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A Study of Cardiovascular Function in Swimming Tuna

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UNIVERSITY OF CALIFORNIA, SAN DIEGO

A Study of Cardiovascular Function in Swimming Tuna

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy in Marine Biology

by

Keith Edward Korsmeyer

Committee in charge:

Jeffrey B. Graham, Chair
Robert E. Shadwick
Richard H. Rosenblatt
Myrl C. Hendershott
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1996

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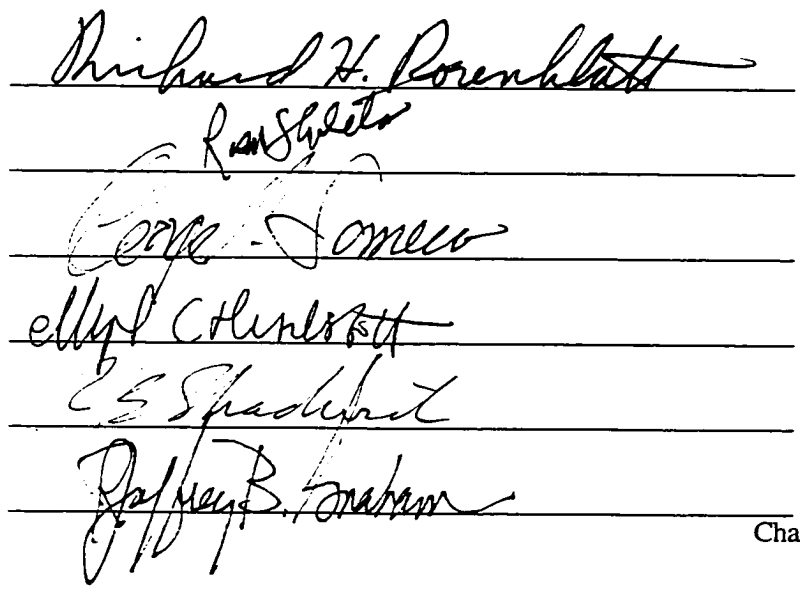

Chair

University of California, San Diego

1996

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LIST OF SYMBOLS AND ABBREVIATIONS

$(a-v)_{O_2}$	arterial-venous oxygen content difference
$(a-v)_{O_2}$ RM	red muscle arterial-venous oxygen content difference
BP	blood pressure
BP_{da}	dorsal-aortic blood pressure
BPva	ventral-aortic blood pressure
C_{O_2}	oxygen content
$C_{a.O_2}$	oxygen content of arterial blood
$C_{\bar{v}.O_2}$	oxygen content of venous blood
f_H	heart rate
FL	fork length
Hb	hemoglobin concentration
Hb_a	hemoglobin concentration of arterial blood
Hb_v	hemoglobin concentration of venous blood
Hct	hematocrit
MCHC	mean cell hemoglobin concentration
$MCHC_a$	mean cell hemoglobin concentration of arterial blood
$MCHC_v$	mean cell hemoglobin concentration of venous blood
$\dot{M}_{O_2}$	rate of oxygen consumption (in mass units)
M_{RM}	mass of red muscle
M_T	total body mass
O_2	oxygen
ODC	oxygen dissociation curve
pHa	pH of arterial blood
pH_v	pH of venous blood

P_{50}	blood oxygen partial pressure at 50% blood oxygen saturation
P_{O_2}	oxygen partial pressure
P_{a,O_2}	oxygen partial pressure in arterial blood
$P_{\bar{v},O_2}$	oxygen partial pressure in venous blood
P_{CO_2}	carbon dioxide partial pressure
P_{a,CO_2}	carbon dioxide partial pressure in arterial blood
$P_{\bar{v},CO_2}$	carbon dioxide partial pressure in venous blood
Q_{10}	ratio of values at temperatures 10°C apart
$\dot{Q}$	cardiac output, or blood flow
$\dot{Q}_{RM}$	red muscle blood flow
R_{branch}	branchial vascular resistance
R_{total}	total vascular resistance
R_{syst}	systemic vascular resistance
R	total aerobic metabolic rate
R_A	metabolic rate attributable to aerobic locomotor activity
R_D	metabolic rate attributable to oxygen debt recovery
R_F	metabolic rate attributable to digestion and assimilation of food
$R_{G\&R}$	metabolic rate attributable to growth and reproduction
R_S	standard metabolic rate
SDA	specific dynamic action
SMR	standard metabolic rate
S_{O_2}	percentage of blood oxygen saturation
$S_{\bar{v},O_2}$	oxygen saturation of venous blood
S_{a,O_2}	oxygen saturation of arterial blood
SV	cardiac stroke volume
U	swimming velocity

U_{crit}	critical swimming velocity
U_{max}	maximal swimming velocity
$\dot{V}_{O_2}$	rate of oxygen consumption (in volume units)
$\dot{V}_{O_2\,maint}$	maintenance oxygen consumption associated with exercise
$\dot{V}_{O_2\,RM}$	red muscle oxygen consumption
$\dot{V}_{O_2\,swim}$	total swimming oxygen consumption

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The text of Chapters II and III, in full, are reprints of the material as it appears in Comparative Biochemistry and Physiology. The dissertation author was the primary researcher and author, and the co-authors listed in those publications assisted with and/or supervised the research which forms the basis for those chapters.

This dissertation is 100% dolphin-safe.

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Korsmeyer, K. E. (1991). Acute temperature effects on the heart rates of kelp bass (*Paralabrax clathratus*) and spotted sand bass (*P. maculatofasciatus*). *Am. Zool.* **31**, 57A. (Abstract)

Korsmeyer, K. E., Graham, J. B. and Shadwick, R. E. (1992). Cardiac performance in swimming yellowfin tuna. *Am. Zool.* **32**, 57A. (Abstract)

Korsmeyer, K. E., Lai, N. C., Knower, T., Dewar, H., Shadwick, R. E. and Graham, J. B. (1993). Cardiovascular function in swimming yellowfin tuna (*Thunnus albacares*). *Am. Zool.* **33**, 44A. (Abstract)

Knower, T., Shadwick, R. E., Biewener, A. A., Korsmeyer, K. and Graham, J. B. (1993). Direct measurement of tail tendon forces in swimming tuna. *Am. Zool.* **33**, 30A. (Abstract)

Knower, T., Shadwick, R. E., Wardle, C. S., Korsmeyer, K. and Graham, J. B. (1993). The timing of red muscle activation in swimming tuna. *Am. Zool.* **33**, 30A. (Abstract)

Korsmeyer, K. E., Lai, N. C., Dewar, H., Knower, T., Shadwick, R. E. and Graham, J. B. (1994). Cardiovascular responses to exercise in yellowfin tuna. *The Physiologist* **37**, A-93. (Abstract)

Graham, J. B., Dewar, H., Lai, N. C., Korsmeyer, K. E., Fields, P. A., Knower, T., Shadwick, R. E., Shabetai, R. and Brill, R. W. (1994). Swimming physiology of pelagic fishes. In *Mechanics and Physiology of Animal Swimming*, (ed. L. Maddock, Q. Bone and J. M. V. Rayner), pp. 63-74. Cambridge: Cambridge University Press.

Lai, N. C., Korsmeyer, K. E., Graham, J. B., Shabetai, R. and Ziegler, M. G. (1994). Catecholamine and potassium ion concentrations in swimming yellowfin tuna. *The Physiologist* **37**, A-93. (Abstract)

Korsmeyer, K. E., Lai, N. C., Dewar, H., Knower, T., Shadwick, R. E. and Graham, J. B. (1995). Oxygen transport and cardiovascular responses to exercise in yellowfin tuna (*Thunnus albacares*). *Physiol. Zool.* **68**, 55. (Abstract)

Graham, J. B., Korsmeyer, K. E., Lai, N. C., Shadwick, R. E., Knower, T., Dewar, H. and Dickson, K. A. (1995). Biomechanics, energetics, and physiology of tuna swimming. *Physiol. Zool.* **68**, 54. (Abstract)

Korsmeyer, K. E., Dewar, H., Lai, N. C. and Graham, J. B. (1996). Tuna aerobic swimming performance: physiological and environmental limits based on oxygen supply and demand. *Comp. Biochem. Physiol.* **113B**, 45-56.

Korsmeyer, K. E., Dewar, H., Lai, N. C. and Graham, J. B. (1996). The aerobic capacity of tunas: adaptation for multiple metabolic demands. *Comp. Biochem. Physiol.* **113A**, 17-24.

In review:

Lai, N. C., Korsmeyer, K. E., Katz, S., Holts, D. B., Laughlin, L. M. and Graham, J. B. Hemodynamics and blood properties of the shortfin mako shark, *Isurus oxyrinchus*. *Copeia*

In preparation:

Korsmeyer, K. E., Lai, N. C., Shadwick, R. E. and Graham, J. B. Oxygen transport and cardiovascular responses to exercise in the yellowfin tuna *Thunnus albacares* (Bonnaterre).

Korsmeyer, K. E., Lai, N. C., Shadwick, R. E. and Graham, J. B. Stroke volume and heart rate contributions to the regulation of cardiac output in swimming yellowfin tuna: response to exercise and temperature.

ABSTRACT OF THE DISSERTATION

A Study of Cardiovascular Function in Swimming Tuna

by

Keith Edward Korsmeyer
Doctor of Philosophy in Marine Biology
University of California, San Diego, 1996
Professor Jeffrey B. Graham, Chair

Oxygen transport and cardiovascular responses to exercise and environmental changes were studied in swimming yellowfin tuna (*Thunnus albacares*), a high-performance teleost. Use of a large water tunnel permitted measurements of heart rate (f_H), relative stroke volume (SV), and arterial and venous blood-gas variables during increases in swimming velocity or rapid changes in ambient temperature and oxygen content that simulated vertical migrations through the thermocline.

In addition, a model of aerobic swimming performance for a 50 cm yellowfin was developed, based on the oxygen delivery potential of the cardiovascular system, and oxygen demand during swimming. This model predicts a lower maximal sustained (aerobic) velocity (~3-4 fork lengths [FL]/s) compared to previous estimates. This model also predicts that the rate of total oxygen consumption is not maximal at the maximal sustained velocity, suggesting that additional aerobic scope is available for other high-oxygen demand metabolic functions (e.g., digestion, oxygen debt recovery, and growth).

Measured f_Hs, although highly variable among individuals, were generally lower than previously reported, averaging from 68 to 102 bpm with a velocity increase from 1.0 to 2.9 FL/s. These f_Hs are comparable to other active teleosts at similar velocities. A significant bradycardia developed at an oxygen tension of 16.1 kPa O_2, and f_H was very sensitive to changes in temperature (Q_{10} = 2.37, 28°C to 18°C).

Relative SV measurements revealed a slight decrease with exercise (mean 3.9%), indicating that, unlike most other fishes, cardiac output in the yellowfin is regulated primarily through changes in f_H. However, SV increased with a drop in temperature, moderating the effect of f_H on cardiac output (Q_{10} = 1.52).

Yellowfin blood has a high-oxygen carrying capacity (16 to 18 ml O_2/dl) and a low *in vivo* oxygen affinity (P_{50} = 5.3 kPa). Exercise caused in rise in arterial (74 to 88%) and a decline in venous oxygen saturation (48 to 44%), increasing the arterial-venous oxygen content difference [$(a - v)_{O_2}$], but leaving a large venous oxygen reserve. It is estimated that cardiac output and $(a - v)_{O_2}$ contributed equally to the increased oxygen uptake.

CHAPTER I- INTRODUCTION

This study examines the cardiovascular physiology of a high-performance teleost, the yellowfin tuna (*Thunnus albacares* [Bonnaterre], Fig. 1). Yellowfin, and tunas in general, have high metabolic rates that support high levels of activity, and their cardio-respiratory system must meet the necessary oxygen uptake and delivery requirements of the respiring tissues. Because tunas are pelagic, continuous swimmers, with high respiratory requirements, they are difficult to acquire, maintain in captivity, and manipulate in experimental situations. As a result, previous measurements of tuna cardiovascular variables under controlled experimental conditions have been limited.

Using a large water tunnel, it has been possible to investigate tuna cardiovascular responses to changes in oxygen demand induced by increasing swimming velocity, and in response to changes of ambient temperature and ambient oxygen content that mimic those normally encountered by fish in the wild. Chapter II describes a theoretical analysis of the oxygen delivery capabilities of the tuna cardiovascular system during swimming, based on preliminary observations and the results of other workers. This model of aerobic swimming performance is expanded in Chapter III to include other metabolic functions (e.g., digestion, growth, and recovery from anaerobic activity) in an analysis of tuna bioenergetics in relation total aerobic capacity. Chapters IV through VI detail the results of experiments on cardio-respiratory function in swimming yellowfin tuna in response to changes in oxygen demand and environmental oxygen availability.

The objectives of this chapter are to present the background information relating to the significance of these studies on tuna, and to describe the techniques required to carry them out.

General natural history

The 'true' tunas consist of 5 genera (*Thunnus*, 7 species; *Katsuwonus*, 1 sp., *Euthynnus*, 3 sp.; *Auxis*, 2 sp.; *Allothunnus*, 1 sp.) of the tribe Thunnini, within the family Scombridae (Collette *et al.*, 1984). Yellowfin tuna grow to be one of the largest teleosts (over 2 meters and approaching 200 kg) and are highly migratory, inhabiting the tropical and subtropical regions of all the world's oceans (Collette and Nauen, 1983). These fish occur primarily in the upper, mixed layer of the ocean, within the temperature range of 18 to 31°C (Sund *et al.*, 1981). However, yellowfin, and other tunas, make rapid vertical movements through the thermocline, possibly in search of prey, and adults can be found as deep as 380 m (Sund *et al.*, 1981; Carey and Olson, 1982; Holland *et al.*, 1990). These excursions result in exposure to rapid changes in temperature and, in areas such as the eastern tropical Pacific, potentially low oxygen contents (Barkley *et al.*, 1978). Tunas are generally considered to be opportunistic predators, feeding on a variety of fish, crustaceans, and cephalopods, in an environment where prey are rare and patchily distributed (Blackburn, 1968; Olson and Boggs, 1986).

Swimming adaptations

Tunas have numerous morphological and anatomical adaptations enhancing swimming performance (Fig. 1). They are highly streamlined, can fully retract the first dorsal, pectoral, and pelvic fins into grooves to reduce drag, and have a high-aspect-ratio lunate caudal fin for maximal thrust production with minimal drag (Magnuson, 1978). The tuna's skeletal, muscle, and tendon arrangements appear to be uniquely adapted to transfer force production to this caudal fin, resulting in the tuna-specific 'thunniform' swimming mode (Fierstine and Walters, 1968; Magnuson, 1978; Westneat *et al.*, 1993; Dewar and Graham, 1994b). In addition, a corselet of large, thick scales behind the opercula, a series of finlets posterior of the second dorsal and anal fins, and large median keels on the caudal

peduncle may further reduce drag and turbulence during swimming (Magnuson, 1978; Collette and Nauen, 1983)

Tunas not only swim to migrate across large areas of ocean and to catch agile prey, but they have lost the ability to actively pump water over their gills and must swim to force water through the opercular cavity (ram ventilation) (Roberts, 1978). In addition, the buoyancy providing swim bladder is reduced or absent in tunas, requiring forward movement to provide hydrodynamic lift (Magnuson, 1978).

The continuous swimming activity of tuna provides the metabolic basis for another specialization, regional endothermy. Metabolic heat produced by the red, aerobic, swimming muscle is not lost, as in most other fishes, but is retained by a vascular counter-current heat exchanger, the *retia mirabilia* (Carey and Teal, 1966; Graham, 1975). In addition, the red muscle is positioned internally, adjacent to the backbone, as opposed to a subcutaneous lateral wedge as in most other teleosts (Carey and Teal, 1966; Graham *et al.*, 1983). Depending on species, ambient temperature, and activity level, excess muscle temperatures can range from a few degrees to 20°C above ambient (Carey and Teal, 1969; Graham, 1973; Dewar *et al.*, 1994; Dickson, 1994). The ability of tunas to elevate the temperature of swimming muscle is shared with the lamnid sharks (e.g., great white, *Carcharodon carcharias*, mako, *Isurus oxyrinchus*, etc.) and the swordfish (*Xiphias gladius*) (reviewed in Bushnell *et al.*, 1992). The possible advantages of maintaining warm red muscle include increased muscle fiber contraction velocities (Brill and Dizon, 1979a; Johnston and Brill, 1984), increased aerobic metabolic capacity (Dickson, 1995), and conservation of muscle function during exposure to rapid temperature decreases when moving through the thermocline (Dewar *et al.*, 1994). Clearly, tunas have a fundamental and extensive investment in continuous swimming performance.

Cardio-respiratory adaptations

The swimming performance of tuna is complemented with many cardio-respiratory adaptations for an enhanced aerobic capacity (Brill and Bushnell, 1991b; Bushnell and Jones, 1994). Although the cardiovascular system has many functions important to sustained exercise, for example, metabolic substrate delivery and waste removal (Heisler, 1989; Weber, 1992), one of the most immediate and clearly essential functions is the delivery of oxygen (Priede, 1985). A summary of the anatomical and biochemical adaptations in tunas for high rates of oxygen uptake and delivery to the tissues are listed in Table 1.

To overcome the difficulties in working with tunas, most studies of cardio-respiratory physiology have been performed on anesthetized or paralyzed, restrained fish. These studies have found high heart rates, cardiac outputs, blood pressures, ventilation volumes, and an elevated oxygen carrying capacity of the blood compared to other fishes (Stevens, 1972; Lai *et al.*, 1987; White *et al.*, 1988; Bushnell *et al.*, 1990; Brill and Bushnell, 1991a; Bushnell and Brill, 1992; Jones *et al.*, 1993). Although these studies have provided considerable data on tuna physiology, they are difficult to extrapolate to conditions in wild fish because the tuna were not swimming.

A few studies have examined the cardio-respiratory physiology of tunas swimming in a doughnut-shaped tank (Jones *et al.*, 1986; Jones *et al.*, 1990; Bushnell and Brill, 1991; Jones *et al.*, 1993). However, these studies have been limited to measuring only a few variables from fish swimming at slow, voluntary speeds. This has precluded any examination of the effects of swimming velocity (i.e., exercise) on cardio-respiratory function.

SIO water tunnel

The development of the Scripps Institution of Oceanography (SIO) water tunnel, the 'Elasmotunatron' (Fig. 2), has permitted examination of the swimming physiology of large, active, pelagic fishes under controlled velocities and environmental conditions (Graham *et al.*, 1990; Graham *et al.*, 1994). In conjunction with the tuna holding facilities of the National Marine Fisheries Service, Kewalo Research Facility in Honolulu, Hawaii (Brill, 1992), several studies have been conducted on swimming tropical tunas (Dewar and Graham, 1994a, b; Dewar *et al.*, 1994).

The design and function of the SIO water tunnel, and the modifications required for tuna research, are detailed in Graham *et al.* (1990), Dewar (1993), and Dewar and Graham (1994a). Basically, this water tunnel, or flume, operates as a recirculating fish treadmill (Fig. 2). Seawater (3000 l) is driven around an oval loop of PVC pipe (45 cm diameter) by a propeller attached to a 40 hp variable speed motor. Large scale turbulence is reduced as the water passes through the diffuser-contraction section, and then 7 cm of 0.32 cm diameter honeycomb, before entering the working section (WS) where the fish is maintained. Maximal water velocity of this system is over 3 m/s. Two large external reserve water tanks are used to rapidly introduce either hot or cold water, to quickly modulate temperature, or either supersaturated or deoxygenated water, to modulate water oxygen content. This allows simulation of the environmental changes associated with vertical migrations through the thermocline.

Water tunnel studies with tunas have confirmed their high metabolic rates compared to other fishes, and quantified the relationship between oxygen consumption and swimming velocity (Dewar and Graham, 1994a). Although increasing exponentially, the rate of increased oxygen consumption with swimming velocity is less than other fishes, suggesting a greater swimming efficiency. Other studies have examined tuna swimming

kinematics, and thermoregulatory capabilities (Dewar and Graham, 1994b; Dewar *et al.*, 1994).

Handling and anesthesia techniques

Techniques for handling tunas and operation of the water tunnel are described in Dewar (1993) and Dewar and Graham (1994a). However, to make extensive measurements of cardio-respiratory function required techniques for anesthesia, surgery, and recovery of tuna. This has allowed more invasive instrumentation, for example, the attachment of blood-flow probes and cannulation of the major blood vessels. Anesthetizing tunas is problematic due to their sensitivity to handling and high oxygen delivery requirements. Care must be taken to ensure that trauma is minimized and that oxygen requirements are met during all stages of the procedure.

The methods used were a modification of those described in Jones *et al.* (1986). The tuna were quickly netted from the holding tank and guided into a tubular plastic bag containing 5 l of oxygenated (~24 kPa O_2, 24-26°C) seawater and 1 g/l of tricaine methanesulphonate (Finquel®, Argent Chemical Laboratories). The fish was gently sloshed back and forth within the bag to circulate water over its gills until it was sedated (approximately 1 min). The high initial dose of anesthetic quickly subdued the fish, reduced damage associated with struggling, and allowed rapid transport to the surgical table where the fish could be force-ventilated.

The 50 l Lucite tank (Fig. 3), which served as an access port to the working section (WS) during normal operation, was modified into a water operating table by isolating it from the WS with a Lucite plate bolted in place. The tuna was placed in a chamois cradle suspended between two plastic supports over the tank. Chamois-covered sponges supported the fish and allowed slight tail movements without damage to the easily frayed fins. A submersible pump and ventilation hose were used to circulate tank water over the

fish's gills at 10 - 12 l/min. The tank water was supersaturated with O_2 (~24 kPa), cooled (22-24°C), and contained a reduced concentration of anesthetic (0.10 to 0.057 g/l). Seawater pH was maintained by buffering the anesthetic with $NaHCO_3$ or Trizma buffer (Sigma, T-1503).

Following surgery, the anesthetic solution in the Lucite tank was diluted and replaced with an inflow of fresh seawater. The fish was then lowered into the tank while maintaining water flow over the gills. When slight tail oscillations and fin movements were observed, the Lucite plate was removed and the fish guided into the WS. The ventilation hose was removed and the tuna gently held mid-stream (water speed 40 - 70 cm/s) to maintain ventilation until it regained equilibrium and started swimming.

Recovery from anesthesia was most successful using fish that were in captivity at least one week and were feeding. The total time under anesthesia, however, negatively affected swimming performance. Figure 4 shows the affect of anesthesia time on the swimming duration for 22 yellowfin with similar instrumentation. In general, anesthesia longer than 30 min appeared to limit swimming performance.

Research objectives

The focus of this research is the tuna's cardiovascular specializations for sustaining a high aerobic metabolic scope. The high metabolic rate and increases with swimming velocity must be accompanied by changes in the cardio-respiratory system to capture and deliver O_2 at the required rate. One approach to this problem is to examine the physiological parameters affecting tissue O_2 delivery. Oxygen uptake ($\dot{V}_{O_2}$) is determined by the product of cardiac output (blood flow, $\dot{Q}$), and the arterial-venous blood oxygen content difference [$(a - v)_{O_2}$], as described by the Fick equation for convective oxygen transport:

$$\dot{V}_{O_2} = \dot{Q} \cdot (a - v)_{O_2}$$

$\dot{Q}$ is the product of stroke volume (SV) and heart rate (f_H), so the equation becomes:

$$\dot{V}_{O_2} = SV \cdot f_H \cdot (a - v)_{O_2}$$

The objectives of this research were to quantify the changes in each of the parameters of the Fick equation that contribute to increased oxygen delivery during swimming. In addition, the effects of environmental changes in oxygen content and temperature on cardiac performance, which may limit oxygen delivery, were examined.

The experimental portion of this dissertation is divided into three chapters (Chapters IV - VI), each examining a parameter of the Fick equation, in order of increasing invasiveness and experimental difficulty. These studies elucidate the cardiovascular responses to exercise in tuna, resolving speculations about the nature of tuna cardiac function, and clarifying the tuna's place among evolutionary specializations for high metabolic performance.

Table I-1: Anatomical and biochemical adaptations for high aerobic capacity in tunas

Adaptation	References
Large gill surface area	Hughes, 1984
Thin secondary gill lamellae	Hughes, 1984
Large relative heart mass & percentage of compact myocardia	Santer *et al.*, 1983; Poupa *et al.*, 1981; Farrell *et al., 1992*
High heart myoglobin levels	Giovane *et al.*, 1980
High heart aerobic enzyme activities	Moyes *et al.*, 1992a; Dickson, 1995
High muscle myoglobin levels	George and Stevens, 1978; Stevens and Carey, 1981
High muscle mitochondrial density	George and Stevens, 1978; Hulbert *et al.*, 1979
High muscle capillarity	Hulbert *et al.*, 1979; Mathieu-Costello *et al.*, 1992
High muscle aerobic enzyme activities	Guppy *et al.*, 1979; Moyes *et al.*, 1992a; Dickson, 1995

Figure I-1

The yellowfin tuna, *Thunnus albacares* (Bonnaterre). Adapted from Collette and Nauen (1983).

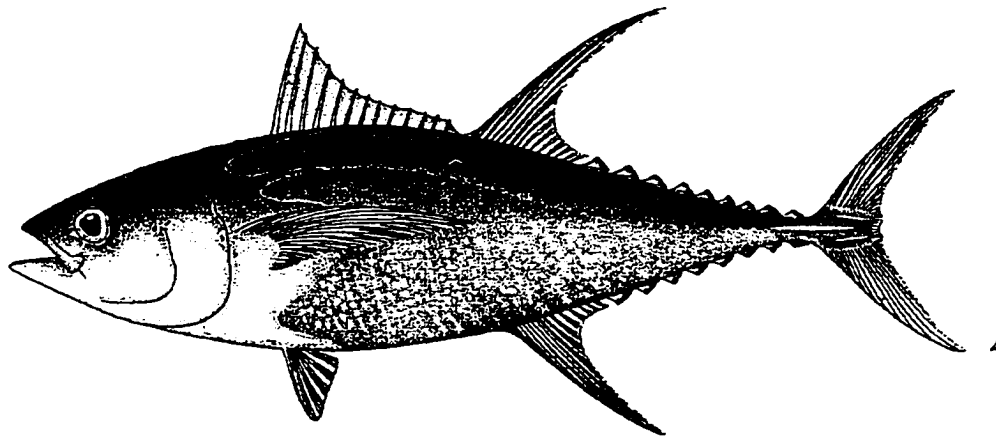


Figure I-2

Schematic diagram of the SIO water tunnel. Arrows indicate the direction of water flow. Adapted from Dewar (1993).

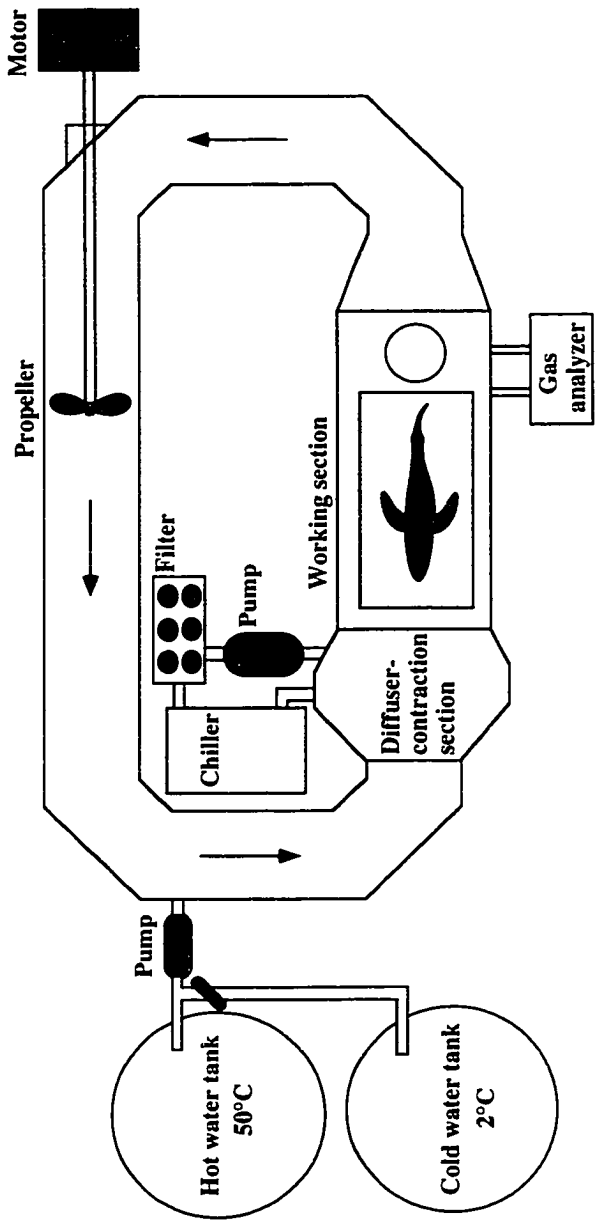


Figure I-3

Schematic of water operating table. The modified Lucite tank (LT), positioned above the working section (WS), and cradle (C) used for surgery are shown. Also indicated are the submersible pumps (P) for ventilation and drainage (D), the oxygen stone, the ventilation hose (V), Lucite plate (Pl) bolted in position, and the seawater input (SW). Arrows indicate the direction of water flow.

