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Cranial endothermy and a putative brain heater in the most basal tuna species, *Allothunnus fallai*

C. A. Sepulveda*†‡, K. A. Dickson§, L. R. Frank‖ and J. B. Graham*

**Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093-0202, U.S.A., †Pfleger Institute of Environmental Research, Oceanside, CA 92054, U.S.A., §Department of Biological Science, California State University Fullerton, Fullerton, CA 92834, U.S.A. and ‖Center for Functional Magnetic Resonance Imaging, University of California, San Diego, La Jolla, CA 92093-0677, U.S.A.*

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Field studies on the slender tuna *Allothunnus fallai* revealed cranial temperatures that were 4·8 ± 0·4° C (mean ± s.d.) above the ambient sea surface temperature. Dissections aimed at documenting the cranial heat source revealed a fused extraocular muscle complex positioned beneath the brain of this basal tuna species. The muscle complex is structurally distinct from that documented for any other fish species. In *A. fallai*, all four extraocular rectus muscle pairs (superior, inferior, medial and lateral rectus) are incorporated into one distinct tissue complex which is positioned between the orbits and in direct contact with the braincase. A combination of morphological, physiological and biochemical techniques were used to characterize the modified muscle tissue, and high-resolution magnetic resonance imaging was used to illustrate its association with the brain and optic nerves. The modified eye muscles lack organized contractile proteins and are perfused by an extensive vascular counter-current system that originates from the internal carotid artery. Vessel diameters, artery–vein configuration, and anatomic position between the systemic circulation and the warm eye muscles all suggest that this system is a heat exchanger. Collectively, these findings suggest that *A. fallai* has evolved extraocular muscles that may function to warm the brain and eye region. This is the first record of a cranial modification comprised of all four rectus muscles and the only documented occurrence of this mechanism for cranial endothermy among the tunas.

Key words: *Allothunnus*; endothermy; heater tissue; Scombridae; temperature; tuna.

INTRODUCTION

Cranial endothermy is the most widespread form of regional endothermy among fishes and has been documented in all but one of the five tuna genera (*Allothunnus*), in the lamnid sharks, and in billfishes (Linthicum & Carey, 1972; Carey, 1982; Block & Carey, 1985; Block, 1986; Block & Finnerty, 1994).

‡Author to whom correspondence should be addressed. Tel.: +1 760 721 1404; fax: +1 760 721 1475; email: chugey@pier.org

Other species suspected of cranial endothermy include the butterfly mackerel *Gasterochisma melampus* Richardson and the bigeye thresher shark *Alopias superciliosus* (Lowe) (Carey, 1982; Finnerty & Block, 1992; Weng & Block, 2004). The convergence upon a brain heating mechanism in several divergent groups has led to the evolution of distinct mechanisms for elevating and maintaining warm cranial temperatures.

The most studied group of cranial endotherms are the billfishes (Xiphiidae and Istiophoridae), which possess a pair of extraocular muscles that have the function of generating heat (Carey, 1982; Block, 1986; Block & Finnerty, 1994). In this group, a portion of each superior rectus muscle is non-contractile and is thought to function solely as a thermogenic tissue. When coupled with a vascular counter-current heat exchanger (retia), this tissue allows warming of the brain and eye region (Carey, 1982; Block, 1986). Muscular modifications for the purpose of heat generation have also been hypothesized to occur in *G. melampus*, the most basal scombrid species, which has modifications in its lateral rectus muscles that resemble the thermogenic tissue of the billfishes (Carey, 1982; Finnerty & Block, 1992). Although structural similarities with the billfish suggest that the lateral rectus modification of *G. melampus* is a brain heater, to date there are no thermal data for this species.

In comparison with the billfishes, the lamnid sharks use an entirely different mechanism to warm the cranial region. The lamnids utilize heat generated in the red, aerobic swimming muscle (RM) to warm the brain (Block & Carey, 1985; Wolf *et al.*, 1988). The blood from the RM vein flows anteriorly through the medially positioned RM where it is warmed, to the myelonal vein, and subsequently into a sinus that houses the sub-orbital rete (Wolf *et al.*, 1988). Less is known about the mechanism used by the bigeye thresher shark, but the presence of a large arterial plexus within the orbital sinus suggests that this species also uses a system whereby warm venous flow bathes the arterial circulation within an orbital sinus (Weng & Block, 2004). Because this species does not possess a medial RM arrangement like the lamnid sharks (Sepulveda *et al.*, 2005) and because there are no cranial temperature measurements for it, cranial endothermy can only be hypothesized.

