), sometimes to more than 3 times the ODBA measured during resting periods in a stationary tank (K.O.L., N.M.W. and A.C.G., unpublished data). While turbulent water will increase resting ODBA, it is not likely to impact the $\dot{M}_{O_2}$ of sharks resting in the field, and thus using a straight ODBA–$\dot{M}_{O_2}$ correlation would provide artificially increased estimates of $\dot{M}_{O_2}$ for such resting intervals. Therefore, the model we adopted uses a mean SMR to predict $\dot{M}_{O_2}$ during all resting intervals, while a linear relationship between ODBA and $\dot{M}_{O_2}$, where the intercept does not necessarily match the SMR, would be used to predict $\dot{M}_{O_2}$ during swimming intervals (see Fig. 4). For both lemon and nurse sharks, the intercepts of these $\dot{M}_{O_2,A}$ relationships tended to be slightly lower than the SMR, though they remained within 1 s.d. of the mean SMR (Tables 1 and 3). Extrapolating power performance curves (swimming speed versus $\dot{M}_{O_2}$) to zero swimming speed is a common method for estimating SMRs of active fish species, and has been validated in several studies (Brill, 1987; Leonard et al., 1999; Dowd et al., 2006). It appears that extrapolating ODBA–$\dot{M}_{O_2}$ curves to zero activity may also be a relatively effective way to estimate SMR, though this method has not been validated before and SMRs calculated using this method, including the SMR reported here for blacktip sharks, should be interpreted with caution.

The Q_{10} values for the intercept of the $\dot{M}_{O_2,A}$ equation were substantially larger than SMR Q_{10} values for both lemon and nurse sharks. Metabolic Q_{10} values in elasmobranchs are generally expected to be between 2 and 3 (Brett and Groves, 1979). The SMR Q_{10} values calculated for each species fall inside this range, but the intercept Q_{10} values are higher. This is because while the difference between the intercepts at the two temperatures is similar to the difference in SMR between the two temperatures, the intercepts fall lower than the SMR and thus the difference constitutes a greater proportion of the intercept compared with the SMR, creating larger Q_{10} values. The magnitude of change in metabolic rate described by these large Q_{10} values is never realized in measurements of metabolism, as the SMR scales with a different Q_{10}, and while the intercept Q_{10} scales up the RMR values, the RMR values related to the intercept are much larger. Therefore, the magnitude of the change in RMR with temperature represents a much smaller proportion of the total value, and would result in a smaller Q_{10}, as is seen with the blacktip RMR Q_{10} (2.67) compared with the intercept Q_{10} (6.27).

One issue arising from the separation of resting and active periods as indicated by our models is that there will be periods of FMR estimation that are shorter than the 20 min periods of respirometry analysis used during the laboratory calibrations. Generally, it is recommended that the same time interval used in calibrations is used to estimate FMR, so that the temporal resolution between the two is held constant and error in FMR analysis of shorter time intervals is not underestimated (Halsey et al., 2009b, 2011a). However,

