

## ONTOGENETIC CHANGES IN CHARACTERISTICS REQUIRED FOR ENDOTHERMY IN JUVENILE BLACK SKIPJACK TUNA (*EUTHYNNUS LINEATUS*)

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### Summary

To characterize better the development of endothermy in tunas, we assessed how the abilities to generate heat and to conserve heat within the aerobic, slow-twitch (red) myotomal muscle using counter-current heat exchangers (retia) change with size in juvenile black skipjack tuna (*Euthynnus lineatus*) above and below the hypothesized minimum size for endothermy of 207 mm fork length (FL). Early juvenile scombrids (10–77 mm FL) collected off the Pacific coast of Panama were raised to larger sizes at the Inter-American Tropical Tuna Commission Laboratory at Achotines Bay, Panama. Evidence of central and lateral rete blood vessels was found in *E. lineatus* as small as 95.9 mm FL and 125 mm FL, respectively. In larger *E. lineatus* juveniles (up to 244 mm FL), the capacity for heat exchange increased with fork length as a result of increases in rete length, rete width and the number of vessel rows. The amount (g) of red muscle increased exponentially with fork length in both *E. lineatus* (105–255 mm FL) and a closely related ectothermic species, the sierra Spanish mackerel *Scomberomorus sierra* (151–212 mm FL), but was greater

in *E. lineatus* at a given fork length. The specific activity (international units g<sup>-1</sup>) of the enzyme citrate synthase in red muscle, an index of tissue heat production potential, increased slightly with fork length in juvenile *E. lineatus* (84.1–180 mm FL) and *S. sierra* (122–215 mm FL). Thus, total red muscle heat production capacity (red muscle citrate synthase activity per gram times red muscle mass in grams) increased with fork length, primarily because of the increase in red muscle mass. Below 95.9 mm FL, *E. lineatus* cannot maintain red muscle temperature ( $T_m$ ) above the ambient water temperature ( $T_a$ ) because juveniles of this size lack retia. Above 95.9 mm FL, the relationship between  $T_x$  ( $T_m - T_a$ ) and FL for *E. lineatus* diverges from that for the ectothermic *S. sierra* because of increases in the capacities for both heat production and heat retention that result in the development of endothermy.

Key words: Scombridae, tuna, *Euthynnus lineatus*, *Scomberomorus sierra*, counter-current heat exchanger, development, endothermy, fish, growth, muscle, ontogeny, retia, scaling, temperature.

### Introduction

Tunas (Family Scombridae) are the only teleost fishes known to maintain the temperature of the slow-twitch, oxidative (red) locomotor muscle ( $T_m$ ) significantly above the ambient water temperature ( $T_a$ ) using metabolically derived heat. They have evolved vascular counter-current heat exchangers (retia mirabilia) to conserve heat generated by myotomal muscle contraction and to limit convective heat loss at the gills (for reviews, see Stevens and Neill, 1978; Bushnell et al., 1992). As a consequence of swimming constantly to ram ventilate and to maintain hydrostatic equilibrium (Magnuson, 1973; Roberts, 1978), tunas produce heat continuously within their red myotomal muscle fibers, which are located in a more axial and anterior position than in other teleosts (Kishinouye, 1923; Carey, 1973; Graham and Dickson, 2000). Thus, tunas have both a metabolic source of heat and a mechanism to retain that heat, the two requirements for endothermy.

Dickson (1994) showed that black skipjack tuna (*Euthynnus lineatus*) longer than 207 mm fork length (FL) can elevate  $T_m$  at least 3 °C above  $T_a$ . On the basis of the presence of axial red muscle and retia in juveniles of this size and the fact that similar muscle temperature measurements from known ectothermic fishes are all  $\leq 2.7$  °C above  $T_a$ , it was hypothesized that the minimum size for endothermy in *E. lineatus* is approximately 207 mm FL and 163 g (Dickson, 1994). The objective of the present study was to gain a better understanding of the ontogeny of endothermy in juvenile black skipjack tuna by determining how the capacities of red muscle to produce heat and of the retia to conserve heat change with size in this species. Myotomal muscle characteristics were also examined in an ectothermic scombrid species, the sierra Spanish mackerel *Scomberomorus sierra*, to focus on differences related to endothermy. It would have been

preferable to have used a member of the sister taxon of the tunas, the bonitos, for this purpose, but none of the appropriate size range was available to us.

### Materials and methods

#### *Fish collection and culture*

Scombrid fishes were raised to various sizes at the Inter-American Tropical Tuna Commission (IATTC) laboratory at Achotines Bay, Republic of Panama, as described previously (Dickson, 1994). Post-flexion larvae and early juveniles (10–30 mm *FL*) of the black skipjack tuna *Euthynnus lineatus* (Kishinouye, 1920) and of the ectothermic scombrid *Scomberomorus sierra* (Jordan and Starks, 1895) were collected by night-lighting. Larger *S. sierra* juveniles (47.8–76.9 mm *FL*) were collected by beach seine. Immediately after capture, fish were placed in polyethylene bags filled with oxygenated sea water and were transported to the IATTC laboratory, where they were held in running, filtered, aerated sea water in outdoor aquaria protected by a roof, and were exposed to natural light:dark cycles. Water temperature, which was not controlled, ranged from 24.3 to 29.1 °C, but did not vary by more than 2 °C during a given day.

Growth rates while in captivity were determined for groups of *E. lineatus* and *S. sierra* collected in November and December 1995 and held at a water temperature of 27.4±0.5 °C (mean ± s.d.; range 26.0–28.5 °C). These *E. lineatus* juveniles were fed wild zooplankton *ad libitum* during the first 1–6 days in captivity, supplemented once with lipid-enriched brine shrimp nauplii, and finely chopped adults or live embryos and yolk-sac larvae of the viviparous poeciliid fishes *Poecilia latipinna* and *Poeciliopsis turubarensis*, while being held in 1.2 m diameter, 300 l or 1.5 m diameter, 900 l fiberglass tanks. When they reached sizes of 30–40 mm *FL*, the tuna were transferred to a 3.6 m diameter, 8200 l or a 4.5 m diameter, 12700 l above-ground plastic-lined tank at densities of up to 50 individuals per tank. The *S. sierra* were held in a 1.5 m diameter, 900 l fiberglass tank, in which they were fed wild zooplankton, prior to transfer to a 3.6 m diameter, 8200 l tank at a density of up to 14 individuals per tank. While in these large tanks, scombrid juveniles were fed chopped or whole fishes to apparent satiation, up to 10 times per day for *E. lineatus* and up to four times per day for *S. sierra*. Until the tuna reached a size of approximately 100 mm *FL*, a mixture of Healthy Mix powdered vitamins, bile powder and/or cod liver oil was added to their food, usually once per day. At each feeding, chopped or whole fishes approximating 5–10% of the total wet mass of the scombrids in a given tank was offered to that tank in several samples. The fish rapidly consumed most, but usually not all, of the food, and the excess was removed within 30 min of feeding. Because the fish attacked the food in a group and consumed it rapidly, and because individuals were not marked, it was not possible to quantify the amount of food that each individual fish ate. Although typical food consumption rates of juvenile scombrids of this size range are not known, those in the present study are almost certainly greater than those in the wild.

Throughout the growth period, mortality rates for the scombrids were high, often as a result of fish hitting the sides of the tank during high-speed swimming bursts when they were startled (K. A. Dickson and M. H. Hansen, personal observation). Dead fish collected within 10 h and individuals used in swimming performance experiments constituted the samples for growth rate calculations. Fish fork length was measured to the nearest 0.1 mm with calipers for individuals up to 150 mm *FL*, and to the nearest 1 mm with a ruler in larger fish. Fish wet mass was measured to the nearest 0.01 g with a top-loading balance. Fish were then either fixed in 10% phosphate-buffered formalin or frozen, and subsequently transported to California State University Fullerton (CSUF). The rate of growth in length during the period of captivity for individual juvenile *E. lineatus* and *S. sierra* was estimated as (final fork length minus estimated fork length at capture)/(days in captivity), which assumes that growth in length is linear during that period. The mean fork length of individuals that did not survive capture and transfer to the laboratory, measured within 12 h of capture and prior to fixation, was used as an estimate of fork length at capture. All *E. lineatus* in the group used for growth rate determinations were caught on one night. The *S. sierra* were from several batches of night-lighted fish, all approximately the same size at capture.