The thermogenic mechanism for cranial endothermy used by the largest group of regional endotherms, the tunas (Scombridae, Thunnini), is also not fully understood (Stevens & Fry, 1971; Linthicum & Carey, 1972; Dickson & Graham, 2004). Linthicum & Carey (1972) offer a thorough description of the heat exchanging retia of *Thunnus thynnus* (L.) and report elevated cranial temperatures for several tuna species [*T. thynnus*, *Thunnus alalunga* (Bonnaterre), *Thunnus obesus* (Lowe) and *Euthynnus alletteratus* (Rafinesque)]. The source of the heat for cranial endothermy in this group is unknown. To date, tunas have not been shown to possess a specialized heater tissue like the billfishes or *G. melampus*, and hypotheses regarding the heat source include contraction of the highly aerobic ocular muscles, the convective transfer from the RM, or possibly heat produced by the central nervous system (Block, 1987; Block & Finnerty, 1994; Dickson & Graham, 2004).

This study focused on the only tuna genus for which there are no data on the capacity for cranial endothermy, *Allothunnus*. *Allothunnus* is a poorly known monotypic genus from the Southern Ocean, and it is phylogenetically classified

as the most basal member of the tuna clade (Thunnini) (Cressey *et al.*, 1983; Graham & Dickson, 2000; Collette *et al.*, 2001). Although recent work on slender tuna *Allothunnus fallai* Serventy has detailed its osteology, vasculature and the arrangement of the aerobic swimming muscles (Graham & Dickson, 2000), cranial endothermy has not been investigated in this species. The objective of this work was to assess whether *A. fallai* has the capacity to elevate its eye and brain temperature, and, if so, to investigate the vasculature and thermal mechanism used for cranial endothermy in this species.

MATERIALS AND METHODS

All experiments were conducted under the guidelines of the University of Otago Animal Ethics Committee, Dunedin, New Zealand, and the Animal Care and Use Committee of the University of California, San Diego (Protocol # S00080), U.S.A. All experiments were initiated following the guidelines of the 2000 Report of the American Veterinarian Medical Association Panel on Euthanasia.

FISH COLLECTION AND *IN SITU* TEMPERATURE MEASUREMENTS

Slender tuna were captured while at the surface by hook and line off of the coast of southern New Zealand aboard a small chartered fishing vessel. The severing of the central nervous system and the cranial temperature measurements were taken immediately upon capture (within 15 s of removal from water) following the protocol of previous tuna thermal studies (Linthicum & Carey, 1972; Dickson, 1994). A digital thermocouple thermometer (Barnant Dual JTEK, model 600, Barrington, IL, U.S.A.) and penetration probe (stainless steel type T) were used for all temperature measurements. The penetration probe doubled as a pithing tool. Upon removal of the fish from the water, the thermocouple probe was inserted into the neurocranium *via* the orbit at a 45° angle to the longitudinal axis of the body and the temperature of the warmest region was recorded (cranium temperature, T_{cranium}). Immediately following, the probe was submersed in the surrounding water to record the ambient sea surface temperature (SST, T_{SST}). For several individuals, the penetration probe was left in place within the neurocranium and the warmest cranial position was subsequently verified by gross dissection.

GROSS DISSECTIONS

After field temperature measurements, the specimens were measured (fork length, L_{F}), weighed, labelled and transported to the Portobello Marine Laboratory, University of Otago, New Zealand, for subsequent morphometric analyses. Gross dissections of the cranial region of 10 freshly caught *A. fallai* were performed to determine the position of the warmest region of the skull, and to identify the presence of any circulatory specializations associated with this area of interest (*i.e.* retia). For purposes of comparison, cranial dissections of other similar-sized tunas [*T. alalunga*, *T. obesus*, *Thunnus albacares* (Bonnaterre), *Katsuwonus pelamis* (L.), *Euthynnus lineatus* Kishinouye and *Auxis thazard* (Lacepède)] were also performed at a later time. An additional 10 *A. fallai* were frozen whole and transported to Scripps Institution of Oceanography, University of California, San Diego, U.S.A., for subsequent analyses which included 2 cm-thick transverse sectioning of the cranial region with a band-saw, magnetic resonance imaging and vascular casting of the circulation.

The cranial circulation of *A. fallai* was examined by both gross dissection and by vascular perfusion using Batson's No. 17 plastic replica kit (Polysciences Inc., Warrington,

PA, U.S.A.). Fine-scale examination of the circulation was performed using stained paraffin embedded preparations under light microscopy.

TISSUE ANALYSES

Frozen tissue was used to prepare histological sections (5 μm thick, stained with haematoxylin and eosin, embedded in paraffin and sectioned; Pacific Pathology, San Diego, CA, U.S.A.) of the cranial tissues near and around the region of maximal thermal excess (the superior, inferior, medial and lateral rectus muscles). A Fontana-Masson silver stain and a modified Giemsa stain (Burnham Institute, San Diego, CA, U.S.A.) were used to identify pigmented regions of the cranial tissue.

Protein composition of the ocular muscles was examined using SDS-PAGE following the protocol of Margossian & Lowey (1982). Briefly, equal amounts (0·1 g) of each *A. fallai* rectus muscle were homogenized, subjected to a myosin extraction procedure and loaded onto a 10% acrylamide gel (BioRad Criterion® PreCast Cat. No. 345-0009) along with high molecular mass standards (SigmaMarker Product No. S8320, Saint Louis, MO, U.S.A.) and run at 100 V for 2 h.