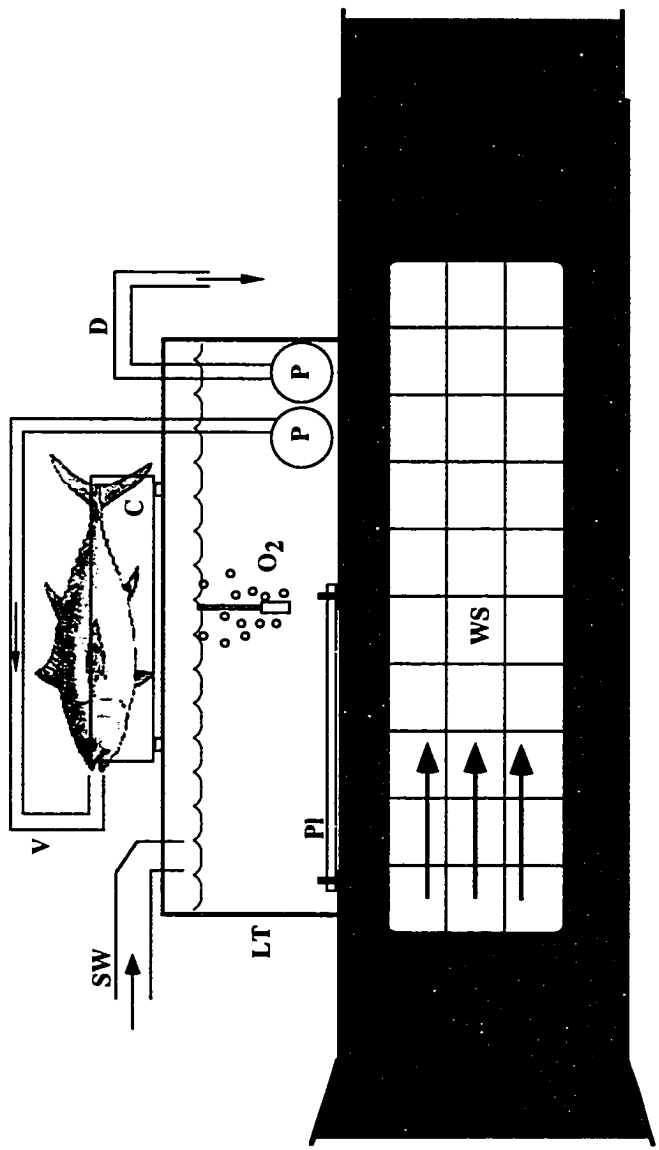
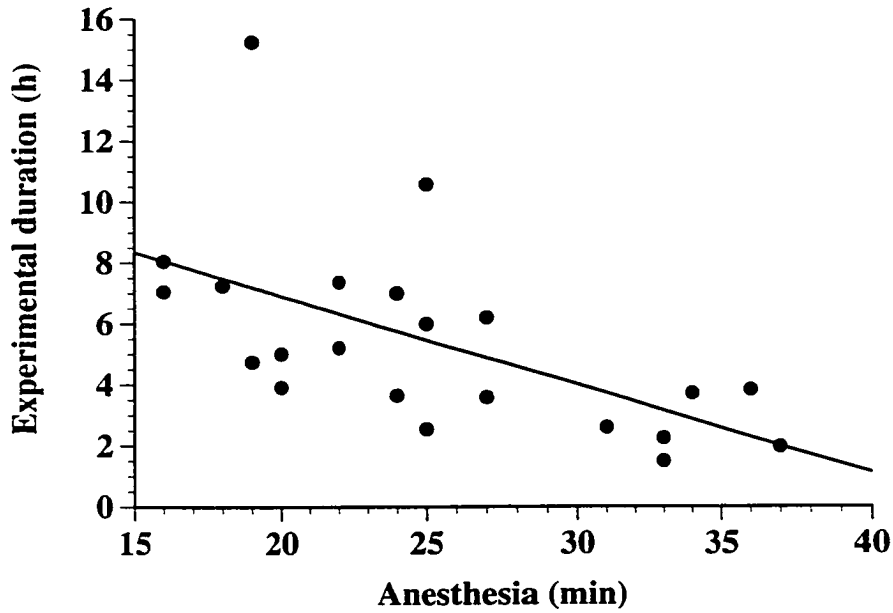


Figure I-4

Experimental duration as a function of time under anesthesia for 22 yellowfin tuna with similar instrumentation (ventral- and dorsal-aortic cannulae) and experimental protocol (see Chapter VI). Regression analysis indicates that the experimental duration decreases with anesthesia ($p<0.005$; $r^2=0.34$).



CHAPTER II: TUNA AEROBIC SWIMMING PERFORMANCE: PHYSIOLOGICAL AND ENVIRONMENTAL LIMITS BASED ON OXYGEN SUPPLY AND DEMAND

Abstract

Sustainable (aerobic) swimming performance in yellowfin tuna (*Thunnus albacares*) is examined through a model estimating red muscle oxygen demand and the cardiovascular system's capacity for oxygen delivery. At maximum oxygen delivery (25°C), red muscle oxygen consumption ($\dot{V}_{O_2}RM$) is estimated to be 0.197 ml O_2/g/min, which is higher than previous estimates of maximum $\dot{V}_{O_2}RM$. However, due to the high costs of swimming at high speeds, maximum sustained velocity at this rate would only be 3.7 fork lengths/s, which is lower than previous estimates for yellowfin. Hypoxia and reduced ambient temperature that may be encountered by yellowfin in the course of their natural movement patterns will decrease oxygen delivery capacity through effects on the cardiovascular system, limiting maximum $\dot{V}_{O_2}RM$. Moderate swimming velocities could, however, be maintained at an oxygen tension of 10 kPa, and at an ambient temperature of 15°C.

Introduction

Tunas (family Scombridae) are highly active, pelagic predators specialized for continuous swimming. A high rate of aerobic metabolism, the capability of regional endothermy, and the need to compensate for relatively rapid changes in environmental oxygen and temperature distinguish tunas from most other fishes. The tuna's many swimming adaptations and high metabolic rates suggest that they are capable of extraordinarily high sustainable (aerobic) swimming speeds (Beamish, 1978; Stevens and Carey, 1981). The elevated metabolic rate of tunas has been well documented. Even when ambient and body temperature differences are taken into account, tunas consume oxygen at 2 to 5 times the rate of other active teleosts, such as salmonids (Gooding *et al.*, 1981; Graham and Laurs, 1982; Boggs and Kitchell, 1991; Brill and Bushnell, 1991b; Dewar and Graham, 1994a). However, there are no direct measurements of maximum sustained swimming speed for tuna.

Skipjack (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) occupy the warm upper waters of tropical-subtropical latitudes (20-30°C) and make periodic sojourns to depth where they may encounter waters that are as much as 10°C cooler than the surface (Dizon *et al.*, 1978; Carey and Olson, 1982; Holland *et al.*, 1990). In many areas including the eastern tropical Pacific, vertical migrations can also bring these tunas into contact with layers of deoxygenated water (Ingham *et al.*, 1977; Barkley *et al.*, 1978; Sund *et al.*, 1981).

Physiological data, fishery catch statistics, and ocean oxygen and temperature profiles have been combined into predictive models for tuna geographical and bathymetric distributions (Sharp, 1978; Sund *et al.*, 1981). Barkley *et al.* (1978) identified suitable skipjack habitats in the eastern tropical Pacific, on the basis of experiments determining susceptibility to relatively mild hypoxia and maximum and minimum temperature limits. Nevertheless, the rapid vertical movements and migratory patterns of tunas invariably

expose them to a range of habitat conditions and may occasionally result in their entry into areas where temperature and oxygen availability limit metabolic performance and preclude long-term residence. The vascular heat-exchangers of tuna conserve metabolic heat production in the swimming muscles and reduce the rate of cooling during decreases in ambient temperature (Dewar *et al.*, 1994). However, other areas of the tuna, including the heart, are not protected from rapid ambient temperature changes. Thus, in the absence of an ability to rapidly compensate for changing habitat conditions, the aerobic swimming performance of a tuna at depth could be restricted by reductions in ambient oxygen and temperature which affect metabolic demand and the cardiorespiratory system's ability to supply oxygen.

For the past several years efforts in this laboratory have focused on estimating the aerobic costs of tuna swimming and on quantifying tuna cardiovascular function in relation to exercise and in response to changes in ambient temperature and oxygen concentration.

The objectives of this paper are to combine our recent findings with the results of other workers in the development of a quantitative model describing oxygen delivery during swimming and showing how this delivery is affected by ambient conditions. Because most fishes power aerobic swimming primarily by red muscle activity, our model emphasizes factors affecting the metabolic performance of tuna red muscle. Recent studies using a large water tunnel (Dewar and Graham, 1994a) have quantified the relationship between swimming velocity and oxygen consumption ($\dot{V}_{O_2}$) for the yellowfin tuna. The model we develop integrates these data with physiological estimates of cardiovascular performance during sustained (aerobic) swimming in order to determine relationships between the total $\dot{V}_{O_2}$ of a tuna and the oxygen consumption of its red muscle. From this analysis, it appears that due to the high costs of swimming at high speeds, red muscle metabolism cannot support extraordinary sustained swimming speeds in yellowfin tuna.

The implications of these results on tuna bioenergetics are discussed in a companion paper (Korsmeyer *et al.*, 1996a).

Materials and Methods

Estimates of red muscle oxygen requirements

Application of measurements of O_2 consumption during swimming

The oxygen requirements of red muscle can be estimated from total oxygen consumption during swimming. A graph relating total $\dot{V}_{O_2}$ of yellowfin tuna to swimming velocity (Fig. 1) illustrates the basic features of this approach. Central to it is the $\dot{V}_{O_2}$-velocity relationship determined by Dewar and Graham (1994a) for 12 yellowfin tuna of approximately 50 cm fork length (FL; fork length is measured from the tip of the snout to the fork of the caudal fin) (mass = 2.1 kg), swimming between 0.5 and 3.0 FL/s. This length and mass of tuna are used in the model to avoid the difficulties of scaling metabolic rate and relative swimming speed, and because the cardiovascular data have been obtained from tuna of this size. The lower boundary condition for $\dot{V}_{O_2}$ in this model is set by extrapolation of the $\dot{V}_{O_2}$-velocity function to "zero" velocity. Although tunas never stop swimming, this extrapolation, in theory, defines yellowfin $\dot{V}_{O_2}$ in the absence of swimming or any other non-essential metabolic costs and this is termed the standard metabolic rate (SMR). The model's upper boundary condition is set by extrapolation to the theoretical maximum $\dot{V}_{O_2}$ of 29 ml O_2/kg/min estimated for yellowfin (Bushnell and Brill, 1991).

From the metabolism-activity relationships described in Fig. 1, it is possible to estimate red muscle oxygen consumption ($\dot{V}_{O_2}$) at any specified aerobic swimming velocity. The difference between SMR and total swimming $\dot{V}_{O_2}$ ($\dot{V}_{O_2 swim}$) at any sustainable velocity (i.e., a velocity powered by aerobic metabolism and not requiring anaerobic activity) is the amount of oxygen required for swimming and is defined here as

the cost of locomotion. While the majority of this oxygen is consumed by the red muscle, a fraction supplies the increased maintenance costs ($\dot{V}_{O_2\,\mathrm{maint}}$) associated with exercise. For example, aerobic exercise generally increases work done by the heart and there are both ventilatory and osmotic costs associated with elevated branchial perfusion (Jones , 1971; Webb, 1975; Jones and Randall, 1978; Stevens and Dizon, 1982). Webb (1975) estimated that these costs could account for up to 37% of maximum aerobic scope in rainbow trout (*Oncorhynchus mykiss*). This value is based on the relative osmoregulatory costs determined for rainbow trout of about 17% of aerobic scope (Rao, 1968) and increased cardiac and ventilatory costs of about 10% each, as estimated by Jones (1971). Because tunas are obligate ram ventilators, their ventilatory costs are integrated with the power requirement of red muscle for sustained swimming. In addition, Farrell and Steffensen (1987) reexamined cardiac costs based on actual myocardial $\dot{V}_{O_2}$ measurements in trout, and from their estimates, cardiac costs would account for only 1.5% of aerobic scope at maximum exercise. This relative cost may be greater in tuna due to a greater relative ventricular mass and cardiac power output (Farrell and Jones, 1992). In the absence of direct estimates of velocity effects on $\dot{V}_{O_2\,\mathrm{maint}}$ in tuna, the model assumes that the increase in maintenance costs (osmoregulatory and cardiac) above that of SMR during swimming is 20% of the cost of locomotion.

Calculations

The equation describing the maintenance component of the swimming $\dot{V}_{O_2}$ of a yellowfin tuna is:

$$\dot{V}_{O_2\,\mathrm{maint}} = \mathrm{SMR} + 0.20 \cdot (\dot{V}_{O_2\,\mathrm{swim}} - \mathrm{SMR}) \qquad (1)$$

As is shown in Fig. 1, the difference between swimming $\dot{V}_{O_2}$ and the corresponding maintenance $\dot{V}_{O_2}$ at any sustainable speed equals total red muscle $\dot{V}_{O_2}$:

$$\text{total } \dot{V}_{O_2\,\mathrm{RM}} = \dot{V}_{O_2\,\mathrm{swim}} - \dot{V}_{O_2\,\mathrm{maint}} \qquad (2)$$

From eq. 1 and 2 it can be shown that:

$$\text{total } \dot{V}_{O_2}\text{RM} = 0.80 \cdot (\dot{V}_{O_2}\text{swim} - \text{SMR}) \quad (3)$$

The relationship between swimming velocity (U) and $\dot{V}_{O_2}$swim is obtained from Dewar and Graham (1994a):

$$\log(\dot{V}_{O_2}\text{swim}) = 0.2142 \cdot U + 0.477 \quad (4)$$

where the units for $\dot{V}_{O_2}$swim are ml O_2/kg/min (all values of oxygen volume are reported at STPD) and U is in FL/s. From eq. 3 and 4, total $\dot{V}_{O_2}$RM (ml O_2/kg/min) can be related to U by the following:

$$\text{total } \dot{V}_{O_2}\text{RM} = 0.80 \cdot [10^{(0.2142 \cdot U + 0.477)} - \text{SMR}] \quad (5)$$

Because SMR can be estimated (3.00 ml O_2/kg/min; Dewar and Graham, 1994a), eq. 5 shows that total $\dot{V}_{O_2}$RM is predicted from U.

Equations 1-5 illustrate the steps required to determine the total red muscle $\dot{V}_{O_2}$ component associated with sustained swimming in yellowfin tuna. Examination of the metabolic intensity of this tissue and comparison with other red muscle $\dot{V}_{O_2}$ estimates require that the units of metabolic activity be converted from ml O_2/kg body mass/min to ml O_2/g red muscle/min. From work by Graham *et al.* (1983), the mass of the red muscle (M_{RM}) can be determined from total body mass (M_T) by:

$$M_{RM} = 0.1177 \cdot (M_T \cdot 1000)^{0.92} \quad (6)$$

where the units of M_{RM} are g, and M_T are kg. From this equation, tissue-specific red muscle $\dot{V}_{O_2}$ ($\dot{V}_{O_2}$RM) is now expressed in ml O_2/g/min:

$$\dot{V}_{O_2}\text{RM} = \frac{(\text{total } \dot{V}_{O_2}\text{RM}) \cdot M_T}{M_{RM}} \quad (7)$$

Equations 5-7 enable estimation of $\dot{V}_{O_2}$RM from SMR and U. The relationship of this $\dot{V}_{O_2}$RM estimate and swimming velocity is shown in Fig. 2. This rate, while approaching zero at zero velocity, increases exponentially with speed. Tuna never stop swimming naturally, however, at zero velocity $\dot{V}_{O_2}$RM would not be zero, but the red muscle would have a basal oxygen consumption associated with SMR. For simplicity, and

because the red muscle contribution to SMR is not known, its component of respiration at zero velocity is not separated from total SMR. Red muscle SMR is not considered to be a large component of $\dot{V}_{O_2}$RM when the red muscle is actively contracting during swimming, especially at maximum swimming speeds. For example, the $\dot{V}_{O_2}$ of aerobic muscle in dog at rest is less than 2% of maximum muscle $\dot{V}_{O_2}$ (Honig *et al.*, 1991).

Red muscle O_2 delivery based on cardiovascular parameters

While the above analysis estimates the oxygen requirements of the red muscle from $\dot{V}_{O_2}$swim, $\dot{V}_{O_2}$RM can also be derived from the oxygen delivered to red muscle by the cardiovascular system.

Experimental

Experiments were carried out to refine measurements of the variables related to oxygen delivery in a swimming yellowfin tuna. Tuna were fitted with ECG (electrocardiogram) electrodes, with a transcutaneous Doppler flow probe mounted over the ventral aorta, and both ventral and dorsal aortic cannulae to permit measurement of arterial and venous O_2 differences and related hematological parameters in swimming yellowfin. Studies were conducted in a large water tunnel to permit control of swimming velocity and both ambient temperature and O_2 content. Methods for handling experimental fishes, instrumenting them, and controlling their swimming performance are described elsewhere (Dewar and Graham, 1994a; Dewar *et al.*, 1994; Dewar *et al.*, in prep.; Chapter I). As will now be detailed, results from these and other studies were incorporated into the red-muscle $\dot{V}_{O_2}$ model through estimates of oxygen delivery based on cardiac output and on differences in arterial and venous O_2 content.

Calculations

Estimates of $\dot{V}_{O_2}RM$ are based on the Fick principle for convective transport:

$$\dot{V}_{O_2}RM = \dot{Q}_{RM} \cdot (a - v)_{O_2}RM \quad (8)$$

where $\dot{Q}_{RM}$ is red muscle blood flow in ml/g/min, $(a - v)_{O_2}$ RM is the difference between arterial and venous O_2 contents in ml O_2/ml, and $\dot{V}_{O_2}RM$ is expressed in ml O_2/g/min. Using radiolabelled microspheres, White *et al.* (1988) estimated a $\dot{Q}_{RM}$ of 36% of cardiac output for lightly anesthetized albacore (*Thunnus alalunga*). We have assumed a $\dot{Q}_{RM}$ of 36% for yellowfin tuna and this is incorporated with red muscle mass (M_{RM}) calculated from eq. 6:

$$\dot{Q}_{RM} = \frac{(\dot{Q} \cdot M_T) \cdot 0.36}{M_{RM}} \quad (9)$$

where $\dot{Q}$ is cardiac output in ml/kg/min.

Integration of estimates of red muscle oxygen demand and delivery

The predicted $\dot{V}_{O_2}RM$ from oxygen delivery (eq. 8) can be incorporated with red muscle oxygen demand through the relation in Fig. 2 describing red muscle oxygen requirements at sustainable swimming speeds (eq. 5 and 7). Thus, from estimates of oxygen delivery to the red muscle, the sustainable swimming speed can be predicted, and the total swimming $\dot{V}_{O_2}$ calculated at that velocity (eq. 4).

Results

Using the above model of $\dot{V}_{O_2}RM$ and sustained swimming performance, cardiovascular parameters will be incorporated into the calculations at two different velocities, ambient oxygen levels, and water temperatures.

i. Slow swimming

It could be expected that minimum O_2 delivery to the red muscle would take place at slow cruising speeds (0.5 to 1.25 FL/s) and Table 1A details the parameters used to estimate the rate of oxygen delivery to red muscle in a 2.1 kg yellowfin swimming at slow speed in 25°C, normoxic water. The rationale for the selected values is now discussed.

Tunas have a high blood oxygen capacity due to hemoglobin concentrations and hematocrits which exceed values in most other teleosts (Brill and Bushnell, 1991b). Oxygen capacity values in tuna range from 0.15 to 0.22 ml O_2/ml (Cech *et al.*, 1984; White *et al.*, 1988; Brill and Bushnell, 1991a) compared to 0.10 ml O_2/ml in rainbow trout (Jones and Randall, 1978). For yellowfin swimming at slow speeds, we measured a mean blood-O_2 carrying capacity of 0.18 ml O_2/ml, an arterial saturation of about 80%, and a venous saturation of 50% (Korsmeyer *et al.*, 1993; Chapter VI). Because it was not possible to determine the venous O_2 contents of the vessels exiting red muscle, our estimate of the amount of oxygen extracted by red muscle [$(a - v)_{O_2\,RM}$] assumes this value is equivalent to the difference between arterial O_2 content (0.144 ml O_2/ml), and ventral aorta content (mixed venous content, 0.090 ml/ml). These blood O_2 contents are similar to those from spinally-blocked yellowfin (Bushnell and Brill, 1992). Table 2 describes cardiovascular variables measured for tropical tunas under various conditions. Measured cardiac outputs in yellowfin tuna range from 54 to 115 ml/kg/min. For slow swimming, we used a cardiac output of 54 ml/kg/min.

The results of the model (Table 1A) are that a 2.1 kg yellowfin swimming at slow speed would have a red muscle $\dot{V}_{O_2}$ of 0.016 ml O_2/g/min and a total $\dot{V}_{O_2}$ of 4.24 ml O_2/kg/min. Based on these values and the swimming speed-$\dot{V}_{O_2}$ relationship in Figs. 1 and 2, its swimming velocity would be 0.74 FL/s. These data are shown in Fig. 3.

ii. Maximum sustained (aerobic) velocity

Table 1B indicates the values used in the model at maximum O_2 delivery to red muscle. Because data at maximum aerobic exercise are not available for tuna, estimates must be made for cardiovascular parameters at maximum O_2 delivery.

Our work shows that arterial oxygen saturation increases with swimming speed in yellowfin (Chapter VI) and presumably would reach 100% as a result of the increased lamellar recruitment and O_2 uptake caused by elevations in ventral aortic blood pressure and cardiac output (Jones and Randall, 1978; Farrell *et al.*, 1980). Based on the difference in arterial and mixed venous oxygen contents during maximum exercise, total oxygen extraction was 86% in trout (Kiceniuk and Jones, 1977), and Randall and Daxboeck (1982) proposed that oxygen extraction was as high as 96% within the red muscle. This extraction rate is probably too high, as the resulting oxygen partial pressure (P_{O_2} = 0.1-0.3 kPa) is unrealistic in terms of the necessary oxygen gradient from the capillary to the muscle mitochondria (Groebe and Thews, 1990). In mammalian muscle at maximum $\dot{V}_{O_2}$, oxygen extraction is reportedly between 75 and 85%, with a mean end-capillary oxygen saturation of between 10 and 20% (Gayeski and Honig, 1988; Honig *et al.*, 1991). For our calculations we assume that at maximum $\dot{V}_{O_2}$, red muscle extracts 90% of the O_2 delivered in arterial blood, resulting in a venous O_2 saturation of 10%.

Table 2 indicates that the cardiac stroke volumes measured for tunas vary by a factor of 2 and that heart rates vary by a factor of 4. We have also shown that yellowfin swimming in the water tunnel respond to moderate increases in velocity by elevating both stroke volume and heart rate (Korsmeyer *et al.*, 1993). However, our results suggest that it is unlikely that, at the maximum heart rates reported for tunas (>200 bpm), maximum stroke volume could be maintained with the limited filling time available (Graham *et al.*, 1994). In addition, Jones *et al.* (1993) found an antagonistic effect of heart rate on stroke volume in yellowfin tuna. The highest cardiac outputs recorded for tuna do not exceed 140

ml/kg/min, however, there are no measurements of absolute cardiac output at maximum exercise. Brill and Bushnell (1991b) predict a maximum cardiac output of between 150 and 200 ml/min/kg for yellowfin and skipjack tuna. For maximum O_2 delivery we assume a 4-fold increase in cardiac output (i.e., from 54 to 216 ml/kg/min).

The calculated values during maximum red muscle O_2 delivery are: Red muscle $\dot{V}_{O_2}$, 0.197 ml O_2/g/min; total swimming $\dot{V}_{O_2}$, 18.68 ml O_2/kg/min; and a corresponding swimming velocity of 3.7 FL/s (Table 1B, Fig. 3).

iii. Maximum sustained velocity: effects of hypoxia

The following model iteration uses the parameters for maximum O_2 delivery, but adjusts for the effects of hypoxia (~10 kPa O_2 tension, 50% saturation). The decrease in the inspired water oxygen tension (P_{O_2}) would preclude blood saturation. In spinally-blocked yellowfin, for example, arterial blood is only approximately 75% saturated at around 12.0 kPa (Bushnell and Brill, 1992).

Most teleosts respond to mild hypoxia with a slowing of heart rate, or bradycardia, while maintaining or limiting decreases in cardiac output through increases in stroke volume (Satchell, 1991; Farrell and Jones, 1992). Skipjack and yellowfin tuna both respond to mild hypoxia (17.3 kPa) with bradycardia, but because there was essentially no change in stroke volume, cardiac output also declined (Bushnell *et al.*, 1990; Bushnell and Brill, 1992).

This agrees with our results for swimming yellowfin, where a drop in ambient O_2 (10 kPa) resulted in a 30% reduction in heart rate (Korsmeyer *et al.*, 1992) and a corresponding reduction of cardiac output (Fig. 4). A decrease in heart rate with no change in stroke volume would reduce estimated maximum cardiac output from 216 to 151 ml/kg/min.

The calculated effects of hypoxia on maximum O_2 delivery are: Red muscle O_2 consumption, 0.100 ml O_2/g/min; total swimming $\dot{V}_{O_2}$, 10.88 ml O_2/kg/min, which corresponds to a maximum swimming velocity of 2.6 FL/s (Fig. 3).

iv. Maximum sustained velocity: effects of reduced temperature

The maximum O_2 delivery parameters were used in the model, with adjustments for a reduction in ambient temperature from 25 to 15°C (at normoxia). Heart rates in swimming yellowfin exposed to acute temperature changes have Q_{10}'s ranging from 1.5 to 2.9 (Korsmeyer *et al.*, 1992) and Fig. 5 shows that this reduction is not fully compensated by an increased stroke volume and cardiac output therefore declines. The average Q_{10} for cardiac output in swimming yellowfin is 1.8 (Chapter V). A reduction in ambient temperature to 15°C would therefore lower maximum cardiac output to 120 ml/kg/min.

As seen in Fig. 3, the overall effects of the change from 25 to 15°C are: Red muscle O_2 consumption, 0.110 ml O_2/g/min; total swimming $\dot{V}_{O_2}$, 11.68 ml O_2/kg/min; maximum swimming velocity, 2.8 FL/s.

Discussion

We have analyzed the aerobic metabolic performance of yellowfin tuna red muscle from two perspectives. First, velocity effects on red muscle $\dot{V}_{O_2}$ were estimated from the measured rate of total swimming $\dot{V}_{O_2}$ by factoring out SMR and the increased maintenance costs associated with swimming (Fig. 1). As expected, the relationship between $\dot{V}_{O_2\,RM}$ and sustainable velocity (Fig. 2) shows that aerobic demand increases exponentially with velocity.

A second red muscle $\dot{V}_{O_2}$ perspective was obtained by integrating measurements of yellowfin cardiovascular performance with estimates of red-muscle blood flow to determine oxygen delivery. This approach estimated the cardiovascular system's capacity for

delivering oxygen to the red muscle, and determined how delivery was affected by changes in ambient temperature and oxygen. Integration of both these approaches allows evaluation of yellowfin aerobic swimming performance (Fig. 3).

Limitations of the model

A number of uncertainties surround the various assumptions needed to estimate red muscle $\dot{V}_{O_2}$ during slow and fast aerobic swimming. As explained in the methods, in the absence of direct data on $\dot{V}_{O_2\,\mathrm{maint}}$ our model assumes that the increase in this cost with exercise is a fixed proportion of 20% of the increase in the cost of swimming. Fig. 2 illustrates the differences in predicted red muscle O_2 demand if this proportion (from eq. 1) is increased to 30%, which would decrease $\dot{V}_{O_2\mathrm{RM}}$ at any given velocity, or decreased to 10%, which would increase predicted $\dot{V}_{O_2\mathrm{RM}}$.

There are no data for $(a - v)_{O_2}$ differences in red muscle blood flow and no data to indicate how cardiac output might be distributed to red muscle with swimming speed. Maximum aerobic conditions were predicted from estimates of maximum cardiovascular performance (Table 1B), and, relative to conditions at the slower velocity, it would be expected that the proportion of cardiac output delivered to red muscle should have increased. However, there are no data comparing $\dot{Q}_{\mathrm{RM}}$ at slow and fast aerobic velocities. The only estimate for tuna $\dot{Q}_{\mathrm{RM}}$ is 36% of cardiac output (White *et al.*, 1988) determined for recently captured albacore, which were lightly anesthetized, and showed some tail movement. Because of the unknown effects of stress and the anesthetic on cardiac output and regional vasodilatation in that preparation, and undetermined effects of activity on the scope of $\dot{Q}_{\mathrm{RM}}$ in tuna, we did not feel comfortable adjusting this value up or down in the model.

How do our estimated red muscle blood flow values compare to values in other species? In trout, resting $\dot{Q}_{\mathrm{RM}}$ is between 6 and 12 % of cardiac output (Neumann *et al.*,

1983; Randall and Daxboeck, 1982; Barron *et al.*, 1987). Trout $\dot{Q}_{RM}$ has been shown to increase 14-fold to 42% of cardiac output at 80% of maximum exercise (Randall and Daxboeck, 1982); the increase measured just after exhaustive exercise was 5.9-fold above resting values, or 30% of cardiac output (Neumann *et al.*, 1983).

