Title
Aspects of the Evolution and Adaptive Significance of Regional Endothermy in Fishes

Author
Sepulveda, Chugey J.A.

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Aspects of the evolution and adaptive significance of regional endothermy in fishes

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in

Marine Biology

by

Chugey Jesus Alejandro Sepulveda

Committee in charge:

Professor Jeffrey B. Graham, Chair
Professor Richard Rosenblatt
Professor Philip Hastings
Professor Frank Powell
Professor Robert E. Shadwick
Professor John R. Hunter
Professor Kathryn A. Dickson

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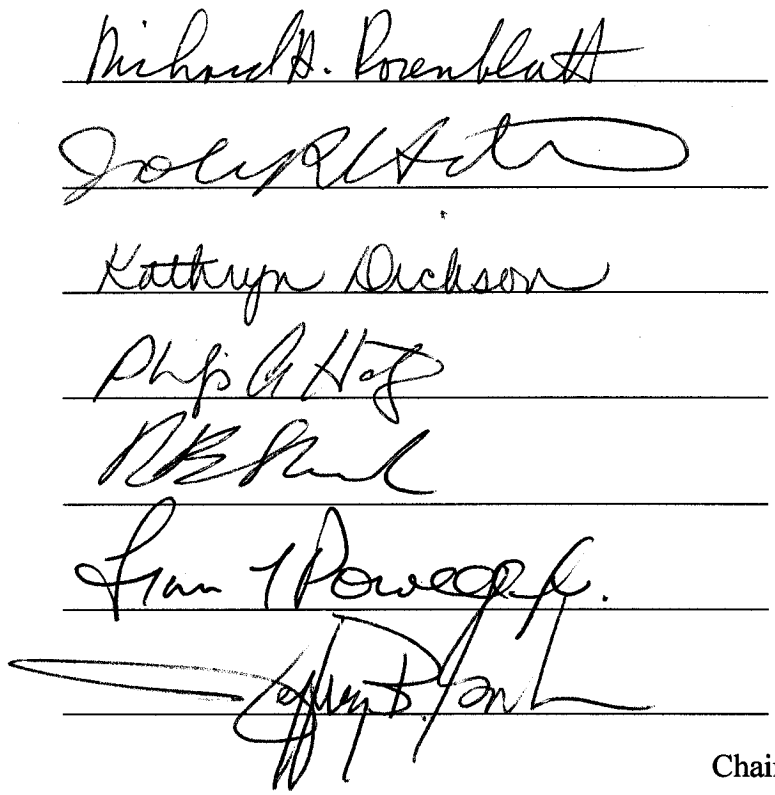

Chair

University of California, San Diego

2005

DEDICATION

To my family and my friends, and in remembrance of Danny Demara.

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I have truly enjoyed my time at SIO. I have met so many amazing individuals, and I feel proud to have been a part of such a great institution. My research would not have been possible if it wasn't for the help I received and the many friendships that I have made over the past few years.

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The text of Chapter IV, in full, is a reprint of the material as it appears in Sepulveda, C. A., Kohin, S., Chan, C. Vetter, R. and Graham, J. B. (2004). Movement patterns, depth preferences, and stomach temperatures of free swimming juvenile mako sharks *Isurus oxyrinchus* in the Southern California Bight. *Marine Biology* 145: 191-199. The dissertation author was the primary author and the co-authors listed in this publication directed and supervised the research, which forms the basis for this chapter.

VITA

Education:

2005	Ph.D. Scripps Institution of Oceanography, University of California, San Diego Field of study: Marine Biology
2004	United States Coast Guard Masters License
2000	M. S. - California State University, Fullerton Field of study: Marine Biology
1997	B. A. - California State University, Fullerton Major field of study: Biology

Publications:

Bernal, D. and Sepulveda, C.A. 2005. Evidence for temperature elevation in the aerobic swimming musculature of the common thresher shark, *Alopias vulpinus*. *Copeia* **2005**: 163-168.

Donley J.M., Sepulveda, C.A., Konstantinidis, P., Gemballa, S. and R.E. Shadwick. 2004. Convergent evolution in mechanical design of lamnid sharks and tunas. *Nature.* **429**: 61-65.

Sepulveda C.A., Kohin, K., Chan, C., Vetter, R. and J.B. Graham. 2004. Movement patterns, depth preferences, and stomach temperatures of free-swimming juvenile mako sharks, *Isurus oxyrinchus*, in the Southern California Bight. *Marine Biology*. **145**: 191-199.

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ABSTRACT OF DISSERTATION

Aspects of the evolution and adaptive significance of regional endothermy in fishes

by

Chugey Jesus Alejandro Sepulveda

Doctor of Philosophy in Marine Biology

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Professor Jeffrey B. Graham, Chair

This dissertation addressed questions related to the selective advantages of regional endothermy and its independent evolution among several fish groups. The chapters integrate laboratory and field physiological measurements to provide a better understanding of the evolution of regional endothermy and its importance as an adaptation to the pelagic environment. Studies on the swimming energetics of the eastern Pacific bonito (*Sarda chiliensis*) revealed that the bonito had a similar cost of transport to that of similar sized yellowfin tuna (*Thunnus albacares*) and that the bonito had a significantly lower standard metabolic rate than tuna. Thermal studies on live slender tuna, *Allothunnus fallai*, were the first to document both red muscle (RM) and eye and brain endothermy for this species and establish regional endothermy as a synapomorphy of the Tribe Thunnini. Additional findings for *A. fallai* revealed this species to possess a brain heater, a thermogenic organ composed of portions of all four of the rectus muscles. These findings demonstrate that the use of a heater tissue to elevate cranial temperatures has evolved independently in at least three fish groups.

Movement studies on the mako shark (*Isurus oxyrinchus*) used acoustic telemetry to record the *in-vivo* stomach temperatures and depth distribution off the coast of Southern California. This work showed that the tracked makos remained in the upper 12 m of the water column during up to 80% of the track period and that larger makos dove to greater depths. Stomach temperature data were recorded for all tracked makos (including young of the year), and these data were used to document feeding events in five of the seven tracking sessions.

A study on the muscle morphology of three thresher shark species (common thresher, *Alopias vulpinus*, pelagic thresher, *A. pelagicus*, and bigeye thresher, *A. superciliosus*) revealed interspecific differences in the RM transverse position and distribution along the body. The common thresher is the only alopiid to possess a RM arrangement similar to that of the endothermic lamnid sharks, suggesting that this species is likely the only thresher to be capable of RM endothermy.

CHAPTER I

Introduction

Regional endothermy, the use of aerobic heat production to warm specific areas of the body, has evolved independently in at least four fish groups: the tunas (Scombridae, tribe Thunnini) (Davy, 1835; Barrett and Hester, 1964; Carey and Teal, 1966), the lamnid sharks (Lamnidae) (Carey et al., 1981; Carey et al., 1985), the thresher sharks (Alopiidae) (Bone and Chub, 1983; Weng and Block, 2004; Bernal and Sepulveda, 2005) and the billfishes (Xiphiidae and Istiophoridae) (Carey, 1982; Block and Finnerty, 1994). Three regions of the body contain endothermic tissues: the red, aerobic swimming musculature (RM), the viscera, and the cranial region (eye and brain).

The most studied form of regional endothermy is the warming of the RM, found in the tunas, lamnid sharks, and the common thresher shark (Carey and Teal, 1966; Carey et al., 1985; Bernal and Sepulveda, 2005). A medial position of the aerobic heat source (RM) and a counter-current heat exchange system (*rete*) are two requisite components for RM endothermy (Carey et al., 1971; Graham et al., 1983; Graham and Dickson, 2000; Bernal et al., 2001; Bernal et al., 2003; Graham and Dickson, 2004). Hypotheses proposed to describe the selective advantages of RM endothermy range from the physiological enhancement of aerobic processes (i.e., muscular, digestive, cellular) to thermal niche expansion (Carey et al., 1971; Graham,

1975; Brill and Dizon, 1979; Johnston and Brill, 1984; Carey et al., 1985; Dewar and Graham, 1994; Block and Finnerty, 1994; Brill, 1996; Altringham and Block, 1997).

Visceral endothermy, documented in the most derived tunas and the lamnid sharks, utililizes the heat produced from the breakdown and synthesis of proteins during digestion (Carey et al., 1981; Carey et al., 1984). The resultant heat is retained in the visceral region by a system of *retia* derived from the celiacomesenteric artery in the tunas and the pericardial arteries in the lamnids (Carey et al., 1985; Fudge and Stevens, 1996; Bernal et al., 2001). Elevating visceral temperatures can increase digestion rates and may play an important role in the feeding ecology of pelagic fishes, especially those heavily reliant on patchily distributed prey (Sund et al., 1981; Stevens and McLeese, 1984; Brill, 1996).

Cranial endothermy is the most widespread form of regional endothermy in terms of both number of fish taxa that display this character and the source of the metabolic heat. It is known in the tunas (Stevens and Fry, 1971; Linthicum and Carey, 1972), lamnid sharks (Block and Carey, 1985; Wolf et al., 1988; Tubbesing and Block, 2000), billfishes (Carey, 1982; Block, 1987), and is suspected for one of the thresher sharks (*A. superciliosus*; Weng and Block, 2004) as well as the butterfly mackerel, *Gasterochisma melampus* (Carey, 1982; Tullis et al., 1991; Block and Finnerty, 1994). Cranial heat can be derived from eye muscle contractions (tunas), the warm venous return from the RM (lamnid sharks), or from a specialized eye muscle modified for heat production (heater tissue) (billfish and butterfly mackerel). Cranial endothermy can increase neuronal transmission rates and enhance visual acuity, two processes involved in escaping predators and capturing prey and thus important to

ecological success in the pelagic environment (Konishi and Hickman, 1964; Friedlander et al., 1976; Fritsches et al., 2005).

The common thread for all endothermic fishes is that they have modified their circulation to retain one of the byproducts of aerobic metabolism, heat. By doing so, their performance is enhanced. Although the enhancement is often difficult to quantify, it is readily apparent in the behavior and performance of these fishes in the pelagic environment, whether it be swordfish using its warm eye and brain to spot prey in the dim waters well below the thermocline, a bluefin tuna (*Thunnus thynnus*) hunting in the cool waters off Nova Scotia maintaining its brain, RM and stomach temperatures more than 10°C above ambient, a salmon shark (*Lamna ditropis*) digesting prey with its warmed (25°C) gut in the cold (9°C) North Pacific, or a bigeye tuna (*Thunnus obesus*) cruising near the equator, frequently diving to 1500 m and 3°C to prey on the deep scattering layer (Carey and Robinson, 1981; Carey and Teal, 1969; Carey and Lawson, 1973; Goldman et al., 2004; Schaefer and Fuller, 2002).

This dissertation examines principles related to all three types of regional endothermy. General questions addressed in this thesis include: (Chapter 2) How do the energetics of endothermic and ectothermic species compare? (Chapter 3) Is the most ancestral tuna species endothermic? (Chapter 4) How do the movement patterns and diving behavior of an endothermic shark affect its elevated muscle and stomach temperatures? (Chapter 5) How does the RM morphology differ among three closely related sharks (Alopiidae), one of which is endothermic?

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CHAPTER II

Swimming performance studies on the eastern Pacific bonito *Sarda chiliensis*, a close relative to the tunas (family Scombridae)

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Swimming performance studies on the eastern Pacific bonito *Sarda chiliensis*, a close relative of the tunas (family Scombridae)

I. Energetics

C. A. Sepulveda[1,*], K. A. Dickson[2] and J. B. Graham[1]

[1]*Center for Marine Biomedicine and Biotechnology and Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0204, USA* and [2]*Department of Biological Science, California State University Fullerton, Fullerton, CA 92834, USA*

*Author for correspondence (e-mail: csepulve@ucsd.edu)

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Summary

A large swim tunnel respirometer was used to quantify the swimming energetics of the eastern Pacific bonito *Sarda chiliensis* (tribe Sardini) (45–50 cm fork length, *FL*) at speeds between 50 and 120 cm s^{-1} and at 18±2°C. The bonito rate of oxygen uptake ($\dot{V}_{O_2}$)–speed function is U-shaped with a minimum $\dot{V}_{O_2}$ at 60 cm s^{-1}, an exponential increase in $\dot{V}_{O_2}$ with increased speed, and an elevated increase in $\dot{V}_{O_2}$ at 50 cm s^{-1} where bonito swimming is unstable. The onset of unstable swimming occurs at speeds predicted by calculation of the minimum speed for bonito hydrostatic equilibrium (1.2 *FL* s^{-1}). The optimum swimming speed (U_{opt}) for the bonito at 18±2°C is approximately 70 cm s^{-1} (1.4 *FL* s^{-1}) and the gross cost of transport at U_{opt} is 0.27 J N^{-1} m^{-1}. The mean standard metabolic rate (SMR), determined by extrapolating swimming $\dot{V}_{O_2}$ to zero speed, is 107±22 mg O_2 kg^{-1} h^{-1}. Plasma lactate determinations at different phases of the experiment showed that capture and handling increased anaerobic metabolism, but plasma lactate concentration returned to pre-experiment levels over the course of the swimming tests. When adjustments are made for differences in temperature, bonito net swimming costs are similar to those of similar-sized yellowfin tuna *Thunnus albacares* (tribe Thunnini), but the bonito has a significantly lower SMR. Because bonitos are the sister group to tunas, this finding suggests that the elevated SMR of the tunas is an autapomorphic trait of the Thunnini.

Key words: energetics, locomotion, swimming, Scombridae, eastern Pacific bonito, *Sarda chiliensis*, standard metabolic rate, cost of transport, tuna.

Introduction

Fishes in the family Scombridae such as the mackerels (tribe Scombrini), bonitos (Sardini) and tunas (Thunnini) exemplify structural design features for maximizing swimming performance (Lighthill, 1969; Webb, 1975; Magnuson, 1978; Nauen and Lauder, 2000; Donley and Dickson, 2000). Tunas, which are the most derived scombrids, possess two additional locomotor-enhancing functions. The first of these is the capacity for regional endothermy, which elevates muscle power (Carey and Teal, 1966; Carey et al., 1971; Graham, 1973; Johnston and Brill, 1984; Altringham and Block, 1997; Graham and Dickson, 2001). The second is the use of the thunniform swimming mode, which reduces drag by minimizing lateral body undulations while generating thrust with rapid oscillations of the high-aspect-ratio caudal fin (Fierstine and Walters, 1968; Lighthill, 1970; Webb, 1975; Lindsey, 1978; Altringham and Shadwick, 2001).

The functional and structural bases for tuna endothermy and thunniform swimming reside in the tunas' myotomal architecture. Red myotomal muscle (RM, the slow-twitch aerobic fibers that power sustained swimming) occurs both more anterior in the body and closer to the vertebral column in tunas than in other fishes (Kishinouye, 1923; Fierstine and Walters, 1968; Graham et al., 1983). Tuna RM position has been hypothesized to have influenced thunniform locomotion through effects on body shape (Magnuson, 1978; Graham and Dickson, 2000) and on the mechanical linkage between myotomes and the caudal fin (Westneat et al., 1993; Shadwick et al., 1998; Ellerby et al., 2000; Graham and Dickson, 2000). In addition, shifts in the pattern of RM vascular supply may have established the basis for counter-current heat transfer and for the origin of endothermy (Graham and Dickson, 2000, 2001).

Although a unique RM position and regional endothermy occur exclusively in the tunas, the sequence of evolutionary changes that took place from the less-derived mackerels and bonitos to the tunas remains unclear. Therefore, testing or

distinguishing among hypotheses about the acquisition sequence of these features requires phylogenetically appropriate structural and functional comparisons with non-tuna scombrids (Magnuson, 1973; Graham, 1975; Collette and Chao, 1975; Collette, 1978; Block et al., 1993; Graham and Dickson, 2000, 2001).

Several works have compared the swimming performance of mackerels to that of tunas. In contrast to the thunniform swimming mode of tunas, both chub (*Scomber japonicus*) and Atlantic (*Scomber scombrus*) mackerels use the carangiform swimming mode (Videler and Hess, 1984; Donley and Dickson, 2000). Although the chub mackerel is not endothermic, rapid swimming does transiently elevate both RM and white muscle temperatures (Roberts and Graham, 1979). Finally, and despite similar costs of locomotion, the standard metabolic rate (SMR, the minimum metabolic rate required for maintenance functions; Videler, 1993) of the chub mackerel is lower than that of the tunas (Sepulveda and Dickson, 2000; Shadwick and Steffensen, 2000).

Although mackerel–tuna comparisons identify key differences between the two groups, most investigators regard the bonitos, which are the tuna sister group (Collette, 1978; Block et al., 1993), as more appropriate for evolutionary comparisons (Block and Finnerty, 1994; Altringham and Block, 1997; Ellerby et al., 2000; Graham and Dickson, 2000). Relative to mackerels, bonitos have many morphological specializations (i.e. body shape, gill surface area, growth rate and maximum size) that are more like those of tunas (Gray, 1954; Campbell and Collins, 1975; Collette, 1978). Bonito RM is also in a more medial position than in the mackerels (Kishinouye, 1923; Graham et al., 1983; Ellerby et al., 2000; Graham and Dickson, 2000), and both bonito myotomal structure and the arrangement of the anterior and posterior oblique tendons (which transmit force from the myotomal musculature to the caudal propeller) are more similar to those of tunas (Ellerby et al., 2000; Westneat et al., 1993; Westneat and Wainwright, 2001; K. A. Dickson and J. B. Graham, unpublished). However, bonitos do not have the anterior RM position found in tunas and they are not endothermic (Carey et al., 1971; Graham, 1975; Graham et al., 1983; Altringham and Block, 1997; Graham and Dickson, 2000, 2001).

While the importance of additional studies with bonitos to increasing our understanding of the swimming adaptations of tunas has long been recognized (Godsil, 1954; Graham, 1975; Block and Finnerty, 1994; Altringham and Block, 1997; Ellerby et al., 2000; Graham and Dickson, 2001), access to live specimens by laboratories capable of conducting physiological studies with them has been rare (Altringham and Block, 1997; Ellerby et al., 2000). The availability of the eastern Pacific bonito *Sarda chiliensis* in southern California coastal waters in the summer of 2000 provided the opportunity to study the swimming energetics and kinematics of this species using the same water tunnel respirometer that had been used for the tuna energetics and kinematics studies reported by Dewar and Graham (1994a,b). This paper reports on bonito swimming energetics, and the companion paper (Dowis et al., 2003) describes bonito swimming kinematics.