Many of the individuals in the groups used to estimate growth rates, in addition to others collected previously (Dickson, 1994), were used to assess ontogenetic changes in the capacity for heat conservation and heat production.

#### *Heat conservation*

The counter-current heat-exchange system perfusing the red muscle of *E. lineatus* consists of a pair of small lateral retia branching from the one lateral subcutaneous artery (LA) and to the one lateral subcutaneous vein (LV) on each side of the body, and a large central rete, within the vertebral hemal arch, which branches from the dorsal aorta (DA) and empties into the post-cardinal vein (PCV) (Graham, 1973). In adult *E. lineatus*, the blood vessels of the retia have been characterized as arterioles and venules on the basis of vessel wall ultrastructure and vessel diameters (Moore, 1998). In the present study, 16 juvenile *E. lineatus* (91.2–244 mm *FL*, 9.0–264.5 g) were examined to determine at what size central and lateral rete blood vessels first appear and how rete dimensions change with fish size. The following rete dimensions were measured: rete length, which affects the magnitude of the gradient that can be maintained between the internalized muscle and water temperature, rete width and the number of rows of rete vessels, which, together with rete length, provide information about the relative surface area for heat exchange within each rete, and rete arteriole and venule diameters.

Transverse sections (3–5 mm thick) of formalin-fixed fish were removed from a number of positions along the body. These were dehydrated in a graded ethanol series, cleared in xylenes and embedded in paraplast by commercial laboratories (Pathology Diagnostics and A Cut Above Histology, both in

San Diego, CA, USA, and Professional Histopathology Services, Anaheim, CA, USA). In addition, to obtain cross sections of the rete blood vessels, segments of central and lateral retia were embedded. Tissue sections (10–15  $\mu\text{m}$  thick) were cut with a microtome, mounted on glass slides and stained with hematoxylin and eosin using standard histological techniques (Humason, 1979). Measurements were made either directly from slides, with a microscope and an ocular micrometer, or with NIH Image software from images captured with a video camera interfaced to a Macintosh computer via a RasterOps 24STV digitizing card. No correction for the shrinkage that occurs during fixation and tissue processing was made.

To identify a putative rete, juxtaposed arterioles and venules had to be present in transverse sections of the retia; the arterioles are distinguished by thicker, more muscular walls. In addition, the criteria of Dickson (1994) were applied to fish transverse sections. For a central rete, several blood vessels in the hemal canal above the DA and PCV, at least one arterial vessel branching from the DA, and at least one venous vessel emptying into the PCV had to be observed. The criteria for a putative lateral rete were the presence of at least one branch from the LA to the red muscle and at least one venous vessel from the red muscle to the LV.

If present and visible in fish transverse sections, central rete length was measured as the distance from the dorsal margin of the DA along the rete vessels to the ventral margin of the red muscle. Lateral rete length was measured from the axial margin of the LA and LV along the rete vessels to the lateral border of the red muscle (see Fig. 1 of Dickson, 1994). Rete widths across all rows of rete vessels, the number of rows of vessels in each rete, and rete arteriole and venule diameters were measured in samples in which the entire rete was sectioned to show blood vessel cross sections. Because the rete arterioles have thicker walls than do the venules, both inner and outer arteriole diameters were measured, but only inner diameter was measured for the venules. For all diameters, two

perpendicular measurements were taken and averaged, and as many as five rete arterioles or venules were measured. In statistical analyses, the means of these multiple measurements, weighted by the inverse of the standard error, were used (Kleinbaum et al., 1988).

#### Heat production

The capacity of juvenile tunas to produce heat for endothermy was assessed by quantifying the amount of red myotomal muscle and the red muscle citrate synthase enzyme activity, an index of red muscle aerobic metabolic capacity.

Transverse sections of individual formalin-fixed or frozen *E. lineatus* and frozen *S. sierra* were removed from various positions along the body. Formalin-fixed sections were prepared as described above. Frozen fish were simply cut into sections while partially frozen. A digital image of each transverse section (Fig. 1) and an appropriate scale (from a stage micrometer or a ruler) for each image were captured to computer as described above. NIH Image software was used to quantify the total cross-sectional area and the area of red myotomal muscle in each section, so that the percentage cross-sectional area composed of red muscle (%RM) could be calculated. In 20 juvenile *E. lineatus* (54.8–288 mm FL, 1.6–418.1 g), %RM was measured at approximately 45–50 % FL, the position where the maximum amount of red muscle occurs. For four *E. lineatus* (105–255 mm FL, 14.9–306.3 g) and five *S. sierra* (151–212 mm FL, 33.2–101.6 g), whole frozen fish were cross-sectioned every 5% of FL along the body beginning at the operculum and ending at 80% of FL near the caudal peduncle. In each section, the area of red muscle and %RM were determined, and the area under the curve of red muscle area ( $\text{mm}^2$ ) versus position along the body (mm) was used as an estimate of total red muscle mass in each fish, by converting  $\text{mm}^3$  to  $\text{cm}^3$  and multiplying by estimates of density from Magnuson (1973).

The specific activity of the citric acid cycle enzyme citrate synthase (CS) correlates with tissue mitochondrial density and

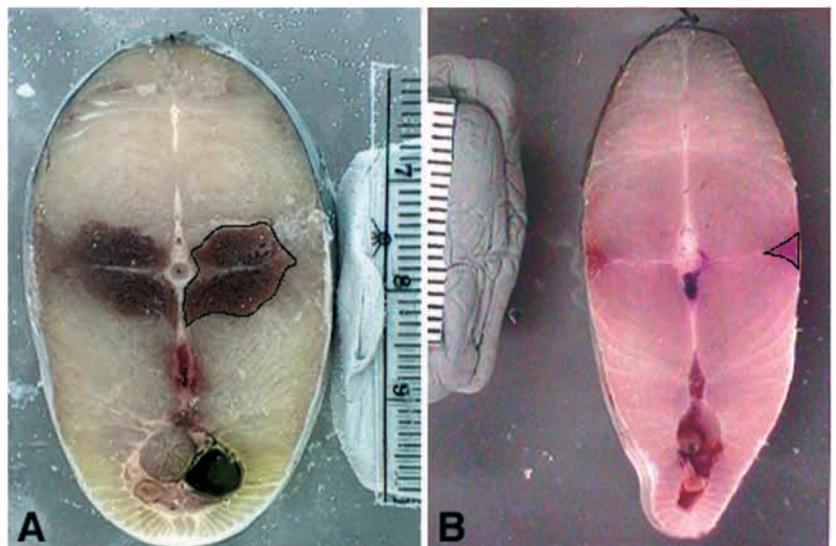


Fig. 1. Transverse sections at a position along the body corresponding to 45% of fork length (FL) in (A) a 201 mm FL juvenile black skipjack tuna *Euthynnus lineatus* and (B) a 197 mm FL sierra Spanish mackerel *Scomberomorus sierra* showing the interspecific difference in the position of the slow-twitch, oxidative (red) myotomal muscle (half of which is outlined in black). The smallest divisions on both scales are millimeters. The different colorations are due to the fact that the fish in A had been fixed, whereas that in B had been frozen.

aerobic metabolic capacity. Because heat for endothermy is produced by aerobic metabolism in the red muscle fibers, total red muscle CS activity provides an index of the fish's capacity to generate the heat used to elevate  $T_m$ . Individual *E. lineatus* (84.1–180 mm FL, 7.9–87.0 g,  $N=13$ ) and *S. sierra* (122–215 mm FL, 12.4–93.3 g,  $N=4$ ) juveniles and two adults of each species, or muscle samples removed from them, were frozen on dry ice or in liquid nitrogen immediately after the fish had been killed. A limited number of frozen specimens was obtained because dry ice or liquid nitrogen was not always available at the remote IATTC laboratory site when fish died or were killed. These samples were transported to CSUF on dry ice or in a liquid nitrogen dry shipper and were stored at  $-80^\circ\text{C}$  until spectrophotometric assays were run under saturating substrate concentrations at  $20^\circ\text{C}$  using methods used previously on adult scombrids (Dickson, 1996). Red muscle tissue was removed from frozen samples, homogenized in imidazole-EDTA buffer and centrifuged at  $4^\circ\text{C}$  at  $10\,000g$ , and supernatants were used in CS assays containing  $0.1\text{ mmol l}^{-1}$  acetyl CoA,  $0.5\text{ mmol l}^{-1}$  oxaloacetate,  $0.1\text{ mmol l}^{-1}$  dithio-bis-nitrobenzoic acid,  $2.0\text{ mmol l}^{-1}$   $\text{MgCl}_2$  and  $80\text{ mmol l}^{-1}$  Tris, pH 8.0 at  $20^\circ\text{C}$ . The relationship between total red muscle CS activity and fish fork length was determined by multiplying the relationship between CS activity per gram of red muscle and fish fork length by that between total mass (g) of red muscle and fish fork length.

