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WHY DO TUNA MAINTAIN ELEVATED SLOW MUSCLE TEMPERATURES? POWER OUTPUT OF MUSCLE ISOLATED FROM ENDOTHERMIC AND ECTOTHERMIC FISH

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Summary

It has been hypothesised that regional endothermy has evolved in the muscle of some tunas to enhance the locomotory performance of the fish by increasing muscle power output. Using the work loop technique, we have determined the relationship between cycle frequency and power output, over a range of temperatures, in isolated bundles of slow muscle fibres from the endothermic yellowfin tuna (*Thunnus albacares*) and its ectothermic relative the bonito (*Sarda chiliensis*). Power output in all preparations was highly temperature-dependent. A counter-current heat exchanger which could maintain a 10 °C temperature differential would typically double maximum muscle power output and the frequency at which maximum power is generated (f_{opt}). The deep slow muscle of the tuna was able to operate at higher temperatures than slow muscle from the bonito, but was more sensitive to temperature change than more superficially located slow fibres from both tuna and bonito. This suggests that it has undergone some evolutionary specialisation for operation at higher, but relatively stable, temperatures. f_{opt} of slow muscle was higher than the tailbeat frequency of undisturbed cruising tuna and, together with the high intrinsic power output of the slow muscle mass, suggests that cruising fish have a substantial slow muscle power reserve. This reserve should be sufficient to power significantly higher sustainable swimming speeds, presumably at lower energetic cost than if intrinsically less efficient fast fibres were recruited.

Key words: endothermy, fish, muscle, power output, swimming, work loops, yellowfin tuna, *Thunnus albacares*, bonito, *Sarda chiliensis*.

Introduction

Tunas (Scombridae, Thunnini) are unique among teleosts because of their ability to elevate the temperature of their locomotor muscle, viscera, brain and eye tissues above that of the water temperature (Carey and Teal, 1966; Carey *et al.* 1971; Graham, 1975; Carey, 1981). Endothermy in tunas is compartmentalised in regions of high metabolic output and coupled with circulatory specialisations to reduce heat loss. Tunas have a high standard metabolic rate and numerous specialisations associated with increased oxygen delivery to the tissues and high metabolic demands (Brill, 1987, 1996). Elevation of slow-twitch (red) muscle temperatures is facilitated by the more axial positioning of the aerobic muscle mass and the presence of counter-current heat exchangers (the rete mirabilia) in the circulatory system, which reduce conductive and convective heat loss at the gills and body surfaces. Many tunas also have a higher proportion of slow-twitch, relative to fast-twitch, myotomal muscle than other teleosts (e.g. Graham *et al.* 1983). The heat generated by muscle contraction and metabolism can thus be conserved, and in these pelagic, continuously swimming fish, the temperature of the more axial slow muscle may be maintained up to 21 °C above ambient water temperature (bluefin tuna, *Thunnus thynnus*, Carey and Lawson, 1973). In *Katsuwonus pelamis*, slow-twitch muscle temperatures can be as much as 12 °C above ambient, and in other *Thunnus* species the steady-state muscle temperature elevation is 6 °C or less (Dizon and Brill, 1979; Graham and Dickson, 1981; Holland *et al.* 1992). Mechanisms for heat retention in aerobic muscle are widespread in large pelagic fish, and telemetry and anatomical studies have shown that heat conservation strategies are present in tunas, lamnid sharks (Carey *et al.* 1971), alopiid sharks (Carey, 1981; Bone and Chubb, 1983), blue sharks (Carey and Scharold, 1991) and swordfish (Carey, 1990). The convergence of similar mechanisms among sharks and fishes suggests that strong selective pressures exist for warming of the locomotor muscles. In tunas and lamnid sharks, it has been suggested by Carey and Teal (1966) and many subsequent authors that this system of regional endothermy has evolved to enhance the

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locomotory performance of the fish by increasing muscle power output. However, this hypothesis has never been tested directly in any endothermic fish.

In addition to their endothermic adaptations, tunas have a suite of morphological characters which appear to be adaptations for efficient, and relatively rapid, sustained swimming. They have a body thickness to length ratio close to the optimum for minimum drag (Hertel, 1966) and a fusiform body which also increases swimming efficiency (Weihs, 1989). The corselet, a zone of modified skin at the deepest point of the body, may introduce microturbulence to prevent breakaway of the boundary layer which would increase drag. A narrow caudal peduncle minimises energy losses due to movement of water, and lateral keels on the peduncle reduce drag and possibly direct water over the middle part of the fin (Walters, 1962; Aleyev, 1977). Dorsal and ventral finlets, in series along the midline of the tapering edges of the body, may limit cross flow between the two sides of the body, reducing drag. Some of the fins fit into slots in the body surface when not in use, and larger fins are often placed behind the deepest part of the body (greatest dorsal to ventral height). The tails of tunas are typically lunate and have a high aspect ratio, both features increasing the efficiency of thrust generation. Kinematically, swimming in tuna is characterised by minimal lateral movement of all but the caudal region of the fish, with virtually all of the thrust coming from the caudal fin. Oxygen consumption measurements confirm the increased swimming efficiency predicted from these adaptations (Dewar and Graham, 1994*a*).

The closest living ectothermic relatives of the tunas are bonitos (tribe Sardini) (Collette, 1978). Few studies are available on vertebrate endothermic and ectothermic species in which physiological performance between taxa with close phylogenetic histories can be directly compared. The presence of extant warm and cold fishes in the scombrid lineage, along with information about their relationships, provides a valuable tool for studies on the evolution of endothermy. The ability to examine the muscle characteristics of both groups in a phylogenetic context is critical for discerning what evolutionary specialisations, if any, have occurred in the endothermic lineage (Block and Finnerty, 1994). Using the work loop technique (Josephson, 1985), we have determined the temperature-dependence of cycle frequency *versus* power output relationships in isolated bundles of slow-twitch muscle fibres from the endothermic yellowfin tuna (*Thunnus albacares*) and its ectothermic relative the bonito (*Sarda chiliensis*).

