

Text S1. Methods

1.1 Specimen data collection

Specimen A was found stranded in Cornwall, southwest England, and Specimen B was live stranded at Scarborough, in the northeast of England. Thirteen approximate half cross-sections were made, without piercing the abdominal cavity, on Specimen A at intervals along the body length every 5% of the fork length of the shark, or every 18 cm (Bernvi 2016). Attempts to re-float Specimen B were unsuccessful and the shark was humanely euthanised with barbiturate drugs by a qualified veterinarian operating under The Wildlife and Countryside Act 1981 (as amended) and The Animal and Welfare Act 2006. A field examination of Specimen B was conducted two days later by the Cetacean Strandings Investigation Programme under license from Natural England (Class License CL01). Five approximate half cross-sections were taken from Specimen B, starting from just anterior of the pectoral fins, between the pectoral fins, under the first dorsal fin, posterior to the first dorsal fin and posterior to the second dorsal fin. Due to logistical and regulatory constraints of the beach dissections, thorough examination of relevant heat retention tissues such as a ‘lamnid specific red muscle vein’ or *rete mirabile* could not be conducted. Beach dissection of Specimen B was exploratory in nature. As a result, full transverse sections could not be taken, and photographs were unable to be taken from 90° to the musculature. Photographic angles were corrected from Specimen B in Adobe Photoshop CC using distortion panel and backgrounds of all photographs were removed using the lasso tool.

1.2 Skeletal red muscle analysis

To confirm putative red muscle, ca. 5 × 5 × 10 cm muscle samples were taken from Specimen B at each half-transverse cross-section to allow for staining of succinate dehydrogenase (SDH), a mitochondrial-bound enzyme found in high abundance in oxidative muscle fibres, and hematoxylin and eosin (H&E) staining (Kielhorn et al. 2013, Nachlas et al. 1957). All muscle samples were initially frozen at -80°C prior to processing. A day later, sub-samples from each of these (measuring ca. 2 × 5 × 10 cm) were placed into 10% neutral buffered formalin and kept at 4°C until H&E staining. Remaining portions of samples were retained at -80°C until analysis.

Within 3 months of the stranding, samples measuring ca. 2 × 2 × 3 cm were taken from deep within the frozen muscle stored at -80°C of each transverse section. SDH staining is routinely conducted on freshly frozen tissue. Consequently, a protocol from Nacalas et al. 1957 was adapted to suit lower tissue quality. Samples of frozen tissue were defrosted allowing for tissue samples measuring ca. 0.5 × 0.5 × 0.5 cm to be cut with a scalpel rather than a cryotome. Sections were incubated in 0.2 mol L⁻¹ phosphate buffer with nitro blue tetrazolium (NBT), menadione solution and 0.2 mol L⁻¹ sodium succinate for 15 min at room temperature and 30 min at 37°C. After sufficient staining was observed, sections were gently rinsed in saline for 3 min, fixed in 10% formalin solution for 10 min and dehydrated in 15% ethanol for 5 min (Nachlas et al. 1957, Kroeger et al. 2020). A control sample was also placed in an NBT solution without the substrate and subjected to the same conditions and fixing protocol. Samples were placed on slides and imaged on an Olympus BX51 light microscope coupled to an Olympus DD73 camera.

Samples of white and putative red muscle measuring ca. 1.0 × 0.5 × 0.5 cm were taken for standard H&E staining (Humason 1979). Samples were dehydrated in a graded ethanol series, cleared in xylene, and then embedded in paraffin wax allowing 5µm sections to be cut on a Leica RM2235

microtome. Sections were mounted on glass slides (SuperFrost®Plus, Menzel-Glaser, Germany) using glycerine mounting jelly. Sections were imaged once again on the Olympus BX51 with the DD73 camera. SDH and H&E staining confirmed red muscle fibre type due to the uptake of NBT stain and small size of fibres.

