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Treading water: respirometer choice may hamper comparative studies of energetics in fishes

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Abstract. Measuring the metabolic rate of animals is an essential part of understanding their ecology, behaviour and life history. Respirometry is the standard method of measuring metabolism in fish, but different respirometry methods and systems can result in disparate measurements of metabolic rate, a factor often difficult to quantify. Here we directly compare the results of two of the most common respirometry systems used in elasmobranch studies, a Steffensen-style flume respirometer and an annular static respirometer. Respirometry trials with juvenile lemon sharks *Negaprion brevirostris* were run in both systems under the same environmental conditions and using the same individuals. Relationships between metabolic rate, swimming speed, overall dynamic body acceleration (ODBA) and tail beat frequency (TBF) were compared between the two systems. The static respirometer elicited higher TBF and ODBA for a given swimming speed compared with the flume respirometer, although it produced relationships between kinematic parameters that were more similar to those observed in free-swimming animals. Metabolic rates and swimming speeds were higher for the flume respirometer. Therefore, although flume respirometers are necessary for many types of controlled laboratory studies, static respirometers may elicit lower stress and produce results that are more applicable to fish in wild systems.

Additional keywords: bioenergetics, elasmobranch, lemon shark, metabolic rate, oxygen consumption, swim tunnel.

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Introduction

Energetic demands of animals drive their life histories, govern ecological interactions and determine how successful an individual will be in a particular environment (McNamara and Houston 1996; Brown *et al.* 2004). As such, measuring the metabolic rates of a species is a crucial part of understanding their ecology and predicting how they may respond to changes in their environment, both natural and anthropogenic (Brown *et al.* 2004; Treberg *et al.* 2016). Measuring oxygen consumption through respirometry has become the most common method of estimating metabolic rate in fishes and, to date, has been used to measure metabolism in more than 20 species of elasmobranchs (Bernal *et al.* 2012) and over 100 species of teleost fish (Killen *et al.* 2016). Recent studies have also started using respirometry procedures to correlate body acceleration and oxygen consumption as a method to estimate field metabolic rates (FMRs) in fish (e.g. Gleiss *et al.* 2010; Yasuda *et al.* 2012; Wright *et al.* 2014; Mori *et al.* 2015; Bouyoucos *et al.* 2017; Lear *et al.* 2017), although this method cannot account for other factors affecting metabolic rate, such as specific dynamic action, the rise in metabolic rate following ingestion of a meal. These calibration studies provide the opportunity for estimates of metabolic rate in nature and *in situ* comparisons of energy expenditure between behavioural patterns, seasons and habitats, expanding our understanding of how energetics drive behaviour and ecology in fishes.

These respirometry studies commonly use several different types of respirometers, which can be split into two general categories: (1) flume or swim-tunnel respirometers; and (2) static respirometers. In flume respirometers, fish swim in place against a current as a motor and propeller push water through the system at a controlled speed (Carlson *et al.* 2004; Clark *et al.* 2013). Static respirometers are circular or rectangular tanks without a current, where fish can rest or swim volitionally (Carlson *et al.* 2004; Clark *et al.* 2013). In teleost studies, static respirometers are generally small rectangular tanks just large

enough for fish to rest, and are used to measure standard metabolic rate (SMR), the metabolic rate of a postabsorptive fish at rest (Carlson *et al.* 2004). Herein we focus instead on annular static systems, or circular tanks, where animals have sufficient room to either swim or rest, which are the most common static respirometry system used for elasmobranchs. Both flume and annular respirometers have benefits and drawbacks depending on the goals of the study and the species under investigation. Flume respirometers allow water velocity, and therefore fish activity level, to be controlled and maintained for long periods. Conversely, fish activity is volitional in a static system, which can make it difficult to collect steady swimming data or elicit a wide range of swimming speeds. Annular respirometers also typically use larger water volumes than flume respirometers for similar sized fish, meaning that the resolution of oxygen decline is generally higher for flume systems. In addition, fish swim in a straight path in a flume, whereas they must swim in a curved path in an annular static system, which can be more energetically costly (Weihs *et al.* 1981; Hughes and Kelley 1996; Carlson *et al.* 2004). However, many past studies have expressed concerns that the forced swimming conditions in flumes alter the swimming behaviour of fish and affect the metabolic rates measured in those systems (e.g. Peake and Farrell 2004, 2006; Gleiss *et al.* 2010; Wright *et al.* 2014), particularly in more sedentary species that typically inhabit low-flow environments and do not adapt well to forced swimming under high flows (Nelson *et al.* 2002; Bouyoucos *et al.* 2017).

Metabolic rates measured in both types of respirometers are regularly compared against each other to facilitate interspecific comparisons of energy expenditure (e.g. Clarke and Johnston 1999; Carlson *et al.* 2004). However, even slight differences in respirometry methods and experimental protocols can produce different measurements of metabolic rates (Carlson *et al.* 2004), and these effects are difficult to account for. Ideally, respirometry methods and protocols would be kept consistent across species to facilitate interspecific comparisons, but it is not always feasible to use the same type of respirometer or the same trial protocols between species or studies. For example, benthic, inactive sharks tend to struggle to swim consistently in flume respirometers (e.g. Brett and Blackburn 1978), whereas more active species may require tank sizes for static respirometry that are too large to permit accurate measurements of oxygen consumption (Carlson *et al.* 2004; Clark *et al.* 2013). Therefore, in order to inform comparative work, it is important to understand how the use of a particular respirometry system may affect the results of a study. For example, a few previous studies in teleosts have shown that using a speed ramp protocol to measure maximum metabolic rate (MMR) in a flume respirometer yields different results than using a chase protocol and static respirometer (Reidy *et al.* 1995; Roche *et al.* 2013; Norin and Clark 2016; Rummer *et al.* 2016; Killen *et al.* 2017). However, there is little information available directly assessing how different respirometry systems affect basic swimming kinematics and their relationship to metabolic rate, particularly in elasmobranchs.

In addition, it is important to understand how metabolic rates and swimming behaviour measured in the laboratory relate to those observed in free-swimming fish, because the overarching goal of many respirometry studies is to use laboratory-measured metabolic rates to estimate the energy expenditure of individuals in natural environments. Unfortunately, the behaviour of animals in captivity is generally not representative of fish behaviour in the wild, and therefore the metabolic rates and swimming behaviour of fish measured during respirometry trials cannot be directly applied to wild fish (Lowe and Goldman 2001). Studies using accelerometry to calibrate body acceleration against metabolic rate in order to estimate FMR in fish have started to address this problem by providing a method to correct laboratory-derived metabolic rates for differences in activity and behaviour in free-ranging fish (for reviews, see Whitney *et al.* 2012; Cooke *et al.* 2016; Metcalfe *et al.* 2016; Treberg *et al.* 2016). Most of these calibration studies have used flume respirometers to correlate body acceleration and metabolism, but many state concerns that the forced swimming conditions affected or biased their results (Gleiss *et al.* 2010; Wright *et al.* 2014; Mori *et al.* 2015; Bouyoucos *et al.* 2017), which would create greater error in the application of these calibrations to wild data.

In the present study we use the lemon shark *Negaprion brevirostris* (Poey) to compare metabolic rates and swimming performance measured in flume and annular static respirometry systems. Lemon sharks were chosen as a model species because of their ability to swim satisfactorily in both a flume and static environment. Respirometry trials were run in both systems, using the same individuals and under the same environmental and holding conditions. Metabolic rates, swimming speeds, and acceleration-derived swimming metrics, including overall dynamic body acceleration (ODBA) and tail beat frequency (TBF), were compared between the two systems. Acceleration data were also compared with data collected from free-swimming individuals to determine which respirometry system produces swimming behaviour that is more applicable to wild fish.

Material and methods

Animal maintenance and trial preparation

All animal collections and captive work were approved by Mote Marine Laboratory Institutional Animal Care and Use Committee (Permit #09-09-NW1).