IMAGING

Magnetic resonance (MR) imaging was used to acquire high-resolution isotropic cranial images of *A. fallai*. MR images were obtained using a 1·5 T GE Clinical Imager (GE Medical Systems, Milwaukee, WI, U.S.A.). A 3D Fast Spin Echo pulse sequence was used to acquire high-resolution, high-contrast, T1-weighted volume images while reducing magnetic susceptibility artefacts caused by air pockets. Image variables were: field-of-view = 140 mm, matrix size 'frequency × phase' = 256 × 192, slice thickness = 0·5 mm, repetition time = 2000 ms, echo time = 7·6 ms, echo train length = 16, number of averages = 4 and number of slabs = 32. Data in the phase encoding direction were interpolated from 192 to 256 to achieve approximately isotropic voxels, frequency, phase, slice = 0·55, 0·55, 0·5 mm. High contrast between the different soft tissues (*i.e.* muscle, brain and connective tissue) enabled a quantitative three-dimensional reconstruction of the modified muscle mass and surrounding structures using the Amira 4.0 software package (Jacobs *et al.*, 2003).

RESULTS

TEMPERATURE MEASUREMENTS

Field studies revealed the *in situ* mean ± S.D. $T_{cranial}$ of *A. fallai* to be 4·8 ± 0·4° C (Table I, $n = 11$) above T_{SST} and pinpointed the warmest region in a gland-like tissue in the posterior myodome directly beneath the brain.

GROSS DISSECTIONS

Cranial dissections revealed the warmest region to be a distinct tissue complex positioned within the medial orbit and in direct contact with the ventral side of the posterior braincase (basisphenoid). A comparison of transverse sections through the cranial region of *A. fallai* and a comparably sized *T. alalunga* showed noticeable differences in the arrangement, configuration and colouration of the extraocular rectus muscles (Fig. 1). In *A. fallai*, there is a region within the medial orbit where the rectus muscles coalesce to form a solid mass of gland-like tissue, which does not segregate into discrete extraocular muscle

TABLE I. Size and body temperature data for *A. fallai*

L_F (cm)	Mass (kg)	T_{SST} (° C)	$T_{cranium}$ (° C)	T_X ($T_{cranium}-T_{SST}$) (° C)
71	5·6	14·3	19·8	5·5
74	8·1	14·8	18·8	4·0
75	4·9	14·3	19·3	5·0
76	5·5	14·9	19·9	5·0
78	6·3	14·9	19·4	4·5
78	6·9	14·9	18·8	3·9
79	8·1	14·3	19·3	5·0
80	7·3	14·3	19·2	4·9
81·5	7·9	14·6	19·6	5·0
85	9·7	14·6	19·3	4·7
85	9·0	14·6	19·9	5·3
Mean ± S.D.		**14·6 ± 0·2**	**19·4 ± 0·4**	**4·8 ±0·4**

L_F, fork length; T_{SST}, sea surface temperature immediately taken after each measurement; $T_{cranium}$, temperature of the cranium.

segments as it does in the other tuna species examined (*T. alalunga*, *T. obesus*, *T. albacares*, *K. pelamis*, *E. lineatus* and *A. thazard*). Whole cranial dissections of *A. fallai* further highlighted these structural differences, showing that the adjacent segments of all four rectus muscles (superior, inferior, medial and lateral) join together to form a single structure that is distinct in texture, colour and pigmentation. Moving medially from the insertion on the eye to the posterior myodome, all four of the rectus muscles appear similar to those of other tunas (striated muscle). At approximately half way to the origin within the myodome, however, the muscles form a single distinct tissue mass. Although the modified tissue is comprised of the rectus muscles, it does not resemble striated muscle; rather it is dark reddish-brown in colour, lacks visible striations and contractile organization (macro and microscopic observations) and has numerous pigmented cells (black in colour) dispersed throughout [Figs 2 and 3(a)]. Other observations made during the cranial dissections of *A. fallai* include large lipid deposits within the orbits and around the eye and a distinct lipid layer along the posterior medial edge of the eyecup, a structure similar to that found in the swordfish *Xiphias gladius* L. (Fritsches *et al.*, 2005; K. A. Fritsches, pers. comm.).

FINE STRUCTURE

Light microscopy was used to examine stained sections of the (1) modified muscle mass, (2) the transition tissue [the border between functional (contractile), non-specialized rectus muscle and the modified tissue] and (3) sections through the non-specialized rectus muscles [Fig. 3(a)]. Unlike the sections of non-specialized rectus muscle, which exhibit obvious myofibrillar organization [inset in Fig. 3(a)], the modified extraocular tissue of *A. fallai* lacks obvious sarcomeric organization (striations). The modified tissue sections also exhibit nuclei that are not located peripherally within cells, as would be expected for striated muscle fibres of rectus muscles.