For yellowfin, Table 1B shows that the predicted increase in $\dot{Q}_{RM}$ during maximum O_2 delivery is 4-fold, not from rest, but from slow swimming (i.e., from 0.31 to 1.22 ml/g/min). This maximum $\dot{Q}_{RM}$ is twice the flow rate measured for trout following exhaustive exercise (0.53 ml/g/min; Neumann *et al.*, 1983) or during 80% maximum sustained swimming (0.64 ml/g/min; Randall and Daxboeck, 1982), and 5.5 times the flow rate measured for anesthetized albacore (0.22 ml/g/min; White *et al.*, 1988). Wilson and Egginton (1994) reported a 27-fold increase in blood flow to trout red muscle from rest to a maximum sustained swimming $\dot{Q}_{RM}$ of 2.08 ml/g/min. This exceptional flow rate appears to be largely due to the high cardiac output (143 ml/kg/min) measured by this group. However, we are skeptical of these values, since this cardiac output is 2 to 3 times higher than previously reported maximum cardiac output for trout at similar temperature (Kiceniuk and Jones, 1977; Keen and Farrell, 1994). In addition, at the reported heart rate (73 bpm), stroke volume would have to be twice as high as previous measurements of maximum stroke volume and maximum ventricular chamber volume in similarly sized trout (Franklin and Davie, 1992; Forster and Farrell, 1994).

Because the maximum $\dot{Q}_{RM}$ estimated for yellowfin (1.22 ml/g/min) is similar to that in mammalian muscle during maximum oxygen consumption (Kayar *et al.*, 1994), we feel it is a reasonable approximation, either at the given perfusion ratio and cardiac output, or possibly a lower cardiac output and greater relative red muscle perfusion. Any changes in the rate of red muscle blood flow will affect our estimate of $\dot{V}_{O_2}RM$ in direct proportion (eq. 8, i.e. a doubling in blood flow will double $\dot{V}_{O_2}$). However, as $\dot{V}_{O_2}RM$ increases, the rate of change of the predicted swimming speed decreases (Fig. 2). Clearly, this is an

area in need of research and the results of our estimated oxygen delivery should be taken with this uncertainty in mind.

Comparative red muscle O_2 consumption data

How do the red muscle O_2 requirements calculated in this study compare to earlier estimates of red muscle aerobic metabolism? Tuna red muscle $\dot{V}_{O_2}$ has been measured *in vitro* and calculated based on calorimetric methods combined with *in vivo* muscle temperatures. Table 3 summarizes these data, shows the estimates for yellowfin tuna obtained in this study, and also contains comparative muscle metabolism data for other teleosts, birds, and mammals.

It can be seen in Table 3 and Fig. 2 that our $\dot{V}_{O_2\,RM}$ value (0.016 ml O_2/g/min), calculated from cardiovascular parameters for a slow-swimming yellowfin, compares favorably with the slow-swimming estimates of Neill *et al.* (1976). Moreover, the estimated velocity of this fish (0.74 FL/s), although less than the minimum velocity for hydrostatic equilibrium determined by Magnuson (1978), is within the velocity range observed for yellowfin in the water tunnel (0.5 FL/s, Dewar and Graham, 1994a, b). The maximum $\dot{V}_{O_2\,RM}$ calculated from the cardiovascular parameters is 0.197 ml O_2/g/min which corresponds to a maximum sustained swimming speed of 3.7 FL/s (Figs. 2 and 3). This velocity is below the maximum value predicted by Bushnell and Brill (1991) for yellowfin (5.6 FL/s), but is similar to their prediction for skipjack (3.5 FL/s) and with the velocity at which white muscle recruitment occurs in skipjack (average 3.9 FL/s, Brill and Dizon, 1979b).

Another $\dot{V}_{O_2\,RM}$ estimate can be obtained from the recent red muscle biochemical energetics data of Moyes *et al.* (1992a). Based on skipjack mitochondrial oxygen consumption *in vitro*, these workers calculated a maximum $\dot{V}_{O_2\,RM}$ of 0.058 ml O_2/g/min at 25°C. This rate can be applied to a swimming fish by correcting it to an assumed

maximum red muscle temperature of 35°C (skipjack red muscle excess temperatures can approach 10°C, Stevens and Neill, 1978). Thus, assuming a probable Q_{10} of 2, the Moyes *et al.* (1992a) value is corrected to 0.116 ml O_2/g/min. This value, while not approaching the maximum rates measured for mammalian and avian muscle (Table 3), is approximately 10 times the rate estimated for tuna red muscle during slow swimming (Neill *et al.*, 1976) and corresponds to a maximum sustained speed of 2.9 FL/s for yellowfin tuna (Fig. 2). However, yellowfin red muscle excess temperatures are less than those of skipjack, approaching only 5°C (Dizon and Brill, 1979; Dickson, 1994). The corrected Moyes *et al.* (1992a) value is of the same magnitude but only 60% of the maximum rate calculated from cardiovascular performance (0.197 ml/g/min), suggesting that the latter may overestimate O_2 delivery. However, mitochondrial respiration rates measured *in vitro* are lower than those estimated *in vivo* by about 40 to 60% (Suarez *et al.*, 1991). With this difference taken into account, the Moyes *et al.* value becomes very similar to our *in vivo* estimate.

Implications of red muscle O_2 consumption estimates for the sustained swimming of tunas

Sustained velocity

Sustained swimming is defined as that which can be maintained for long periods (>200 min) without resulting in fatigue (Beamish, 1978). Although tunas are often reported as capable of sustained swimming speeds as high as 10 FL/s (Yuen, 1970; Beamish, 1978), there are no direct measurements of maximum sustainable swimming speed. Tunas burst swim at speeds approaching 20 FL/s (Walters and Fierstine, 1964; Magnuson, 1978), however, studies of yellowfin tuna swimming in the water tunnel (Dewar and Graham, 1994a, b) showed that only a few fish could sustain a velocity of 3 FL/s for 40 to 60 min. In fact, published values of sustained swimming speeds of tuna tracked in the wild or held in captivity are generally less than 3 FL/s (Yuen, 1970; Dizon, 1977; Dizon *et al.*, 1978; Magnuson, 1978; Gooding *et al.*, 1981; Carey and Olson, 1982;

Jones *et al.*, 1986; Bushnell, 1988; Holland *et al.*, 1990; Boggs and Kitchell, 1991; Bushnell and Brill, 1991; Jones *et al.*, 1993).

Tuna metabolic rate and swimming speed

Gooding *et al.* (1981) measured a maximum $\dot{V}_{O_2}$ of 29 ml O_2/kg/min in skipjack just after capture. The model developed by Bushnell and Brill (1991) predicted a maximum aerobic swimming metabolism of 31.5 ml O_2/kg/min for skipjack and 29 ml O_2/kg/min for yellowfin. The maximum $\dot{V}_{O_2}$ of tunas and most fishes, is generally assumed to correspond to their maximum sustained swimming velocity (Goolish, 1991) and the maximum sustained swimming speeds of tuna have been estimated from relationships between $\dot{V}_{O_2}$ and velocity (Dizon, 1977; Bushnell and Brill, 1991). In the case of yellowfin, this extrapolation indicates a maximum aerobic swimming velocity of 4.6 FL/s at the maximum $\dot{V}_{O_2}$ of 29 ml O_2/kg/min (Fig. 1).

However, from the results of the model, it seems unlikely that a yellowfin could actually sustain a rate of 4.6 FL/s. As shown in Fig. 2, $\dot{V}_{O_2}$RM at this speed would be about 0.33 ml O_2/g/min, which is 66% higher than the maximum $\dot{V}_{O_2}$RM predicted from cardiovascular O_2 delivery and 180% higher than the prediction from mitochondrial $\dot{V}_{O_2}$ (Table 2). Red muscle only accounts for a small fraction of the entire mass of a yellowfin (6.4% or 134 g in a 2.1 kg yellowfin, Eq. 6; Graham *et al.*, 1983), yet our calculations indicate that the total $\dot{V}_{O_2}$ of this tissue at 4.6 FL/s would be 20.9 ml O_2/kg/min, or 72% of the total $\dot{V}_{O_2}$ of the fish. Our findings therefore suggest that neither the intrinsic metabolic capacity of yellowfin red muscle nor its capacity to deliver oxygen to its red muscle are sufficient to allow aerobic swimming at this velocity. The relatively high aerobic potential of tuna white muscle (compared to the white muscle of other fishes) has led to the suggestion that it may be recruited during sustainable aerobic swimming (Guppy and Hochachka, 1978; Hulbert *et al.*, 1979). If this is the case, then speeds above those

predicted here could be sustained. However, tuna white muscle aerobic capacity is much less than that of the red muscle and its metabolism is predominately anaerobic (Hulbert *et al.*, 1979; Moyes *et al.*, 1992a).

Effects of environment on O_2 delivery

Hypoxia

The cardiovascular model shows that yellowfin tuna exposed to an ambient P_{O_2} of 10 kPa (25°C) would be subject to a 50% decrease in maximum red muscle O_2 delivery and a corresponding reduction in maximum sustainable speed to 2.6 FL/s (Fig. 3). Bushnell and Brill (1991) predicted that a P_{O_2} of 11.3 kPa would limit yellowfin maximum speed to only 1.4 FL/s. Skipjack and yellowfin both respond behaviorally and physiologically to moderate hypoxia between 17.3 and 12.0 kPa O_2 (Dizon, 1977; Gooding *et al.*, 1981; Bushnell *et al.*, 1990; Bushnell and Brill, 1991; Bushnell and Brill, 1992). However, Dizon (1977) found no behavioral response in yellowfin exposed to 7.3 kPa O_2, and found that they could survive severe hypoxia (4.0 kPa) for greater than 200 min.

Bushnell and Brill (1992), determined that a P_{O_2} of 12.0 kPa limited O_2 delivery in spinally-blocked skipjack, while for yellowfin, a P_{O_2} of 6.7 kPa was limiting. The reduction in O_2 delivery is caused by a hypoxia induced bradycardia which lowers cardiac output (Fig. 4). Experiments by Graham *et al.* (1989) also showed that the swimming $\dot{V}_{O_2}$ of albacore (0.5-1.4 FL/s) exposed to moderate hypoxia (between 6.7 and 13.2 kPa O_2, 15°C) was lower than rates in normoxia suggesting an inability to maintain O_2 delivery within this range.

Tunas are ram ventilators and may depend on increasing swimming speed in hypoxia in order to increase O_2 supply. An increased swimming speed has been seen in both skipjack and yellowfin in response to hypoxia (Dizon, 1977; Gooding *et al.*, 1981; Bushnell and Brill, 1991). While increases in velocity are relatively less costly to tunas

than other fish (Dewar and Graham, 1994a), they do nevertheless require additional O_2 and it is thus a somewhat paradoxical response to hypoxia. It may be that the velocity increase signifies the "behavioral avoidance" of hypoxia rather than compensation for these conditions (Dizon, 1977; Bushnell and Brill, 1991).

As seen in Fig. 4, we found yellowfin red muscle excess temperatures to increase during exposure to hypoxia, despite a constant swimming speed. The decreased cardiac output in response to hypoxia may increase efficiency of the heat exchanger by increasing blood residence time in the *retial* vessels. The increased metabolic heat retention during hypoxia would be advantageous since hypoxia may be encountered along with a decrease in ambient temperature. However, it will also serve to increase metabolic demand by the warmed muscles at a time when O_2 uptake may be limiting.

Temperature

One of the most distinguishing features of the tunas is their ability to maintain elevated red muscle temperatures. Vascular specializations (*retia mirabilia*) conserve metabolically produced heat in the red muscle and other areas (Linthicum and Carey, 1972; Collette, 1978; Carey *et al.*, 1984). Depending upon ambient temperature and activity level, the excess muscle temperatures of tunas can range from only a few degrees to 20° C above ambient (Carey and Teal, 1969; Graham, 1973; Dewar *et al.*, 1994). Recent work establishes that yellowfin and bigeye (*Thunnus obesus*) tunas can alter thermal conductance to increase thermal inertia in cool water and to facilitate warming when returning to higher temperatures (Holland *et al.*, 1992; Dewar *et al.*, 1994; Brill *et al.*, 1994). Yellowfin can also alter thermal conductance in response to increased activity (and thus heat production) in relation to environmental temperature, presumably to alleviate potential "overheating" in warm waters (Dewar *et al.*, 1994; Brill *et al.*, 1994). The biological advantages of endothermy for tunas likely include elevated metabolic rates, faster and more efficient

muscle contraction and thus swimming speed, enhanced oxygen and metabolite diffusion through tissues, and greater independence from ambient temperature changes (Carey *et al.*, 1971; Graham, 1975; Dizon and Brill, 1979; Stevens and Carey, 1981; Stevens, 1982; Block, 1991). The latter seems especially important because it would minimize thermal effects on vital physiological and metabolic processes (respiration, muscle activity, sensory acuity) during rapid excursions of tunas through a range of ambient temperatures (Dewar *et al.*, 1994).

In terms of O_2 delivery to red muscle, however, it is important to emphasize that only certain regions of the tuna body are insulated from ambient temperature by the action of heat-exchanging *retia*. The heart, for example, is near the body surface and not within the heat-conserving *retial* loop. Thus, the heart must operate at ambient temperature, and is subjected to rapid fluctuations during the vertical movements of tunas. Our model indicates that the decline in yellowfin cardiac output associated with a reduction in ambient temperature from 25 to 15°C, would lower maximum O_2 delivery to the red muscle by 44% and lower maximum sustainable swimming speed to 2.8 FL/s (Fig. 3). This agrees with the finding that swimming speed of yellowfin decreases with decreasing temperature (Dizon *et al.*, 1977). While cardiac output decreases directly with ambient temperature, red muscle temperatures will remain elevated and excess temperature will be greater (Dewar *et al.* 1994). This means that red muscle oxygen demand would remain high at a time when oxygen delivery is reduced by ambient temperature effects on cardiac output. This disparity between O_2 delivery and demand in the red muscle may limit performance in the cooler waters encountered during vertical migration.

Summary

Estimates of yellowfin red muscle $\dot{V}_{O_2}$ at slow swimming speeds obtained with our cardiovascular model are consistent with previous estimates for tuna red muscle.

Whereas this model's estimate of maximum O_2 delivery exceeds all previous estimates of maximum $\dot{V}_{O_2}RM$, the maximum sustainable velocity predicted by this model is less than previously suggested. Our results indicate that, because of the rapid increases in the cost of locomotion at higher velocities, O_2 delivery and the metabolic capacity of red muscle are insufficient to support aerobic swimming velocities above about 3.7 FL/s.

Our model also predicts that both moderate hypoxia (P_{O_2} = 10 kPa) and cool temperatures (15°C) will limit oxygen delivery to the red musculature and thus reduce maximum sustained velocity. However, sufficient O_2 delivery will be available for moderate swimming velocities under these conditions. In areas such as the eastern tropical Pacific, hypoxia is likely to be encountered along with a decrease in temperature and the combined effects on metabolic capacity will probably be more severe.

This chapter, in full, is a reprint of the material as it appears in Comparative Biochemistry and Physiology, Vol. 113B, No. 1, Korsmeyer, K. E., Dewar, H., Lai, N. C., and Graham, J. B., Elsevier Science, Inc., 1996. The dissertation author was the primary investigator and author of this paper.

Table II-1: Model of O_2 delivery to red muscle

A: Slow Swimming

Variables:		Calculated:	
Total mass (kg):	2.1	Red muscle mass (g):	134
Blood carrying capacity (ml O_2/ml):	0.18*	Arterial O_2 content (ml O_2/ml):	0.144
Arterial % saturation:	80*	Venous O_2 content (ml O_2/ml):	0.090
Venous % saturation:	50*	Red muscle $(a-v)_{O_2}$ (ml O_2/ml):	0.054
Cardiac output (ml/kg/min):	54*	Red muscle blood flow (ml/g/min):	0.305
% Cardiac output to red muscle:	36‡	Red muscle $\dot{V}_{O_2}$ (ml O_2/g/min):	0.016
SMR (ml O_2/kg/min):	3.00*	Swimming $\dot{V}_{O_2}$ (ml O_2/kg/min):	4.24
		Swimming velocity (FL/s):	0.74

B: Maximum O_2 Delivery

Variables:		Calculated:	
Total mass (kg):	2.1	Red muscle mass (g):	134
Blood carrying capacity (ml O_2/ml):	0.18*	Arterial O_2 content (ml O_2/ml):	0.180
Arterial % saturation:	100†	Venous O_2 content (ml O_2/ml):	0.018
Venous % saturation:	10†	Red muscle $(a-v)_{O_2}$ (ml O_2/ml):	0.164
Cardiac output (ml/kg/min):	216†	Red muscle blood flow (ml/g/min):	1.218
% Cardiac output to red muscle:	36‡	Red muscle $\dot{V}_{O_2}$ (ml O_2/g/min):	0.197
SMR (ml O_2/kg/min):	3.00*	Swimming $\dot{V}_{O_2}$ (ml O_2/kg/min):	18.68
		Swimming velocity (FL/s):	3.72

*from actual measurements, †estimated, ‡from anesthetized albacore (see text).

Table II-2. Tuna cardiovascular variables

Species	f_H bpm	SV ml/kg	$\dot{Q}$ ml/kg/min	T	Notes	References
Skipjack	46 - 168	--	50 - 80	25°C	anesthetized	Stevens, 1972
	80 - 240	--	--	25°C	free-swimming, telemetry	Kanwisher *et al.*, 1974
	230	--	--	25°C	paralyzed	Brill, 1987
	76.8	--	--	25°C	swimming, 1.6 FL/s	Bushnell and Brill, 1991
	125.9	1.1	132.3	25°C	spinally-blocked	Bushnell and Brill, 1992
	154	0.68	84.5	25°C	*in vitro* perfused heart, maximum	Farrell *et al.*, 1992
Kawakawa	206	--	--	25°C	paralyzed	Brill, 1987
	128	0.54	67	25°C	swimming, 1.3 FL/s	Jones *et al.*, 1993
Yellowfin	132	--	--	25°C	paralyzed	Brill, 1987
	67.9	--	--	25°C	swimming, 1.2 FL/s	Bushnell and Brill, 1991
	96.7	1.3	115.4	25°C	spinally-blocked	Bushnell and Brill, 1992
	137.6	0.87	108.0	25°C	*in vitro* perfused heart, maximum	Farrell *et al.*, 1992
	86 - 139	0.43 - 0.95	53.8 - 95.0	25°C	anesthetized	Jones *et al.*, 1993
	102 - 130	--	--	25°C	swimming	Jones *et al.*, 1993
	62 - 98	--	--	25°C	swimming, 1.0-2.2 FL/s	Korsmeyer *et al.*, 1993

f_H = heart rate, SV = stroke volume, $\dot{Q}$ = cardiac output, T = ambient temperature. Kawakawa (*Euthynnus affinis*).

Table II-3. Estimates of muscle oxygen consumption ($\dot{V}_{O_2}$)

Species	$\dot{V}_{O_2}$ ml O_2/g/min	T_{RM}	Notes	Method	References
Skipjack tuna	0.025	25°C		*in vitro* tissue $\dot{V}_{O_2}$	Gordon, 1968
(red muscle)	0.012 - 0.016	25.5 - 26.5°C	slow swimming	calorimetry	Neill *et al.*, 1976
	0.082	27 - 30°C	maximum	calorimetry	Neill *et al.*, 1976
	0.058	25°C	maximum	mitochondrial $\dot{V}_{O_2}$	Moyes *et al.*, 1992a
	(0.116)	(35°C)	(assuming Q_{10}=2)		
Yellowfin tuna (red muscle)	0.016	> 25°C	slow swimming, 0.74 FL/s	see text	this study
	0.197	> 25°C	maximum, 3.7 FL/s	see text	this study
Carp (red muscle)	0.017	15°C	maximum	mitochondrial $\dot{V}_{O_2}$	Moyes *et al.*, 1992a
Trout (red muscle)	0.065	10°C	80% maximum sustained swimming	calculated O_2 uptake *in vivo*	Randall and Daxboeck, 1982
Cat (soleus)	0.31	37°C	maximum	mitochondrial $\dot{V}_{O_2}$	Schwerzmann *et al.*, 1989
Hummingbird (pectoralis)	2.1	40°C	during hovering flight	calculated O_2 uptake *in vivo*	Suarez *et al.*, 1991

T_{RM} is red muscle temperature. Carp (*Cyprinus carpio*), Trout (*Oncorhynchus mykiss*), Cat (*Felis domesticus*), hummingbird (*Selasphorus rufus*).

Figure II-1

Components of red-muscle $\dot{V}_{O_2}$ model based on the $\dot{V}_{O_2}$ - velocity relationship for 2.1 kg (50 cm) yellowfin tuna. See text for description.

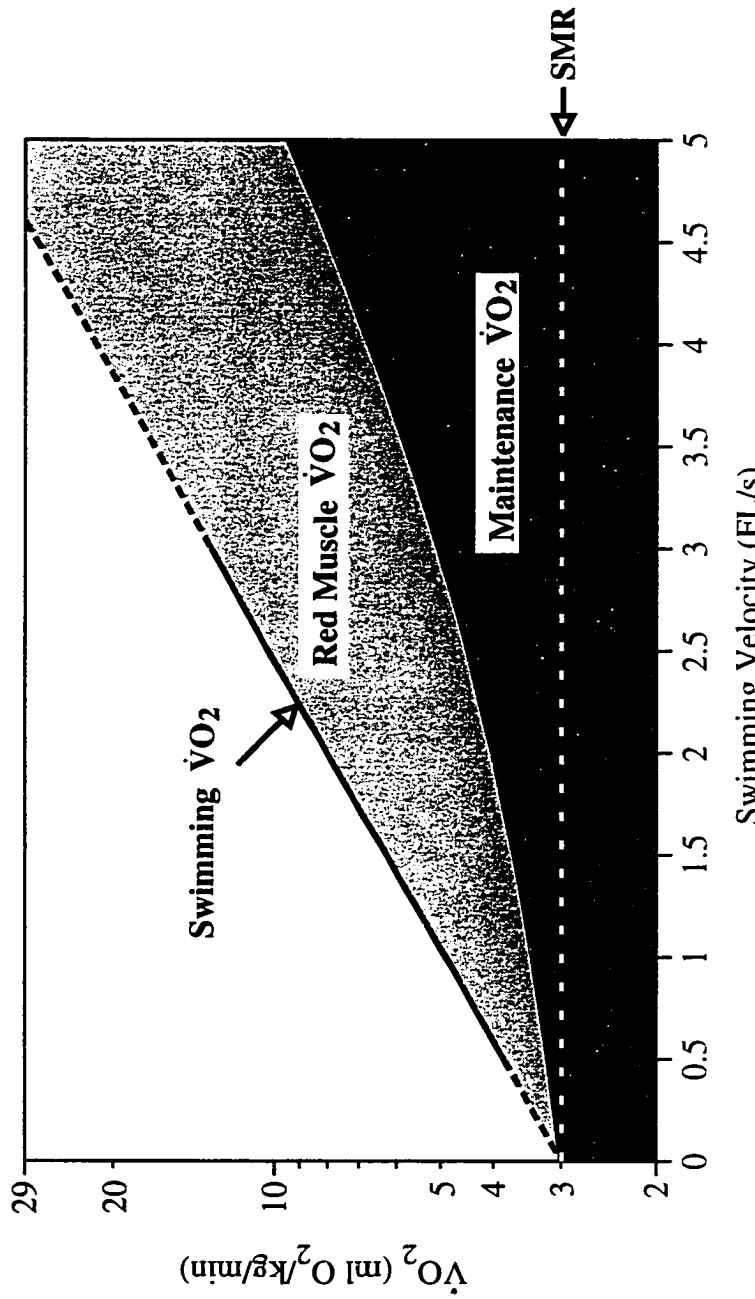


Figure II-2

Predicted red-muscle oxygen demand at sustainable swimming velocities from model depicted in Fig. 1. Estimates of red muscle $\dot{V}_{O_2}$ are plotted to show corresponding swimming velocity. A: red muscle $\dot{V}_{O_2}$ at slow swimming, B: maximum $\dot{V}_{O_2}$ predicted from Moyes *et al.* (1992a) (Table 2), C: predicted maximum O_2 delivery, D: red muscle $\dot{V}_{O_2}$ required for sustained swimming at estimated maximum total $\dot{V}_{O_2}$ of 29 ml O_2/kg/min. Dashed lines indicate predicted red muscle oxygen demand with changes in the assumed increase in maintanence costs ($\dot{V}_{O_2\,\mathrm{maint}}$) with exercise (eq. 1). Solid line for 20% of locomotory costs, upper dashed line for 10%, and lower dashed line, 30%.

Figure II-3

Predicted red mucle $\dot{V}_{O_2}$'s at slow swimming and at maximum O_2 delivery rates in relation to the model (Fig. 1). Maximum O_2 delivery rates shown for normoxia (25°C), hypoxia (10 kPa O_2), and decreased temperature (15°C).

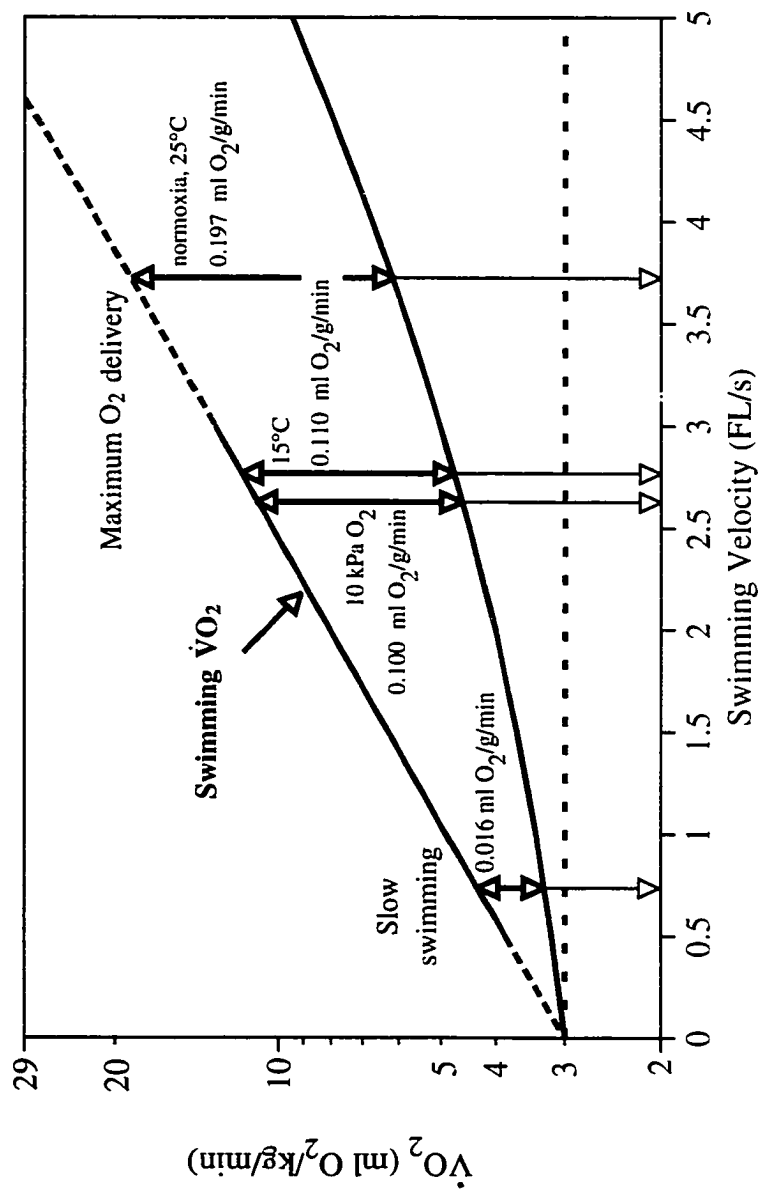
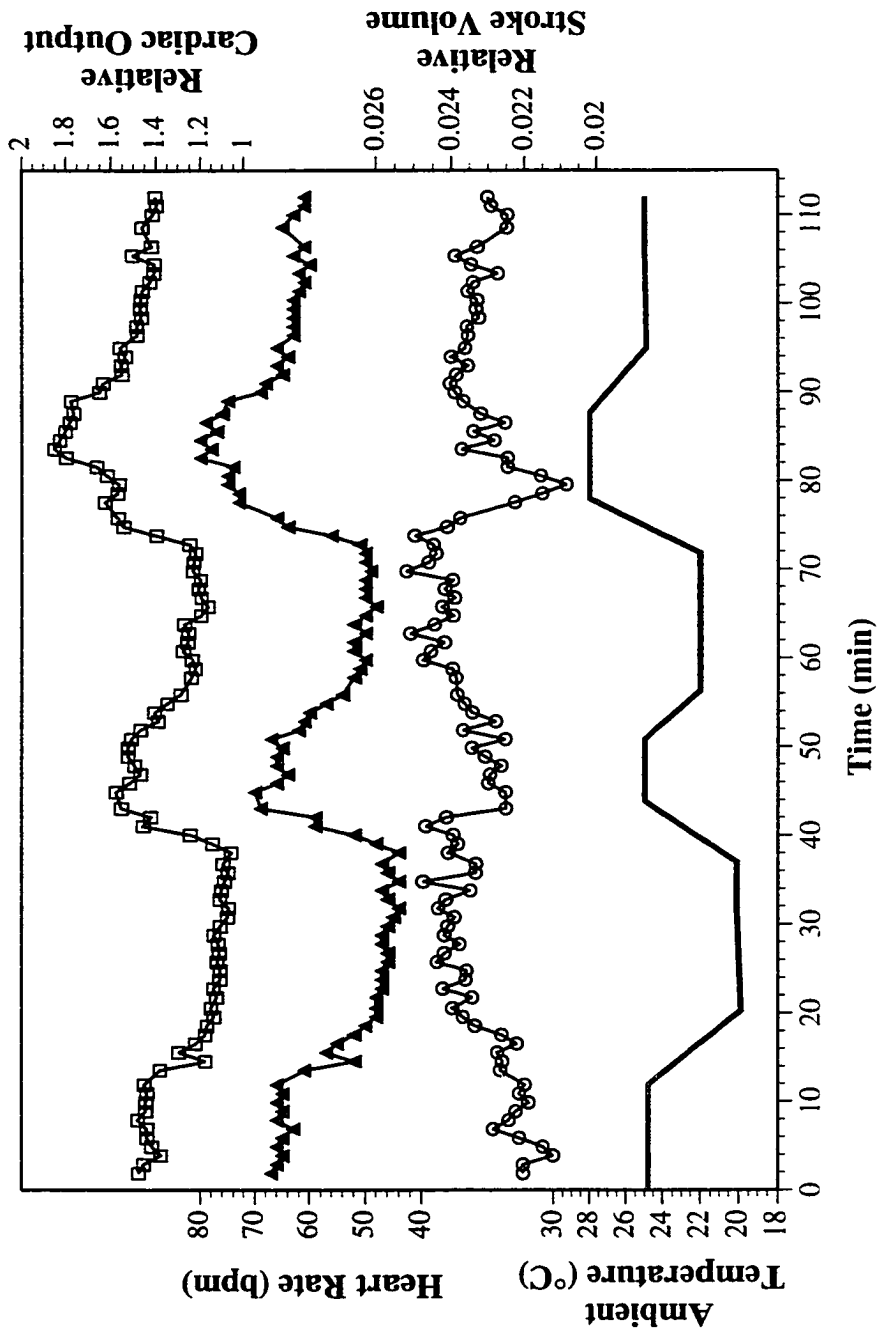


Figure II-4

The effects of water oxygen tension (P_{O_2}, —) on cardiac output (o) and excess red-muscle temperature (▲, T_X = muscle temperature - ambient temperature) for a yellowfin tuna (1805 g, 48.5 cm) swimming 1.6 FL/s at 25°C.

