J. exp. Biol. **111**, 171–177 (1984)
Printed in Great Britain © *The Company of Biologists Limited 1984*

POWER OUTPUT AND FORCE-VELOCITY RELATIONSHIP OF RED AND WHITE MUSCLE FIBRES FROM THE PACIFIC BLUE MARLIN (*MAKAIRA NIGRICANS*)

BY IAN A. JOHNSTON* AND JOHN SALAMONSKI

Pacific Gamefish Foundation, PO Box 3189, Kailua-Kona, Hawaii, 96740, U.S.A.

Accepted 12 March 1984

SUMMARY

Single white fibres and small bundles (two to three) of red fibres were isolated from the trunk muscle of Pacific Blue Marlin (50–121 kg body weight). Fibres were chemically skinned with 1 % Brij. Maximum Ca^{2+}-activated force production (P_o) was 57 kN m^{-2} for red fibres and 176 kN m^{-2} for white fibres at 25 °C. The force-velocity (P-V) characteristics of these fibres were determined at 15 and 25 °C. Points below 0·6 P_o on the P-V curve could be fitted to a linear form of Hill's equation. The degree of curvature of the P-V curve was similar at 15 and 25 °C (Hill's constant $a/P_o = 0{\cdot}24$ and 0·12 for red and white fibres respectively). Extrapolated maximum contraction velocities (V_{max}) were 2·5 muscle lengths s^{-1} ($L_o\,s^{-1}$) (red fibres) and 5·3 $L_o\,s^{-1}$ (white fibres) at 25 °C. $Q_{10(15–25\,°C)}$ values for V_{max} were 1·4 and 1·3 for red and white fibres respectively. Maximum power output had a similar low temperature dependence and amounted to 13 W kg^{-1} for red and 57 W kg^{-1} for white muscle at 25 °C. The results are briefly discussed in relation to the locomotion and ecology of marlin.

INTRODUCTION

Billfishes are large pelagic fish that range throughout the world's temperate and tropical seas. Carey & Robison (1981) studied the vertical migrations of Atlantic and Pacific populations of swordfish *Xiphius gladius* using acoustic telemetry. Swordfish occur in surface waters at night, descending to depths of up to 600 m during the day. During these vertical migrations, individuals may experience changes in water temperature of up to 19 °C in under 2 h (Carey & Robison, 1981). A specialized heat producing tissue has been reported to be associated with one of the eye muscles of *Xiphius* (Carey, 1982*a*). This tissue, which is rich in mitochondria, is supplied with blood by a highly developed rete mirabile. Since the venous and arterial flows in the retial vessels are in opposite directions, metabolic heat is conserved, warming the brain and eye by up to 10–14 °C above ambient (Carey, 1982*a*). It has been suggested

* Usual address: Department of Physiology, University of St Andrews, St Andrews, Fife, Scotland, KY16 9TS, U.K.

Key words: Skeletal muscle, fish, contractile properties.

that such heat-exchanges maintain brain temperatures during vertical migration stabilizing the various sensory and integrative functions and learned behaviour associated with prey capture. Similar structures are found associated with the brains of other billfishes including sailfish (*Istiophorus platupterus*), shortbilled spearfish (*Tetrapturus angustirostris*) and blue marlin (*Makaira nigricans*).

In addition to the retial vessels in the brain, tuna and lamnid sharks possess heat exchangers in the red body muscles (Carey & Teal, 1969*a*,*b*). These function to conserve metabolic heat during activity establishing a temperature gradient of up to 10 °C between the deep red muscle and the surface of the body (Stevens & Neill, 1978). Comparable heat-exchangers are not found in the muscles of billfish and their swimming muscles operate close to ambient temperatures (Carey, 1982*b*).

The present study describes the effects of temperature on the power output and force-velocity relationship of red and white fibres isolated from the trunk muscles of the Pacific blue marlin (*Makaira nigricans*).

MATERIALS AND METHODS

Fish

Pacific blue marlin (*Makaira nigricans* Wakiya), (14 fish; 50–121 kg body weight) were obtained during the 25th Hawaiian International Bill Fish Tournament held at Kailua-Kona, Hawaii, during August 1983. Muscle biopsies were taken from freshly caught fish (14–45 min) and transferred on ice to the nearby Pacific Gamefish Foundation Research Laboratories.