Previous work on bonito energetics includes indirect $\dot{V}_{O_2}$ estimates derived from mouth gape and swimming distance (Mendo and Pauly, 1988) and estimates of routine metabolic rate (RMR, which includes both spontaneous movements and locomotion; Beamish, 1978) made for bonito swimming inside of a small annular respirometer, without speed control (Freund, 1999). The objectives of the present study were to quantify bonito swimming energetics and obtain swimming–$\dot{V}_{O_2}$ data over a range of controlled speeds. We also wanted to compare bonito swimming to that of its sister group, the tunas, by contrasting the relationship between $\dot{V}_{O_2}$ and swimming speed in similar-sized fish to estimate the cost of transport and SMR. This approach has provided new insight into two important questions about scombrid physiology. (1) How do the costs of locomotion compare for tunas and bonitos? (2) Is the high SMR of the tunas a unique tuna trait or a synapomorphy of the tribes Sardini and Thunnini?

Materials and methods

Fish collection and maintenance

Eastern Pacific bonito *Sarda chiliensis* Cuvier 1832 (size range: 30–40 cm fork length *FL*, mass 500–1000 g) were caught in waters off La Jolla, California, in July and August of 2000 and transported to the laboratory at Scripps Institution of Oceanography (SIO) in a 378 liter cylindrical tank containing oxygenated seawater. Fish were captured using barbless hooks and transferred untouched into the transport tank. In the laboratory, bonito were transferred, without touching, to an 8 m diameter, 1.2 m deep, above-ground, plastic-lined holding tank supplied with continuously flowing aerated seawater (18±2°C). Fish were held in this tank over the course of the study (90–150 days). They were conditioned to feed with live guppies (Scientific Hatcheries, Huntington Beach, CA, USA) and, once feeding, were fed daily to satiation (about 5–10% body mass) using a mixture of chopped fish and squid.

The respirometer

The variable-speed water-tunnel respirometer used in this study has been described previously (Graham et al., 1990; Dewar and Graham, 1994a; Graham et al., 1994; Bernal et al., 2001). It consists of a 2.3 m×6.3 m (length×width) oval of 46 cm-diameter polyvinylchloride pipe with an in-series diffuser-contraction section and a 100 cm×51 cm×42 cm (length×width×height) working section. It has a total volume of 3000 liters and is powered by a 40 hp variable-speed electric motor with a fixed, low-pitch propeller. Initial flow analyses (Graham et al., 1990) using attached threads, observations of particle motion and dye streaming all confirmed a uniform speed and laminar flow field in the center and first half of the working section, which is where swimming fish spend most of their time (see below).

Experimental protocol

Care was taken not to touch the bonito during all steps involved in capture and transfer to the water tunnel. Prior to all experiments, food was withheld from the entire tank for 24 h. The experimental bonito was obtained by fishing with a baited, barbless hook. The hooked fish was immediately placed in the cylindrical transport tank containing oxygenated seawater and the manufacturer's recommended dose of Fritzyme®, a mucus coat protecting agent. We found that the presence of other fish in the transfer tank minimized the introduced bonito's swimming speed and the frequency of its collisions with the tank wall. Standard procedure therefore was to place between one and five 'companion fish' (either chub mackerel *Scomber japonicus*, or topsmelt *Atherinops affinis*) in the tank prior to introducing the experimental bonito.

After 15 min the bonito and the water around it were scooped up in a soft plastic bucket liner and transferred into the working section of the respirometer. We found that the transferred bonito could better orient to the flow and had fewer erratic movements and bursts if a 'companion fish' was also present in the working section at the time of introduction. Both chub mackerel and topsmelt, which swim well in water tunnels (Sepulveda and Dickson, 2000; C. Sepulveda, personal observation) were used as 'working-section companions'. The companion fish was removed after about 60 min, once the bonito had begun to swim steadily. Covering the working section with a dark cloth and placing a light at its front end helped to keep the bonito swimming in the center of the channel. A mirror positioned at 45° over the working section facilitated remote observation of the fish for the duration of the study.

The bonito was then given a 4 h period of slow, steady swimming to allow its recovery from capture and tunnel placement stress. Previous scombrid respirometry studies have noted a marked change in fish behavior and $\dot{V}_{O_2}$ after about 2 h in the working section (Dewar and Graham, 1994a; Sepulveda and Dickson, 2000). During recovery the bonito swam slowly (about 60 cm s^{-1}) while fresh seawater flowed through the tunnel to maintain temperature and ambient O_2 levels.

Following the recovery period, water inflow to the tunnel was stopped and the system was sealed. Measurements of bonito $\dot{V}_{O_2}$ were then made at different swimming speeds by recording water-tunnel O_2 declination rate over a 30–60 min period of steady swimming. Tunnel water speeds were determined by calibration with a flow meter (model 2035, General Oceanics Inc., FL, USA). The respirometer-water O_2 level was monitored with a polarographic O_2 electrode (Yellow Springs Instrument, OH, USA) connected to a model 52 meter. For each bonito energetics study, ProComm data acquisition software was used to record both water speed and O_2 concentration.

During a given experiment, the respirometer water temperature was maintained within ±1°C of the bonito holding tank, which was supplied with a continuous flow of fresh seawater and subject to subtle environmental fluctuations in temperature (i.e. solar heating, seasonal fluctuation). Collectively for all tests, the experimental temperature was 18±2°C. Respirometer water O_2 concentration was maintained at or above 80% saturation. When fish respiration reduced chamber O_2 levels to near the 80% saturation limit, the system was re-oxygenated by seawater flow and application of a gentle stream of bubbles from a compressed O_2 cylinder.

$\dot{V}_{O_2}$ measurements were made on bonito swimming between 50 and 120 cm s^{-1}. Tests of each fish began by swimming it at the lowest speed it could maintain (50–60 cm s^{-1}) for 30–60 min while O_2 declination was measured. Following this determination, speed was increased by 10 cm s^{-1} and $\dot{V}_{O_2}$ again measured. This protocol was repeated until the bonito could no longer swim steadily or maintain position. Most studies terminated at this point. However, in two cases, replicate $\dot{V}_{O_2}$ measurements were repeated at a lower speeds. Video records of the bonito swimming at the different test speeds were then made for a kinematics analysis (Dowis et al., 2003).

After each individual swimming test, the bonito was removed and the empty respirometer was resealed to measure the background bacterial respiration rate and instrumental drift. Throughout the background measurements, the respirometer motor was circulating the water at approximately 50 cm s^{-1} and the $\dot{V}_{O_2}$ was recorded for approximately 3–6 h. The mean background $\dot{V}_{O_2}$ value for all experiments was 62±2.3 mg O_2 h^{-1} or 6–14% of the maximum measured oxygen consumption rate; the percentage was greatest at lowest speeds. All reported bonito $\dot{V}_{O_2}$ data are background-corrected. After each experiment, the water tunnel was bleached and cleaned with fresh water.

Plasma lactate

Bonito plasma lactate levels were measured to provide an indication of the level of anaerobic metabolic stress associated with three different phases of the respirometry study: (1) pre-experiment, just after capture from the holding tank, (2) at the end of the 15 min period in the transfer tank, (3) at the end of all swimming tests. Phases 1 and 2 used bonito that, although not used in the respirometer, were handled in exactly the same way as were the respirometer fish. Bonito taken directly from the respirometer following the completion of all swimming studies were used for phase 3 measurements.

Blood samples were taken by quickly grasping the bonito behind the operculum, and holding it firmly while removing approximately 2 ml of blood *via* cardiac puncture with a 20-gauge needle attached to a 3 ml syringe. Both the needle and syringe had been flushed with heparinized saline, which also filled the dead space. Plasma obtained by immediately centrifuging the blood (5 min at 3000 *g*) was stored at –80°C. Lactate assays were performed spectrophotometrically using a Sigma Diagnostics kit (Procedure 735). This method did not employ a deproteination step and may thus have indicated slightly higher lactate levels than actually occurred. The tests do nevertheless provide a relative index for the change in plasma lactate concentrations at the different experimental phases.

Results

Swimming performance

Swimming $\dot{V}_{O_2}$ data were obtained for 12 bonito ranging in body size from 0.98 to 1.49 kg (45-50 cm *FL*) (Fig. 1, Table 1). Swimming speeds ranged from 50 to 120 cm s^{-1}, with all of the fish studied swimming at two or more test speeds, and eight fish swimming at four or more speeds. The best performing fish was bonito 10, which swam from 60 to 120 cm s^{-1}. Replicate $\dot{V}_{O_2}$ tests were performed for two individuals, bonitos 3 and 7, to validate the repeatability of the $\dot{V}_{O_2}$ measurements. The first $\dot{V}_{O_2}$ measurement for bonito 3 was 275 mg O_2 kg^{-1} h^{-1} at 60 cm s^{-1}. After swimming at two higher speeds (70 and 90 cm s^{-1}) for 2 h, the $\dot{V}_{O_2}$ of this fish remeasured at 60 cm s^{-1} was 250 mg O_2 kg^{-1} h^{-1}. The initial $\dot{V}_{O_2}$ of bonito 7 at 50 cm s^{-1} was 259 mg O_2 kg^{-1} h^{-1}; after swimming for 1 h at 60 cm s^{-1}, its $\dot{V}_{O_2}$ at 50 cm s^{-1} was 243 mg O_2 kg^{-1} h^{-1}.

Energetics

Covariance analysis (ANCOVA; Minitab version 12) indicated a significant ($P<0.05$) positive relationship between swimming speed and $\dot{V}_{O_2}$, but no effect of either body mass or *FL* on swimming $\dot{V}_{O_2}$. Absence of a body size effect allowed pooling of data for individual bonito and facilitated comparisons with $\dot{V}_{O_2}$ values obtained for tuna (Dewar and Graham, 1994a).

Fig. 1 shows the expected exponential increase in $\dot{V}_{O_2}$ with increasing speed for each bonito (Videler, 1993; Webb, 1998), but also indicates a trend for bonito $\dot{V}_{O_2}$ to level off or even increase at 50–60 cm s^{-1}. In fact, five of the six bonito for which swimming $\dot{V}_{O_2}$ data were obtained at 50 cm s^{-1} actually had a lower $\dot{V}_{O_2}$ at 60 cm s^{-1}. The tendency for bonito $\dot{V}_{O_2}$ to remain higher than expected at slower speeds is evident in the combined $\dot{V}_{O_2}$-speed relationship for all bonito at each test speed (Fig. 2). This curve is U-shaped; the mean $\dot{V}_{O_2}$ at 50 cm s^{-1} is higher than the $\dot{V}_{O_2}$ at 60 cm s^{-1}, and the value at 60 cm s^{-1} is only slightly less than the rate at 70 cm s^{-1}. Two additional features of bonito low-speed swimming are indicated in Table 1. First, nine of the 12 fish had an elevated $\dot{V}_{O_2}$ at the lowest speed tested (either 50 or 60 cm s^{-1}) than at the next higher test speed. Second, records for these same fish at the time of study all noted erratic swimming (i.e. bursting, lateral movements and rapid fluttering of the pectoral fins) at these lower speeds.

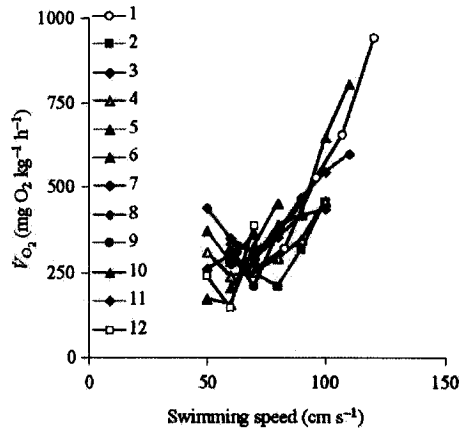

Fig. 1. Relationships between $\dot{V}_{O_2}$ and swimming speed for 12 bonito (44–50 cm *FL*, 0.98–1.49 kg) swimming at 18±2°C. Fish numbers are the same as those in Table 1.

Table 1. *Body size, swimming speed range, rate of oxygen uptake regression equations and low-speed observations for the 12 bonito studied*

Fish number	*FL* (cm)	Mass (g)	Speed range (cm s^{-1})	Regression equation for $\dot{V}_{O_2}$ (exponential fit)	*r*	Slow speed observations: Elevated $\dot{V}_{O_2}$*	Slow speed observations: Erratic swimming
1	45.0	1130	60–120	$y=66.0e^{0.0214x}$	0.94	+	+
2	46.5	1180	60–100	$y=123e^{0.011x}$	0.59	+	+
3	49.0	1430	60–90	$y=0.90e^{0.0168x}$	0.67	–	–
4	45.5	1270	50–100	$y=155e^{0.0091x}$	0.72	+	+
5	44.5	1021	50–70	ND	–	+	+
6	46.5	1150	50–80	$y=3.99e^{0.0361x}$	0.92	+	+
7	46.0	1110	50–60	ND	–	–	–
8	50.0	1490	60–100	$y=188e^{0.0083x}$	0.90	+	+
9	49.5	1280	60–90	$y=0.90e^{0.0165x}$	0.67	+	+
10	46.0	1060	60–110	$y=41.3e^{0.027x}$	0.97	–	–
11	47.5	1190	50–110	$y=225e^{0.008x}$	0.70	+	+
12	45.0	980	50–70	ND		+	+

FL, fork length; $\dot{V}_{O_2}$, rate of oxygen uptake.
*Elevated $\dot{V}_{O_2}$: the $\dot{V}_{O_2}$ at the slowest speed was not the lowest value recorded for that fish. For details, see text.
ND, Equation and *r*-value were not determined for individuals swimming at fewer than 3 different speeds.

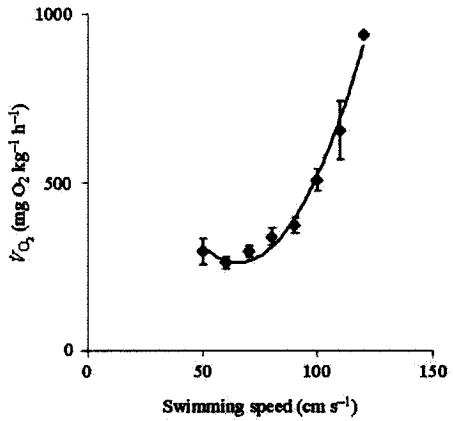

Fig. 2. U-shaped curve obtained by plotting the mean combined $\dot{V}_{O_2}$ (means ± S.E.M.) for all bonitos (N=12) at each test speed.

A higher than expected $\dot{V}_{O_2}$ at low swimming speeds affects estimates of both cost of transport (determined from the instantaneous slope of the $\dot{V}_{O_2}$–speed regression) and SMR (estimated by extrapolation of the regression function to zero speed) (Videler, 1993). It was therefore important to quantify the effect of an elevated $\dot{V}_{O_2}$ at low speed by contrasting a semi-logarithmic regression equation for the complete bonito data set with a 'corrected' regression (i.e. calculated after the selective removal of approximately one high-$\dot{V}_{O_2}$, low-speed data point from each fish). The criteria used to justify the selective removal of a high-$\dot{V}_{O_2}$, low-speed data point were: occurrence of a lower $\dot{V}_{O_2}$ at the next highest test speed and the occurrence, in notes made at the time of study, of observations of erratic swimming (Table 1).

Fig. 3 shows the effect of selective data removal: the exponential form of the regression equation for the complete data set is $y=107e^{0.015x}$. The corrected data set regression equation is $y=70e^{0.020x}$. The corrected function has a higher correlation coefficient (r=0.84 *versus* 0.74); however, there is considerable overlap of the 95% confidence intervals of the slope and y-intercept values of the two functions, indicating that they are not significantly different. These two functions do nevertheless demonstrate the marked effect of an elevated $\dot{V}_{O_2}$ at low speed both in lowering the regression equation's slope (from 0.02 to 0.15, a 25% reduction) and increasing its y-intercept, by 53%. Furthermore, because the y-intercept value at zero speed is an estimate of SMR (Videler, 1993), this selective data removal reduces the calculated bonito SMR from 107 to 70 mg O_2 kg^{-1} h^{-1}.

Bonito cost of transport

Although Fig. 3 indicates differences between the complete and corrected bonito swimming speed–$\dot{V}_{O_2}$ regressions, a valid comparison of the bonito data with published tuna energetics data necessitated use of the complete bonito data set because no high-$\dot{V}_{O_2}$, low-speed data points were removed in the tuna study.

To calculate gross cost of transport (GCOT), the mass-specific $\dot{V}_{O_2}$ values were first converted to weight-specific values (by dividing by 9.8 m s^{-2}, the acceleration due to gravity), and these were converted from mg O_2 to Joules (J), using the oxycalorific coefficient of 14.1 J mg^{-1} O_2 (Tucker, 1975; Videler, 1993; Dewar and Graham, 1994a). Then, these values were divided by the speed at which the value was determined, to obtain GCOT in units of J N^{-1} m^{-1}. Because J=N m, this expression of GCOT is dimensionless: (mg O_2 h^{-1} kg^{-1}) (9.8 m s^{-2})$^{-1}$ (14.1 J mg^{-1} O_2) (cm s^{-1})$^{-1}$ (1 h 3600 s^{-1}) (100 cm m^{-1}) (kg m s^{-2} N^{-1}) = J N^{-1} m^{-1}. The net cost of transport (COT_{net}) at each speed was calculated in the same manner, starting with the rate of oxygen consumption above SMR (mass-specific $\dot{V}_{O_2}$ at each speed – SMR).

The graph showing bonito GCOT in relation to speed (Fig. 4A, note speed units are m s^{-1}), has a U-shaped function. The function's minimum defines the optimal swimming speed (U_{opt}, i.e. the speed with the lowest energy cost per unit distance) (Videler and Nolet, 1990; Videler, 1993; Korsmeyer and Dewar, 2001). The U_{opt} of the bonito at both 18°C and

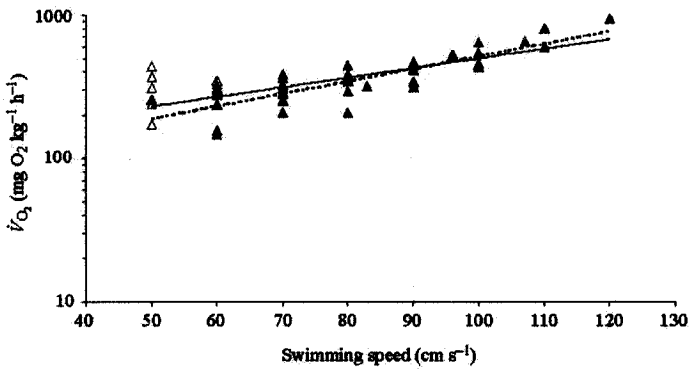

Fig. 3. Semi-logarithmic plots comparing the complete (open triangles, solid line) and corrected (filled triangles, broken line) bonito swimming speed–$\dot{V}_{O_2}$ data sets. [Note: the corrected data set was obtained by the selective removal of 10 elevated-$\dot{V}_{O_2}$, slow-speed data points (see text).] The two data sets are described by the best-fit equations: $y=107e^{0.015x}$, r=0.74, for the complete data set; $y=70e^{0.020x}$, r=0.84, for the corrected data set. Extrapolation of these equations to zero speed estimates bonito SMR as 107±22 mg O_2 kg^{-1} h^{-1} for the complete data set; 70±10 mg O_2 kg^{-1} h^{-1} for the corrected data set.