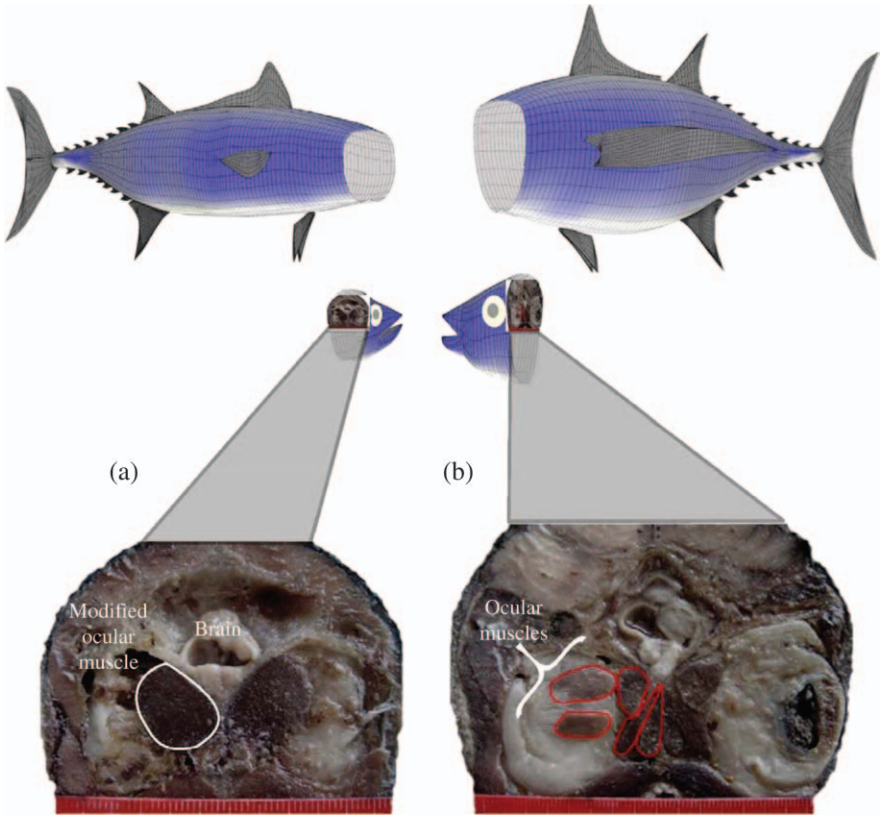

FIG. 1. Transverse sections through the mid-brain, posterior eye regions (vertical dashed line in the fish-head side view) of the heads of (a) a slender tuna, *Allothunnus fallai* and (b) an albacore tuna, *Thunnus alalunga*, contrasting the discrete bands of the extraocular rectus muscles in the albacore and their fusion to form the modified extraocular muscle or heater tissue beneath the brain in the slender tuna.

Other observations from the stained sections of modified muscle include dense clusters of vascular tissue (arteries and veins) and the presence of pigmented cells that are distributed throughout the tissue [Fig. 3(a)]. A Fontana-Masson silver stain and a modified Giemsa stain were used to identify the pigmented regions as chromaffin cells [Fig. 3(a)], non-contractile, pigmented cells associated with modulation of cardiovascular and a respiratory function.

Electrophoretic studies confirmed the absence of a prominent myosin band (205 kd) in the modified rectus muscle homogenate of *A. fallai*, and its presence in the adjacent, functional portion of the same rectus muscles from the same individual [Fig. 3(c)].

VASCULAR SUPPLY

A pair of dorso-ventrally flattened vascular bundles was found to perfuse the modified muscle mass (Fig. 2). Each vascular bundle is comprised of hundreds

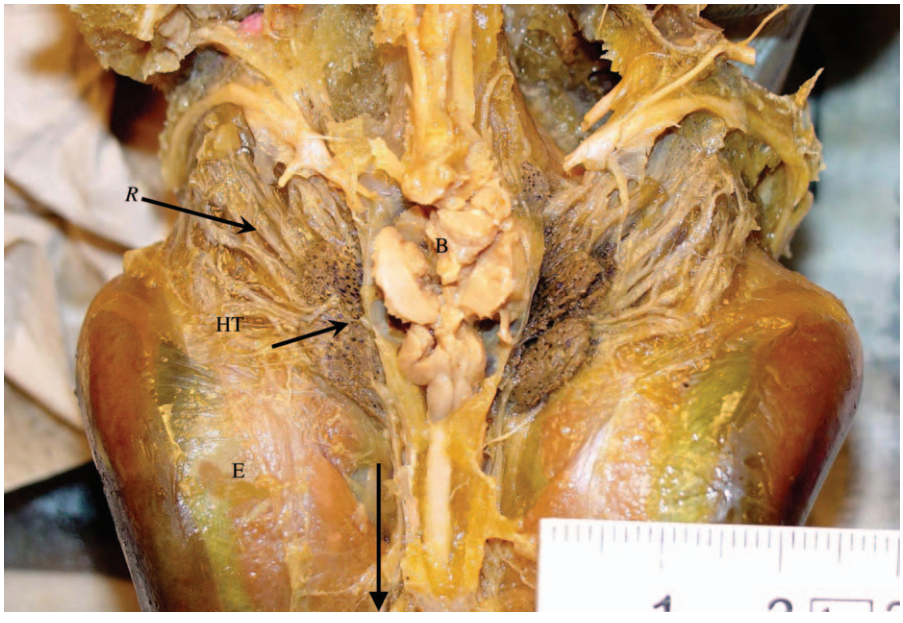

FIG. 2. Dorsal view of the cranial dissection of a preserved slender tuna, showing the brain (B), eyes (E), heater tissue (HT) and putative vascular heat exchanging retia (R). The dorsal portion of the skull has been removed to expose the brain (arrow points to the anterior). Scale in cm.

