

Mammal-like muscles power swimming in a cold-water shark

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Effects of temperature on muscle contraction and powering movement are profound, outwardly obvious, and of great consequence to survival^{1,2}. To cope with the effects of environmental temperature fluctuations, endothermic birds and mammals maintain a relatively warm and constant body temperature, whereas most fishes and other vertebrates are ectothermic and conform to their thermal niche, compromising performance at colder temperatures^{2,3}. However, within the fishes the tunas and lamnid sharks deviate from the ectothermic strategy, maintaining elevated core body temperatures^{4,5} that presumably confer physiological advantages for their roles as fast and continuously swimming pelagic predators. Here we show that the salmon shark, a lamnid inhabiting cold, north Pacific waters, has become so specialized for endothermy that its red, aerobic, locomotor muscles, which power continuous swimming, seem mammal-like, functioning only within a markedly elevated temperature range (20–30 °C). These muscles are ineffectual if exposed to the cool water temperatures, and when warmed even 10 °C above ambient they still produce only 25–50% of the power produced at 26 °C. In contrast, the white muscles, powering burst swimming, do not show such a marked thermal dependence and work well across a wide range of temperatures.

An anatomical feature that sets the lamnid sharks apart from ectothermic fishes but reveals evolutionary convergence with tunas is the location of the red, aerobic, locomotor muscle (RM), being deep in the body next to the vertebral column and concentrated in the mid-body region, in contrast with the lateral and subcutaneous position of RM in other fishes⁶. Coupled with vascular heat exchangers, tunas and lamnid sharks have evolved the ability to retain metabolic heat in RM and attain temperatures that are significantly above that of the surrounding water^{5,6}. Furthermore, the white locomotor muscle (WM) is also significantly warmed by conductive heat transfer arising from its proximity to the RM, with progressive cooling towards the skin⁷. Thus, two of the most striking differences between endothermic and ectothermic fishes are the position of the RM and the capacity to warm both the RM and a portion of the WM.

What are the functional consequences of these thermal and anatomical modifications? Previous work on the swimming physiology and metabolic biochemistry of muscles in warm-bodied lamnid sharks^{7,8} and tunas^{9,10} suggests that these fishes have the potential to sustain a higher aerobic swimming metabolism, and have an increased potential for burst swimming relative to that of other fishes. However, there is no direct experimental evidence demonstrating how a change in the operating temperature of these tissues might affect the contractile properties of shark muscle, if the maintenance of high temperatures in RM is necessary for

satisfactory function, or if warm-bodied sharks are capable of enhanced swimming performance¹¹. To address these questions we examined thermal effects on the contractile properties of RM and WM in salmon sharks (*Lamna ditropis*), a species inhabiting the cold waters of the north Pacific Ocean¹² but noted for maintaining elevated body temperatures^{6,13,14} and prized as a sport-fish for its impressive swimming power and speed¹⁵.

Within minutes of landing the sharks we mapped the thermal gradient in the muscle mass by measuring temperatures along the body. Measurements taken just anterior to the first dorsal fin, where the RM is most abundant, showed that all sharks had a core temperature about 18–20 °C above the surrounding sea surface temperature, which ranged from 6 °C to 8 °C; this gradient is even larger than those reported previously^{6,13,14}, probably due to the somewhat cooler surface temperature at the time of this study. Regardless of variations in surface temperatures, tagging studies have shown that salmon sharks routinely dive to 200 m and encounter water temperatures of 6 °C during most of the year¹². The maximum temperature of the deeply positioned RM was 26 °C, which was 16 °C warmer than the superficial WM at this position and about 20 °C warmer than the ambient water. A smaller thermal gradient was present at the level of the second dorsal fin, a narrower region of the body, with the deep RM temperature being 10–13 °C above ambient. In Fig. 1, the thermal data from all specimens are shown superimposed on a three-dimensional reconstruction of the distribution of locomotor muscle in the salmon shark, providing a perspective of the operating temperatures *in situ* for the RM and WM along both the longitudinal and transverse axes of the body. Temperatures selected from this reconstruction were used to determine thermal effects on muscle contractile properties.

