Thermal dependence of contractile properties of the aerobic locomotor muscle in the leopard shark and shortfin mako shark

Jeanine M. Donley[1], Robert E. Shadwick[2,*], Chugey A. Sepulveda[3] and Douglas A. Syme[4]

[1]*Biology Department, MiraCosta College, Oceanside, CA 92056, USA, [2]Marine Biology Research Division, Scripps Institution of Oceanography University of California, San Diego, La Jolla, CA 92093-0202, USA, [3]Pfleger Institute of Environmental Research, Oceanside, CA 92054, USA and [4]Department of Biological Sciences, University of Calgary, Calgary, Alberta, T2N 1N4, Canada*

*Author for correspondence at present address: Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC, V6T 1Z4, Canada (e-mail: shadwick@zoology.ubc.ca)

Summary

The work loop technique was used to examine contractile properties of the red aerobic locomotor muscle (RM) in the ectothermic leopard shark *Triakis semifasciata* and endothermic shortfin mako shark *Isurus oxyrinchus*. The effects of axial position and temperature on the twitch kinetics, and the stimulus duration and phase producing maximum net positive work and power output were investigated. Contractile performance was measured over the temperature range of 15 to 25°C for *Triakis* and 15 to 28°C for *Isurus* at cycle frequencies (analogous to tailbeat frequencies) ranging from 0.25 to 3 Hz using muscle bundles isolated from anterior (0.4 *L* where *L* is total body length) and posterior (0.6–0.65 *L*) axial positions. Pairwise comparisons of twitch times for anterior and posterior muscle samples indicated that there were no significant differences related to body position, except in mako sharks at unphysiologically cool temperatures (<20°C). We found no significant differences in optimal stimulus duration, phase, net work or power output between anterior and posterior bundles in each species. With increasing cycle frequency the stimulus duration yielding maximum power decreased while optimal phase occurred earlier. The cycle frequency at which peak power was generated in leopard shark RM was only affected slightly by temperature, increasing from about 0.6 to 1.0 Hz between 15 and 25°C. In contrast, mako RM showed a much more dramatic temperature sensitivity, with the peak power frequency rising from <0.25 to 2.25 Hz between 15 and 28°C. These data support the hypothesis that the contractile properties of RM are functionally similar along the body in both species. In addition, our data identify a significant difference in the effect of temperature on net work and power output between these two shark species; at 15°C muscle from the ectothermic leopard shark performs relatively well in comparison with mako, while at higher temperatures, which reflect those normally experienced by the mako, the optimal cycle frequency for power is nearly double that of the leopard shark, suggesting that the mako may be able to maintain greater aerobic swimming speeds.

Key words: red muscle, temperature, contractile properties, work loop, lamnid, *Isurus*, *Triakis*.

Introduction

The leopard shark *Triakis semifasciata* and the shortfin mako *Isurus oxyrinchus* represent two ends of a spectrum of locomotor performance among sharks. The leopard shark is an ectothermic subcarangiform swimmer with laterally positioned red muscle and an elongated body, whereas the mako is an endothermic thunniform swimmer with internal red muscle and a fusiform body shape. Previous studies examining muscle dynamics during steady swimming reveal that in both species neither the phase nor duration (duty cycle) of red muscle (RM) activation varies along the body (Donley and Shadwick, 2003; Donley et al., 2005). This is in contrast to most bony fishes, in which decreases in duty cycle and advances in the phase of onset of RM activation relative to the muscle strain cycle typically occur from anterior to posterior (Altringham and Ellerby, 1999; Gillis, 1998; Coughlin, 2002). Since changes in activation timing can have a significant effect on the net work and power produced by a muscle [e.g. scup *Stenotomus chrysops* (Rome et al., 1993); saithe *Pollachius virens* white muscle (Altringham et al., 1993); rainbow trout *Oncorhynchus mykiss* (Hammond et al., 1998); largemouth bass *Micropterus salmoides* (Coughlin, 2000)], many studies on bony fishes have concluded that the longitudinal phase shift in activation timing leads to regional variation in muscle function (for reviews, see Altringham and Ellerby, 1999; Coughlin, 2000) which may be important in undulatory swimming. The lack of such changes in sharks, including the undulatory leopard shark, might suggest a divergent reliance on force and power for swimming.

A number of physiological properties of muscle will influence power output, including activation kinetics, force–velocity characteristics, and the relative timing of onset and offset of activation during movement. (Josephson, 1999). A key difference between mako and leopard sharks swimming in 20°C water is that the phases of onset and offset of RM activation are approximately 30° later in the strain cycle in the mako (Donley et al., 2005). This later activation phase predicts several potential differences in muscle function. First, it suggests that RM in the mako may be faster, allowing it to develop force and relax faster than the leopard shark. Thus, mako RM may absorb proportionally less lengthening work and produce more net positive work in each contraction cycle. Second, shorter activation and relaxation times allow muscle to operate at higher cycle frequencies, so the mako may have a greater range of aerobic swimming speeds than the leopard shark. Third, due to the relatively late activation of RM in the mako, there is virtually no period during which posterior fibers perform lengthening work (i.e. active while being lengthened). In the leopard shark, by contrast, active lengthening of the mid- and posterior muscle coincides with active shortening in the anterior for about 10% of each tailbeat cycle (Donley and Shadwick, 2003). During this time the posterior fibers may develop high force and act to stiffen the body, facilitating power transmission from the anterior muscle along the body to the tail, as has been hypothesized for some teleosts (Altringham et al., 1993). Transmission of force in the mako may simply rely on tendons rather than on transient properties of the posterior musculature (Donley et al., 2004).

This study examines whether muscle function is indeed constant along the body in elasmobranchs by testing the hypothesis that the power production and contractile properties of the red, aerobic, locomotor muscles do not vary longitudinally in the leopard shark and shortfin mako. Furthermore, we identify potential differences in the contractile properties between ecto- and endothermic sharks, including the effects of temperature on optimal stimulus duration, phase, net work and power output. Based on later EMG onset and offset times measured in swimming mako sharks mentioned above, it is hypothesized that red muscle from mako sharks will produce power at greater cycle frequencies than the red muscle of leopard sharks, at a given temperature.