Single white fibres, and small bundles (two to three) of red fibres, were dissected under silicone oil (BDH MS 550) using a high power binocular microscope. The sample sites are shown in Fig. 1. Fibre segments 2–3 mm length (50–120 μm diameter) were transferred directly to the apparatus using jewellers' forceps. The thin coating of oil helps prevent dehydration during the 15–30 s required to transfer and mount fibres. Fibres were attached to two small glass hooks using Perspex acetone glue (Altringham & Johnston, 1982). The apparatus consisted of three water-jacketed Perspex chambers (1 ml capacity) containing respectively skinning, relaxing and activating solutions. The basic relaxing solution contained 20 mM-imidazole-HCl, 110 mM-KCl, 3 mM-$MgCl_2$, 5 mM-EGTA [ethylene glycol bis-(β-aminoethylether

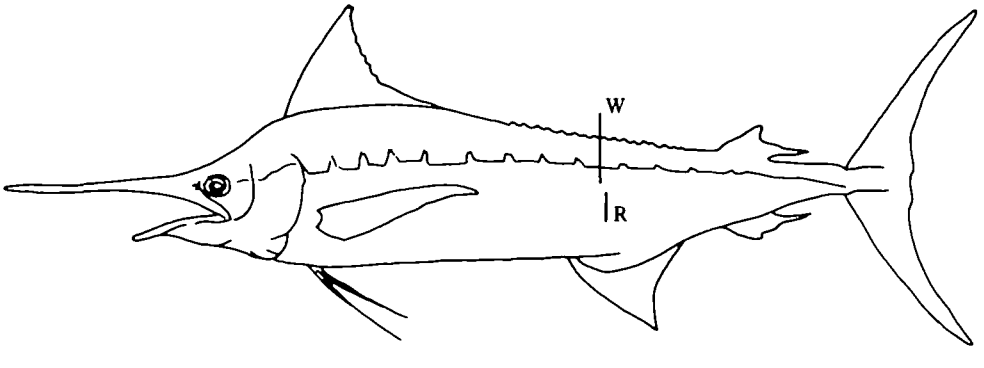

Fig. 1. Sample sites for isolation of bundles of red (R) and white (W) muscle fibres.

N^1 tetra acetic acid), 10 mM-phosphocreatine, 2·5 mM-ATP, 20 units ml^{-1} creatine phosphokinase, pH 7·2 at 20 °C. Skinning solution was prepared by addition of 1 % Brij 58 (polyoxyethylene 20 cetyl ether), a non-ionic detergent, to relaxing solution. Activating solutions were prepared by addition of a 1 M volumetric solution of $CaCl_2$ to relaxing solution to give a final concentration of 4·0–5·0 mM. Ionic compositions were determined using an iterative computer programme written in BBC Basic based on that described by Fabiato & Fabiato (1979). The programme was modified to correct for changes in ionic composition with alterations in temperature and pH. Ionic strength of activating solution was 0·17 M, free concentrations of Ca^{2+}, Mg^{2+} and MgATP were 5–10 μm, 0·5–0·54 mM and 2·3–2·5 mM respectively. (Stability constant CaEGTA/Ca.EGTA = $8{\cdot}84\times10^{10}$ at 22 °C.) Ca^{2+} concentrations required to give maximum isometric tensions at each temperature were determined in preliminary experiments.

Fibres were chemically skinned *in situ* by a 10-min soak in 1 % Brij. Initial sarcomere length was measured by laser-diffraction and set to 2·3 μm. Muscle length and diameter were measured *in situ* using a high power microscope. Force was measured with a