Figure II-5

The effects of changing ambient temperature (—) on cardiac output (□), heart rate (▲), and stroke volume (o) for a yellowfin (1430 g, 43.5 cm) swimming 1.2 FL/s.



CHAPTER III: THE AEROBIC CAPACITY OF TUNAS: ADAPTATION FOR MULTIPLE METABOLIC DEMANDS

Abstract

Tunas are pelagic, continuous swimmers, with numerous specializations for achieving a high aerobic scope. Tunas must maintain a high rate of energy turnover, and therefore require elevated levels of aerobic performance in multiple physiological functions simultaneously. Based on a model of oxygen demand and delivery to the swimming musculature, the yellowfin's total oxygen consumption at the predicted maximum sustainable (aerobic) swimming velocity is well below estimates of its maximum oxygen consumption. This suggests that the high aerobic scope of tunas may be a specialization that permits continuous swimming in addition to supplying oxygen to other metabolic functions. Estimates of the metabolic costs of oxygen-debt repayment, growth, and specific dynamic action have been combined with this model of aerobic swimming performance to evaluate the total energy budget in relation to the aerobic scope of the yellowfin tuna. Repayment of the oxygen debt incurred during burst swimming is potentially a large component of tuna respiratory metabolism and the relatively high aerobic capacity of tuna white muscle may be a specialization for rapid lactate clearance.

Introduction

Tunas have been succinctly described as "energy speculators" based on their high rates of energy turnover in a nutrient poor pelagic environment (Stevens and Neill, 1978). As an adaptive mechanism, energy speculation can be defined as the gambling of high rates of energy expenditure on potentially high rates of energy return (Stevens and Neill, 1978; Stevens and Dizon, 1982; Brill, 1987). In a tuna's pelagic environment, prey are patchily distributed and feeding success depends upon the ability to find, capture, and process food items as rapidly as possible. This high rate of consumption, in turn, supports the high metabolic investment in continuous swimming and other energy consuming processes associated with these activities. The search for prey extends from the surface to considerable depth and thus can cross significant thermal and oxygen gradients which may limit oxygen uptake (Dizon *et al.*, 1978; Sund *et al.*, 1981; Carey and Olson, 1982; Holland *et al.*, 1990, 1992, Korsmeyer *et al.*, 1996b). Feeding in schools on aggregations of prey may also result in burst (anaerobic) swimming during a "feeding frenzy." After feeding the tuna must be able to repay any oxygen debt incurred during prey capture and rapidly complete the processes of digestion and assimilation in order to prepare the gut for the next prey encounter. These tasks must be conducted while the tuna also sustains a level of swimming sufficient for hydrostatic equilibrium, gill ventilation, and an optimal prey search. Whereas the aerobic capacity of most fishes would be overtaxed by the simultaneous requirements for swimming, digestion, and recovery from an oxygen debt (Priede, 1985), this may be a normal occurrence for tunas. The high aerobic capacity of tunas may have therefore evolved to meet the simultaneous demands of the multiple physiological functions needed to sustain the high performance requirements of energy speculation.

Previous work in this laboratory has focused on measurements of the rate of oxygen consumption ($\dot{M}_{O_2}$) and cardiovascular functions of tunas swimming in a water

tunnel at controlled velocities, temperature, and oxygen levels (Korsmeyer *et al.*, 1992, 1993, 1994; Dewar and Graham, 1994a; Graham *et al.*, 1994). Synthesis of this information with other work on tunas has yielded a model relating the oxygen requirements of yellowfin tuna (*Thunnus albacares*) red (aerobic) muscle during swimming to the oxygen delivery potential of its cardio-respiratory system (Korsmeyer *et al.*; 1996b). Based on this model, estimates of the aerobic swimming performance of a 50 cm (2.1 kg) yellowfin indicate that both maximum sustainable swimming speed and maximum swimming $\dot{M}_{O_2}$ which are below the maximum performance and $\dot{M}_{O_2}$ predicted for tuna of this body size (Fig. 1). This finding calls into question the supposition that the high oxygen uptake capabilities of tuna permit exceptionally high sustainable swimming speeds (Stevens and Carey, 1981). The purpose of this paper is to expand our model of tuna aerobic capacity by considering the aerobic energy requirements of the several additional metabolic processes necessary for sustained high performance in the pelagic environment.

Components Of Aerobic Metabolism

As reviewed by Priede (1985), the respiratory or aerobic metabolism of an animal can be described in terms of its component parts:

$$R = R_S + R_F + R_A \qquad (1)$$

where R is the total aerobic metabolic rate, R_S is the standard metabolic rate, R_F is the metabolic rate attributable to the activities of digestion and assimilation, also termed specific dynamic action (SDA), and R_A is metabolism due to aerobic locomotor activity. To describe tuna aerobic metabolism, eq. 1 is expanded to include terms for recovery from oxygen debt (R_D) and growth and reproduction ($R_{G\&R}$):

$$R = R_S + R_F + R_A + R_D + R_{G\&R}. \quad (2)$$

The following sections will discuss what is known about each of these metabolic components in tuna and, by attempting an estimate of the magnitude of each parameter, determine the relative importance of each to tuna aerobic metabolism. This information is summarized in Table 1.

Total Aerobic Capacity (R)

Recent work has documented the elevated metabolic rate of tunas (Fig. 2). Even when ambient and body temperature differences are taken into account, tunas consume oxygen at 2 to 5 times the rate of other active teleosts, such as salmonids (Gooding *et al.*, 1981; Graham and Laurs, 1982; Boggs and Kitchell, 1991; Brill and Bushnell, 1991b; Dewar and Graham, 1994a).

A high maximum $\dot{M}_{O_2}$ is suggested by the tuna's many cardio-respiratory adaptations for oxygen uptake and delivery (reviewed by Brill and Bushnell, 1991b and Bushnell and Jones, 1994). For skipjack tuna (*Katsuwonus pelamis*), Gooding *et al.* (1981) measured a maximum $\dot{M}_{O_2}$ of 2500 mg O_2/kg/h (1.8 to 2.2 kg, 24°C) immediately after capture, and Dewar and Graham (1994a) measured $\dot{M}_{O_2}$'s up to 2200 mg O_2/kg/h (1.7 ± 0.26 kg, 24°C). Similarly, the oxygen uptake model developed by Bushnell and Brill (1991) predicts a maximum aerobic metabolism of 2700 mg O_2/kg/h for skipjack and 2500 mg O_2/kg/h for yellowfin tuna (1.4 kg, 24°C). These rates are almost 3 times the maximum $\dot{M}_{O_2}$ of similarly sized sockeye salmon (*Oncorhynchus nerka*) at 20°C (Brett and Glass, 1973).

Standard Metabolic Rate (R_S)

R_S is defined as the metabolic rate of an unstressed, post-absorptive ectotherm at rest and should therefore reflect the minimum aerobic energy expenditure required for maintenance. R_S is generally higher in fishes with high metabolic capacities and is presumed to reflect the energetic cost associated with maintaining the metabolic machinery necessary for a high aerobic capacity (Priede, 1985; Brill, 1987). For example, the large surface area and thin epithelium of tuna gills (Muir and Hughes, 1969; Hughes, 1984), while aiding gas exchange, results in a greater potential for ion and water exchange and thus raises osmoregulatory costs. The proportion of total $\dot{M}_{O_2}$ used directly by the gills in spinally blocked (i.e., non-swimming) skipjack and yellowfin is very high (68% and 54% respectively; Bushnell and Brill, 1992) relative to that of rainbow trout (*Oncorhynchus mykiss*, 27% of R_S; Daxboeck *et al.*, 1982). Tuna cardiac power outputs relative to body mass can approach 10 times the maximum value for rainbow trout (Farrell *et al.*, 1992) due to the tuna's high cardiac output, high blood pressure, and a larger heart size relative to other teleosts (Lai *et al.*, 1987; White *et al.*, 1988; Bushnell and Brill, 1992; Farrell *et al.*, 1992). This high cardiac performance will entail higher relative costs for cardiac function. In addition, the high mitochondrial concentrations found in tuna muscle (Moyes *et al.*, 1992a), will add to the elevated maintenance costs.

Tuna R_S is difficult to quantify because these fish swim continuously and their body temperature can vary regionally and in relation to ambient temperature (Dewar *et al.*, 1994). Yellowfin R_S (Fig. 1) was estimated by extrapolating the $\dot{M}_{O_2}$-velocity relationships of swimming fish to zero velocity (Dewar and Graham, 1994a). The result (257 mg O_2/kg/h for a 2 kg fish at 24°C) is similar to the "stasis" $\dot{M}_{O_2}$ (213 mg O_2/kg/h) measured for immobilized yellowfin (Brill, 1987). Whereas these values are approximately 10% of the estimated maximum $\dot{M}_{O_2}$ of yellowfin, they are considerably higher than other teleosts (Brill, 1987).

Specific Dynamic Action (R_F)

A certain percentage of ingested energy is lost due to the costs of the mechanical and biochemical processes associated with digestion and assimilation, and this is defined as specific dynamic action (R_F)(Jobling, 1981). R_F in fishes accounts for about 15% of ingested energy and can be measured by the increase in $\dot{M}_{O_2}$ following feeding (Brett and Groves, 1979; Jobling, 1981). In some fishes the $\dot{M}_{O_2}$ due to R_F can equal the maximum active $\dot{M}_{O_2}$ (Beamish, 1974; Priede, 1985).

Although no direct measurements have been made for tunas, R_F is likely to be a large component of aerobic metabolism because these fishes have both high rates of consumption and digestion. Tunas can consume up to 30% of their body mass per day (Kitchell *et al.*, 1978). The daily ration for skipjack in captivity was about 15% of body mass (Magnuson, 1969). Olson and Boggs (1986) estimated a daily ration of 4 to 7% for yellowfin in the wild.

Digestion rates are also high in tunas. Based on post-feeding stomach contents, captive skipjack and yellowfin (24°C) were found to empty their gut in an average of 12 and 10.4 h, respectively (Magnuson, 1969; Olson and Boggs, 1986). This rate of gut clearance is 4 to 5 times faster than for other piscivores of comparable size (Magnuson, 1969). In addition, some species of tunas have the ability to elevate visceral temperature with vascular retia (Collette, 1978; Carey *et al.*, 1984), presumably to aid the rapid digestion and assimilation of food. Stevens and McLeese (1984) found that the elevated gut temperatures of bluefin tuna (*Thunnus thynnus*) could increase the activity of digestive peptidases by three-fold. The ability to consume large amounts and rapidly clear the gut enables tunas to take advantage of encounters with prey aggregations that are patchily distributed, and still meet their high metabolic demands (Olson and Boggs, 1986).

The $\dot{M}_{O_2}$ due to R_F in tuna can be estimated from the caloric intake during feeding. Average meal size for captive skipjack and yellowfin is about 8.6% of body weight (Magnuson, 1969; Olson and Boggs, 1986). Using the mean caloric density of tuna prey determined by Olson (1982; 4995 J/g), a 2.1 kg tuna would consume 902,000 J/meal. Assuming 15% of this energy is lost as R_F, and an oxycalorific equivalent of 13.6 J/mg O_2, then R_F would be 9950 mg O_2/meal. If digestion and assimilation are completed in 12 h, then the average increase in $\dot{M}_{O_2}$ would be 829 mg O_2/h or 395 mg O_2/kg body mass/h (Table 1). This value must be considered a first approximation and it probably underestimates maximum R_F because $\dot{M}_{O_2}$ following feeding in fishes is not constant but increases to a maximum and then declines over many hours (Beamish, 1974; Jobling, 1981).

Growth and Reproduction ($R_{G\&R}$)

Tunas have relatively high rates of growth (Brill, 1996) and the energy required for this can be estimated as the caloric content of the biomass gain per time. A 2.1 kg yellowfin adds about 14 g of mass per day; a relative growth rate of 0.66% (Wild, 1986). Relative growth rates are higher in younger tuna and decrease with age. The average caloric density of yellowfin is 6030 J/g (Boggs and Kitchell, 1991), therefore 84,420 J are added per day, or 40,200 J/kg body mass/day. Assuming growth is constant, this rate is 1675 J/kg/h or 123 mg O_2/kg/h (Table 1).

This estimate will be low because of the many energy requiring steps between ingestion of prey and the consequential conversion of the assimilated materials into tuna biomass (Sharp and Francis, 1976). On the other hand, some of this added cost is reflected in the R_F, a large part of which results from the synthesis of protein from absorbed amino acids (Jobling, 1981; Brown and Cameron, 1991).

Yellowfin do not attain sexual maturity until over 50 cm in length, and may not mature until over 120 cm (Wild, 1994). Therefore, we have not included reproductive costs for a 2.1 kg, 50 cm fish. However, sexually mature female tunas devote considerable energy into egg production. Skipjack produce about 100,000 eggs per kilogram per spawning (equal to approx. 2% of body mass) and at times may spawn on a near daily basis, producing tens of millions of eggs annually (Forsbergh, 1980; Hunter *et al.*, 1986).

Aerobic Locomotion (R_A)

Tunas must swim continuously for both ram gill ventilation and, because they are negatively buoyant, to provide the hydrodynamic lift required to maintain stability and position in the water column (Roberts, 1978; Magnuson, 1978). Thus, the metabolic cost associated with sustained swimming, although varying with velocity, is a permanent feature of tuna aerobic metabolism.

Oxygen consumption has been measured for yellowfin tuna (50 cm) swimming from 0.5 to 3.0 fork lengths per second (FL/s) (Dewar and Graham, 1994a). As shown in Fig. 2, tuna $\dot{M}_{O_2}$ is elevated compared to other teleosts. However, the rate of increase with swimming velocity is less, suggesting an increased swimming efficiency, a conclusion that is consistent with features of tuna morphology, including streamlining, thunniform swimming mode, and elevated muscle temperatures (Dewar and Graham, 1994a, b). A low increase in aerobic costs with swimming speed has also been shown for albacore (*Thunnus alalunga*; Graham *et al.*, 1989) and skipjack (Gooding *et al.*, 1981).

The lower slope of the log($\dot{M}_{O_2}$)-velocity relationship would mean that swimming costs at higher velocities (i.e., achieved during burst swimming) may be less than in other fish and, along with morphological and physiological adaptations for enhanced oxygen uptake, implies that tunas are capable of sustaining extraordinarily high swimming speeds.

Sustained swimming is defined as that which can be maintained for long periods (> 200 min) without resulting in fatigue (Beamish, 1978) and is thus a function of the red, aerobic, musculature (Rayner and Keenan, 1967; Jones and Randall, 1978). Tuna red muscle, relative to other fishes, has a high capillarity (Mathieu-Costello *et al.*, 1992), high mitochondrial protein concentration (Moyes *et al.*, 1992a), and a high myoglobin content (George and Stevens, 1978). These features, combined with the ability to maintain elevated muscle temperatures (Carey *et al.*, 1971), suggest a high aerobic potential and metabolic performance and agrees with the general conception that tunas are capable of high sustainable swimming speeds.

Although tunas are often cited as capable of sustaining velocities of 6 to 10 FL/s (Yuen, 1970; Beamish, 1978; Dizon *et al.*, 1978), no direct measurements of maximum sustainable swimming speed have been made on them, and yellowfin swimming in a water tunnel could only sustain velocities of up to 3 FL/s for 40 to 60 min (Dewar and Graham, 1994a, b). In addition, sustained swimming speeds of tuna tracked in the wild or held in captivity are generally less than 3 FL/s (Yuen, 1970; Dizon, 1977; Dizon *et al.*, 1978; Magnuson, 1978; Gooding *et al.*, 1981; Carey and Olson, 1982; Jones *et al.*, 1986; Bushnell, 1988; Wardle *et al.*, 1989; Holland *et al.*, 1990; Boggs and Kitchell, 1991; Bushnell and Brill, 1991; Jones *et al.*, 1993). Thus, while we would not expect tuna to constantly swim at their maximum sustainable swimming speeds, there is little direct evidence for the excessively high cruising speeds often attributed to tuna.

Several investigations now suggest that the high aerobic capacity of tuna does not necessarily translate into extraordinary sustainable swimming speeds. Based on a model of oxygen uptake, Bushnell and Brill (1991) predicted maximum sustained swimming speeds of 5.6 and 3.5 FL/s for 40 cm yellowfin and skipjack tuna, respectively (but see Brill, 1996). A recently developed model for yellowfin (Korsmeyer *et al.*, 1996b) predicts a maximum aerobic swimming velocity between 3 and 4 FL/s (length = 50 cm, Fig. 1),

which is similar to or slightly higher than other comparably sized teleosts (Beamish, 1978; Videler, 1993). This model takes into account the effects of swimming velocity on total $\dot{M}_{O_2}$ and cardiorespiratory function to predict red-muscle oxygen demand and maximum aerobic swimming performance. Maximum delivery of oxygen to red muscle is predicted to be 0.28 mg O_2/g red muscle/min (Korsmeyer *et al.*, 1996b). For a 2.1 kg yellowfin (which has 134 g of red muscle, Graham *et al.*, 1983), this maximum red muscle $\dot{M}_{O_2}$ is 1075 mg O_2/kg/h (Table 1). Added to this are the increased maintenance costs associated with exercise (i.e., increased osmoregulatory and cardiac costs, assumed to be 20% of locomotory costs or 269 mg O_2/kg/h; Korsmeyer *et al.*, 1996b) and these raise maximum aerobic swimming costs (R_A) to 1344 mg O_2/kg/h (Table 1). When this maximum R_A is added to R_S, the total metabolic costs for swimming is 1601 mg O_2/kg/h. It can be seen from Fig. 1 that this $\dot{M}_{O_2}$ corresponds to a velocity of 3.7 FL/s.

Oxygen Debt Recovery (R_D)

Tuna white muscle is predominantly anaerobic and fueled by glycogen (Hulbert *et al.*, 1979). Compared to the white muscle of other fishes, however, it also has a relatively high aerobic capacity, as reflected in a high intracellular lipid content, mitochondrial abundance, and capillarity (Hulbert *et al.*, 1979), and high concentrations of aerobic enzymes (Guppy and Hochachka, 1978; Moyes *et al.*, 1992a).

The aerobic potential of tuna white muscle has led to the suggestion that it has a role in sustained aerobic swimming (Guppy and Hochachka, 1978; Hulbert *et al.*, 1979; Brill and Dizon, 1979b; Bushnell and Jones, 1994). If this is the case, a tuna could sustain swimming speeds above the predicted range of 3 to 4 FL/s. Brill and Dizon (1979b) found white muscle recruitment in skipjack to occur at around 4 FL/s, however, these velocities were only maintained for a short time (< 3 min) and, as was indicated above, both

laboratory and field measurements of swimming velocity suggest that sustainable speeds of tunas are within the range of red muscle activity alone.

The high anaerobic potential of tuna white muscle, supported by a high lactate dehydrogenase (LDH) activity and buffering capacity (Guppy and Hochachka, 1978; Dickson and Somero, 1987) allow extremely high, short-duration, "burst" swimming speeds of 10 to 20 FL/s (Walters and Fierstine, 1964; Magnuson, 1978). This anaerobic activity produces large amounts of white muscle lactate (over 100 μmol/g; Guppy *et al.*, 1979; Arthur *et al.*, 1992), most of which is resynthesized to glycogen *in situ* (Batty and Wardle, 1979; Weber *et al.*, 1986; Milligan and McDonald, 1988; Arthur *et al.*, 1992; Buck *et al.*, 1992; Moyes *et al.*, 1992b; Schulte *et al.*, 1992), a process that requires ATP provided by mitochondrial (oxidative) metabolism (Moyes *et al.*, 1993). White muscle citrate synthase (CS) activity, a measure of mitochondrial oxidative capacity, strongly correlates with the rate of lactate disappearance in fishes (Moyes *et al.*, 1993). In skipjack white muscle, CS activity is 5 times higher than in carp (Moyes *et al.*, 1992a), and lactate clearance rates are up to 20 times faster than trout (Weber *et al.*, 1986; Arthur *et al.*, 1992).

All of this suggests that the high aerobic capacity of tuna white muscle may be a specialization for rapid repayment of oxygen debt. Bushnell and Jones (1994) were able to estimate the increase in $\dot{M}_{O_2}$ during oxygen debt recovery based on rates of lactate clearance and creatine rephosphorylation reported for skipjack by Arthur *et al.* (1992). Correcting for a relative white muscle mass of approximately 54% (Graham *et al.*, 1983; a value of 80% was used by Bushnell and Jones), $\dot{M}_{O_2}$ would increase by 840 mg O_2/kg/h in association with anaerobic recovery (Table 1). This value may underestimate the increased oxygen consumption following exhaustive exercise as ion, acid-base, and fluid volume disturbances will also be readjusted (Wood, 1991) in addition to possible catecholamine mediated metabolic effects (Pörtner *et al.*, 1994).

Discussion

Fig. 3 combines the costs of aerobic swimming (from Fig. 1) with the additional components of aerobic metabolism (from Table 1) to show the total estimated aerobic metabolic costs for a yellowfin tuna in relation to swimming velocity and the predicted maximum $\dot{M}_{O_2}$ of 2500 mg O_2/kg/h. Maximum $\dot{M}_{O_2}$ is determined by the capacity for oxygen transport to respiring tissues, and the difference between this rate and R_S is defined as the aerobic scope (Fry, 1947). The sum of all the aerobic metabolic components (i.e., R_F, R_A, R_D, and $R_{G\&R}$), can never exceed aerobic scope. It is clear from Fig. 3 that aerobic swimming and R_S are major metabolic costs for yellowfin, particularly at higher velocities. Nevertheless, it is also apparent that the aerobic swimming costs at the predicted maximum sustainable swimming speed of a yellowfin (3.7 FL/s, based on oxygen delivery rate to red muscle) do not approach its predicted maximum $\dot{M}_{O_2}$. Thus, it would be possible for a yellowfin to simultaneously sustain all of its various aerobic costs up to a swimming velocity of about 3 FL/s. At the maximum aerobic swimming speed (3.7 FL/s) total aerobic capacity (R, 2959 mg O2/kg/h, Table 1) exceeds the predicted maximum $\dot{M}_{O_2}$, thus some, but not all, additional aerobic costs can be sustained at this velocity (Fig. 3).

The maximum aerobic capacity of most fishes, has been assumed to occur during maximum sustained swimming (Goolish, 1991). For this reason, the maximum sustained swimming speed of tuna has been estimated by extrapolating the $\dot{M}_{O_2}$-velocity relationship to the estimated maximum $\dot{M}_{O_2}$ (Dizon, 1977; Bushnell and Brill, 1991). Our model, however, suggests that the maximum sustained speed is achieved before the estimated maximum aerobic capacity has been reached (Korsmeyer *et al.*, 1996b). This both reduces estimates of sustained swimming speeds and indicates that the metabolic demand of other tissues can be met in conjunction with high speed swimming. Actual measurements of maximum $\dot{M}_{O_2}$ are needed to quantify the aerobic scope of tunas.

Why aren't sustainable velocities higher for tuna? While increases in velocity are less costly for tunas, the absolute swimming costs are higher than other fish at sustainable swimming speeds, due in part to an elevated R_S (Fig. 2). Furthermore, while tunas have many adaptations for enhanced oxygen uptake and delivery, not all of the oxygen extracted by the gills can be delivered directly to the red muscle. Red muscle comprises only a small portion of the total body mass of tuna (6.4% in a 50 cm yellowfin tuna, Graham *et al.*, 1983) and only a fraction of total cardiac output will perfuse it.

The much larger proportion of white muscle relative to red muscle in all fishes suggests that most of the "total muscle aerobic capacity" is actually found in the white muscle and that maximum $\dot{M}_{O_2}$ may be associated with activities other than sustained swimming (Goolish, 1991). The $\dot{M}_{O_2}$ of the cod (*Gadus morhua*) during oxygen-debt repayment or during digestion can exceed the maximum active $\dot{M}_{O_2}$ (Soofiani and Hawkins, 1982; Soofiani and Priede, 1985). In tunas, white muscle accounts for about 54% of total body mass or 89% of total muscle mass (Graham *et al.*, 1983). While CS activity in skipjack red muscle is 5 times higher than in white muscle (Moyes *et al.*, 1992a), there is 9 times as much white muscle, therefore total aerobic capacity is potentially 1.8 times higher for the white muscle as a whole than the red muscle.

There is not, however, strong evidence for the use of white muscle by tunas during aerobic swimming and nothing is presently known about the innervation and muscle recruitment pattern in these fishes. Many of the more derived teleosts have multiply innervated white muscle fibers, a pattern that is considered synonymous with function at sustainable speeds (Bone *et al.*, 1978; Johnston, 1981). However, not all modern teleosts have this innervation pattern (Videler, 1993) and some having it do not display a graded muscle recruitment (Johnston, 1981).

Anaerobic metabolism can elevate energy expenditure beyond the aerobic scope, but ultimately will result in the aerobic costs associated with R_D. An oxygen debt may develop

as a result of swimming into deeper water that is cooler and may also be hypoxic relative to surface waters (Korsmeyer *et al.*, 1996b). However, the greatest anaerobic activity in tunas results from burst swimming. The high white muscle aerobic capacity allows rapid recovery from the bouts of burst swimming associated with feeding frenzies or predator avoidance. From Fig. 3, it can be seen that while oxygen debt repayment is potentially a large component of total aerobic metabolism, the high aerobic scope will permit this function to occur during high sustainable swimming speeds. Most of the physiological measurements on tuna are from juveniles, and the increased costs of locomotion with size (Wardle, 1977; Webb, 1977) and the scaling of anaerobic and aerobic potential may alter the relationship between the various metabolic components in large adults (Somero and Childress, 1985; Goolish, 1991). Nevertheless, it seems that adaptations for high rates of oxygen uptake permit a large amount of burst swimming and subsequent recovery without compromising continuous swimming.

The ram gill ventilation of tunas means that ventilation rate, and therefore oxygen uptake, is dependent to some degree on swimming speed. Bushnell and Brill's (1991) model of oxygen extraction by tuna gills indicated that high rates of oxygen uptake could be maintained even at relatively slow swimming speeds (1.5 FL/s). We agree with Bushnell and Brill that the main benefit of the high aerobic scope of tunas is to allow rapid repayment of oxygen debt during continuous swimming at moderate speeds (Brill and Bushnell, 1991b; Bushnell and Brill, 1991). It should be pointed out, that while maximum aerobic swimming speed may only be 3 to 4 FL/s, the ability to maintain this speed while repaying an oxygen debt from intermittent burst swimming (> 4 FL/s) would result in a higher speed averaged over time.