24°C is approximately 0.7 m s^{-1} (about 1.4 *FL* s^{-1}). The corresponding GCOT at U_{opt} is 0.17 J N^{-1} m^{-1} at 18°C and about 0.27 J N^{-1} m^{-1} at 24°C. Also shown in Fig. 4A are regressions describing bonito COT_{net} at both 18°C and 24°C. These functions are nearly parallel ($y=0.061e^{0.885x}$, $r=0.99$, at 18°C; $y=0.0951e^{0.916x}$, $r=0.99$, at 24°C); the difference in the y-intercepts reflects the assumed increase in SMR at 24°C (see below).

Interspecific comparisons

Most tuna respirometry has been done at 24°C. Because we lacked the heating and temperature-control equipment required to acclimate bonito to 24°C, the 18°C bonito $\dot{V}_{O_2}$ data were adjusted to 24°C, assuming a Q_{10} of 2.0 (Brill, 1979, 1987; Dewar and Graham, 1994a), for comparison with tuna data.

Fig. 4B compares the GCOT and COT_{net} values for the bonito and yellowfin tuna at 24°C (data from Dewar and Graham, 1994a). At all speeds compared, the GCOT is higher in the yellowfin tuna than in the bonito. Due to its higher SMR (Korsmeyer and Dewar, 2001), the yellowfin also has a higher U_{opt}, approximately 1 m s^{-1} (2.3 *FL* s^{-1}) *versus* 0.7 m s^{-1} (1.4 *FL* s^{-1}) for the bonito. The GCOT at U_{opt} for the yellowfin tuna is greater than that of the bonito (0.46 J N^{-1} m^{-1} *versus* 0.27 J N^{-1} m^{-1}). The COT_{net} functions for the bonito ($y=0.0951e^{0.916x}$, $r=0.99$) and yellowfin tuna ($y=0.0375e^{2.07x}$, $r=0.98$) differ, with the yellowfin having both a greater slope and a lower intercept. However, because the 95% confidence intervals of these parameters overlap, the two functions are not significantly different.

The bonito standard metabolic rate (SMR), as determined by the complete data set (Fig. 3), does not differ significantly (i.e. there is overlap in the 95% confidence intervals) from that of similar sized teleosts (Fig. 5), the yellowtail (*Seriola quinqueradiata*) at 20°C (Yamamoto et al., 1981) or the salmon (*Oncorhynchus nerka*) at 20°C (Brett and Glass, 1973). However, when adjusted for ambient temperature differences, the bonito SMR (161±33 mg O_2 kg^{-1} h^{-1}) is significantly lower ($P<0.05$, t-test) than that of similar sized yellowfin, skipjack and kawakawa *Euthynnus affinis* tunas (Brill, 1979, 1987; Dewar and Graham, 1994a) and dolphinfish *Coryphaena hippurus* (Benetti et al., 1995).

Plasma lactate concentrations

Bonito plasma lactate levels were determined for three phases of this respirometry study: just after capture from the holding tank, after 15 min in the round tank with companion fish, and after the swimming tests. The lactate concentration measured in the post-transfer period (2.84±0.88 mmol l^{-1}, $N=4$) was much greater than the pre-experiment (baseline-

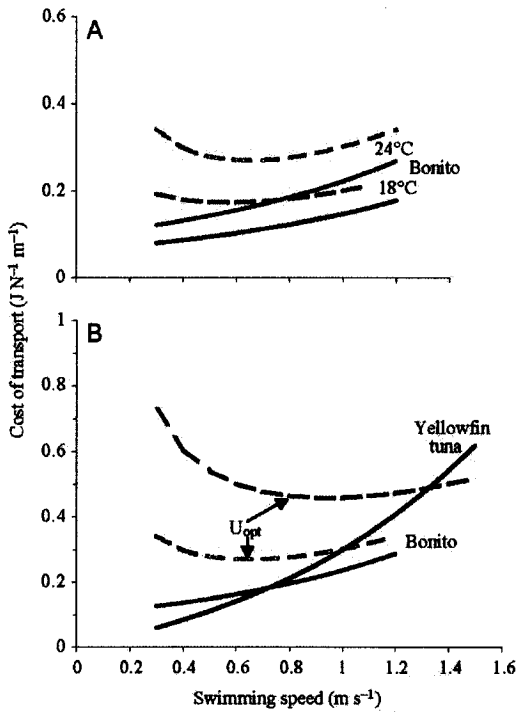

Fig. 4. (A) The gross cost of transport (GCOT, broken lines) and net cost of transport (COT_{net}, solid lines) estimates for bonito swimming at 18°C (blue) and 24°C (red). [Note: 24°C values are estimated from 18°C values, based on a Q_{10} of 2.0 (see text).] (B) Comparison of the GCOT (broken lines) and COT_{net} (solid lines) values predicted for the bonito (red) at 24°C with estimates for similar sized yellowfin tuna (black) at 24±1°C (Dewar and Graham, 1994a).

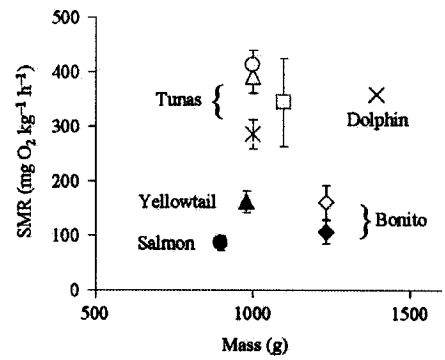

Fig. 5. Comparison of bonito mass-specific standard metabolic rate (SMR) at 18°C and 24°C with values published for other comparably sized species at similar test temperatures: yellowtail *Seriola quinqueradiata* (filled triangle) (20°C; Yamamoto et al., 1981), salmon *Oncorhynchus nerka* (filled circle) (20°C; Brett and Glass, 1973), skipjack *Katsuwonus pelamis* (open circle) (25°C; Brill, 1979), yellowfin *Thunnus albacares* (asterisk) and kawakawa *Euthynnus affinis* (open triangle) tunas (25°C; Brill, 1987), yellowfin tuna (open square) (24±1°C; Dewar and Graham, 1994a) and dolphinfish *Coryphaena hippurus* (cross) (Benetti et al., 1995).

control) lactate concentration (0.11±0.03 mmol l^{-1}, N=2) and significantly higher (two sample t-test, P<0.05) than the post-swimming experiment levels (0.26±0.08 mmol l^{-1}, N=4).

Discussion

This study quantifies the swimming energetics of the eastern Pacific bonito at nearly constant temperature and over a range of sustained speeds and provides the first comparative metabolic performance data for a species in the tuna sister group, tribe Sardini. We found that the bonito COT_{net} is similar to that of both tunas and other active teleosts, including another scombrid, the chub mackerel (*Scomber japonicus*, tribe Scombrini). The SMR of the bonito was also found to be similar to values in similar sized yellowtail and salmon, but was significantly lower than that of size-matched yellowfin, skipjack and kawakawa tunas and dolphinfish (Fig. 5). Although we recognize the need for bonito and tuna comparisons at the same experimental temperature, this study offers strong evidence that the elevated SMR of tunas, and not their locomotory costs, distinguishes the tunas from other scombrids and most other pelagic, high performance fishes.

Validation of the bonito $\dot{V}_{O_2}$–speed relationship

Both capture and handling stress have the potential to increase fish metabolic intensity to the point of elevating muscle and plasma lactate levels, followed by a period of elevated aerobic metabolism required for the reconversion of lactate to glycogen (Milligan and Girard, 1993; Milligan, 1996). Validation of the bonito swimming $\dot{V}_{O_2}$ data therefore required confirmation that handling and confinement in the respirometer working section did not result in an excessive build-up of lactate. Our findings that bonito plasma lactate levels were elevated during the handling phases of the experiments, but then fell to low levels during the swimming tests, indicate that the metabolic costs of lactate reconversion were not a significant component of the bonito swimming-$\dot{V}_{O_2}$ determinations.

Unstable swimming

Bonito $\dot{V}_{O_2}$ was determined over speeds ranging from 50 to 120 cm s^{-1}. Although the exponential rise in $\dot{V}_{O_2}$ occurring with incremental speed increases above 60 cm s^{-1} is entirely consistent with theory (Videler, 1993; Webb, 1998), Fig. 2 shows that the mean $\dot{V}_{O_2}$ for bonito is minimal at 60 cm s^{-1} and elevated at 50 cm s^{-1}. Because erratic fin fluttering and unsteady swimming were documented for slow-swimming bonito (Table 1), we attribute the increase in mean $\dot{V}_{O_2}$ at speeds of 50 cm s^{-1} (and, to some extent, 60 cm s^{-1}) to the metabolic costs associated with the additional movements required for low-speed stability. This interpretation is consistent with findings for other fishes at low speeds (He and Wardle, 1986; Lucas et al., 1993; Webb, 1998; Freund, 1999; Sepulveda and Dickson, 2000), as well as for other animals (Withers, 1992; Schmidt-Nielsen, 1993).

Most scombrids, including bonitos, are negatively buoyant and must swim continuously, both for hydrodynamic lift (Magnuson, 1973) and ram ventilation (Roberts, 1975). Magnuson (1973) used comparative morphometric and body density data to estimate the minimum speeds required for bonitos and tunas to maintain hydrostatic equilibrium. Application of the Magnuson (1973) minimum speed equation to bonito (45–55 cm FL) in this study yields a minimum speed of approximately 1.2 FL s^{-1} or about 54–66 cm s^{-1}, which corresponds to the range where unsteady motion and increased swimming costs occurred for the bonito in this study (Fig. 2).

Unstable swimming and COT and SMR estimates

Comparison of the complete $\dot{V}_{O_2}$–speed data set to one modified by the selective removal of the ten high-$\dot{V}_{O_2}$, low-speed measurements (Fig. 3) documents how unsteady swimming artificially increases the SMR estimate and lowers the estimated swimming cost (regression parameters of the $\dot{V}_{O_2}$–speed relationship). In the case of the bonito, this data manipulation was warranted because of our direct observations of unsteady swimming at low speeds (Table 1) and the known correspondence between unsteady swimming and the bonito's minimum speed requirements for hydrostatic equilibrium (Magnuson, 1973, 1978). While this correction did not significantly change the parameters of either regression function (i.e. there is overlap in the 95% confidence intervals), it did improve the correlation coefficient for the $\dot{V}_{O_2}$–speed function. Although the selected data set appeared to be more meaningful biologically, comparisons with data for other species, for which selective removal was not done, required that we operate with the complete data set.

Comparisons of $\dot{V}_{O_2}$ in bonito

Two previous studies provide swimming $\dot{V}_{O_2}$ estimates for the eastern Pacific bonito. Mendo and Pauly (1988) used the observational swimming speed data of Magnuson and Prescott (1966) to estimate the routine metabolic rate (RMR) [defined by Beamish (1978) as the combination of normal and spontaneous movements] for a 2.5 kg, 57 cm FL bonito swimming in a large aquarium at 22°C. By combining mean swimming speed (1.4 FL s^{-1}) with the estimated volume of water traversing the gills (based on mouth aperture or gape), and assuming the extraction of 50% of the O_2 in the water contacting the gills, these workers arrived at an estimated RMR of 160 000 cal day^{-1}, or 800 mg O_2 kg^{-1} h^{-1}. This value is three times the $\dot{V}_{O_2}$ that we measured for bonito swimming at 1.4 FL s^{-1} (260 mg O_2 kg^{-1} h^{-1}) and doubtlessly reflects the numerous assumptions underlying the Mendo and Pauly (1988) $\dot{V}_{O_2}$ estimate.

Freund (1999) reported similar RMR values for bonito and skipjack tuna swimming in an annular respirometer at 20±1°C. However, speed was not controlled in that study and unsteady swimming was noted for both species. The mean RMR estimated for the bonito was 427 mg O_2 kg^{-1} h^{-1}, and a speed estimate for one bonito was 50 cm s^{-1} (Freund, 1999). Although bonito size and experimental temperature were similar in that study to ours, it is difficult to compare the two.

Inspection of Fig. 2 shows that 50 cm s^{-1} is too slow for stable swimming in bonito of the size studied, and this may account for the 1.4-fold higher $\dot{V}_{O_2}$ measured by Freund (1999).

Bonito and tuna comparisons

Swimming efficiency

Although much of our understanding of tuna morphology and physiology suggests that they should swim more efficiently than other fishes, no study, including this work with the eastern Pacific bonito, has successfully documented this difference (Sepulveda and Dickson, 2000; Korsmeyer and Dewar, 2001). This is paradoxical because tuna specializations, including a high degree of body streamlining, a unique RM position, tendon arrangement, thunniform swimming mode and endothermy, have all been hypothesized to augment aerobic swimming performance and presumably swimming efficiency (Carey and Teal, 1966; Fierstine and Walters, 1968; Graham, 1975; Johnston and Brill, 1984; Westneat et al., 1993; Altringham and Block, 1997; Graham and Dickson, 2000, 2001; Donley and Dickson, 2000; Ellerby et al., 2000; Altringham and Shadwick, 2001; Dowis et al., 2003).

Studies of tuna swimming efficiency, however, have of necessity been carried out on juvenile or sub-adult fishes in swimming chambers (Gooding et al., 1981; Graham and Laurs, 1982; Graham et al., 1989; Dewar and Graham, 1994a; Freund, 1999; Sepulveda and Dickson, 2000; Donley and Dickson, 2000). In the case of water tunnels, it may be that the requirement to maintain position by matching swimming thrust production to water flow speed obscures the utility of structural and physiological specializations for swimming that, in a more natural setting, increase swimming efficiency. A free-swimming tuna, for example, has options for turning, gliding and changing depth, as well as responding behaviorally to physical features in the environment, such as density gradients and thermal fronts. In addition, schooling may augment swimming efficiency (Weihs, 1973). Magnuson's finding (Magnuson, 1973) of allometric changes that lower the minimum speed requirements of larger tunas further suggests that increased size, in combination with swimming specializations, increases swimming efficiency in larger tunas.

Standard metabolic rates

Extrapolation of the Fig. 3 regressions to zero speed yields SMR estimates for the bonito that are comparable to those of the salmon (Brett and Glass, 1973) and yellowtail (Yamamoto et al., 1981), but significantly lower than both similar-sized yellowfin and skipjack tunas swimming in the same respirometer system (Dewar and Graham, 1994a) and spinally blocked (paralyzed) yellowfin, skipjack and kawakawa tunas (Brill, 1979, 1987) and dolphinfish (Benetti et al., 1995) (Fig. 5). The dolphinfish is the only fish species shown to have an SMR comparable to that of the tunas (Benetti et al., 1995). Although it lacks an elevated tissue temperature, the dolphinfish is a highly active species with several specializations (i.e. rapid growth rate, high heart rate) related to its high-energy-demand, pelagic existence (Brill, 1996). Similarity between the bonito SMR and that of the chub mackerel (Shadwick and Steffensen, 2000) suggests that, among the scombrids thus far studied, the tunas are unique in having a high SMR.

The basis for the tuna's elevated SMR has been the topic of considerable discussion (Brill, 1979, 1987, 1996; Gooding et al., 1981; Dewar and Graham, 1994a; Brill et al., 2001; Sepulveda and Dickson, 2000; Korsmeyer and Dewar, 2001; Graham and Dickson, 2001). First, an elevated SMR is consistent with higher maintenance costs associated with the tunas' relatively large gills and heart and with the elevated metabolic requirements of the tunas' warm muscle and other tissues. In addition, there may be functional costs requiring a high SMR, such as the osmoregulatory load imposed by a larger gill surface area (Brill, 1987, 1996; Benetti et al., 1995; Brill et al., 2001). Tunas also have the added costs associated with the aerobic requirements of a larger and thicker-walled ventricle that has both a high cardiac output and a high systolic pressure (Graham et al., 1983; Brill and Bushnell, 1991, 2001; Bushnell and Jones, 1994; Graham and Dickson, 2000). Added to these may be a higher aerobic cost associated with the more aerobic white muscle of tunas. As in other fishes, white muscle accounts for the greatest percentage of body mass, and tuna white muscle has a greater aerobic capacity than in other species (as indicated by the high activity of the aerobic enzyme, citrate synthase) (Dickson, 1995, 1996; Korsmeyer and Dewar, 2001). Finally, there are the additional metabolic costs for processes such as gastric evacuation, somatic and gonadal growth rates, and lactate processing, which all appear to be augmented in tunas relative to other scombrids and other pelagic teleosts (Magnuson, 1969; Schaefer, 1984; Perry et al., 1985; Weber et al., 1985; Hunter et al., 1986; Arthur et al., 1992; Bushnell and Jones, 1994; Brill, 1996; Korsmeyer and Dewar, 2001). The high SMR of tunas therefore appears important in enabling them to sustain high energy production rates required by the combinations of endothermy and a high aerobic scope for activity (Priede, 1985; Bushnell and Jones, 1994; Brill, 1996; Brill et al., 2001; Bushnell and Brill, 2001; Korsmeyer and Dewar, 2001).

Conclusions

The objectives of this study were to obtain swimming energetics data for the eastern Pacific bonito in the same respirometer that has been used to study tunas, and to compare bonito and tuna locomotor costs and SMR. Because the bonito is in the scombrid tribe Sardini, which is the sister group of the tunas (tribe Thunnini), comparisons of tuna–bonito swimming performance have the potential to delineate the pattern of character acquisition leading to tuna locomotor specializations, including endothermy. Although our swimming performance comparisons necessitated a temperature adjustment (from 18°C to 24°C) of the bonito swimming–$\dot{V}_{O_2}$ data, the resulting net cost of transport (COT_{net}) estimate for the bonito significantly overlaps with that of the yellowfin tuna and thus indicates comparable swimming costs over the range of speeds tested.

On the other hand, the finding of a lower SMR in both the

bonito and the chub mackerel relative to tunas implies that, in tuna, elevated SMR is an apomorphy linked to this group's numerous structures and functions requiring high metabolic maintenance. It may be that studies on the more basal species of tunas, for example, the slender tuna, *Allothunnus fallai*, will elucidate the linkages between SMR, aerobic function and endothermy. *Allothunnus* has less RM than other tunas and some of the circulatory modifications required for endothermy, but whether or not it is endothermic is not presently known (Graham and Dickson, 2000).