Materials and methods

Fish

Thunnus albacares (Bonaterre) and *Sarda chiliensis* (Cuvier) were caught using lift poles with barbless hooks off the coast of California in September 1995 and held in fish wells on board ship, before being transferred to holding facilities in San Diego, California, within 2–3 h of capture. Fish were then transferred *via* a specialised transport tank placed on board a flatbed truck to the Tuna Research and Conservation Center at Pacific Grove, California, a joint facility of Hopkins Marine Station (Stanford University) and the Monterey Bay Aquarium. Tuna were maintained in three tanks. The largest tank (T1) was 13 m in diameter and 3.3 m deep, and two smaller tanks (T2 and T3) were 10 m in diameter and 2 m deep. Tuna were held from October 1995 to May 1996 (the start of experiments) in T1 at 20± 0.5 °C and in T2 for the same period at 18±0.5 °C and in T3 at 24±0.5 °C. Recirculated, filtered and aerated sea water from Monterey Bay passed through all three tanks. Tuna in tanks T1 and T2 were fed three times per week on a mixed diet of fish and squid at a constant level 115 kJ kg^{-1} day^{-1}. To compensate for a higher metabolic rate in the warmer T3 tank, these fish were fed 165 kJ kg^{-1} day^{-1}. Bonito were maintained at 20 °C in a tank 6 m in diameter and 1.5 m deep. Their diet consisted of chopped fish and squid. All experiments were carried out during May and June 1996.

Muscle mechanics

Fish were captured using nets (tuna) or barbless hooks (bonito), and killed by decapitation and pithing. Blocks of slow muscle 3–5 myotomes long were rapidly dissected from the chosen region of the fish (see below) and immersed in a large volume of Ringer's solution (composition, in mmol l^{-1}: NaCl, 175.7; KCl, 7; $CaCl_2$, 1.9; $MgCl_2$, 1.1; sodium pyruvate, 10; Hepes, 10; pH 7.8 at 25 °C), bubbled with oxygen, at 20 °C. The Ringer's solution was changed frequently during the initial dissection, which was carried out with the aid of a stereomicroscope. The aim was to rapidly remove a bundle of fibres from the central myotome of the block, approximately 1–3 mm in diameter, and place it in a large volume of fresh oxygenated Ringer. The preparation was reduced in diameter to 0.5–1.5 mm by removing fibres from the outside of the bundle. A piece of myoseptum was retained at both ends, and this was trimmed to shape and mounted in a small aluminium foil clip (Altringham and Johnston, 1990*a*,*b*).

Preparations were transferred to a chamber through which Ringer's solution flowed at 20±0.1 °C. One end of the preparation was attached to an isometric force transducer (AE801, SensoNor, Horten, Norway), the other to a servo motor. The preparation was lengthened to remove slack and left for a minimum of 30 min before experimentation. Stimulation amplitude was altered to maximise twitch force, using 2 ms duration stimuli, and the length of the muscle was adjusted to place the preparation on the plateau of the length–force relationship, defined as l_0. Twitch parameters were then determined for each preparation: time from stimulus to peak force (twitch rise time, t_a), and time from peak force to half-maximum force (half-relaxation time, $t_{r0.5}$). 1 s tetani were used to determine the stimulation frequency for maximum tetanic force, and this frequency was used in all subsequent experiments on that preparation.

Maximum power output was then determined at a range of cycle frequencies using the work loop technique (Josephson, 1985; Altringham and Johnston, 1990*a*,*b*). Briefly,

preparations were subjected to sinusoidal strains symmetrical about l_0 and stimulated during part of each strain cycle. A plot of muscle length against force yields a hysteresis loop for each cycle, the area of which is the net work performed during the cycle. Previous experiments on fish muscle (Altringham and Johnston, 1990*b*) have established that, over the range of cycle frequencies yielding close to absolute maximum power output, the strain yielding maximum power output is approximately ±5 % l_0 (10 % peak to peak). Kinematic studies of swimming fish (e.g. Hess and Videler, 1984; van Leeuwen *et al.* 1990; Rome *et al.* 1993) have shown that *in vivo* strains range from ±3 to ±6 % l_0 over that part of the body believed to generate most of the power for swimming. A strain of ±5 % l_0 was used in all experiments in the present study. The number of stimuli and the phase shift between the onset of stimulation and the strain cycle were manipulated to maximise power at each frequency. Stimulation phase shift typically increased from 20 ° to 60 ° between 15 °C and 30 °C. Phase shift is defined from the strain cycle: 0/360 ° is muscle at l_0 and lengthening, one complete cycle is 360 °. In each experimental run, a preparation performed eight work loops and was then allowed to recover for 6 min before the next run. The power output was calculated from the seventh loop of each run: power typically rose by 5–20 % (depending upon cycle frequency) over the first 4–6 loops of a run, before stabilising.

A complete power *versus* frequency relationship was determined at 20, 25, 15 and 30 °C for each preparation, in the order given. To monitor any change in performance during the course of the experiment, which could take up to 10 h, regular controls were taken. At a given temperature, every third or fourth run was a repeat of a standard set of parameters: those giving maximum or near-maximum power. Runs with the same control parameters were made at 20 °C as the temperature was lowered from 25 to 15 °C, and at 20 and 25 °C as the temperature was raised to 30 °C. After experiments at 30 °C, controls were again made at 20 °C. Corrections for changes in performance over time were made, where necessary (usually only at the highest temperatures, see Results), by multiplying power by (power of initial control experiment/power of control run closest to the experimental run). At the end of each experiment, the preparation was removed from the apparatus and weighed, after removal of the clips and myosepta.

Fibres were studied from two locations along the length of the body of tuna, 0.40 and 0.65 body lengths (*BL*, snout to fork) from the snout (Fig. 1A). At both locations, fibres were taken from the centre of the broadest region of the more axially distributed slow muscle (‘deep’) (Fig. 1B). At 0.65 *BL*, fibres were additionally taken from within the superficial slow muscle zone (‘superficial’), delineated by the septa of the ‘lateral line triangle’, and from the deepest region of the slow muscle (‘very deep’), close to the vertebral column (Fig. 1B). The ‘superficial’ fibres studied were more axial to a distinct layer of mixed red fibres and were histochemically homogeneous (E. Freund, unpublished observations). Because of the nested cone arrangement of the myotomes, sites sampled at increasing depth were taken from different myotomes. We were unable to determine exactly which myotome each preparation was from, but no more than 3–4 myotomes separated the most anterior and most posterior preparations. Bonito slow muscle fibres were taken from 0.65 *BL*, at a depth of 8–10 mm, beyond at least half the total depth of the slow muscle.