Journal of Experimental Biology (2017) 220, 397-407 doi:10.1242/jeb.146993

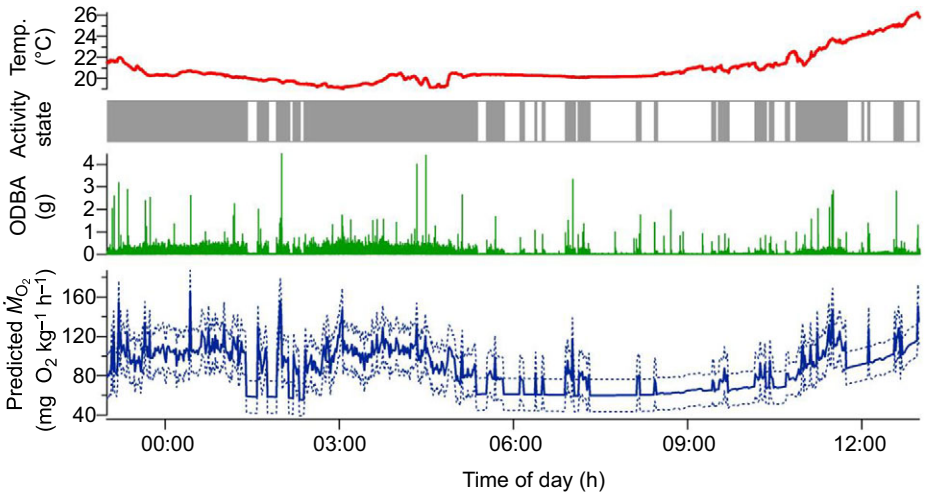

Fig. 4. Example prediction of the $\dot{M}_{O_2}$ of a free-swimming lemon shark in the field based on the laboratory ODBA calibration. The standard metabolic rate (SMR) of the shark and the intercept of the calibration relationship are determined by the temperature during the deployment (red trace) using the appropriate Q_{10} values. Activity state is determined as active (grey bars) or resting (white bars), here done using *k*-means cluster analyses (see Sakamoto et al., 2009; Whitney et al., 2010). Using the chosen predictive model, resting intervals are assigned an SMR based on the mean temperature during the interval, and active periods are assigned a routine metabolic rate (RMR) based on ODBA and temperature. The predicted $\dot{M}_{O_2}$ resulting from this method is shown in the bottom panel (blue), ±standard error of the estimate (s.e.e.), with s.e.e. calculated separately for active and resting periods.

parcelling field data into 20 min intervals for analysis requires combining resting and active periods to produce a mean ODBA value every 20 min, which would incorporate the variable resting ODBA values due to water movement into the estimate. While separating resting and active periods and using shorter intervals for FMR analysis may under-represent the error associated with the estimate, it is likely that this method would still produce more accurate estimates of FMR because the SMR will not be consistently over-estimated.

Model validation

The error calculated through the model validation exercise was generally low, with the overall algebraic error, which takes into account the sign of the difference between predicted and measured values, within 2% of the measured value for all species (Table 3). The s.e.e. and resulting COV for each species, calculated using the total error without taking the sign of the difference into account, were higher: 7.7% for blacktip sharks, 12.3% for nurse sharks and 14.9% for lemon sharks. However, when only active data were considered, these errors remained below 10% in all three species (Table 3), demonstrating that ODBA as a method to estimate $\dot{M}_{O_2}$ has higher predictive power when the animal is active compared with when it is resting. This trend has also been shown in other studies, which determined that ODBA is a less effective predictor of $\dot{M}_{O_2}$ during inactivity compared with activity (Green et al., 2009; Halsey et al., 2011a). Arguably the most important factor affecting $\dot{M}_{O_2}$ during inactivity is temperature, which is included in the present calibrations. However, other factors also increase variation in $\dot{M}_{O_2}$ during inactivity, including individual variation in SMR due to age, sex, body size or other life history characteristics (White and Seymour, 2004; Burton et al., 2011).

Prediction errors were also much larger for some individuals compared with the overall species error, up to 25.3% (Table 3), meaning that it is likely that the application of these laboratory calibrations to estimates of FMR will be more accurate for populations than for individuals (Green, 2011; Halsey et al., 2009b, 2011a). However, there were no significant differences in mean $\dot{M}_{O_2}$ predictions compared with measured $\dot{M}_{O_2}$ values for any species overall or for any individual of any species, and the algebraic error remained low for all species. The higher COV compared with algebraic error is due to the fact that the s.e.e. is based on the total error of the prediction regardless of the sign of the difference between the measured and predicted value, while the algebraic error takes the sign of this difference into account. Therefore, while the COV describes the difference between measured and predicted values accumulated from each point of the calibration, algebraic error describes the overall difference between measured and predicted values, allowing high and low estimates to cancel each other out. This suggests that while there may be higher error in pairing metabolic rate estimates with specific time intervals, estimates of metabolic rate spanning over a larger time period that includes several analysis intervals are likely to sustain a high enough degree of accuracy to maintain statistical integrity.

Application to estimates of FMR

In air-breathing species, several methods of measuring metabolic rate, including DLW and recording respiration timing, can be employed as an additional validation technique to directly compare with field estimates of metabolic rate made through accelerometry calibrations (e.g. Elliott et al., 2012; Stothart et al., 2016; Roos et al., 2016). This type of concomitant validation in the field is unfortunately not possible for water-breathing taxa; instead, calibrations in the laboratory represent the most comprehensive validation possible for the method in fish. However, the strong relationships between ODBA and $\dot{M}_{O_2}$ during activity and relatively low error found in this and previous studies of fish (e.g. Gleiss et al., 2010; Wright et al., 2014), comparisons of ODBA and heart rate methods in fish (Clark et al., 2010) and air-breathing taxa (Green et al., 2009) together suggest that this method can estimate activity-specific energy expenditure with a high level of accuracy.

