Function of the medial red muscle during sustained swimming in common thresher sharks: Contrast and convergence with thunniform swimmers ☆

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ABSTRACT

Through convergent evolution tunas and lamnid sharks share thunniform swimming and a medial position of the red, aerobic swimming musculature. During continuous cruise swimming these muscles move uniformly out of phase with local body curvature and the surrounding white muscle tissue. This design results in thrust production primarily from the caudal fin rather than causing whole-body undulations. The common thresher shark (Family Alopiidae) is the only other fish known to share the same medial red muscle anatomy as the thunniform swimmers. However, the overall body shape and extremely heterocercal caudal fin of the common thresher is not shared with the thunniform swimmers, which have both fusiform bodies and high aspect-ratio, lunate caudal fins. Our study used sonomicrometry to measure the dynamics of red and white muscle movement in common thresher sharks swimming in the ocean to test whether the medial position of red muscle is associated with uncoupling of muscle shortening and local body bending as characteristic of thunniform swimmers. Common threshers (~60–100 kg) instrumented with sonomicrometric and electromyographic (EMG) leads swam alongside of the vessel with a tail-beat frequency of ~0.5 Hz. EMG signals confirmed that only the red muscle was active during sustained swimming. Despite the more medial position of the red muscle relative to the white muscle, its strain was approximately 1.5-times greater than that of the overlying white muscle, and there was a notable phase shift between strain trajectories in the red muscle and adjacent white muscle. These results suggest an uncoupling (shearing) of the red muscle from the adjacent white muscle. Although the magnitude of the phase shift between red and white muscle strain was relatively constant within individuals, it varied among sharks, ranging from near zero (red and white in phase) to almost 180° out of phase. This extent in variability has not been documented previously for thunniform swimmers with a medial red muscle position and may be a characteristic of the thresher's unique body and caudal fin morphology. Nonetheless, the uncoupling of red and white muscle strain remains a consistent character associated with fishes having a medially positioned red muscle.

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1. Introduction

Fishes are a diverse group of vertebrates (>25,000 species) in which the majority swim using undulations of the whole-body (Lighthill, 1969). Locomotion is predominantly powered by two, perhaps three, morphologically distinct myotomal muscle fiber types that differ in quantity, size, ultrastructure, contractile and biochemical metabolic capacities (Bone, 1978; Bone et al., 1986; Rome et al., 1988; Sanger and Stoiber, 2001). Red, oxidative myotomal muscles (RM) typically comprise less than 10% of body mass and are relatively small, well-vascularized, myoglobin-rich fibers that power aerobic, sustained (continuous) cruise swimming. White myotomal muscles (WM) comprise approximately 50% of body mass and are larger, less-vascularized, myoglobin-poor fibers recruited to power anaerobic, short duration, burst swimming. In fishes, not only are these muscle fibers different in abundance, morphology, and contractile capacity, but they have a discrete spatial separation within the myotomes such that the fiber types do not mix. In nearly all extant fishes the WM makes up the bulk of the myotomal muscles while the RM occurs as a relatively thin layer directly beneath the skin (subcutaneous), and becomes more abundant in the posterior regions of the body (Greer-Walker and Pull,

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1975). However, the tunas (Family Scombridae) and lamnid sharks (Family Lamnidae) possess a unique myotomal framework in which the RM has shifted to a more medial (closer to the vertebrae) and anterior body position (Bernal et al., 2001; Carey et al., 1985; Graham and Dickson, 2000, 2001; Graham et al., 1983). In addition, the vascular supply to the RM in tunas and lamnids is through a set of lateral vessels which give rise to a counter-current heat exchange system that allows for conservation of metabolically derived heat and for the temperature of the RM to be elevated significantly above ambient (i.e., RM endothermy) (Carey et al., 1971, 1985). The only other group of fish that share these muscle and vascular specializations are the thresher sharks (Family Alopiidae).

Thresher sharks are comprised of three species, the common thresher, *Alopias vulpinus*, pelagic thresher, *A. pelagicus*, and bigeye thresher, *A. superciliosus* that are readily distinguished from other sharks by having a caudal fin with an extremely elongate upper lobe, which can be as long as the length of the rest of the body (Gruber and Compagno, 1981). Superficially, all three thresher species appear to share a similar body morphology, but recent work has shown that the architecture of the myotomal muscles and the layout of the vasculature supplying the muscles of the common thresher are surprisingly distinct from that of the other two thresher species (Sepulveda et al., 2005). The RM of *A. vulpinus* is condensed into a solid piston-like muscle mass that is distributed predominantly toward the anterior of the body and in a medial position, an anatomical specialization that until recently was only known to exist in the lamnid sharks and tunas. In addition, the common thresher has a vascular supply to the RM that, in a manner similar to tunas and lamnids, relies on lateral vessels that give rise to counter-current heat exchangers (Bone and Chubb, 1983) that allow for RM endothermy (Bernal and Sepulveda, 2005). By contrast the RM in the pelagic and bigeye threshers is subcutaneous as in most fishes (excluding tunas and lamnids).

Considerable information has been gathered on the swimming biomechanics and physiology of lamnid sharks and tunas, and recent findings have shed new insight into the remarkable evolutionary convergence between these two groups in functional and mechanical design for high performance locomotion (Bernal et al., 2001; Donley et al., 2004, 2005). In particular, the contraction of the subcutaneous RM of most fish is in phase with local body bending, such that RM contractions result in bending of the body at that position (Katz and Shadwick, 1998). By contrast, the medial RM of tunas and lamnid sharks is only loosely connected to the surrounding WM fibers (which are not recruited during slow speed swimming) and transmit their forces toward the tail via tendons, resulting in shearing between these two muscle groups during sustained swimming (Bernal et al., 2001; Donley et al., 2004; Shadwick et al., 1999). Thus, when this unique anterior-medial RM shortens its strain is uncoupled from local body bending and induces body bending at more posterior locations. This transmission of force from the anterior RM towards the tail functionally separates the area of the body which produces the force from that which undergoes lateral, thrust-producing movements and minimizes the degree of lateral undulation by the anterior body, thereby reducing drag and providing a biomechanical advantage for performance at sustained, aerobic cruise swimming speeds (Altringham and Shadwick, 2001; Bernal et al., 2001; Donley et al., 2004; Graham et al., 1983; Westneat et al., 1993). This system allows these fishes to produce thrust primarily by caudal oscillation, a "thunniform swimming mode", as opposed to whole-body undulations (Fierstine and Walters, 1968; Knower, 1998; Lighthill, 1970; Magnuson, 1978; Webb, 1975).