As put forth by Priede (1985), all fishes have a potential power budgeting problem in that the combined capacities for R_A, R_D, and R_F, in addition to R_S, will likely exceed maximum aerobic capacity. In tunas this problem is made especially acute by a high R_S,

and potentially high metabolic rates due to each of the other aerobic components. In addition, all of these various metabolic oxygen requirements can and do occur simultaneously, heightening potential conflicts in oxygen demand. The high aerobic scope in tunas may thus reflect accommodation for each aerobic component, and Fig. 3 shows that each of the components of aerobic metabolism can be sustained at the optimal swimming velocity for yellowfin (~2 FL/s, Dewar and Graham, 1994a). The highest measured $\dot{M}_{O_2}$ in tuna was from skipjack (with an estimated average swimming speed of 3.5 FL/s) that were just caught from a feeding frenzy and were most likely repaying an oxygen debt and had a high R_F (Gooding *et al.*, 1981). In addition, the tuna's large metabolic scope will limit conflicting demands for energy to growth and reproduction, which, ultimately, will determine survival.

The analysis presented here and in the recent works of several investigators (Dickson, 1988; Block, 1991; Brill, 1996) support the idea that the high aerobic scope and physiological performance of tuna "energy speculation" is probably not unique, but may represent the bioenergetic scenario of scombrids and other active pelagic fishes that are convergent with tunas in many of the same physiological, behavioral, and ecological characteristics.

This chapter, in full, is a reprint of the material as it appears in Comparative Biochemistry and Physiology, Vol. 113A, No. 1, Korsmeyer, K. E., Dewar, H., Lai, N. C., Graham, J. B., Elsevier Science, Inc., 1996. The dissertation author was the primary author of this paper.

Table III-1: Potential rates of oxygen consumption for each aerobic metabolic component of a 2.1 kg yellowfin tuna at 25°C

	$\dot{M}_{O_2}$ mg O_2/kg/h
R_S	257
R_F	395
$R_{G\&R}$	123
R_A (3.7 FL/s)	1344
red muscle	(1075)
maintenance	(269)
R_D	840
TOTAL (R)	2959
	(Max. $\dot{M}_{O_2}$=2500)

Figure III-1

Model of aerobic swimming performance for a 2.1 kg (50 cm) yellowfin at 25°C (Korsmeyer *et al.*, 1996b). The swimming $\dot{M}_{O_2}$- velocity relationship from 0.5 to 3.0 FL/s is extrapolated to zero velocity for the standard metabolic rate (R_S) and upwards to predicted maximum $\dot{M}_{O_2}$. Red muscle oxygen demand is estimated by subtracting from swimming $\dot{M}_{O_2}$ the R_S and the increased maintenance costs associated with exercise (assumed to be 20% of the difference between swimming $\dot{M}_{O_2}$ and R_S). Maximum sustainable swimming speed was estimated by predicting the potential of the yellowfin cardio-respiratory system to deliver oxygen to the red muscle.

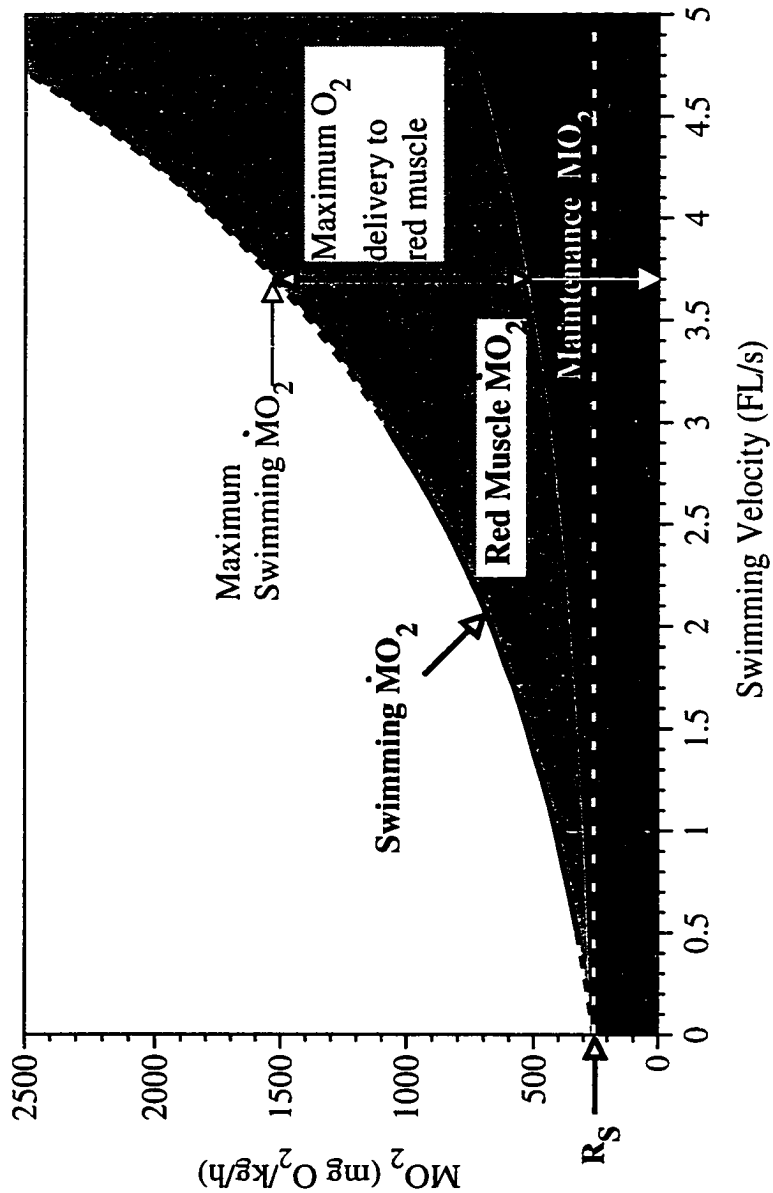


Figure III-2

Summary of $\dot{M}_{O_2}$-velocity relationships for tunas in comparison with sockeye salmon (*Oncorhynchus nerka*, 15°C; Brett and Glass, 1973) and Atlantic mackerel (*Scomber scombrus*, 9.9°C; Lucas *et al.*, 1993). Yellowfin (*Thunnus albacares*) data at 24°C from Dewar and Graham (1994a). Skipjack (*Katsuwonus pelamis*) at 24°C from Gooding *et al.* (1981). Pacific albacore (*T. alalunga*) at 13.5 to 16.9°C from Graham *et al.* (1989).

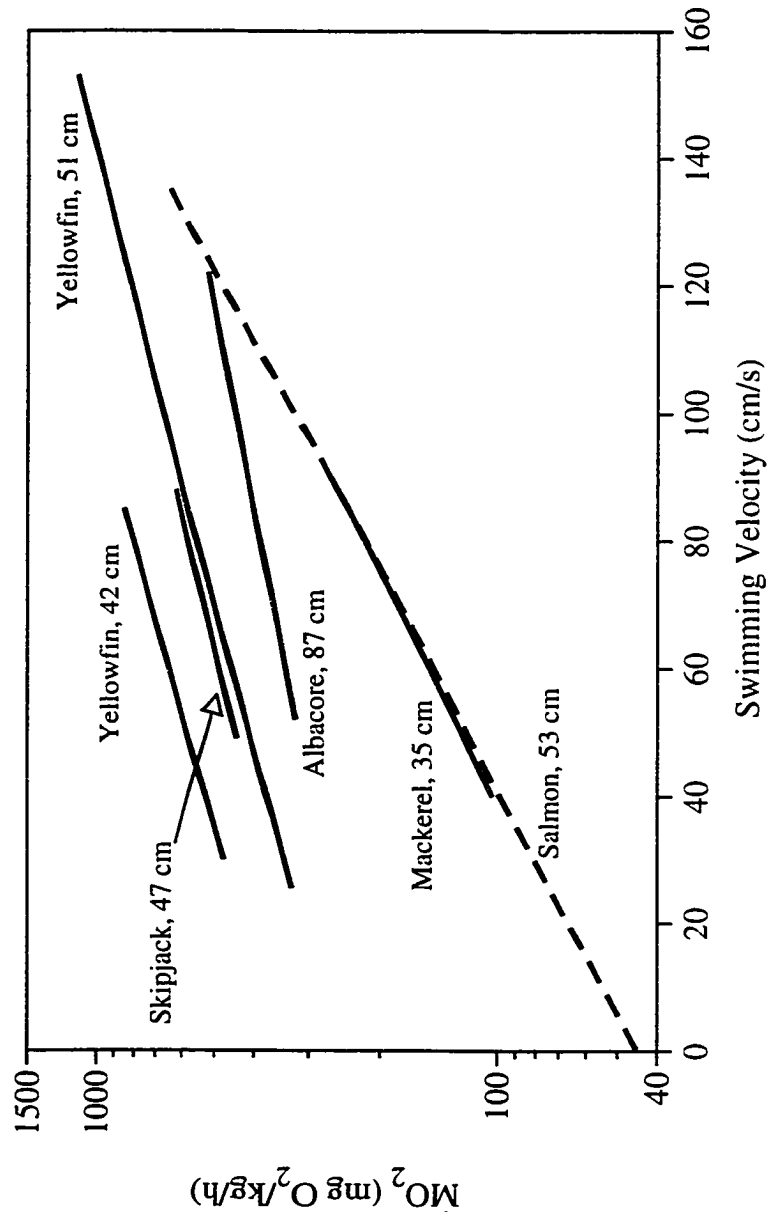
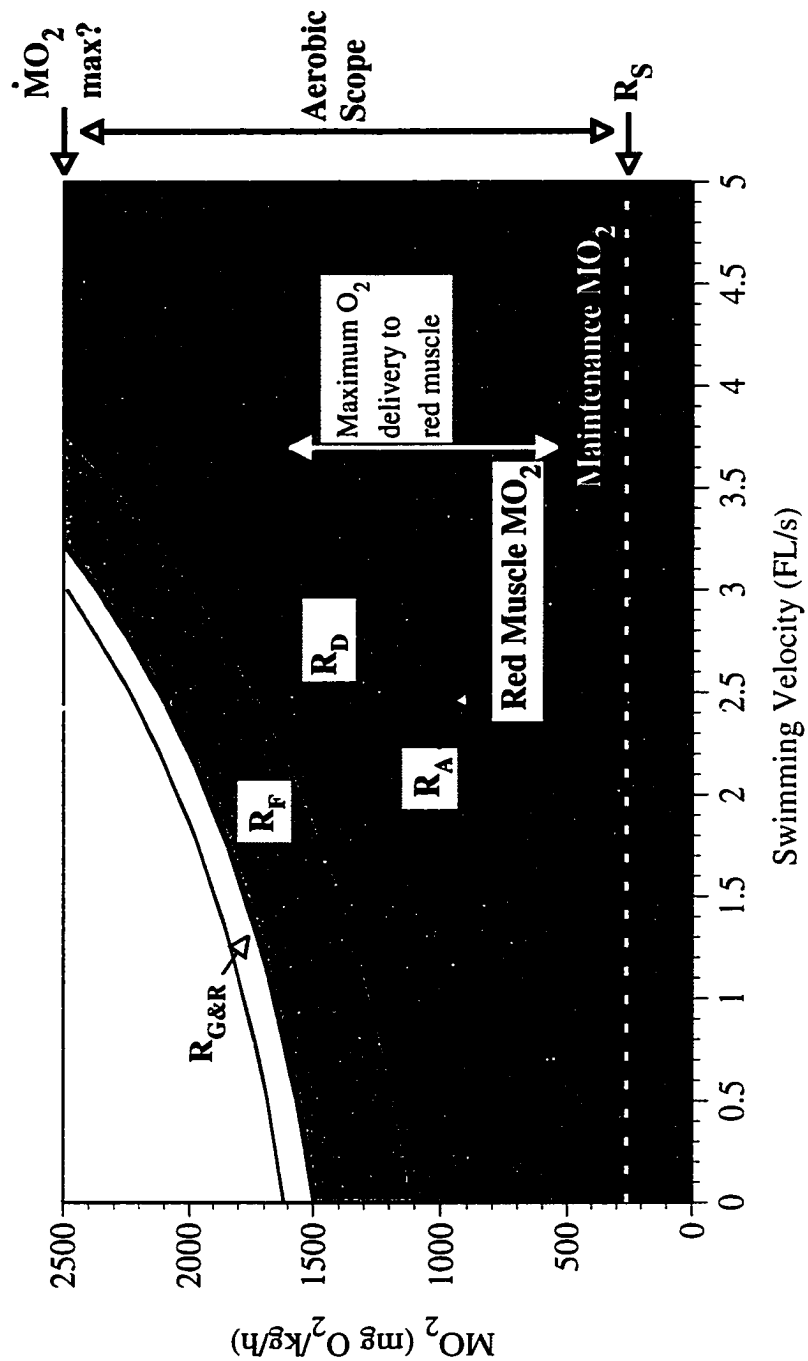


Figure III-3

Total aerobic metabolic cost estimates for a 2.1 kg yellowfin tuna at 25°C in relation to swimming velocity. The potential aerobic costs for oxygen debt recovery (R_D), specific dynamic action (R_F), and growth (R_G) (from Table 1), were added to total swimming $\dot{M}_{O_2}$(R_A) from the model in Fig. 1.



CHAPTER IV: HEART RATE IN SWIMMING YELLOWFIN TUNA: RESPONSE TO EXERCISE, TEMPERATURE, AND HYPOXIA

Abstract

Heart rates (f_H) of yellowfin tuna (*Thunnus albacares*, 945 ±204 g, 38.3 ±2.6 cm fork length [FL]) swimming in a large water tunnel were measured during changes in swimming velocity (0.9 to 3.1 FL/s), temperature (18 to 28°C), and hypoxia (20.5 to 10.0 kPa O_2). Heart rates at 24 ±1°C (30 to 130 bpm) were lower on average than previous measurements with non-swimming (restrained) tunas, and comparable to other active teleosts at similar relative swimming velocities. Although highly variable between individuals, f_H increased with velocity (U, in FL/s) in all fish [$f_H = 49.93 + 17.93(U)$]. Heart rate was very sensitive to changes in temperature (mean $Q_{10} = 2.37$) and hypoxia. A significant bradycardia developed at an oxygen tension of 16.1 kPa O_2, and f_H at 10.0 kPa had decreased by 28.5 ±4.4%. However, f_H increased in hypoxia with increases in swimming velocity. In normoxia, the contribution (48%) of the increased f_H in meeting the estimated increase in oxygen demand during swimming is within the range reported for other teleosts.

Introduction

The nature of cardiovascular responses to exercise in tunas (family Scombridae, tribe Thunnini) remains largely speculative. Until recently, the difficulty in working with tunas has prevented detailed physiological measurements under the controlled swimming conditions necessary to examine changes in oxygen demand. Swimming is a constant and integral factor in tuna biology. Tunas possess many morphological adaptations for efficient swimming, conserve metabolically produced heat in aerobic swimming musculature, and must swim continuously for ram gill ventilation and to maintain hydrostatic equilibrium (Carey *et al.*, 1971; Magnuson, 1978; Roberts, 1978; Dewar and Graham, 1994a).

In addition, tunas are highly active, pelagic predators with metabolic rates 2 to 5 fold greater than other teleosts (Boggs and Kitchell, 1991; Brill and Bushnell, 1991b; Dewar and Graham, 1994a). This high rate of oxygen consumption is supported by many cardiovascular and respiratory adaptations for enhanced oxygen uptake and delivery (Brill and Bushnell, 1991b; Bushnell and Jones, 1994).

Previous studies on tuna cardiovascular physiology have been conducted on anesthetized, paralyzed, and spinally-blocked tuna (Stevens, 1972; Breisch *et al.*, 1983; Brill, 1987; Lai *et al.*, 1987; White *et al.*, 1988; Bushnell *et al.*, 1990; Bushnell and Brill, 1992; Jones *et al.*, 1993) or on tuna swimming at slow, uncontrolled speeds (Jones *et al.*, 1986; Bushnell and Brill, 1991; Jones *et al.*, 1993). Reported heart rates (f_H) from paralyzed and spinally-blocked, restrained tunas are high and cover a wide range compared to other teleosts (90 to 240 bpm) (Brill, 1987; Bushnell *et al.*, 1990; Brill and Bushnell, 1991b), while f_H in swimming tunas have been found to be significantly lower (averaging around 70 bpm, and in some fish as low as 35 bpm; Bushnell and Brill, 1991). Despite these data from swimming fish, the overwhelming f_H data from non-swimming tunas has resulted in continued emphasis on the tuna's high heart rates, with "normal" values reported as being between 90 and 130 bpm (Brill and Bushnell, 1991b; Farrell, 1991;

Bushnell and Jones, 1994). Recent work on spinally-blocked tunas also suggests that earlier results from non-swimming fish may have overestimated routine f_H values (Keen *et al.*, 1995).

The large range of f_Hs reported for tunas has also resulted in conflicting conclusions about the importance of f_H changes during exercise. It has been hypothesized that in tunas, unlike other fishes, cardiac stroke volume is relatively fixed *in vivo*, suggesting that a large scope for modulating f_H is important in increasing cardiac output during changes in oxygen demand (Farrell, 1991; Farrell *et al.*, 1992). Alternatively, the wide range of f_H observed in both swimming and non-swimming (spinally-blocked and anesthetized) tunas has led to the conclusion that "obviously, heart rate does not appear to correlate well with the fish's level of activity" (Jones *et al.*, 1993).

In addition to the effects of exercise, environmental changes experienced by tunas will affect cardiac function. Many tunas make rapid vertical movements through the thermocline in search of prey, and as a result may encounter variable ambient conditions of oxygen tension and temperature (Dizon *et al.*, 1978; Sund *et al.*, 1981; Carey and Olson, 1982; Holland *et al.*, 1990). The effects of these environmental changes on the cardiovascular system, particularly f_H, may limit oxygen uptake and transport at a time when oxygen demand may be high (i.e., during prey capture or predator avoidance).

The objective of this study was to measure f_H in swimming yellowfin tuna (*Thunnus albacares* [Bonnaterre]), without the use of anesthesia or invasive surgery, under conditions of normoxia, hypoxia and altered environmental temperature. These data provide baseline values to which more invasive studies can be compared, show how f_H in yellowfin is affected by swimming speed, and indicate to what extent the acute changes in temperature and dissolved oxygen that may be encountered during normal vertical excursions can affect f_H.

Materials and methods

Animals, equipment and instrumentation

Yellowfin tuna were purchased from local commercial fishermen and held in outdoor tanks (24 ±1°C) at the Kewalo Research Facility in Honolulu, Hawaii (Southwest Fisheries Science Center Honolulu Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration) (Nakamura, 1972). Fish were fed once daily, although experimental subjects were not fed for 24 h prior to testing. All fish used in this study were healthy, feeding, and in captivity for at least 1 week, but not more than 6 weeks.

Experiments were conducted using the large (3000 l) water tunnel described by Dewar and Graham (1994a). The size of the swimming section in these experiments was fixed at 113 x 22.5 x 32.5 cm (length, width, height). Oxygen content and temperature were continuously monitored with a YSI (Yellow Springs Instruments) temperature-compensated oxygen probe (5450/5758, model 54A meter) mounted in a flow-through cuvette, and a thermocouple within the water tunnel connected to a Physi-Temp digital thermometer (model BAT-12). All swimming velocities were corrected for the solid-blocking effect of the fish as described by Bell and Terhune (1970).

Fish handling and water tunnel procedures are described in Dewar and Graham (1994a); instrumentation and procedures for experiments in this study are described briefly. Thirteen yellowfin tuna (673 to 1415 g, 33 to 44.5 cm fork length [FL]) were instrumented with electrocardiogram (ECG) electrodes (30 gauge insulated wire) to monitor f_H. The tuna were quickly dip-netted from the holding tank, placed ventral side up in a water-filled, padded, plastic trough and ventilated with seawater. One electrode was inserted subcutaneously adjacent to the ventricle, and a second electrode wire was placed caudally, below the pectoral fin. The electrodes were held in place near the insertion point by small fish hooks (#15 Mustad) attached to the wire (Bushnell and Brill, 1991). The pre-braided

electrode wires were then looped through the skin near the insertion of the second dorsal fin and self-tied in position. This procedure was completed in 1 to 3 minutes. Once the electrodes were in place, the fish was rushed in the plastic trough to the water tunnel where it was lowered into the swimming section. Between 1.5 and 2 hours were allowed for the fish to acclimate to the tunnel and water velocity was adjusted to find a stable swimming speed for the fish (between 0.9 and 1.9 FL/s).

The ECG wires were connected to a pre-amplifier (Grass model P15D) and the signal was recorded with a computer data acquisition system (486 PC-compatible, Axotape and Cyberamp, Axon Instruments).

Experimental protocol

Following acclimation to the water tunnel, water velocity was increased step-wise by 5 to 15 cm/s. Upon reaching the new velocity, f_H was recorded during 5 min of stable swimming or until the fish could no longer maintain position in the water tunnel. After a series of velocity increases, velocity was reduced to a resting level, which was maintained until f_H stabilized. This procedure was followed by additional velocity tests or by acute changes in temperature or hypoxia.

Acute temperature change experiments were conducted by rapidly introducing hot (40°C) or cold (2°C) seawater from external reservoirs into the water tunnel until the desired experimental temperature was achieved (~1°C/min). Temperature was first reduced from 24°C to 18°C, and then increased to 28°C (±1°C). Limited velocity tests were conducted at each temperature. Four yellowfin (757 to 1155 g, 37 to 40 cm FL) were used in these experiments.

For hypoxia experiments, deoxygenated (4 kPa O_2) or supersaturated (28 kPa) seawater was introduced into the water tunnel to modulate oxygen tensions (P_{O_2}) from normoxia (20.5 kPa) to 10.0 kPa. Seven yellowfin (824 to 1415 g, 37.5 to 44.5 cm FL)

were used in these experiments. Ambient P_{O_2} was decreased in steps and then returned to normoxia, with ECG recordings made for 5 to 10 minutes at each stable O_2 level. Velocity was held constant during the change in P_{O_2}, except for 2 of the 7 fish, in which it was adjusted slightly (by no more than 0.2 FL/s) to maintain steady swimming. At the lowest O_2 level (approximately 10.0 kPa), a limited velocity test was conducted (as described above) followed by a return to the previous swimming velocity before increasing P_{O_2}.

Data analysis

Digitized ECG records were analyzed by a Turbo Pascal program to identify the position of the QRS complexes. QRS identification was presented graphically to verify correct triggering. An additional program calculated f_H (bpm) by counting the number of QRS complexes over one minute intervals.

For f_H and velocity data, least-squares linear regressions were applied to each fish, and a combined regression was calculated from pooled data for all fish. Regressions were tested for significance with regression analysis of variance (ANOVA). Comparison of regressions for individual fish was made with analysis of covariance (ANCOVA) and a post hoc multiple comparison test. For f_H measured at different temperatures, Q_{10} values were determined from the mean f_H recorded at the same velocities. Q_{10} values between 18 to 24°C and 24 to 28°C were compared with a paired t-test. The effects of hypoxia on f_H for each fish were compared as a percentage change from the mean, pre-hypoxia f_H using ANOVA and multiple comparison tests following arcsine transformation (Zar, 1984). A fiducial limit of 5% was used for all statistical tests. Mean values are presented with standard error of the mean (S.E.M.).

Results

Exercise

Linear regressions of f_H and velocity for 13 yellowfin at 24 ±1 °C are shown in Fig. 1A, with the combined regression for all fish presented in Fig. 1B. Regression parameters are listed in Table 1. All individual regressions had highly significant positive slopes ($p<0.0001$). Heart rate varied considerably among individuals and there were significant differences in the rate of increase with velocity. However, results of the multiple comparison of slopes were ambiguous, in that distinct groups of fish with similar slopes were not found. Elevations (i.e., the f_H values over the measured range of velocities) were significantly different among regressions of common slope.

The combined regression of f_H on velocity (U) for pooled data was also highly significant ($p<0.0001$):

$$f_H = 17.93(U) + 49.93 \quad (1)$$

Due to the variability in f_H between fish, however, the coefficient of determination ($r^2 = 0.14$) was much less than for individual regressions (Table 1). The low r^2 value indicates that the combined regression cannot be used as a predictor of f_H for individual fish. However, the regression can be used to estimate the average f_H for the population of yellowfin (Fig. 1B).

The highest velocity achieved in these tests (24°C) was 2.9 FL/s and f_H did not exceed 130 bpm. Multiple regression found no effect of body mass on f_H, however, the range of masses in this study was relatively small (673 to 1415 g).

Temperature

Heart rate responded rapidly (within 1 min) and closely matched changes in ambient temperature (Fig. 2). Temperature and velocity effects on the f_H of 4 yellowfin are shown in Fig. 3, and regression parameters for f_H on velocity at 18 and 28°C are presented in

Table 2. At 18°C, the f_H of all fish had a significant positive linear regression with velocity ($p<0.0001$). At 28°C, fish nos. 2 and 3 (Fig. 3A, B) had significant positive regressions between f_H and velocity ($p<0.0001$), however, that of fish no. 4 (Fig. 3C) did not differ from zero, and that for no. 9 (Fig. 3D) was negative ($p<0.0001$). The 28°C regression for fish no. 4 is probably not significant due to insufficient data over a range of velocities (Fig. 3C). However, these data could be used for calculation of Q_{10}. In fish no. 9, f_H briefly rose to a maximum of 205 bpm (for less than a minute) immediately after the increase in temperature to 28°C, and then declined despite increases in velocity (Fig. 3D). This fish had higher f_Hs at 24°C than the other fish (Fig. 1A).

Mean Q_{10} values for each fish are presented in Table 3. Over the two temperature ranges, 18° to 24° (25°C for one fish) and 24 to 28°C (27°C for one fish), Q_{10} was not significantly different (mean $Q_{10} = 2.37 \pm 0.17$).

Hypoxia

The sequence of experimental changes in ambient oxygen tension (P_{O_2}) is shown in Fig. 4A. Mean (±S.E.M.) values of P_{O_2} at each step in hypoxia and the percentage change in f_H for each fish are shown in Fig. 4B. Average swimming velocity during the hypoxia test was 1.6 ±0.12 FL/s, and differences in swimming velocity did not correlate with the degree of bradycardia. The average f_H during the control period (20.5 kPa) was 69.9 ±5.7 bpm. All fish showed a significant decrease in f_H (10.3 ±2.7%) with a drop in P_{O_2} from 20.5 to 16.1 kPa. At the lowest experimental P_{O_2} (10.0 kPa), f_H decreased between 14.6 and 44.4% (28.5 ±4.4%). Heart rates during increasing P_{O_2} were typically higher than at similar P_{O_2}s during the oxygen decrease, and in several fish, f_H exceeded control values upon return to normoxia (Fig. 4).

Results of the velocity test at 10.0 kPa O_2 are shown in Fig. 5 and regression parameters are listed in Table 4. Linear regressions for the 7 yellowfin were all positively

correlated with velocity ($p<0.02$). ANCOVA found differences in slopes between individuals and, as in the normoxic velocity tests, discrete groups of fish with similar slope were not found. Elevations were significantly different among regressions of common slope. The combined regression in hypoxia for all f_Hs on velocity was significantly positive ($p<0.0001$, $r^2 = 0.59$, Fig. 5B):

$$f_H = 32.80(U) - 3.46 \quad (2)$$

Discussion

Heart rates of yellowfin compared to other species

Previously reported f_Hs for non-swimming tunas are high compared to other teleosts and cover a wide range (Table 5). We have found, however, that f_H in swimming yellowfin is generally lower than expected, based on these earlier studies, particularly if the f_H-velocity relationship is extrapolated to zero velocity (Fig. 1B). In addition, heart rates measured in this study are lower than those reported by Jones *et al.* (1993) (Table 6) for slowly swimming (velocities not reported) yellowfin, which may be due to the added stress of anesthesia and surgery, and the towing of multiple cannulae in that study. However, the mean f_H reported by Bushnell and Brill (1991) for yellowfin swimming in a circular tank at an average velocity of 1.2 FL/s was 67.9 bpm, which is very similar to the f_H predicted from the combined regression in Fig. 1B (71.4 bpm). Bushnell and Brill (1991) suggest that the higher f_Hs measured in non-swimming, spinally-blocked tuna may reflect over-ventilation in these preparations. In addition, it may be that swimming tuna are less stressed compared to restrained fish. These differences suggest that physiological variables measured in non-swimming tunas should not be considered equivalent to a "resting" state.

Although generally lower than other measurements for tuna, there was considerable variability in f_H between individuals (Fig. 1A). Significant intraspecific differences in f_H have also been found in leopard shark (*Triakis semifasciata*) (Scharold *et al.*, 1989), lemon

shark (*Negaprion brevirostris*) (Scharold and Gruber, 1991), Atlantic salmon (*Salmo salar*) (Lucas, 1994), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and cod (*Gadus morhua*) (Priede and Tytler, 1977). The individual variability in f_H in this study may be due to unknown differences in either physical condition or response to handling stress. A similar amount of variability, both between and within individual fish, was reported by Bushnell and Brill (1991) for yellowfin swimming over slow, voluntary speeds in a circular tank (see Fig. 1 in Bushnell and Brill, 1991).