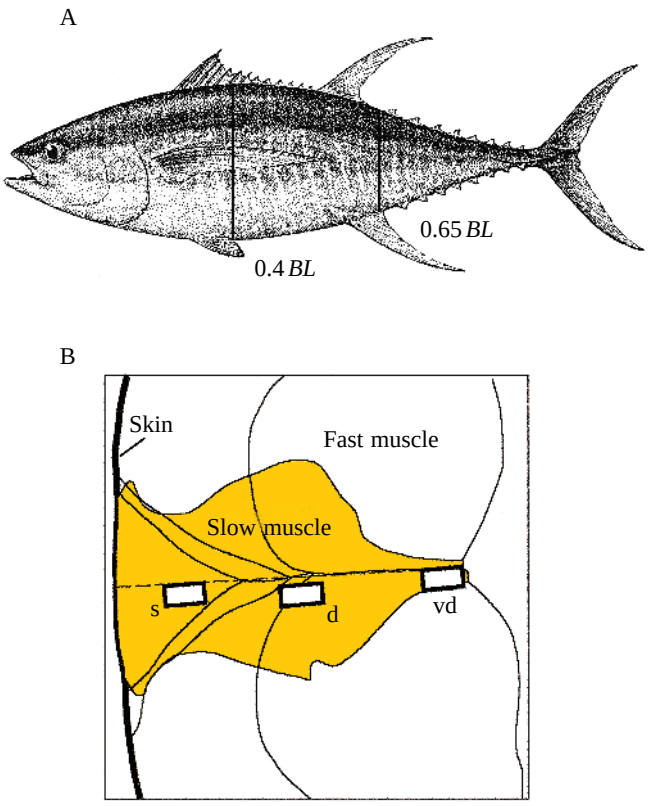

Fig. 1. (A) Location of sampling sites on the yellowfin tuna. (B) Sampling sites within the slow muscle of tuna in relation to the organisation of the slow muscle. s, superficial slow muscle; d, deep slow muscle; vd, very deep slow muscle. *BL*, body length.

Swimming kinematics

Swimming kinematics of the 18 °C- and 24 °C-acclimated tuna and the 20 °C-acclimated bonito were studied in undisturbed fish on a day on which they were not routinely fed, and during feeding. Fish, of the same size range as those used in mechanical experiments, were recorded using a Canon EX2Hi, Hi8 video camcorder, which was mounted directly above the tanks. The field of view was typically four by five fish lengths. Tracings were made from video sequences of the paths of the snout of the fish and the caudal puduncle, where the presence of the lateral keels enabled the same point to be followed with some precision from frame to frame. Twenty randomly chosen sequences were analysed for each group/condition and, from each, mean tailbeat frequency and swimming speed were determined from straight swimming sequences of 3–5 tailbeats. Analysis of alternate frames of the 50 Hz video sequences gave a resolution of 40 ms, or 2 % or better, for tailbeat frequency. Speed is expressed in

Table 1. *Body sizes of fish used in the study*

	Number	Length (m) Range Mean ± S.E.M.	Mass (kg) Range Mean ± S.E.M.
Yellowfin tuna (*Thunnus albacares*)	13	0.585–0.81 0.667±0.022	3.45–8.9 5.05±0.54
Bonito (*Sarda chiliensis*)	4	0.42–0.47 0.44±0.122	1.02–1.45 1.14±0.12

body lengths s^{-1} (*BL* s^{-1}) because the exact lengths of most fish were not known and could not be determined from the video sequences, as their vertical position in the 2 m deep water column was not known.

Results

Fish

The body sizes of the fish used in the study are summarised in Table 1.

Isometric mechanical properties

No trends were found in twitch kinetics data from differently sized yellowfin tuna: analysis of variance (ANOVA) and correlation analysis revealed no significant (P<0.05) size-related changes. In addition, no significant differences or consistent trends were observed in twitch kinetics from fish maintained at 18 °C (four fish), 20 °C (four fish) and 24 °C (five fish). Although the number of replicates was often small, variation was low: all standard errors were less than 10 % of the mean value. Data from all yellowfin tuna were therefore pooled where appropriate. Fig. 2 summarises the isometric twitch kinetics of all preparations from both fish species at 20 °C. The location of the muscle fibres in the body had a significant (P<0.05) effect on twitch kinetics in the yellowfin tuna, and the results of one-way ANOVAs are summarised in Fig. 2. The small data set meant that activation time results failed normality tests, even after transformation, and a less sensitive ANOVA on ranks was performed. Both activation and half-relaxation times of deep fibres were greater at 0.65 *BL* than at 0.4 *BL*, although only the latter was statistically significant (P<0.05). The small data set suggests caution in interpreting the results. At 0.65 *BL*, there was a tendency for twitch kinetics to become more rapid with increasing depth. Twitch parameters for bonito muscle are shown in Fig. 2C.

The effects of temperature on twitch kinetics are summarised in Fig. 3, for 0.40 *BL* muscle from the tuna and 0.65 *BL* muscle for the bonito: locations with the largest data