In contrast to tunas and lamnids, there exists little information on the morphological and physiological specializations for locomotion in the thresher sharks, the only other group known to include species with a lamnid/tuna-like RM position and RM endothermy. Although the position of RM in the common thresher is similar to that of lamnids and tunas, the thresher's overall body morphology is markedly different and lacks several of the hallmark characters present in fish well adapted for cruise swimming (e.g., fusiform body and caudal keels; Bernal et al., 2001). In addition, the extremely long caudal fin of threshers differs radically from the lunate, high aspect-ratio caudal fin of tunas and lamnids. Taken together, the body design of the common thresher shares the medial RM but not the fusiform, keeled, lunate caudal anatomy exemplified by the thunniform swimmers, leading to questions about the functional basis of swimming and muscle physiology in these two groups.

Despite obvious differences in external appearance between the common thresher and thunniform swimmers, they appear to share a similar RM anatomy and it is not known whether they share similarities in their muscle mechanics (i.e., RM shearing). Thus, we tested the hypothesis that shearing also occurs between the medial red and white myotomal muscles in the common thresher shark during cruise swimming. The potential uncoupling of RM strain from local body bending (i.e., RM shearing) may indicate that body bending is produced at more posterior locations along the body, which is suggestive of thrust production primarily by caudal oscillations as opposed to whole-body undulations. Alternatively, uncoupling of RM strain from local body bending in a fish that does employ whole-body undulations may suggest that such shearing is ubiquitous and obligatory amongst fishes with medial RM, perhaps suggesting a unique biomechanical strategy. Ultimately, elucidation of the muscle mechanics of the common thresher sharks and a comparison with those of lamnid sharks will allow us to better understand if the shared anatomy of a medial RM in the common thresher is associated with a shared thunniform swimming mode.

2. Methods

2.1. Experimental subjects and capture

Experiments were conducted on the common thresher shark (*Alopias vulpinus*) at sea near the port of Oceanside, CA, USA (33° 10′N and 117° 30′W) during mid-May through early June from 2006 to 2009. Common thresher sharks were collected by hook and line off the Southern California coast using methods described by Bernal and Sepulveda (2005). Briefly, sharks were caught using trolled 36-kg conventional tackle (Penn 30VSW) and brought to the vessel as quickly as possible (average time ~20 min) to minimize fatigue. Once boatside, the sharks were restrained using a submersible sling and allowed to swim and ventilate as the boat moved slowly forward. For conservation purposes, all field experiments were selectively performed on smaller, non-gravid individuals ([males<184 cm fork length (FL), females<226 cm FL; Kohler et al., 1995)]. All procedures followed the guidelines of the University of Massachusetts, Dartmouth Animal Care Protocol (#05–06) and University of Calgary protocol BI 2007-31.

2.2. Sonomicrometry and electromyography

The measurement of length changes in the muscle segments (strain) and electromyographic (EMG) activation patterns was performed at two positions along the body (anterior: 40% *FL;* posterior: 60% *FL;* Fig. 1A), which encompass most of the longitudinal distribution of RM in the common thresher shark (Bernal et al., 2003; Sepulveda et al., 2005). Similar to previous studies on mako sharks (*Isurus oxyrinchus*; Donley et al., 2005), we noted that in positions anterior to 35% *FL* or posterior to 65% *FL* there either was a relatively small amount of RM or its transverse position precluded the accurate placement of the probes that measured *in situ* strain and EMG.

All surgical procedures were performed alongside the vessel as the sharks remained mostly submerged. Once boatside and gently restrained in the sling the sharks were rolled on their side (eliciting what appeared to be tonic immobility) and remained docile, not
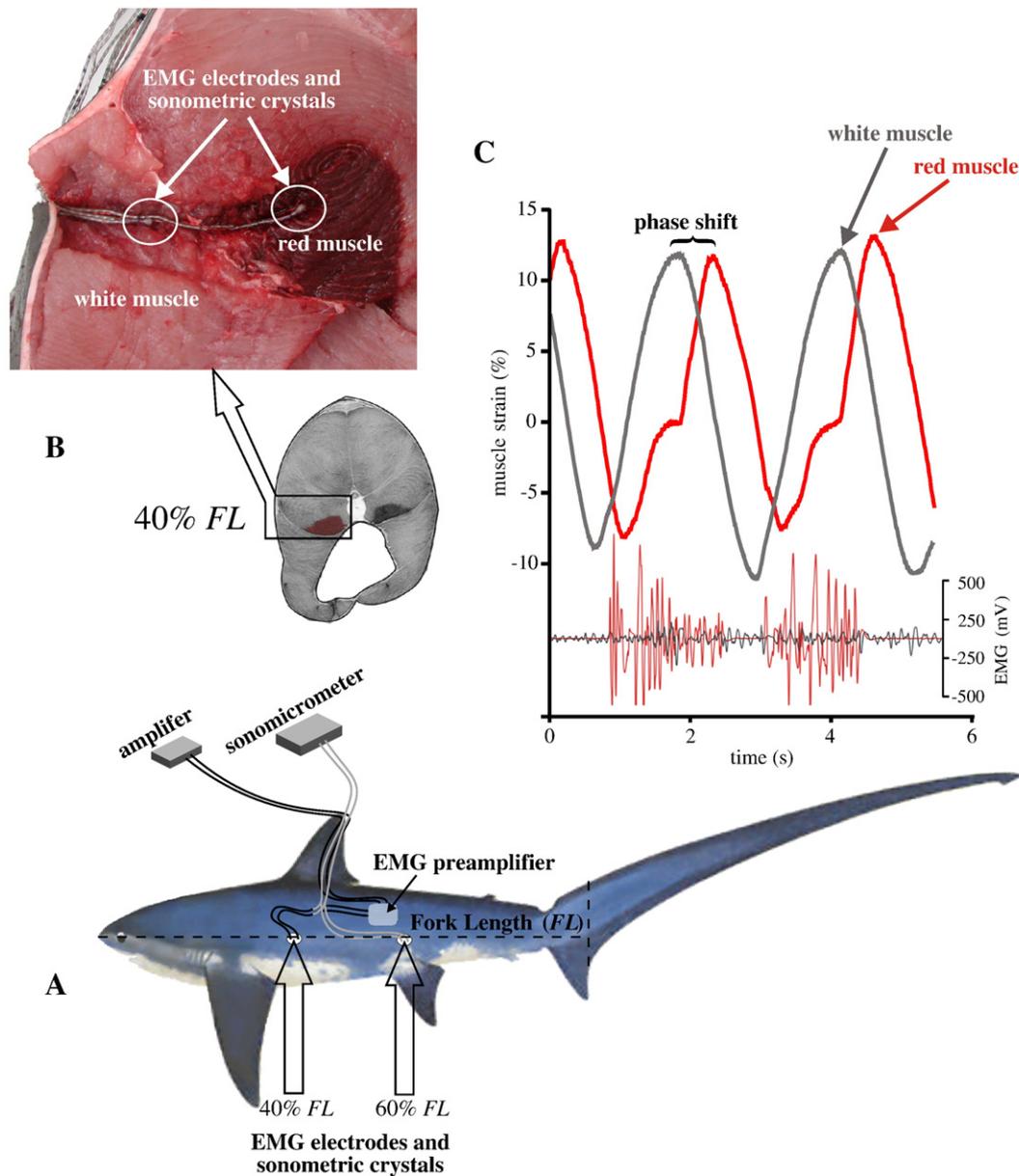
Fig. 1. A) Thresher shark showing the two positions along the length of the body (i.e., 40 and 60% fork length, *FL*, dashed line) where pairs of sonomicrometry crystals and EMG leads were implanted into the medial red (RM) and overlying white myotomal muscles (WM). Preamplified EMG signals and sonomicrometry cables were connected to amplifiers and the sonomicrometer on board the vessel. B) Transverse section of a thresher shark at 40% *FL* showing the position of sonomicrometer crystals and EMG leads in the RM and WM. A second pair of crystals was located about 15 mm posterior to these (not shown). C) RM and WM strain recorded with sonomicrometry, illustrating the amplitude and phase shift between the muscles. EMG records during cruise swimming showing activity in RM but not in WM.