Heart rates for yellowfin reported in this study are not very different from those reported for other active teleosts, such as rainbow trout or Atlantic salmon. Heart rates for these species at 1.0 and 2.0 FL/s are presented in Table 6 for comparison, and are very similar despite a 9°C difference in temperature. The measured f_H reported for one yellowtail (*Seriola quinqueradiata*, Carangidae) at 24°C increases from about 80 to 100 bpm with an increase in swimming velocity from approximately 1.0 to 2.6 FL/s (Hanyu *et al.*, 1979), a similar response to that of the yellowfin (Fig. 1B). It appears, therefore, that during routine aerobic activity yellowfin have f_Hs similar to other active fishes, despite the capacity for much higher f_Hs (Table 5).

Skipjack (*Katsuwonus pelamis*) and kawakawa (*Euthynnus affinis*) tunas appear to have a higher maximal f_H than yellowfin and thus may have higher routine f_Hs. The maximum reported f_Hs for yellowfin at 25°C are approximately 160 bpm (Brill, 1987; Farrell *et al.*, 1992), while maximum f_Hs in skipjack and kawakawa are well over 200 bpm (Table 5 and 6). The mean f_H in swimming kawakawa (1.3 FL/s) was 128 bpm (Jones *et al.*, 1993). However, the average f_H reported for skipjack swimming at 1.6 FL/s (76.8 bpm; Bushnell and Brill, 1991) is very similar to that predicted for yellowfin from the combined regression in Fig. 1B (78.6 bpm).

Exercise

Oxygen consumption ($\dot{V}_{O_2}$) in fishes increases exponentially with swimming velocity (Beamish, 1978; Dewar and Graham, 1994a). The increased oxygen delivery to the working muscles must be met with increases in f_H, stroke volume (SV), or increased oxygen extraction according to the Fick equation:

$$\dot{V}_{O_2} = SV \cdot f_H \cdot (a - v)_{O_2} \quad (3)$$

where $(a - v)_{O_2}$ is the difference between arterial and venous oxygen content. Although reports differ with respect to the response of f_H to increased exercise in fishes, cardiac output typically increases as a result of increased f_H, and an equal or greater increase in SV (as reviewed in Jones and Randall, 1978; Satchell, 1991; Farrell and Jones, 1992). Based on the performance of *in vitro*, perfused tuna hearts, Farrell (1991; Farrell *et al.*, 1992) has suggested that tunas may operate differently from most other fish by regulating f_H with a relatively fixed SV.

Heart rate in fishes has often been described as increasing exponentially with fish swimming speed (Priede, 1974; Priede and Tytler, 1977; Scharold and Gruber, 1991; Lucas, 1994). We, however, found that a linear regression provided a better fit based on the r^2 values for yellowfin in this study. Despite considerable intraspecific differences in the specific f_H, each fish showed a significant increase with velocity. Although $\dot{V}_{O_2}$ was not measured in this study, the contribution of f_H in meeting the increased oxygen demand can be estimated using the relationship between $\dot{V}_{O_2}$ (mg O_2/kg/h) and velocity (FL/s) determined previously under similar experimental conditions for 42 cm yellowfin (Dewar and Graham, 1994a):

$$\log \dot{V}_{O_2} = 2.537 + 0.1932(U) \quad (4)$$

The percentage contribution of f_H to increased oxygen consumption (%con f_H) is given by the following equation (Scharold and Gruber, 1991; Lucas, 1994):

$$\%\text{con } f_H = \log(f_{H_2}/f_{H_1})/\log(\dot{V}_{O_{2\,2}}/\dot{V}_{O_{2\,1}}) \times 100 \quad (5)$$

where the subscripts 1 and 2 represent two different levels of oxygen demand. With an increase in swimming velocity from 1.0 to 2.9 FL/s, $\dot{V}_{O_2}$ is predicted to increase from 537 to 1251 mg O_2/kg/h (eq. 4) and f_H from 67.9 to 101.9 bpm (eq. 1). These values give a %con f_H of 48%, which is within the range of values calculated for rainbow trout (8.7 to 49.7%), similar to Atlantic salmon (45 to 47%), and less than in pike (*Esox lucius*, 122.1%) or mammals and birds (55.3 to 171.0%) (Scharold and Gruber, 1991; Lucas, 1994). Thus, the relative contribution of yellowfin f_H to increased metabolic demands is similar to other active teleosts.

Heart rate in the absence of nervous and humoral modulation is set by the intrinsic rhythm of the pacemaker (Farrell and Jones, 1992). The intrinsic f_H of yellowfin is reportedly 119 bpm and under a large vagal (cholinergic) inhibitory tone (Keen *et al.*, 1995). This agrees with results from the injection of the cholinergic blocker, atropine, in swimming yellowfin, which caused f_H to increase to between 120 to 130 bpm (K. E. Korsmeyer and N. C. Lai, unpub. obs.). Thus during aerobic exercise, f_H could be increased through decreased vagal inhibition alone. For free-swimming tuna, it is not clear what conditions may initiate exceptionally high f_Hs (>120 bpm, Tables 5 and 6). High-speed anaerobic swimming is usually accompanied by bradycardia in fishes, but f_H may increase during the oxygen debt recovery from such activity (Farrell and Jones, 1992). During intense exercise or stress, plasma catecholamine levels are elevated, which would aid the increase in f_H above the intrinsic value, in addition to potential neural adrenergic stimulation (Satchell, 1991; Farrell and Jones, 1992).

Temperature

Yellowfin f_H was rapidly and strongly affected by temperature (Fig. 2, Table 3). This effect is likely due to the direct action of temperature on the heart's pacemaker cells (Randall, 1970). At the low (18°C) and high (28°C) temperatures used in this study, f_H of

most fish increased with velocity, similar to the response at 24°C (Fig. 3). One fish had unusually high f_Hs at 24°C (Fig. 3D) and may have been more stressed during handling than the other yellowfin. At 28°C, f_H in this fish appeared to reach a maximum level of just over 200 bpm, followed by a rapid drop in f_H, which continued to decline, despite increases in velocity. The highest previous report of f_H in yellowfin is 200 bpm at 30°C (Brill, 1987). This appears to be the maximum f_H in yellowfin at this temperature, and the decline in f_H with increasing velocity in Fig. 3D is likely due to these unusually high f_Hs, which could not be sustained.

Yellowfin make regular rapid excursions between the warm surface waters and the cooler waters of the upper thermocline; spanning about 10°C (Holland *et al.*, 1990). In most ectotherms, the decrease in f_H with temperature is accompanied by a decrease in total metabolic rate, however, tuna red (aerobic) muscle metabolism may not be affected to the same degree as cardiac metabolism. Tunas are capable of elevating red muscle temperatures above ambient through the use of vascular counter-current heat exchangers (*retia mirabilia*) (Carey *et al.*, 1971). Yellowfin maintain excess red muscle temperatures (the difference between muscle and ambient temperature) of 1 to 5°C (Dewar *et al.*, 1994; Dickson, 1994). In addition, the insulating effect of the *retia* attenuates red muscle heat loss when the fish moves into cooler waters (Dewar *et al.*, 1994). Although blood delivered to the red muscle is warmed by counter-current exchange in the *retia*, these same structures cool the venous blood returning to the heart (Brill *et al.*, 1994). Because of the efficiency of the *retial* heat exchangers, and venous return from areas of the body that were never heated, the temperature of the mixed venous blood entering the lumen of the heart is unlikely to be more than a few tenths of a degree C above ambient (Graham, 1973; Neill *et al.*, 1976). In addition, the heart is located close to surrounding water, and is fed with a coronary blood supply that is in thermal equilibrium with ambient conditions in the gill. The rapid response of f_H to ambient temperature changes (Fig. 2) suggests that the

temperature of the heart quickly comes into equilibrium with the ambient water. In addition, despite the elevated muscle temperatures of swimming yellowfin, the Q_{10} for f_H (Table 3) is similar to those reported for paralyzed, non-swimming tunas, and other fishes (Brill, 1987; Farrell and Jones, 1992). During rapid decreases in ambient temperature, the heart of yellowfin experiences the full effect of the temperature change, while the red muscle *excess* temperature increases (Dewar *et al.*, 1994). This disparity may result in insufficient oxygen delivery to the red muscle during movements below the thermocline.

Hypoxia

In areas such as the eastern tropical Pacific Ocean, oxygen content declines rapidly at relatively shallow depths, and yellowfin may encounter hypoxic conditions during dives to depth in search of prey. This shallow hypoxic condition appears to limit some tunas to the near surface waters (Barkley *et al.*, 1978; Sharp, 1978; Sund *et al.*, 1981).

A significant bradycardia was observed in yellowfin at relatively mild hypoxic conditions (16.1 kPa, Fig. 4). This is consistent with the P_{O_2} at which f_H declined in spinally-blocked tuna (16.5-14.0 kPa; Bushnell *et al.*, 1990), and in swimming yellowfin (17.3 kPa; Bushnell and Brill, 1991). Although this P_{O_2} threshold for bradycardia is higher than for rainbow trout (10 to 13 kPa; Randall and Smith, 1967), it is within the range reported for other marine teleosts (Davis, 1975; Bushnell *et al.*, 1990).

At 10.0 kPa, the degree of bradycardia (28.5%), although highly variable among individuals, was greater than previously reported for spinally-blocked or swimming yellowfin (~15-20%), but similar to spinally-blocked skipjack and bigeye tuna (*Thunnus obesus*) (Bushnell *et al.*, 1990; Bushnell and Brill, 1991). The hypoxic bradycardia in yellowfin, and other fishes, appears to be elicited in response to chemoreceptors sensitive to environmental P_{O_2} and located on the first gill arch (Milsom and Brill, 1986; Burleson *et al.*, 1992).

Many fishes respond to hypoxia with bradycardia, but cardiac output is maintained, or increased, through increases in stroke volume (Satchell, 1991; Farrell and Jones, 1992). Spinally-blocked tuna show a decline in cardiac output with hypoxia because SV does not change significantly (Bushnell *et al.*, 1990; Bushnell and Brill, 1992). Thus, the decrease in f_H in hypoxia may limit oxygen delivery.

Bushnell and Brill (1991) found that at hypoxic levels below 13 kPa, f_H in swimming tuna increased or remained the same despite further decreases in P_{O_2}. The increases in swimming velocity with hypoxia apparently offset the decline in f_H at lower P_{O_2} levels. This agrees with our results of the velocity test at 10.0 kPa (Fig. 5), where f_H increased significantly with swimming speed, despite the hypoxia induced bradycardia.

Heart rate increased rapidly with the return to normoxia and exceeded control values in some fish (Fig. 4). A similar tachycardia in response to a rapid increase in P_{O_2} from hypoxia was reported for dogfish (*Scyliorhinus canicula*) (Butler and Taylor, 1971). In addition, cardiac output in the trout was elevated above original values after hypoxic exposure, although it was a result of increased SV and not f_H (Wood and Shelton, 1980). The extended hypoxic exposure in the present study (approximately 100 min, with 20 min at 10.0 kPa, Fig. 4A), and the velocity test in hypoxia, may have resulted in an oxygen debt that caused an increase in cardiac output upon to return to normoxia. Dizon (1977), however, found no behavioral response in yellowfin exposed to as low as 4.0 kPa O_2 for 200 min, suggesting that this level of hypoxia was not stressful. Circulating catecholamine levels often increase in fishes due to hypoxic exposure (Boutilier *et al.*, 1988; Ristori and Laurent, 1989; Fritsche and Nilsson, 1990) and this could result in an elevated f_H once vagal inhibition due to hypoxia was removed.

Summary

These results establish f_H responses to exercise in minimally stressed yellowfin tuna. Although capable of a very high f_H, swimming yellowfin have f_Hs that are less than those previously reported for restrained, non-swimming tuna, and are comparable to other active teleosts. The exceptionally high f_Hs reported for tunas, while not found at the swimming velocities obtained at 24°C in this study, can occur at elevated temperatures.

Temperature and ambient oxygen levels have a strong effect on f_H in yellowfin, and will likely affect the ability of this fish to deliver oxygen to active swimming muscles during rapid vertical movements in the wild.

Heart rate increases with exercise in yellowfin, and the contribution of f_H to increased oxygen demand during swimming appears within the range reported for other teleosts. The remainder of the increased oxygen demand must be met with elevations in SV or $(a-v)_{O_2}$ (eq. 3).

Further measurements of f_H are needed during high-speed anaerobic swimming and during oxygen debt recovery. However, the present results provide a comparative basis for evaluating more invasive studies needed to examine the changes in SV and blood oxygen extraction [$(a-v)_{O_2}$] during swimming in yellowfin tuna.

Table IV-1: Summary of parameters for the regression of heart rate (bpm) on relative swimming velocity (FL/s), including sample size (*N*) and coefficient of determination (r^2), for 13 yellowfin tuna in normoxia (24 ±1°C)

Fish No.	Mass (g)	FL (cm)	*N*	Slope	Intercept	r^2
1	673	33	22	26.75	40.30	.82
2	859	37	126	15.55	47.54	.59
3	774	39	167	7.15	68.22	.38
4	757	37	80	11.94	62.97	.66
5	875	37	36	4.99	55.17	.54
6	933	38.5	38	16.25	30.85	.58
7	861	37.5	31	31.78	26.23	.59
8	973	39	53	5.45	60.14	.49
9	1155	40	113	13.08	91.67	.44
10	993	37.5	29	15.65	70.28	.64
11	1190	40	61	20.57	41.52	.67
12	824	38	60	19.90	39.92	.68
13	1415	44.5	69	29.66	14.77	.63
Combined	945	38.3	885	17.93	49.93	.14
± S.E.M.	57	0.7		1.48	2.43	

Table IV-2: Summary of parameters for the regression heart rate (bpm) on relative swimming velocity (FL/s) for 4 yellowfin at 18 and 28°C

Fish No.	*N*	Slope	Intercept	r^2
18°C				
2	16	33.33	-5.68	.94
3	21	10.83	22.54	.79
4	22	14.94	24.37	.86
9	26	20.90	34.49	.52
28°C				
2*	33	23.74	51.81	.54
3	45	16.21	68.84	.56
9	45	-10.65	191.78	.30

N = sample size
*actual temperature 27°C

Table IV-3. Summary of Q_{10} values for heart rate in yellowfin over 18 to 24°C and 24 to 28°C

Fish No.	Q_{10} (18°-24°C)	Q_{10} (24°-28°C)
2	2.08	1.82*
3	2.24†	2.27‡
4	2.26	3.35
9	2.16	2.81
Mean ± S.E.M.	2.19 ±0.04	2.56 ±0.33

*24 to 27°C
†18 to 25°C
‡25 to 28°C

Table IV-4: Summary of parameters for the regression of heart rate (bpm) on relative swimmng velocity (FL/s) for 7 yellowfin in hypoxia (10.0 ±0.2 kPa O_2, 24 ±1°C)

Fish No.	*N*	Slope	Intercept	r^2
6	37	29.30	-0.44	.94
7	16	13.57	25.04	.69
8	14	23.33	10.83	.61
10	13	23.21	34.73	.43
11	18	17.22	32.11	.90
12	19	35.49	-7.90	.86
13	25	26.83	0.31	.90
Combined	142	32.80	-3.46	.59
± S.E.M.		2.29	3.90	

N = sample size

Table IV-5: Heart rates in non-swimming (anesthetized, paralyzed, or spinally-blocked) tunas

Species	mean f_H bpm (range)	Condition	Mass kg	T ℃	References
Skipjack	70 (46-168)	anesthetized	1.7	23-25	Stevens, 1972
	230 (191-237)	paralyzed	0.63	25	Brill, 1987
	125.9	spinally-blocked	1.64	25	Bushnell and Brill, 1992
	79.4	spinally-blocked	1.12	25	Keen *et al.*, 1995
Kawakawa	206 (175-253)	paralyzed	1.3	25	Brill, 1987
Yellowfin	132 (90-160)	paralyzed	1.4	25	Brill, 1987
	96.7	spinally-blocked	1.4	25	Bushnell and Brill, 1992
	112 (86-139)	anesthetized	1.4	25	Jones *et al.* 1993
	75.5	spinally-blocked	1.59	25	Keen *et al.*, 1995
Albacore	120 (60-150)	paralyzed	10.3	20-24	Breisch *et al.* 1983
	87 (50-120)	anesthetized	9.1	16.5	Lai *et al.* 1987
	115 (69-150)	anesthetized	9.3	21.9	White *et al.* 1988

f_H = heart rate, T = ambient temperature. Skipjack (*Katsuwonus pelamis*), kawakawa (*Euthynnus affinis*), yellowfin (*Thunnus albacares*), albacore (*T. alalunga*).

Table IV-6: Heart rates in swimming tunas and other teleosts

Species	f_H min^{-1}	Velocity FL s^{-1}	Fork length cm	T ℃	References
Skipjack	80-240	na	na	25	Kanwisher *et al.* 1974
	76.8	1.6	44.7	25	Bushnell and Brill, 1991
Kawakawa	128	1.3	46	25	Jones *et al.* 1993
Yellowfin	67.9	1.2	46.1	25	Bushnell and Brill, 1991
	108.8	na	na (1.4 kg)	25	Jones *et al.* 1993
	67.9	1.0	38.3	24	Present study
	85.8	2.0	38.3	24	Present study
	101.9	2.9	38.3	24	Present study
Rainbow trout	57.2	1.0	23.0-28.2	15	Priede, 1974
	89.4	2.0	23.0-28.2	15	Priede, 1974
Atlantic salmon	60.3	1.0	50.7	15	Lucas, 1994
	81.3	2.0	50.7	15	Lucas, 1994

f_H = heart rate, T = ambient temperature, na = data not available. Skipjack (*Katsuwonus pelamis*), kawakawa (*Euthynnus affinis*), yellowfin (*Thunnus albacares*), rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*).

Figure IV-1

Effect of swimming velocity on heart rate in normoxia. A) Least-squares regressions of heart rate on swimming velocity for 13 yellowfin tuna at 24 ±1°C. Numbers adjacent to each line identify individual fish as presented in Table 1. All regressions were significantly positive ($p<0.0001$). B) Combined regression from data for all 13 fish (heart rate = 49.93 + 17.93[velocity], $p<0.0001$, $r^2 = 0.14$). Dashed lines are 95% confidence limits. Points are heart rate recorded over 1 min intervals. Note that multiple points occupying the same location are not indicated.

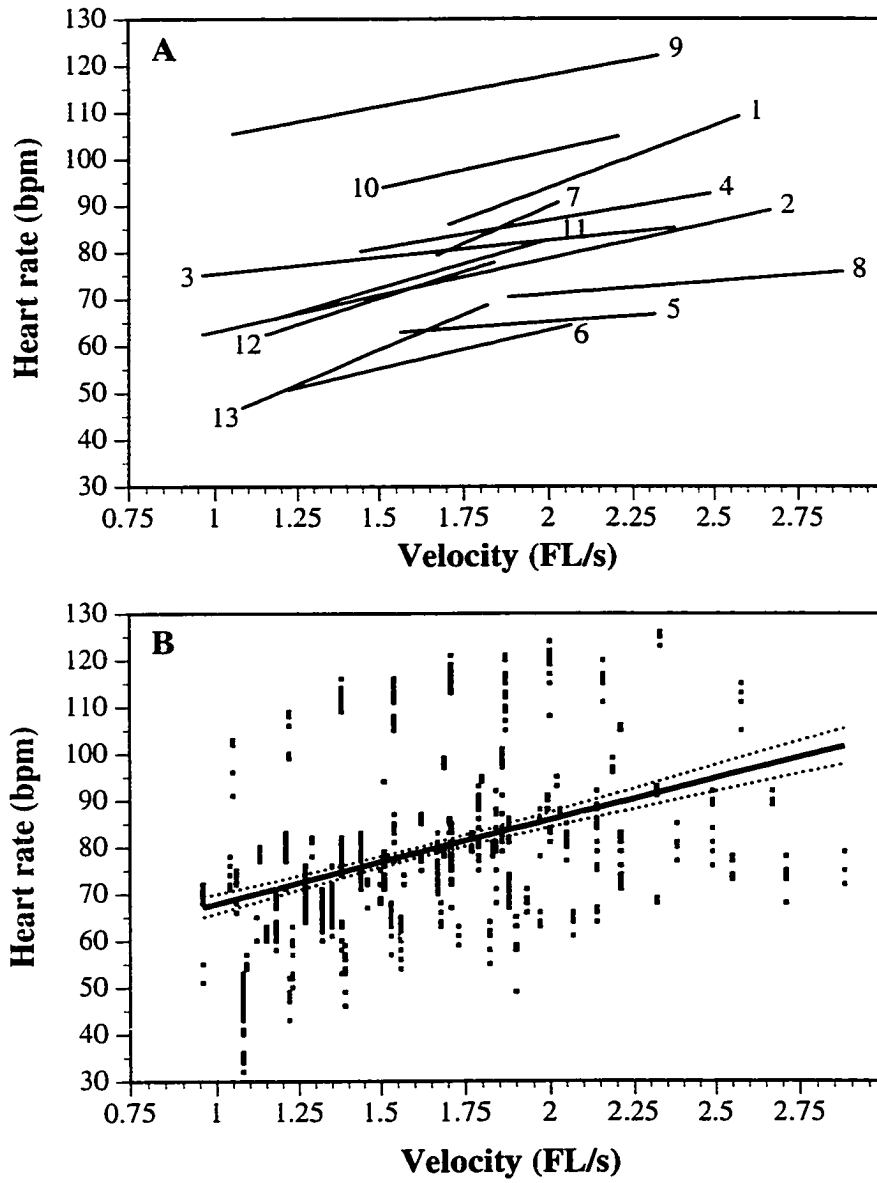


Figure IV-2

Response of heart rate (circles) to acute decrease in ambient temperature (solid line) in yellowfin (Fish No. 4). Swimming velocity was constant at 1.4 FL/s. Heart rate values were recorded over 1 min intervals.

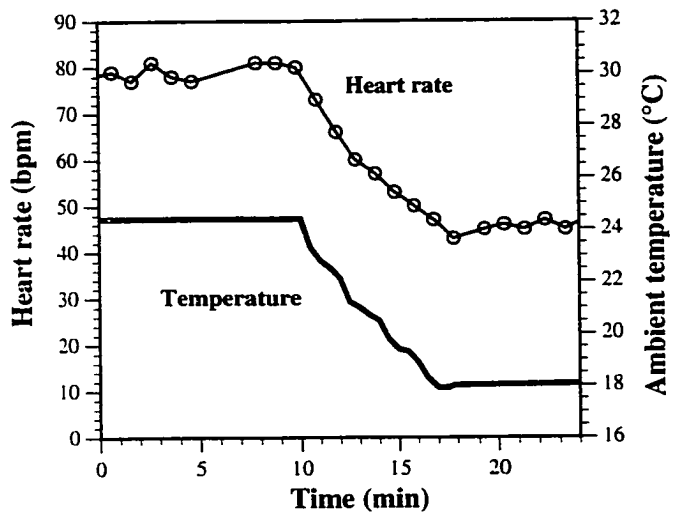


Figure IV-3

Effect of swimming velocity on heart rate at different temperatures in 4 yellowfin tuna. Points are heart rate measured over 1 min intervals and lines are least-squares regression. Fish identification numbers are indicated on each graph and correspond to those in the tables and other figures. The 24-25°C lines are also shown in Fig. 1. Note that multiple points occupying the same location are not indicated.

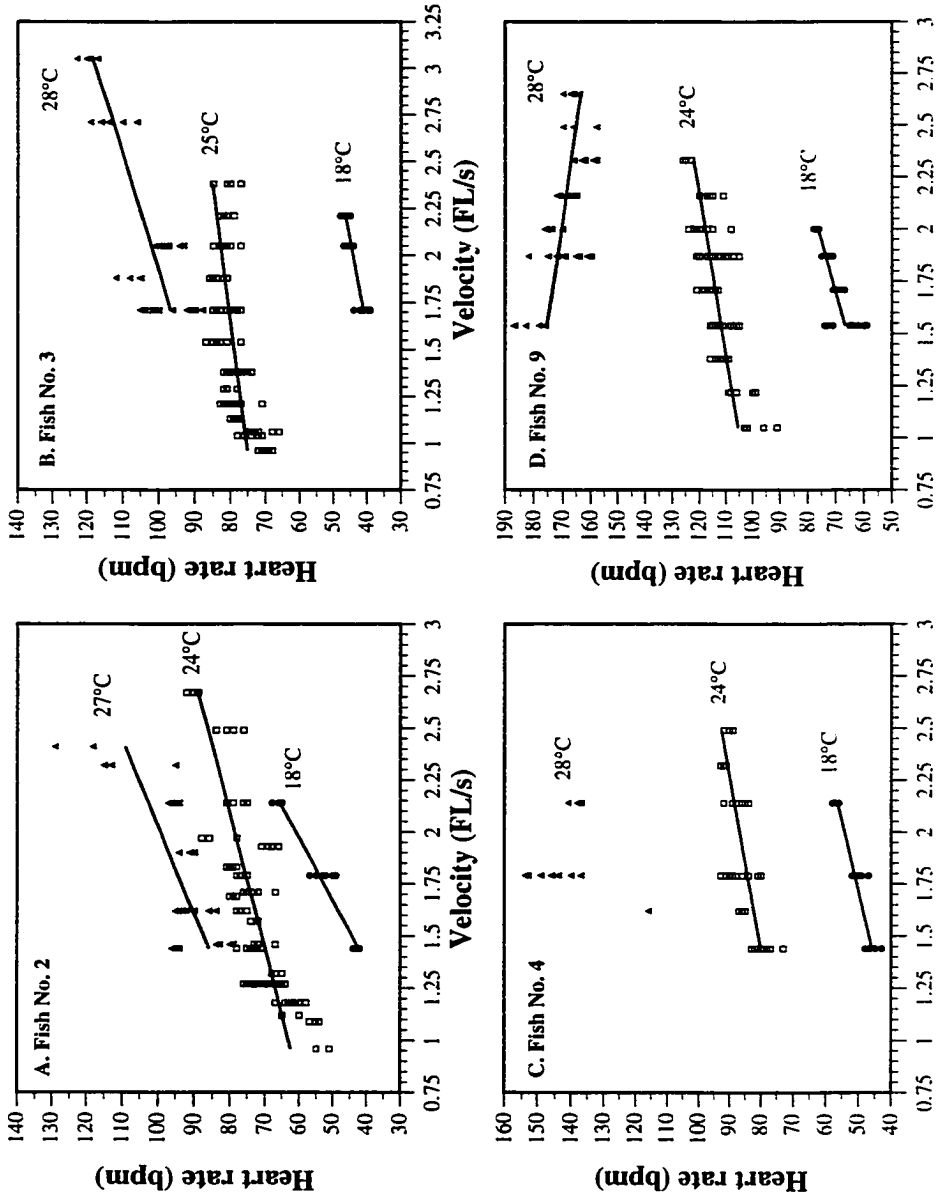


Figure IV-4

Heart rate response to hypoxia in swimming yellowfin tuna. A) Typical trace of ambient (= inspired) oxygen tension (P_{O_2}) with time during acute hypoxia experiments. B) Mean percentage change in heart rate from pre-hypoxia control values for 7 yellowfin swimming at 1.6 ±0.12 FL/s. Heart rates were measured during periods when P_{O_2} was stable in both the decreasing and increasing experimental phases of P_{O_2} change. Mean P_{O_2} ±S.E.M. for each level of hypoxia are indicated above each group. Fish identification numbers correspond to those in the tables and other figures. Error bars are ±S.E.M. * indicates significant difference from control values. na = data not available.