Using a portable stimulator/transducer to make measurements of muscle twitches *in situ*, we next determined that the duration of twitches from superficial WM was invariant along the length of the body (see Supplementary Fig. 1) but decreased significantly (that is, the twitches got faster) with depth along the temperature gradient of WM from the cool skin to the warm body core. Contractile tests were then conducted on small bundles of living muscle fibres isolated from a midbody position (that is, at the level just anterior to the first dorsal fin), where the RM and WM temperatures were the most elevated. Durations of isometric twitches measured from WM ranged from 143 ms at 10 °C to 51 ms at 26 °C, yielding an average thermal rate coefficient (Q_{10}) of 2.0 (Fig. 2), a typical temperature response for muscle from an ectothermic vertebrate¹. In contrast to WM, the twitch times of RM had both much higher Q_{10} values (up to 3.7) and exceedingly long twitch durations, with a time to reach peak force exceeding 3 s at 10 °C, and even longer relaxation times (see Fig. 2). These observations indicate that the RM is extremely sensitive

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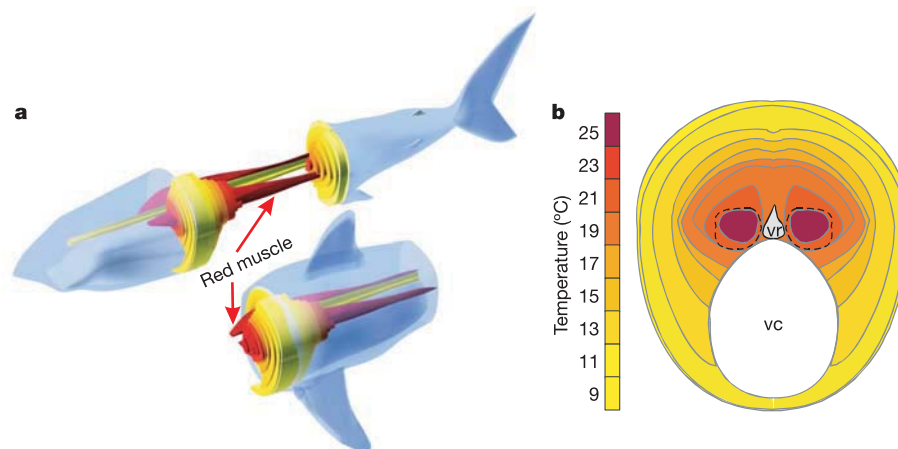


Figure 1 | Thermal gradients superimposed on a three-dimensional map of RM and WM distribution in salmon sharks (*Lamna ditropis*). **a**, Isotherms shown are the mean value for two specimens (216 and 222 cm FL, about 140 and 160 kg body mass) at two positions along the body (40% and 63% of FL). **b**, The body section just anterior to the first dorsal fin (lower section in

a) that contains the highest cross-sectional area of RM. Dashed circles in transverse images indicate red muscle position. The external surface is at the same temperature as the water, 8.5 °C. Abbreviations: vc, visceral mass; vr, vertebrae.

to temperature, particularly at cooler temperatures, and would probably be incapable of contracting in a way that would result in effective swimming if allowed to cool even to 15 °C (a temperature still 9 °C above ambient).

To understand how temperature might limit the ability of these muscles to power swimming, we characterized their power-producing potential by using work-loop analysis¹⁶ at temperatures encompassing those measured in these sharks and over a range of cycle (that is, tail-beat) frequencies that they might use during swimming. The temperature-dependent power spectra of WM indicate a retained ability to produce high power at tail-beat frequencies of 4–6 Hz between temperatures of 10 °C and 26 °C, with power output being maintained up to a frequency of 8 Hz at 26 °C (Fig. 3a). Thus, WM of salmon sharks shows a thermal sensitivity typical of that observed in most other temperate and cold-water fishes^{2,17}, allowing WM to function readily at temperatures between 10 and 26 °C, well within the 18–20 °C thermal gradient noted between the cold surface and the warm interior of the shark's body. In marked contrast, the fastest tail-beat frequency at