showing any apparent signs of discomfort or reaction to the minimally invasive implantation (needle puncture) of fine-wire EMG's and sonomicrometry crystals. As such the animals could be subsequently released to swim with minimal stress from the procedure and without the complications, additional delay or need to recover from anesthesia. Two small (~2 mm) incisions were made in the skin approximately 15 mm apart along the length of the fish at both 40% or 60% *FL* to allow implantation of the sonomicrometer crystals and EMG leads (Fig. 1A). Each EMG lead pair (34 gauge insulated copper with 1 mm bared and hooked tips) was attached in tandem to the lead of a 2 mm piezoelectric sonomicrometer crystal (Sonometrics Corp, Ontario, Canada) with a 15 m lead. A second such pair of EMG leads and sonomicrometer crystal was then attached about 3–5 cm up the lead from the first pair such that when implanted into the shark the first EMG/crystal pair would enter the medial RM and the second EMG/crystal pair would reside in the adjacent WM a known distance

from the RM (Fig. 1B). Both crystals and EMG leads could thus be implanted simultaneously to reduce time and minimize stress on the fish. For implantation, the leads were placed into a 15 cm long and 2.5 mm diameter stainless-steel tube which was inserted through the incision in the skin and used to guide the crystals and EMG electrodes to the desired position, after which the stainless-steel tube was withdrawn leaving the crystals and EMG electrodes in place. A second set of sonomicrometry crystals was then inserted via the second incision about 15 mm posterior from the first, so that a pair of crystals was positioned in the deep RM and a second pair was positioned in the overlying WM. The crystals and EMG leads were then secured to the skin via sutures providing strain relief such that lateral body bending during swimming did not result in traction on the leads. A 0.95 mm polyester cord was anchored to the first dorsal fin using an 8/0 hook and served as both a leash and as a guide to attach all leads in order to keep them free from the caudal fin during swimming. Postmortem examinations

confirmed the location of crystal and EMG electrodes within the RM and WM.

2.3. Data collection

Following surgery fish were released from the sling and allowed to swim alongside the vessel. Vessel speed was adjusted to match the natural swimming speed of the fish, and gentle traction was applied to the cord on the dorsal fin if needed to keep the fish within the limits of the electrode leads. Several (e.g., 2–10) periods of active, steady swimming were recorded, each approximately 30 to 120 s in duration (e.g., 15 to 60 tail-beat cycles in each period). Upon completion of these recordings fish were euthanized by a rapid severing of the central nervous system and pithing. Passive 'swimming' was then recorded with the boat moving forward at the same speed as during active swimming while the head of the fish was moved side to side to induce lateral undulatory movements that closely mimicked the amplitude and frequency of those during active swimming (Fig. 2).