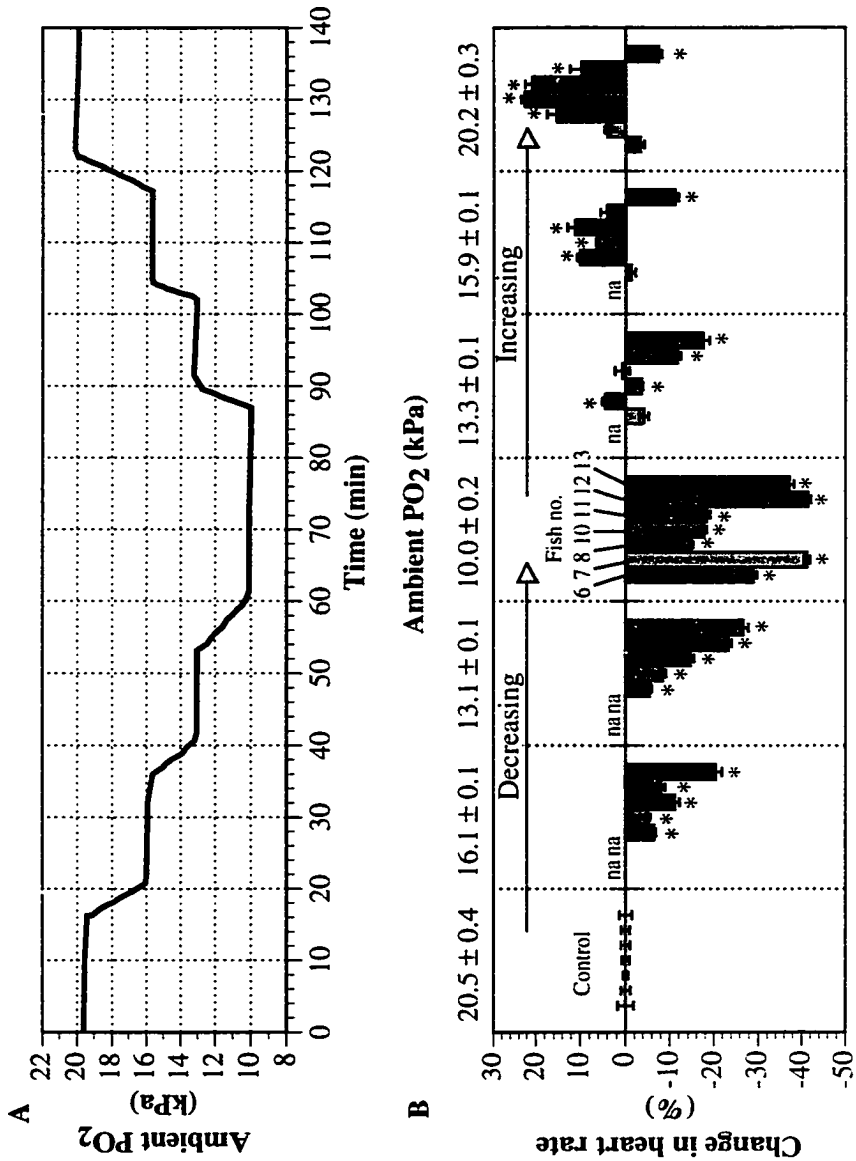
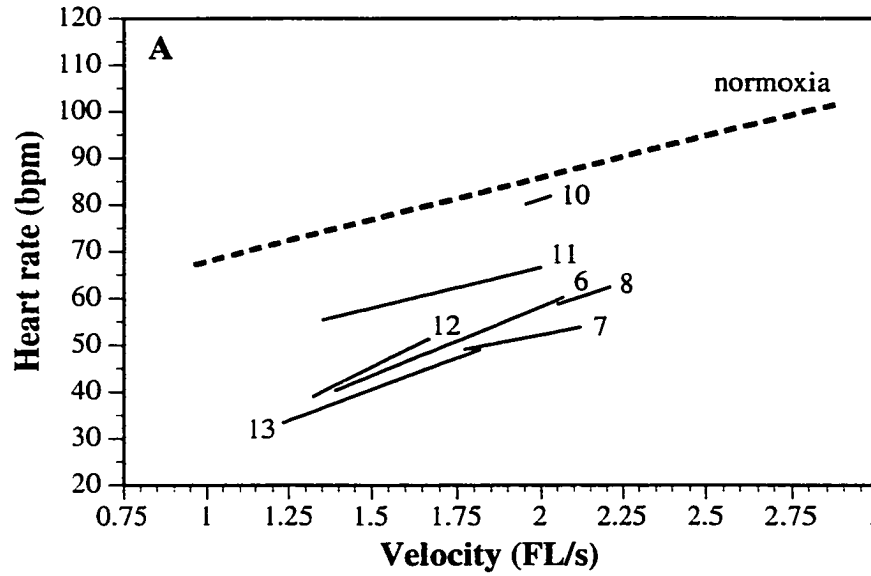
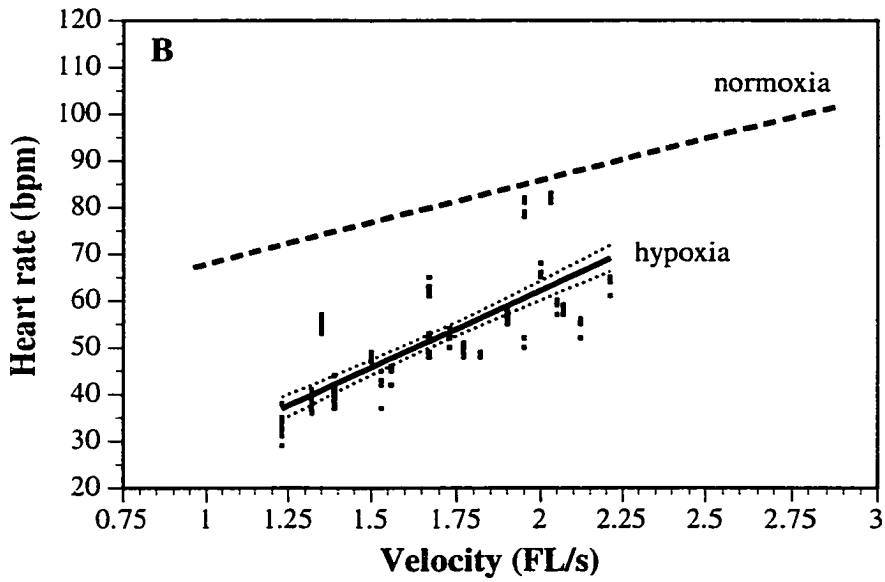


Figure IV-5

Effect of swimming velocity on heart rate in hypoxia. A) Least-squares regressions of heart rate on swimming velocity for 7 yellowfin tuna in hypoxia (P_{O_2} = 10.0 ±0.2 kPa, 24 ±1°C). All regressions were significantly positive ($p<0.02$). Numbers adjacent to each line identify individual fish as in the tables and other figures. Dashed line is the combined regression for 13 yellowfin in normoxia (from Fig. 1B). B) Combined regression for all 7 fish in hypoxia (heart rate = -3.46 + 32.80[velocity], $p<0.0001$, $r^2 = 0.59$). Thin dashed lines indicate 95% confidence limits. Heavy dashed line is normoxia regression as in A. Points are heart rates measured over 1 min intervals. Note that multiple points occupying the same location are not indicated.





CHAPTER V: STROKE VOLUME AND HEART RATE CONTRIBUTIONS TO THE REGULATION OF CARDIAC OUTPUT IN SWIMMING YELLOWFIN TUNA: RESPONSE TO EXERCISE AND TEMPERATURE

Abstract

Changes in cardiac output were measured in swimming yellowfin tuna (*Thunnus albacares*, 1430 -2470 g) instrumented with a transcutaneous Doppler flow probe. Increases in swimming velocity (mean, 1.23 to 2.10 fork lengths/s) resulted in a 13.6% increase in cardiac output caused by an 18.8% increase in heart rate and 3.9% decrease in stroke volume. These results indicate that, unlike most other fishes, cardiac output is regulated primarily through increases in heart rate. In addition, stroke volume appears to have a strong dependency on cardiac filling time. Acute reductions in ambient temperature (25 to 18°C) resulted in decreases in cardiac output (Q_{10} = 1.52) and heart rate (Q_{10} = 2.16), but increases in stroke volume (Q_{10} = 0.78). This observation suggests that the lack of an increase in stroke volume during exercise is not due to the tuna heart operating at maximal anatomical limits, and that maximal stroke volume is not achieved during swimming exercise.

Introduction

The adaptations for enhanced cardiac performance in tunas have been of considerable interest. Tunas (family Scombridae, tribe Thunnini) must swim continuously, are capable of very high burst speeds, and are thus one of the most athletic fishes ever studied (Walters and Fierstine, 1964; Magnuson, 1978). The relative heart size of these fishes is 3 to 10 times that of other teleosts and the tuna heart has an extensive coronary system that delivers oxygenated blood to both the compact and spongy myocardia (Tota, 1978; Farrell and Jones, 1992). Tunas have a relatively high percentage of compact myocardium and the myocardial fibers appear to be arranged to maximize pumping efficiency (Santer *et al.*, 1983; Sanchez-Quintana and Hurle, 1987; Farrell and Jones, 1992). In addition, the hearts of tuna have high levels of myoglobin and aerobic enzyme activities (Giovane *et al.*, 1980; Moyes *et al.*, 1992a; Dickson, 1995). Unlike other fishes, tuna myocardium has a significant dependence on intracellular calcium stores, which may permit rapid contraction frequencies (Keen *et al.*, 1992; Tibbits and Kashihara, 1992). These morphological and biochemical features support the tuna's potential for extraordinary heart rates, cardiac outputs, and blood pressures compared to other fishes (Breisch *et al.*, 1983; Lai *et al.*, 1987; Brill and Bushnell, 1991b; Jones *et al.*, 1993; Bushnell and Jones, 1994).

Two disparate hypotheses of *in vivo* cardiac function propose that the tuna heart either operates differently from the hearts of most other fishes (Farrell, 1991; Farrell *et al.*, 1992) or responds similarly to exercise (Bushnell and Jones, 1994). Based on the performance of *in vitro* perfused tuna hearts (Farrell *et al.*, 1992) and stroke volumes measured in restrained, spinally-blocked tuna (Bushnell and Brill, 1992), Farrell (1991; Farrell *et al.*, 1992) concluded that the tuna heart operates at near maximal stroke volume, and therefore cannot increase stroke volume during exercise (i.e., increases in swimming

velocity). This is supported by cardiac responses to hypoxia in spinalized tuna, in which bradycardia is not accompanied by increases in stroke volume, unlike the response in most other fishes (Bushnell *et al.*, 1990; Bushnell and Brill, 1992). It is further hypothesized that the large range of heart rates reported for tuna (35 to 250 bpm) (Kanwisher *et al.*, 1974; Brill, 1987; Bushnell and Brill, 1991; Chapter IV), is sufficient to account for all the modulation of cardiac output necessary to achieve maximal oxygen consumptions (Farrell *et al.*, 1992). This is in contrast to most other fishes, which increase stroke volume by an equal or greater extent than heart rate in order to elevate cardiac output (Farrell and Jones, 1992).

An alternative hypothesis is that stroke volume increases two-fold, along with elevations in heart rate, during exercise in tuna (Bushnell and Jones, 1994). This view is based on the wide range of stroke volumes measured in different tuna species, under various experimental conditions (anesthetized, spinalized, or slow swimming).

In addition to the changes in oxygen demand with swimming velocity, the cardiovascular system of tunas must maintain adequate performance during a wide range of ambient temperatures. Yellowfin tuna (*Thunnus albacares*) make regular rapid excursions between the warm surface waters and the cooler waters of the upper thermocline; spanning about 10°C (Holland *et al.*, 1990). Although tuna maintain elevated red muscle temperatures which limit the effect of ambient temperature changes on this tissue (Dewar *et al.*, 1994), the heart is subjected to the full range of ambient conditions (Chapter IV). A failure to maintain cardiac output during drops in ambient temperature may result in a disparity between oxygen demand and delivery to the warm muscles of tuna.

The objectives of this study were to determine how stroke volume is altered during exercise and acute temperature change, by measuring *in vivo* changes in cardiac output in unanesthetized and unrestrained yellowfin tuna, swimming in a large water tunnel. These data show how heart rate and stroke volume contribute to the changes in oxygen demand

associated with swimming velocity and variations in ambient temperature that may be encountered in the wild.

Materials and methods

Experimental animals and equipment

Yellowfin tuna (1430 - 2470 g, mean = 1768 ±131.3 g, 42.5 - 53.5 cm, mean = 46.2 ±1.4 cm fork length, FL) were purchased from local commercial fishermen and maintained at ambient temperature (24 - 26°C) in large outdoor tanks (76,000 l) at the Kewalo Research Facility, Honolulu, Hawaii (Southwest Fisheries Science Center Honolulu Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration) (Nakamura, 1972). The tuna were fed once daily, *ad libitum*, with chopped squid and fish, but food was withheld at least 20 h prior to a study to allow for gut clearance (Magnuson, 1969). All fish were healthy, feeding, and in captivity for at least 1 week, but not more than 6 weeks.

Experiments were conducted using the large (3000 l) water tunnel described by Dewar and Graham (1994a). The swimming section was 113 cm long, 32.5 cm high, and the width was adjusted to either 22.5 cm or 28 cm depending on fish size. Ambient oxygen content and temperatures were continuously monitored with a YSI (Yellow Springs Instruments) temperature-compensated oxygen probe (5450/5758, model 54A meter) mounted in a flow-through cuvette, and a thermocouple within the water tunnel connected to a Physi-Temp digital thermometer (model BAT-12), respectively.

Anesthesia and instrumentation

Fish handling and anesthesia procedures are described in Dewar and Graham (1994a) and Chapter I. Yellowfin tuna were quickly subdued in oxygenated seawater containing 1.0 g/l of tricaine methanesulfonate (Finquel®, Argent Chemical Laboratories)

buffered with $NaHCO_3$ or Trizma (Sigma, T-1503). Anesthesia was maintained during instrumentation by ventilation with oxygenated seawater at 0.100 to 0.077 g tricaine/l.

The fish were fitted with a transcutaneous Doppler blood-flow probe over the ventral aorta. This type of flow probe was chosen because it is non-invasive, reducing stress and surgical time. The subminiture piezoelectric transducer (Titronics Medical Instruments, Iowa City, Iowa) was mounted with silicone cement to a patch of latex rubber (approx. 3 cm^2 and 1 mm in thickness). This patch was glued (transducer side down) to the epithelial lining of the gill cavity, directly over the ventral aorta, with Vetbond™ tissue adhesive (3M) (Bushnell *et al.*, 1990). The Doppler probe leads were sutured in place, along the side of the fish, and trailed off the second dorsal fin. The attachment procedure was completed in 10 to 20 min.

The blood-flow probe was driven by a directional pulsed Doppler flowmeter (model 545C-4, Bioengineering, The University of Iowa). The instantaneous blood flow signal (Fig. 1) was digitized at 50 Hz and recorded to computer hard disk (Axotape and Cyberamp, Axon Instruments). Zero flow was verified during brief, spontaneous, periods of bradycardia (Fig. 1). Attempts to calibrate the flow probe postmortem were unsuccessful; therefore cardiac output is reported in relative measurements.

Experimental protocol

Following surgery and the return of swimming ability (~20 min after removal of anesthesia), the velocity was adjusted to find a stable swimming speed for the fish (between 0.8 to 1.5 FL/s). Two hours were allowed for recovery from anesthesia and acclimation to the water tunnel. Swimming exercise was induced by increasing water velocity step-wise by 5 to 15 cm/s. Upon reaching the new velocity, blood flow was recorded during 5 min of stable swimming or until the fish could no longer maintain position in the water tunnel. After a series of velocity increases, velocity was reduced to a

resting level, which was maintained until cardiac variables stabilized. The velocity test was followed by an acute change in temperature.

Acute temperature change experiments were conducted by rapidly introducing hot (40°C) or cold (2°C) seawater from external reservoirs into the water tunnel until the desired experimental temperature was achieved (~1.0 °C/min). Temperature was first reduced from 24-25°C to 18°C (in two initial experiments the temperature was reduced to only 20°C), and then increased to 28°C. Blood-flow recordings were made over 10 min at each temperature at a constant swimming velocity

Data analysis

Heart rate and relative cardiac output were determined by counting the peaks in blood flow, and averaging instantaneous flow and over one minute intervals, respectively (DADiSP Worksheet, DSP Development Corporation). Stroke volume was calculated by dividing cardiac output by heart rate. Because the transcutaneous Doppler flow probe measures blood velocity, changes in cardiac output and stroke volume were calculated assuming the cross-sectional area of the ventral aorta was constant. Although the increases in mean blood-pressure during exercise (Chapter VI) will increase vessel cross-sectional area, potentially resulting in an underestimation of the change in flow, this change is predicted to be less than 5% based on pressure-volume and pressure-diameter curves obtained from yellowfin ventral aortae (Bushnell *et al.*, 1992; R. E. Shadwick, unpublished observations).

Significant difference from values at the lowest velocity (exercise tests) or 24-25°C (temperature tests) were tested with ANOVA and a post-hoc multiple comparison test. Q_{10} values were determined from mean values at each temperature. A fiducial limit of 5% was used for all statistical tests. Mean values are reported with standard error of the mean (S.E.M.) unless otherwise noted.

Results

Exercise

Figure 2 shows the effects of velocity on cardiac output, heart rate, and stroke volume in seven yellowfin. Heart rate accounted for all of the increase in cardiac output during exercise. The mean initial swimming speed was 1.23 ±0.08 FL/s, increasing to 2.10 ±9.3 FL/s. Heart rate increased by an average 18.8 ±5.4%, from 67.7 ±7.1 to 80.5 ±9.3 bpm. Stroke volume decreased by 3.9 ±2.3%, resulting in increase in cardiac output of only 13.6 ±3.0%. The effects of exercise on stroke volume varied among individual fish, with significant decreases in four (Fig. 2A,C,E,G), no change in two (Fig. 2B,D), and an initial increase in one, although at the highest velocity it was not significantly different from the initial value (Fig 2F). This same fish was the only one found to have a dorsal-aortic infection with the larval cestode, *Dasyrhynchus talismani* (Brill *et al.*, 1987).

Temperature

The effects of temperature on cardiac output, heart rate, and stroke volume in six yellowfin tuna are shown in Fig. 3. Changes in both cardiac output and heart rate were directly related to temperature. Stroke volume increased with a decrease in temperature from 24-25°C to 20 or 18°C in all fish. With increases in temperature from 24-25°C to 28°C, stroke volume decreased in three fish (Fig. 3C,D,E), did not change significantly in two (Fig. 3A,F), and in one it increased slightly (7%, Fig. 3B). Q_{10} values for cardiac output, heart rate, and stroke volume were not significantly different over the two temperature ranges (paired t-test) and averaged 1.52 ±0.08, 2.16 ±0.25, and 0.78 ±0.07, respectively.

Discussion

Cardiac performance during exercise

Although Keen *et al.* (1995) suggested that use of a Doppler flow probe resulted in elevated heart rates in tuna, we did not find this to be the case in our study. Heart rates were very similar to previous measurements on swimming yellowfin that had never been anesthetized (Chapter IV). For example, at the mean initial (1.23 FL/s) and maximal velocity (2.10 FL/s), heart rates averaged 67.7 and 80.5 bpm, respectively. This compares with 72.0 and 87.6 bpm determined from the heart rate-velocity regression for yellowfin (Chapter IV). Previous measurements of heart rate, stroke volume, and cardiac output in tuna are reported in Table 1. The heart rates measured in this study are generally lower than those from both non-swimming and swimming tuna for which stroke volume has also been measured (Table 1).

Increases in heart rate during exercise in the yellowfin tuna had the greatest influence on cardiac output, increasing blood flow, often despite opposing changes in stroke volume (Fig. 2). In general, stroke volume either did not change, or declined initially, with little change during further increases in velocity (Fig. 2). Intrinsic regulation of cardiac stroke volume occurs through the Starling mechanism: myocardial stretching caused by an increased end-diastolic volume (preload) will augment the force of contraction, increasing stroke volume. Increases in stroke volume through a decreased end-systolic volume appear to be less important in fishes than mammals, with the normal ventricular ejection fraction nearly complete (Franklin and Davie, 1992). The end-diastolic volume is a function of venous filling pressure and filling time. In the yellowfin, there appears to be an inverse relationship between heart rate and stroke volume. The fish with the largest increases in heart rate exhibited the largest decreases in stroke volume (Fig. 2). This can be explained by the influence of filling time on end-diastolic volume, limiting stroke volume at higher contraction frequencies.

Maintenance of stroke volume in some of the tuna, and following the initial drop in others, may occur through an elevated venous filling pressure associated with the increase in muscular activity, and a decreased vascular resistance (Chapter VI). Central venous pressures increase during swimming in leopard shark (*Triakis semifasciata*) (Lai *et al.*, 1990) and rainbow trout (*Oncorhynchus mykiss*) (Kiceniuk and Jones, 1977) which likely contributes to the increase in stroke volume in these species. In addition, filling of the heart by suction, or "vis-a-fronte" mechanism, may assist the maintenance of stroke volume. Using *in vitro* perfused yellowfin hearts, Farrell *et al.* (1992) showed that physiological stroke volumes could be obtained with sub-ambient filling pressures, suggesting that this is an important mechanism in tuna. In addition, sub-ambient pericardial pressures were measured in anesthetized albacore (*Thunnus alalunga*) (Lai *et al.*, 1987), however, as these fish were not swimming, the contributions of muscular contractions to venous return were absent, possibly abnormally reducing central venous pressure.

The results for yellowfin tuna support the hypothesis of Farrell (1991; Farrell *et al.*, 1992) that cardiac output is increased primarily through heart rate. This is different from most other fish species studied, in which increases in cardiac output during exercise occur through the contributions of both an elevated stroke volume and heart rate. Depending on species and acclimation temperature, stroke volume accounts for 30 to 80% of the increases in cardiac output in other teleosts (Farrell, 1991; Kolok *et al.*, 1993; Kolok and Farrell, 1994). However, another group of fishes, the red-blooded Antarctic nototheniids, appears to regulate heart rate much more than stroke volume (Axelsson *et al.*, 1992). Preliminary stroke volume measurements on swimming yellowfin suggested that, like most other fishes, stroke volume increased with exercise (Korsmeyer *et al.*, 1993; Graham *et al.*, 1994). However, further examination revealed significant increases in stroke volume during recovery from anesthesia that masked exercise effects if velocity was increased too soon after surgery (unpublished observations). Although stroke volume may increase in

some circumstances (Fig. 2F), the present study suggests that heart rate is the most important contributor to increased cardiac output during exercise in tuna.

Measurements of absolute stroke volume in tuna under various conditions cover a two-fold range (Table 1). Although cardiovascular variables in spinally-blocked tuna have been considered to be "resting" values (Brill and Bushnell, 1991b; Bushnell and Brill, 1992), it has been shown that this preparation results in values for total oxygen consumption, heart rate, and cardiac output that exceed those in swimming tuna (Bushnell and Brill, 1991; Jones *et al.*, 1993; Dewar and Graham, 1994a; Chapter IV). Similarly, the 1.3 ml/kg stroke volume measured in spinalized yellowfin (Table 1) may not represent those in swimming fish. *In vitro* perfused heart preparations were unable to achieve equivalent stroke volumes (Table 1), most likely due to insufficient oxygen delivery to the myocardia (the coronary artery was not perfused) (Farrell *et al.*, 1992). The lower heart rates in swimming yellowfin may permit higher stroke volumes through increased filling time, although the high variability in heart rate suggests that stroke volume may be equally variable (Chapter IV). Stroke volume in swimming kawakawa (0.54 ml/kg) is half that reported for spinalized skipjack or yellowfin, although at similar heart rates (Table 1) (Jones *et al.*, 1993). The absolute range of stroke volume and cardiac output in swimming tunas remains to be determined.

It has been proposed that the tuna heart is adapted as a high-pressure pump, moving small volumes of blood at high rates (Agnisola and Tota, 1994). Ventral aortic blood pressures in yellowfin are high relative to other fishes, and increase with exercise (see Chapter VI). To generate these pressures, the tuna heart is relatively large and has a high percentage of compact myocardium (Farrell and Jones, 1992). Cardiac stroke work is the product of stroke volume and the pressure against which the blood is pumped (afterload). Increases in afterload (i.e., ventral aortic pressure) during exercise may limit increases in

stroke volume. This will minimize the increase in stroke work, and cardiac output can be increased instead through higher heart rates (Agnisola and Tota, 1994).

In addition, the lack of a stroke volume increase may be explained in part by the absence of adrenergic stimulation in tuna at the levels of exercise observed in this study. The intrinsic heart rate in yellowfin at 25°C is 119 bpm, and the lower "basal" rates in spinalized tuna are under a large cholinergic (inhibitory) tonus and a very low level of adrenergic stimulation (Keen *et al.*, 1995). Therefore, the increases in heart rate observed during prolonged exercise can be achieved solely through a reduced vagal inhibition, suggesting that adrenergic stimulation is less important in tuna than other teleosts (Keen *et al.*, 1995; Chapter IV). Cholinergic effects on heart contractility in fishes have not been determined, but it is suggested that adrenergic stimulation has a greater affect on the contractility of the ventricle (Farrell, 1984; Farrell and Jones, 1992). Without adrenergic stimulation during prolonged swimming, stroke volume may be limited.

It is likely that without the influence of potential adrenergic stimulation, the antagonistic affects of filling time on cardiac output will be more severe at higher heart rates. Heart rates in tuna have been reported as high as 240 bpm, and in yellowfin specifically, 160 bpm at 25°C (Kanwisher *et al.*, 1974; Brill, 1987; Chapter IV). In addition, myocardial contraction force may be reduced at high heart rates. For example, peak tension development in skipjack atrial muscle strips decreases at stimulation frequencies above 1.6 Hz, or the equivalent of 96 bpm (Keen *et al.*, 1992). However, exceptionally high heart rates, above intrinsic values, must be achieved through increased circulating catecholamines or direct sympathetic nervous stimulation during intense exercise or stress, and these mechanisms may also serve to maintain or increase stroke volume by increasing myocardial contractility (Farrell and Jones, 1992).

Cardiac performance during acute temperature change

As in the response to exercise, changes in heart rate had the greatest influence on cardiac output in yellowfin tuna during acute temperature change (Fig. 3). The Q_{10} for heart rate in this study (2.16) is similar to that for other fishes and for swimming yellowfin that were never anesthetized (Q_{10} = 2.37) (Farrell, 1984; Chapter IV). The lower Q_{10} for cardiac output (1.52) reflects the opposing changes in stroke volume.

The inverse relationship between heart rate and stroke volume during acute temperature change has been demonstrated with *in vitro* fish hearts, resulting in no change (Bennion, 1968; Yamamitsu and Itazawa, 1990) or an increase in cardiac output with an increase in temperature (Graham and Farrell, 1985). Heart rate changes are attributed to the direct effect of temperature on the pacemaker cells (Randall, 1970), and, as a decrease in temperature does not directly affect myocardial force development (Driedzic and Gesser, 1994), the opposing changes in stroke volume reflect alterations in filling time. *In vivo* cardiac responses to acute temperature change in teleosts show that heart rate dominates changes in cardiac output. With increasing temperature, both heart rate and cardiac output increase, with little change in stroke volume, in the lingcod (*Ophiodon elongatus*) and flounder (*Pseudopleuronectes americanus*) (Stevens *et al.*, 1972; Cech, 1976), or in the case of the red-blooded Antarctic fish, *Pagothenia bernacchii*, it caused a slight decrease in stroke volume (Axelsson *et al.*, 1992).

The Q_{10} measured for cardiac output (1.52) is close to the Q_{10} for oxygen consumption in swimming yellowfin (1.67; Dewar and Graham, 1994a), suggesting that changes in cardiac output can almost fully compensate for variations in metabolic rate due to temperature change. It should be noted, however, that the Q_{10} of oxygen consumption was determined at relatively slow velocities (~1 FL/s) and after 30 min of acclimation to allow for stabilization of red muscle temperature (Dewar and Graham, 1994a). With rapid drops in ambient temperature, the vascular counter-current heat exchangers reduce the rate

of heat loss from the red muscle, and increase the excess temperature above ambient (Dewar *et al.*, 1994). Thus, the resulting temperature change experienced by the red muscle will be less than the ambient change experienced by the heart (Dewar *et al.*, 1994; Chapter IV). This disparity will be particularly evident at higher swimming velocities, when metabolism of the warmed red muscle will have the greatest contribution to total oxygen consumption (Korsmeyer *et al.*, 1996b), effectively reducing Q_{10} under these conditions. Without compensatory changes in blood flow, maximal swimming performance may be limited during the tuna's rapid movements through the thermocline.

Although it has been suggested that stroke volume is maximal, and therefore relatively fixed, at 25°C in tuna (Farrell *et al.*, 1992), decreases in heart rate with temperature were accompanied by an increase in stroke volume (Fig. 3). This indicates that the anatomical limits of ventricular volume have not been reached (i.e., there is a preload reserve). These results differ from the response to hypoxia in yellowfin, in which bradycardia occurred with no increase in stroke volume (Bushnell *et al.*, 1990; Bushnell and Brill, 1992). The lack of a stroke volume change in spinalized tuna may be due to the higher heart rates, and therefore reduced filling time, caused by that preparation (Bushnell *et al.*, 1990; Bushnell and Brill, 1992). However, preliminary results suggest that, despite lower heart rates, stroke volume does not increase during hypoxia in swimming tuna (Korsmeyer *et al.*, 1996b). In hypoxia, peripheral vascular resistance increases (Bushnell and Brill, 1992), which may reduce venous filling pressure, negating the positive effect of an increased filling time. In addition, tuna hearts are dependent on coronary oxygen delivery in order to maintain performance (Farrell *et al.*, 1992) and a reduced arterial oxygen saturation in hypoxia may adversely affect cardiac output.

Conclusions

Our results support the hypotheses of a primarily frequency-modulated regulation of cardiac output in tuna (Farrell, 1991), and that the tuna heart is adapted for the development of high pressure at relatively high rates and low volumes (Agnisola and Tota, 1994). However, increases in stroke volume during acute temperature change suggest that the lack of an increase during exercise is not due to the heart operating at maximal anatomical limits (Farrell, 1991; Farrell *et al.*, 1992). The increases in stroke volume during a drop in temperature partially offset the decline in cardiac output caused by the decrease in heart rate. As a result, cardiac output appears to fully compensate for changes in oxygen demand during slow swimming and steady-state conditions.

The results of this study are for a relatively narrow range of exercise, and further work is needed to determine cardiac function during intense exercise or other conditions (e.g., oxygen debt recovery) when total oxygen consumption may be highest, and therefore the demands of the cardiovascular system will be greatest.

Table V-1. Heart rate (f_H), stroke volume (SV), and cardiac output ($\dot{Q}$) measurements from tunas at 25°C

Species	f_H bpm	SV ml/kg	$\dot{Q}$ ml/kg/min	Condition	References
Yellowfin	96.7	1.3	115.4	spinally-blocked	Bushnell and Brill, 1992
	137.6	0.87	108.0	*in vitro* perfused heart, maximum	Farrell *et al.*, 1992
	112	0.67	71.9	anesthetized	Jones *et al.*, 1993
Skipjack	125.9	1.1	132.3	spinally-blocked	Bushnell and Brill, 1992
	154	0.68	84.5	*in vitro* perfused heart, maximum	Farrell *et al.*, 1992
Kawakawa	128	0.54	67	swimming, 1.3 FL/s	Jones *et al.*, 1993

Skipjack (*Katsuwonus pelamis*), kawakawa (*Euthynnus affinis*)

Figure V-1

Instantaneous blood flow trace from a swimming yellowfin tuna (1.73 FL/s). Arrow indicates verification of zero flow during spontaneous bradycardia.