#### Statistical analyses

Statistical analyses were performed with SPSS version 9.0 or Minitab release 10, using a significance level of  $P=0.05$ . Correlation and regression analyses were used to determine whether the relationship between a given variable and fish fork length was significant. Scaling coefficients that describe the effects of fish mass on a given variable were determined from least-squares best-fit linear regressions of log/log-transformed data. To compare the means of two independent samples or the slopes of two linear regressions, a Student's  $t$ -test or a Mann-Whitney  $U$ -test was used when variances were equal or unequal, respectively. Paired  $t$ -tests were used to compare two non-independent samples.

## Results

### Growth rates

Growth rates during the time in captivity were determined for 65 *E. lineatus* (estimated mean  $\pm$  S.D. size at capture  $15.8\pm 1.7$  mm FL) and for 11 *S. sierra* juveniles (estimated mean  $\pm$  S.D. size at capture  $26.4\pm 4.6$  mm FL). These *E. lineatus* were held for up to 33 days and reached a size of 204 mm FL; the *S. sierra* were held for up to 56 days and grew as large as 219 mm FL. All individuals were assumed to be juveniles on the basis of the size at first maturity of 400 mm total length for *E. lineatus* females in the eastern Pacific (Schaefer, 1987) and 260–320 mm FL for *S. sierra* in Colombia (Collette and Nauen, 1983). When the entire life span is included, growth curves (size versus age) for fishes are usually sigmoidal to hyperbolic

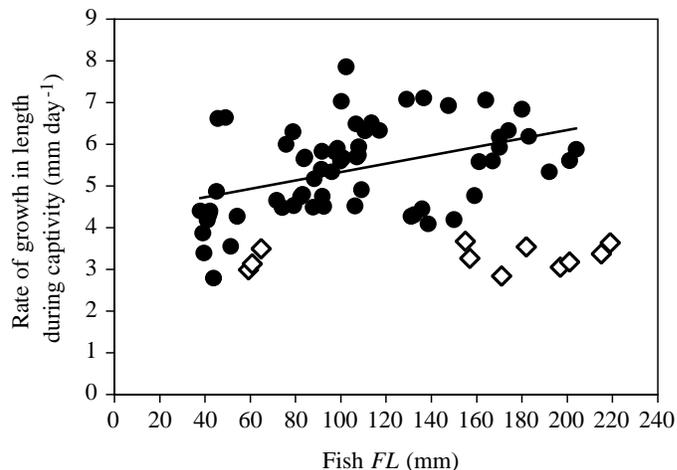


Fig. 2. Rates of growth in length for individual juvenile *Euthynnus lineatus* (37.8–204 mm FL,  $N=65$ ) (filled circles and solid line) and *Scomberomorus sierra* (60.8–219 mm FL,  $N=11$ ) (open diamonds) captured by night-lighting and raised in the laboratory. In *E. lineatus*, there was a significant positive correlation ( $r=0.431$ ,  $P<0.05$ ) between growth rate ( $G$ ,  $\text{mm day}^{-1}$ ) and fish fork length, FL; the best-fitting linear regression is  $G=0.010FL+4.322$ . Growth rate did not vary with fork length in the *S. sierra*.

in shape because they include the slower-growing larvae and adults, with a faster, near-linear growth phase during the juvenile stage (e.g. Ricker, 1979). Because all the fish in the present study were juveniles, the assumption of a linear rate of growth in fork length during the period of captivity is reasonable.

Growth rates during captivity ranged from  $2.79$  to  $7.86\text{ mm day}^{-1}$  ( $5.38\pm 1.05\text{ mm day}^{-1}$ ; mean  $\pm$  S.D.) for *E. lineatus* and from  $2.84$  to  $3.67\text{ mm day}^{-1}$  ( $3.29\pm 0.28\text{ mm day}^{-1}$ ; mean  $\pm$  S.D.) for *S. sierra* (Fig. 2). Individual growth rate did not vary significantly with time in captivity in either species (data not shown), but did correlate significantly ( $P<0.05$ ) with final fish fork length in *E. lineatus* ( $r=0.431$ ,  $P<0.05$ ), but not in *S. sierra* (Fig. 2).

### Heat conservation

The smallest *E. lineatus* in which evidence of central rete vessels was found was 95.9 mm FL. In that specimen, both arterial and venous vessels were detected in rete cross sections at several positions along the body, even though retia were not apparent in transverse sections of fish. The putative central rete vessels were present in two patches, separated by the bone of the vertebra (Fig. 3A). In the next smallest individual available, 91.2 mm FL, no evidence of retia was found. In all individuals examined larger than 95.9 mm FL, a putative central rete was present; in those more than 108.5 mm FL, central rete vessels were also evident in transverse sections of fish. The smallest *E. lineatus* containing putative lateral rete arterial and venous vessels, on the basis of both fish and rete transverse sections, was 125 mm FL, and all specimens above that size that were examined had putative lateral retia. In the

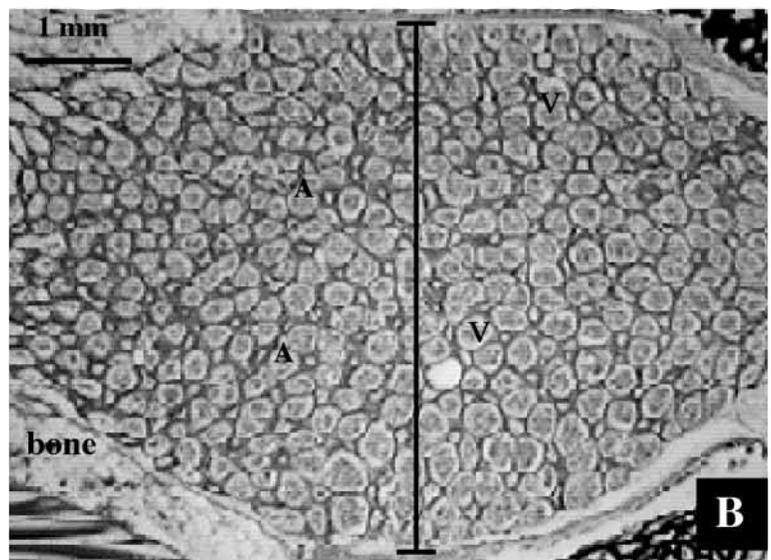
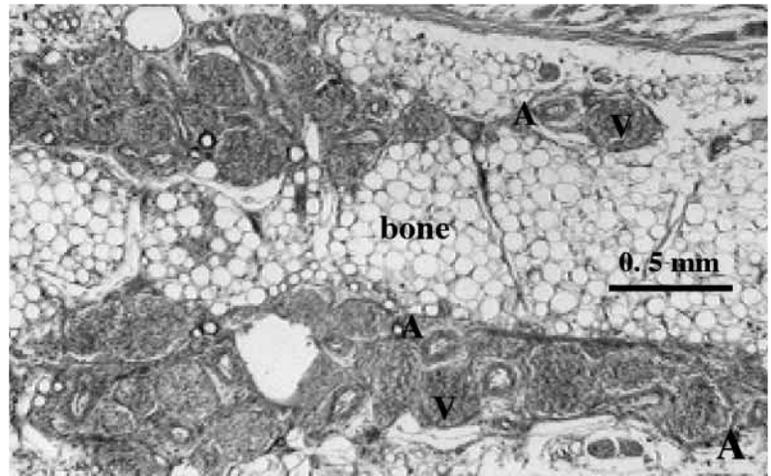


Fig. 3. Putative central retia from juvenile black skipjack tuna *Euthynnus lineatus* sectioned within the body's frontal plane, just ventral to the vertebral centra, to show central rete vessel cross sections. (A) Sample from a 95.9 mm *FL* specimen (the smallest *E. lineatus* with evidence of a putative central rete), taken at a position along the body of 41.7% of fork length (*FL*). (B) Sample from a 182 mm *FL* specimen at 52.7% *FL*. A, rete arterioles (the letter is positioned to the left of the arteriole); V, rete venules. The bone of the vertebra is indicated. The line in B shows how rete width was measured.

next smallest specimen, 117.4 mm *FL*, lateral rete venules, but not arterioles, were found, and in all smaller specimens, from 108.5 mm *FL*, no lateral rete vessels were detected.