of small (0·05–0·10 mm diameter), juxtaposed, parallel arteries and veins (arteries determined by their thickened walls) [Fig. 3(b)]. The relative size of the vascular network is much larger than that observed in similar-sized *T. alalunga* and *T. albacares* and the location of this vascular complex is further anterior in *A. fallai*, in direct contact with the anterior and ventral surface of the large prootic bones. Gross dissections and vascular casting revealed that the arteries supplying the vascular network originate on the internal carotid artery, which branches from the first efferent branchial, a major arterial supply to the cranial region in most fishes (Harder, 1975). The venous circulation of the vascular complex flows from the modified muscle tissue into the anterior cardinal vein. The position of the vascular complex (between the cold arterial supply coming directly from the first gill arch and the warm brain region), the anatomic arrangement of the arteries and veins, as well as the vessel diameters, suggest that this structure is a counter-current heat exchange system.

IMAGING

Magnetic resonance imaging (MRI) was used to examine the three-dimensional position of the modified tissue within the intact cranium of *A. fallai* and to approximate its size. MR images show that the modified muscle fully encompasses the ventral surface of the brain and surrounds both optic nerves as they progress from the posterior eye to the midline of the body (Fig. 4). Volume estimates of the modified muscle mass in an 86 cm L_F slender tuna showed the