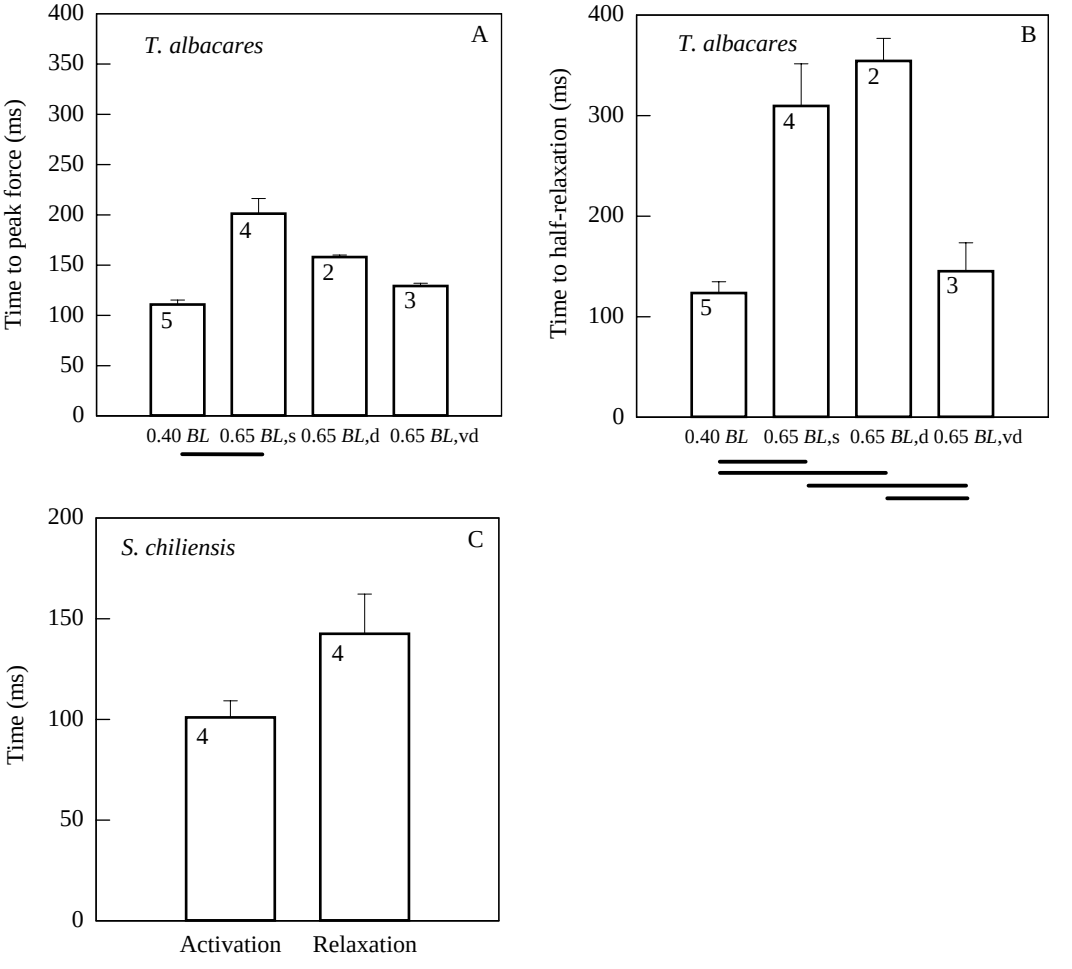

Fig. 2. Time to peak twitch force (A) and half-relaxation time (B) for yellowfin tuna preparations at different locations in the body: 0.4 *BL*=0.4 body lengths from snout. s, superficial slow muscle; d, deep slow muscle; vd, very deep slow muscle. See Fig. 1B for a full description of the locations. (C) Time to peak force (activation) and half-relaxation time for bonito muscle preparations. All data are presented as means + S.E.M.; the number of fish is given within the columns, at 20 °C. The solid lines below the figures connect columns that are significantly different from each other (ANOVA, P<0.05).

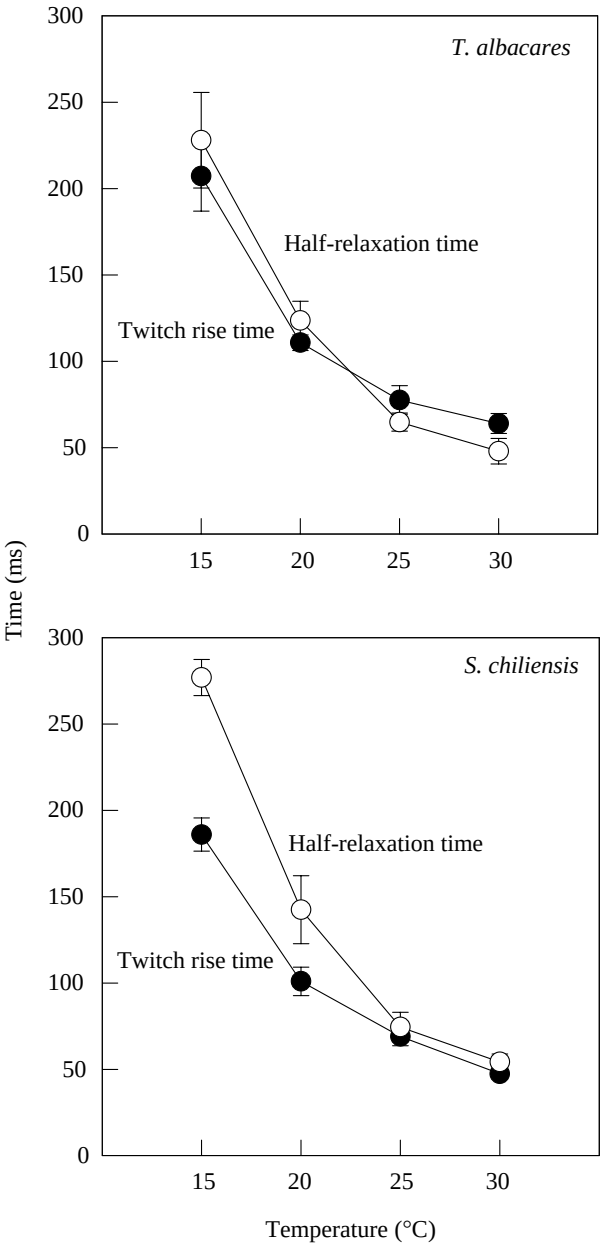

Fig. 3. Temperature-dependence of twitch rise time and half-relaxation time in yellowfin tuna *Thunnus albacares* (at 0.4 *BL*) and bonito *Sarda chiliensis* (at 0.65 *BL*) muscle. Data are presented as means ± S.E.M., N=5 tuna and N=4 bonito.

sets. Similar results were obtained from other locations on the tuna. Both twitch rise time (t_a) and half-relaxation time ($t_{r0.5}$) decreased markedly with increasing temperature. The rate of change of both parameters over 5 °C temperature increments decreased with increasing temperature (rates are expressed as a coefficient: time at x °C/time at x+5 °C; e.g. activation time at 20 °C/activation time at 25 °C). However, despite this consistent change, ANOVA showed that only the change in activation time coefficients in the tuna was significant (P<0.05). Activation times and half-relaxation times had very similar temperature coefficients, and coefficients were very similar for the two species: these results are summarised in Table 2.