The area for heat exchange in both the central rete and the smaller lateral retia increased with fish size in juvenile *E. lineatus* because of significant ( $P < 0.004$ ) increases in length, width and the maximum number of rows of rete vessels (Figs 3, 4). The central rete is wider, with more vessel rows, than the lateral retia. The central rete increased from a maximum of six rows of vessels in the 95.9 mm *FL* individual to 28 rows in the largest specimen examined (244 mm *FL*); the lateral retia increased from one row in the 125 mm *FL* specimen to a maximum of three rows in the four largest individuals (193, 209, 226 and 244 mm *FL*).

The number of both central and lateral rete vessels varied with position along the body in juvenile *E. lineatus*, and this accounts for much of the variability in rete width measurements from individual fish (Fig. 4B). Rete vessel distribution along the body corresponded with the distribution of the red muscle that it perfuses. This is shown for a 159 mm *FL* juvenile *E. lineatus* in Fig. 5 and parallels previous

findings in larger *E. lineatus* and in the skipjack tuna *Katsuwonus pelamis* (Stevens et al., 1974; Graham and Diener, 1978).

Mean rete vessel diameters in juvenile *E. lineatus* were  $17.05 \pm 4.65 \mu\text{m}$ ,  $30.61 \pm 8.82 \mu\text{m}$  and  $53.61 \pm 17.38 \mu\text{m}$  (means  $\pm$  S.D.) for the central rete inner arteriole, outer arteriole and venule, respectively (95.9–244 mm *FL*,  $N=15$ ) and were  $12.17 \pm 3.07 \mu\text{m}$ ,  $23.11 \pm 5.96 \mu\text{m}$  and  $59.96 \pm 13.79 \mu\text{m}$  (means  $\pm$  S.D.) for the lateral rete inner arteriole, outer arteriole and venule, respectively (125–244 mm *FL*,  $N=9$ ). Within each rete type, the venule diameter was significantly greater than both arteriole diameters (paired  $t$ -test,  $P < 0.0001$ ). For each vessel type, diameters in the central and lateral retia did not differ significantly (paired  $t$ -test,  $P > 0.13$ ). Central rete vessel diameters did not vary significantly with fish fork length, but all three diameters measured in the lateral retia increased significantly with fork length (regression analysis,  $P < 0.05$ ).

#### Heat production

Juvenile *E. lineatus* have the axial and anterior red muscle position characteristic of tunas, whereas juvenile *S. sierra* have

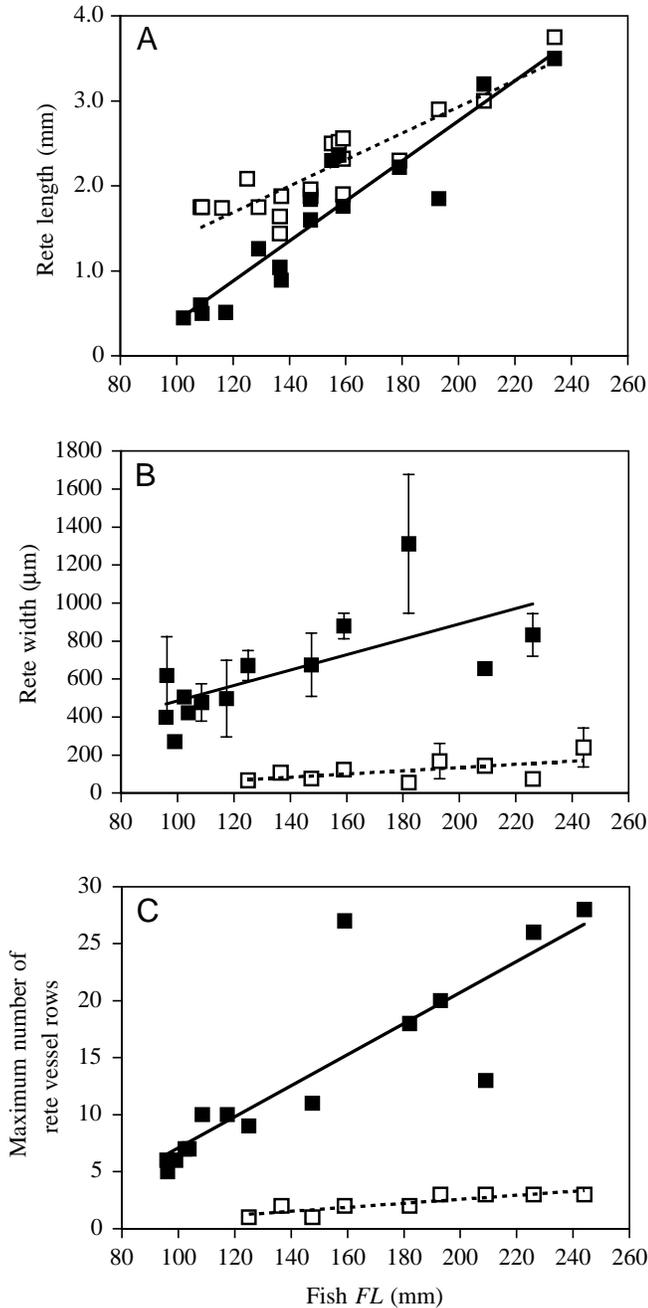


Fig. 4. Dimensions of the central rete (filled squares and solid lines) and lateral retina (open squares and dashed lines) versus fish fork length (FL) in juvenile black skipjack tuna. (A) Rete length (including data from Dickson, 1994); (B) rete width (mean  $\pm$  S.E.M.,  $N=1-4$  samples along the body); (C) maximum number of rete vessel rows. Lines are least-squares linear regressions fitted to the data. All variables increased significantly with fork length.

a red muscle distribution typical of ectothermic teleosts (Figs 1, 6). All *E. lineatus* individuals examined, including the smallest fish of 14 mm FL that was serially sectioned longitudinally, had axially positioned red muscle. The distribution of red muscle along the body in all individuals studied was compared by plotting the percentage of the cross-

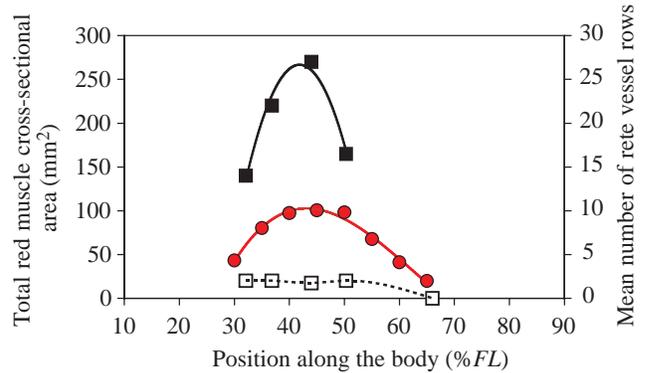
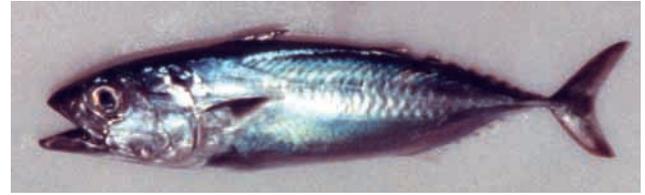


Fig. 5. Mean number of vessel rows in central (filled squares) and lateral (open squares) retia in a 159 mm FL black skipjack (right y-axis) and cross-sectional area of red muscle (red circles) in a 161 mm FL black skipjack (left y-axis) versus relative position along the length of the body (% of fork length, FL), with reference to a photograph of a 159 mm FL black skipjack, to show the correspondence between rete and red muscle distributions along the body.