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CHAPTER III

The discovery of a cranial furnace in a tuna species

Abstract

Selection for endothermy among vertebrates has led to the evolution of a variety of mechanisms for both production and retention of metabolic heat (Bakker, 1971; Crompton et al., 1978; Bennett and Ruben, 1979; Block, 1994; Ruben, 1995). Only two endothermic fish taxa, the billfishes (Istiophoridae and Xiphiidae) and the butterfly mackerel, *Gasterochisma melampus* (Scombridae), have eye muscles modified specifically for heat production (Carey, 1982; Block, 1986). Until this study, convergence on a brain heater was thought to have occurred independently only within these two groups (Block and Finnerty, 1994). Here we show a never before described thermogenic organ situated beneath the brain of the slender tuna, *Allothunnus fallai*. We used a combination of physiological, morphological, and biochemical techniques to characterize the thermogenic tissue and magnetic resonance imaging to illustrate its association with the brain and optic nerves. Four of the extraocular muscles have been modified for heat generation. We report on elevated cranial and red aerobic muscle temperatures, eye muscles lacking contractile proteins and the presence of a counter-current heat exchange system in direct contact with the heater tissue. These findings document the first case of non-shivering thermogenesis in a tuna species (Tribe Thunnini), and the third group to have independently evolved a brain heater.

Introduction

The body temperature of most fishes is similar to that of the surrounding water due to the heat loss associated with both thermal conductance and convection. A few highly specialized groups of marine fishes have evolved mechanisms to reduce heat loss and significantly elevate tissue temperatures above ambient conditions (regional endothermy). The tunas, the largest group of endothermic fish, regionally elevate body temperatures using vascular counter-current heat exchangers (*retia*) that conserve metabolic heat from aerobic muscle contractions and digestion (Carey et al., 1971). Tunas warm their red oxidative swimming muscle, stomach, and eye and brain regions (Carey et al., 1966; Linthicum and Carey, 1972; Graham, 1975; Graham and Diener, 1978; Carey et al., 1984; Stevens and Fry, 1971; Schaefer, 1985). Endothermy has been documented for all tunas except for the most basal member of the clade (tribe Thunnini), the monotypic genus of the Southern Ocean, the slender tuna, *Allothunnus fallai* (Graham and Dickson, 2000). The objectives of this study were to record in vivo body temperatures for *A. fallai*. .

Methods

Species

Slender tuna were captured by hook and line off of the coast of southern New Zealand. Temperature measurements were taken immediately upon capture with a digital thermocouple thermometer (Barnant model 600, IL, USA). The thermocouple was inserted into the neurocranium via the orbit at a 45° angle to the axis of the body, the warmest region was recorded and subsequently verified with gross dissections. Temperature measurements were also taken for the red, aerobic locomotor muscle (RM) at the body position of maximum RM (just beneath the first dorsal fin) (Graham and Dickson, 2000).

Imaging

Magnetic resonance imaging was used to acquire high-resolution isotropic data with high tissue contrast using a 3D T1-weighted sequence. A 3D Fast Spin Echo (3D-FSE) pulse sequence was used to reduce the effect of possible air pockets. Image parameters were: field-of-view = 140mm, matrix size (frequency x phase) = (256 x 192), slice thickness = 0.5mm, repetition time = 2000ms, echo time = 7.6ms, echo train length = 16, number of averages = 4, number of slabs = 32. Data in the phase encoding direction were interpolated from 192 to 256 to achieve approximate isotropic resolution (frequency, phase, slice) = (0.55mm, 0.55mm, 0.5mm). Three dimensional reconstruction and volumetric calculations were made using the Amira® software package (Jacobs et al., 2003).

Tissue analyses

Histological sections (5μm) stained with hematoxylin and eosin were prepared from fresh and frozen *A. fallai* functional and specialized extraocular muscles. A Fontana-Masson silver stain and a modified Giemsa stain were used to identify chromaffin cells (Rindi et al., 2004). Protein composition of the ocular muscles was examined using SDS-PAGE (Margossian and Lowey, 1982).

Results

Table 1 presents the morphological and *in vivo* temperature data for 30 slender tuna. The field studies on live *A. fallai* demonstrated it has warm RM (8.21 $\pm$ 0.18, n=30; above the ambient sea surface temperature, SST) and eye and brain temperatures (4.80 $\pm$ 0.18, n=11; above SST) (Figure, 1). Cranial dissections targeting the region of elevated temperature revealed the presence of a notable alteration to the extraocular muscles; a modified segment of all four rectus muscles that functions to warm the brain and eye region.

Discussion

Cranial dissections exposed a modified eye-muscle complex unlike that of any other tuna species (Stevens and Fry, 1971; Linthicum and Carey, 1972) (Figure 2). It is positioned in the posterior orbit in direct contact with the ventral side of the brain case (Figure 3). The tissue is formed at the point where the extraocular muscles converge, and is composed of portions of all four of the rectus muscles (superior, inferior, medial and lateral). As the rectus muscles proceed from their insertion on the eye to their medial origin, they coalesce and undergo a morphological transition from red, aerobic striated muscle to a darkened, fused mass of gland-like tissue. The heater tissue lacks visible myofibril organization, is highly vascularized, and has large pigmented bodies dispersed throughout. As this tissue extends medially beneath the braincase, it unites with the similar extraocular tissue that is formed from the opposite eye muscles.

Magnetic resonance (MR) imaging was used to acquire high-resolution isotropic cranial images for *A. fallai* (Figure 4). High contrast between the different soft tissues (i.e., muscle, brain, and connective tissue) enabled a three-dimensional reconstruction of the heater tissue and surrounding region (Jacobs et al., 2003). The thermogenic organ of an 86 cm fork length slender tuna represents a significant portion of the cranial region: 5,043 mm^3, 3.5 times the volume of the brain (1,434 mm^3).

The MR images also allowed us to track the trajectory of the optic nerves as they pass from the eye to the brain. Along this path the nerves enter the heater and become completely surrounded by the tissue (Figure 4, tiles a, b, and c). The intimate arrangement of the heater tissue effectively warms both the brain and the optic nerves,

a process documented to enhance physiological performance (i.e., neuronal transmission and temporal resolution) (Konishi and Hickman, 1964; Friedlander et al., 1976; Fritsches et al., 2005).

The braincase of *A. fallai* is uniquely configured to accommodate the heater tissue and provides a skeletal framework unlike that of any other member of the tribe Thunnini. The distinct cranial architecture creates a skeletal arch between the orbits, which provides a unique chamber for the heater tissue (Collette and Chao, 1975). Another structural modification of the braincase of *A. fallai* is the reduced thickness of the basisphenoid, which provides only a thin membrane-like separation between the heater tissue and the brain. The parallel evolution of the skeletal elements and the modified ocular muscles has reduced the thermal barrier between the two tissues, allowing maximal conduction of heat.

A. fallai also has a highly specialized vascular supply to the cranium, a counter-current system notably different from that of the other tunas (Linthicum and Carey, 1972). Instead of the carotid rete, the counter-current heat exchange system used for eye and brain endothermy in the other tunas, *A. fallai* has a dorsally-flattened, bilateral mass of vascular tissue that feeds directly into the dorsal surface of the heater tissue (Figure 2). The arterial component of the complex originates from the internal carotid artery, which in turn branches off the first efferent branchial, a major arterial supply to the head region in most fishes (Harder, 1975). The venous return is composed of a comparable number of veins, which drain into the anterior cardinal vein. Collectively, the vascular structure is made up of hundreds of arteries and veins positioned in a counter-current arrangement (Figure 4b). We propose that the proximity and intimate

association of the venous and arterial circulation results in counter-current heat exchange and enables the maintenance of elevated cranial temperatures.

We tested whether the brain heater of *A. fallai* shared characteristics with the heater tissue of the swordfish, *Xiphias gladius*, one of the most derived cranial endotherms (Block, 1994; Carey, 1982; Block, 1986; Block and Finnerty, 1994; DeMetrio et al., 1997). Under light microscopy, the ultrastructure of the tissue appeared similar, with both species exhibiting highly vascularized sections with a non-peripheral distribution of nuclei. Further examination revealed the absence of myofibrillar organization within the thermogenic region of the extraocular muscle, which contrasts markedly with the prominent striations occurring throughout the functional, non-specialized region (Figure 5a). Electrophoretic studies confirmed the absence of a myosin band (205 kd) in the heater tissue homogenate of *A. fallai*, and its presence in the non-specialized, neighboring portion of the same rectus muscle (Figure 5c). Stained preparations showed the pigmented regions of the heater to be chromaffin cells (Rindi et al., 2004), which have a role in modulating cardiovascular and respiratory function (Reid et al., 1998).

The thermogenic organ of *A. fallai* possesses several physical attributes that distinguish it from the brain heaters of both billfish and *Gasterochisma*. Most notable is the inclusion of all four rectus muscles into the heater organ of *A. fallai*, whereas heater modifications occur in only one muscle pair in both billfish (superior rectus) and *Gasterochisma* (lateral rectus) (Carey, 1982; Khono, 1984). Differences in cranial configuration (Block, 1986; Collette and Chao, 1975; Khono, 1984), the presence of chromaffin cells only in *A. fallai*, the position of the counter-current heat exchange

system (Carey, 1982; Linthicum and Carey, 1972), and the respective phylogenetic relationships (Block and Finnerty, 1994; Graham and Dickson, 2000) establish that cranial endothermy evolved independently in these three groups.

We propose that the eye muscles of *A. fallai* have been modified into a thermogenic organ that functions to warm the brain and optic nerves. Heat produced by this tissue is locally conserved, by the intricate counter-current arrangement of the blood supply. Brain warming is facilitated by the reduced thickness and unique architecture of the cranial bones. Similarities in physical appearance, protein composition, and the documentation of elevated temperatures suggest that the heat generating mechanism used by *A. fallai* is convergent with that of the billfishes and *Gasterochisma*. These findings represent the first ever case of non-shivering thermogenesis in a tuna species (Thunnini).

Table 1. Morphological and body temperature data for *A. fallai*.

A. fallai	FL (cm)	Mass (kg)	T_{RM} (°C)	T_{water} (°C)	T_{XRM} (°C)	T_C (°C)	T_{XC} (°C)
1	76.0	7.5	22.7	14.6	8.1	-	-
2	75.5	7.0	23.2	14.7	8.5	-	-
3	81.5	8.5	21.9	14.8	7.1	-	-
4	86.5	10.3	24.0	14.9	9.1	-	-
5	84.0	8.5	22.4	14.9	7.5	-	-
6	76.0	7.5	22.2	14.9	7.3	-	-
7	80.0	8.0	24.3	15.0	9.3	-	-
8	79.0	8.2	23.2	15.8	7.4	-	-
9	80.5	9.0	25.8	15.8	10	-	-
10	81.0	7.8	23.0	15.9	7.1	-	-
11	82.0	8.2	23.5	16.0	7.5	-	-
12	79.0	9.3	24.1	16.0	8.1	-	-
13	80.0	8.0	23.3	16.3	7.0	-	-
14	78.5	8.0	24.7	16.4	8.3	-	-
15	85.0	10.6	24.7	16.4	8.3	-	-
16	80.0	8.0	24.6	16.4	8.2	-	-
17	80.0	9.0	24.8	16.2	8.6	-	-
18	80.5	9.3	23.4	16.3	7.1	-	-
19	83.5	9.5	24.7	16.3	8.4	-	-
20	76.0	5.5	21.7	14.9	6.8	19.9	5.0
21	78.0	6.9	21.6	14.9	6.7	18.8	3.9
22	74.0	8.2	21.6	14.8	6.8	18.8	4.0
23	78.0	6.4	24.0	14.9	9.1	19.4	4.5
24	71.0	5.6	23.7	14.3	9.4	19.8	5.5
25	79.0	8.2	23.6	14.3	9.3	19.3	5.0
26	75.0	4.9	23.8	14.3	9.5	19.3	5.0
27	80.0	7.3	22.3	14.3	8.0	19.2	4.9
28	-	-	-	-	-	-	-
29	85.0	9.8	23.9	14.6	9.3	19.3	4.7
30	81.5	7.9	23.4	14.6	8.8	19.6	5.0
31	85.0	9.0	24.3	14.6	9.7	19.9	5.3
Mean +/- S.E.M					8.21 ± 0.18		4.80 ± 0.18

Fork length (FL); Red muscle temperature (T_{RM}); Sea surface temperature (T_{water}); Red muscle temperature elevation above ambient sea surface temperature (T_{XRM}); Cranial temperature (T_C); Cranial temperature elevation above T_{water} (T_{XC}).

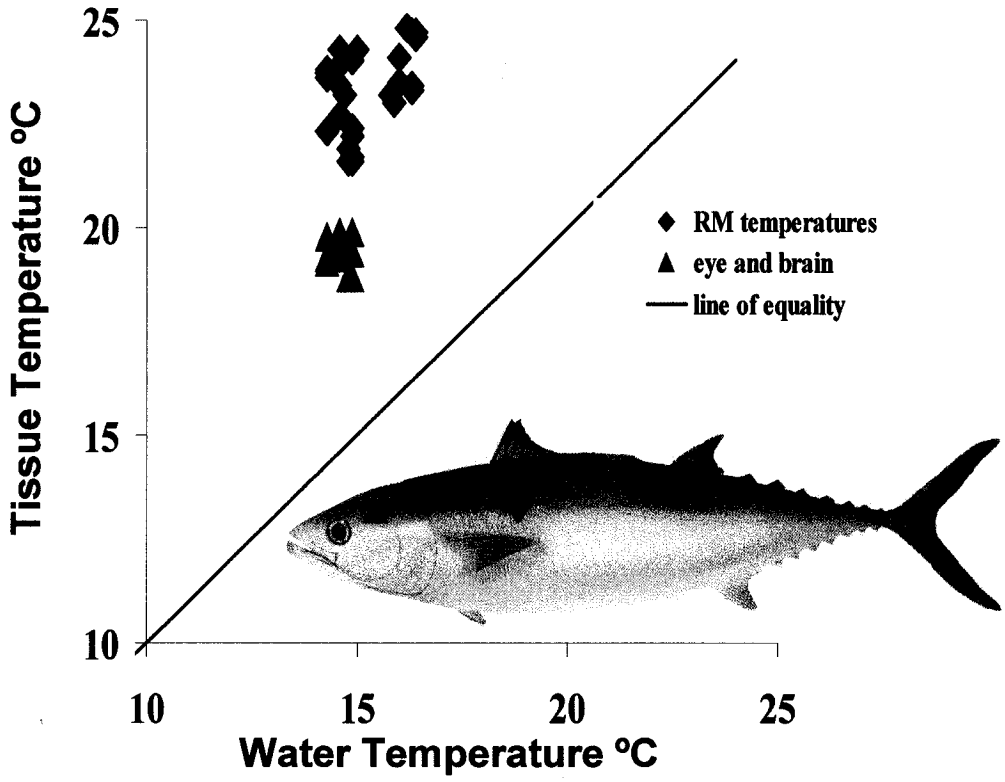

Figure 1. *A. fallai* red muscle (red diamonds) and eye and brain (triangles) temperature measurements plotted against the ambient sea surface temperature.

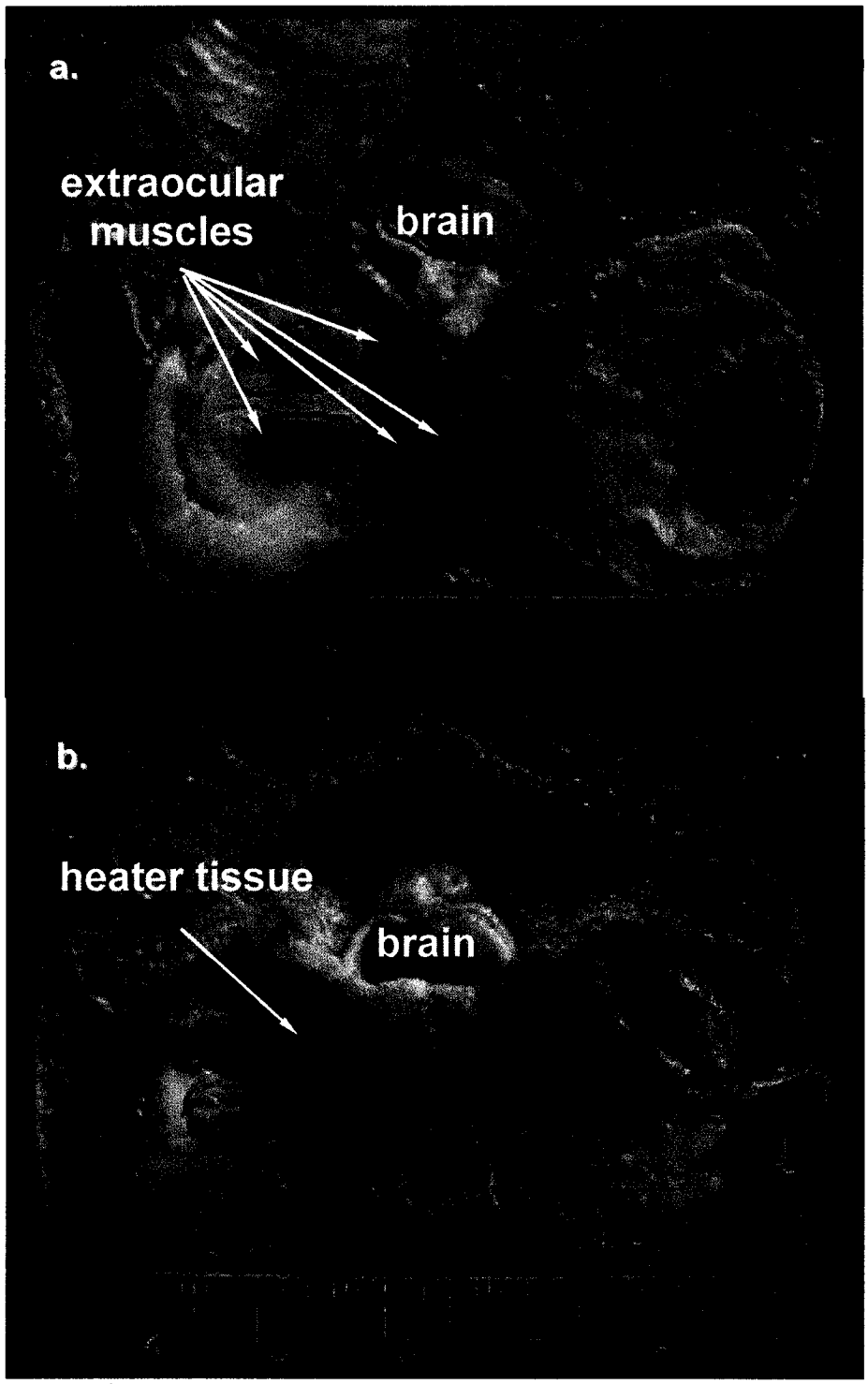

Figure 2. Transverse section through the cranial region (mid brain) of (a) an albacore tuna, *Thunnus alalunga*, and (b) a slender tuna, *Allothunnus fallai*, illustrating the arrangement of the extraocular muscles in the albacore and the presence of the fused mass of extraocular muscle tissue (heater tissue) beneath the brain of *A. fallai*.