Sonomicrometer crystals were connected to a Sonometrics TRX Series 6 Digital Ultrasonic Measurement System and associated PC running Sonoview software (ver. 3.3.4) (Sonometrics, London, ON, Canada) allowing the change in distance between the emitting and receiving crystals to be measured in both the RM and WM during steady active and passive swimming. The EMG leads were connected to a 300× gain high-impedance amplifier (YO3 EMG Preamplifier, Motion Lab Systems, Baton Rouge, LA, USA) in a small waterproof case attached to the skin of the shark. The amplified signal was carried to an A.C. amplifier (model P55 Grass Instrument Co., West Warwick, RI, USA) located on the boat. The EMG signal was band pass filtered between 3 and 300 Hz to remove movement artifacts and other interference and recorded simultaneously with the sonomicrometer signals at 500 Hz. Sonomicrometer and EMG data were subsequently smoothed or filtered, if required, using AcqKnowledge 3.5 (Biopac Systems Inc., Santa Barbara, CA, USA) sufficiently to allow clear resolution of the timing of the peaks and amplitudes of the muscle strain patterns from sonomicrometry and the presence of EMG activity. Because of the
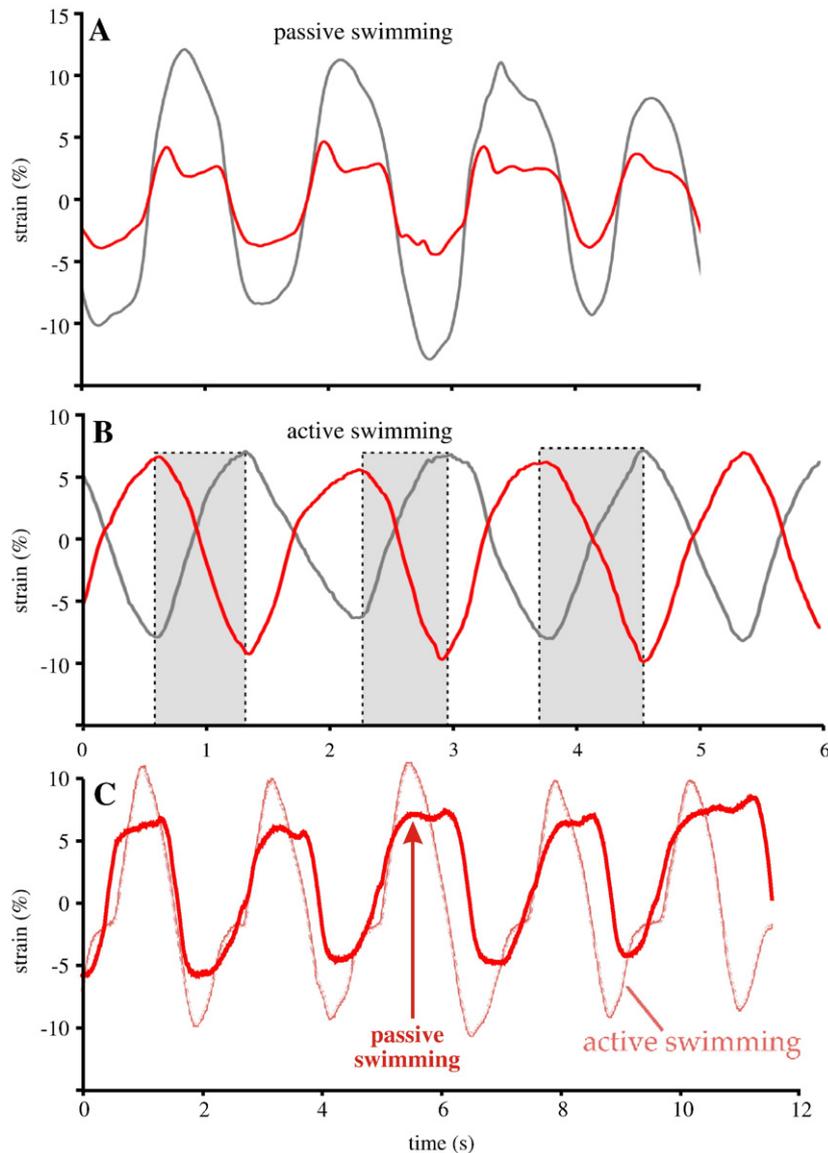
Fig. 2. Trajectories of red muscle (red-line) and white muscle (grey-line) strain during active and passive swimming in common thresher sharks. A) During passive swimming strain of RM and WM are in phase, whereas B) during active swimming in the same fish the strain trajectories are out of phase. Shaded boxes indicate the degree of phase shift (i.e., peak RM strain to peak WM strain). C) Comparison of strain trajectories of RM from another individual during active (fine trace) and passive swimming (bold trace), illustrating the prominent double peak often observed during passive swimming (see also panel A).

challenging experimental, electrical and marine environment, useable EMG data were only collected from two fish.

2.4. Data analysis

For each individual specimen, muscle strain amplitude was calculated for at least 15 tail-beat cycles from as many of the recording sessions as were available. Strain amplitude was calculated as the ratio between peak-to-peak amplitude and median muscle segment length, and expressed as a percentage (as described in Shadwick et al., 1999; Donley and Shadwick, 2003). The phase shift between RM and WM strain trajectories at a given longitudinal position of the body was calculated by dividing the difference in time between the peaks of the WM and RM strain patterns by the period of the associated tail-beat cycle. Phase was then expressed as degrees of a tail-beat cycle, such that a phase of zero degrees signifies the peaks of the RM and WM strain patterns were simultaneous (i.e., both commenced shortening together), and a phase other than zero degrees indicates that either the peak of RM strain preceded the peak of WM strain by up to 180°, or if greater than 180° then the peak of WM strain was considered to precede the peak of RM strain by up to 180° (i.e. RM led WM or WM led RM by up to half a cycle). The presence of active EMG was defined by a notable increase in amplitude of the EMG signal (Fig. 1C). Only data from cycles in which there was steady swimming were used for analysis, as confirmed by consistent waveform shape and amplitude with adjacent cycles. All measurements of a given parameter from each animal were averaged such than only one value per animal for each parameter was used for statistical tests.

A two-sample Student's t-test was used to compare mean values (e.g., tail-beat frequency, relative strain, phase) and a paired test was used to compare between parameters during active and passive swimming. For all statistical tests, significance was set at $\alpha = 0.05$. Values given are mean ($\pm$SD) unless otherwise indicated.

3. Results

3.1. Tail-beat frequency

A total of 10 thresher sharks were captured for the present study ranging in size from 155 to 190 cm fork length (FL) (body mass ~61–103 kg). The number of tail-beat cycles analyzed for each individual shark ranged from 30 to 344, with the exception of the 180 cm FL individual for which only 3 tail-beat cycles yielded usable data (Table 1). The mean tail-beat frequency (TBF) during active swimming for all 10 sharks (total of 1148 tail-beat cycles) was 0.56 ± 0.10 Hz (range 0.42–0.68 Hz) and the mean TBF during passive swimming for 6 sharks (total of 197 tail-beat cycles) was 0.50 ± 0.14 Hz (range 0.39–0.76 Hz). There was no significant difference in the TBF during active and passive swimming in the six individuals for which TBFs were recorded during both active and passive swimming (Table 1), negating differences in TBF as a factor when comparing strain amplitude and phase shifts between the two groups.

3.2. EMG in active and passive swimming

EMG signals were examined in two individuals (Table 1) to assess activation of the RM versus WM in both active and passive swimming. During active swimming, EMG signals were present in the RM and absent in the WM (Fig. 1C). By contrast, during passive swimming EMG signals were not detected in either muscle tissue (data not shown). These results indicate that the RM was the only myotomal muscle being recruited during active cruise swimming.

3.3. Position effects on RM and WM strain and phase

Muscle strain and phase were measured at two positions along the body (i.e., 40% and 60% FL) in the initial three sharks used in the study (170, 180, and 190 cm FL). When compared with each other, body position had no notable effect on the RM and WM strain and phase. For this reason, we focused our analysis on the 40% FL position that has the highest RM abundance (i.e., 40% FL; Bernal et al., 2003), and for which we have the most data.