Power output

Power output under optimal stimulation conditions was 12.0±1.7 W kg^{-1} (N=13) at 25 °C for tuna muscle and 13.8±1.9 W kg^{-1} (N=4) at 25 °C for bonito muscle. No significant differences were found in power output measured for tuna muscle from different body locations, so the above value is the mean of all results. Maximum power decreased with decreasing temperature in all preparations, but some notable differences were seen in temperature-dependence. Temperature coefficients (power at x °C/power at x–5 °C) are summarised in Table 3. In 'deep' and 'very deep' tuna muscle fibres, the temperature-dependence of power was greatest at lower temperatures. This trend was observed in *all* individual preparations. ANOVA showed that temperature sensitivity at 15–20 °C for tuna fibres from 0.40 *BL* was significantly greater than at 20–25 °C and 25–30 °C (ANOVA P<0.001; Student–Newman–Keuls *post-hoc* tests P<0.05). There were insufficient data at other locations for meaningful comparisons). Furthermore, although the data are sparse for some locations and temperatures, there was a clear trend towards increased sensitivity with increasing depth into the tuna. Finally, power output in 'superficial' fibres from both tuna and bonito had a low thermal dependence, which did not increase with decreasing temperature. At 15–20 °C, significant differences were found between deep tuna fibres at 0.4 *BL* and superficial tuna fibres and bonito fibres (overall ANOVA P<0.001; *post-hoc* tests P<0.05). The temperature sensitivities of deep fibres at 0.40 and 0.65 *BL* were not significantly different. A similar comparison at 20–25 °C revealed no significant differences, but the small data set means that the results should be interpreted with caution.

Power–frequency relationships

Very similar power–frequency curves were obtained from all preparations, and representative curves for tuna and bonito are shown in Fig. 4. Fig. 5 summarises all the data, normalised to maximum power at 25 °C. The greater temperature-dependence of the deep slow fibres of the tuna, relative to the superficial fibres from tuna and bonito, is clearly evident. In the physiological temperature range (20–30 °C), a 10 °C increase in temperature increased maximum power, and the frequency at which it was produced, by 50–100 % in both tuna and bonito. The frequency which produces maximum power output (f_{opt}) was in the range 2–9 Hz, depending upon muscle type and temperature. Attempts to determine the power–frequency curve for bonito preparations at 30 °C were largely unsuccessful (Fig. 5C). All bonito preparations began to deteriorate (revealed by a decline in power output) within 15 min of raising the temperature to 30 °C, and the construction of all curves relied on correcting for this fall in power output after the initial points had been collected. The 30 °C data shown in Fig. 5C are therefore unreliable and serve primarily to indicate the instability of the preparation. After 30 min at

Table 2. *Temperature coefficients of twitch kinetics for yellowfin tuna* Thunnus albacares *and bonito* Sarda chiliensis *muscle preparations*

	Temperature coefficients (time at x °C/time at x+5 °C)					
	For activation time t_a			For half-relaxation time $t_{r0.5}$		
	15–20 °C	20–25 °C	25–30 °C	15–20 °C	20–25 °C	25–30 °C
Yellowfin tuna *Thunnus albacares* 0.40 *BL* (*N*=5)	1.87±0.19	1.48±0.13	1.30±0.03	1.87±0.21	1.98±0.32	1.44±0.16
Bonito *Sarda chiliensis* 0.65 *BL* (*N*=4)	1.88±0.22	1.47±0.12	1.45±0.11	2.08±0.38	1.91±0.20	1.38±0.12

Values are means ± S.E.M.
BL, body length.

30 °C, power output declined by up to 50 %, but recovery was half complete within 30 min of reducing the temperature to 20 °C. In contrast, the deep (Fig. 5A) and very deep (not shown) slow fibres of the tuna were stable at 30 °C, but deteriorated at 35 °C. Unfortunately, no experiments were performed at 30 °C on superficial fibres from tuna. Tuna preparations were very stable at 30 °C or below for many hours: controls under given conditions were typically within 1–3 % of initial values, even after 8 h or more of experimentation. Bonito preparations were similarly stable at or below 25 °C.

Swimming kinematics

Undisturbed tuna and bonito swam primarily in schools, around the perimeter of the tanks, occasionally reversing direction or breaking formation to swim more randomly. When feeding, the schools broke up and fish swam singly, often bursting rapidly to take food. Swimming speeds and tailbeat frequencies are summarised in Table 4.

Undisturbed, 'cruising' tuna swam at a mean speed of approximately 1 $BL\,s^{-1}$, with a tailbeat frequency of approximately 1.6 Hz, much lower than the frequency which produced maximum power output in isolated slow fibres (f_{opt}). In rapid bursts during feeding, tailbeat frequency (8–12 Hz) was much greater than f_{opt}, and fast fibres were presumably recruited. The limited time resolution of the video recording, and the water turbulence created by feeding tuna, meant that burst tailbeat frequency could only be estimated roughly. The bonito had a similar cruising tailbeat frequency to the yellowfin

Table 3. *Temperature coefficients of muscle power output for yellowfin tuna* Thunnus albacares *and bonito* Sarda chiliensis *muscle preparations*

	Temperature coefficients for power (power at x °C/power at x–5 °C)		
	15–20 °C	20–25 °C	25–30 °C
Yellowfin tuna *Thunnus albacares*			
Very deep fibres, 0.65 *BL*	2.00 (1)	1.52±0.08 (2)	1.27 (1)
Deep fibres, 0.40 *BL*	1.84±0.09 (5)	1.40±0.04 (5)	1.31±0.05 (5)
Deep fibres, 0.65 *BL*	1.55±0.21 (2)	1.35±0.03 (2)	1.09 (1)
Superficial fibres, 0.65 *BL*	1.20±0.08 (4)	1.22±0.07 (4)	
Bonito *Sarda chiliensis* 0.65 *BL*	1.31±0.04 (4)	1.37±0.05 (4)	

Values are means ± S.E.M. (*N*).
BL, body length.