1.3 Electronic biologging tag package

The Lotek LAT1810S tags were calibrated by the manufacturer before deployment and concurrent data collected by the tag after release from individual sharks allowed for calibration between the external and internal temperature sensors to be calculated post-deployment. DeltaT (the difference in temperature from one timepoint to the next) was calculated as the difference between the internal and external temperature sensors post- deployment (while the tag was floating on the water surface) for each individual shark. The mean value of deltaT was calculated and added to each body temperature recording of the relevant individual (0.08, 0.01, 0.19 and 0.02°C for the shark measuring 7.0, 5.5, 8 and 5 m respectively) to correct the internal body temperature recording.

Figure 2A schematic was created in Procreate® software (version 5.3, Savage Interactive Pty Ltd.)

1.4 Heat transfer coefficient model

Changes in whole-body heat transfer over time were investigated using the following established equation (Nakamura et al. 2020, Watanabe et al. 2021):

$$\frac{dT_m(t)}{dt} = k(T_a(t) - T_m(t)) + \dot{T}0$$

where k is the whole-body heat transfer (degrees Celsius per second per degree Celsius, the resolution of our system), T_a is the ambient water temperature (degrees Celsius) at time (t), T_m is the subcutaneous white muscle temperature (degrees Celsius) at time (t) and $\dot{T}0$ is a temperature elevation term arising from heat production and retention (degrees Celsius per second).

The equation above was applied to a hypothetical ectothermic basking shark based on temperature data collected by ‘basking shark 1’, which measured approximately 7.0 m total length (Figure 2B), as well as two ectothermic whale sharks of similar body size (approximately 7.0 m total length). Whale shark constant k estimates were taken from Nakamura et al. 2020, while basking shark constant k estimates were assumed from an allometric plot of body size and calculated k values from ectothermic and regionally endothermic fish species after we corrected for the scale bar errors present in the original Nakamura et al. 2020 publication. The term $\dot{T}0$ was assumed to be 0.0°C due to ectotherms lacking the anatomical and physiological traits to retain body heat and because heat transfer models provide a better fit for ectothermic sharks without the term included (Nakamura et al. 2020). Model sharks were assumed to have the same starting muscle temperature as ‘basking shark 1’. An ODE solver model [‘deSolve’ (Soetaert et al. 2020) and ‘tidyverse’ (Wickham et al. 2019) packages in R Studio (R Development Core Team, 2019)] that minimized the fit of k and $\dot{T}0$ using least squares method over time was used to produce hypothetical ectothermic subcutaneous white muscle temperatures at the same sampling resolution as our system (0.1 Hz). Results from the models were plotted against time, once again in R Studio.