3.4. Strain and phase

The mean strain amplitude in the RM of all sharks at 40% FL during active swimming was $16.8 \pm 8.3\%$ (range 8.2–31.8%, $n = 10$) and during passive swimming was $12.3 \pm 5.5\%$ (range 7.6–22.7%, $n = 6$) (Fig. 3, Table 1). For the six individuals in which RM strain was recorded during both active and passive swimming, the mean strain amplitude decreased significantly (by ~24%) during passive swimming (Table 1). The mean strain amplitude in the WM of all sharks during active swimming was $10.9 \pm 5.8\%$ (range 3.0–21.4%, $n = 10$) and during passive swimming was $15.1 \pm 5.6\%$ (range 8.4–21.8%, $n = 6$). For the six individuals in which WM strain was recorded during both active and passive swimming, the mean strain amplitude increased significantly (~24%) during passive swimming (Table 1, Fig. 2).

Table 1

Comparison of tail-beat frequency (TBF) and strain amplitude (peak-to-peak) of red muscle (RM) and white muscle (WM) in common thresher sharks during active cruise swimming and during passive swimming in which the head was moved side to side to induce lateral undulatory movements that closely mimicked the amplitude and frequency of those during active swimming. Values are mean ± SD. The fork length (FL), mass of the fish (estimated from FL; Kohler et al., 1995), and number of tail-beat cycles analyzed (n) are given. Asterisk indicates individuals on which EMG traces were recorded. The percent change in RM and WM strain amplitude between passive and active swimming (Dstrain) is shown, where negative numbers indicate a decrease during passive swimming. The relative phase shift between peak RM and WM strain during active swimming is given as a percentage of tail-beat cycle (see Section 2.4).

FL (cm), ~mass (kg), sex	Active swimming				Passive swimming						n	Phase shift (%)
	n	TBF (Hz)	Strain amplitude (%)		n	TBF (Hz)	Strain amplitude (%)		Dstrain (%)			
			RM	WM			RM	WM	RM	WM		
155, ~61, M	142	0.49 ± 0.03	17.9 ± 2.4	11.7 ± 2.0							142	1.1 ± 2.0
160, ~67, M	344	0.63 ± 0.06	13.8 ± 1.5	8.7 ± 1.4	39	0.47 ± 0.10	8.8 ± 2.5	8.4 ± 2.9	−36	−3	344	0.9 ± 0.6
166, ~73, F	109	0.64 ± 0.09	30.3 ± 4.5	8.1 ± 1.8	26	0.52 ± 0.10	22.7 ± 4.6	13.5 ± 2.6	−25	67	137	−132.6 ± 34.5
*169, ~77, F	126	0.67 ± 0.06	16.6 ± 1.2	17.6 ± 2.4	33	0.76 ± 0.11	7.6 ± 1.0	21.8 ± 3.6	−54	24	119	−167.7 ± 18.4
170, ~78, F	30	0.42 ± 0.03	8.2 ± 0.6	3.9 ± 0.9							30	−14.6 ± 3.6
175, ~84, F	58	0.68 ± 0.08	14.0 ± 2.3	7.3 ± 1.1	32	0.39 ± 0.03	12.4 ± 1.8	10.3 ± 2.2	−12	42	104	27.5 ± 13.2
*177, ~86, M	40	0.64 ± 0.06	8.3 ± 1.9	13.0 ± 2.0	28	0.39 ± 0.08	8.9 ± 2.6	14.5 ± 2.5	7	11	69	−5.9 ± 46.2
180, ~90, M	3	0.43 ± 0.01	9.6 ± 0.7	3.0 ± 0.3							3	−6.3 ± 0.5
188, ~100, F	79	0.43 ± 0.04	17.7 ± 2.4	21.4 ± 3.8	39	0.46 ± 0.08	13.6 ± 2.3	21.7 ± 3.3	−23	2	25	25.0 ± 3.4
190, ~103, F	217	0.58 ± 0.06	31.8 ± 3.4	14.1 ± 2.1							200	5.4 ± 1.2
Mean (±SD)		0.56 ± 0.10	16.8 ± 8.3	10.9 ± 5.8		0.50 ± 0.14	12.3 ± 5.5	15.1 ± 5.6				
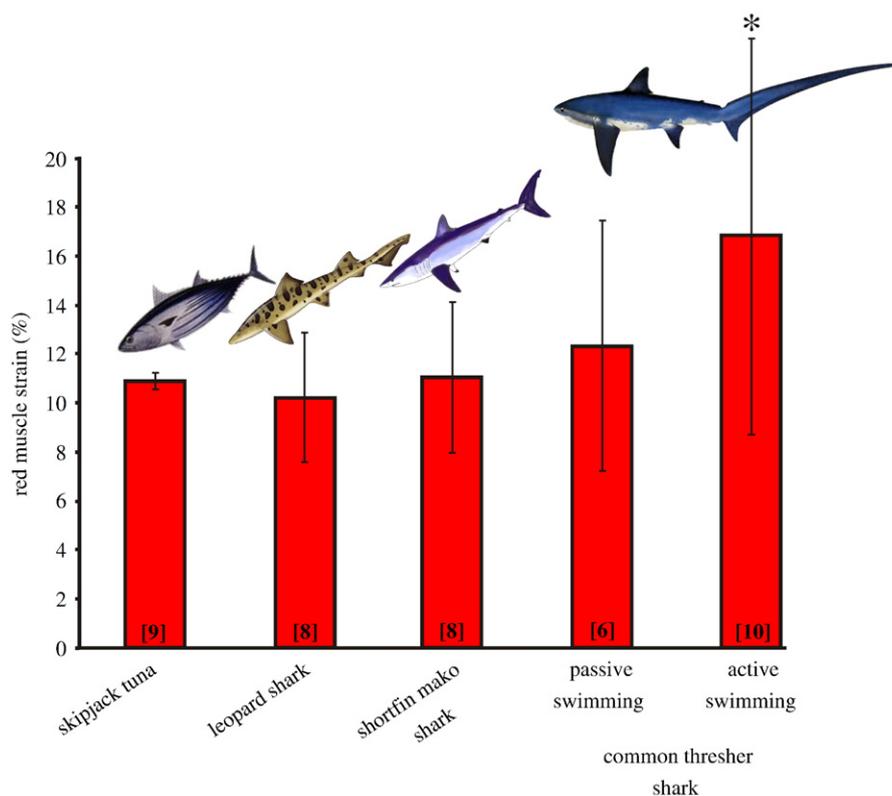
Fig. 3. Red muscle strain amplitude (peak-to-peak) during active swimming in select species of fish. Data for passive swimming is also presented for the common thresher shark. *indicates significant difference between active and passive swimming. Values are mean ± SD and sample size. Data for skipjack tuna (*Katsuwonus pelamis*), a thunniform swimmer with medial RM, from Shadwick et al. (1999), shortfin mako shark (*Isurus oxyrinchus*), a thunniform swimmer with medial RM, from Donley et al. (2005), and leopard shark (*Triakis semifasciata*), a subcarangiform swimmer with subcutaneous RM, from Donley and Shadwick (2003).