Juvenile lemon sharks (72–97-cm total length, 2.05–4.18 kg) were captured from Cape Canaveral, (FL, USA; $n = 20$) and transported to Mote Marine Laboratory in Sarasota (FL, USA), where they were kept in a 151 000-L recirculating tank for the duration of the experiments. Animals were fed to satiation on a diet of herring, squid and shrimp every other day, but were fasted for 72 h before the start of trials to ensure that they were in a postabsorptive state (Cortés and Gruber 1992). Sharks were seasonally acclimated to trial water temperatures, which ranged between 19.2 and 22.1°C.

At least 24 h in advance of a trial, sharks were tagged with an acceleration data logger (ADL; Model G6A+; Cefas, Lowestoft, UK). An acoustic transmitter (Model V9; Vemco, Nova Scotia, Canada) was epoxied to the ADL so that the tags matched the weight and drag of tags used in field studies as part of a separate project. The complete tag package measured 37 × 36 × 15 mm and weighed 23 g in air (0.05–1.1% body-weight of study individuals), with a frontal cross-sectional area of 4.3 cm^2 (~3–9% of the cross-sectional area of study animals based on estimates made from a length–girth conversion from

animals of similar size; L. R. Brewster, unpubl. data). ADLs were attached to the first dorsal fin at two points using monofilament and recorded triaxial acceleration at 25 Hz (Lear *et al.* 2017).

Each individual first underwent one training flume trial, followed by one static trial and one flume trial in random order on subsequent days (see respirometry protocols below). To compare swimming kinematics of sharks in the respirometers with those of free-swimming sharks, acceleration data were collected from two sharks swimming in a large 150 000-L holding tank and three wild sharks in Bimini, Bahamas, at water temperatures similar to trial water temperatures. Swimming speeds of sharks in the holding tank were measured by timing how long sharks took to swim steadily in a straight line between two points of known distance.

Static respirometry system and trial protocol

The static respirometry system used here is described in detail in Whitney *et al.* (2016). Briefly, the system was built from a modified holding tank with a diameter of 2.45 m, and was sealed with a lid made from a polyvinyl chloride (PVC) ring with transparent polyethylene sheeting of ~150 μm thickness stretched across it (Dowd *et al.* 2006). The sealed respirometer volume was 2494 L. Dissolved oxygen (DO) was measured using a multiparameter meter (Model Pro Plus; Yellow Springs Instruments, Yellow Springs, OH, USA) placed in the centre of the respirometer, where a pump shuttled water from the outside of the tank past the multiparameter meter to facilitate accurate DO measurements and water mixing. The pump and multiparameter meter were enclosed in a circular cage of plastic mesh in order to protect the instruments from the animals and to encourage sharks to swim in full circles around the outside of the respirometer (Whitney *et al.* 2016; Fig. 1).

Lemon sharks were placed into the static respirometer at least 12 h in advance of the start of trials to allow them to acclimate to the system. At the beginning of trials, the respirometer was sealed with the lid and the trials were monitored remotely via a live video feed to limit disturbance to the animal. DO and water temperature were recorded every 5 min, and the behaviour of the shark was monitored constantly. Sharks tended to swim in consistent laps around the outer edge of the respirometer, and swimming speed was measured by recording the time the shark took to make a full lap of the circumference of the respirometer three times in every 5-min period of the trial. Trials began near 100% air saturation, and were run until 80% saturation was reached. Within a trial, periods of at least 20 min of consistent behaviour were used to assess metabolic rate, so that each full trial resulted in several analysis intervals for metabolic rate and swimming activity (for details of analytical methods, see below). To assess background respiration, a blank respirometer (i.e. without an animal) was measured for 4 h at the beginning of each week of the 3-week period used to run respirometry trials. The ODBA–metabolic rate relationship produced by the animals in the static system is published in Lear *et al.* (2017).

Flume respirometry system and protocol

The flume respirometer used in this study was a Steffensen-style 992-L flume with a working section of 45 × 45 × 135 cm

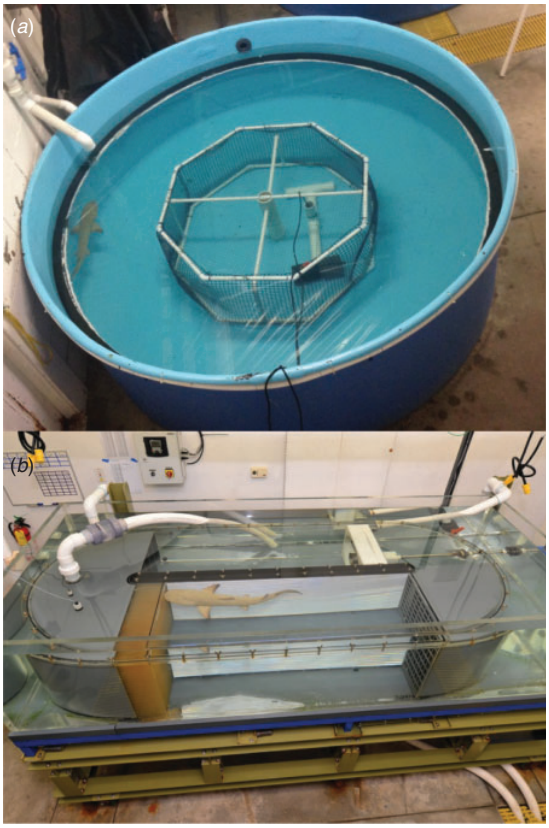

Fig. 1. (*a*) Static respirometry set-up and (*b*) the flume respirometer used in this study, with an 80-cm (total length) lemon shark.

(Loligo Systems, Viborg, Denmark; Fig. 1). To promote rectilinear water movement, flow straighteners were placed in the curves before and after the swimming chamber, and a 20-cm collimator consisting of straws 1 cm wide was placed directly in front of the swimming chamber. DO was measured using a galvanic oxygen probe (Loligo Systems). The flume worked on a rotating 35-min cycle for the duration of all trials, where the system was flushed for 10 min to restore DO, followed by a 5-min wait period to allow the reoxygenated water to fully mix in the respirometer and a 20-min measurement phase before the next flush. Oxygen levels never dropped below 80% air saturation, and typically varied between 90 and 100% saturation.

Sharks were introduced to the flume in an initial training trial where they were not equipped with ADLs. The dual purpose of this trial was to acclimate the animals to the flume and to determine the range of swimming speeds that individuals could maintain. During these training trials, sharks swam at a range of speeds starting at 0.6 body lengths (BL) s^{-1} and increasing by 0.1 BL s^{-1} during each subsequent flush cycle. Trials ended when the shark could no longer maintain its position in the flume, determined by excessive turning or drifting to the back end of the working section. These training trials were completed prior to the static and flume trials used to measure metabolic rate.

During the measurement trial, sharks were placed into the flume and were acclimated to the system for 4 h at the lowest

speed where they would reliably maintain steady swimming, generally around 0.6 BL s^{-1}. This 4-h acclimation period was adopted following a series of 12- to 48-h flume trials run at constant speeds, which showed that oxygen consumption rates ($\dot{M}O_2$) levelled off after 4 h, and that sharks forced to swim in the flume for long periods (≥20 h) showed signs of fatigue. Following the 4-h acclimation period, two speed sets were conducted, separated by a 2-h rest period where the water velocity was returned to the acclimation speed. During the two speed sets, sharks were swum at three speeds ranging from the lowest 0.05 BL s^{-1} increment above the acclimation speed to the highest swimming speed the shark maintained during its training trial, swimming at each speed for one 35-min cycle. These three speeds were presented in a random order, and the water velocity changed gradually for each speed set during the preceding flush period (10–15 min before measurement). During each 20-min measurement phase, the DO and temperature were recorded every 5 min, and the swimming behaviour of the shark was constantly observed. To assess background respiration, a blank respirometer was measured for 4 h without flushing the system at the beginning of each week of the 3-week period used to run flume trials.