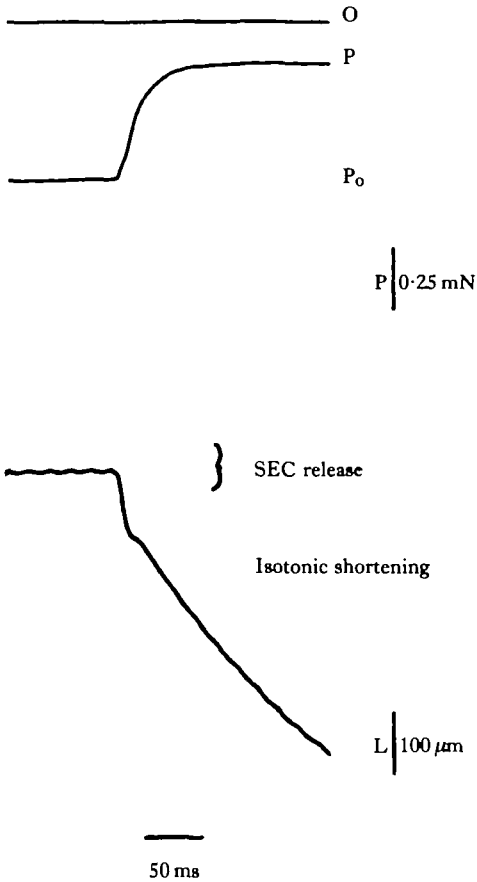

Fig. 2. A typical isotonic release. P, tension; P_o, maximum isometric tension prior to release; O, zero tension; L, muscle length. Mean contraction velocity was determined over the second 50-ms interval following release (SEC release).

silicone beam tension transducer (sensitivity 40 mV mg^{-1}) (Akers AME 801, Horte[illegible] Norway). The force-velocity relationship for maximally activated fibres was determined by giving step tension releases using a low inertia isotonic lever attached to a galvanometer coil (Altringham & Johnston, 1982). Different after-loads were applied to the fibre by increasing the galvanometer coil current. A typical step tension release is shown in Fig. 2. Following each release, fibres were re-extended to their original lengths. Force velocity curves were fitted by Hill's equation (1938) for muscle shortening

$$V(P + a) = b(P_o - P),$$

in which P is force, P_o maximum isometric tension, V the velocity of shortening and a and b are constants.

The maximum load at which maximum power output is achieved was determined directly from Hill's equation and is at a value of force corresponding to $(a^2+aP_o)^{1/2}-a$.

RESULTS

In spite of the large size of the fish, (~4–5 m), the average length of muscle fibres in the region sampled was only around 1 cm (Fig. 1). Superficial white fibres from the dorsal epaxial muscle (Fig. 1) were orientated at angles >80° with the longitudinal axis of the fish. This is substantially greater than has been reported for most other teleosts (Alexander, 1969).

The contractile properties of marlin muscle fibres are summarized in Table 1 and Fig. 3. Maximum Ca^{2+}-activated force production was around three times higher in white than red fibres and similar at 15 and 25 °C. Maximum contraction velocity (V_{max}) was obtained from a linearized form of Hill's equation $(1-P/P_o)/V$ *versus* P/P_o. V_{max} was two times greater for white than red fibres. $Q_{10(15-25\,°C)}$ for V_{max} was 1·4 and 1·3 for red and white fibres respectively. Values for Hill's constant a/P_o and b (Table 1) are comparable to those obtained for homologous fibre types in other teleosts (Altringham & Johnston, 1982).

Table 1. *Contractile properties of red and white fibres isolated from the myotomal muscle of pacific blue marlin* Makaira nigricans

Parameter	Red fibres		White fibres	
	15 °C	25 °C	15 °C	25 °C
Maximum Ca^{2+}–activated force (P_o, kN m^{-2})	55 ± 6 (11)	57 ± 9 (11)	153 ± 10 (10)	176 ± 21 (13)
V_{max} (L_o s^{-1})	1·8 (9)	2·5 (11)	4·0 (10)	5·3 (10)
a/P_o	0·24	0·23	0·12	0·12
b (L_o s^{-1})	0·43	0·58	0·48	0·64
Load for maximum power output	0·31P_o	0·31P_o	0·25P_o	0·25P_o
Maximum power output (W kg^{-1})	9·7	13·1	37·2	57·2

Number of samples is given in parentheses.