sectional area composed of red muscle (%RM) versus relative position along the body (%FL) (Fig. 6). The mean position at which %RM reaches a maximum is 45 %FL in *E. lineatus* and 74 %FL in *S. sierra*. When red muscle distribution is plotted as red muscle area (mm<sup>2</sup>) versus position along the body (mm) for individual fish (not shown), the integrated area under the curve approximates the total red muscle mass of the fish. Red muscle mass increased exponentially with fork length in both *E. lineatus* and *S. sierra* (Fig. 7A), largely because of the increase in fish mass with fork length (Fig. 7B). Power equations describing the relationship between red muscle mass and fish mass gave significant scaling coefficients of  $1.17 \pm 0.22$  for juvenile *E. lineatus* and  $1.37 \pm 0.45$  (means  $\pm$  S.D.) for juvenile *S. sierra*; these values do not differ significantly and neither is significantly different from 1.0 (*t*-test,  $P > 0.05$ ). The percentage of red muscle based on mass, calculated by dividing the estimated red muscle mass values by total fish mass, was  $5.71 \pm 1.54\%$  (mean  $\pm$  S.D.,  $N=4$ ) for *E. lineatus* and  $2.51 \pm 0.57\%$  ( $N=5$ ) for *S. sierra*; the mean for *E. lineatus* is significantly greater than that for *S. sierra* (Mann-Whitney *U*-test,  $P=0.016$ ). In addition, %RM at one position (approximately 45–50 %FL) in *E. lineatus* ranged from 8.3 to 18.6% ( $12.8 \pm 2.5\%$ , mean  $\pm$  S.D.,  $N=20$ ) in juveniles ranging in size from 54.8 to 288 mm FL. For these fish, %RM at approximately 45–50 %FL increased slightly but significantly with fish size according to the best-fitting linear regression:  $\%RM = 0.025FL + 9.016$  ( $r=0.665$ ,  $P < 0.01$ ).

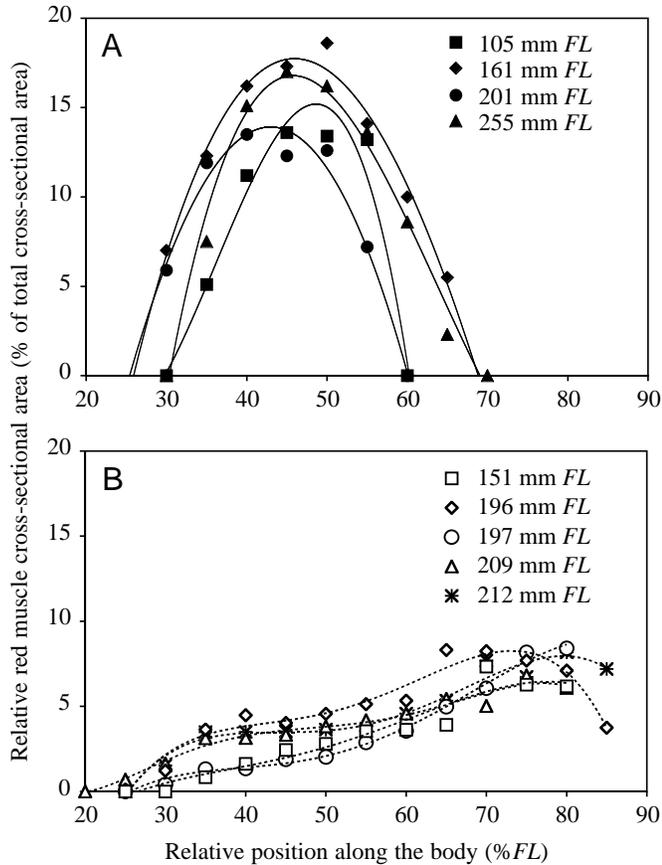


Fig. 6. Distribution of red muscle along the body in four *Euthynnus lineatus* juveniles (A) and in five *Scomberomorus sierra* juveniles (B). The percentage of total cross-sectional area composed of red muscle is plotted versus relative position along the body (% of fork length, FL). Lines represent the best-fitting polynomial equations for individual fish. Comparison of A and B, plotted on the same x- and y-axis scales, shows the greater amount of more anteriorly placed red muscle in the black skipjack (A).

The specific activity of CS in red muscle, measured at 20 °C, increased significantly with fish fork length (Fig. 7C) and with fish mass in juveniles of both species, with mass scaling coefficients (means  $\pm$  S.D.) of  $0.24 \pm 0.21$  for *E. lineatus* and  $0.16 \pm 0.07$  for *S. sierra*. However, when the two adults of each species are included, the scaling coefficients decreased to  $0.18 \pm 0.11$  for *E. lineatus* and  $-0.082 \pm 0.093$  for *S. sierra*; the value for *E. lineatus*, but not that for *S. sierra*, is significantly different from zero, and CS activity is greater in adult *E. lineatus* than in *S. sierra* (see also Dickson, 1996). When the relationship between red muscle CS specific activity (international units  $g^{-1}$ ) and fork length (Fig. 7C) is combined with the relationship between the amount of red muscle (g) and fork length (Fig. 7A), the relationship between total red muscle heat production potential (total units of CS activity in red muscle) and fork length is obtained (Fig. 7D). This value increased with fish size in both species, primarily because of the increase in red muscle mass with fork length (Fig. 7A), and is greater in