Table 4. *Swimming performance in yellowfin tuna* Thunnus albacares *and bonito* Sarda chiliensis

	Swimming speed ($BL\,s^{-1}$)	Tailbeat frequency (Hz)	Stride length
Yellowfin tuna (*Thunnus albacares*)			
18 °C-acclimated			
Undisturbed (*N*=20)	1.01±0.03	1.52±0.04	0.67±0.03
Feeding bursts		8–12	
24 °C-acclimated			
Undisturbed (*N*=20)	0.93±0.03	1.66±0.05	0.56±0.02
Feeding bursts		8–12	
Bonito (*Sarda chiliensis*)			
20 °C-acclimated			
Undisturbed (*N*=15)	1.21±0.02	1.55±0.04	0.79±0.02
Feeding bursts		>12	

Tailbeat frequency was significantly (P<0.05) higher in 18 °C-acclimated tuna than in 24 °C-acclimated fish.

Estimated ranges are given for tailbeat frequency during feeding bursts.

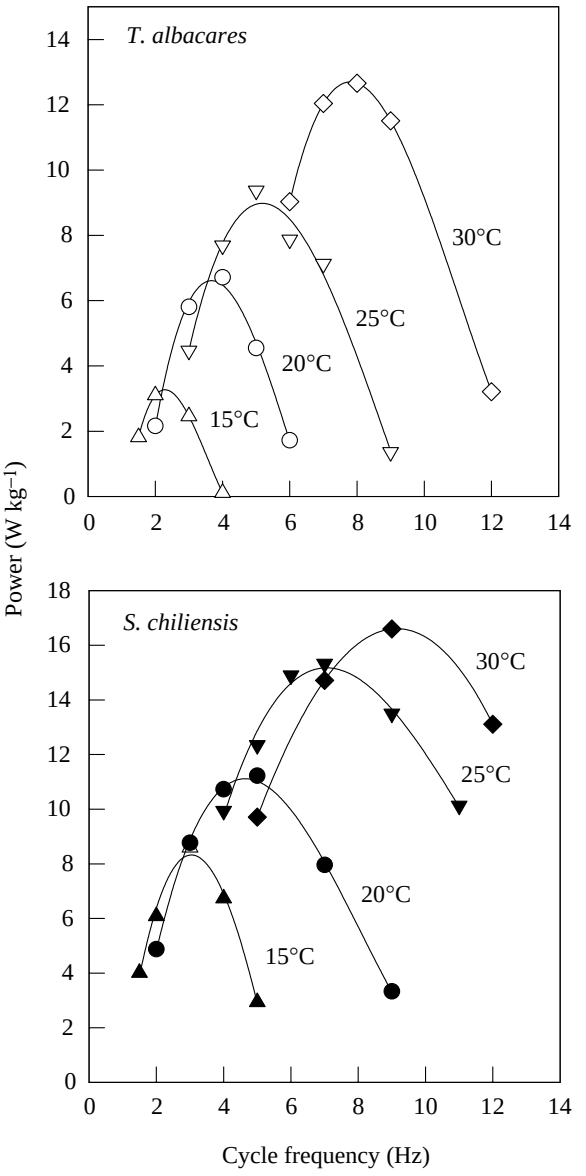

Fig. 4. Representative power *versus* frequency curves for yellowfin tuna *Thunnus albacares* and bonito *Sarda chiliensis*. Lines were fitted by third-order regressions. The bonito preparation was the only one of four to show an increase in power when the temperature was raised to 30 °C but, as with other preparations, power declined rapidly when the high temperature was maintained.

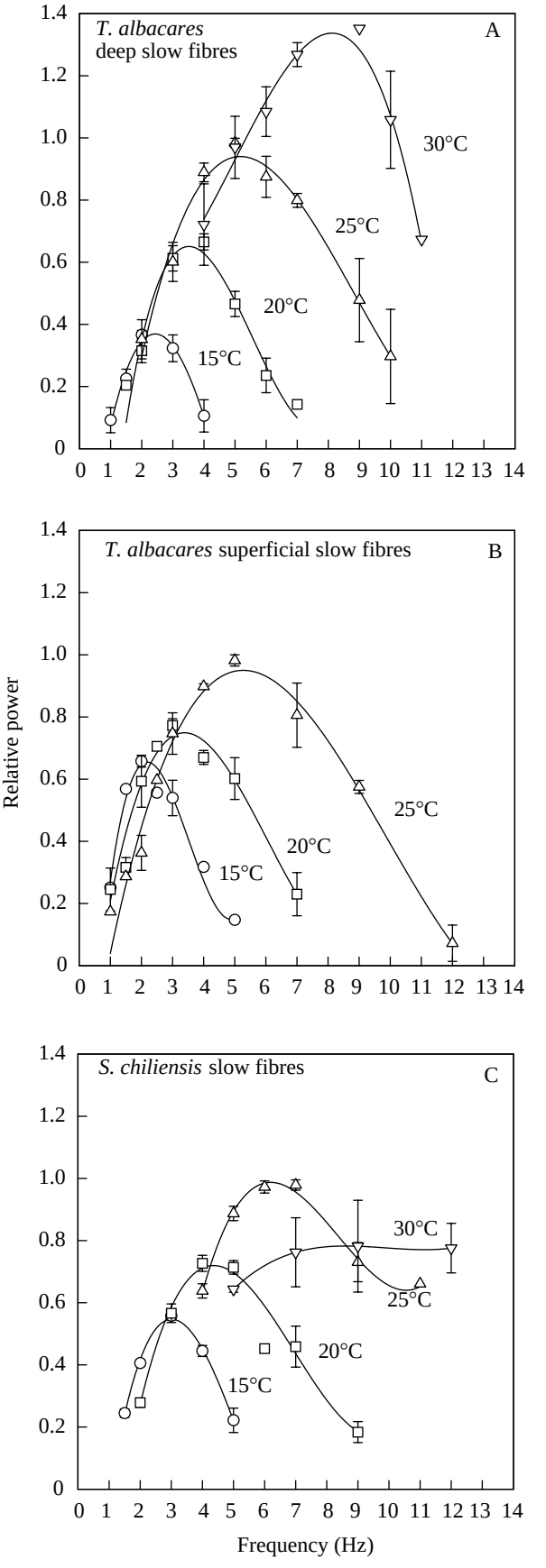

Fig. 5. Relative power *versus* frequency curves for (A) deep (N=5) and (B) superficial slow fibres (N=4) from the yellowfin tuna *Thunnus albacares* and slow fibres (C) from the bonito *Sarda chiliensis* (N=4). Data are presented as means ± S.E.M. Power is normalized to maximum power at 25 °C.