Data analysis

Metabolic rate was calculated for each 20-min measurement phase during flume trials ($n = 6$ per trial) and each period during static trials where animals showed consistent swimming behaviour for at least 20 min (n = 1–13 per trial, depending on shark behaviour). These periods were designated analysis intervals, and mass-specific oxygen consumption rate ($\dot{M}O_2$; mg O_2 kg^{-1} h^{-1}) was calculated for each one of these intervals using Eqn 1:

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

where S represents the slope of oxygen decline over time (mg O_2 L^{-1} min^{-1}), b is the slope of the affiliated background respiration rate, V is the volume of the respirometer (L) and M is the mass of the shark (kg). Shark volume was calculated from weight measurements using the mean lemon shark density reported by Baldridge (1970), and was subtracted from respirometer volume for $\dot{M}O_2$ calculation. Swimming speeds in the flume were corrected for a solid blocking effect using eqn 15.3 from Ellerby and Herskin (2013). Because swimming in a circular path results in increased energy expenditure compared with swimming in a straight line, metabolic rates measured in the static respirometry system were corrected to straight swimming estimates following the methods described by Weihs *et al.* (1981), as used by Dowd (2003). Mean routine metabolic rate (RMR), the metabolic rate during volitional swimming activity, was calculated for the static system by averaging the metabolic rates measured during all analysis intervals in that system.

Acceleration data were analysed using Igor Pro (Wavemetrics, Lake Oswego, OR, USA), and the Ethographer extension of Igor Pro (Sakamoto *et al.* 2009). Following methods outlined by Shepard *et al.* (2008), the static component of acceleration was isolated from the dynamic component by using a 3-s box smoother, because the dominant stroke period for these small sharks was <3 s. This smoothing interval was sufficient to remove the tail beat signal from the static acceleration traces (Lear *et al.* 2017). The absolute values of the three dynamic acceleration axes were summed to produce ODBA (Fig. 2). TBF was calculated using a continuous wavelet transformation of the sway axis in Ethographer. ODBA and TBF were averaged to produce one mean value of ODBA and TBF for each analysis interval. For static trials, all swimming speed measurements made during an analysis interval were averaged to produce a mean swimming speed for each interval.

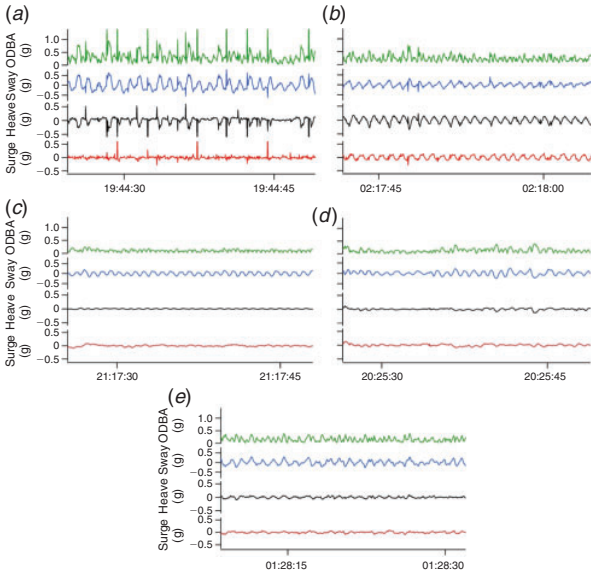

Fig. 2. Acceleration data collected from sharks swimming in the (*a*) flume respirometer at a relatively slow water flow speed of 0.65 body lengths (BL) s^{-1}, (*b*) flume respirometer at a relatively high flow speed of 0.85 BL s^{-1}, (*c*) static respirometer, (*d*) large, 150 000-L holding tank and (*e*) in the wild. Overall dynamic body acceleration (ODBA) was calculated as the sum of the absolute value of the three dynamic acceleration axes: sway (tail beats), surge and heave.

Further analyses were conducted in R (ver. 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) to compare metabolic rates and swimming kinematics measured in the flume and static systems. Linear mixed effects models (LMEs), built using the lme4 package (Bates *et al.* 2015), were used to compare $\dot{M}O_2$ between the static and flume respirometers, swimming speed between both respirometers and the large holding tank, and TBF and ODBA between the respirometers, holding tank and wild sharks. All models included individual as a random effect, and were followed by Tukey's honest significant difference (HSD) tests to determine where differences lay between specific environments. LMEs were also used to analyse the relationships between metabolic rate, TBF, ODBA and swimming speed. Respirometry system was included as a factor in these models and individual was included as a random effect, allowing for a random intercept. Interactions were included between predictor variables and respirometer type to allow for separate intercepts and slopes for the relationships in each system. These interactions were retained in the model if the corrected Akaikes

information criterion (AICc) score of the model was >2 lower than the more parsimonious model without the interaction effect. To ensure that sharks were acclimated to the respirometers during measurement phases and that the different acclimation times in the static and flume respirometry trials did not affect comparative results, the time since the beginning of the trial for each $\dot{M}O_2$ measurement was initially included as a covariate. However, this was not significant in any model and was subsequently removed. LMEs were also compared with null models to test whether swimming metrics were related in the flume and static systems, with the null models rejected and the relationships between metrics retained if the AICc decreased by >2.

To compare metabolic rates measured here with those in previous studies, RMR values were temperature corrected using a Q_{10} temperature coefficient of 1.69. This Q_{10} was calculated from RMR data reported at 20.6 and 29.5°C by Lear *et al.* (2017) using the Van't Hoff equation, presented as eqn 2 in Lear *et al.* (2017). This new RMR Q_{10} was calculated because RMRs are larger than SMRs, and therefore the proportional scaling rate and Q_{10} value for RMR data will be different than the SMR scaling Q_{10} originally reported by Lear *et al.* (2017).

To assess which acceleration-derived parameters (ODBA or TBF) and respirometry system offered the most accurate predictive model for $\dot{M}O_2$, a jack-knife approach was used to estimate the $\dot{M}O_2$ prediction error of each relevant model (Halsey *et al.* 2009; Enstipp *et al.* 2016). The resulting prediction error was used to calculate the standard error of the estimate (s.e.e.) for the flume and static ODBA–$\dot{M}O_2$ and TBF–$\dot{M}O_2$ calibrations. This s.e.e. was converted into a coefficient of variability (COV), or the s.e.e. as a percentage of the mean estimated $\dot{M}O_2$ value in each system. These error metrics, in addition to the AICc, log likelihood and R^2 of the models, were used to determine which model and system was best for predicting $\dot{M}O_2$ from acceleration data. Unless noted otherwise, data are reported as the mean ± s.d.

Results

Active swimming data were successfully collected from 11 of 20 sharks tested in the static respirometer (9 sharks did not demonstrate swimming behaviour), and from 16 of 20 sharks tested in the flume respirometer (4 sharks did not acclimate satisfactorily to the flume environment). However, to ensure that no bias was introduced into the analyses by including sharks that performed in one system and not the other, further comparative analyses only included individuals that performed in both systems ($n = 10$). Mean trial water temperature was 20.5 ± 0.5°C in the static respirometer and 20.6 ± 0.6°C in the flume. Mean body mass of fish used in comparative analyses was 2.73 ± 0.52 kg. The R^2 of oxygen decline in all respirometry intervals used for analysis was >0.9, indicating that both systems measured consistent declines in oxygen despite fairly high fish-to-respirometer volume ratios. Background respiration was consistent during the 3-week trial period, at 0.0005 and 0.0007 mg O_2 L^{-1} h^{-1} in the static and flume systems respectively, equating to ~5.5 and 11.8% of the average oxygen decline observed in the respective systems.

The mean RMR in the static system was 152 ± 30 mg O_2 kg^{-1} h^{-1}, with volitional swimming speeds ranging from 0.44 to 0.70 BL s^{-1} (mean 0.59 ± 0.08 BL s^{-1}) or 33–57 cm s^{-1}. The mean straight line-corrected RMR estimate for the static system was 134 ± 26 mg O_2 kg^{-1} h^{-1}. Mean $\dot{M}O_2$ measured in the flume was 181 ± 40 mg O_2 kg^{-1} h^{-1}, with swimming speeds higher as well, with a mean of 0.76 ± 0.06 BL s^{-1}. Animals were not able to maintain steady swimming behaviour at speeds <0.65 or >0.9 BL s^{-1} (<53 and >74 cm s^{-1} respectively), despite repeated efforts at eliciting consistent behaviour at slower swimming speeds. Swimming speeds in the 150 000-L holding tank were between those measured in the flume and static, ranging from 32 to 70 cm s^{-1} (Fig. 3). Mean TBF was not significantly different between sharks in the flume, static, holding tank and wild (Tukey's HSD, $P > 0.05$), but ODBA was different between all environments except compared between the static respirometer and holding tank (Tukey's HSD, $P < 0.001$), with ODBA lowest in the wild (mean 0.10 ± 0.03 g), intermediate in the flume (0.14 ± 0.03 g) and highest in the holding tank and static respirometer (0.17 ± 0.02 and 0.18 ± 0.04 g respectively; Fig. 3).