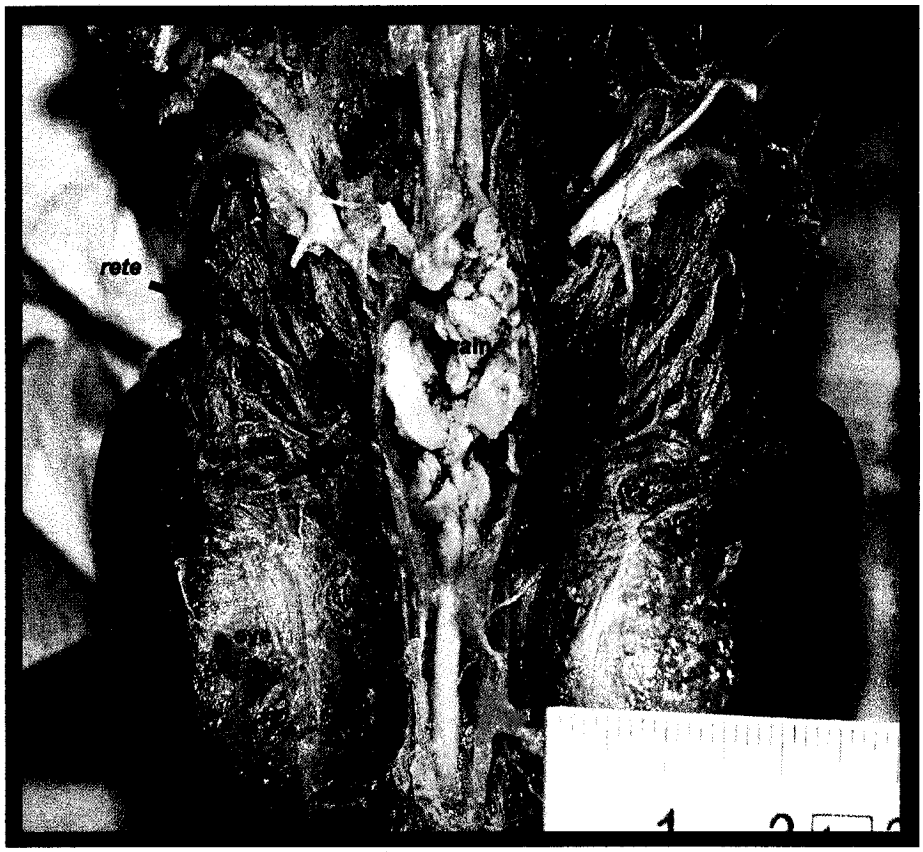

Figure 3. Dorsal view of the dissected cranial region of a preserved slender tuna, showing the brain, eyes, heater tissue and vascular heat exchangers (*retia*) (arrow on the left indicates anterior and the units on the scale are in centimeters).

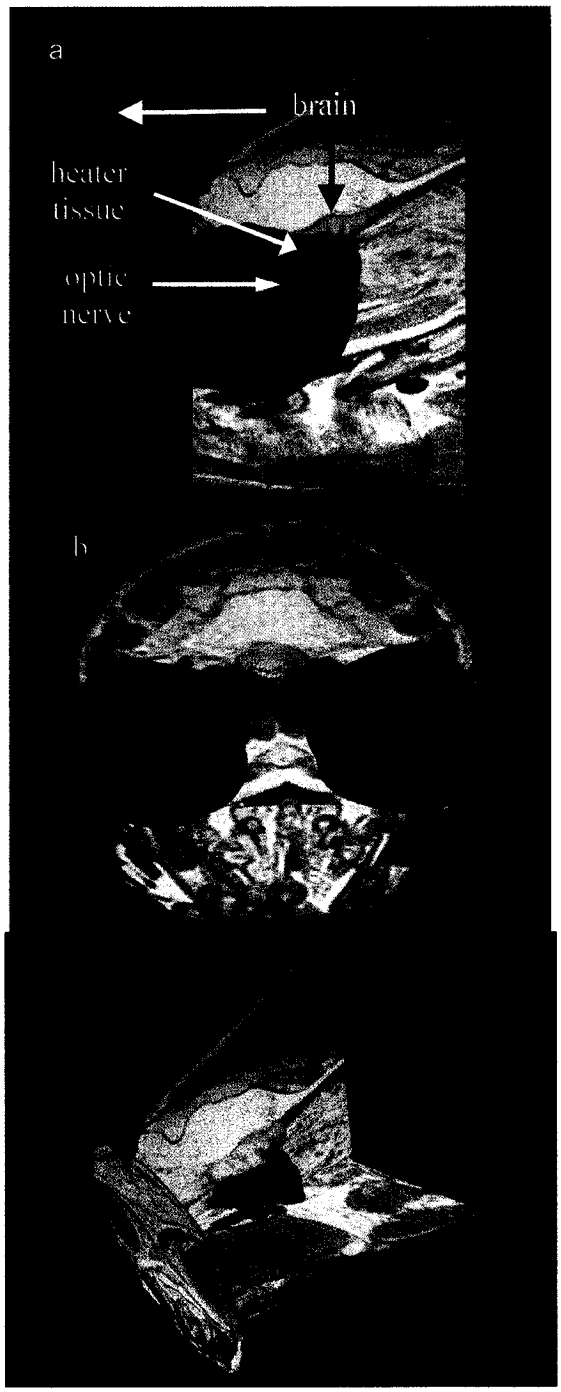

Figure 4. Three-dimensional reconstructions of magnetic resonance images of the cranial region of an 86 cm fork length slender tuna illustrating the proximity and orientation of the heater tissue to the brain and the optic nerves. The brain and optic nerves are yellow and the heater tissue is red. a, sagittal section. b, axial section. c, overlay of the sagittal, coronal and axial sections. (White arrows indicate the anterior direction).

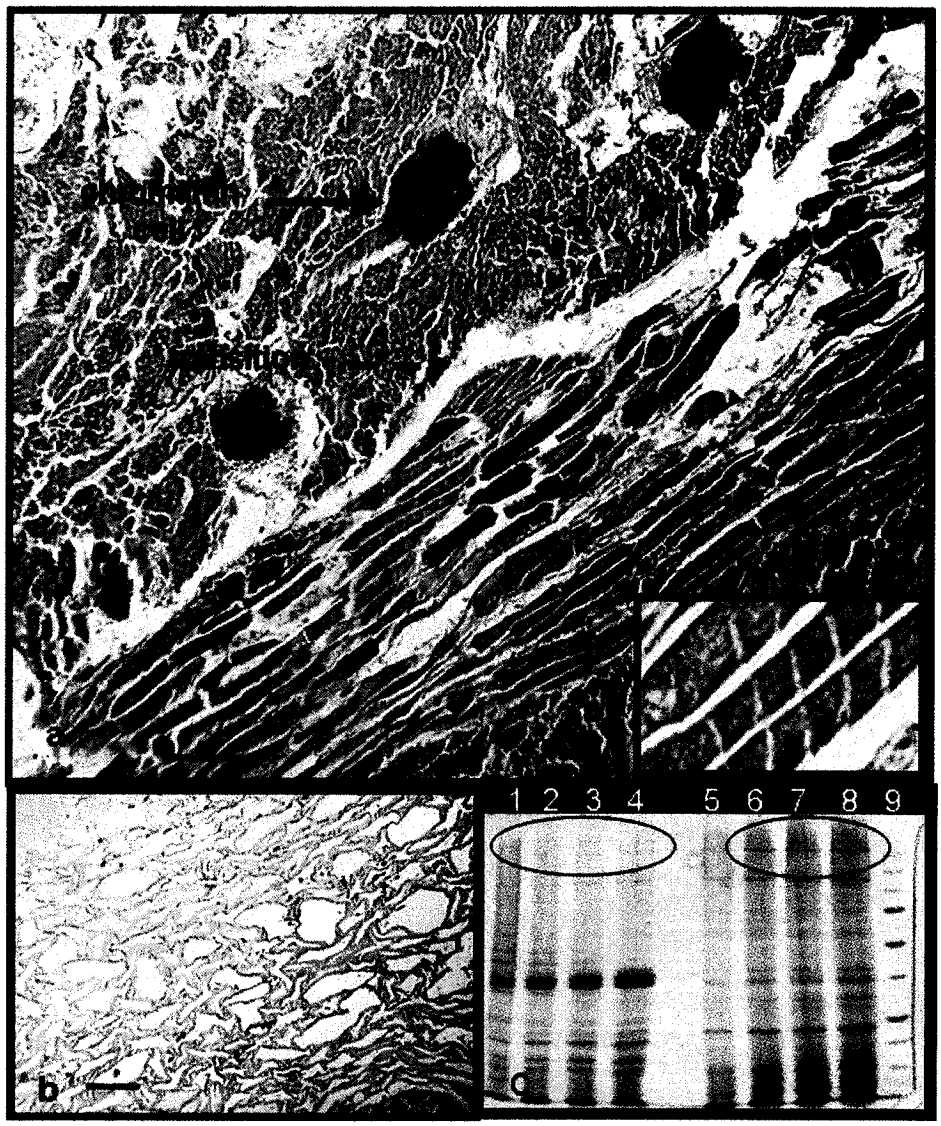

Figure 5. Light microscope sections (5 μm) of (a), the heater tissue transition region showing: the specialized, non-striated tissue (upper left) and the functional rectus muscle; chromaffin cells in the heater region (solid arrow); the transition zone which separates the two tissue types (dashed arrow); and myofibril striations in the non-specialized rectus muscle (lower right). (b), the vascular supply to the heater of *A. fallai* illustrating the presence of both arteries and veins. (c), SDS PAGE of the heater tissue and functional rectus muscle homogenates; lanes 1-4 are from different portions of the heater tissue, lane 5 from the transition region, lane 6 from the non-specialized portion of the functional superior rectus, lane 7 from the non-specialized region of medial rectus, lane 8 from the non-specialized portion of lateral rectus, and lane 9 is the molecular weight ladder.

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CHAPTER IV

Movement patterns, depth preferences, and stomach temperatures of free swimming juvenile mako sharks *Isurus oxyrinchus* in the Southern California Bight

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RESEARCH ARTICLE

C. A. Sepulveda · S. Kohin · C. Chan · R. Vetter
J. B. Graham

Movement patterns, depth preferences, and stomach temperatures of free-swimming juvenile mako sharks, *Isurus oxyrinchus*, in the Southern California Bight

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Abstract Acoustic telemetry was used to track vertical and horizontal movement patterns and to monitor the stomach temperatures of seven juvenile shortfin mako sharks (*Isurus oxyrinchus* Rafinesque) in the Southern California Bight from July to November 2002. Makos (80 145 cm fork length, FL) were attracted to the tracking vessel, where they were fed a mackerel containing an acoustic transmitter that reported temperature and pressure. Tracks ranged from 6.8 45.4 h. Collectively, the mako sharks spent 80% of the track record at 0 12 m, 15% at 12 24 m, and 5% at depths >24 m. The average horizontal swimming speed was 2.3 km h^{-1} or 0.55 FLs s^{-1}, and the greatest distance traveled was 145 km in 45.4 h. For the six tracks >21 h, there was a positive correlation between body size and maximum depth. Makos used more of the water column during daylight hours. Mean stomach temperature was 3.8 ± 1.5°C above ambient, and body size was positively correlated with both maximum and average stomach temperature. Stomach content analyses of four makos captured at the end of tracking verified the occurrence of feeding events as indicated by changes in stomach temperature.

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C. A. Sepulveda (✉) · C. Chan · J. B. Graham
Center for Marine Biotechnology and Biomedicine and Marine Biology Research Division, Scripps Institution of Oceanography, 9500 Gilman Drive, La Jolla, CA 92093-0204, USA
E-mail: csepulve@ucsd.edu
Fax: +1-858-5341304

S. Kohin · R. Vetter
Southwest Fisheries Science Center,
National Marine Fisheries Service,
La Jolla, CA 92037, USA

Introduction

This study examined the movements of the shortfin mako shark (*Isurus oxyrinchus*) in the Southern California Bight (SCB). The mako belongs to the family Lamnidae, a group that is capable of elevating the temperatures of their aerobic locomotor musculature (red muscle, RM), brains, and stomachs above ambient (Carey and Teal 1969; Carey et al. 1971; McCosker 1987; Goldman 1997; Bernal et al. 2001a, 2001b). The mako, like the other lamnids, is an active species with a relatively high metabolic rate (Graham et al. 1990).

Makos occurring within the SCB are of special concern because this region is one of the few documented nursery habitats in the North Pacific (Hanan et al. 1993). Makos harvested in the SCB are predominantly juveniles [year classes 0 3 (13 27 kg, 1 1.5 m fork length, FL)] (Cailliet and Bedford 1983; Cailliet et al. 1983; Pratt and Casey 1983; Holts and Bedford 1993; Hanan et al. 1993; O'Brien and Sunada 1994), which are present seasonally from May to October. Although commercially fished, there is little information about the population dynamics, migratory patterns, or fine-scale movements of makos within the SCB.

Mako movement patterns have been studied through conventional tagging (release and recapture) (Casey and Kohler 1992; California Department of Fish and Game 2001), commercial catch information (Stillwell and Kohler 1982; Cailliet and Bedford 1983), and acoustic telemetry (Carey et al. 1981; Holts and Bedford 1993; Klimley et al. 2002). The latter technique has been used to document fine-scale movements in relation to temperature, depth, and other physical factors. However, in the present study, to eliminate the possibility that capture and transmitter attachment may affect the shark's behavior (Carey et al. 1981; Holts and Bedford 1993), we used a non-invasive tag attachment technique, whereby the sharks volitionally ingested the transmitter.

This study addresses the following questions. How do physical conditions within the track area (i.e. submarine

topography, sea surface temperature, time of day) and factors such as body size and stomach temperature relate to mako swimming depth and habitat utilization? How do the track results from this study compare with previous studies (Carey et al. 1981; Holts and Bedford 1993; Klimley et al. 2002)? Are there differences in habitat utilization among the different mako year classes (years 0, 1, 2) most frequently found in the SCB?

Materials and methods

Mako shark capture and tagging procedure

Seven mako sharks (*Isurus oxyrinchus* Rafinesque) were tracked from July to November 2002. Makos were attracted to the 6-m tracking vessel, R.V. "Spilunky", using ground chub mackerel (*Scomber japonicus*) as chum. Once near the vessel, the size of the mako to be tracked was estimated and it was offered a mackerel in which an acoustic transmitter was embedded. The transmitter package consisted of a 6/0 fishing hook (which was intended to prevent immediate regurgitation) that was set in the mackerel and attached to a 10-cm-long (100 kg) monofilament leader spliced to a light (10 kg) main line. The heavy monofilament prevented a premature severing of the line until the package was at least 10 cm into the mako's esophagus. During ingestion, care was taken not to put any tension on the main line or to make any sudden movements that might affect the shark's behavior. Once the transmitter was swallowed, tracking commenced.

Four of the tracked makos were captured at the end of the study. Necropsy results provided data on gut contents, transmitter position, and body size (mass and FL).

Transmitters, receiving platform, and thermal profiles

We used Vemco Electronics (Armdale, Nova Scotia) V22 TP (22 mm diameter, temperature and pressure) 50-kHz transmitters, with the extra power option and an external reed switch. The rated accuracy of the transmitters was ±6.12 m (3% of total scale, 204 m) and temperature at ±1°C.

Signals were received and decoded by a Vemco VR60 receiver or a VR28 tracking system. The former used a V10 hydrophone (150 of the 167 total tracking hours) that was attached to a 2.5-cm-diameter steel pipe that extended 1.5 m below the lowest point of the hull of the tracking vessel. The pipe could be rotated 360° as to determine the shark's relative bearing. The VR28 used a V41 hydrophone side-mounted approximately 1 m below the hull of the 7-m tracking vessel (R.V. "Ernest"). The horizontal position estimates for each track were recorded periodically (approximately every 3 30 min) using a handheld GPS (Garmin model 72) and downloaded using the Map Source software package.

Water column thermal profiles and ambient track conditions were measured with a Sea Bird Electronics micro-bathythermograph (model SBE-39), which was deployed with a fishing reel at the initial tagging site and at 1- to 3-h intervals over the duration of the track.

Data analysis

Three terms are used to describe mako stomach temperatures. T_S is the observed stomach temperature and T_{SX} is the difference between T_S and the ambient water temperature at which the shark was swimming (T_A, extrapolated from the thermal profile data). In order to compare our study to previous work (Carey et al. 1981; Bernal et al. 2001b), we also used T_X, which is defined as the difference between T_S and the sea surface temperature (SST; $T_X = T_S - T_{SST}$).

Two methods were used to estimate swimming speeds. The first was an over-ground estimate determined from the GPS data from the tracking-vessel position. The second, which incorporated the shark's horizontal and vertical movements, required calculation of each vertical vector and combining them with the over-ground distance estimate to construct two legs of a right triangle, of which the calculation of the hypotenuse and the track time provided the combined speed estimate (Marcinek et al. 2001).

Daylight hours were defined as 30 min before sunrise to 30 min after sunset. Diurnal movement patterns for each shark were analyzed for differences in average depth using a two-sample *t*-test, and depth range was analyzed using a paired Wilcoxon rank test, one-sided. All values are indicated as means ± SE.

The presence of acoustic noise (i.e. cetaceans, scattering layer organisms, and depth recorders that use 50 kHz) and interruptions in data transmission (spurious data points) provided occasional erroneous temperature and depth measurements. These data points accounted for <4% of the entire data set and were filtered from the records. The criteria for the removal of such data points included: (1) if the recorded values were not within the transmitter specifications [depth (0 204 m) and temperature range (−5°C to 35°C)]; (2) if consecutive data points in the depth record deviated by >12 FLs s^{-1}; and (3) if consecutive temperature data points deviated by >2°C s^{-1}.