The phase shift between the strain patterns of RM and WM were measured in 10 thresher sharks during active swimming. Data for 5 of those 10 sharks showed that on average the phase of the RM lagged the WM with a mean difference of 12.0 ± 13.1°, and varied considerably ranging from 27.5° to only 0.91° (Table 1, Fig. 4). For the other 5 individuals the average RM phase led the WM with a mean difference of 65.4 ± 62°, and again varied considerably from 167° to 5.9° (Table 1, Fig. 4). Taken together, all sharks showed a phase shift between strain peaks of RM and WM, regardless of which muscle led the phase shift.

Of the ten sharks for which a phase shift between RM and WM was measured during active swimming, we also were able to measure the phase shift between the strain patterns during passive swimming in six of them. In four of those individuals there was a significant decrease in the magnitude of the phase shift between RM and WM during passive swimming compared with active, such that the strain patterns became more synchronized during passive swimming (Fig. 2). Moreover, the RM lagged the WM by 12.9 ± 14° in four of those sharks, while in the other two specimens the RM led the WM by 47.9° and 154.8°, average 101.3°.

4. Discussion

Red muscle in most fishes is used to power body/caudal fin undulatory cruise swimming, yet different swimming modes are identified including anguilliform, sub/carangiform, and thunniform, categorized based on body shape, caudal fin morphology and the degree of body bending (Thomson and Simanek, 1977; Wardle et al., 1995; Webb, 1998). Because the degree to which the body of thresher sharks bends during cruise swimming remains unquantified, catego-

rization of their swimming mode is based solely on their body shape, which may be problematic given how different they appear from other sharks. Threshers are considered to exhibit a subcarangiform swimming mode, intermediate between anguilliform and carangiform, and similar to many other sharks (e.g., leopard shark, *Triakis semifasciata*; blue shark, *Prionace glauca*) in which the degree of body bending increases rapidly toward the posterior half of the body during cruise swimming (Webb, 1975). However, this classification is based solely on body shape, ignoring that the different thresher species differ radically from each other in their myotomal architecture (i.e., subcutaneous vs medial RM position), and may be misleading in light of recent findings on swimming sharks. In leopard sharks the subcutaneous RM contracts in-phase with the WM and causes local body bending, resulting in a subcarangiform swimming mode, while in lamnids the medial location of RM appears to form the basis for their thunniform swimming mode (Bernal et al., 2001; Donley et al., 2004), minimizing lateral movements of the anterior body and concentrating the thrust-producing undulations towards the tail region. This uncommon medial RM position potentially poses mechanical constraints on both bending and the ability of RM to shorten and do work. Recent investigations in swimming lamnids and tunas have revealed that the medial RM arrangement facilitates stiff-bodied, thunniform locomotion (Donley et al., 2004) via robust, elongated longitudinally-directed tendons that transfer force from RM down the body to the caudal propeller (Donley et al., 2004; Gemballa et al., 2006). In this system, RM contraction is uncoupled from local body bending, thereby reducing drag along the body during swimming. Only recently has it come to light that despite 400 million years of independent evolution, lamnids and tunas have converged both structurally and functionally in this regard. More recently it has
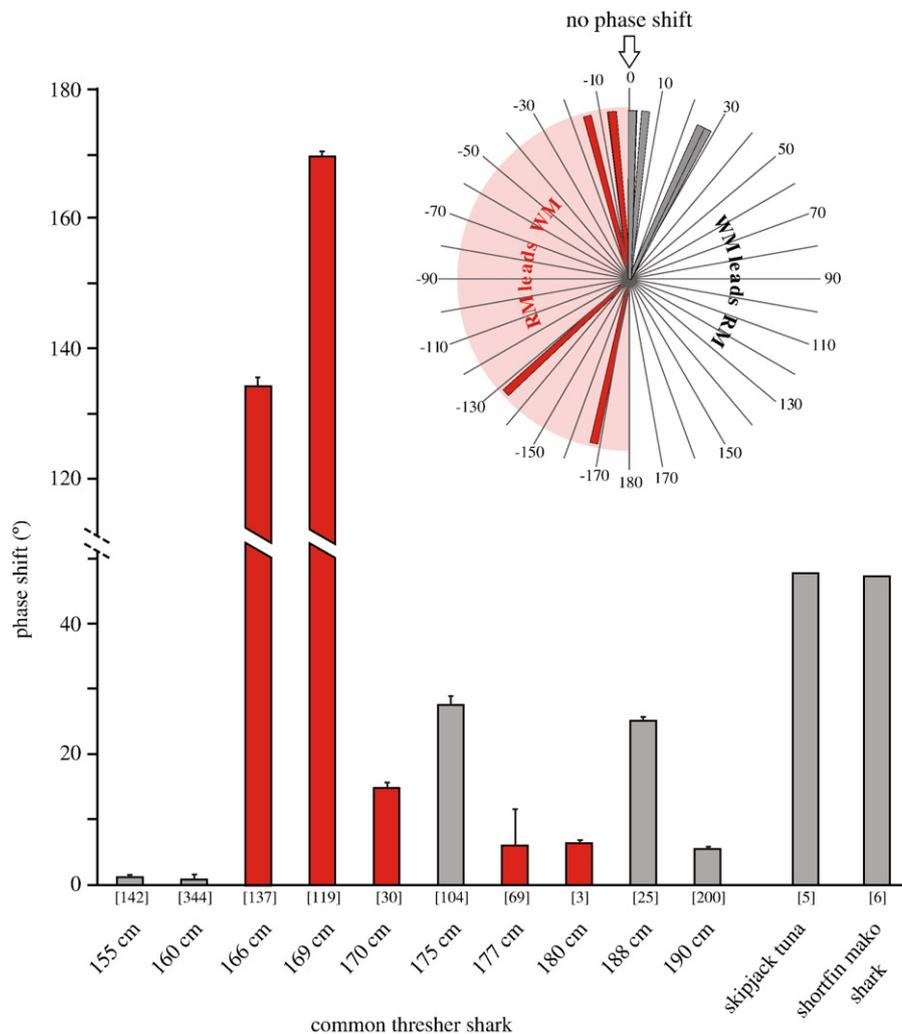
Fig. 4. Phase shift between peak of red (RM) and white muscle (WM) strain trajectories (see Fig. 1C) in the common thresher sharks during active cruise swimming. Phase shift expressed as degrees of the tail-beat cycle in a linear scale and on a circular plot (inset). Instances in which the WM leads the RM are shown in grey, and where RM leads the WM are shown in red. The fork length of each individual thresher shark is noted below the bars and values are mean ± SE and sample size. Data for skipjack tuna (*Katsuwonus pelamis*) from Shadwick et al. (1999) and for mako shark (*Isurus oxyrinchus*) from Donley et al. (2005). Detailed morphometric data for each individual thresher shark in the inset are given in Table 1.