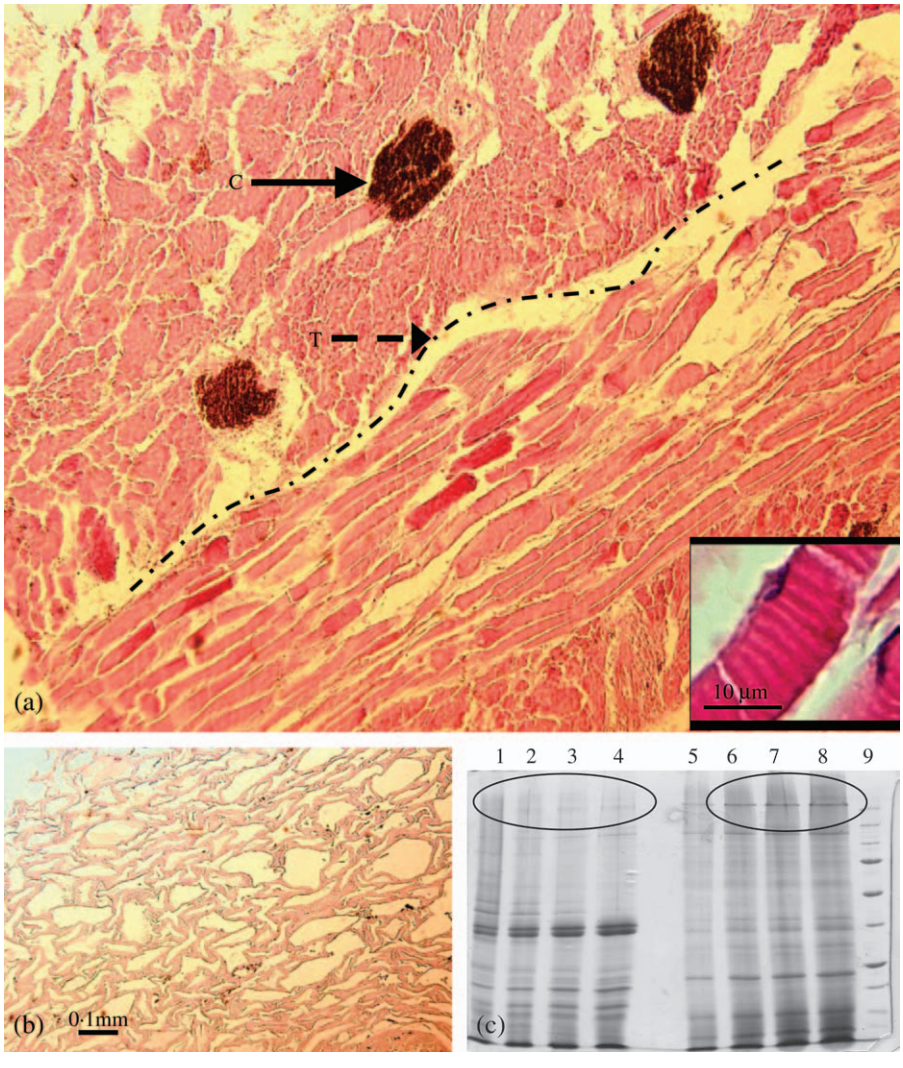

FIG. 3. (a) Microscopic section of the slender tuna heater organ showing the transition (T, - · -) region between non-striated heater tissue (upper left) and the functional rectus muscle composed of typical striated muscle cells (lower right). Chromaffin cells (C) occur throughout the heater tissue. Lower right box inset is an enlarged view of the functional rectus muscle, showing striations and contractile elements. (b) Microscopic cross-section showing the arteries (smaller, thick walled vessels) and veins (larger, thin walled vessels) contained within the vascular counter-current complex that perfuses the modified muscle tissue. (c) SDS-PAGE gel of the modified rectus muscle and functional rectus muscle homogenates, with a circle around the 205 kd (myosin) portion of the gel. Lane identifications: 1–4, different regions of the heater tissue; 5, heater-muscle transition region; 6, functional superior rectus; 7, functional medial rectus; 8, functional lateral rectus; 9, molecular mass ladder (Sigma High Range S8320). Equal amounts of tissue were prepared and loaded into each lane of the gel.

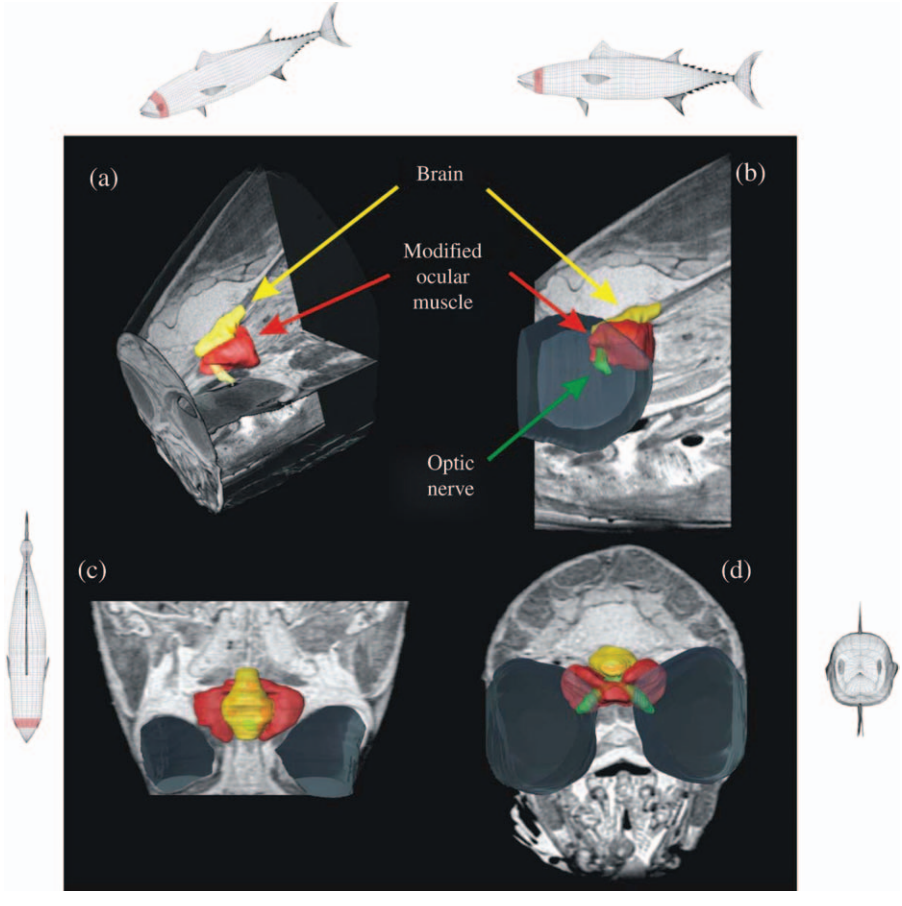

FIG. 4. Three-dimensional reconstructions of magnetic resonance (MR) images of the cranial region of an 86 cm fork length slender tuna showing the proximity and orientation of the heater tissue (red) to the brain (yellow) and the optic nerves (green). (a) Sagittal section, (b) axial section, (c) coronal section and (d) overlay of the sagittal, coronal and axial sections. Fish illustration indicates the orientation of each MR image and the shaded portion indicates the area of focus (arrows indicate anterior).

structure to occupy a significant volume of the cranium: 5043 mm^3 or 3·5 times the volume of the brain (1434 mm^3).