Materials and methods
Experimental animals

Five leopard sharks (*Triakis semifasciata*; family Triakidae) ranging in size from 70 to 115 cm total length (*L*) and seven mako sharks (*Isurus oxyrinchus*; family Lamnidae) ranging in length from 75 to 120 cm were examined. Both species were collected by hook and line off the coast of Southern California and transported to the Scripps Institution of Oceanography (SIO) in a transport tank with circulating seawater according to methods developed earlier (Bernal et al., 2001). At SIO, the leopard sharks were maintained in a 3-m diameter tank with continuous flow of seawater and aeration for several weeks

prior to experimentation at a water temperature that reflected the ambient coastal conditions (15–20°C). These sharks were fed squid and chopped fish weekly until satiation. Because to date mako sharks cannot be maintained in aquaria for prolonged periods, the muscle tissue samples were taken from each test specimen immediately upon arrival at SIO. All procedures in capture, maintenance and experimentation followed the guidelines of our University of California, San Diego animal care protocol.

Muscle preparation

Animals were killed by severing of the spinal cord. Small blocks of red muscle (RM) were collected from two axial positions (0.4 L anterior and 0.6–0.65 L posterior) and immediately placed into chilled (5–10°C), oxygenated elasmobranch saline (composition in mmol l^{-1}: NaCl, 292; KCl, 3.2; CaCl$_2$, 5.0; MgSO$_4$, 1.0; Na$_2$SO$_4$, 1.6; NaHCO$_3$, 5.9; TMAO, 150; Urea, 300; Glucose, 10; pH 7.8 at 20°C). The muscle blocks were then dissected to isolate small bundles spanning a single myomere (~0.5 mm in diameter). The preparations were rinsed with chilled oxygenated saline frequently during dissection. Bundles were then transferred to a temperature-controlled chamber filled with circulating oxygenated saline. Braided silk (5-0) surgical suture was used to attach the collagenous myosepta on the ends of each muscle bundle to the force transducer (Grass model FTO3C; Quincy, MA, USA) and the servomotor lever arm (model 305B-LC, Aurora Scientific, Aurora, Ontario, Canada).

Assessment of contractile properties

Once each bundle was attached to the force transducer and servomotor lever arm, the preparation was stretched to remove visible slack. A pair of platinum-tipped stimulating electrodes was positioned next to the muscle and the voltage adjusted to 150% of that required to produce maximum, isometric twitch force. A force–length curve was then constructed by stimulating the muscle preparation with short duration (1 ms) stimuli and measuring isometric twitch force over a range of muscle lengths. The length of the bundle was adjusted by increments of 2–5%L between each stimulus. The peak in the force–length curve was not always distinct, so the criteria for establishing optimal length were that at which developed force was at or near maximal while passive tension just began to rise. The minimum stimulus frequency that produced maximum, isometric, tetanic force was about 60 Hz at all experimental temperatures (15–28°C) and was used in all experiments. The stimulus duration required to elicit maximum, isometric, tetanic force was typically 600 ms. Isometric tetanic force was recorded routinely throughout the experiments to monitor stability of the preparation, and remained stable in all preparations, declining by no more than 5%.

To measure mechanical work and power output (Josephson, 1985; Josephson, 1993), each muscle bundle was subjected to a series of sinusoidal length changes centered about the optimal length and stimulated to contract under conditions of strain (% length change) recorded *in vivo*

[±4.0% for the anterior and ±6.0% for the posterior muscle in the leopard shark; ±5.5% anterior and ±9.0% posterior muscle in the mako (Donley and Shadwick, 2003; Donley et al., 2004)]. Work loop experiments were performed over a series of cycle frequencies ranging from 0.25 to 3 Hz, encompassing the range over which the RM could produce substantial net positive work. At each combination of strain and cycle frequency, stimulus duration and phase were adjusted in increments of ~25 ms and 5–10°, respectively, until net work production was maximal, requiring up to 10 work loops for each cycle frequency. Stimulus phase, defined as the point in the strain cycle when the stimulus begins, is expressed in degrees of the sinusoidal length change cycle ranging from 0° to 360°, where 0° is mean length during the lengthening portion of the cycle and 90° is peak muscle length. Stimulus duration is the period of muscle activation. Stimulus phase, duration, the amplitude of length change, and cycle frequency were controlled using custom software written in Labview (National Instruments, Austin, TX, USA). Net work of each complete strain cycle was calculated as the integral of force with respect to length. Power output was calculated as the product of work per cycle and cycle frequency.

The effects of temperature on twitch kinetics, work and power were also examined. Temperatures encompass those normally experienced in the water by both the leopard and mako sharks, typically ranging from 14 to 20°C (Cailliet, 1992; Sepulveda et al., 2004). Because the leopard shark is ectothermic, its body (and superficial RM) temperature conforms to that of the surrounding environment and thus normally fluctuates within this range. For each axial position examined in the leopard shark we present data from seven RM bundles (from five sharks) measured at 15, 20 and 25±1°C. The mako, like other lamnid sharks, is regionally endothermic. In free-swimming makos, the temperature of the deep RM has been shown to be maintained up to 8°C above ambient (Carey and Teal, 1969; Carey et al., 1971). So despite cooler ambient conditions, mako RM may typically fluctuate between ~20 and 28°C. For the mako, we present data from nine RM bundles (from seven sharks) from the anterior position and eight RM bundles from the posterior position measured at 15, 20 and 28±1°C. The sequence of temperatures was varied between experiments and all work loop experiments were repeated at each temperature to quantify any changes in the force production capacity over the course of the experiment.