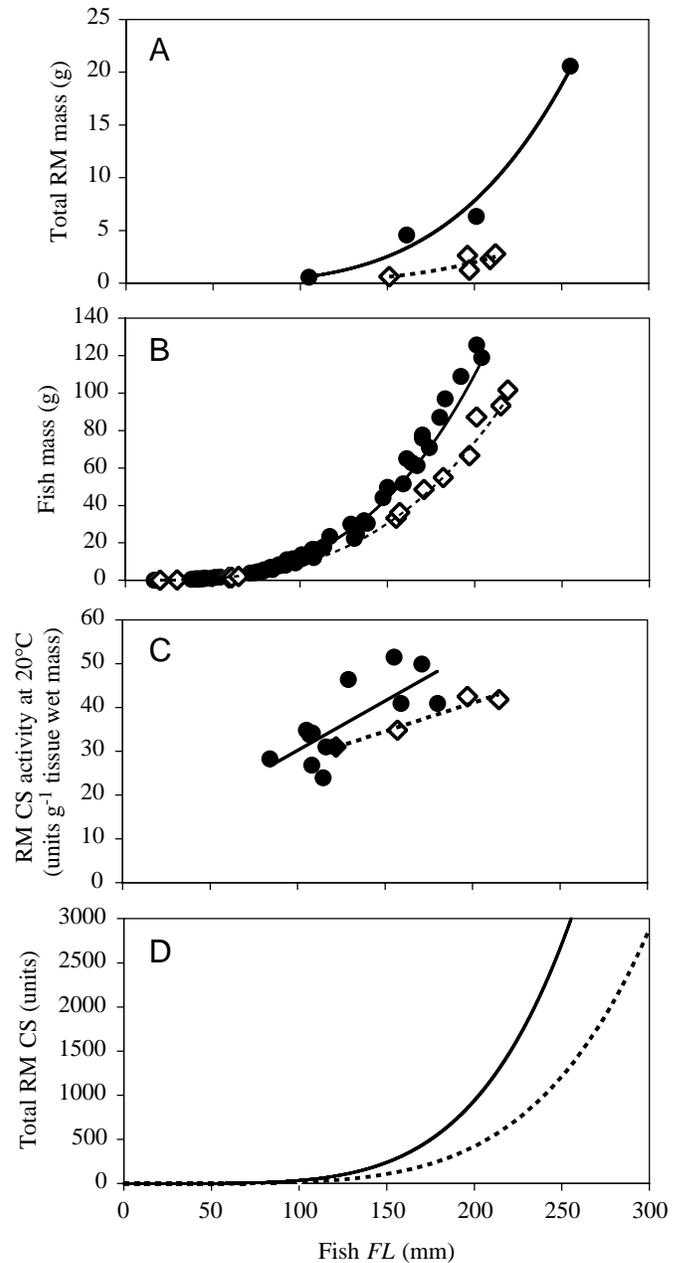


Fig. 7. Changes in the potential for heat production in red myotomal muscle (RM) with fish fork length (FL) in juvenile *Euthynnus lineatus* (filled circles and solid lines) and in the ectothermic *Scomberomorus sierra* (open diamonds and dashed lines). (A) Red muscle mass versus FL, with the best-fitting power equations ( $y=7 \times 10^{-9} FL^{3.926}$ ,  $r^2=0.976$ ,  $P<0.05$ ) for *E. lineatus* and ( $y=5 \times 10^{-10} FL^{4.182}$ ,  $r^2=0.812$ ,  $P<0.05$ ) for *S. sierra*. (B) Fish mass versus FL, with the best-fitting power equations ( $y=3 \times 10^{-6} FL^{3.189}$ ,  $r^2=0.996$ ,  $P<0.001$ ) for *E. lineatus* and ( $y=5 \times 10^{-6} FL^{3.099}$ ,  $r^2=0.997$ ,  $P<0.001$ ) for *S. sierra*. (C) The specific activity of citrate synthase (CS) in red muscle at 20 °C (international units  $g^{-1}$ ) versus FL, with the best-fitting linear regressions ( $y=0.226FL+7.59$ ,  $r^2=0.561$ ,  $P<0.01$ ) for *E. lineatus* and ( $y=0.130FL+15.04$ ,  $r^2=0.945$ ,  $P<0.05$ ) for *S. sierra*. (D) Total red muscle citrate synthase activity (international units), used as an estimate of a fish's total red muscle heat production capacity, versus FL. The curves from A and C for each species were mathematically combined to produce the corresponding curves in D.

*E. lineatus* than in *S. sierra* primarily because of the greater %RM in *E. lineatus*.

### Discussion

The growth rates estimated for individual captive juvenile *E. lineatus* in the present study (maximum  $7.86 \text{ mm day}^{-1}$ ;  $5.38 \pm 1.05 \text{ mm day}^{-1}$ , mean  $\pm$  S.D.) are greater than the rate of  $3.91 \text{ mm day}^{-1}$  recorded by Clemens (1956) for *E. lineatus* juveniles (18.5–75 mm FL) raised on board ship and are greater than the maximum growth rate ( $4.8 \text{ mm day}^{-1}$ ) of individual *E. lineatus* (50–120 mm standard length, SL) previously raised at the IATTC laboratory and postulated to represent the maximum for this species (Olson and Scholey, 1990). They are also higher than almost all other reported growth rates for pelagic fishes, except those for juveniles of the blue marlin *Makaira nigricans* ( $16.6 \text{ mm day}^{-1}$ ; Prince et al., 1991), the sailfish *Istiophorus platypterus* ( $11 \text{ mm day}^{-1}$ ; de Sylva, 1957) and the dolphin fish *Coryphaena hippurus* (up to  $5.88 \text{ mm day}^{-1}$ ; for a review, see Oxenford and Hunte, 1983). The high growth rates of the captive *E. lineatus* juveniles in the present study may be a result of several factors, including high water temperatures, high food consumption rates and because rates of growth in length are probably highest in juvenile tunas of the size range studied (see Olson and Scholey, 1990). The mean growth rate measured for individual *S. sierra* juveniles in the present study ( $3.29 \pm 0.28 \text{ mm day}^{-1}$ ) is only slightly higher than the only other measurement that we know of for *S. sierra* juveniles (12.0–54.0 mm SL) of  $1.92\text{--}3.16 \text{ mm day}^{-1}$  (Clemens, 1956) and is similar to previous measurements on similarly sized juveniles (79–320 mm FL) of the congener *S. cavalla* ( $2.9 \text{ mm day}^{-1}$ ; Collins et al., 1988). How growth rate may affect the ontogeny of characteristics related to endothermy in tunas is unknown. We do know that, regardless of growth rates, the *E. lineatus* raised in captivity had a mass *versus* fork length relationship (Fig. 7B) that was very similar to those for wild-caught skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) tuna juveniles of similar sizes (K. A. Dickson, unpublished results).

The smallest *E. lineatus* with putative rete vessels was found to be 95.9 mm FL for the central rete and 125 mm FL for lateral retia. This extends the minimum size for the presence of putative retia in a tuna beyond those reported in Dickson (1994), because the present study examined rete cross sections, in addition to fish transverse sections, at several positions along the body. The next smallest *E. lineatus* studied, which had no evidence of any rete vessels, was 91.2 mm FL. Although that was the smallest fish examined in the present study, Dickson (1994) could find no evidence of retia in smaller *E. lineatus* (35.5–87.2 mm FL). The difference in age between 91.2 and 95.9 mm FL may be 1 day or less, given the mean growth rate of  $5.38 \text{ mm day}^{-1}$  measured in the present study. Only approximately 5.4 days of growth separates the individuals in which the first appearance of central rete vessels (95.9 mm FL) and first appearance of lateral rete vessels (125 mm FL) were noted.

The central rete is larger than the lateral retia, apparently develops first and demonstrates a greater increase with fish size in surface area available for heat exchange when compared with the lateral retia (Fig. 4). The central rete is likely to be a much more effective heat exchanger than the lateral retia in *E. lineatus* because of the larger number of vessel rows present. In the lateral retia, the maximum number of lateral rete vessels found in any juvenile *E. lineatus* was three rows. Only the center row is flanked on all sides by other rete vessels, and the other two rows, which are adjacent to muscle fibers, cannot contribute to retial heat exchange along their outer edges. Graham and Dickson (2000) proposed that, in the tuna clade, the central rete evolved first and that the lateral retia represent a more derived character. *E. lineatus* is one of the less-derived tuna species (Collette and Nauen, 1983; Block et al., 1993; Carpenter et al., 1995), so the pattern of development of central and lateral retia that we have described in this species may reflect historical phylogenetic constraints.

The diameters of rete vessels measured for juveniles in the present study are within the range characteristic of fish arterioles and venules, which are smaller than  $300 \mu\text{m}$  (Rhodin, 1972), and greater than capillary outer diameters of  $4\text{--}10 \mu\text{m}$  (Satchell, 1992). Central rete arteriole and venule inner diameters in juvenile *E. lineatus* are significantly less than those measured in one adult (492 mm FL, 1.66 kg) by Graham and Diener (1978), who reported mean ( $\pm$  S.E.M.;  $N=20$ ) values of  $53.9 \pm 4.56 \mu\text{m}$  and  $109.5 \pm 6.21 \mu\text{m}$ , respectively ( $t$ -test,  $P < 0.01$ ). The only known previous measurements of *E. lineatus* lateral rete vessel diameters are  $11.2 \mu\text{m}$  and  $74.8 \mu\text{m}$  for inner arteriole and venule diameters, respectively, in an individual weighing approximately 3 kg (Moore, 1998), values similar to those reported here. In the present study, lateral rete vessel diameters increased with fish size, but there was no significant change in central rete vessel diameters with fish size. This should result in ontogenetic differences in relative blood flow through the two rete types (Johnson, 1999). However, the vessel diameter data should be interpreted cautiously, because the muscular layer of the arterioles in particular, but also of the venules, contracts upon fixation, possibly to different degrees in different individuals. Furthermore, we did not measure or account for the shrinkage that occurs during tissue processing. Therefore, the absolute values reported here are probably not what they are *in vivo*, and thus cannot be used to predict blood flow within the retia accurately. We need to know more about tuna rete geometry and hemodynamics to determine rete blood flow and heat-exchange effectiveness and how these may differ in central *versus* lateral retia.

Although *E. lineatus* individuals smaller than 95.9 mm FL do have axial anterior red muscle, we found no evidence that they have counter-current heat exchangers serving this tissue. Thus, below 95.9 mm FL, *E. lineatus* juveniles cannot elevate  $T_m$  significantly above  $T_a$  because the convective loss of heat from the red muscle cannot be prevented. At those sizes, the tuna's body is more laterally compressed than it is in larger individuals (K. A. Dickson, personal observations), and the

amount of heat produced in the small mass of red muscle present at that stage would also be lost rapidly *via* conduction across the body surface. Thus, below 95.9 mm *FL*,  $T_x$  for *E. lineatus* is similar to that of the ectothermic scombrid *S. sierra* (Dickson, 1994; Fig. 8).