been noted that common thresher sharks also appear to share this musculotendinous anatomy (S. Gemballa, C. Sepulveda, D. Bernal, unpublished data).

Although these studies suggest a clear relationship between the position of RM and the swimming mode of sharks (and tunas), where fish with medial RM exhibit thunniform swimming, the common thresher differs from lamnids and tunas in its body shape and has a caudal fin that does not resemble the symmetrical hydrofoil of thunniform swimmers. Relative to thunniform swimmers, the extremely elongate caudal fin of threshers will assuredly alter both the hydrodynamics and physiology of sustained swimming. Therefore, it seems likely that the obvious differences in body and tail morphology between lamnids and threshers could result in significant differences in swimming kinematics between these groups, despite the shared RM anatomy. We examined muscle mechanics during steady swimming in the common thresher shark, which has medially placed RM but whose body shape may not suggest a clear tendency toward thunniform swimming, to test the hypothesis that the medial position of RM is consistently associated with uncoupling of RM shortening from local body bending characteristic of thunniform swimmers.

4.1. Strain

Because no effect of body position on RM or WM strain or phase was found, a standard body position of 40% *FL* was used as a reference point for comparison amongst all individuals. The mean strain amplitude in the RM of all sharks at 40% *FL* during active swimming was greater than that during passive swimming (16.8 ± 8.3%, active; 12.3 ± 5.5%, passive) (Fig. 3, Table 1). Further, the strain of RM was on average 60% greater than that of the adjacent WM (Table 1). Both observations suggest that the strain of RM is not consistent with that expected in a fish bending as a homogenous beam, such that RM strain is not tightly coupled to that of WM and is not constrained to be less than that of WM despite the more medial position of RM. In contrast, strain in the WM of all sharks was lower during active swimming than during passive (10.9 ± 5.8%, active; 15.1 ± 5.6%, passive). This observation suggests that active shortening of RM may influence the strain of WM, perhaps by altering the degree of body curvature beyond that which occurs during passive bending. Alternatively, the bending imposed on the fish during passive swimming may have been greater than during active swimming, although from subjective observation it did not appear to be different by the nearly 40% difference in muscle

strain. Along with the recordings of RM and WM strain, EMG recordings were used to verify the relationship between muscle activation and resultant patterns of strain. Both active and passive simulated swimming movements were associated with a lack of an EMG signal within the WM, confirming that only RM was contributing to movement (Fig. 1C).

Comparison of mean RM strain amplitude between the common thresher and the thunniform swimming shortfin mako shark (Donley et al., 2005) and skipjack tuna (Shadwick et al., 1999), as well as the subcarangiform leopard shark (Donley and Shadwick, 2003) reveals that the values are similar, although perhaps slightly greater in the threshers (Fig. 3). The absolute distance of the RM from the backbone may impact the magnitude of strain, where the RM from large threshers examined in the present study may thus exhibit slightly greater strain when compared to values from studies on other smaller fishes with a medial RM position (e.g. ~61–103 kg for threshers vs ~3.5–10 kg for makos and for skipjack tuna ~1–1.5 kg; Donley et al., 2005; Shadwick et al., 1999). However, the ability of the RM to shear relative to the surrounding WM may dissociate the amplitude of RM strain from its proximity to the backbone, and so RM of common threshers may simply experience greater strain than in the mako and skipjack tuna, perhaps related to the more undulatory swimming mode of threshers (see below). The common thresher and both the mako and tuna species have a medial RM position, while the leopard shark does not. Despite this, the strain amplitudes appear similar, and similar to what is commonly observed to result in near maximal work output from skeletal muscles operating at near normal cycling (tail-beat) frequencies (Syme, 2006).

The relatively large strain amplitude of RM in thunniform swimming fish with medially located RM, and now including the common thresher shark which overtly appears to display a subcarangiform swimming mode, supports the uncoupling of RM strain from adjacent WM and local body bending as a consistent characteristic in fish with medial RM.

4.2. Phase

Our results indicate that although the magnitude and polarity of the phase shift between RM and WM strain trajectories varied among individuals (i.e., RM leading or lagging WM), a notable phase shift was almost always present (Fig. 4) and so clearly RM shears the WM in the common thresher. Similar to results observed in the mako shark (Donley et al., 2005), in several thresher individuals shortening of the RM lagged the WM, but a surprising observation was that in several threshers the RM led the WM (Fig. 4). We interpret this to suggest that the biomechanics of swimming in the threshers are perhaps more flexible than in the thunniform swimming makos. This may be related to the need to employ the tail via pronounced body flexion during feeding in threshers (Aalbers et al., in press). Furthermore, several individuals displayed a significant decrease in the magnitude of the phase shift between RM and WM during passive swimming when compared with active swimming, such that the strain patterns became more synchronized during passive swimming (Fig. 2A, B). These observations were also noted in mako sharks and suggest that the medial RM position does contribute to a thunniform-like mode of locomotion in the common thresher shark. However, based on casual observation of the sharks swimming alongside the vessel, the threshers observed in the present study appeared to swim with a more undulatory mode than that characteristic of thunniform swimmers. Thus, while the phase shift between RM and WM shortening during active swimming and the synchronization of phase during passive swimming suggest that the RM contributes to force production and lateral movement of a more posterior region of the body as in thunniform swimmers, the resultant lateral movement of the body in threshers suggests the forces may not be localized at the tail as in thunniform swimmers. This, in turn, may have important

implications for expectations about the magnitude and polarity of the phase shift.