Results

Data for the seven tracked mako sharks (*Isurus oxyrinchus*) are given in Table 1 and Appendix 1 of the Electronic Supplementary Material to this paper. The mean over-ground speed was 2.3 ± 0.2 km h^{-1}or 0.55 ± 0.04 FLs s^{-1} and the combined vertical and horizontal speed estimate was 6.7 ± 1.2 km h^{-1}. The com-

Table 1 *Isurus oxyrinchus*. Details for seven mako sharks tracked in the Southern California Bight in 2002 (*M* male; *F* female; *n/a* data not available)

Fish no.	Date	Fork length (m)	Mass (kg)	Gender	Start location (DD)	Track duration (h)	Distance traveled (km)	Mean speed (km h^{-1})	Max. depth (m)	Capture
1	25 Jul	~1.15	~16	n/a	32.839N; 117.357W	6.8	18	2.6	17	No
2	20 Sep	1.15	16.0	F	32.833N; 117.376W	25	49	2.0	101	Yes
3	29 Sep	1.2	18.0	F	32.888N; 117.405W	23	55	2.4	168	Yes
4	8 Oct	~1.25	~20	n/a	32.854N; 117.499W	21	32	1.5	80	No
5	15 Oct	0.83	7.0	M	32.899N; 117.389W	23.2	50	2.15	32	Yes
6	22 Oct	~1.45	~32	n/a	33.108N; 117.454W	45.4	145	3.2	201	No
7	6 Nov	0.80	5.0	F	32.846N; 117.442W	23.2	37	1.6	35	Yes

bined depth records show that 80% of the track record (given as a percent of total data points recorded) was spent at depths from 0 to 12 m, 15% at 12–24 m, and 5% at depths >24 m (Fig. 1). For the six tracks spanning both day and night hours (makos 2–7), the average swimming depth and overall depth range were significantly greater during the day than at night (paired Wilcoxon rank test; $P<0.05$). The greatest distance traveled by any mako was 145 km in 45.4 h (mako 6), and the greatest depth penetrated was 201 m (mako 6). Makos 2–7 showed a positive correlation ($r^2=0.86$; $P<0.05$) between body length and maximum depth penetration (Fig. 2). The mean T_{SX} for each shark ranged from 1.7°C to 5.7°C, with a mean of 3.8±1.5°C. When the T_{SX} data were examined in relation to body size, positive correlations were found between body mass and maximum T_{SX} ($T_{SXmax}=2.847e^{0.029mass}$; $r^2=0.56$) and average T_{SX} ($T_{SX}=0.568e^{1.62mass}$; $r^2=0.61$).

Individual tracks

Mako 1 remained at depths between 0 and 12 m for 99% of the track record (Fig. 3a). The average T_S was 25.9±1.2°C, and the SST was approximately 21.3°C.

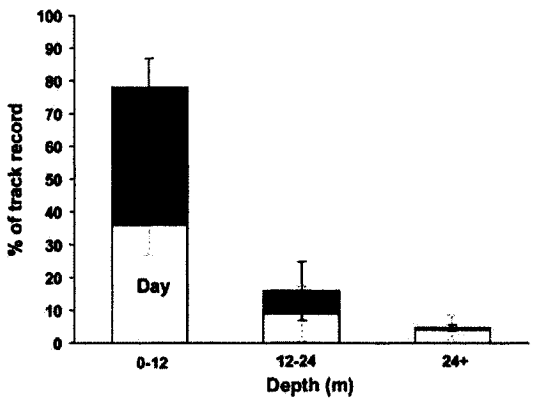


Fig. 1 *Isurus oxyrinchus*. Combined depth distribution data (presented as percentage of track record) for six makos (makos 2–7). Day (*white*) and night (*shaded*) hours indicated for three depth categories: surface–12 m, 12–24 m, and >24 m

The maximum T_X for this mako was 5.8°C. (The thermal profile of the water column was not measured during this tracking session.) This shark was fed a mackerel (ca. 300–400 g) near the end of the track session (~1620 hours) in order to quantify the reduction in T_S caused by food ingestion. A T_S decrease of 0.12°C min^{-1} was recorded immediately after swallowing the mackerel (Fig. 3a, 1620 hours). For all subsequent tracks, we interpreted abrupt T_S changes of this magnitude or greater as indicative of feeding. We identified six presumptive feeding events.

Mako 2 spent 91% of its track record shallower than 12 m, 7% at 12–24 m, and 2% deeper than 24 m (Fig. 3b). Diurnal trends were not obvious in the track record of mako 2. The mean T_S was 23.6±1.4°C at an average SST of 20.3±0.05°C. Its average T_{SX} was 4.2±1.7°C. The T_S record indicated two feeding events. The first was at 1840 hours, about 2.5 h after the track began. The second occurred the following afternoon when, at approximately 1340 hours, it abruptly descended to 101 m. This shark was captured soon thereafter, and we found six (~0.4 kg total mass) nearly undigested Pacific saury (*Cololabis saira*). We also found the transmitter to be in the posterior portion of the stomach and that the transmitter hook had not pierced the stomach wall.

Mako 3 spent ~82% of the track record at 0–12 m, 4% at 12–24 m, and 14% deeper than 24 m (Fig. 3c). The depth record indicated a strong diurnal pattern in

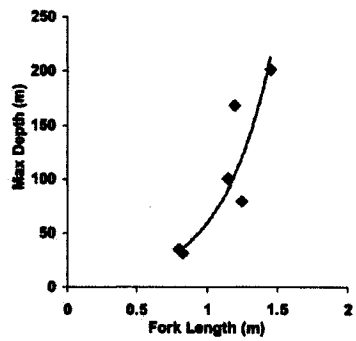
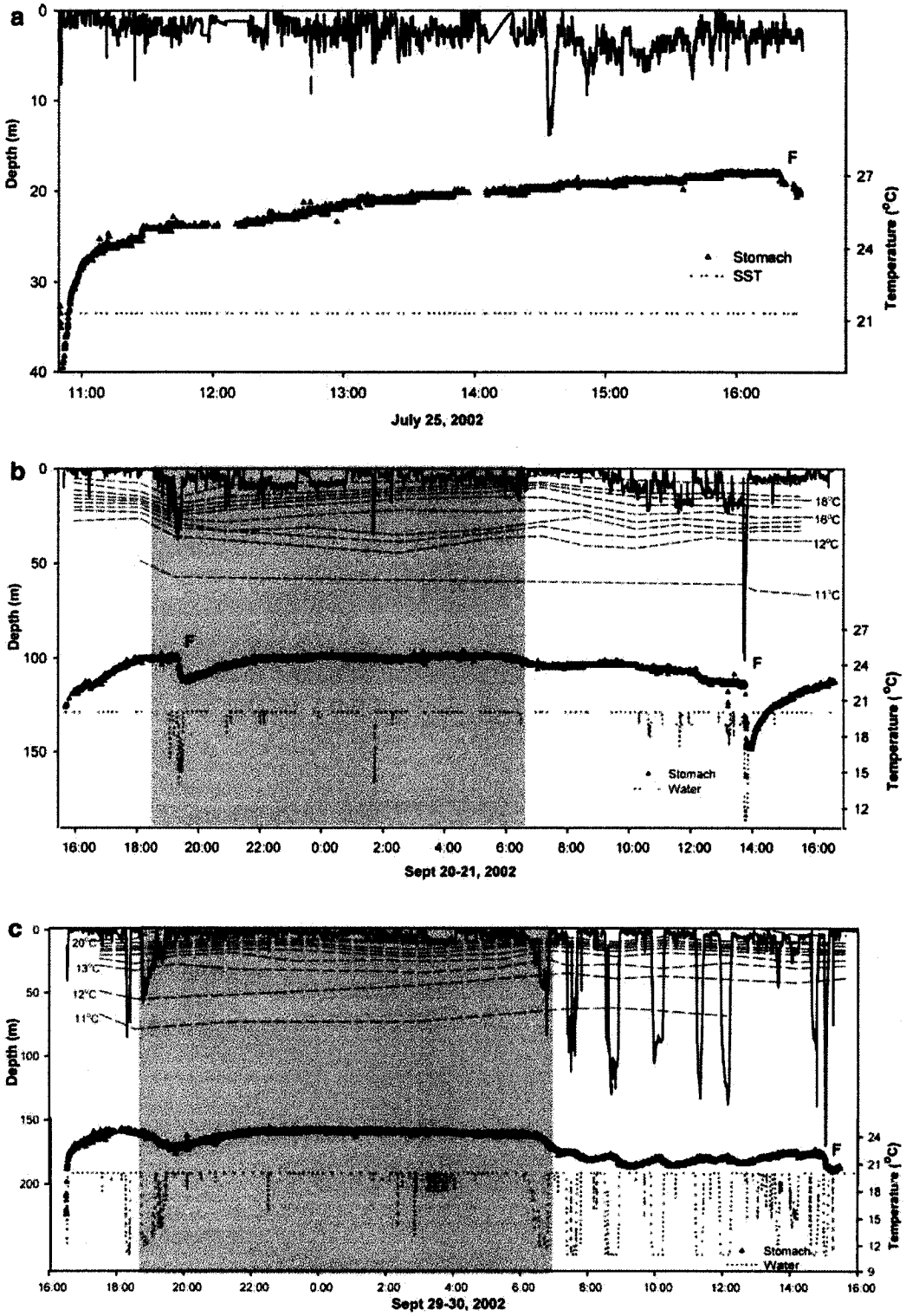
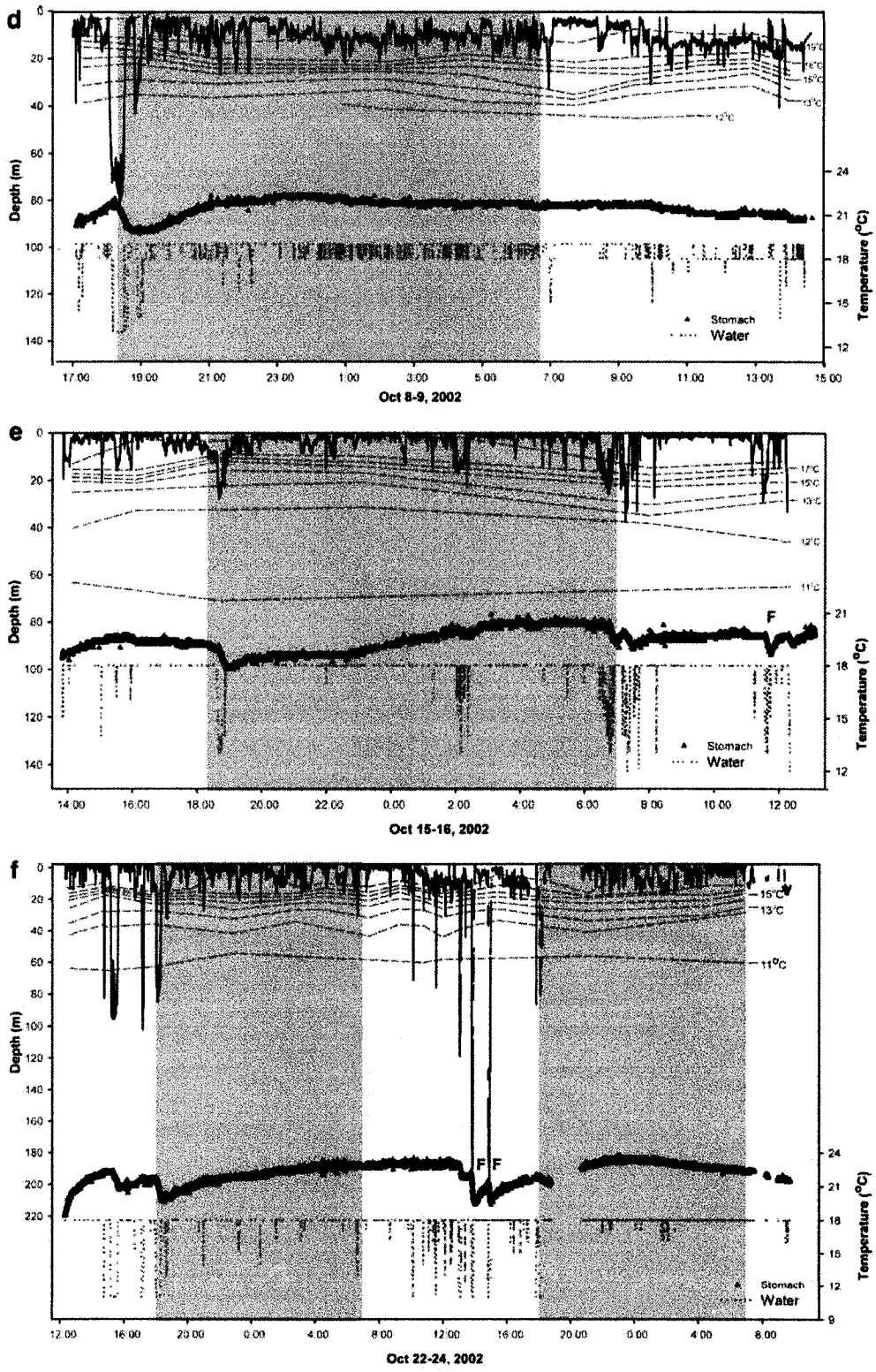


Fig. 2 *Isurus oxyrinchus*. Maximum depth penetration (D, m) as a function of fork length (FL, m) ($D=3.539e^{2.825FL}$; $r^2=0.86$)





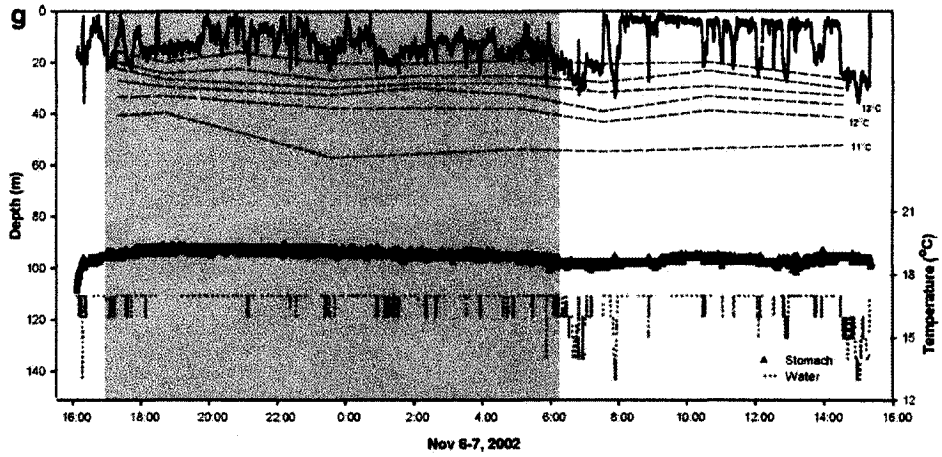


Fig. 3a–g *Isurus oxyrinchus*. Vertical movements and stomach temperatures for seven sharks (a–g: makos 1–7, respectively) tracked in the Southern California Bight. *Top to bottom*: swimming depth (*solid line*), ambient isotherms (*dashed lines*), stomach temperature (*triangles*) and corresponding water temperature (*dotted lines*) plotted over time. *Shaded areas* indicate hours between sunset and sunrise. Suspected feeding events (*F*)

which the shark remained at or near the surface at night and went deeper during the day. Mako 3 had an average T_S of 23.3±1.3°C (SST 19.7±0.4°C), and the average T_{SX} was 5.1±2.7°C. The T_S record suggested only one feeding event at ~1500 hours (30 min before capture). Necropsy results revealed one 90 g nearly undigested Pacific sardine (*Sardinops sagax*) and, as with mako 2, that the transmitter was in the posterior stomach and that the hook had not penetrated the stomach wall.

Mako 4 ranged between the surface and 80 m (Fig. 3d). It spent 60% of the track record at 0–12 m, 36% at 12–24 m, and 4% deeper than 24 m, and did not demonstrate any clear diurnal dive patterns. The average T_S was 21.5±0.5°C at an average SST of 19.6±0.4°C. The average T_{SX} was 3.3±1.4°C, and there were no feeding events.

Mako 5 spent 93% of the track record at 0–12 m, 6% at 12–24 m, and 1% deeper than 24 m (Fig. 3e). While there were no diurnal trends, the shark did spend ~50% of the track within 1 m of the surface. The average T_S was 19.5±0.6°C (SST 18.3±0.3°C), and the average T_{SX} was 1.7±1°C. One feeding event occurred at 1130 hours (~1.5 h before capture), which was a 100 g Pacific sardine. The necropsy revealed that the transmitter was in the posterior stomach and that there was no hook penetration.

Mako 6 was the largest shark tracked. It ranged from the surface to 201 m (Fig. 3f) and spent 89% of its track record at 0–12 m, 5% at 12–24 m and 6% deeper than 24 m. Mako 6 exhibited a diurnal dive pattern similar to that of mako 3, in that it remained near the surface throughout the night and undertook deeper dives during the day. The average T_S was 22.1±1°C (SST 18.2±0.2°C), and the average T_{SX} was 4.5±1.9°C. The T_S record indicated two feeding events (at ~1400 and 1500 hours on day 2). Mako 6 was not captured at the end of the study.

Mako 7 spent 50% of the track record at 0–12 m, 45% at 12–24 m, and 5% deeper than 24 m (Fig. 3g). Although it made frequent vertical excursions between 0 and 35 m, mako 7 did not display any obvious diurnal depth trends. The average T_S was 18.9±0.3°C (SST 17.5±0.2°C), and the average T_{SX} was 2.1±0.6°C. Mako 7 did not feed during the time that it was followed. Necropsy results indicated the absence of prey, the transmitter in the posterior stomach, and that the hook had not pierced the stomach wall.

Discussion

This study provides information on the movement patterns and stomach temperatures of seven juvenile mako sharks (*Isurus oxyrinchus*) tracked in the SCB using a non-invasive tagging procedure. Using the T_S we were able to record feeding events for free-swimming makos. The post-track capture and subsequent gut content analyses of four of the sharks verified the feeding signal in the T_S record. This work on non-stressed makos suggests that juveniles may spend as much as 80% of the time in surface waters (< 12 m depth) and that excursions into deeper, cooler waters are more frequent during the day. When coupled with earlier mako telemetry studies (Holts and Bedford 1993; Klimley et al. 2002), it is evident that SCB juveniles frequent the upper mixed layer. Our study also confirms previous observations that makos undergo frequent vertical oscillations (Carey et al. 1981; Holts and Bedford 1993; Klimley et al. 2002), routinely entering waters cooler (< 11°C) than those previously described as the mako thermal boundary (14°C; Klimley et al. 2002). Additionally, this study indicates that larger makos swim to greater maximum depths than do smaller makos (Fig. 2).