Once the retia have developed, the capacity for heat conservation increases with fish size in juvenile *E. lineatus* because both central and lateral retia increase in length, width and number of rows of rete vessels (Fig. 4). Thus, the maximum thermal gradient between  $T_m$  and  $T_a$  (i.e.  $T_x$ ) that juvenile *E. lineatus* can maintain, which is determined by rete length, and the surface area for heat exchange, which depends on both the length and number of rete vessels, should increase with fish fork length. The capacity for heat production also increases with fish size in juvenile *E. lineatus* because the amount of red muscle and its CS activity both increase with fish size. Almost all the increase in red muscle heat production capacity can be attributed to the exponential increase in red muscle mass, which corresponds to the increase in fish mass with fork length (Fig. 7). Furthermore, as juvenile tuna add more red muscle mass, the body is also increasing in girth. Thus, the core of the red muscle mass is farther from the body surface in larger individuals, as reflected in the observed increase in lateral rete length with fork length (Fig. 4A). This, together with the decrease in surface area to volume ratio with increasing mass and girth, favors reduced conductive heat loss relative to heat production. The size at which the retia first appear and at which the total red muscle CS activity *versus* fork length curves for *E. lineatus* and *S. sierra* start to diverge corresponds to the point at which the  $T_x$  *versus* fork length curves of these two species diverge, at approximately 100–125 mm *FL* (Figs 7, 8). Thus, it seems that concomitant increases in heat production, driven primarily by the increase in red muscle mass with fork length, and in heat conservation, due to the growth of the retia, especially the larger central rete of *E. lineatus*, contribute to the development of endothermy in juvenile tunas.

On the basis of the data in Fig. 8, and the fact that all similar acute temperature measurements from known ectothermic

Table 1. Rete dimensions estimated from the regressions in Fig. 4 for a juvenile *Euthynnus lineatus* of 207 mm *FL*, the proposed minimum size for endothermy

Rete dimensions	Central rete	Lateral rete
Length (mm)	3.01	3.13
Width ( $\mu\text{m}$ )	918	140
Maximum number of rete vessel rows	22	3

species do not exceed a  $T_x$  of 2.7°C, Dickson (1994) hypothesized that 207 mm *FL* is the minimum size for endothermy in *E. lineatus*. It is not possible to model heat exchange within tuna retia accurately because no data on blood flow within the retial vessels exist. However, we did estimate the minimum rete size necessary for *E. lineatus* to elevate  $T_m$  significantly above  $T_a$  by calculating the rete dimensions for a 207 mm *FL* individual using the regression equations in Fig. 4 that relate rete dimensions to fish fork length (Table 1). In a 207 mm *FL* *E. lineatus*, total red muscle mass would be 8.7 g (Fig. 7A), which could generate 4.35–43.5 J min<sup>-1</sup>, based on the range of heat production rates for tuna red muscle (0.50–4.98 J min<sup>-1</sup> g<sup>-1</sup>) in Stevens and Neill (1978). Additional information about rates of convective and conductive heat loss will be necessary to determine whether the development of endothermy in juvenile tunas is limited by rete size, red muscle heat production or body size and shape. Also, it should be noted that there is only one  $T_x$  data point for *E. lineatus* juveniles between 121 and 207 mm *FL* (Fig. 8) and no data on muscle temperatures during voluntary swimming for any tuna near this size range. Thus, 207 mm *FL* is only an estimate of the minimum size for endothermy in tunas.

Interspecific comparisons were made between *E. lineatus* and *S. sierra* because it was the only ectothermic scombrid species in which juveniles over a similar size range were available. Among the scombrids, the body shape of the Spanish mackerels is the least like that of the tunas; *S. sierra* has a more elongated and laterally compressed body than do the mackerels, bonitos and tunas (Collette and Nauen, 1983). A

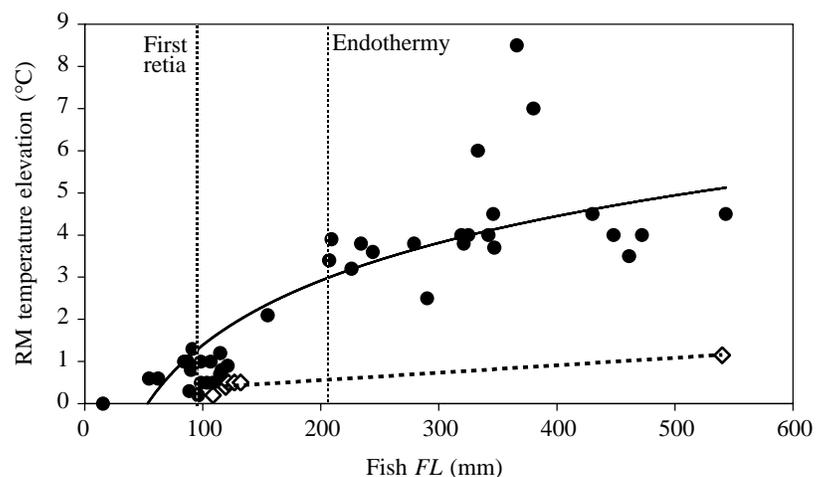


Fig. 8. Maximum red muscle (RM) temperature elevation ( $T_x$ =muscle temperature minus ambient water temperature) *versus* fish fork length (*FL*) in juvenile *Euthynnus lineatus* (filled circles and solid line) and *Scomberomorus sierra* (open diamonds and dashed line). The fork length at which putative retia were first observed and the hypothesized minimum size for endothermy are indicated. Modified from Dickson (1994, 1996).

more appropriate comparison would be with the bonitos, the sister group to the tuna clade. In larger individuals, the eastern Pacific bonito (*Sarda chiliensis*) has a higher red muscle CS activity (mean  $\pm$  S.D.,  $41.2 \pm 9.3$  international units  $g^{-1}$ ; Dickson, 1996) and a larger relative amount of red muscle (mean  $\pm$  S.D.,  $4.51 \pm 2.57$  %RM; Graham et al., 1983) than does *S. sierra*, so the red muscle heat production potential of juvenile *S. chiliensis* may be more similar to that of *E. lineatus*. However, since the bonitos lack retia, they lack the ability to maintain muscle temperatures elevated significantly above water temperature.

Although the difference between  $T_m$  and  $T_a$  (i.e.  $T_x$ ) increases with fork length in juvenile *E. lineatus*,  $T_x$  values level off in adults (Fig. 8), which follows the trend for relative amount of red muscle and red muscle CS specific activity. Red muscle mass continues to increase with size, but does so at a lower rate in larger *E. lineatus*: Graham et al. (1983) reported a mass scaling coefficient for red muscle mass of  $0.93 \pm 0.16$  (mean  $\pm$  S.D.) for 14 individuals of 289–570 mm FL, which is significantly less than the value of  $1.17 \pm 0.22$  found in the present study (*t*-test,  $P < 0.05$ ). The red muscle mass scaling coefficients were also less than or equal to 1.0 in four other tuna species (individuals 310–812 mm FL), but were significantly greater than 1.0 in two ectothermic scombrids (the chub mackerel *Scomber japonicus* and *S. chiliensis*) (Graham et al., 1983); red muscle mass has not been reported for larger *S. sierra*. The mean percentage of red muscle by mass, measured by weighing all red muscle dissected from individual fish, was  $11.11 \pm 1.44$  % (mean  $\pm$  S.D.) in the *E. lineatus* studied by Graham et al. (1983). Thus, it seems that there is a leveling off in the rate of increase of red muscle mass with fish mass at larger sizes in *E. lineatus*, but not until a size greater than 255 mm FL, the largest individual so examined in the present study, whose red muscle mass was 6.72 % of body mass. It also appears that red muscle CS activity levels off in larger individuals. In the two *E. lineatus* adults, red muscle CS values were greater than in the juveniles, but the scaling coefficient was reduced when these individuals were included. In *S. sierra*, red muscle CS declined in adults, resulting in an overall scaling coefficient not significantly different from zero. We do not know how the capacity to conserve heat changes in the adults, and nothing is known about the process of angiogenesis of the rete vessels, but tunas represent an excellent system for future study of this process for arterioles and venules because of the rapid growth of the retia.