which the RM produced power was restricted to a relatively slow 2 Hz even at a very warm temperature of 31 °C, with power production being very low and further limited to a tail-beat frequency of only 0.5 Hz at 15 °C (Fig. 3b). The RM of salmon sharks seems to be designed for high power production only at very high body temperatures (26 °C or above) and could not produce useful work and power if allowed to cool below about 20 °C (Fig. 3b), a mere 6 °C below its operating temperature *in vivo* but still considerably more than 10 °C warmer than the water. Thus, the superficial WM can function well at cold temperatures, but like mammalian skeletal muscle¹ the internalized RM has become an obligate endothermic tissue and functions only at high body temperatures. Further, the elevated temperature of WM close to the warm RM near the body core would result in an approximately threefold increase in its ability to produce power, probably further enhancing the burst swimming capabilities of these fish.

Our results show that WM from salmon sharks can effectively power burst swimming at relatively high tail-beat frequencies across a wide range of temperatures; it is therefore well-suited to work in the

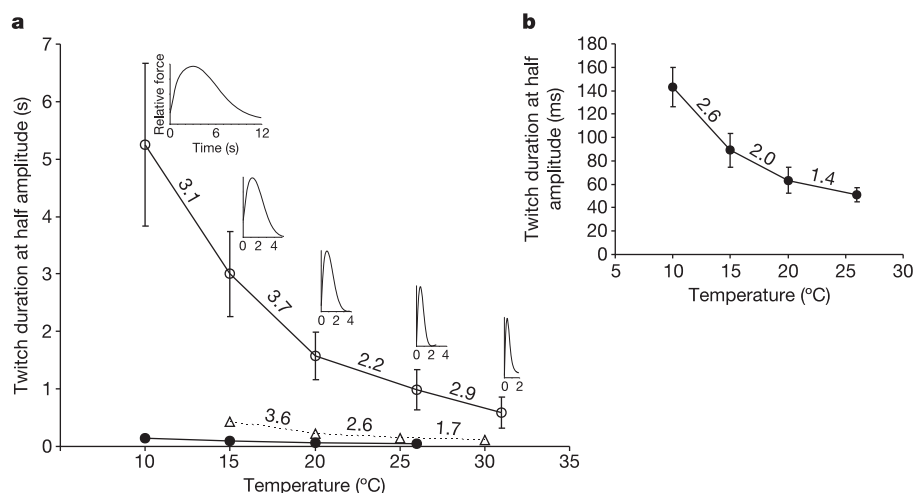


Figure 2 | Muscle twitch duration at different temperatures. **a**, Twitch in RM (open circles) and WM (filled circles) from *Lamna ditropis* and in RM from *Thunnus albacares* (open triangles). The thermal rate coefficients (Q_{10}) for twitch duration are shown at each temperature interval. Insets show single-twitch profiles at each temperature for RM from *L. ditropis*.

b, Twitch durations for WM from *L. ditropis*, with expanded axes for clarity. Values shown are means \pm s.d. Each point of twitch data for salmon sharks is the period for which the force was greater than 50% of maximal, and for RM from yellowfin tuna (*T. albacares*) it is the time from stimulus to half relaxation¹⁸.

cold but probably realizes a considerable benefit in being warmed through its close proximity to the RM. In contrast, the RM used to power sustained swimming is specialized for use at warm temperatures only, perhaps minimally 15 °C warmer than the water that the fish inhabits, and the shark's highly endothermic nature is essential to the functioning of these muscles. In this sense the RM of salmon sharks is analogous to that of mammals in functioning only at relatively warm temperatures and having Q_{10} values for twitch speed that are higher than those typically observed in ectothermic muscles and are more similar to those for endothermic muscle¹. This tissue is unlike muscle found in any other fish so far, including that of the endothermic tunas. Both salmon sharks and tunas seem to be similar in having very thermally sensitive RM, particularly in the deeper, warmer regions of the fish¹⁸, but differ in that the twitches of tuna RM are about sixfold faster¹⁸ than those noted in salmon sharks at similar temperatures (Fig. 2). Further, unlike salmon sharks, tuna RM can continue to power relatively fast swimming (for example with a tail-beat frequency of 2–4 Hz) even at a cool temperature of 15 °C (ref. 18). Whereas the large size of the salmon sharks may contribute in part to the slow contractions of its RM, evidence from dogfish sharks¹⁹ and tuna RM¹⁸ indicate that scaling with body size might not be an important influence, and even after correcting for potential effects of body size using scaling coefficients from studies on other species²⁰, the twitch speeds of RM in salmon sharks remain about fourfold slower than those from RM of tuna.