tuna, and a similar f_{opt} was found for the two species. However, the bonito had a significantly higher length-specific swimming speed (and stride length), suggesting that there may be differences in their kinematics. During feeding bursts, the tailbeat frequency of bonito exceeded 12 Hz. No evidence was found in this simple analysis for a substantial acclimation response: there was a small but significant difference in tailbeat frequency (and hence stride length, $P<0.05$, *t*-test) but not in swimming speed between 18 °C- and 24 °C-acclimated tuna.

Discussion

Twitch kinetics

Change in kinetics with muscle position along the body

A number of other studies have shown that muscle contraction kinetics slow from anterior to posterior along the length of many fish. Wardle *et al.* (1995) and L. Hammond, J. D. Altringham and C. S. Wardle (in preparation) have discussed possible reasons for this trend.

Change in twitch kinetics with muscle depth

Twitch kinetics tended to become faster with increasing depth at 0.65 *BL* in the tuna (Fig. 2). If twitch kinetics become slower in an anterior to posterior direction (Fig. 2), as many studies have now shown, then depth and position along the body will interact in a complex way to determine twitch kinetics, produced by the complex three-dimensional myotomal structure (Alexander, 1969). Changes in kinetics due to sampling from different myotomes are unlikely to be more than a small component of the observed changes with depth. Jayne and Lauder (1995) have shown in largemouth bass that the propagation of muscle activity is by the sequential activation of myotomes, but that not all of a particular myotome is necessarily active: different zones within the fast fibre population may be activated independently. We know too little about any particular fish to propose a mechanism for how this is achieved or to give it functional significance, but regional variations in mechanical properties must be very important to an understanding of swimming, and require further study.

Temperature sensitivity of twitch kinetics

Temperature has been shown previously to have a large effect on muscle function in vertebrates (e.g. Bennett, 1984). In the tuna and bonito, both activation and relaxation times were temperature-dependent, and sensitivity was greatest at low temperatures. The muscle of the two species showed a similar temperature sensitivity, with temperature coefficients within the range reported for muscles of other ectothermic animals (reviewed by e.g. Johnston and Altringham, 1989; Rome, 1990). Regional endothermy in the tuna does not appear to have led to major adaptations in muscle kinetics.

Muscle power output and temperature

Maximum power outputs are comparable to values reported for the muscles of other ectotherms at similar temperatures and operating frequencies (for a summary, see James *et al.* 1995, their Fig. 8). This is in agreement with observations on the metabolic properties of yellowfin tuna slow-twitch muscle: citrate synthase activities measured at 20 °C were not significantly different between yellowfin tunas and the Eastern Pacific bonito *S. chiliensis* (Dickson, 1996). The effects of temperature on slow muscle citrate synthase activity in tunas and bonitos have also been shown to have a similar Q_{10} (1.91 for tuna, 1.98 for bonito from 10 to 20 °C). Power output in all muscle preparations was highly temperature-dependent. A counter-current heat exchanger which could maintain a 10 °C temperature differential would typically double the maximum muscle power output and the frequency at which it was obtained (f_{opt}). A similar Q_{10} has been reported for scup slow muscle power output (derived using the work loop technique) (Rome and Swank, 1992). Notably, there is a trend towards increasing temperature sensitivity with increasing depth into the body of the tuna. If the deep muscle has undergone some adaptation to the stable, elevated temperatures, then we might expect to see not only this greater temperature-dependence, but also a more marked difference at the lowest temperatures, and this is indeed the case: the highest temperature coefficients for power output were in the deep, slow muscle of tuna at 15–20 °C. Evidence for an adaptation to a higher operating temperature is seen the ability of deep tuna muscle to function at 30 °C, developing almost 40 % more power than at 25 °C. Slow muscle from bonito showed impaired performance at 30 °C and was to some degree irreversibly damaged. Do these differences reflect a functional adaptation of the deep slow muscle of the tuna for endothermic operation? The geographical distribution of yellowfin tuna takes them into slightly warmer waters than the Eastern Pacific bonito. Yellowfin collected from the same area as the tunas used in this study (the most northern part of their range) have been followed using acoustic telemetry and were found to spend most of their time between the top of the thermocline at 17.5 °C and surface waters of 20 °C (Block *et al.* 1997). The tuna frequently dive for short periods (1–5 min) into the cool waters beneath the thermocline. In warmer regions such as Hawaii, the yellowfin tuna are found between the top of the thermocline and the surface and experience temperatures of 19–28 °C (Holland *et al.* 1992). Surface water temperature where the bonito is thought to occur most often is within the range 16–23 °C. *S. chiliensis* is uncommon north of Point Conception, but has been caught as far north as Alaska, although it is unclear whether these northern occurrences are associated with El Nino events (Yoshida, 1980). Measurements on the superficial fibres of the tuna at 30 °C would have helped to confirm these hypotheses but, for logistical reasons and owing to the limited supply of fish, this was unfortunately not done.

Muscle properties and swimming performance

The frequency for maximum power output, f_{opt}, of slow muscle was higher than the tailbeat frequency of undisturbed cruising tuna, and this, together with the high intrinsic power

output of the large slow muscle mass, suggests that cruising fish have a substantial slow muscle power reserve. This may be sufficient to power significantly higher sustainable swimming speeds, presumably at lower energetic cost than if the intrinsically less efficient fast fibres were recruited. During feeding, the brief, rapid swimming bursts observed (8–12 Hz tailbeat frequency) were presumably driven by fast fibres, but between these bursts tuna swam with a tailbeat frequency of approximately 3 Hz when searching for food, well within the range of slow fibre recruitment (see below). The smaller bonito cruised with a similar tailbeat frequency, but their greater stride length gave them a higher length-specific swimming speed.