The variation in the polarity and magnitude of the phase of RM strain relative to WM strain is a striking result that has not been observed in previous studies on thunniform swimmers. Fifty percent of sharks showed that on average the phase of the RM lagged the WM, whereas in the other 50% the average RM phase led the WM (Fig. 4), with great variability across (but not within) individual fish. Although crystal placement was verified and was consistent in axial position for every individual, it is possible that slight variations of their location within the myotomal cone of the RM between individuals results in different phase shift patterns. Based upon previous work on makos, variation in crystal placement (in the periphery versus center of the RM cone) resulted in some variations in the magnitude of strain as well as the phase in RM shortening relative to the WM (Donley et al., 2005). Indeed, crystals placed in the lateral periphery of the RM mass in makos resulted in phases nearly synchronous with the surrounding WM; whereas, crystals placed in the center of the RM cone showed the distinctive phase shift in RM and WM shortening characteristic of thunniform swimmers. Further analysis of the fiber orientation and tendinous architecture within the RM cone in the common thresher may shed light on the contrast in phase polarity seen amongst individuals. Furthermore, because threshers appear to display a greater degree of undulation of the body than thunniform swimmers (Shadwick et al., 1999), it is likely that thrust is not being generated exclusively at or near the tail but rather along the posterior half of the fish. Lacking a detailed anatomical description of the tendinous architecture in common thresher sharks, the pattern of strain trajectory during passive swimming provides indirect evidence of force transmission to more posterior locations. It was commonly observed during passive swimming, when the wave of body bending dictates muscle strain, that the RM strain pattern displayed a prominent double peak (Fig. 2A, C). The first peak was closely associated with local body bending (i.e. the peak of the WM strain trajectory), while the second peak occurred between 12 and 20% of a cycle later, as would be associated with bending of the body more caudal to the location of the RM. The double hump was either absent or much less prominent during active swimming (Fig. 2B, C), when muscle strain causes body bending, and it might not be expected that a muscle that was only loosely coupled to adjacent tissue would experience strain in response to local body curvature.

The phase shift in common threshers was noted to be similar to makos/tunas in some instances, but very dissimilar in others. If the RM in common thresher sharks exerts force more caudal on the body, but not necessarily at the tail as in thunniform swimmers, the magnitude of the phase shift between RM and WM (Fig. 4) will not necessarily be the same as in thunniform swimmers (mako shark 36–61°, skipjack tuna ~48°; Donley et al., 2005; Shadwick et al., 1999). Furthermore, during steady swimming in which the RM is active and exerting force at a more posterior location, while the WM bends passively and presumably in phase with local body curvature, if the thresher changes the waveform of body bending in any way, including with changes in speed or the relative stiffness of the body, the phase shift between RM and WM will likely change. In other words, if the WM is always in phase with local body bending during steady swimming and the RM exerts its forces somewhere caudal to its longitudinal location (i.e. its pull is distributed along the posterior portions of body length to cause undulations), then if the waveform of body bending changes the phase shift between RM and WM will also change. This potentially important difference between the mode of swimming between the common thresher and the more thunniform tuna or lamnid sharks may account for the considerable variation in phase lag between RM and WM observed amongst individual threshers.

Another important difference in the myotomal architecture between the common thresher and lamnid sharks is that the latter have a well-developed lubricative sheath around the RM (Donley et al., 2005). The

presence of this lubricative sheath is hypothesized to allow the RM to become physically uncoupled from the surrounding WM during sustained swimming and thus allow the RM to move freely past the surrounding WM and effectively lead to RM shearing (Bernal et al., 2001; Donley et al., 2005). In addition, the lubricative sheath in lamnids is contiguous with extensive tendons designed to transfer force from the RM bundle to the tail region (Gemballa et al., 2006). This RM sheath has also been documented in the tunas and is believed to have the same functional implications. By contrast, common thresher sharks do not appear to have a well-developed loosely-bound layer of connective tissue surrounding the RM that may act as this lubricative sheath, thus the shearing observed between RM and surrounding WM occurs regardless of the presence of this sheath. The lack of a lubricative sheath in the common thresher may be related to the fact that force transmission by shortening of the RM is not solely focused at the tail as it is in lamnids and tunas but rather is hypothesized to span over a greater posterior axial region. Muscle strain recordings (Figs. 2 and 4) clearly show that during active swimming all threshers had a shift in the phase of peak strain between the RM and WM, and the magnitude of RM strain was greater than WM and not less than that observed in RM of lamnids and tunas, indicating that RM is indeed shearing the WM to a notable extent. Therefore, the common thresher appears to be achieving RM shearing in a manner perhaps different from that which has been documented for lamnid sharks and tunas. It is possible that the non-thunniform swimming mode of the common thresher is a result of this unusual myotomal architecture and the lack of the lubricative sheath surrounding the RM.

In addition to the potential biomechanical advantage that RM shearing may offer to tunas, lamnids and the common thresher, the medial RM of all three groups has a specialized vascular arrangement in which counter-current perfusion of the RM allows for the retention of metabolically produced heat that in turn warms the locomotor muscles above ambient temperatures (RM endothermy) (Carey et al., 1971). There is, therefore, an added benefit to a medial RM position, one in which an increased RM temperature will potentially elevate muscle performance and ultimately affect cruise swimming. Previous work on tunas hypothesized that the medial RM position arose in response to selective pressure to increase biomechanical performance during cruise swimming (i.e. thunniform swimming) and that subsequent to this derived muscle arrangement came RM endothermy (Graham and Dickson, 2000; Westneat et al., 1993). The findings from the present study, that the medial RM position in the threshers may offer a biomechanical advantage despite the threshers not adopting a stiff-bodied, thunniform swimming mode, coupled with the finding that these sharks also exhibit RM endothermy, leaves unanswered the question as to whether endothermy or swimming mechanics was the selective advantage that may have resulted in a medial RM phenotype. However, these observations do question the adoption of thunniform swimming as the sole adaptation responsible for medially placed RM. The three lineages that have remarkably converged upon a similar myotomal framework in having a medial RM position all present high RM strain through shearing and heat retention that allow for RM endothermy (Bernal and Sepulveda, 2005; Carey and Teal, 1966, 1969). Further studies on the swimming mechanics, functional anatomy of RM, and thermal sensitivity of contractile characteristics of RM in the closely related but anatomically disparate thresher species may hold important clues to answering these questions.

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