Feeding the transmitter to the mako sharks avoided capture and restraint for tag attachment. Capture and handling can alter blood and body biochemistry (i.e. elevated plasma and muscle lactate; catecholamines; glucose), which may influence the shark's behavior (Arthur et al. 1992; Milligan 1996; Bernal et al. 2001b). Telemetry studies on both blue (*Prionace glauca*) and mako sharks that were hooked and restrained prior to their tracking have shown a distinct post-handling dive immediately upon release (Carey and Scharold 1990; Holts and Bedford 1993; Klimley et al. 2002), something we did not observe in this study. This suggests that there was little stress induced by the initial feeding of the transmitter to the sharks. Other indications that these makos were not adversely affected by the tagging procedure were the records of natural feeding events, the relatively slow swim speeds observed throughout the tracks (even immediately after tag ingestion), and the subsequent capture of post-track individuals when presented with a baited hook.

The maximum T_X (T_S–T_{SST}) range for the seven tracked makos (1.9 5.7°C) was slightly warmer than those determined by Bernal et al. (2001b; 1 3°C) for similar sized makos in a swim tunnel, but less than the values of Carey et al. (1981; 6 8°C) for larger makos in the West Atlantic. The degree to which lamnids can control T_S is not known. However, Goldman (1997) has determined that large white sharks (*Carcharodon carcharias*) can maintain a relatively constant T_S (24°C) in cool water (~14°C SST). In the present study, we observed T_S records that remained relatively stable over periods as long as 23 h (Fig. 3g), but abrupt changes in the T_S records were also common. These abrupt changes were associated with both verified feeding events and penetration into cooler waters. An unstable T_S record may be more representative of wild juvenile (0.80 1.45 m) makos that are feeding and regularly traversing the thermocline.

The criterion used to define the T_S feeding signal was based on the temperature change associated with the ingestion of a 300 400 g mackerel. Therefore, we may be underestimating the number of actual feeding events, in that smaller prey undoubtedly result in a smaller decrease in T_S. Six suspected feeding events were recorded using our conservative criteria, three of which (makos 2, 3, and 5) were confirmed through gut content analyses.

The swimming behaviors and diurnal patterns we observed resemble those previously recorded for the blue shark in the Atlantic and Pacific (Carey and Scharold 1990), for makos in the Atlantic (Carey et al. 1981), and a juvenile white shark fitted with a "pop-off" satellite tag in the SCB (Dewar et al. 2004). For all tracked makos the greatest depth always occurred during daylight hours, and, for makos 3 and 6, there was a consistent pattern of repetitive deeper dives during the day, with the shark remaining near the surface at night. Although these trends are consistent with those described for other pelagic species known to follow and prey on the vertically migrating deep scattering layer (DSL) (Carey and Robinson 1981; Holland et al. 1990; Dagorn et al. 2000; Schaefer and Fuller 2002), all but one of the suspected mako feeding events took place during the day and gut inspection did not reveal mesopelagic prey (species common to the DSL). One explanation for the diurnal movements may be that makos rely heavily on vision for locating prey and, thus, penetrate to greater depths during the daylight hours when visibility is the greatest.

Other trends in the dive records included repeated vertical oscillations throughout the tracks. Oscillatory swimming is well described for fishes (Carey et al. 1981; Carey and Scharold 1990; Holland et al. 1992; Holts and Bedford 1993; Brill et al. 2002; Klimley et al. 2002). Despite individual variation, the oscillatory swimming we observed seemed to fit into two distinct categories: one in which the swimming pattern included repeated shallow oscillations as if the sharks were searching a small section of the water column and the second in which the oscillations seemed to be directed descents into deeper waters followed by rapid ascents, or "bounce dives". These "bounce dives" were frequently associated with successful feeding events.

Several hypotheses have been put forth as to why fish might make frequent vertical oscillations. Carey and Scharold (1990) suggested that this pattern might provide for optimal foraging of the water column. In the case of the mako, frequent excursions may enable them to use their counter-shading and burst capabilities to overtake prey from below, as has been shown for the white shark (Klimley 1994). The makos may also undertake frequent excursions to deeper, colder waters and use the warmer surface waters to regain lost heat and hence behaviorally thermoregulate (Holland et al. 1992; Bernal et al. 2001a; Klimley et al. 2002). Weihs (1973) has proposed that swimming in an oscillatory manner (bursting upward and gliding downward) is a more efficient form of locomotion as opposed to swimming in a straight line. However, Weihs' (1973) model requires a shallower angle of descent than ascent, the opposite of what we observed in this study. Other explanations of oscillatory swimming include a potential role in navigation (Klimley et al. 2002), or possibly in sensing different water masses for directional information (Westerberg 1982; Klimley 1993; Klimley et al. 2002).

All track records were plotted on a high-resolution topographical map to determine if their horizontal movements tracked the submarine topography of the SCB (Appendix 1, Electronic Supplementary Material). Although we observed our tracked makos associating with topographic features and at times following specific bathymetric contours, we cannot conclude whether these movements were directed towards these features, or if the intricate topography of the SCB is such that they encountered these by chance.

Recent management decisions to close part of the central and northern California coastline to the gillnet fleet (2001 temporal and regional closures to the California gillnet fishery for swordfish and thresher shark,

Federal Register 2001) have redistributed the gillnet effort within the SCB. This fishery is more than 20 years old and accounts for more than half of the California annual mako harvest (Taylor and Bedford 2001). Better understanding of mako fine-scale movement patterns may provide managers with the habitat utilization data necessary for managing the target species of the fishery as well as reducing the bycatch. Our data suggest that juvenile makos spend up to 80% of their time in surface waters (< 12 m) and that excursions into deeper, cooler waters are more frequent during the day. Considering the current gillnet depth restrictions employed to reduce interactions with marine mammals [36-foot sub-surface extenders and daytime fishing prohibited (California Department of Fish and Game 2003)] it may be that the management decisions already in place cause the gillnet fishery to have a reduced juvenile mako catch. Further study of a larger size range of makos and tracking of the target species of the gillnet fishery (thresher sharks, *Alopias* spp., and swordfish) are needed to better understand how these apex predators partition the SCB.

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The text of Chapter IV, in full, is a reprint of the material as it appears in Sepulveda, C. A., Kohin, S., Chan, C. Vetter, R. and Graham, J. B. (2004). Movement patterns, depth preferences, and stomach temperatures of free swimming juvenile mako sharks *Isurus oxyrinchus* in the Southern California Bight. *Marine Biology* 145: 191-199. The dissertation author was the primary author and the co-authors listed in this publication directed and supervised the research, which forms the basis for this chapter.

CHAPTER V

The red muscle morphology of the thresher sharks (Alopiidae)

Abstract

The red, aerobic locomotor muscle (RM) in endothermic (regionally warm) and ectothermic sharks differs in transverse position and distribution along the body. The RM of ectothermic sharks is located in a lateral, subcutaneous position and is distributed over most of the length of the body, whereas the RM of endothermic sharks is located in a more medial and anterior position. Recent works have described the RM distribution and endothermy in the common thresher shark (*Alopias vulpinus*), but little is known about the other two members of the thresher shark family Alopiidae (*A. superciliosus*, bigeye thresher, and *A. pelagicus*, pelagic thresher). This study tested whether the bigeye and pelagic thresher sharks also possess the specialized RM position exhibited by their sister species, the common thresher. Total RM volume, transverse position and longitudinal distribution were quantified for both the bigeye and pelagic thresher sharks, and the findings were compared to previous studies on the common thresher. The RM in the bigeye and pelagic threshers is located in a lateral, subcutaneous position. The RM longitudinal distribution showed that the maximum RM is located in a more posterior body position in both the pelagic and bigeye thresher (75% and 55% fork length, respectively) compared to the common thresher (45% fork length). There was no significant difference in the total relative RM mass between the pelagic thresher (mean $\pm$ S.E.M., 3.01$\pm$ 0.10 % of body mass) bigeye thresher (2.31 $\pm$ 0.11% of body mass) and the common thresher (2.34% $\pm$ 0.21 of body mass). These data show that the common thresher has a RM position similar to other endothermic sharks, and is likely the only alopiid capable of RM endothermy.

Introduction

The thresher sharks (i.e., common thresher, *Alopias vulpinus*, pelagic thresher, *A. pelagicus*, and bigeye thresher, *A. superciliosus*) comprise the family Alopiidae and are easily recognized by their elongate upper caudal lobe (Compagno, 1990). Although these three species display several external morphological similarities, most of what is known regarding the muscle morphology for this group is based on studies of the common thresher.

The morphology of the red, aerobic locomotor muscle (RM) in the common thresher is similar to that of the endothermic lamnid sharks (Carey et al., 1971; Carey et al., 1985; Bone and Chub, 1983; Bernal et al., 2003). These two groups possess a medial RM arrangement that is primarily distributed over the anterior body (Bone and Chub, 1983; Bernal et al., 2003). Other lamnid similarities include the predominant use of a lateral as opposed to a central circulation, and the presence of vascular heat exchangers (retia) adjacent to the RM (Bone and Chub, 1983). As in the lamnids, the aerobic specializations of the common thresher enable metabolic heat conservation and elevate RM temperatures above the ambient seawater (Bernal and Sepulveda, 2005).

Although the muscle morphology and endothermic status of the common thresher have been documented, there are no studies that describe the interspecific differences in RM arrangement among the alopiids. To our knowledge, there are no temperature data substantiating RM endothermy for the pelagic and bigeye threshers, nor are there studies describing the RM position, and to what extent it is specialized

for endothermy (i.e., medial and anterior arrangement). Therefore, the objective of this study were to quantify the transverse position and longitudinal distribution of RM in the pelagic and bigeye thresher sharks and compare these data to the previous work on the common thresher, to ultimately determine which species possess the aerobic specializations associated with RM endothermy.

Materials and Methods

All animal collection and experiments were performed under the guidelines of the Animal Care and Use Committee of the University of California, San Diego, Protocol # S00080 and with permission from the California Department of Fish and Game (permit # 803019).

Specimen collection and identification

Morphometric data for the sharks used in this study are presented in Table 1. The interspecific comparison utilized data from six common thresher sharks (*Alopias vulpinus* Bonnaterre 1788) that were previously examined by Bernal et al.(2003). The three bigeye thresher sharks (*Alopias superciliosus* Lowe, 1841) were purchased whole from the California drift-gillnet fishers operating out of San Diego, CA, and their identification confirmed based on morphological characters (Compagno, 2001). The largest pelagic thresher (AP1; *Alopias pelagicus* Nakamura, 1935) was captured using a long line aboard the R/V David Starr Jordan (National Marine Fisheries Service Eastern Tropical Pacific shark census, 2004) using methods described in O'Brien and Sunada (1994). Two additional pelagic threshers, captured near Guymas, Mexico, were purchased from Chesapeke Fish Co., San Diego, CA. Because these specimens were not intact (heads, tails and viscera are typically discarded at sea) it was necessary to verify correct species identification, by sequence analysis of the mitochondrially encoded 16S and 12S genes. DNA extraction and sequencing protocols followed Craig et al. (2004). Total genomic DNA was isolated using the Qiagen DNEasy isolation kit and the polymerase chain reaction (PCR) was used to

amplify a 594 base pair fragment of the 16s gene and a 424 base pair fragment of the 12s rDNA gene. Sequences were aligned using ClustalX and visually optimized using MacClade. Percent sequence divergence was estimated in PAUP*4b10. Among the three individuals there was no genetic difference at the 16S locus and no appreciable difference was found at the 12S locus (0.2%), hence confirming the species identification. Both genes showed a 4.5 and 6.6% percent difference from *Alopias superciliosus* as determined by comparisons with sequences available on GenBank.

Body size

For the two pelagic threshers, and for individuals that were not weighed, body size parameters were estimated using established fork length to total length and total length to body mass regressions. Pelagic thresher alternate-length (insertion of first dorsal to insertion of second dorsal) was converted to total-length using data from the California Drift Gillnet Fishery database (D. Holts, National Marine Fisheries Service, unpublished) and total-length to body mass relationships were determined using regressions from Liu et al. (1999). Bigeye thresher body mass was determined using data from Kohler et al. (1995).

Sectioning, RM quantification, and three-dimensional reconstruction

Muscle sectioning and RM quantification were performed using methods similar to those described in Bernal et al. (2003). Sharks were frozen whole, in a position that avoided any bending of the body, and transverse sections approximately 3-4 cm thick were cut along the entire length of the shark using a large band saw. Sectioning was also performed over the entire dressed carcass length, and for at least one specimen of each species, observations were made on the presence of RM in the

upper lobe of the caudal fin. For all sections, slice thickness was measured and high-resolution digital images taken (Canon, PowerShot A80) of the anterior surface of each slice. The cross-sectional area (cm^2) of each image was determined using the NIH Image J software©. The longitudinal distribution of RM was determined using the methods described in Bernal et al. (2003) with the RM surface area (cm^2) at 50% *FL* being used as a relative value of 1, and this value used as a reference point for all other positions along the body. For each slice, RM volume was calculated from the product of RM surface area and slice thickness and the mass determined using a RM density of 1.05 g cm^{-3} (Bernal et al., 2003).

Three-dimensional reconstructions of muscle morphology were obtained by using each high-resolution two-dimensional image along the body and building a vector-based outline of the area of interest (i.e., whole body, visceral mass, spine, and RM). Outlines were then skinned together using morphometric data for one specimen of each of the three species and final images rendered using Strata 3D Pro.

Results

RM arrangement and distribution

Morphometric data for all thresher sharks are shown in Table 1. The pelagic and bigeye thresher sharks have their RM concentrated in a lateral, subcutaneous position (i.e., directly beneath the skin). The RM extends as a thin layer from the epaxial to hypaxial position (Figure 1A and B). In contrast, the RM in the common thresher is medially positioned (i.e., closer to the vertebrae) along the length of the body (Figure 1C) (Bernal et al., 2003).

The total relative RM mass (mean ±SE; RM mass as a % of total body mass) for the pelagic thresher ($3.10 \pm 0.10\%$), bigeye thresher ($2.31 \pm 0.11\%$), and common thresher ($2.34\% \pm 0.21$) are not significantly different (one-way ANOVA). The RM longitudinal distribution for the three thresher sharks shows an anterior progression from the pelagic to the bigeye to the common thresher (Figure 2). Most of the RM in the pelagic thresher is between 35 to 100% fork length, *FL*, and maximal at 75% *FL* (Figure 2A). RM in the bigeye thresher also extends over much of the body (predominantly from 35 to 100% *FL*), but maximum RM occurs in a more anterior position (50% *FL*) (Figure 2B). When the RM longitudinal distribution of both the pelagic and bigeye threshers are compared to that of the common thresher (Figure 2C) the latter has the most pronounced anterior RM distribution (mostly between 30-70% *FL*) with the greatest amount of RM at 40-50% *FL*.

Discussion

This study quantified the arrangement and distribution of the RM in the three thresher shark species (Alopiidae), and demonstrates two distinct patterns of RM organization within the genus *Alopias*. The bigeye and pelagic thresher sharks have a lateral RM position similar to that of most ectothermic shark and bony fish species (Carey et al., 1971; Graham et al., 1983; Bernal et al., 2003). In contrast, the common thresher has a medial RM arrangement comparable to the endothermic lamnid sharks (Carey et al., 1971; Bone and Chub, 1983; Bernal et al., 2003). Notable interspecific differences were also found in the longitudinal distribution of the RM, with the maximum amount of RM located in a more anterior position in both the bigeye and common threshers relative to the pelagic thresher. Although additional RM temperature studies are warranted for the bigeye and pelagic threshers, this study shows that only the common thresher has the requisite RM position for RM endothermy.

Red muscle

Quantity

The range of total RM volume in the three thresher species (2.4-3.1% body mass) is within the narrow range of values recorded for other pelagic sharks (1.8-3.1% body mass) (Bernal et al., 2003). Collectively, the range for pelagic sharks is lower and much more consistent than that recorded for scombrid fishes (4-13% body mass) (Graham et al., 1983; Graham and Dickson, 2000). Attempts to explain the divergence in RM volume between sharks and bony fishes must consider how much

more diverse teleosts are in terms of both number of species and morphology (Nelson, 1994). Further, it may be that sharks lack the range of aerobic swimming speeds of teleosts, or that differences in muscle function limit their scope for aerobic performance (Donley and Shadwick, 2003). From the sharks that have been studied, it appears that, despite differences in activity level, body size, and caudal propeller shape (i.e., lunate, heterocercal), they all possess a relatively similar amount of RM.

RM position and distribution

All fish taxa capable of elevating the temperature of their RM, including the common thresher, have their RM in an anterior and medial position (a condition that isolates the RM from the surrounding water and positions it such that it is at the thickest part of the body) (Graham et al., 1983; reviewed by Dickson and Graham, 2004). In the endothermic lamnid sharks, as well as tunas, it is hypothesized that the internal RM position evolved for reasons of locomotor efficiency (reviewed by Bernal et al., 2001). An internal RM coupled with an intricate tendon system has enabled these groups to swim using the thunniform mode of locomotion (a system in which shortening of the anterior RM results in bending of the posterior body) (Donley et al., 2004). Although the common thresher shares this anatomy, little is known regarding the swimming kinematics and muscle dynamics of this species.

Data on the longitudinal distribution of the RM in threshers showed that maximum RM is located in a more anterior position in the common and bigeye threshers than in the pelagic thresher. The marked anterior RM position of the common thresher resembles closely that of the lamnid sharks (Bernal et al., 2003), while the distribution of the bigeye is somewhat intermediate between the pelagic and

the common thresher (Figure 2). Although maximum RM in the bigeye (55% *FL*) is more anterior than the pelagic (75% *FL*), both species exhibit RM that is broadly distributed over much of the body, just as it is in other ectothermic shark species (Bernal et al., 2003). When compared to endothermic and ectothermic teleosts of the family Scombridae (Graham et al., 1983; Graham and Dickson, 2000), a similar, but much more pronounced shift in RM distribution is observed, with the ectothermic scombrids having their RM located in a more posterior body position than the tunas.

Phylogenetic relationships

The phylogenetic relationship of the thresher sharks has been examined using both morphological and molecular techniques (Maisey, 1985; Compagno, 1990; Eitner, 1995; Martin and Naylor, 1997; Naylor et al., 1997). Using morphological characters, Compagno (1990) hypothesized that the three thresher species comprise a monophyletic family (Alopiidae) in the Order Lamniformes, and that *A. vulpinus* is the sister taxa to *A. pelagicus* + *A. superciliosus*. This hypothesis is also supported by the molecular based analysis of Eitner (1995). Other hypotheses based on molecular data fail to provide a monophyletic origin for the threshers, and force relationships based on the strong morphological data (Martin and Naylor, 1997; Naylor et al., 1997). Because the internal and anterior RM morphology is only found in the common thresher, this character state alone cannot be used to distinguish the relatedness of the alopiids, but rather suggests this trait to be an autapomorphy of the common thresher.