Values represent the mean ± standard error (s.e.m.). To avoid errors associated with estimating viable muscle mass in the preparations, and because an assessment of absolute work output was not required for the goals of this study, net work and power output are presented as a percentage of the maximum values recorded over the range of frequencies for each preparation (Syme and Shadwick, 2002). Anterior *vs* posterior twitch times, activation phase and duration, and the cycle frequency at which power was maximal, were compared using *t*-tests.

Results

Twitch kinetics

In both the leopard and mako sharks, the trajectory of isometric twitches of RM appeared similar at the two axial positions (Fig. 1). In both species the time required for force development and relaxation decreased as temperature increased (Figs 1, 2), with the effect being more pronounced in the mako shark. At 15 and 20°C, leopard shark RM exhibited faster twitches than mako RM, but this difference did not exist at the higher temperatures (Fig. 2). Pairwise comparisons of twitch times for anterior and posterior muscle samples indicated that there were no significant differences ($P>0.05$) related to body position in leopard shark at all temperatures, or in mako shark at 20°C and 28°C. However, at 15°C mako RM appeared to be significantly slower in the posterior position ($P<0.05$). Over the temperature ranges studied, Q_{10} values for rates of force development were 3.1 in the leopard shark and 2.9 in the mako, and for force relaxation were 4.5 for leopard sharks and 5.2 for the mako.

Work loops; stimulus duration and phase

Work loops measured using optimized conditions of stimulus duration and phase across a range of cycle frequencies at 15°C are shown for leopard and mako shark RM in Fig. 3 and Fig. 4, respectively. Net work is large and positive at low frequencies and decreases with increasing cycle frequency. Within each species, anterior and posterior muscle samples have similar responses. Furthermore there appeared to be a pronounced effect of cooling on mako RM, as expected from the slower twitch kinetics at this low temperature: net work was much reduced at cycle frequencies above 1 Hz in the mako RM compared with the leopard shark RM.

In all cases, optimal stimulus duration and phase were not different for anterior and posterior muscle samples at each temperature within both leopard and mako sharks ($P>0.05$; Tables 1, 2). Consequently, stimulus duration and phase data from anterior and posterior RM bundles were pooled for each species (Fig. 5). The stimulus duration that produced maximum net positive work (optimal duration) decreased with increasing cycle frequency at all three temperatures, remaining approximately 20–40% of the cycle period (Fig. 5A,B). In all cases optimal stimulus phase occurred between 0° and 90° (i.e. during the lengthening portion of the strain cycle) and was lower (i.e. earlier in the strain cycle) with increasing cycle frequency (Fig. 5C,D). While, in general, stimulus duration and phase tended to decrease with temperature, this effect was much less evident in the leopard shark RM compared with the mako RM.

Net work and power output

There was no significant longitudinal variation in the cycle frequencies producing maximum net work or power output at any temperature in RM from leopard or mako sharks ($P>0.05$). Therefore, as with stimulation parameters, data for anterior and posterior work and power output were pooled and normalized (Fig. 6). In the case of net work, data are expressed as a
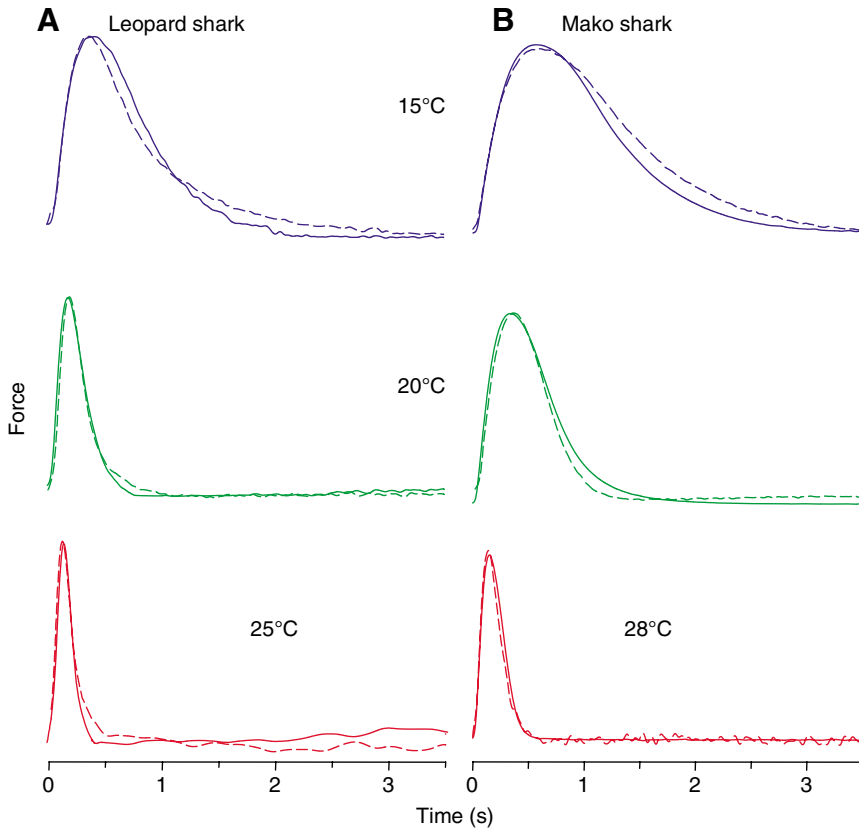
Fig. 1. Isometric twitches recorded in anterior (solid lines) and posterior (broken lines) red muscle from leopard and mako sharks at three temperatures (blue, 15°C; green, 20°C; red, 25/28°C), indicating the close similarity in contraction and relaxation between the two body positions at each temperature. Note also the greater effect of reduced temperature exhibited by the mako samples.