The AICc values of models comparing swimming metrics suggested that an interaction between the predictor variables and respirometer type should be included for all models, meaning that the relationships between $\dot{M}O_2$, swimming speed, ODBA and TBF have different slopes and intercepts in the flume and static systems (Table 1). The slopes of these relationships are similar between the two systems (Table 2), but the intercepts are substantially different, with $\dot{M}O_2$ higher in the flume compared with the static system in relation to all swimming metrics, and ODBA and TBF higher in the static system than in the flume system in relation to swimming speed (Table 2). In addition, AICc did not recommend rejection of null models for comparisons of ODBA, TBF and swimming speed in the flume, indicating that these kinematic parameters were not related in the flume system. However, null models were rejected for all comparisons in the static system, retaining all kinematic and metabolic relationships (Fig. 4). Relationships between ODBA and TBF were also retained in the large holding tank and in the wild, with slopes and intercepts similar to those observed in the static respirometer.

Error estimation and model selection criteria all indicated that the best model for predicting $\dot{M}O_2$ from acceleration data was the ODBA–$\dot{M}O_2$ correlation formed in the static respirometer. This model had the lowest AICc, highest log likelihood and R^2 and lowest error of any of the models, with a s.e.e. of 26.4 mg O_2 kg^{-1} h^{-1}, or a COV of 17.0% of the average static $\dot{M}O_2$ (Table 2). Both the flume relationships represented a substantial drop in model fit compared with their static counterparts (minimum ΔAICc of 166), although a small drop in the calculated error (Table 2).

Discussion

Much of our understanding of the factors that drive physiology, behaviour and metabolism in fish has come from comparative studies based on respirometry data (e.g. Clarke and Johnston 1999; Gillooly *et al.* 2001; Killen *et al.* 2010, 2016). Therefore, the ability to compare metabolic rates across different species of fish and to apply these measured rates to wild systems is an integral component to understanding the ecology of these animals. As such, determining how respirometry systems affect or bias metabolic rates and swimming performance is critical. Our

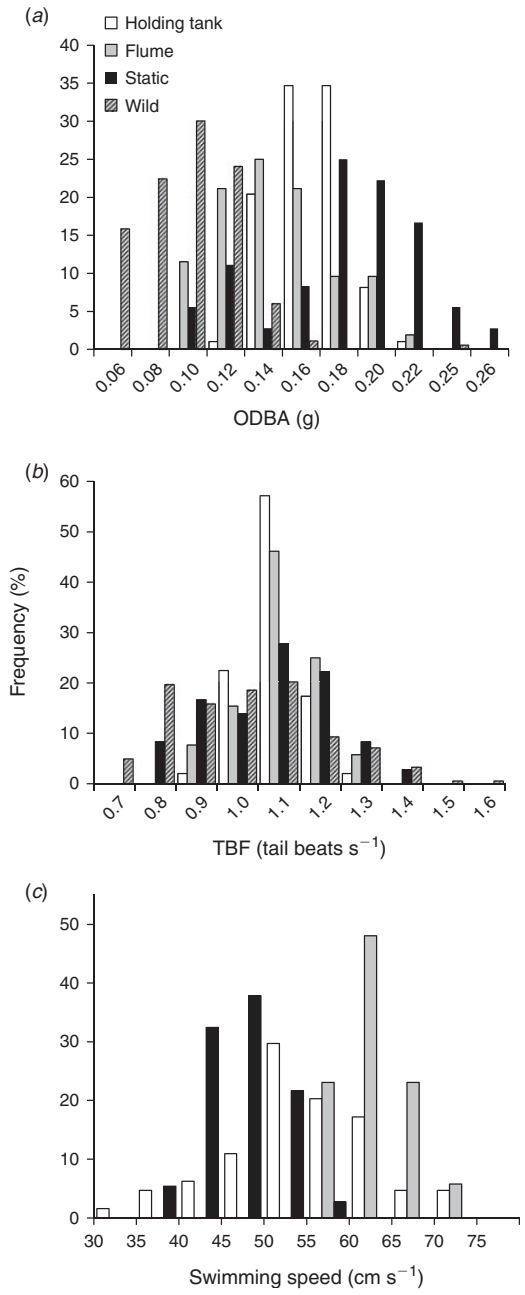

Fig. 3. Frequency distributions of (*a*) overall dynamic body acceleration (ODBA), (*b*) tail beat frequency (TBF) and (*c*) swimming speed of sharks swimming in the static respirometer, flume respirometer, 150 000-L holding tank and in the wild.

results show that the choice of respirometer significantly affected both the metabolic rates and swimming kinematics of lemon sharks. Because the same individuals were used in both flume and static respirometry trials, it is unlikely that the differences in metabolic rate observed between the two systems are due to interindividual variation or are a result of the captive environment. Instead, these discrepancies are driven by differences inherent in the respirometry systems themselves, including the forced swimming conditions in the flume respirometer and the curved path swimming necessary in the static system. The results of this study in lemon sharks are not necessarily universally applicable to other elasmobranchs, which may differ in their swimming behaviours and ability to acclimate to enclosed systems. However, these results demonstrate several general trends in swimming behaviour and metabolism measured in static and flume respirometry systems, and highlight the importance of considering the effect these systems have on the metabolic rates and swimming kinematics they measure.

Lemon shark metabolic rates

The RMRs measured for lemon sharks in this study (at 152 ± 30 and 181 ± 37 mg O_2 kg^{-1} h^{-1} in the static and flume respirometry systems respectively) were generally similar to those measured in previous lemon shark studies in that they were higher than those of benthic species, such as nurse sharks *Ginglymostoma cirratum* (RMR 95.3 mg O_2 kg^{-1} h^{-1} at 23°C; Whitney *et al.* 2016), and lower than active ram-ventilating species, such as blacknose sharks *Carcharhinus acronotus* (RMR 395 mg O_2 kg^{-1} h^{-1} at 28°C; Carlson *et al.* 1999), scalloped hammerhead sharks *Sphyrna lewini* (RMR 275 mg O_2 kg^{-1} h^{-1} at 26°C; Lowe 2001) and bonnethead sharks *Sphyrna tiburo* (RMR 235 mg O_2 kg^{-1} h^{-1} at 26°C; Carlson and Parsons 2003). There is some variation in lemon shark metabolic rates reported in previous studies and the present study (Table 3), although differences in respirometry and data analysis methods make direct comparisons difficult. This variation could be due to interpopulation differences in metabolism (Bouyoucos *et al.* 2017); for example, due to divergent growth rates or variation in other life history characteristics arising from the use of different habitats or behavioural strategies. The different respirometry protocols used in these studies could also contribute to the variation in measured metabolic rates. In addition, comparing RMR data from a wide temperature range can be problematic, because ectotherms may change their activity levels with temperature (Halsey *et al.* 2015). Therefore, comparisons of RMRs made over a large temperature range will include differences in metabolic rate driven by changing activity levels, which will persist even after metabolic rates are temperature corrected.