Regional endothermy

There has been previous speculation as to the endothermic status of all three thresher sharks (Carey et al., 1971; Gruber and Compagno, 1981; Bone and Chub,

1983; Block and Finnerty, 1994; Weng and Block, 2004), with recent evidence concluding that the common thresher has warm RM temperatures (Bernal and Sepulveda, 2005) and that the bigeye thresher has a large, orbital *rete* highly suggestive of cranial endothermy (Weng and Block, 2004). To our knowledge, there are no temperature data substantiating endothermy for the pelagic thresher and, because of its RM morphology, lack of a highly modified cranial circulation (Block and Carey, 1985), and its existence in predominantly warm tropical and sub-tropical waters (Liu et al., 1999), it is likely that the bigeye (brain and eye region) and common (RM) threshers are the only two regional endotherms of the Alopiidae.

Has regional endothermy affected the geographic distribution of the thresher sharks? Because comparable depth data are not available for all three species (i.e., location, season), it is not possible to fully assess the thermal niche of this group; however, from the latitudinal and depth-distribution data, it is evident that the common and bigeye threshers inhabit cooler waters than the pelagic thresher. While all three species, at times, occupy similar habitats (Hanan et al., 1993), the common thresher has been shown to have the greatest overall latitudinal distribution, ranging in the Eastern Pacific from 58°N to 55°S (Compagno, 2001). Although the bigeye thresher's latitudinal distribution is not as extensive as that of the common thresher (approximately 35°N to 35°S; Ivanov, 1986; Compagno, 2001), its thermal niche may exceed that of the other threshers when considering the deep waters it has been shown to inhabit. Satellite tagging and acoustic telemetry studies have shown that the bigeye thresher spends much of the daylight hours at depth in waters between 6 and 12°C (Nakano et al., 2003; Weng and Block, 2004). Similar archival tagging data for the

common thresher also show this species to frequent waters below the thermocline, but the range of temperatures encountered is less than that of the bigeye (9-13°C; C. Sepulveda, unpublished). There are no movement studies on the pelagic thresher, but fishery data suggest this species resides predominantly in the upper water column with most of the catch occurring above the thermocline (Liu et al., 1999).

Conclusions

This study compared the muscle morphology of the three thresher sharks and described the degree to which their RM is specialized for endothermy. The common thresher is the only alopiid having the RM morphology typically associated with endothermy. Based on the phylogeny proposed by Compagno (1990) and Eitner (1995), the medial RM position of the common thresher must be an autapomorphic trait that was evolved after the separation of the common thresher from the bigeye+pelagic ancestor.

Table 1. Shark fork length, body mass, red muscle (RM) position, and relative RM mass.

Species, RM position* (common name)	Fork length (cm)	Mass‡ (kg)	RM cross-sectional area at 50% FL (cm^2)	RM mass (% body mass)
Alopias pelagicus, L (pelagic thresher)	132†	29‡	8.79	2.81
	144†	38‡	10.80	3.11
	163†	56‡	14.97	3.11
				mean ± S.E.M. 3.01 ± 0.10
Alopias superciliosus, L (bigeye thresher)	153	49‡‡	14.03	2.31
	162	58‡‡	14.00	2.12
	175	74‡‡	15.23	2.51
				mean ± S.E.M. 2.31 ± 0.11
Alopias vulpinus†‡, M (common thresher)	85	4.5	5.61	2.02
	105	20.9	8.82	1.98
	120	24.9	13.40	2.83
	123	37.2	15.31	2.11
	124	34.9	12.91	1.96
	163	70.4	35.13	3.14
				mean ± S.E.M. 2.34 0.21

* Lateral (L), directly under the skin or Medial (M), closer to the vertebrae
† calculated from D. Holts, National Marine Fisheries Service, unpublished
‡ calculated from Liu et al. (1999)
†‡ from Bernal et al. (2003)
‡‡ calculated from Kohler et al. (1995)

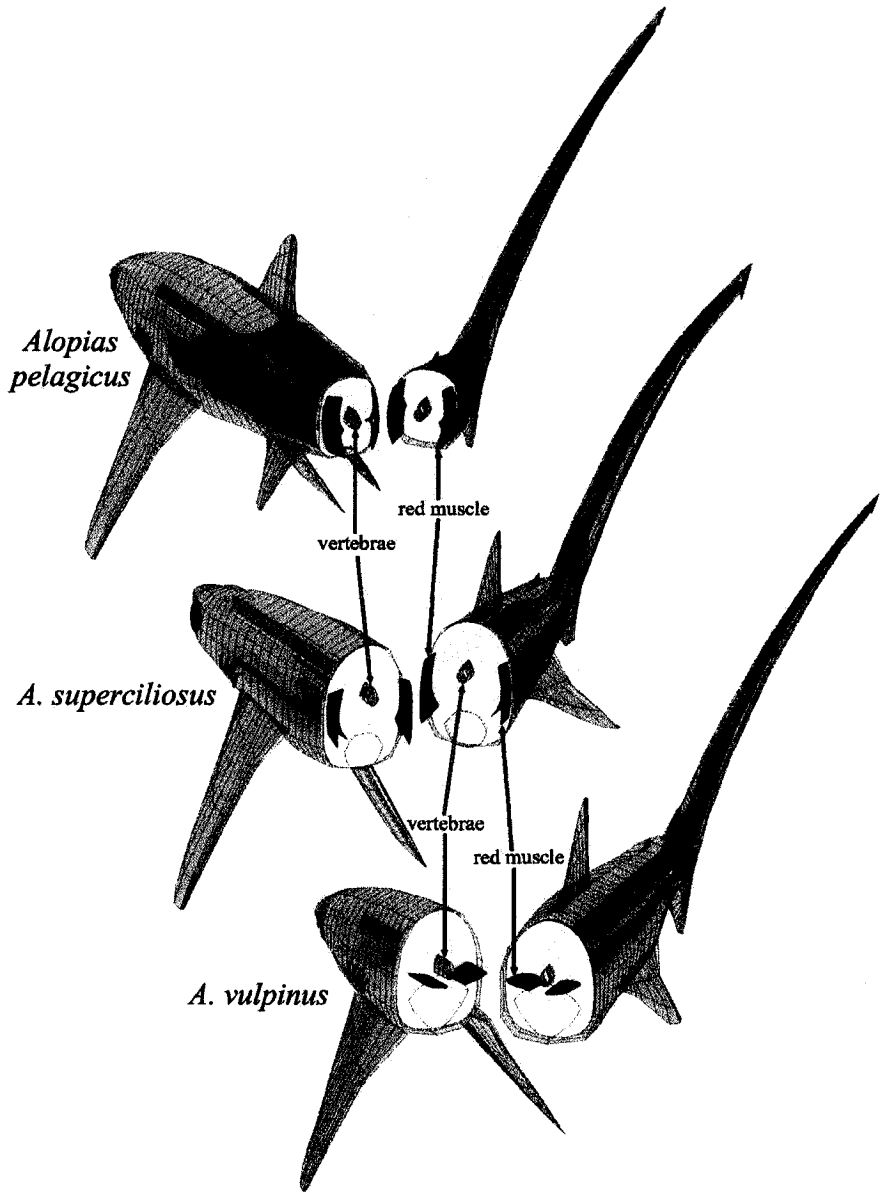

Figure 1

Figure 1. Whole-body reconstructions of the three thresher sharks showing the position of the red, aerobic locomotor muscle (RM) (red) and vertebral column (yellow). Top, pelagic thresher, *Alopias pelagicus* (56 kg), middle, bigeye thresher, *A. superciliosus* (58 kg) and bottom, common thresher, *A. vulpinus* (70 kg) (from Bernal et al., 2003). Location of the transverse section of each species corresponds to the position of maximum RM.

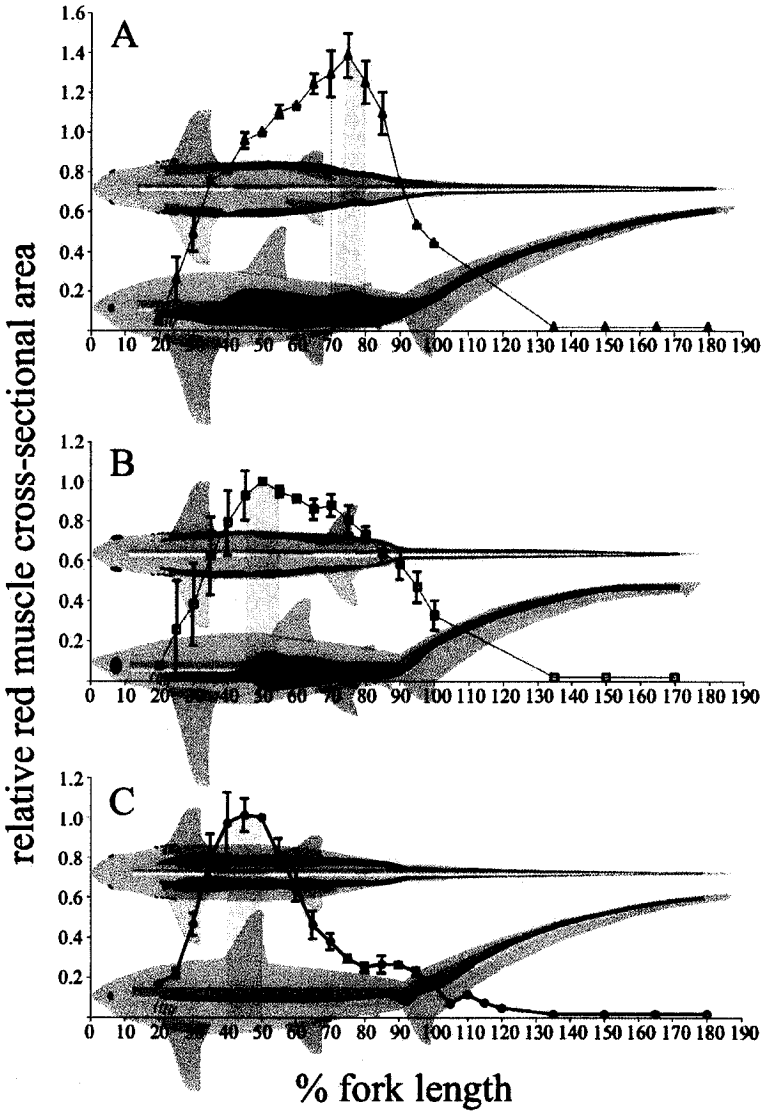

Figure 2

Figure 2. Longitudinal distribution of the red, aerobic locomotor muscle (RM) for the three thresher shark species. A) pelagic thresher, *Alopias pelagicus* (n=3), B) bigeye thresher, *A. superciliosus* (n=3), C) common thresher, *A. vulpinus* (n=6) (from Bernal et al., 2003). The relative amounts of RM in the different positions along the body are expressed as a proportion of the RM cross-sectional area equal to 1 at 50% fork length (see Table 1 for maximum surface area in cm^2).

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CHAPTER VI

Conclusions

This dissertation addressed questions related to the selective advantages of regional endothermy and its independent evolution among several fish groups. The chapters integrate laboratory and field physiological measurements to provide a better understanding of the evolution of regional endothermy and its importance as an adaptation to the pelagic environment. The following questions summarize the main findings of this thesis and present new ideas for future directions in the study of regional endothermy in fishes.

How do the energetics of endothermic and ectothermic species compare?

Chapter II investigated the adaptive significance of elevated red muscle temperature in tunas. This work stemmed from previous energetics studies showing tunas have higher standard metabolic rates than most other fishes (SMR, the minimum metabolic rate required for maintenance functions; Videler, 1993) (Brill, 1979; Graham and Dewar, 1994; Sepulveda and Dickson, 2003). I quantified the swimming energetics of the eastern Pacific bonito (*Sarda chiliensis*, tribe Sardini), the ectothermic sister group to the tunas, and compared these data to similar-sized skipjack (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) (Brill, 1979; Dewar and Graham, 1994). Specific hypotheses tested included: (1) tunas swim more efficiently than the bonito and, (2) bonito and tunas have similar SMRs.

The findings were that bonito had a similar cost of transport but a significantly lower SMR than tunas. These results support previous studies on smaller scombrids (Sepulveda and Dickson, 2003) and suggest that, despite similar costs of locomotion, the aerobic specializations unique to tunas require an increased maintenance cost (SMR).

Is the slender tuna endothermic?

Chapter III tested whether the slender tuna (*Allothunnus fallai*) is capable of regional endothermy or if it is the only ectothermic species of the Tribe Thunnini. Previous investigations of *A. fallai* were limited to fresh and preserved specimens and included primarily morphological analyses of the skeletal elements (Serventy, 1948; Collette and Chao, 1975; Collette, 1978). More recent studies on the circulatory system (i.e., vascular plexus or putative heat exchanger) and RM showed *A. fallai* to possess several of the morphological specializations necessary for RM endothermy but to a much lesser extent than that of the other tunas (Graham and Dickson, 2000). With this in mind, I set out to determine the endothermic status of *A. fallai*. This is the first study on live *A. fallai* and represents a significant contribution towards understanding the progression of character states enabling regional endothermy in the tunas.

Although *A. fallai* does not possess the full suite of derived characters present in the other tunas (Serventy, 1948; Cressey et al., 1983; Graham and Dickson, 2000; Collette et al., 2001), it proved to be highly specialized, exhibiting both elevated temperatures in the RM as well as brain and eye region. In addition, I found that the mechanism used by *A. fallai* to elevate and maintain eye and brain temperatures is not like that of the other tunas, which are thought to use heat produced from the metabolic

function of ocular muscles and retained by the carotid rete (Stevens and Fry, 1971; Linthicum and Carey, 1972). Rather, *A. fallai* has four highly modified extraocular muscles that generate heat and effectively warm the brain and eye region. The brain heater is composed of portions of all four of the rectus muscles (superior, inferior, lateral and medial) and is positioned such that it is in direct contact with the brain case and optic nerves. I describe the modified muscles, show that they lack contractile proteins, and describe the counter-current heat exchange system required to conserve metabolic heat within the skull. The discovery of a brain heater in *A. fallai* is the first such finding for any tuna species. In this respect, *Allothunnus* is convergent with the billfishes and *Gasterochisma* (Carey, 1982; Block and Finnerty, 1994). However, the use of different muscles (Carey et al., 1982), differences in cranial configuration (Collette and Chao, 1975; Kohno, 1984; Block, 1986), the position of the counter-current heat exchange system (Carey, 1982; Block, 1986), and the respective phylogenetic relationships (Kohno, 1984; Cressey et al., 1983; Graham and Dickson, 2000; Collette et al., 2001) confirm that cranial endothermy has evolved independently in these three groups.

How does endothermy affect the movement patterns of an endothermic shark?

Chapter IV examined regional endothermy in free-swimming mako sharks (*Isurus oxyrinchus*). Specifically, this work focused on measuring the stomach-temperature elevation and fine-scale movements of juvenile makos off the coast of southern California.

Acoustic telemetry and a novel tag attachment technique (whereby the shark volitionally swallowed the transmitter) were used to record the *in-vivo* stomach temperatures and depth distribution of seven juvenile makos for periods of up to two days. This work showed that the tracked makos spent the vast majority of the time (up to 80%) in the upper 12 m of the water column, while larger makos dove to greater depths. In addition, elevated stomach temperatures were recorded for all makos (including young of the year) and these data were used to document feeding events in five of the seven tracking sessions.

How does the red muscle morphology compare among three closely related sharks, one of which is endothermic?

Chapter V examined the muscle morphology of the thresher sharks (common, *Alopias vulpinus*, bigeye, *A. superciliosus* and pelagic thresher, *A. pelagicus*) and described the degree to which their RM is specialized for endothermy. Although the threshers are commercially fished, little is known regarding their physiological adaptations to the pelagic environment, and there is debate concerning the occurrence of RM endothermy in both the pelagic, and bigeye threshers (Carey et al., 1971; Bone and Chub, 1983; Block and Finnerty, 1994; Bernal and Sepulveda, 2005). My work compared the volume, transverse position, and longitudinal distribution of RM in the pelagic and bigeye threshers to that of the common thresher, and has demonstrated two distinct patterns of RM organization within the Alopiidae.

The main findings were that the common thresher is the only alopiid to possess a RM arrangement similar to the endothermic lamnid sharks (i.e., medial and more anteriorly positioned) (Carey et al., 1971; Bernal et al., 2003). This study also noted

differences in the RM longitudinal distribution among the three species, with both the common and bigeye threshers having their RM in a more anterior position than that of the pelagic thresher. When coupled with phylogenetic studies, these data help formulate hypotheses regarding the sequence of events in the evolution of RM endothermy in the common thresher.

Future directions

This dissertation has answered several questions related to the biology and ecology of pelagic fishes but, has also set the foundation for future studies. The energetics chapter revealed that skipjack and yellowfin tuna have a greater SMR than bonito. Subsequent studies could test whether *A. fallai*, also has an increased SMR and whether it shares the aerobic specializations that are thought to account for the heightened metabolic rate of tunas (i.e., increased gill surface area, enzyme activities, growth rates). The discovery of a brain heater in *Allothunnus* has inspired a whole new set of questions, ranging from those associated with its natural history, to its unique endothermic specializations. Whether *A. fallai* uses a heat-generating mechanism similar to that of the billfishes, and the extent to which it warms its brain and eye in the wild are not yet known. Also, it is not known whether eye mobility has been sacrificed by the alteration of all four extraocular muscles, and if this alteration in eye mobility is associated with the evolution of filter feeding in this species.

The mako tracking work showed how movement patterns can be used to answer questions related to conservation and fishing gear selectivity. Additionally, by feeding the transmitter to the shark, this work was able to apply a novel twist to the

current technology used to monitor the fine-scale movements of pelagic species. Future research should focus on similar movement studies for other pelagic species in the Southern California Bight (including the common thresher shark and swordfish).

The thresher RM study revealed major anatomical differences in the muscle morphology of the three species and sets the stage for studies of their phylogeny, and comparisons of their circulation, muscle dynamics, and contractile kinetics.

Logistically, these were all difficult projects. The bonito work involved establishing a healthy population at the laboratory and running 24-hour experiments. The *Allothunnus* work involved searching for an uncommon species in a foreign country on a limited budget. The mako tracking work required countless hours of monotonous tracking from a small skiff, and the thresher work necessitated the acquisition of all three thresher species, which was no small task. Despite these difficulties, I feel fortunate to have had the opportunity to work on such a diverse group of fishes and am eager to continue in this field.

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