percentage of the maximum work recorded at each temperature to emphasize the sensitivity of work to cycle frequency at different temperatures in the two sharks. The cycle frequency producing maximum net work was nearly invariant with temperature in the leopard shark (at about 0.5 Hz), but increased from 0.25 Hz at 15°C to 1.0 Hz at 28°C in the mako (Fig. 6A,B). Power output for all temperatures is expressed as a percentage of the maximum power recorded at 20°C to highlight the differences in temperature sensitivity of power in the two species (Fig. 6C,D). Power output was relatively high at low temperatures but reduced at high temperature in the leopard shark, whereas power was relatively poor at low temperature and high at high temperatures in the mako. Muscle power output in the mako was greatest at 28°C, reaching 120% of the peak at 20°C and remaining above 80% up to at least 3 Hz. Even at 20°C, where the pattern of power output was most similar between the leopard and mako sharks, power output stayed elevated in the mako shark (>60% of peak) up to
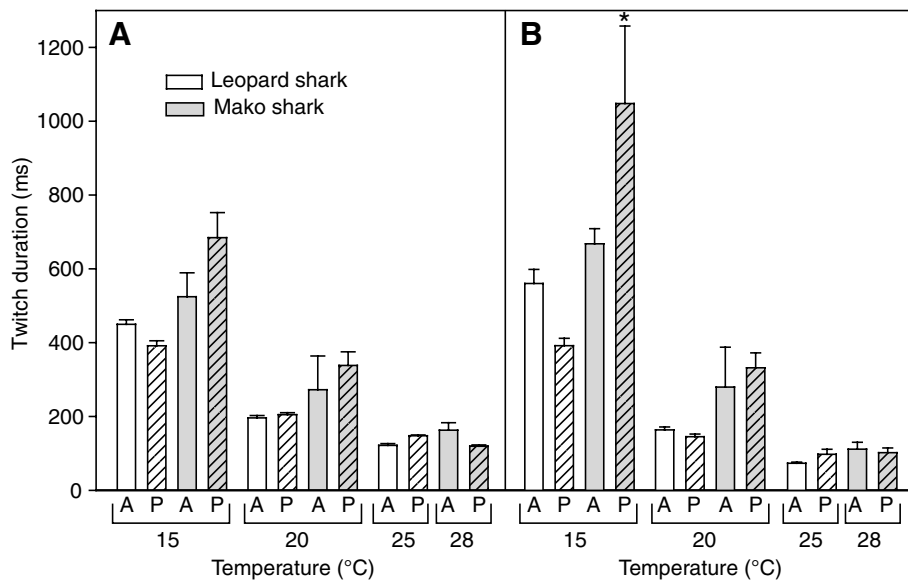

Fig. 2. Isometric twitch kinetics as a function of temperature and axial location in leopard and mako shark red muscle. (A) Time to peak force development after stimulus; (B) relaxation time to 50% of peak force. Pairwise comparisons of times at each temperature indicate no significant differences between axial location ($P>0.05$), except for the mako at 15°C where posterior muscle (hatched bars) appeared to be slower than anterior muscle (*$P<0.05$). Values shown are means ± s.d. (leopard shark, $N=3$; mako shark, $N=3$–6). A, anterior location; P, posterior location.
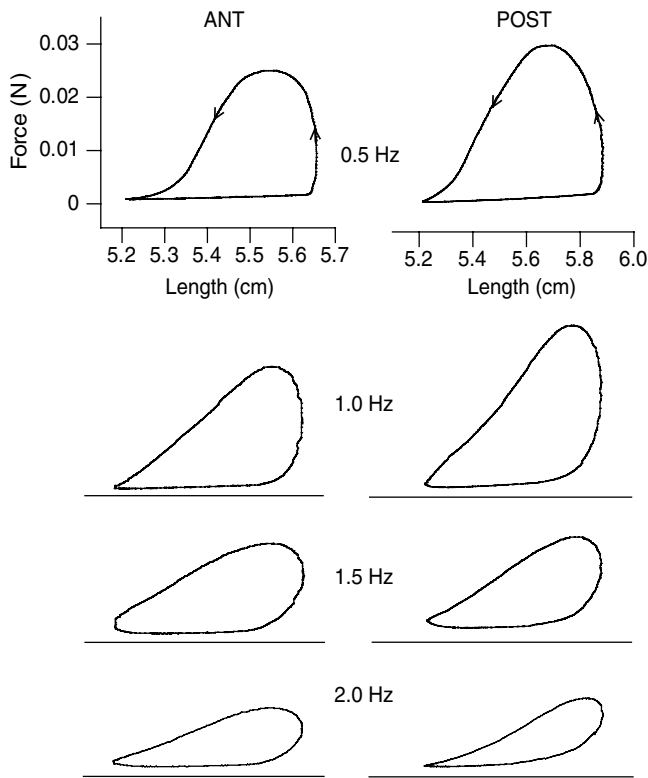
Fig. 3. Sample work loops from anterior (ANT) and posterior (POST) red muscle of the leopard shark operating under optimized conditions of stimulus duration and phase of activation at 15°C. Strains used were based on those recorded *in vivo* (±4% anterior, ±6% posterior). Arrows indicate the trajectory of the work loops.
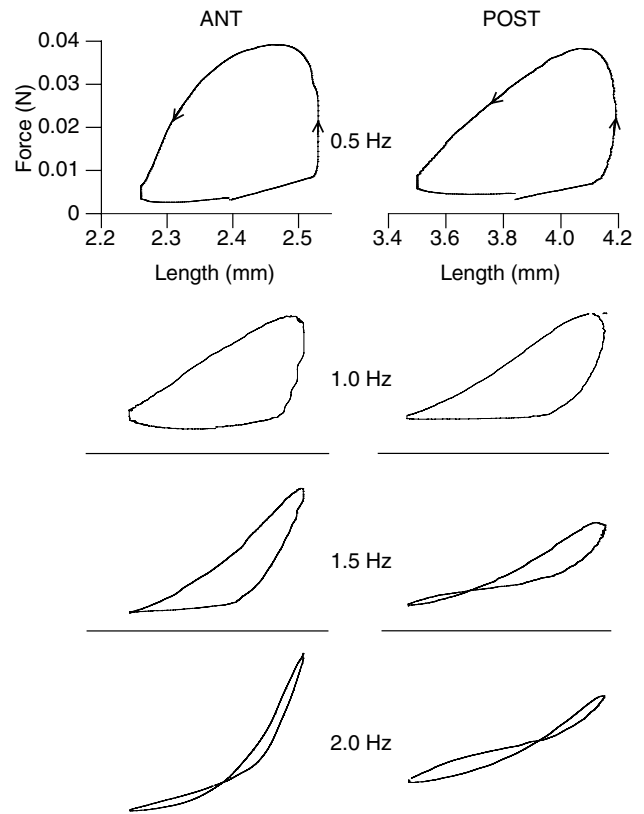
Fig. 4. Sample work loops from anterior (ANT) and posterior (POST) red muscle of the mako shark operating under optimized conditions of stimulus duration and phase of activation at 15°C. Strains used were based on those recorded *in vivo* (±5.5% anterior, ±9% posterior). Arrows indicate the trajectory of the work loops.