The size or age at which each tuna species can maintain elevated muscle temperatures and thus gain some independence from environmental temperature has important implications for tuna ecology and life-history strategy. Because all tuna species spawn in warm waters (Nakamura, 1969; Bayliff, 1980), the larvae and juveniles may require high water temperatures for survival, and juveniles may not be able to move into waters of lower temperature below the thermocline or at higher latitudes until they become endothermic. Characterizing the distribution and migration patterns of the juveniles will help determine whether tunas must be able to

maintain a certain  $T_x$  before they can exploit regions of cooler waters, which will provide a test of the hypothesis that thermal niche expansion was an important selective force in the evolution of endothermy in tunas (Block et al., 1993; Graham and Dickson, 2000). An attempt to do this using present collection and distribution records for tunas was unsuccessful because of the paucity of data available for tuna juveniles smaller than 300 mm FL (Johnson, 1999). Future efforts in this regard should focus on the bluefin tuna *Thunnus thynnus*, which reaches the largest adult size of any tuna species (Collette and Nauen, 1983), inhabits water as cool as 6 °C as an adult (Carey and Teal, 1969) and is known to have lateral retia at a size of 200 mm FL (Funakoshi et al., 1983).

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## References

- Bayliff, W. H. (1980). (ed.) *Synopsis of Biological Data on Eight Species of Scombrids*. La Jolla, CA: Inter-American Tropical Tuna Commission. 530pp.
- Block, B., Finnerty, J. R., Stewart, A. F. R. and Kidd, J. (1993). Evolution of endothermy in fish: mapping physiological traits on a molecular phylogeny. *Science* **260**, 210–214.
- Bushnell, P. G., Jones, D. R. and Farrell, A. P. (1992). The arterial system. In *Fish Physiology*, vol. XII, part A, *The Cardiovascular System* (ed. W. H. Hoar and D. J. Randall), pp. 89–139. New York: Academic Press.
- Carey, F. G. (1973). Fishes with warm bodies. *Scient. Am.* **228**, 36–44.
- Carey, F. G. and Teal, J. M. (1969). Regulation of body temperature by the bluefin tuna. *Comp. Biochem. Physiol.* **28**, 205–213.
- Carpenter, K. E., Collette, B. B. and Russo, J. L. (1995). Unstable and stable classifications of scombrid fishes. *Bull. Mar. Sci.* **56**, 379–405.
- Clemens, H. B. (1956). Rearing larval scombrid fishes in shipboard aquaria. *Calif. Fish Game* **42**, 69–79.
- Collette, B. B. and Nauen, C. E. (1983). *Scombrids of the World*. F.A.O. Species Catalog, vol. 2. Rome: F.A.O. 137pp.
- Collins, M. R., Schmidt, D. J., Waltz, C. W. and Pickney, J. L. (1988). Age and growth of king mackerel, *Scomberomorus cavalla*, from the Atlantic coast of the United States. *Fish. Bull.* **87**, 49–61.

- de Sylva, D. P.** (1957). Studies on the age and growth of the Atlantic sailfish, *Istiophorus americanus* (Cuvier), using length–frequency curves. *Bull. Mar. Sci. Gulf Caribb.* **7**, 1–20.
- Dickson, K. A.** (1994). Tunas as small as 207 mm fork length can elevate muscle temperatures significantly above ambient water temperature. *J. Exp. Biol.* **190**, 79–93.
- Dickson, K. A.** (1996). Locomotor muscle of high-performance fishes: What do comparisons of tunas with ectothermic sister taxa reveal? *Comp. Biochem. Physiol.* **113A**, 39–49.
- Funakoshi, S., Suzuki, T. and Wada, K.** (1983). Anatomical observation on the *rete mirabile* of young bluefin tuna *Thunnus thynnus*. *Bull. Natl. Res. Inst. Aquacult., Tamaki, Japan* **4**, 87–98.
- Graham, J. B.** (1973). Heat exchange in the black skipjack and the blood–gas relationship of warm-bodied fishes. *Proc. Natl. Acad. Sci. USA* **70**, 1964–1967.
- Graham, J. B. and Dickson, K. A.** (2000). The evolution of thunniform locomotion and heat conservation in scombrid fishes: New insights based on the morphology of *Allothunnus fallai*. *Zool. J. Linn. Soc.* **129**, 419–466.
- Graham, J. B. and Diener, D. R.** (1978). Comparative morphology of the central heat exchangers in the skipjacks *Katsuwonus* and *Euthynnus*. In *The Physiological Ecology of Tunas* (ed. G. D. Sharp and A. E. Dizon), pp. 113–133. New York: Academic Press.
- Graham, J. B., Koehn, F. J. and Dickson, K. A.** (1983). Distribution and relative proportions of red muscle in scombrid fishes: consequences of body size and relationships to endothermy. *Can. J. Zool.* **61**, 2087–2096.
- Humason, G. L.** (1979). *Animal Tissue Techniques*, 4th edition. pp. 118–122. Baltimore: Johns Hopkins University Press.
- Johnson, N. M.** (1999). Development of *rete mirabilia* in the black skipjack, *Euthynnus lineatus*. Master's thesis, San Francisco State University. 72pp.
- Kishinouye, K.** (1923). Contributions to the comparative study of the so-called scombroid fishes. *J. Coll. Agric., Tokyo Imperial Univ.* **8**, 293–475.
- Kleinbaum, D. G., Kupper, L. L. and Muller, K. E.** (1988). *Applied Regression Analysis and Other Multivariable Methods*, 2nd edition, pp. 219–220. Boston: PWS-Kent Publishing Company.
- Magnuson, J. J.** (1973). Comparative study of adaptations for continuous swimming and hydrostatic equilibrium of scombroid and xiphoid fishes. *Fish. Bull.* **71**, 337–356.
- Moore, J. A.** (1998). Possible nervous innervation and characterization of the blood vessels of the counter-current heat exchangers in three species of tuna. Master's thesis, California State University Fullerton. 156pp.
- Nakamura, H.** (1969). *Tuna Distribution and Migration*. London: Fishing News (Books) Ltd. 76pp.
- Olson, R. J. and Scholey, V. P.** (1990). Captive tunas in a tropical marine research laboratory: growth of late-larval and early-juvenile black skipjack tuna *Euthynnus lineatus*. *Fish. Bull.* **88**, 821–828.
- Oxenford, H. A. and Hunte, W.** (1983). Age and growth of dolphin, *Coryphaena hippurus*, as determined by growth rings in otoliths. *Fish. Bull.* **81**, 906–909.
- Prince, E. D., Lee, D. W., Zweifel, J. R. and Brothers, E. B.** (1991). Estimating age and growth of young Atlantic blue marlin *Makaira nigricans* from otolith microstructure. *Fish. Bull.* **89**, 441–459.
- Rhodin, J. A. G.** (1972). Fine structure of elasmobranch arteries, capillaries and veins in the spiny dogfish, *Squalus acanthias*. *Comp. Biochem. Physiol.* **42A**, 59–64.
- Ricker, W. E.** (1979). Growth rates and models. In *Fish Physiology*, vol. XIII (ed. W. H. Hoar, D. J. Randall and J. R. Brett), pp. 677–743. New York: Academic Press.
- Roberts, J. L.** (1978). Ram gill ventilation in fish. In *The Physiological Ecology of Tunas* (ed. G. D. Sharp and A. E. Dizon), pp. 83–88. New York: Academic Press.
- Satchell, G. H.** (1992). The venous system. In *Fish Physiology*, vol. XII, part A, *The Cardiovascular System* (ed. W. H. Hoar and D. J. Randall), pp. 141–183. New York: Academic Press.
- Schaefer, K. M.** (1987). Reproductive biology of black skipjack, *Euthynnus lineatus*, an eastern Pacific tuna. *Bull. Inter-Am. Trop. Tuna Comm.* **19**, 169–260.
- Stevens, E. D., Lam, H. M. and Kendall, J.** (1974). Vascular anatomy of the counter-current heat exchanger of skipjack tuna. *J. Exp. Biol.* **61**, 145–153.
- Stevens, E. D. and Neill, W. H.** (1978). Body temperature relations of tunas, especially skipjack. In *Fish Physiology*, vol. VII (ed. W. S. Hoar and D. J. Randall), pp. 315–359. New York: Academic Press.