Comparison of flume and static respirometry systems

The flume and static respirometry systems showed significant differences in metabolic rate measurements and the relationships between metabolic rate and TBF, ODBA, and swimming speed. TBF and ODBA were both higher in relation to swimming speed in the static system, likely due to the nature of curved-path swimming in the round static tank. Turning requires more body movement and mechanical effort than swimming in a straight path (Weihs *et al.* 1981), resulting in increased ODBA and TBF for a given swimming speed. Theoretically, the increased body movement necessary for curved swimming should also result in higher energy expenditure for sharks swimming in the static system. However, the opposite proved to be the case, with oxygen consumption rates significantly higher in the flume compared with static system in relation to all

Table 1. Model results for respirometry-derived relationships

Details are included for relationships between oxygen consumption ($\dot{M}O_2$), overall dynamic body acceleration (ODBA), tail beat frequency (TBF), swimming speed (U) and respirometry system, with interactions included between predictor variables and respirometry system in all models (designated by ':'). Model results for models without interactions are not shown, but consistently had an Akaike's information criterion corrected for small sample size (AICc) >10 greater than models with interactions. For equations marked with an asterisk (*), AICc did not recommend retention of relationships between kinematic metrics for the flume respirometer

Model	Log likelihood	AICc	R^2	Flume equation	Static equation
$\dot{M}O_2$ ~ODBA : system	−737.8	893.7	0.42	352.8(ODBA) + 128.5	376.7(ODBA) + 79.6
$\dot{M}O_2$ ~SS : system	−444.7	901.4	0.42	217.5(U) + 21.1	92.03(U) + 80.35
$\dot{M}O_2$ ~TBF : system	−707.7	1427.3	0.52	180.8(TBF) − 8.5	106.0(TBF) + 39.9
TBF ~SS : system	76.5	−140.9	0.41	0.58(U) + 0.63*	1.1(U) + 0.43
TBF ~ODBA : system	72	−132	0.35	−0.38(ODBA) + 1.12*	1.12(ODBA) + 0.81
ODBA ~SS : system	177.5	−342.9	0.39	0.002(U) + 0.14*	0.27(U) + 0.03

Table 2. Model selection criteria for oxygen consumption ($\dot{M}O_2$) prediction models

Selection criteria are shown for predictive models using overall dynamic body acceleration (ODBA) and tail beat frequency (TBF) to estimate $\dot{M}O_2$ in the static and flume respirometers. s.e.e., standard error of the estimate; COV, coefficient of variability, or s.e.e. as a percentage of the estimated value; AICc, Akaike's information criterion corrected for small sample size

System	Model	Log likelihood	AICc	ΔAICc	R^2	s.e.e. (mg O_2 kg^{-1} h^{-1})	COV (%)
Static	$\dot{M}O_2$~ODBA	−158.8	325.5	–	0.33	26.4	17.0
Static	$\dot{M}O_2$~TBF	−163	334.1	8.6	0.2	29.9	19.4
Flume	$\dot{M}O_2$~ODBA	−242.1	492.3	166.8	0.07	42.8	23.5
Flume	$\dot{M}O_2$~TBF	−244	496.1	170.6	0.07	37.7	20.7

swimming metrics. This can be attributed, in part, to the sharks swimming at higher velocities in the flume, because sharks did not maintain steady swimming behaviour when water velocity was similar to the volitional swimming speeds observed in the static respirometer and holding tank. However, the relationship between $\dot{M}O_2$ and swimming speed also had a higher intercept in the flume compared with the static respirometer, a trend mirrored in the relationship between $\dot{M}O_2$ and ODBA (Fig. 4). These higher intercepts suggest that the minimum metabolic rate of fish in the flume is higher than in the static system. Increased stress can elevate metabolic rate in fish (Wendelaar Bonga 1997; Sloman *et al.* 2000), and this is the most likely explanation for the increased minimum metabolic rate in the flume, with stress caused by the confined space and forced swimming inherent in flume respirometers (Carlson *et al.* 2004). Previous studies have also cited concerns that stress in flumes elevated metabolic rates of fish (e.g. Lowe 1996; 2001; Wright *et al.* 2014).

The occurrence of faster swimming speeds in the flume respirometer is important to note in and of itself. Although swimming speed in the flume is experimentally controlled, the range of swimming speeds used in the present study represented the entire range of swimming speeds where sharks were able to maintain steady swimming in the flume. However, the slowest flume swimming speed was close to the highest volitional swimming speed observed in the static system (Fig. 3). Considering that both ODBA and TBF were significantly lower in wild sharks compared with both respirometry systems, average swimming speeds are likely lower in the wild as well, meaning the swimming data that can be collected in the flume are unlikely to represent a substantial proportion of the swimming behaviour observed in wild sharks. At speeds lower than the test range used in the flume, sharks tended to swim erratically, using substantial dorsoventral and lateral movement in order to maintain their position in the water column and turning around often within the working section. This type of erratic swimming has also been reported in several past elasmobranch flume studies (e.g. Lowe 1996, 2001; Gleiss *et al.* 2010; Bouyoucos *et al.* 2017), as well as in some teleost studies (e.g. Wright *et al.* 2014; Mori *et al.* 2015).

Although swimming speed ranges barely overlapped between the two systems, ODBA showed almost a complete overlap (Fig. 3). Therefore, the higher metabolic rates observed in the flume system are of particular importance for studies correlating ODBA and $\dot{M}O_2$, because the correlations made in the flume will result in substantially higher estimates of FMR than correlations made in the static system. Erratic swimming at low water velocities in the flume also changed the way that ODBA, TBF, and swimming speed related, with no relationships between kinematic parameters retained in the flume respirometer. Although dorsoventral and lateral movement were limited at the speeds used during flume trials, slower water velocities still elicited more erratic swimming behaviour, with sharks often swimming in the corners of the flume where water velocity was slowest due to wall effects (Lowe 2001), with an angled body and uneven tail beats. This erratic swimming can be seen in the acceleration data, with flume animals typically producing choppy tail beat movements with substantial heave, where extraneous body movements would substantially contribute to ODBA independent of TBF (Fig. 2). This was especially true at