2 Hz, while declining in the leopard shark to about 25% of peak at 2 Hz. Leopard shark RM showed only a small increase in the cycle frequency for maximum power output when temperature increased from 15 to 25°C (Fig. 6C, Fig. 7). In mako shark RM the frequency for maximum power increased markedly with temperature over the entire range studied, from 0.25 Hz at 15°C to >2 Hz at 28°C (Fig. 6D, Fig. 7).

Discussion

The findings support our hypothesis that RM contractile properties are constant along the body in the leopard shark *T. semifasciata* and shortfin mako shark *I. oxyrinchus*. The effects of temperature on power production and twitch kinetics of RM in these two species of shark were substantially different. The endothermic mako shark exhibited a more pronounced influence of temperature on its red muscle contractile properties, with an enhanced ability to function at warm temperatures. In contrast the leopard shark performed well across a wide range of temperatures, with only a relatively small decrease in function at the coldest temperature.

Contractile properties do not vary longitudinally

Longitudinal variation in RM function is common, although not ubiquitous, among bony fishes (Gillis, 1998; Altringham and Ellerby, 1999; Coughlin, 2002). Studies have shown differences in patterns of power production and variation in isometric twitch kinetics along the body in several species of bony fish. Slower rates of relaxation [scup *S. chrysops* (Coughlin et al., 1996); yellowfin tuna *T. albacares* (Altringham and Block, 1997); largemouth bass *M. salmoides* (Coughlin, 2000)] and slower rates of activation in the posterior musculature relative to the anterior [rainbow trout *O. mykiss* (Hammond et al., 1998; Coughlin et al., 2001) have been documented. In contrast, the current study noted no significant differences in the twitch kinetics between anterior and posterior RM in leopard sharks from 15–25°C, or in mako sharks from 20–28°C (Figs 1, 2). Furthermore, in both species there were no significant differences in optimal stimulus duration or phase for work from RM at the two axial positions (Fig. 5). Consequently, patterns of work and power production as a function of cycle frequency were similar in the anterior and posterior positions for both shark species (Fig. 6). These observations and the consistency in activation duty cycle and phase recorded along the body during steady swimming (Donley and Shadwick, 2003; Donley et al., 2005) all support the hypothesis that contractile properties and thus muscle function is constant along the body in the leopard and mako

sharks (at least within the normal physiological temperature range).

Our findings lead us to conclude that longitudinal variation in contractile performance of muscle may occur only in bony fishes; at this point, there is no evidence to the contrary.

Perhaps sharks utilize a lower diversity of locomotor behaviors and thus may not require such differential muscle function along the body. Despite an apparent lack of difference in axial performance, there is an increase in red muscle strain from anterior to posterior in both sharks during steady swimming

Table 1. *Optimal stimulus duration in the anterior and posterior muscle as a function of cycle frequency at three experimental temperatures in T. semifasciata and I. oxyrinchus*

		Stimulus duration (ms)								
		15°C			20°C			25°C/28°C*		
Species	f (Hz)	ANT	POST	P	ANT	POST	P	ANT	POST	P
Leopard shark	3		25 (1)			50 (1)		100±0 (3)	87±17 (4)	0.39
	2	62±13 (6)	61±13 (6)	0.96	93±13 (7)	88±14 (7)	0.72	127±21 (5)	150±18 (6)	0.44
	1.5	133±25 (6)	150±22 (6)	0.63	193±17 (7)	192±17 (7)	1.0	200±32 (5)	242±20 (6)	0.30
	1	248±20 (6)	322±61 (6)	0.23	275±18 (7)	293±20 (7)	0.52	300±35 (5)	342±27 (6)	0.38
	0.5	475±57 (6)	517±60 (6)	0.62	532±54 (7)	586±52 (7)	0.49	435±100 (5)	613±86 (6)	0.21
	0.25	500±41 (3)	600±58 (3)	0.49	675±22 (4)	700±73 (6)	0.76	550±100 (2)	800 (1)	
Mako shark	3							82±9 (6)	58±12 (5)	0.16
	2	44±4 (7)	34±5 (6)	0.16	56±7 (9)	73±9 (8)	0.15	146±21 (6)	150±16 (5)	0.88
	1.5	52±9 (7)	61±12 (6)	0.56	125±16 (9)	137±23 (8)	0.87	214±21 (6)	254±10 (5)	0.22
	1	103±28 (7)	64±12 (6)	0.17	246±19 (9)	273±18 (8)	0.31	350±32 (5)	315±10 (5)	0.34
	0.5	293±73 (7)	263±56 (6)	0.74	439±25 (9)	453±35 (8)	0.75	525±25 (4)		
	0.25	543±38 (7)	520±58 (5)	0.75	600 (1)	650±150 (2)				

*25°C, leopard shark; 28°C, mako shark.

f, cycle frequency; ANT, anterior; POST, posterior.