Supplemental references

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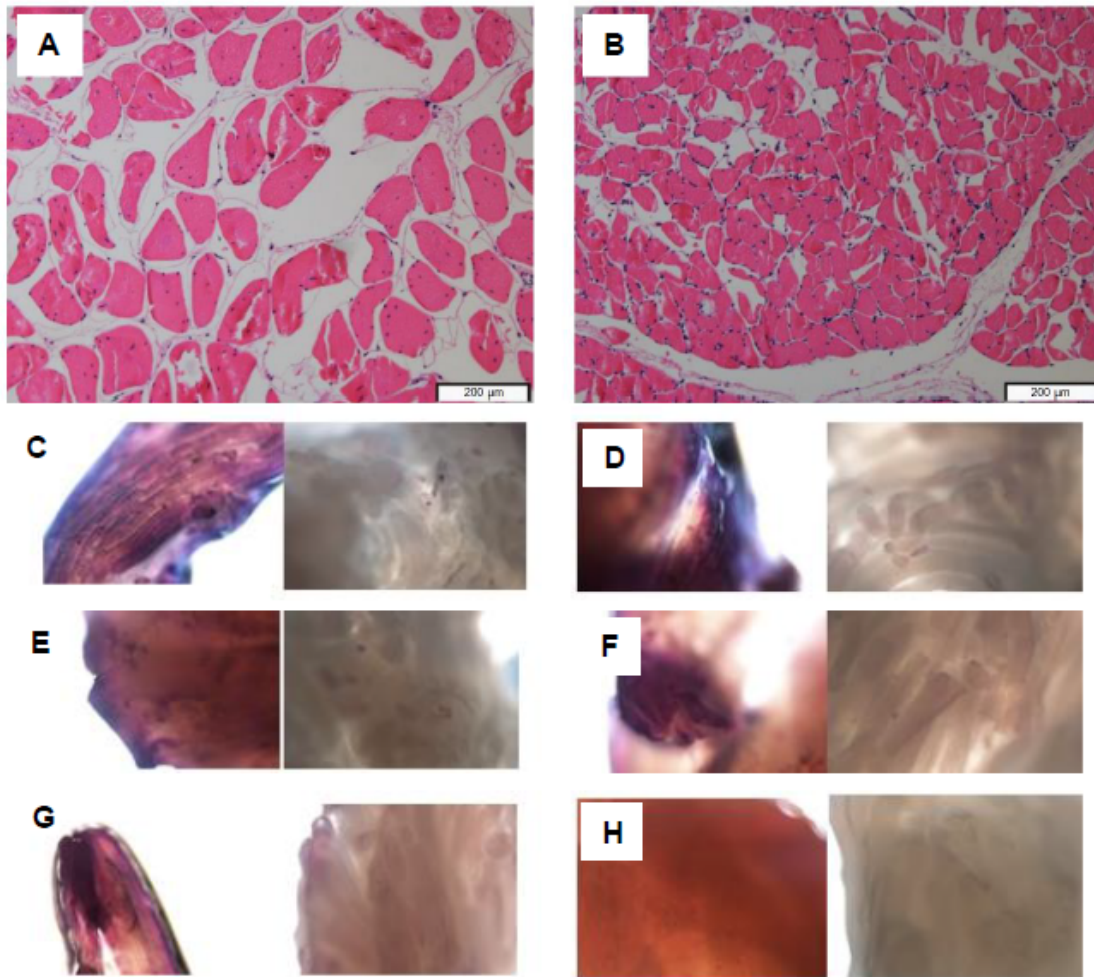


Figure S1. Histological staining of red and white muscle in basking sharks. (A) Hematoxylin and eosin staining of white muscle and (B) red muscle, where the fibres are stained pink and nuclei are stained blue. (C – G) Nitro blue tetrazolium staining of red and white fibres from each transverse section collected from specimen B; location also show by the white curved horizontal lines on the dorsal profile of Figure 1A. Nitro blue tetrazolium stains for the mitochondrial enzyme, succinate dehydrogenase, which is abundant in oxidative fibres, and thus a dark blue stain is produced in red muscle fibres and a sparse speckled stain in white muscle fibres. (H) Control sample for nitro blue tetrazolium stain was also taken from Specimen B.