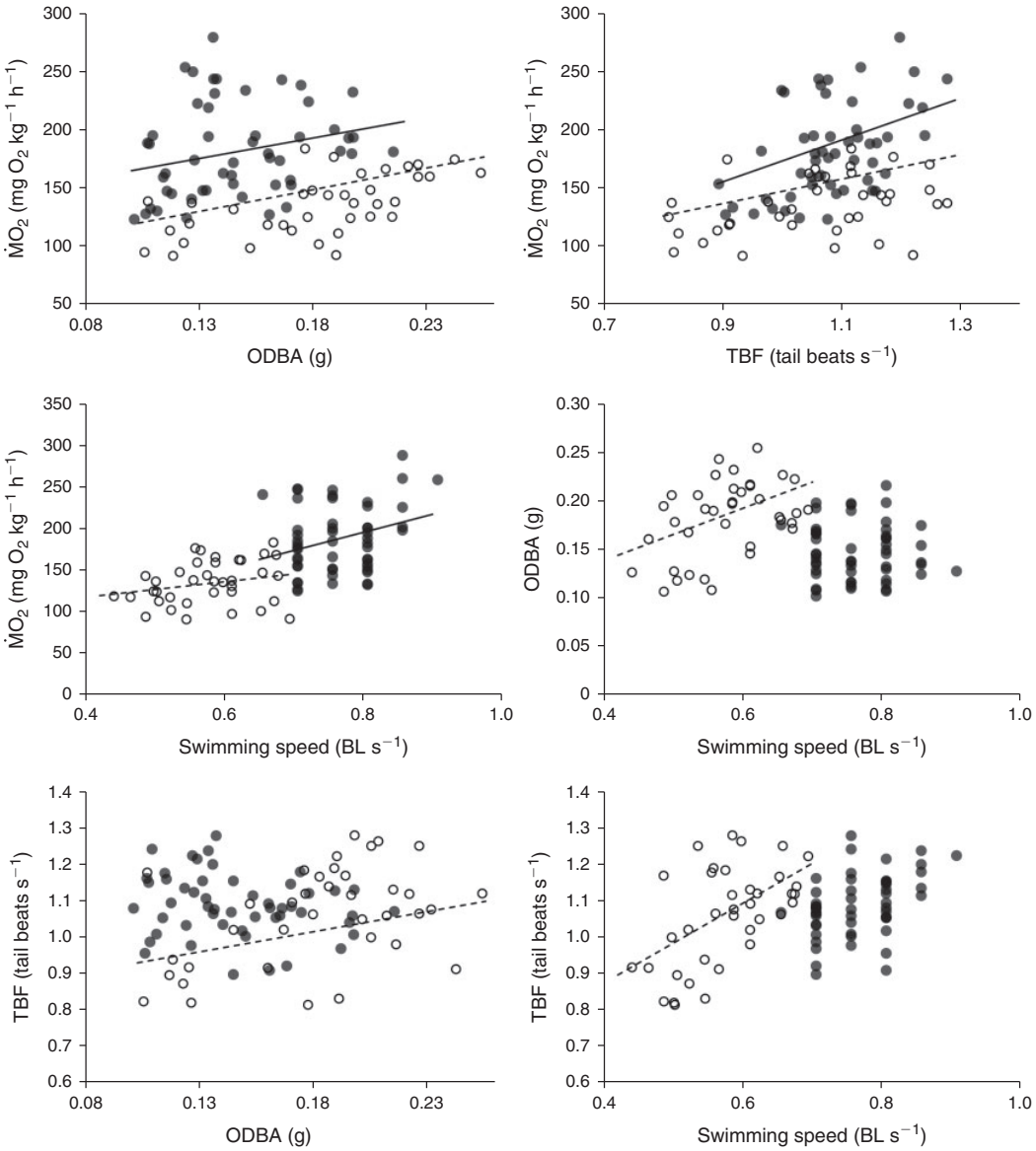

Fig. 4. Relationships between oxygen consumption rate ($\dot{M}O_2$), tail beat frequency (TBF), swimming speed, and overall dynamic body acceleration (ODBA) measured in the flume respirometer (filled circles, solid lines) and static respirometer (empty circles, dashed lines). Regression lines are not shown for the flume ODBA–TBF, ODBA–swimming speed and TBF–swimming speed relationships because model results did not recommend considering these relationships. The static $\dot{M}O_2$ plotted against swimming speed is the straight line-corrected $\dot{M}O_2$. All other $\dot{M}O_2$ values are uncorrected. BL, body lengths.

low water velocities. At higher velocities sharks were forced to adopt more streamlined swimming techniques, generally swimming consistently in the centre of the working section, where the majority of ODBA originated from tail beat movements. Animals in the static respirometer produced smoother, consistent tail beats regardless of swimming speed (Fig. 2), maintaining relationships between ODBA, TBF, and swimming speed.

Acceleration data collected from the large holding tank and from wild individuals also showed a relationship between ODBA and TBF, with slopes and intercepts similar to those observed in the static respirometer. However, sharks in the holding tank showed variation in swimming speeds and ODBA compared with respirometer-confined fish, and wild sharks had lower values of ODBA and TBF than all captive animals (Fig. 3), showing that no captive environment accurately mirrors the behaviour of wild sharks. Even so, the similarity in kinematic relationships between the static respirometer and free-swimming animals indicate that despite the constant turning, the static respirometer elicited more natural swimming behaviour than the flume respirometer.

Table 3. Oxygen consumption rates ($\dot{M}O_2$) of lemon sharks measured in the present and previous studies

$\dot{M}O_2$ values were temperature corrected using a Q_{10} of 1.69. Swimming speed (U) is reported as a range for all studies except for Bouyoucos *et al.* (2017), where a mean ± s.d. is reported. Note that swimming speeds during flume studies were experimentally controlled, and therefore care must be taken when comparing values with volitional activity $\dot{M}O_2$ measured in static systems. BL, body lengths

References	System	U (BL s^{-1})	Reported $\dot{M}O_2$ (mg O_2 kg^{-1} h^{-1})	Study temperature (°C)	$\dot{M}O_2$ at 20.6°C (mg O_2 kg^{-1} h^{-1})
Nixon and Gruber (1988)	Static	0.0–0.6	193[A]	23	170.2
Bushnell *et al.* (1989)	Static	0.0–0.7	145.9[A]	22	135.6
Scharold and Gruber (1991)	Static	0.31–0.57	240.2	25	190.7
Graham *et al.* (1990)	Flume	1.0–1.3	318	22.4	289.3
Bouyoucos *et al.* (2017)	Flume	0.19 ± 0.01[B]	249.7[B]	30.8	146.2
Present study	Static	0.44–0.70	152	20.6	152
Present study	Flume	0.65–0.90	181	20.6	181

[A]The $\dot{M}O_2$ values reported by Nixon and Gruber (1988) and Bushnell *et al.* (1989) are mean daily $\dot{M}O_2$, which includes resting behaviour.
[B]The swimming speed and $\dot{M}O_2$ reported by Bouyoucos *et al.* (2017) are estimated from accelerometers deployed on sharks swimming in a mesocosm, based on a calibration between activity and metabolic rate conducted in a flume respirometer.

Recommendations for future studies

For most types of respirometry studies, metabolic rates could feasibly be measured by either a static or a flume respirometer, and both systems have inherent advantages and disadvantages. The volitional swimming activity in static respirometers means that collecting metabolic rate data is contingent upon study animals voluntarily maintaining consistent behaviour and swimming speeds for extended time periods. This may prove particularly problematic for sharks capable of buccal pumping, including lemon sharks, where swimming and resting often occur in short bursts, making collecting steady swim data potentially difficult and time consuming. For example, in order to collect the ~19 h of steady swimming data observed here, over 250 h of trials were run in the static system, meaning only ~7.5% of trial time could be used for RMR estimation. In contrast, because water velocity can be controlled in a flume respirometer, a shark's behaviour is more easily regulated, here with 20 of every 35 min of flume trial time resulting in useable swimming data for $\dot{M}O_2$ estimation (57% of trial time), assuming animals swum in an appropriate manner.

Although flume respirometers may be more efficient for collecting active swimming data, there are also several drawbacks to these systems. First, it can be difficult to get sharks to swim in flumes. Although 80% of lemon sharks in our study acclimated satisfactorily to the flume, this is fairly unusual, and many past studies have reported high failure rates for acclimating sharks to flumes. For example, Bouyoucos *et al.* (2017) reported a 40% success rate for lemon sharks, Gleiss *et al.* (2010) experienced a 23% success rate for scalloped hammerhead sharks and Lowe (1996, 2001) reported high mortality for scalloped hammerheads left to acclimate to a flume overnight. We have also experienced 0% success rates in acclimating blacktip sharks *Carcharhinus limbatus* ($n = 10$) and bonnethead sharks *S. tiburo* ($n = 16$) to a flume respirometer, and although nurse sharks *G. cirratum* acclimated successfully to the flume, steady swimming behaviour could not be elicited (N. Whitney, K. Lear and A. Gleiss, unpubl. data). In addition, testing a large number of individuals and choosing only animals that are able to swim satisfactorily in flumes can bias results towards animals that swim in a certain way or respond more favourably to stress or confinement.

Even for animals that acclimate satisfactorily to the flume, reluctance to swim in flumes for extended periods generally forces shorter acclimation times for flume respirometry studies than are usually advised. In past studies, these have included a 6-h acclimation period for lemon sharks (Bouyoucos *et al.* 2017), 4-h acclimation periods for mako sharks *Isurus oxyrinchus* (Sepulveda *et al.* 2007), 30-min acclimation periods for scalloped hammerheads (Lowe 1996, 2001), and respirometry trials started as soon as sharks reached steady swimming in scalloped hammerheads (Gleiss *et al.* 2010) and mako, lemon and leopard sharks *Triakis semifasciata* (Graham *et al.* 1990). Reluctance of animals to swim in the flume also necessitated shorter acclimation times in the present study, where the 4-h flume acclimation time, although determined sufficient here for lemon sharks, was shorter than the post-handling acclimation times generally advised for elasmobranchs (Carlson *et al.* 2004) and shorter than the acclimation time adopted for the static respirometer.

In addition, our results indicate that even after acclimation the forced swimming conditions in the flume produced elevated metabolic rates and kinematic relationships that were inconsistent with those observed in free-swimming lemon sharks. Conversely, relationships between kinematic parameters in the static tank mirrored those observed in free-swimming animals, meaning that static respirometers are likely to produce estimates of metabolic rates and swimming behaviour that are more representative of wild lemon sharks. This is particularly relevant for studies correlating metabolic rate and ODBA to provide a metric to estimate FMR. ODBA decoupled with swimming speed and TBF in the flume, showing that sharks tune body movements in flumes compared with free-swimming environments, and thus potentially change or weaken the relationship between swimming metrics and metabolic rate. This is demonstrated by the weaker correlations between $\dot{M}O_2$ and ODBA and $\dot{M}O_2$ and TBF in the flume compared with their counterparts in the static system, and the greater error observed in these calibrations (Table 2). In addition, if metabolic rates measured in flume systems are elevated by stress, applying these rates to

free-swimming, non-stressed animals in the field will overestimate their FMRs. The RMR measured here in the flume was 19% higher than the RMR measured in the static system, meaning that using the ODBA–$\dot{M}O_2$ calibration produced by the flume respirometer would substantially inflate FMR estimates. For example, applying the slopes and intercepts of the ODBA–$\dot{M}O_2$ relationships established here to wild acceleration data using the recommended predictive model outlined by Lear *et al.* (2017), a juvenile 3.1-kg lemon shark would use ~25.6 kcal day^{-1} according to the static calibration, whereas the flume calibration yields an estimate of 39.5 kcal day^{-1} (mean field temperature 21.3°C). This represents an increase in estimated energy use of 54% when using the flume calibration compared with the static calibration, which would substantially affect estimates of energy expenditure and bias their application to ecosystem or bioenergetics modelling.