Unlike mammals and birds, which do not depend solely on locomotor activity to warm their muscles and can use other sources of metabolic heat during times of inactivity, sharks have no known non-locomotor-associated thermogenic tissues and therefore salmon

sharks must rely on the constant contractile activity of their RM to elevate this tissue's temperature. Thus, these sharks live in a physiological extreme in that if the aerobic RM were to become inactive (that is, if the fish stopped swimming) and ceased to produce heat, allowing the temperature to fall below even 20 °C, which is still about 15 °C warmer than the surrounding water, the RM would fail to function and possibly could not recover. Obligate, continuous locomotion therefore seems to be essential in allowing these sharks to maintain warm RM temperatures that in turn permit them to exploit, all year round, the north Pacific's energy-rich cold waters as apex pelagic predators.

METHODS

Fish collection. Three salmon sharks (*Lamna ditropis*) were captured by hook and line in May and June off Prince William Sound in the Gulf of Alaska, USA. Specimens ranged from 2.16 to 2.22 m fork length (FL), with a body mass ranging from about 130 kg to 160 kg. All capture and experimental procedures followed the guidelines of the University of California, San Diego IACUC.

In situ temperature and twitch measurements. A thermocouple was used to measure tissue temperatures at several positions along the body in freshly caught specimens; recordings began within 60 s of landing. Two small incisions were made at 45% of FL, the first just anterior to the first dorsal fin and the second along the side of the body between the epaxial and hypaxial musculature, and a thermocouple probe was advanced in an orthogonal direction towards the vertebrae, eventually reaching the spine. Temperature data were recorded every 10 mm along these trajectories. Similar measurements were obtained near the second dorsal fin; thermal data for both positions along the body were mapped on a three-dimensional model of salmon shark locomotor muscles and isotherms were interpolated at intervals of 2 °C.

Muscle preparation. Segments of RM and WM (about 1 cm³) were removed from directly under the first dorsal fin (about 45% of FL) shortly after capture. A small bundle of fibres about 1 mm in diameter and spanning several myotomes in length was then isolated under a dissecting microscope on a cooled stage (about 4 °C) with frequent rinses of chilled shark saline (292 mM NaCl, 3.2 mM KCl, 5 mM CaCl₂, 0.6 mM MgSO₄, 1.6 mM Na₂SO₄, 300 mM urea, 150 mM trimethylamine N-oxide, 10 mM glucose, 6 mM NaHCO₃; total osmolality 1,080 mosM, pH 7.7 at 20 °C). These preparations were immersed in oxygenated saline on ice during transport back to the laboratory (about 180 min). Muscle bundles were then further dissected to remove damaged fibres and reduced to a single myotome. Preparations were transferred to a temperature-controlled experimental chamber filled with shark saline; the saline was circulated through a temperature-controlled reservoir and bubbled with pure oxygen. The myoseptum on one end of the bundle was tied to a stainless-steel pin connected to a servomotor arm (model 350 for RM and model 305B for WM; Cambridge Technology) and the myoseptum on the other end was tied to a pin connected to a force transducer (a model BG-50G (Kulite Semiconductor Products Inc.) for RM and a model FT03C (Grass Telefactor) for WM). Muscle length was adjusted to remove visible slack. Platinum-tipped electrodes or plates were positioned alongside the muscle and the stimulus voltage was adjusted to 150% of that required to elicit a maximal isometric twitch (1-ms stimulus pulse). Muscle length was then adjusted systematically until maximal twitch force was attained. All data were recorded at 1 kHz on National Instruments PCI MIO 16-E4 analogue-to-digital cards using custom software written in LabView (National Instruments). Isometric twitches were recorded over the range of temperatures as noted below. Twitch duration was measured as the period for which force was greater than 50% of the maximum recorded.