It is important to note, however, that ODBA accounts only for the portion of metabolic rate due to movement, and while this may be the most variable factor contributing to metabolic rate in active species, there are several other elements that determine FMR as well. Arguably the most important of these is temperature, which, as discussed, significantly changes the SMR and the intercept of

ODBA–$\dot{M}_{O_2}$ relationships. Temperature can be accounted for relatively easily in FMR estimates as long as the data logger includes a temperature sensor and the Q_{10} of the SMR of the study species is known or is included in the laboratory calibrations, as was done here. Salinity and DO are other abiotic variables that, to a lesser extent, may influence metabolism in the field (Carlson et al., 2004) and were not included in these calibrations. Deploying water quality sensors alongside accelerometers in the field may help to extrapolate some of these effects (Cooke et al., 2016).

Other biotic factors, including specific dynamic action (SDA, the proportion of energy dedicated to food processing and digestion) and recovery from anaerobic exercise, are more difficult to account for using the ODBA method, and have not been incorporated into the present calibrations. Like temperature, SDA will affect SMR and the intercept of the ODBA–$\dot{M}_{O_2}$ relationship in the hours following feeding events, increasing to over 2 times SMR in sharks depending on the size of the meal and other factors (Ferry-Graham and Gibb, 2001; Sims and Davies, 1994). As the success and magnitude of feeding events generally cannot be discerned from accelerometer data, elevated metabolic rates due to SDA can be difficult to incorporate into FMR estimates. Additionally, when anaerobic respiration is used during short bouts of increased activity, such as during prey capture, predator avoidance or mating, animals incur an oxygen debt that leads to increased metabolism during post-exercise recovery periods, which again is difficult to directly account for using accelerometry (Cooke et al., 2016), though with additional calibrations anaerobic respiration can be successfully quantified from acceleration data (e.g. Robson et al., 2012). Using heart rate telemetry simultaneously with accelerometry may increase the accuracy of metabolic rate estimates (Clark et al., 2010; Cooke et al., 2016), as heart rate may scale more closely with $\dot{M}_{O_2}$ during physiological challenges, particularly during resting periods (Green et al., 2009; Halsey et al., 2011a).

Regardless of the method of estimation, there will always be variation in FMR as a consequence of external influences that cannot be directly accounted for through laboratory calibrations. Despite these challenges, accelerometry is likely to provide accurate estimates of FMR, particularly during activity, and offers additional benefits over other methods. These benefits include ready commercial availability and inexpensive costs compared with other types of sensors (Whitney et al., 2012; Lear and Whitney, 2016), as well as relatively easy and non-invasive attachment mechanisms. However, external tag attachments can sometimes affect energy expenditure and swimming behaviour through increased drag and negative buoyancy, particularly in small animals (e.g. Lowe et al., 1998; K.O.L., A.C.G. and N.M.W., submitted), which should be considered when extrapolating energetic and behavioural data from tagged individuals. In addition, acceleration data loggers provide high temporal resolution of metabolic rate estimates. Recording movement data at high sample rates allows the estimation of energy expenditure over short time periods, and thus the costs of short-lived behaviours, such as prey capture and mating events, can be estimated. Finally, the fine-scale sampling of acceleration data loggers allows the concurrent measurement of energy expenditure and behaviour. Specific behaviours can be readily identified from acceleration data collected from free-ranging fish without direct observation (e.g. Tsuda et al., 2006; Whitney et al., 2010, 2012; Gleiss et al., 2013; Broell et al., 2013). These behaviours can then be paired with metabolic rate estimates through ODBA, allowing the construction of high-resolution time–energy budgets for free-ranging fish. This task has not been possible using other methods of estimation, and is essential to provide a clear picture of the relative energetic and fitness costs associated with the behavioural decisions that individuals make. Using acceleration data loggers to estimate FMR in fish can provide the information necessary to comparatively assess the activity-specific energy expenditure and bioenergetics of sharks across individuals, populations and seasons, providing data crucial to understanding how environmental factors drive the physiological ecology and fitness of these animals.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
N.M.W., A.C.G. and R.E.H. conceived the study and provided logistical support throughout laboratory trials and analysis. J.J.M. constructed the respirometry facilities, managed shark husbandry and assisted with laboratory trials. N.M.W. oversaw laboratory trials, and K.O.L. managed and conducted laboratory trials and performed the analysis with A.C.G. L.R.B. collected and analysed field data for use in Fig. 4. All authors contributed to the writing of the manuscript.

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Data availability
Data are available from the BCO-DMO digital repository: http://www.bco-dmo.org/project/555866.

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