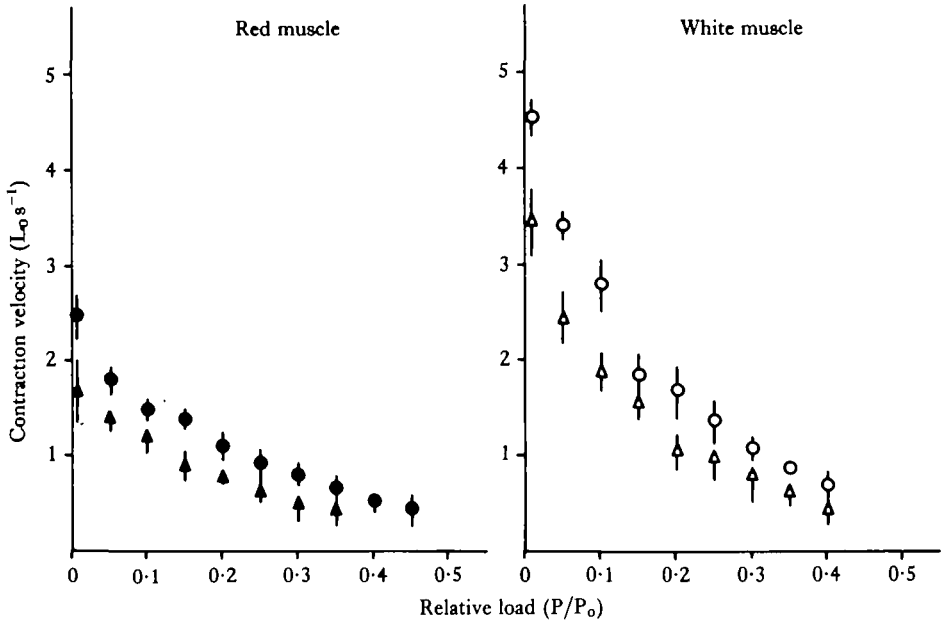

Fig. 3. Force-velocity curves for red and white muscle fibres from Pacific blue marlin at 15 °C (triangles) and 25 °C (circles).

Values for a/P_o were identical at 15 and 25 °C, indicating that the degree of curvature of the force velocity relationship is independent of temperature over this range (Fig. 3). Maximum power output had a similar low temperature dependence, and was around four times higher for white than red fibres.

DISCUSSION

Relatively little is known about patterns of vertical migration in Pacific blue marlin. It is noteworthy, however, that members of the *Xiphiidae, Scombridae* and *Lamnidae* all have heat exchangers associated with their eyes and brain (Carey, 1982*b*). It seems likely that both marlin and tuna experience significant changes in water temperature during migrations from cooler, deeper (~15 °C) to warmer, surface waters (24–28 °C) (Carey & Robison, 1981).

Analyses of stomach contents have shown that marlin are important predators of the fast swimming small tunas, such as the skipjack, *Katsuwonus pelamis* (Carey, 1982*a*). Skipjack tuna are specialized for high speed cruising as evidenced by their streamlined shape, adoption of ram-ventilation and high proportion of red muscle fibres (~33 % in 1 kg fish) which are maintained at above ambient temperatures during intense activity (Stevens & Neill, 1978). The $Q_{10(15-30\,°C)}$ for maximum contraction velocity (V_{max}) of the deep red muscle of tuna (3·1) is substantially higher than for white muscle (2·0) and exceeds that for muscles from a variety of shallow water North Pacific reef fish (1·8–2·1) (Johnston & Brill, 1984). In contrast, marlin muscle is primarily composed of white, fast glycolytic fibres which are adapted for sprint activity. The large body size of marlin (>100 kg) undoubtedly contributes to their success as sprint predators. Marlin have

adopted an alternative thermal strategy to tuna in that they do not maintain elevated muscle temperatures, and selection has favoured contractile proteins which function relatively independently of temperature over the range 15–25 °C.

Maximum power output for marlin white muscle, calculated from the P-V relationship (Table 1) is comparable to values obtained for the white muscle of other relatively athletic fish using other methods. For example, Alexander (1977) applied hydrodynamic equations to the experimental data obtained by Bainbridge (1962) and calculated a mechanical power output for rainbow trout muscle equivalent to 50 W kg^{-1} at 12 °C. The value of 37 W kg^{-1} obtained for marlin is almost certainly an underestimate. Maximum isometric forces obtained from skinned fibres can be as much as 50 % lower than those obtained from intact preparations (Ramsey & Street, 1940; Hellam & Podolsky, 1969) so a value of 74 W kg^{-1} would be more realistic. Since only one half of the musculature is active at any one time, then mean power would be 37 W kg^{-1} at 15 °C, comparable to Alexander's figures. Phosphocreatine concentrations are typically around 20 mmol kg^{-1} for teleost white muscles (Walesby & Johnston, 1980). This would be sufficient for 14·5 s at maximum power output assuming a ΔG° for ATP hydrolysis equivalent to 60 kJ mol^{-1}. Bainbridge (1962) observed that 28 cm trout could swim at 2·8 m s^{-1} . for 1 s, but that slightly longer sprints were rarely faster than 1·5 m s^{-1}. It would appear that phosphocreatine concentrations are more than sufficient to meet the energy requirements involved in very short sprints at maximum speed.