Values are means ± s.e.m. Number of muscle bundles shown in parentheses.

All pairwise comparisons indicate no significant difference between stimulus duration for anterior and posterior muscle from both leopard and mako sharks at each temperature (P values are shown for anterior–posterior comparisons within each species at each temperature/tailbeat combination).

Table 2. *Optimal stimulus phase in the anterior and posterior muscle as a function of cycle frequency at three experimental temperatures in T. semifasciata and I. oxyrinchus*

		Stimulus phase (deg.)								
		15°C			20°C			25°C/28°C*		
Species	f (Hz)	ANT	POST	P	ANT	POST	P	ANT	POST	P
Leopard shark	3		2 (1)					3±0.5 (4)	−2±4 (4)	0.37
	2	7±4 (4)	6±3 (5)	0.90	7±2 (7)	5±3 (7)	0.67	12±4 (5)	6±4 (6)	0.53
	1.5	8±3 (5)	6±2 (5)	0.62	11±3 (7)	13±4 (7)	0.63	11±2 (5)	8±3 (6)	0.74
	1	20±2 (5)	14±2 (6)	0.21	23±3 (7)	22±4 (7)	0.92	30±4 (5)	28±2 (6)	0.65
	0.5	38±3 (6)	42±3 (5)	0.86	50±3 (7)	52±2 (7)	0.55	41±3 (3)	40±3 (5)	0.91
	0.25	43±4 (3)	52±3 (5)	0.09	54±0 (3)	57±2 (6)	0.18		61 (1)	
Mako shark	3							0±4 (6)	−0.7±4 (5)	0.09
	2	6±5 (7)	10±3 (6)	0.42	−4±4 (9)	1±5 (8)	0.61	10±4 (6)	5±1 (5)	0.27
	1.5	3±5 (7)	9±6 (6)	0.70	5±1 (9)	7±2 (8)	0.70	22±7 (6)	12±3 (5)	0.24
	1	18±3 (7)	13±9 (6)	0.62	19±4 (9)	14±3 (8)	0.42	36±3 (5)	34±4 (5)	0.67
	0.5	43±3 (7)	36±6 (6)	0.33	51±5 (9)	46±7 (8)	0.55	70±2 (4)		
	0.25	64±7 (7)	68±6 (5)	0.67	83 (1)	85±2 (2)				

*25°C, leopard shark; 28°C, mako shark.

f, cycle frequency; ANT, anterior; POST, posterior.

Values are means ± s.e.m. Number of muscle bundles shown in parentheses.

All pairwise comparisons indicate no significant difference between stimulus phase for anterior and posterior muscle from both leopard and mako sharks at each temperature (P values are shown for anterior–posterior comparisons within each species at each temperature/tailbeat combination).
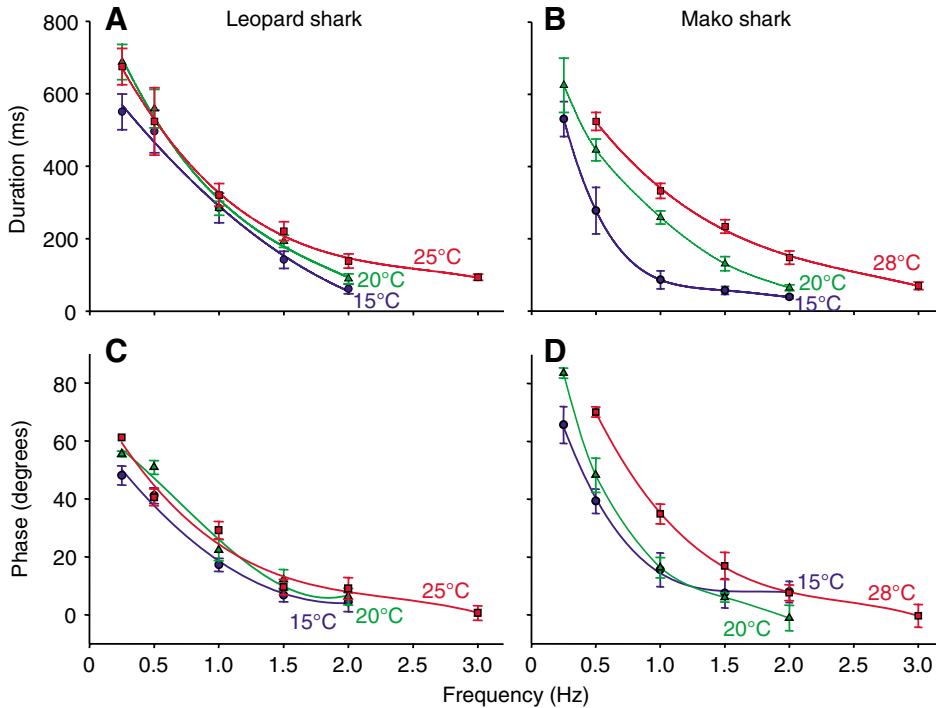
Fig. 5. Optimal stimulus duration (A,B) and activation phase (C,D) as a function of cycle frequency and temperature in leopard (A,C) and mako (B,D) sharks. Data for anterior and posterior locations (Tables 1, 2) have been pooled in this graph. Values shown are means ± s.e.m. (*N* values are given in the tables).

[4% *vs* 6% leopard, 5.5% *vs* 9% mako (Donley and Shadwick, 2003; Donley et al., 2005)], a characteristic likely to increase total power output during cyclical contractions (Altringham and Johnston, 1990; Syme and Shadwick, 2002). In addition to increased strain, muscle fiber length increases dramatically along the body in both species (J.M.D., unpublished data) and therefore larger absolute length changes occur in the posterior muscle fibres. Therefore, these sharks may produce greater power output in the posterior due to a combination of greater fiber lengths and strains even though the muscle contractile properties are constant.