However, this is not to say that flume respirometers are not a valuable tool to study both elasmobranchs and teleosts. For example, in studies examining swimming kinematics or biomechanics, swimming speed needs to be controlled so that measurements can be accurately compared across species or across flow rates. Flume respirometers also allow for metabolic rates to be measured and compared across specific speeds, providing information on the cost of added activity, optimum swimming speeds and critical swimming speeds, among other parameters. These studies provide data that are important to understanding how fish interact with their aquatic environments, and are only possible in flume respirometers where precise selection of swimming speed offers a controlled setting for such comparative work.

Conclusions

The results of the present study indicate that the annular static system produced swimming behaviour similar to that of free-swimming animals, whereas the flume respirometer produced elevated metabolic rates and swimming behaviour and kinematic relationships that diverge from those observed in free-swimming lemon sharks. Therefore, whereas flume respirometers are necessary for comparative kinematic or biomechanical studies and can provide valuable data in a controlled setting, static respirometers may produce estimates of swimming behaviour and metabolic rates that more accurately reflect wild systems, and are likely preferable for studies measuring metabolic rates with the purpose of applying estimates to free-swimming animals. This is particularly relevant for studies correlating ODBA and metabolic rate to provide calibrations for estimating FMRs, because calibrations produced in the flume may not only result in greater prediction errors, but may also substantially overestimate metabolic rates, compromising the integrity of any subsequent bioenergetics or ecosystem models. These results also emphasise the importance of considering the effects that respirometry systems have on the metabolic rates they measure, particularly for comparative studies. Caution must be exercised when evaluating metabolic rates measured in divergent systems in order to promote accurate physiological comparisons across species.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

Baldridge, H. D. (1970). Sinking factors and average densities of Florida sharks as functions of liver buoyancy. *Copeia*, 744–754. doi:10.2307/1442317

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1–48. doi:10.18637/JSS.V067.I01

Bernal, D., Carlson, J. K., Goldman, K. J., and Lowe, C. G. (2012). Energetics, metabolism, and endothermy in sharks and rays. In 'Biology of Sharks and their Relatives', 2nd edn. (Eds J. C. Carrier, J. A. Musick, and M. C. Heithaus.) pp. 211–237. (CRC Press: Boca Raton, FL, USA.)

Bouyoucos, I. A., Montgomery, D. W., Brownscombe, J. W., Cooke, S. J., Suski, C. D., Mandelman, J. W., and Brooks, E. J. (2017). Swimming speeds and metabolic rates of semi-captive juvenile lemon sharks (*Negaprion brevirostris*, Poey) estimated with acceleration biologgers. *Journal of Experimental Marine Biology and Ecology* **486**, 245–254. doi:10.1016/J.JEMBE.2016.10.019

Brett, J. R., and Blackburn, J. M. (1978). Metabolic rate and energy expenditure of the spiny dogfish, *Squalus acanthias*. *Journal of the Fisheries Research Board of Canada* **35**, 816–821. doi:10.1139/F78-131

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., and West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789. doi:10.1890/03-9000

Bushnell, P. G., Lutz, P. L., and Gruber, S. H. (1989). The metabolic rate of an active, tropical elasmobranch, the lemon shark (*Negaprion brevirostris*). *Experimental Biology* **48**, 279–283.

Carlson, J. K., and Parsons, G. R. (2003). Respiratory and hematological responses of the bonnethead shark, *Sphyrna tiburo*, to acute changes in dissolved oxygen. *Journal of Experimental Marine Biology and Ecology* **294**, 15–26. doi:10.1016/S0022-0981(03)00237-5

Carlson, J. K., Palmer, C. L., and Parsons, G. R. (1999). Oxygen consumption rate and swimming efficiency of the blacknose shark, *Carcharhinus acronotus*. *Copeia* **1999**(1), 34–39. doi:10.2307/1447382

Carlson, J. K., Goldman, K. J., and Lowe, C. G. (2004) Metabolism, energetic demand, and endothermy. In 'Biology of Sharks and Their Relatives', 1st edn. (Eds J. C. Carrier, J. A. Musick, and M. C. Heithaus.) pp. 203–224. (CRC Press: Boca Raton, FL, USA.)

Clark, T. D., Sandblom, E., and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *The Journal of Experimental Biology* **216**, 2771–2782. doi:10.1242/JEB.084251

Clarke, A., and Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* **68**, 893–905. doi:10.1046/J.1365-2656.1999.00337.X

Cooke, S. J., Brownscombe, J. W., Raby, G. D., Browll, F., Hinch, S. G., Clark, T. D., and Semmens, J. M. (2016). Remote bioenergetics

measurements in wild fish: opportunities and challenges. *Comparative Biochemistry and Physiology – A. Molecular & Integrative Physiology* **202**, 23–37. doi:10.1016/J.CBPA.2016.03.022

Cortés, E., and Gruber, S. H. (1992). Gastric evacuation in the young lemon shark, *Negaprion brevirostris*, under field conditions. *Environmental Biology of Fishes* **35**(2), 205–212. doi:10.1007/BF00002195

Dowd, W. W. (2003). Metabolic rates and bioenergetics of juvenile sandbar sharks (*Carcharhinus plumbeus*). M.Sc. Thesis, College of William and Mary, Williamsburg, VA, USA.

Dowd, W. W., Brill, R. W., Bushnell, P. G., and Musick, J. A. (2006). Standard and routine metabolic rates of juvenile sandbar sharks (*Carcharhinus plumbeus*), including the effects of body mass and acute temperature change. *Fishery Bulletin* **104**, 323–331.

Ellerby, D. J., and Herskin, J. (2013). Swimming flumes as a tool for studying swimming behavior and physiology: current applications and future developments. In 'Swimming Physiology of Fish. (Eds A. P. Palstra and J. V. Planas.) pp. 345–373. (Springer Science & Business Media: Berlin, Germany.)

Enstipp, M. R., Ballorain, K., Ciccione, S., Naraxaki, T., Sato, K., and Georges, J.-Y. (2016). Energy expenditure of adult green turtles (*Chelonia mydas*) at their foraging grounds and during simulated oceanic migration. *Functional Ecology* **30**, 1810–1825. doi:10.1111/1365-2435.12667

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* **293**, 2248–2251. doi:10.1126/SCIENCE.1061967

Gleiss, A. C., Dale, J. J., Holland, K. N., and Wilson, R. P. (2010). Accelerating estimates of activity-specific metabolic rate in fishes: testing the applicability of acceleration data-loggers. *Journal of Experimental Marine Biology and Ecology* **385**, 85–91. doi:10.1016/J.JEMBE.2010.01.012

Graham, J. B., Dewar, H., Lai, N. C., Lowell, W. R., and Arce, S. M. (1990). Aspects of sharks swimming performance determined using a large water tunnel. *The Journal of Experimental Biology* **151**, 175–192.