Assuming muscle comprises 75 % of body weight in marlin and that 93 % is composed of white fibres (derived from relative areas of fibre populations in transverse sections of whole fish), the total mechanical power output available is equivalent to 2·8 kW for a 70 kg fish, rising to 20 kW for a 455 kg specimen. Estimated power outputs for marlin and trout white muscle are higher than those calculated for more sluggish species from P-V data. For example, maximum power output for cod white muscle is only equivalent to 16 W kg^{-1} (Altringham & Johnston, 1982). Differences in the intrinsic power outputs of the muscle in these species may contribute to differences in their value as sports fish.

This work would not have been possible without the enterprise and enthusiastic support of Dr Charles Daxboeck, Scientific Director of the Pacific Gamefish Foundation. We are also grateful to the Governors of the Pacific Gamefish Foundation and the Hawaiian International Billfish Angling Tournament for their support and cooperation. Dr Ian A. Johnston gratefully acknowledges receipt of the Royal Society John Murray Travelling Studentship in Oceanography and Limnology for 1983.

John Salamonski thanks the Russell Trust, the Company of Biologists and the Dale Fund of The Physiological Society for travel grants.

This work was supported by grant GR3/4936 from the Natural Environment Research Council.

REFERENCES

ALEXANDER, R. MCN. (1969). The orientation of muscle fibres in the myomere of fishes. *J. mar. biol. Ass. U.K.* **49**, 263–290.

ALEXANDER, R. MCN. (1977). Swimming. In *Mechanics and Energetics of Animal Locomotion*, (eds R. McN. Alexander & G. Goldspink), pp. 222–248. London: Chapman & Hall.

[illegible]RINGHAM, J. D. & JOHNSTON, I. A. (1982). The pCa-tension and force-velocity characteristics of skinned fibres isolated from fish fast and slow muscles. *J. Physiol., Lond.* **333**, 421–449.

BAINBRIDGE, R. (1962). Training, speed and stamina in trout. *J. exp. Biol.* **39**, 537–555.

CAREY, F. G. (1982*a*). A brain heater in the swordfish. *Science, N.Y.* **216**, 1327–1329.

CAREY, F. G. (1982*b*). Warm fish. In *A Companion to Animal Physiology*, (eds C. R. Taylor, K. Johansen & L. Bolis), pp. 216–233. Cambridge, New York, Melbourne: Cambridge University Press.

CAREY, F. G. & ROBISON, B. H. (1981). Daily patterns of swordfish observed by acoustic telemetry. *Fishery Bull. Fish Wildl. Serv. U.S.* **79**, 277–292.

CAREY, F. G. & TEAL, J. M. (1969*a*). Mako and porbeagle: warm bodied sharks. *Comp. Biochem. Physiol.* **28**, 199–204.

CAREY, F. G. & TEAL, J. M. (1969*b*). Regulation of body temperature by the bluefin tuna. *Comp. Biochem. Physiol.* **28**, 205–213.

FABIATO, A. & FABIATO, F. (1979). Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *J. Physiol., Paris* **75**, 463–505.

HELLAM, D. C. & PODOLSKY, R. J. (1969). Force measurements in skinned muscle fibres. *J. Physiol., Lond.* **201**, 49–59.

HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B* **126**, 136–195.

JOHNSTON, I. A. & BRILL, R. (1984). Thermal dependence of contractile properties of single skinned muscle fibres isolated from Antarctic and various warm-water marine fishes including skipjack tuna (*Katsuwonus pelamis*) and Kawakawa (*Euthynuus affinis*). *J. comp. Physiol.* (in press).

RAMSEY, R. W. & STREET, S. F. (1940). Isometric length-tension diagrams of isolated skeletal muscle fibres of the frog. *J. cell. comp. Physiol.* **15**, 11–34.

STEVENS, E. D. & NEILL, W. H. (1978). Body temperature relations of tunas especially skipjack. In *Fish Physiology*, Vol. 7, (eds W. S. Hoar & D. J. Randall), pp. 316–359. New York: Academic Press.

WALESBY, N. J. & JOHNSTON, I. A. (1980). Temperature acclimation of brook trout muscle. Adenine nucleotide concentrations, phosphorylation state and adenylate energy charge. *J. comp. Physiol.* **139**, 127–133.