Temperature effects

Temperature has a significant effect on power output of fish muscle (see Syme, 2006). To accommodate swimming at low temperatures, fish may decrease stimulus duration (duty cycle), decrease activation phase so that muscle is activated earlier in its strain cycle, recruit more muscle fibers, or simply swim at
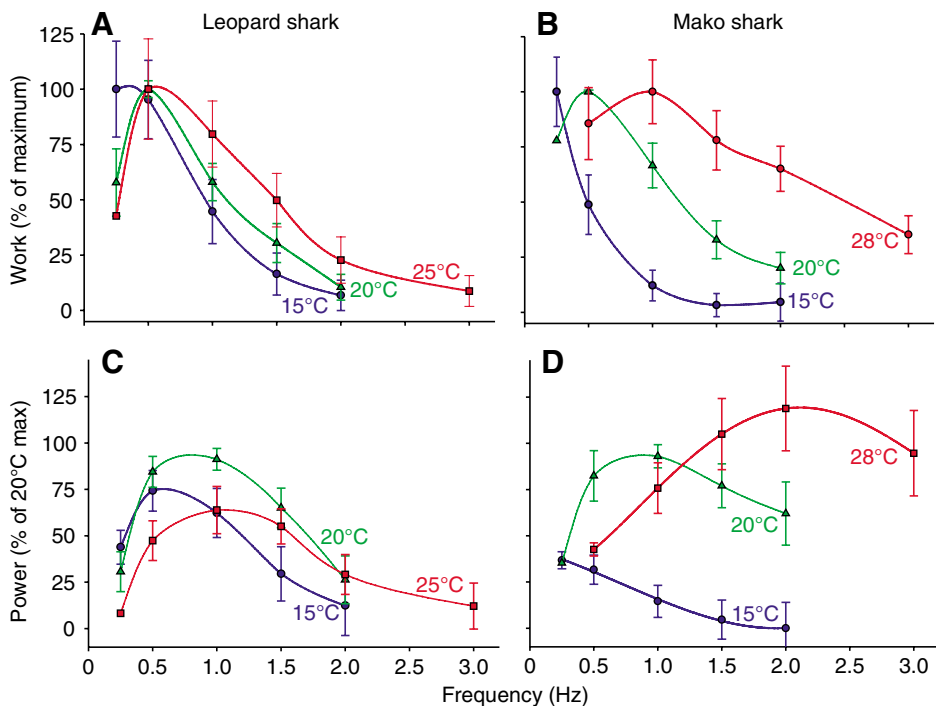

Fig. 6. Maximal net work (A,B) and power (C,D) as a function of cycle frequency and temperature in leopard (A,C) and mako (B,D) sharks. Values of net work are normalized to the maximum value at each temperature. Values of power are normalized to the maximum at 20°C for each species. Values shown are means ± s.e.m. (*N* values are given in the tables).
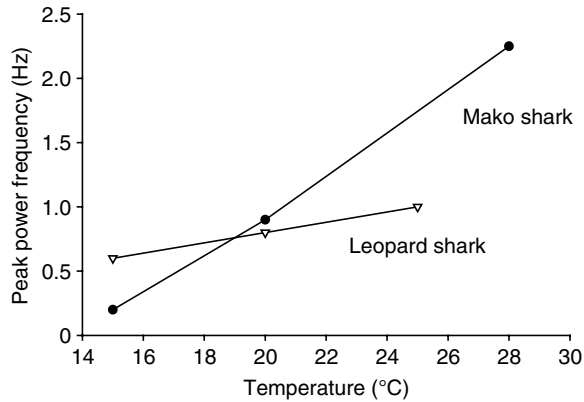
Fig. 7. The cycle frequency at which maximal power is developed as a function of temperature for RM from leopard and mako sharks.

a slower tailbeat frequency (Swank and Rome, 1999). Temperature modulated increases in muscle power output are also common in teleosts fishes: e.g. sculpin *Myoxocephalus scorpius* WM (Johnson and Johnston, 1991); scup RM (Rome and Swank, 1992); tuna and bonito RM (Altringham and Block, 1997).

Optimal activation of RM from the ectothermic leopard shark required only a small decrease in the duty cycle, from 35% to 20% of a cycle, between 25 and 15°C, while the most significant temperature effect was on the RM of the endothermic mako, particularly at the lowest temperature, where the duty cycle decreased from about 40% down to 10% or less, when cooled from 28°C to 15°C. Stimulus phase for maximum work was largely insensitive to temperature changes, particularly in the leopard shark, although a trend to lower phases with decreasing temperature was observed, as would be expected from the concomitant slowing of twitch kinetics.

There was a much greater effect of temperature on work and power output of red muscle in the mako than in the leopard shark (Fig. 6). In the mako, performance was significantly enhanced with temperatures up to 28°C, while in the leopard shark power actually declined slightly at the warm temperature. When cooled to 15°C mako RM bundles did not perform well even when stimulation was optimized at slow frequencies. For example, the net work at 1 Hz was only about 10% of the work generated at 0.25 Hz, and this fell to zero by 1.5 Hz (Fig. 6B). But in the leopard shark power output was not markedly depressed by cool temperature. The poor performance of mako RM at 15°C suggests that this temperature may be below the range in which it can function effectively. In contrast, leopard shark RM showed a broad temperature tolerance with perhaps a lower thermal maximum.

Increases in temperature yielded a much more pronounced increase in the cycle frequency producing peak RM power output in the mako than in the leopard shark. If the cycle frequency is considered a proxy for tailbeat frequency, and therefore swim speed, then the results in Fig. 7 predict that the mako may be able to power swimming at greater sustained

tailbeat frequencies (and speeds) than the leopard shark, as long as its RM temperature remains above about 20°C, but not below. Interestingly, recent experiments on the endothermic salmon shark *Lamna ditropis* (Bernal et al., 2005) showed an even more pronounced temperature sensitivity in RM, such that power production was greatly enhanced with temperatures up to at least 30°C but was virtually zero below 20°C, even though these fish spend much of their time at water temperatures below 10°C (Weng et al., 2005). While not so extreme in the mako, it appears that the RM of endothermic sharks may share this requirement for sustained, elevated temperatures to function well.