Recording work and power. Mechanical work and power were measured with the work-loop method¹⁶, in which muscle preparations were subjected to sinusoidal strain centred on the length at which force was maximal. The frequency of the strain oscillations was varied as noted below, and the amplitude of the muscle strain oscillation was fixed at $\pm 5\%$ of the reference length, similar to that recorded *in vivo* from RM of swimming tuna²¹ and mako sharks²². Preparations were stimulated by means of platinum electrodes during the strain cycle, and the timing of the stimulus was adjusted such that force was produced primarily during shortening and the muscle was relaxed during lengthening. Net work done by the muscle was calculated as the integral of force with respect to change in muscle length over a complete cycle, and power was calculated as the product of work done per cycle and cycle frequency. At each combination of cycle frequency and temperature, the stimulus parameters were adjusted to maximize the net work done during the length cycles. Work was measured over a range of cycle frequencies (equivalent to tail-beat frequencies) encompassing 0.5–3 Hz for RM and 2–7 Hz for WM. Isometric twitches were recorded

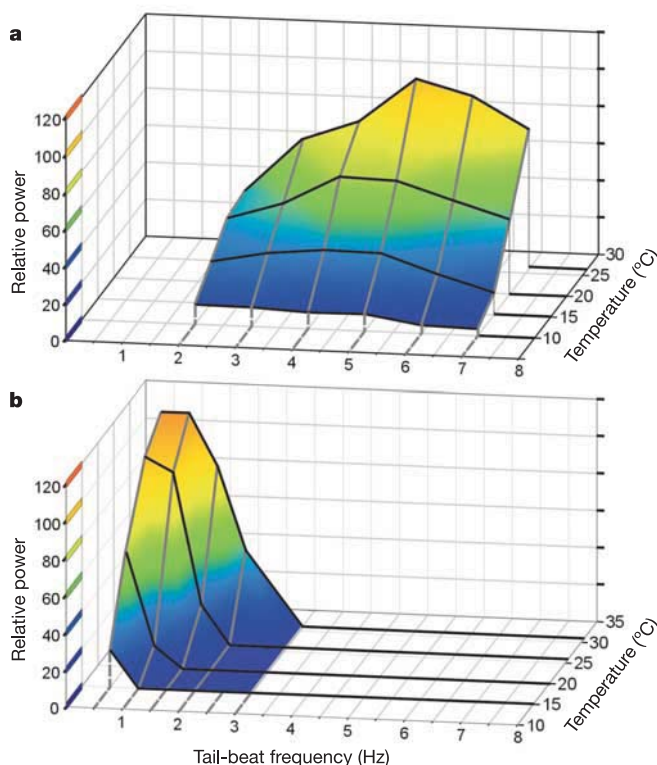


Figure 3 | Relative power output in myotomal muscle of *Lamna ditropis* at different cycle (that is, tail-beat) frequencies and temperatures, measured by work-loop analysis. a, WM; b, RM. Amplitude of muscle strain oscillation was fixed at $\pm 5\%$ of sample reference length. Stimulation parameters were optimized at each combination of temperature and cycle frequency to maximize net power output. Note the limited frequency range over which red muscle can produce power, and its reliance on high temperatures.

systematically throughout the experiments to monitor the stability of the preparations, and any muscles that exhibited a marked decline in force were discarded (this occurred in only one preparation).

Temperature effects. Isometric twitches, work and power were recorded in muscle preparations exposed to temperatures of 10, 15, 20 and 26 °C for WM and 15, 20, 26 and 31 °C for RM; twitches were also recorded at 10 °C in two preparations of RM. These temperature ranges encompassed values recorded in freshly caught specimens, with additional measurements at the lower and higher ends to determine the full extent of the thermal effects on muscle contractile properties.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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