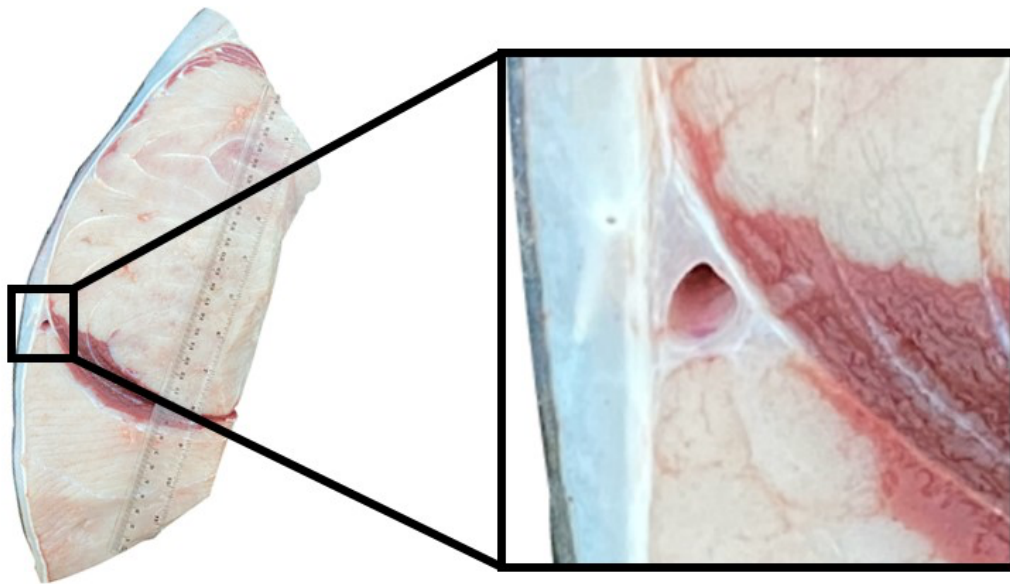


Figure S2. Lateral subcutaneous vessels of basking sharks. Within the connective tissue there appears a small lateral subcutaneous artery and large lateral subcutaneous vein located near the lateral extents of the red muscle. Image taken from specimen A from near the pectoral fin region.

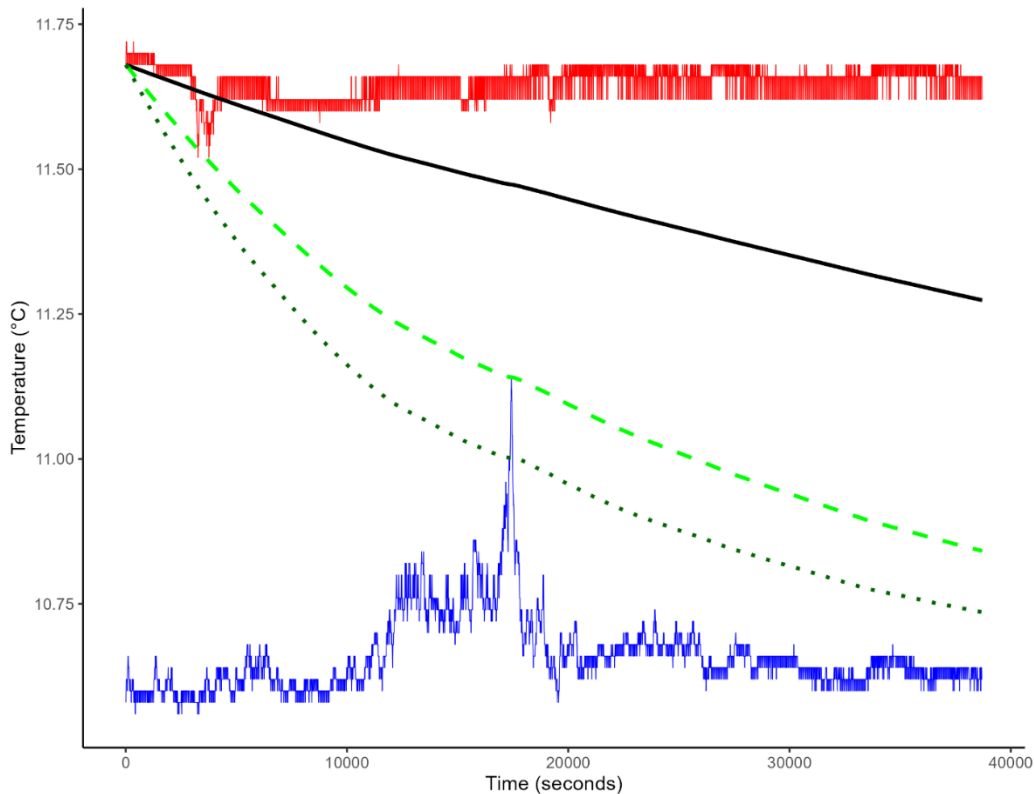


Figure S3. Measured basking shark subcutaneous white muscle temperature and modelled hypothetical ectothermic sharks of a similar body size. Red and blue lines represent measured subcutaneous white muscle temperature and ambient temperatures collected by ‘basking shark 1’, respectively. The black line, dashed light green line and dotted dark green line represent a modelled hypothetical fully ectothermic basking shark as well as two whale sharks (7.0 and 7.2 m total length), respectively.