The cycle frequency at which maximum contractile efficiency occurs is typically slower than that for peak power output (Curtin and Woledge, 1993a; Curtin and Woledge, 1993b; Curtin and Woledge, 1996). Thus, to ascribe swimming behaviour solely on the basis of the tailbeat frequencies that produce maximum power could be misleading. It is a reasonable expectation that fish most frequently cruise at tailbeat frequencies near optimal for economy (i.e. at the speed of minimum cost of transport) (Videler, 1993). In the leopard shark, peak power occurred at relatively slow cycle frequencies (Fig. 5) that approximate those commonly observed during free-swimming (0.5–1 Hz) (Donley and Shadwick, 2003). This suggests that, for leopard sharks, tailbeat frequencies used most often may be nearly optimal in terms of both power and efficiency, and that scopes and range of cruise swimming speeds for maximal power and efficiency are narrow in a given temperature range. In the mako there was a broad range of cycle frequencies producing high power at 28°C, with maximum power occurring above 2.0 Hz. This is at least twice the tailbeat frequency normally observed in free-swimming mako sharks, and may indicate that in mako sharks power peaks at greater frequencies than does efficiency (Sepulveda et al., 2004). Thus, the mako may be able to sustain greater aerobic swimming speeds at higher temperatures but at increased metabolic cost.

Onset of RM activation while swimming occurs significantly later in the strain cycle in the mako than in the leopard shark (Donley et al., 2005). Based on this later phase of activation we predicted that mako RM would be faster to develop force and faster to relax than RM of the leopard shark. Comparison of the twitch kinetics at each temperature in the two sharks (Fig. 2) revealed that this was not the case; if anything the RM of mako sharks is marginally slower, except at warm temperatures where the two species are similar. The later phase of RM activation recorded in the mako shark *in vivo* may simply reflect the faster twitch kinetics associated with an elevated RM temperature rather than an intrinsic difference in contractile properties. As stated above, RM temperatures measured in free-swimming mako sharks are up to 8°C above that of the ambient water (Carey and Teal, 1969; Carey et al., 1971). In contrast, the leopard shark, like most sharks, is ectothermic so its RM temperature matches that of the ambient water. Therefore, although these two sharks inhabit comparable water temperatures, their RM may be operating at significantly different temperatures. When the leopard shark experiences

water of 20°C, for example, its RM will produce maximal power at a tailbeat frequency of 0.75 Hz (Fig. 7). In the same ambient conditions, mako RM operates near 28°C and produces maximal power at >2.0 Hz, affording the mako an advantage in maintaining higher aerobic swimming speeds.

How do optimal stimulus parameters compare to those predicted from in vivo EMG recordings?

The duration of muscle activation recorded during swimming (i.e. duty cycle) has been shown to closely match the optimal stimulus duration for cyclic work in only a few fish species [e.g. in RM of skipjack tuna (Syme and Shadwick, 2002) and in RM of scup at warm temperatures (Rome and Swank, 1992)]. A close agreement was also noted here in both the leopard and mako sharks. The duration of muscle activation recorded during swimming was similar at the anterior and posterior axial positions in both the leopard and mako sharks (Donley and Shadwick, 2003; Donley et al., 2005), being roughly 30% of the strain cycle in the leopard shark and 33% in the mako at a tailbeat frequency of 0.5–1.0 Hz and a water temperature of 15°C. Given a 30% duty cycle measured in the ectothermic leopard shark and assuming muscle temperature also to be 15°C, the stimulus duration would be ~600 ms at 0.5 Hz and 300 ms at 1 Hz, which agrees well with the optimal durations at 15°C (Fig. 5A). As in some teleosts (Grillner and Kashin, 1976; Altringham and Johnston, 1990) the optimal stimulus duration at all three temperatures in the leopard shark remained close to a constant proportion of the strain cycle across the range of cycle frequencies that produced net positive work. Assuming the RM of the mako shark was operating about 8°C warmer than ambient water temperature (i.e. near 23°C when in 15°C water), the optimal stimulus durations for RM of the mako (Fig. 5B at 20–28°C) were again close to the range measured in swimming fish (33% of the cycle period at 0.5 and 1.0 Hz tailbeat frequency).

Onset of RM activation in leopard sharks swimming at 14–15°C and tailbeat frequencies of 0.5 to 1.0 Hz typically occurs at ~55° of the strain cycle (Donley and Shadwick, 2003). Mean values for optimal stimulus phase measured in the present study were only 20–40° at this temperature and tailbeat frequencies (Fig. 5C). Assuming the RM of mako sharks is operating near 23°C in 15°C water, the values for optimal phase (Fig. 5D) are close to the onset of activation recorded in swimming fish (~84°; swimming at ~0.5–1.0 Hz) (Donley et al., 2005) at 0.5 Hz tailbeat frequency, but not at 1 Hz. At this time we have no explanation for these discrepancies.

Conclusion

These data support the hypothesis that the contractile properties of RM are constant along the body in both the leopard and mako sharks, and that the muscles are used to produce maximal power during swimming under most, but not all, circumstances. This study also illustrates differences in the effect of temperature on optimal cycle frequencies for power output in these two sharks. While these species may experience the same ambient conditions, the RM of mako sharks operates

at significantly higher temperatures than the leopard shark and maximal power output occurs at a higher cycle frequency in the mako. In turn the RM of leopard sharks appears to do better at cooler temperatures and slower cycle frequencies. Ectothermic species like the leopard shark may thus be able to inhabit cold water but at the expense of a decline in muscle power output and overall activity. In endothermic sharks like lamnids, the ability to maintain RM temperatures elevated above that of the ambient water has perhaps allowed them to expand their thermal niche and exploit waters of a greater temperature range while maintaining the potential for relatively high power output and swimming speeds.

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