From oxygen consumption measurements, Dewar and Graham (1994*a*) calculated that, at 24 °C, a 51 cm (2.2 kg) yellowfin tuna, swimming at 2 *BL* s^{-1} (at a tailbeat frequency of approximately 3.2 Hz, Dewar and Graham, 1994*b*), consumed 0.67 W. Assuming a metabolic efficiency of 50 %, and a mechanical muscle efficiency of 50 %, then this would yield 0.17 W of mechanical power [see recent measurements on fish (Curtin and Woledge, 1993) and mammalian (Barclay, 1994) slow muscle under work loop conditions]. Slow muscle wet mass for yellowfin tuna is 6.5 % of body mass (Graham *et al.* 1983): 0.143 kg in a 2.2 kg fish. From the present study, this mass of slow muscle could produce 30–40 % of maximum power at 3 Hz and 24 °C, or 0.5–0.7 W. Thus, only 50–65 % of the slow muscle would be needed to power swimming at 2 *BL* s^{-1}, and at this swimming speed the slow muscle is working well below its f_{opt} (Figs 4, 5). By recruiting more of the slow muscle, at higher tailbeat frequencies, significantly higher swimming speeds should be possible using only slow muscle. The fish used in the present study were larger than those used by Dewar and Graham (1994*a*,*b*) (mean lengths 0.67 and 0.48–0.53 m respectively: a difference in mass of more than twofold). At a given length-specific speed, tailbeat frequency in the smaller fish is approximately 40 % higher, and this is probably reflected in faster muscle kinetics and a shift to a higher f_{opt} (e.g. Altringham and Johnston, 1990*b*; Anderson and Johnston, 1992), which would perhaps increase the power reserve of slow muscle still further. Electromyographic studies, to determine the swimming speed at which the different fibre types are recruited, would help validate these calculations.

The above discussion assumes that the slow fibres operate *in vivo* under conditions which yield maximum power output, and that *in vivo* strain is ±5 % l_0. Neither of these assumptions can be verified directly as yet, but they are consistent with many published studies on other fish species, as discussed above.

Swimming speeds

One hypothesis for heat retention in the slow muscles of tunas is that warming of the locomotory muscles permits increases in maximum sustained and burst swimming speeds, thus aiding in the search for prey in the patchy oceanic environment. Testing this is difficult because of the problems of measuring swimming speeds of free-swimming tunas. Although direct telemetry of swimming speed has not been accomplished on any tuna species, improved acoustic tracking, with regular global positions *via* satellite to a ship, provides a reasonable means of assessing average swimming speeds. Recent data on small tunas indicate mean swimming speeds for cruising ranging from 0.5 to 1.0 *BL* s^{-1} and maximum sustained speeds of up to 3.5 *BL* s^{-1} for over an hour (Block *et al.* 1997). Telemetry studies on blue marlin (Block *et al.* 1992), which lack central or lateral heat exchangers, indicate sustained speeds similar to the slower speeds observed for the yellowfin tuna, but the higher continuous speeds were not observed. It remains possible that the observed differences are due to the warming of the slow-twitch muscles. Tunas in captivity have displayed remarkable bursts of speed that are estimated to range up to 8 *BL* s^{-1} (B. A. Block, unpublished observation), and comparisons with ectothermic taxa such as bonito should be possible.

Regional endothermy in tuna

The entire *Thunnus* clade is hypothesized to have radiated from a pantropical distribution to a more temperate and subpolar niche (Collette, 1978; Sharp and Pirages, 1978). Under this scenario, warming of the slow-twitch muscles would have evolved in a warm tropical ocean. Recent studies suggest inconsistencies between molecular and morphological phylogenies. Morphologists have separated tunas into a warm-water clade consisting of of the yellowfin tuna (*T. albacares*), blackfin tuna (*T. tonggol*) and longtail tuna (*T. atlanticus*), which possess both central and lateral heat exchangers in the slow muscle. The cold-water clade includes the bigeye tuna (*T. obesus*), albacore (*T. alalunga*), northern bluefin (*T. thynnus*) and southern bluefin (*T. maccoyii*), which have either lost or reduced the central heat exchanger (Gibbs and Collette, 1967; Collette, 1978). The changes in vascular retia among the two subgroups of tunas are paralleled by a shift in their latitudinal distribution patterns (Graham, 1975; Sharp, 1978). The yellowfin, blackfin and longtail tunas occur in subtropical and tropical waters, primarily in warmer waters above the thermocline (Carey and Olson, 1982; Holland *et al.* 1990; Block *et al.* 1997), whereas the bluefins, albacore and big-eye tunas have extended their range to cooler waters at higher latitudes or below the thermocline (Carey and Teal, 1969; Laurs *et al.* 1978; Block *et al.* 1997). The thermal excess for yellowfin tuna (2.2–6 kg) ranges from 1.4 to 4 °C (Dizon and Brill, 1979; Dewar *et al.* 1994), but in northern bluefin (220 kg) has been reported to be up to 21 °C (Carey and Lawson, 1973).

The major distinction between morphological and molecular phylogenetic analyses is that genetic studies indicate that the cold-water tunas (albacore, bluefins, big-eye) are earlier offshoots of the *Thunnus* radiation than the warm-water tunas (Sharp and Pirage, 1978; Block *et al.* 1993; Finnerty and Block, 1995; Chow and Kishino, 1995; Bremer *et al.* 1997). These data imply that the most recent common ancestor of *Thunnus* evolved endothermy in cold seas. Albacore, big-eye and bluefin have a wider thermal niche (7–25 °C) relative to the warm tropical tunas. From the results presented here, it is

clear that the evolution of mechanisms for retaining metabolic heat in slow muscles would have enabled these species to improve their muscle power output and contraction frequency when foraging or migrating in cooler waters. We have shown here that the retention of these mechanisms in the slow muscle of the yellowfin tuna, which occurs in waters that range from 17.5 to 28 °C, would also provide an increase in muscle performance.

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