Halsey, L. G., Green, J. A., Wilson, R. P., and Frappell, P. B. (2009). Accelerometry to estimate energy expenditure during activity: best practice with data loggers. *Physiological and Biochemical Zoology* **82**, 396–404. doi:10.1086/589815

Halsey, L. G., Matthews, P. G. D., Rezende, E. L., Chauvaud, L., and Robson, A. A. (2015). The interactions between temperature and activity levels in driving metabolic rate: theory, with empirical validation from contrasting ectotherms. *Oecologia* **177**, 1117–1129. doi:10.1007/S00442-014-3190-5

Hughes, N. F., and Kelley, L. H. (1996). A hydrodynamic model for estimating the energetic cost of swimming maneuvers from a description of their geometry and dynamics. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 2484–2493. doi:10.1139/F96-204

Killen, S., Atkinson, D., and Glazier, D. S. (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters* **13**, 184–193. doi:10.1111/J.1461-0248.2009.01415.X

Killen, S. S., Glazier, D. S., Rezende, E. L., Clark, T. D., Atkinson, D., Willener, A. S. T., and Halsey, L. G. (2016). Ecological influences and morphological correlates of resting and maximal metabolic rates across teleost fish species. *American Naturalist* **187**, 592–606. doi:10.1086/685893

Killen, S. S., Norin, T., and Halsey, L. G. (2017). Do method and species lifestyle affect measures of maximum metabolic rate in fishes? *Journal of Fish Biology* **90**(3), 1037–1046. doi:10.1111/JFB.13195

Lear, K. O., Whitney, N. M., Brewster, L. R., Morris, J. J., Hueter, R. E., and Gleiss, A. C. (2017). Correlations of metabolic rate and body acceleration in three species of coastal sharks under contrasting temperature regimes. *The Journal of Experimental Biology* **220**, 397–407. doi:10.1242/JEB.146993

Lowe, C. G. (1996). Kinematics and critical swimming speed of juvenile scalloped hammerhead sharks. *The Journal of Experimental Biology* **199**, 2605–2610.

Lowe, C. G. (2001). Metabolic rates of juvenile scalloped hammerhead sharks (*Sphyrna lewini*). *Marine Biology* **139**, 447–453. doi:10.1007/S002270100585

Lowe, C. G., and Goldman, K. J. (2001). Thermal and bioenergetics of elasmobranchs: bridging the gap. *Environmental Biology of Fishes* **60**, 251–266. doi:10.1023/A:1007650502269

McNamara, J. M., and Houston, A. I. (1996). State-dependent life histories. *Nature* **380**, 215–221. doi:10.1038/380215A0

Metcalfe, J. D., Wright, S., Tudorache, C., and Wilson, R. P. (2016). Recent advances telemetry for estimating the energy metabolism of wild fishes. *Journal of Fish Biology* **88**, 284–297. doi:10.1111/JFB.12804

Mori, T., Miyata, N., Aoyama, J., Niizuma, Y., and Sato, K. (2015). Estimation of metabolic rate from activity measured by recorders deployed on Japanese sea bass *Lateolabrax japonicus*. *Fisheries Science* **81**, 871–882. doi:10.1007/S12562-015-0910-7

Nelson, J. A., Gotwalt, P. S., Reidy, S. P., and Webber, D. M. (2002). Beyond U_{crit}: matching swimming performance tests to the physiological ecology of the animal, including a new fish 'drag strip'. *Comparative Biochemistry and Physiology Part A* **133**, 289–302. doi:10.1016/S1095-6433(02)00161-7

Nixon, A. J., and Gruber, S. H. (1988). Diel metabolic and activity patterns of the lemon shark (*Negaprion brevirostris*). *The Journal of Experimental Zoology* **248**, 1–6. doi:10.1002/JEZ.1402480102

Norin, T., and Clark, T. (2016). Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology* **88**(1), 122–151. doi:10.1111/JFB.12796

Peake, S. J., and Farrell, A. P. (2004). Locomotory behaviour and post-exercise physiology in relation to swimming speed, gait transition, and metabolism in free-swimming smallmouth bass. *The Journal of Experimental Biology* **207**, 1563–1575. doi:10.1242/JEB.00927

Peake, S. J., and Farrell, A. P. (2006). Fatigue is a behavioural response in respirometer-confined smallmouth bass. *Journal of Fish Biology* **68**, 1742–1755. doi:10.1111/J.0022-1112.2006.01052.X

Reidy, S., Nelson, J., Tang, Y., and Kerr, S. (1995). Post-exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. *Journal of Fish Biology* **47**(3), 377–386. doi:10.1111/J.1095-8649.1995.TB01907.X

Roche, D. G., Binning, S. A., Bosiger, Y., Johansen, J. L., and Rummer, J. L. (2013). Finding the best estimates of metabolic rates in a coral reef fish. *The Journal of Experimental Biology* **216**(11), 2103–2110. doi:10.1242/JEB.082925

Rummer, J. L., Binning, S. A., Roche, D. G., and Johansen, J. L. (2016). Methods matter: considering locomotory mode and respirometry technique when estimating metabolic rates of fishes. *Conservation Physiology* **4**(1), cow008. doi:10.1093/CONPHYS/COW008

Sakamoto, K. Q., Sato, K., Ishizuka, M., Watanuki, Y., Takahashi, A., Daunt, F., and Wanless, S. (2009). Can ethograms be automatically generated using body acceleration data from free-ranging birds? *PLoS One* **4**, e5379. doi:10.1371/JOURNAL.PONE.0005379

Scharold, J., and Gruber, S. H. (1991). Telemetered heart rate as a measure of metabolic rate in the lemon shark, *Negaprion brevirostris*. *Copeia* (4), 942–953. doi:10.2307/1446090

Sepulveda, C. A., Graham, J. B., and Bernal, D. (2007). Aerobic metabolic rates of swimming juvenile mako sharks, *Isurus oxyrinchus*. *Marine Biology* **152**, 1087–1094. doi:10.1007/S00227-007-0757-2

Shepard, E. L. C., Wilson, R. P., Halsey, L. G., Quintana, F., Laich, A. G., Gleiss, A. C., Liebsch, N., Myers, A. E., and Norman, B. (2008). Derivation of body motion via appropriate smoothing of acceleration data. *Aquatic Biology* **4**, 235–241. doi:10.3354/AB00104

Sloman, K. A., Motherwell, G., O'Connor, K. I., and Taylor, A. C. (2000). The effect of social stress on the standard metabolic rate (SMR) of brown

trout, *Salmo trutta*. *Fish Physiology and Biochemistry* **23**, 49–53. doi:10.1023/A:1007855100185

Treberg, J. R., Killen, S. S., MacCormack, T. J., Lamarre, S. G., and Enders, E. C. (2016). Estimates of metabolic rate and major constituents of metabolic demand in fishes under field conditions: methods, proxies, and new perspectives. *Comparative Biochemistry and Physiology – A. Molecular & Integrative Physiology* **202**, 10–22. doi:10.1016/J.CBPA.2016.04.022

Weihs, D., Keyes, R. S., and Stalls, D. M. (1981). Voluntary swimming speeds of two species of large Carcharhinid sharks. *Copeia* (1), 219–222. doi:10.2307/1444062

Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews* **77**, 591–625. doi:10.1152/PHYSREV.1997.77.3.591

Whitney, N. M., Papastamatiou, Y. P., and Gleiss, A. C. (2012). Integrative multisensor tagging: emerging techniques to link elasmobranch behavior, physiology, and ecology. In 'Biology of Sharks and Their Relatives', 2nd edn. (Eds J. C. Carrier, J. A. Musick, and M. R. Heithaus.) pp. 265–289. (CRC Press: Boca Raton, FL, USA.)

Whitney, N. M., Lear, K. O., Gaskins, L. C., and Gleiss, A. C. (2016). The effects of temperature and swimming speed on the metabolic rate of the nurse shark (*Ginglymostoma cirratum*, Bonaterre). *Journal of Experimental Marine Biology and Ecology* **477**, 40–46. doi:10.1016/J.JEMBE.2015.12.009

Wright, S., Metcalfe, J. D., Hetherington, S., and Wilson, R. (2014). Estimating activity-specific energy expenditure in a teleost fish, using accelerometer loggers. *Marine Ecology Progress Series* **496**, 19–32. doi:10.3354/MEPS10528

Yasuda, T., Komeyama, K., Kato, K., and Mitsunaga, Y. (2012). Use of acceleration loggers in aquaculture to determine net-cage use and field metabolic rates in red sea bream *Pagurus major*. *Fisheries Science* **78**, 229–235. doi:10.1007/S12562-011-0446-4

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