DISCUSSION

CRANIAL THERMAL EXCESS

Thermal excess is defined as the difference between $T_{cranial}$ and T_{SST}, $T_{Xcranium} = T_{cranium} - T_{SST}$. When compared to other cranial endotherms, the thermal excess exhibited by *A. fallai* (mean ± S.D. 4·8 ± 0·4° C) is within the range of other comparably sized tunas (1·6–5·5° C, Stevens & Fry, 1971; 4·2–7·5° C, Linthicum & Carey, 1972) as well as those of lamnid sharks *Isurus oxyrinchus* Rafinesque and *Lamna nasus* (Bonnaterre) (3·0–6·0° C, Block & Carey, 1985) and billfishes

(Istiophoridae and Xiphiidae; 3·2–4·7° C, Carey, 1982). Because of the difficulty and inevitable struggle involved in capturing and handling pelagic fishes, most body temperature measurements available are from exercised fishes, a condition that has been shown at times to increase (Stevens & Fry, 1971) or decrease (Carey, 1982; Block & Carey, 1985; Bernal *et al.*, 2001) *in situ* body temperature. Although this may be the case, Dickson (1994) reviewed the thermal biology of the swimming musculature of both regionally endothermic and ectothermic species to show that, even after exhaustive exercise, there were no ectothermic species which possessed body temperatures in excess of 2·7° C above T_{SST}.

The use of SST to determine the thermal excess of a species may underestimate the true temperature differential between the fish and its surroundings, especially if there is little or no information about its depth in the water column prior to capture. For example, free swimming swordfish at depth can have a cranial thermal excess of up to 14·0° C relative to the water temperature, while thermal studies at the surface report the thermal excess of these fish to be much less (mean ± S.D. 4·7 ± 2·0° C; Carey, 1982). Therefore, the measurements of T_X in *A. fallai*, which are in reference to SST, may underestimate the true thermal excess, but not by a great extent because the fish in this study were caught near the surface while they were filter feeding on euphausids (C. A. Sepulveda, pers. obs.).

THERMAL EFFECTS

Warming of the brain and eye region in *A. fallai* may enhance physiological processes such as synaptic transmission, postsynaptic integration, conduction and, in the eye, temporal resolution (Konishi & Hickman, 1964; Friedlander *et al.*, 1976; Montgomery & Macdonald, 1990; Fritsches *et al.*, 2005; Van den Burg *et al.*, 2005). Fritsches *et al.* (2005) recently showed that the swordfish eye is extremely temperature-sensitive, having a flicker fusion frequency Q_{10} of 5·1, and that warming the retina significantly improves temporal resolution. Warming the retina probably enhances the swordfish's ability to process information and hunt fast moving prey. In the case of *A. fallai*, a small filter feeding tuna, the warming of the retina may increase the ability to avoid fast moving predators, like the swordfish, or it may be that cranial warming counters the harsh thermal conditions experienced by this species in its southern distribution (20–50° S; Watanabe *et al.*, 1966; Yatsu, 1995).

OSTEOLOGY AND CRANIAL STRUCTURE

Discovery of the extraocular muscle modifications in *A. fallai* explains why its cranial architecture is so different from that of the other scombrids (Nakamura & Mori, 1966; Collette & Chao, 1975). When compared to its closest relatives, *A. fallai* has been noted to possess marked differences in the configuration of the basisphenoid, pterosphenoid and prootic bones of the ventral neurocranium (Collette & Chao, 1975). In both tunas and bonitos (Tribe Sardini, the tuna sister group) the basisphenoid connects dorsally to both the pterosphenoids

and prootics and bisects the orbital chamber as it extends ventrally to the parasphenoid. In *A. fallai*, the basisphenoid curves posteriorly and does not contact the parasphenoid, thus providing a larger open space under the braincase. A pouch-like, concave groove in the ventral surface of the prootic also expands this space, which is where the modified muscle mass of *A. fallai* occurs. The pterosphenoids, paired bones forming the anterior portion of the ventral braincase, also occur above the heater organ and, compared to other scombrids, these bones are thinner and do not meet along the medial ventral line. This results in the formation of a relatively large pterosphenotic window, a condition that would also favour heat transfer to the brain. In addition, the observations of the overall thickness of the bones that constitute the base of the braincase suggest that they are reduced in *A. fallai*, so that there is only a thin membrane-like separation between the modified muscle tissue and the brain. A similar condition has been described for the swordfish, in which the pterosphenoid and basisphenoid are largely reduced, most probably to allow increased thermal conductance from the heater tissue to the brain (De Metrio *et al.*, 1997). The anatomic position of the modified extraocular complex in *A. fallai* is also similar to that of the swordfish brain heater, transverse sections through the mid-brain region of each illustrate the proximity of the muscle mass to the brain (Fig. 5; Carey, 1982). The parallel evolution of the neurocranial skeleton and the modified ocular muscles in *A. fallai* increases the contact area between the modified muscle complex and the brain and reduces the thermal barrier between the two tissues.

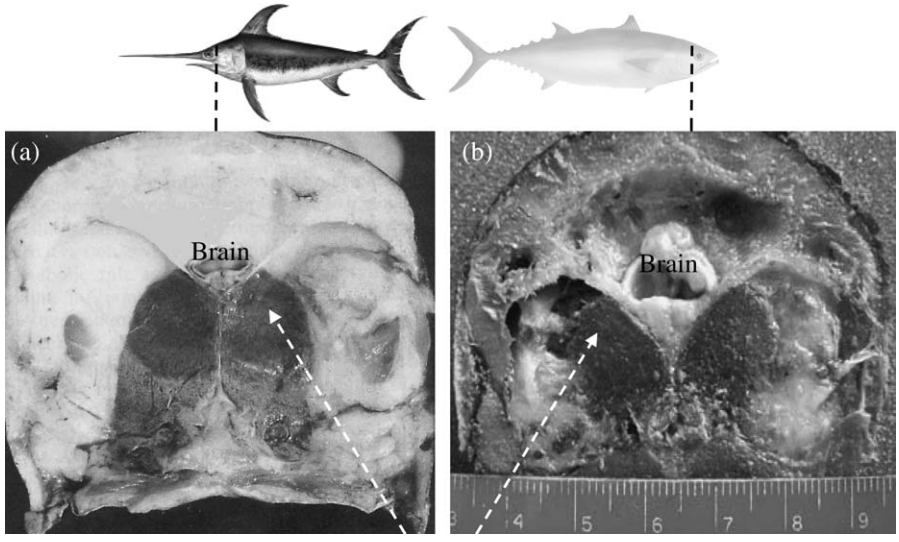

FIG. 5. Transverse section through the mid-brain region of (a) a 14 kg swordfish *Xiphias gladius* (Carey, 1982) and (b) a 9·3 kg slender tuna *Allothunnus fallai* showing the extraocular modifications (dashed white arrow) and their proximity to the brain. Scale in cm. Black dashed lines through the swordfish and slender tuna images illustrate the longitudinal position of the transverse section.

ECOLOGY

The convergence on cranial endothermy in several divergent groups suggests strong selection for this trait among pelagic fishes. Unlike the other cranial endotherms, *A. fallai* has modified all four of its rectus muscles, a condition that probably affects the extent to which this species can move its eyes. Selection for heat production and the modification of all four rectus muscles may relate to the very different ecological niche occupied by *A. fallai* when compared to other cranial endotherms. Specifically, *A. fallai* is the only cranial endotherm to feed primarily as a filter-feeder, a feeding strategy that probably does not require a high degree of ocular mobility. Because little is known regarding the movements (diurnal or longer term) or biology of this species, it is not clear how the muscular modification affects its behavioural ecology.

MUSCULAR MODIFICATION

The findings from this work show that the extaocular rectus muscles of *A. fallai* are structurally distinct from those of the other tuna species. The rectus muscles coalesce to form one tissue mass that is intimately situated in the posterior myodome and in direct contact with the ventral braincase which is highly reduced in thickness in this species. The modified tissue does not have the sarcomeric organization present in other fish eye muscles, but rather is composed of a highly vascularized glandular mass that is similar in appearance to the heater tissue of the billfishes (Carey, 1982; Block, 1986; De Metrio *et al.*, 1997). Cranial dissections of the region show that the vascular supply to this tissue is through a large counter-current (artery and vein) system with vessels that are similar in size and orientation to the heat exchangers of other regionally endothermic species (De Metrio *et al.*, 1997). The position of the vascular exchange system is directly between the cold arterial flow from the first efferent branchial and the warm modified muscle tissue and brain and suggest heat exchange. Similarly, the anatomic position of the vessels against the prootic bone and not the retina, suggest that the system is not an oxygen multiplier for the eye but that it most probably functions for heat exchange. Other structural features observed in the cranial region of *A. fallai* were large lipid deposits around the orbit and along the eyecup. A similar insulation layout has also been described for the swordfish eye and brain region (Fritsches *et al.*, 2005), and is believed to be necessary for maintaining the cranial thermal excess especially because the eye itself provides little to no insulation from the surrounding water temperature.

The unavailability of properly fixed tissues for electron microscopy prevented further comparison with billfish brain heaters on a cellular level, however, elevated temperatures, the counter-current vascular network, ocular muscles lacking protein components that allow for muscle contraction, the position of the modified muscle within the cranium and the unique osteology of *A. fallai* all suggest that this structure plays a role in warming the cranial region of this species.

Collectively the appearance, structure and protein composition of the modified muscle in *A. fallai* suggest a functional convergence with the heater organs

of the billfishes (Xiphiidae and Istiophoridae). The modified muscle of *A. fallai*, however, differs from that of the other fish brain heaters in a number of ways. Most notable is the inclusion of all four pairs of rectus muscles, whereas heater modifications occur in only one muscle pair in the billfishes (superior rectus) and *G. melampus* (lateral rectus) (Carey, 1982; Block, 1987; Finnerty & Block, 1992). Differences in skull structure (Collette & Chao, 1975), in the location and dimensions of the counter-current heat exchanger, and the presence of chromaffin cells also distinguish the muscular modification of *A. fallai*. These differences, together with the separate phylogenies (Collette & Chao, 1975; Kohno, 1984; Block & Finnerty, 1994; Collette *et al.*, 2001), probably indicate the independent evolution of cranial endothermy in *A. fallai*.

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