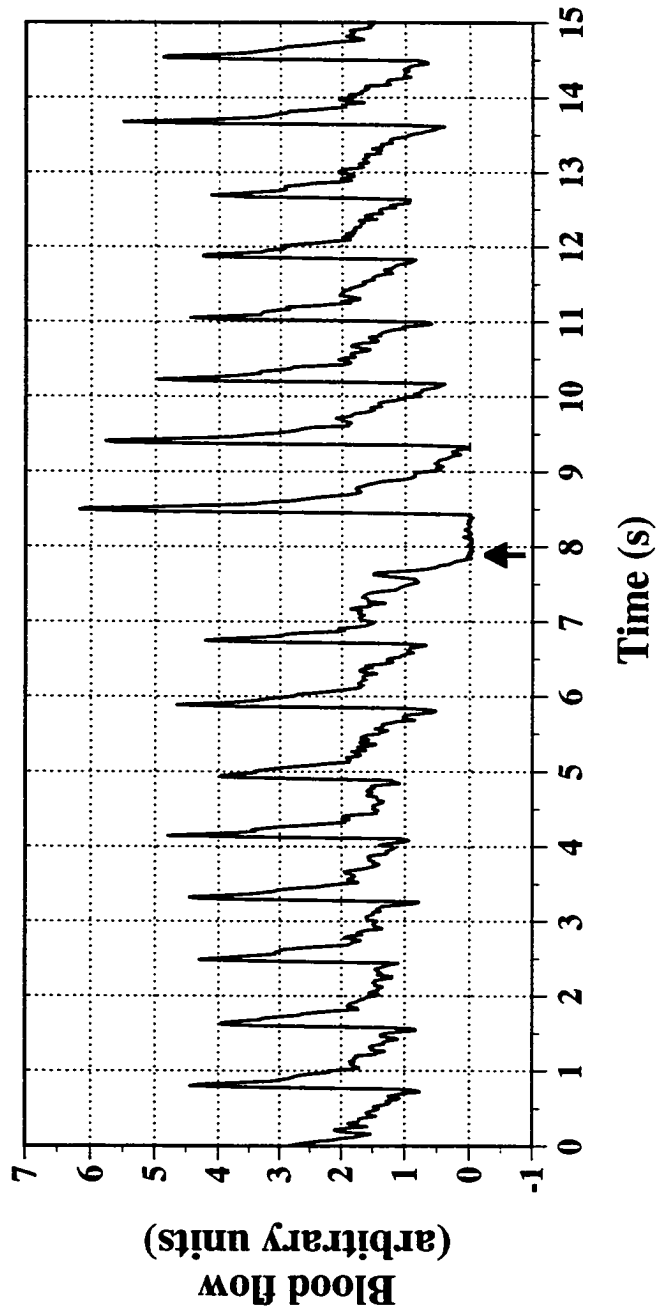


Figure V-2

Relative changes in heart rate (f_H, ■), cardiac output ($\dot{Q}$, O) and stroke volume (SV, ▲) with increases in swimming velocity in seven yellowfin tuna at 24–25°C. Mean values at the lowest velocity were set to a value of 1.0 for each fish. Error bars are standard deviation. Note the change in the ordinate and coordinate scaling in (G). Initial mean heart rates (bpm) for each fish were: A) 108.3, B) 66.6, C) 49.6, D) 60.3, E) 59.5, F) 66.2, G) 63.5. *indicates significant difference from lowest velocity value.

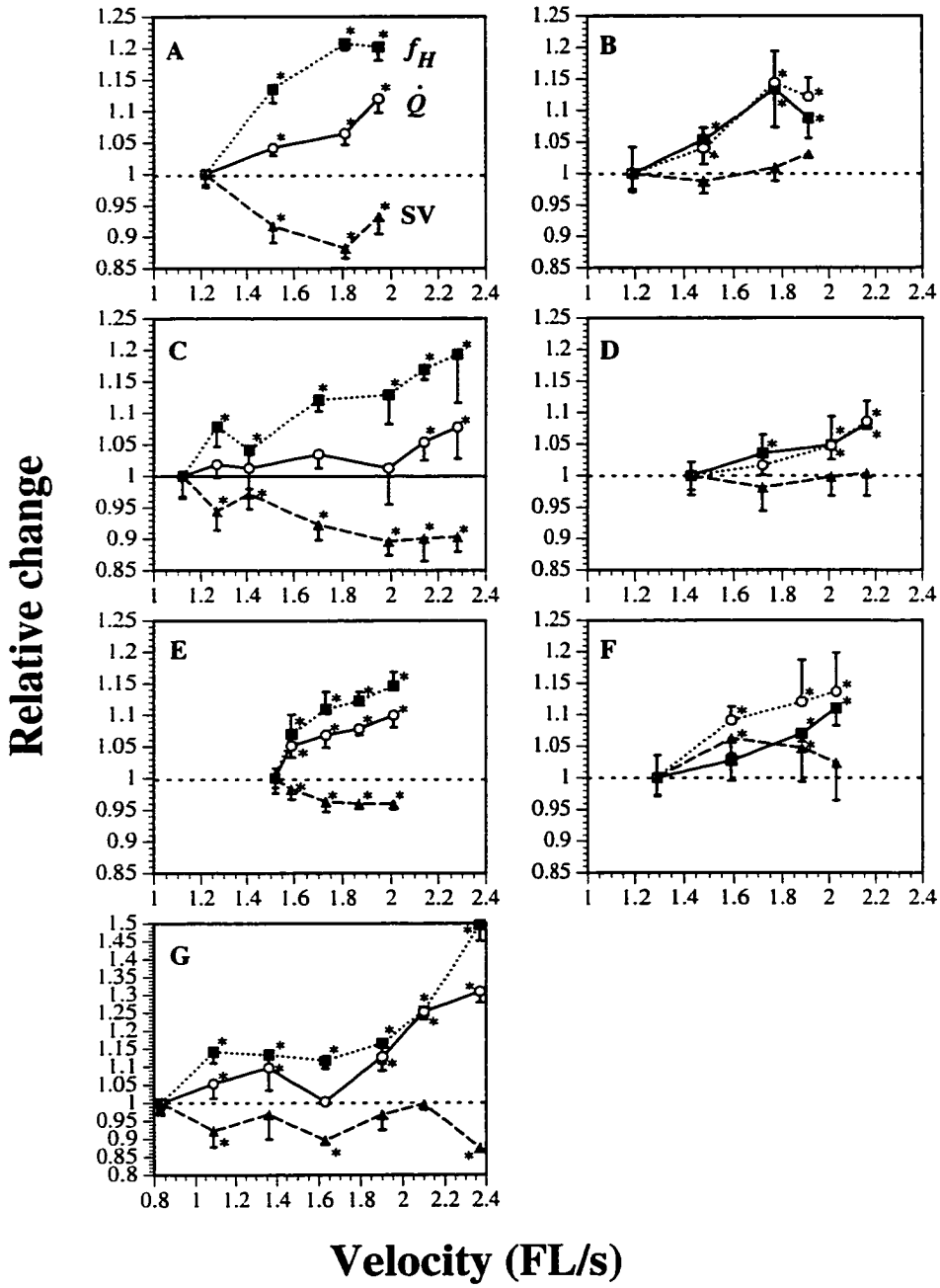
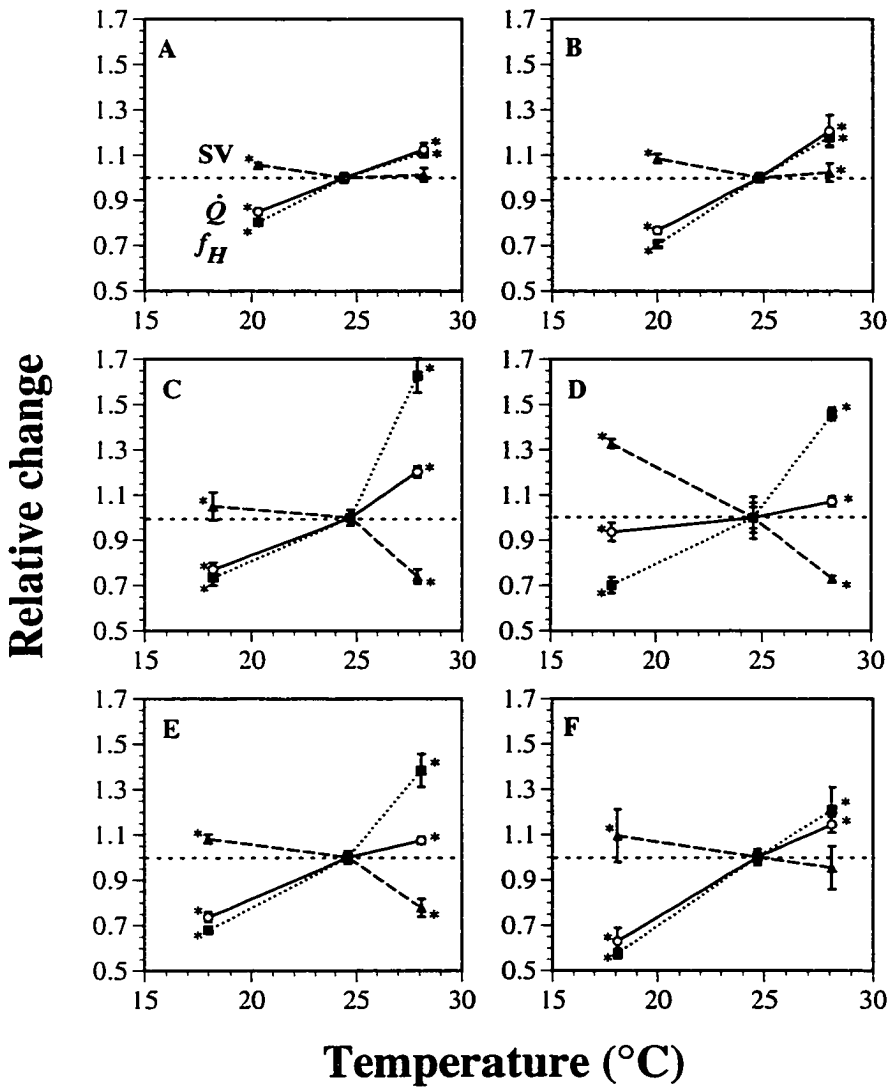


Figure V-3

Relative changes in heart rate (f_H, ■), cardiac output ($\dot{Q}$, O) and stroke volume (SV, ▲) with acute changes in ambient temperature in six yellowfin at constant swimming velocities. Mean values at 24-25°C were set to a value of 1.0 for each fish. Error bars are standard deviation. Swimming velocities and initial mean heart rates for each fish were: A) 1.51 FL/s, 122.7 bpm; B) 1.19 FL/s, 65.5 bpm; C) 1.12 FL/s, 49.6 bpm; D) 1.72 FL/s, 66.5 bpm; E) 1.59 FL/s, 63.5 bpm; F) 1.29 FL/s, 66.2 bpm. *indicates significant difference from 24-25°C value.



CHAPTER VI: OXYGEN TRANSPORT AND CARDIOVASCULAR RESPONSES TO EXERCISE IN THE YELLOWFIN TUNA

Abstract

Yellowfin tuna (1400-2175 g) instrumented with ECG electrodes and pre- and post-branchial catheters were subjected to incremental swimming velocity tests. Increasing velocity, from a minimal speed of 1.0 FL/s, resulted in a 1.4 fold increase in heart rate (61.4 to 84.6 bpm), an elevated ventral-aortic blood pressure (10.8 to 12.2 kPa), and a decreased systemic vascular resistance. Relative branchial vascular resistance at minimal speed ranged from 24.4 to 40.0% of total vascular resistance and increased with velocity. Yellowfin blood has a high oxygen carrying capacity (16 to 18 ml O_2/dl), and a low *in vivo* oxygen affinity (P_{50} = 5.3 kPa). Exercise caused a rise in arterial saturation (74 to 88%) and a decline in venous saturation (48 to 44%), resulting in a 1.3 fold increase in tissue oxygen extraction from the blood (arterial-venous oxygen content difference). Whereas arterial oxygen partial pressure (P_{O_2}) increased with exercise, venous P_{O_2} remained unchanged (~5.3 kPa). Thus, the observed decrease in venous oxygen content was brought about by a lowered blood pH (7.80 to 7.76) and a large Bohr shift. The large venous oxygen reserve still available to yellowfin at maximal prolonged velocities suggests that the maximal oxygen delivery potential of the cardiovascular system in this species is not fully utilized during aerobic swimming. This reserve may be needed during forays into deeper, hypoxic waters, during recovery from oxygen debt, or for sustaining other aerobic metabolic processes in addition to continuous swimming.

Introduction

The yellowfin tuna (*Thunnus albacares* [Bonnaterre]), is a large, tropical, pelagic predator with a high metabolic rate, and numerous anatomical and morphological adaptations for high-performance swimming (Magnuson, 1978; Dewar and Graham, 1994a, b; Brill, 1996). In addition, all tuna need to swim continuously to provide hydrodynamic lift and to ram ventilate their gills (Magnuson, 1978; Roberts, 1978). High metabolic capacities in tuna are supported by elevated anaerobic and aerobic enzyme activities (Dickson, 1995), partial endothermy (Carey *et al.*, 1971; Dewar *et al.*, 1994), and by cardiorespiratory adaptations for enhanced oxygen uptake and transport (Brill and Bushnell, 1991b; Bushnell and Jones, 1994). Tuna cardiorespiratory specializations include a large gill surface area and thin gill epithelium (Hughes, 1984), a large relative heart mass, high cardiac output, and a high blood oxygen-carrying capacity (Brill and Bushnell, 1991a; Bushnell and Brill, 1992; Farrell and Jones, 1992; Jones *et al.*, 1993).

In addition to supplying oxygen for a high standard metabolism, the cardiorespiratory system of a tuna must also increase oxygen delivery to its swimming musculature during increases in aerobic (sustainable) swimming speed. Increased muscle oxygen demand could be supplied by either or both an increased cardiac output ($\dot{Q}$) or an increased oxygen extraction from the blood, as described by the Fick equation:

$$\dot{V}_{O_2} = \dot{Q} \cdot (a - v)_{O_2} \qquad (1)$$

where $\dot{V}_{O_2}$ is total body oxygen demand, $\dot{Q}$ is the product of heart rate (f_H) and stroke volume (SV), and $(a - v)_{O_2}$ is the difference between arterial and venous oxygen content.

Although extensive cardiorespiratory measurements were made on paralyzed (spinally-blocked) tuna by Bushnell and Brill (1992), studies of swimming tuna have been limited to only a few variables from fish in which control of velocity, and therefore oxygen demand, was impossible (Jones *et al.*, 1986; Bushnell and Brill, 1991; Jones *et al.*, 1993). Consequently, tuna cardiorespiratory performance during exercise has remained a matter of

speculation (Brill and Bushnell, 1991b; Farrell, 1991; Bushnell and Jones, 1994). Brill and Bushnell (1991b), for example, estimated that the maximal aerobic metabolic rate of tuna is 8 to 9 times higher than its standard metabolic rate and suggested this increase is achieved by a 3 to 4 fold increase in oxygen extraction [$(a - v)_{O_2}$]. Thus, the elevated oxygen demands during exercise could be met by utilizing the high oxygen carrying capacity of the blood, without an extraordinary increase in $\dot{Q}$.

Recently, the development of a water tunnel for tunas has enabled *in vivo* physiological measurements under controlled swimming conditions (Dewar and Graham, 1994a; Dewar *et al.*, 1994; Chapter IV). The objectives of this paper are to report measurements of both pre-branchial (venous) and post-branchial (arterial) hemodynamic, blood-gas, and hematological variables in unrestrained and unanesthetized yellowfin tuna, and to examine the relationship between exercise (i.e., swimming velocity) and tissue oxygen extraction. This study is one of only a few to measure changes in both arterial and venous blood oxygen contents during prolonged swimming in fishes.

Materials and methods

Experimental animals and equipment

Yellowfin (mean mass 1850 ±71 g, range 1400 - 2175 g, mean fork length [FL] 47.6 ±0.6 cm, range 43 - 50 cm) were purchased from local commercial fishermen and maintained at ambient temperature (24 - 26°C) in large outdoor tanks (76,000 l) at the Kewalo Research Facility, Honolulu, Hawaii (Southwest Fisheries Science Center Honolulu Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration) (Nakamura, 1972). The tuna were fed once daily, *ad libitum*, with chopped squid and fish. Experimental subjects were not fed for approximately 20 h prior to use to allow for gut clearance (Magnuson, 1969). All fish were healthy, feeding, and in captivity for at least 1 week, but not more than 6 weeks.

Experiments were conducted using the large (3000 l) water tunnel described by Dewar and Graham (1994a). The size of the swimming section was fixed at 113 x 22.5 x 32.5 cm (length, width, height). Ambient oxygen content (6.9 to 8.0 mg O_2/l, 20 to 23 kPa O_2) and temperatures (25 ±1°C) were continuously monitored with a YSI (Yellow Springs Instruments) temperature-compensated oxygen probe (5450/5758, model 54A meter) mounted in a flow-through cuvette, and a thermocouple within the water tunnel connected to a Physi-Temp digital thermometer (model BAT-12), respectively.

Anesthesia and instrumentation

Anesthesia and surgical procedures were similar to those described in Jones *et al.* (1986). Tuna were quickly dip-netted from the holding tank and rapidly subdued in a plastic bag of oxygenated seawater containing 1.0 g/l of tricaine methanesulfonate (MS 222, Finquel®, Argent Chemical Laboratories) buffered with $NaHCO_3$ or Trizma buffer (Sigma, T-1503). After about 1 min, the tuna was prepared for surgery by transferring it to a chamois cradle where it was ventilated with a reduced concentration of anesthetic (0.057 g/l) in cooled, oxygenated seawater (~24 kPa O_2, 23°C).

The dorsal and ventral aortae were catheterized using 18 gauge thin-walled needles advanced percutaneously under manometric guide. The dorsal vessel was entered through the roof of the mouth and the ventral aorta was accessed through the ventral body wall. Once access to the vessel was confirmed by pulsatile pressure recordings, a heparinized (100 I.U.), saline-filled PE 50 tubing was advanced through the hub and beyond the tip of the needle into the vessel. The dorsal aorta was catheterized beyond the confluence (1 to 2 cm) of the efferent branchial arteries of the first and second gill arches, and the catheter was fed 2 to 3 cm down the vessel so that its tip was posterior of the union of the third and fourth branchial arteries. The ventral-aortic catheter entered the bulbus arteriosus and was fed 1.5 to 2 cm anteriorly into the ventral aorta. After proper placement was confirmed by

checking blood pressure and the ease of blood aspiration, the needle was carefully withdrawn and passed over the end of the tubing, leaving the catheter in place. The dorsal-aortic catheter was sutured to the roof of the mouth and passed out through the snout via a hole formed by an 18 gauge needle.

Electrocardiogram (ECG) electrodes (30 gauge insulated wire) were inserted subcutaneously adjacent to the ventricle. The two catheters and ECG wires were secured with sutures along the body of the fish leading to the second dorsal fin, and the free ends were tied together at regular intervals to prevent entanglement once the fish was swimming. The catheters and ECG leads were long enough to extend from the fish to outside the water tunnel (approximately 150 cm). The entire procedure was completed within 30 min, as it was found that longer times under anesthesia resulted in poor recovery (see Chapter I).

Following surgery, the anesthetic was diluted with fresh seawater until the fish exhibited the return of tail and fin movements. At this point the tuna was carefully lowered into the swimming section of the water tunnel, and the ventilation hose was removed. The tuna was gently held mid-stream (water speed 40 to 70 cm/s) to maintain sufficient ventilation until swimming ability returned (15 to 75 min). The catheters and ECG leads were fed through an open slot in the hatch of the swimming section. Fish handling and water tunnel procedures are described further in Dewar and Graham (1994a).

Experimental protocol

Following the return of swimming ability, the velocity was adjusted to approximately 1 FL/s, the predicted minimal swimming speed for hydrodynamic equilibrium (Magnuson, 1978). Blood pressure and ECG recordings were taken after 15 min, followed by an initial post-anesthesia blood sample. Recordings were made every 30 min and blood samples were taken each hour for 2 h during recovery from anesthesia and acclimation to the water tunnel. The final sample of this series (taken 2 h after the first

sample) is considered the pre-exercise control value. A 2 h recovery time has been shown to be sufficient for tuna to restore acid-base equilibrium, clear lactate buildup, and stabilize cardiorespiratory parameters following anesthesia (Perry *et al.*, 1985; Bushnell *et al.*, 1990; Arthur *et al.*, 1992; Bushnell and Brill, 1992). This period of time is also sufficient for stabilization of $\dot{V}_{O_2}$ in yellowfin, following introduction into the water tunnel (Dewar and Graham, 1994a).

The recovery period was followed by an incremental velocity test (Beamish, 1978), with increases of approximately 0.25 FL/s (10 to 14 cm/s). The new velocity was maintained for 1 h and then was increased again. This procedure was repeated until the fish could no longer maintain position in the swimming section. When the tuna fell against the downstream screen, velocity was temporarily reduced. If the fish continued to swim after the velocity was increased to the previous level, then the first failure was ignored. However, at the second failure to maintain position, velocity was reduced to some intermediate velocity that permitted stable swimming for a 1 h recovery period.

Pressure and ECG recordings were made at 30, 45 and 59 min of each 1 h velocity period. Blood samples were taken at 50 min, or if the velocity could not be sustained for the entire hour period, a blood sample was taken before reducing velocity.

Following the experiment, blood samples were taken from each fish to determine oxygen carrying capacity as described in Lai *et al.* (1990). The correct positioning of the catheters was verified post-mortem.

Analytical procedures

Blood pressure was recorded with calibrated Statham pressure transducers (Gould model P231D), with zero determined through a PE tubing connection to the sea water in the tunnel. Pressure signals were amplified (Gould model 13-4615-50) and recorded on a strip chart recorder (Gould model 2400) as was the ECG signal (Grass model P15D and Gould

model 13-4615-58). The pressure and ECG signals were simultaneously digitized (200 Hz) and saved to computer hard disk (486 PC-compatible, Axotape data acquisition system, Axon Instruments).

Venous and arterial blood samples (0.8 ml) were drawn in sequence for immediate analysis of hematological and blood-gas parameters. An additional 1 ml sample was taken less frequently for a separate study on catecholamine levels (Lai *et al.*, 1994). The size and large blood volume of these fish resulted in a total blood loss of less than 10% (based on a blood volume of 8% of body mass; Laurs *et al.*, 1978). Blood oxygen and carbon dioxide partial pressures (P_{O_2} and P_{CO_2}) and pH were determined using a Radiometer blood gas microsystem (BMS-3MK2) thermostatted at 25°C. The P_{O_2} electrode was calibrated with a standard zero-P_{O_2} solution and air-saturated water. Precision-mixed gases for calibration of the P_{CO_2} electrode were available for only a few experiments. The pH electrode was calibrated with Radiometer precision buffer solutions.

Total O_2 content (C_{O_2}) was determined as described by Tucker (1967). A small sample of blood (7 µl) was introduced into a chamber filled with ferricyanide and monitored with an oxygen electrode to record P_{O_2}. The hemoglobin in the blood is converted to the methemoglobin form by the ferricyanide, releasing bound oxygen into solution. The resulting P_{O_2} change is then a measure of C_{O_2}.

Hematocrit (Hct) was determined by centrifugation and hemoglobin concentration (Hb) was measured using a Radiometer OSM2b Hemoximeter. The remaining blood was centrifuged and the plasma immediately frozen on dry ice for transfer back to Scripps Institution of Oceanography, La Jolla, CA (within 4 weeks) where it was stored at -80°C until analyses were made of lactate and glucose concentrations (YSI Stat2000).

Data analysis and statistics

Mean ventral and dorsal-aortic blood pressures (BP_{va} and BP_{da}) were calculated by averaging the digitized pulsatile recording (DADiSP Worksheet, DSP Development Corporation). Typical frequency response for the system was approximately 25 Hz with damping between 10 and 25% of critical damping. However, only mean pressures were analyzed because of apparent changes in response during the course of the experiments from partial blood clotting or movement of the catheters. In addition, fidelity of the signals decreased at high swimming speeds due to catheter vibration. Heart rates were determined using a Turbo Pascal program that counted the number of QRS complexes over 1 min intervals. Blood pressure and heart rates measured during the last half hour of each hour measurement period were averaged for each fish. Branchial vascular resistance (R_{branch}) was calculated relative to total peripheral resistance (R_{total}) as $[(BP_{va}-BP_{da})/BP_{va}] * 100\%$, with the assumption that venous pressure was negligible (Farrell, 1991).

Mean cell hemoglobin concentration (MCHC) was calculated as Hb/Hct. The percentage oxygen saturation (S_{O_2}) was calculated from the oxygen carrying capacity and Hb determined for each fish. For the two fish for which Hb was not measured (the equipment was unavailable), S_{O_2} was calculated based on an estimate of Hb from Hct measurements and the mean MCHC determined for the other fish.

All swimming velocities were corrected for the solid-blocking effect of the fish as described by Bell and Terhune (1970). To normalize for differences in swimming performance, swimming velocities were converted to a percentage of the critical swimming velocity (U_{crit}, FL/s) (Beamish, 1978):

$$U_{crit} = U_i + (t_i/t_{ii} * U_{ii})$$

where U_i is the highest velocity maintained for the entire prescribed time, U_{ii} is the velocity increment (~0.25 FL/s), t_i is the length of time at the velocity that produced fatigue (U_{max}), and t_{ii} is the prescribed time between velocity increments (60 min).

Due to the high degree of variability among individual fish and differences in variances between samples, nonparametric statistics were used in the analysis. Statistical comparisons between measurements during recovery from anesthesia were made with Friedman's ANOVA followed by a nonparametric multiple comparison test (Zar, 1984). Comparisons between the venous and arterial blood samples, and among the pre-exercise, exercise (maximal velocity, U_{max}), and post-exercise values were made with the Wilcoxon paired-sample test. Correlation analysis was carried out with the procedures in StatView (Abacus Concepts, Inc.) and tested for significance with Bartlett's test of sphericity and Fisher's r to z transformation. A fiducial limit of 5% was used for all statistical tests. Mean values are presented with standard error of the mean (S.E.M.) and the sample size in parenthesis.

Results

Tables 1-3 report mean values for fish swimming velocity and cardiorespiratory parameters during five experimental sampling periods: 0 h and 1 h post-anesthesia, pre-exercise (2 h), at maximal velocity (U_{max}), and post-exercise. Subsequent sections will describe the observed arterial-venous blood differences, changes that occurred during recovery from anesthesia (i.e., from post-anesthesia to pre-exercise), and during prolonged exercise (i.e., from pre-exercise to U_{max}).

Of 22 attempted studies, data for only 11 yellowfin were of sufficient quality to warrant reporting due to the difficulty in handling tuna and their sensitivity to respiratory stress. Data were eliminated if fish were not swimming well at the end of the 2 h recovery period. In general, these fish had higher plasma lactate levels (some greater than 15 mM), and a lower blood pH (< 7.6) than those that survived and swam in the experiment (see below). Results for one fish were eliminated because of a dramatic drop in Hct (from 40 to 12%) during the experiment.

In addition, ventral- and dorsal-aortic catheterizations were not always successful for each fish. In some cases the vessel could not be accessed in the limited surgical time, or following surgery the catheter became inoperable, most commonly due to the catheter tip abutting the vessel wall. As a result, four fish had both functional catheters, four fish had a ventral-aortic catheter only, and three fish had a dorsal-aortic catheter only.

Arterial-venous blood differences

To determine if there were significant changes in blood parameters between venous and arterial samples, irrespective of changes between experimental periods, comparisons were made using only paired data from yellowfin which had both catheters. These comparisons, combining data from all experimental sampling periods, revealed no significant arterial-venous differences in Hct, lactate, or glucose. However, significant differences were found between arterial and venous pH, Hb, and MCHC, and therefore these values were reported separately for the larger data set including all fish in Tables 2 and 3. The mean arterial-venous differences for these parameters at each experimental sample in the four tuna are shown in Table 4. In addition, Table 4 reports the relative branchial vascular resistances determined from paired BP_{va} and BP_{da} measurements and the calculated $(a - v)_{O_2}$ values from the four tuna. There were no significant changes between experimental periods for the arterial-venous differences, but this may be a result of the small sample sizes (n=3 to 4).

Recovery from anesthesia: changes from post-anesthesia to pre-exercise

Over the 2 h period from the end of anesthesia to the pre-exercise sample, swimming velocity was relatively constant (Table 1). The observed changes in cardiorespiratory parameters therefore reflect recovery from the effects of the brief (<1 min) struggle before the fish was subdued by the anesthetic, and from potential respiratory

distress caused by anesthesia and surgery. During this recovery period, significant increases occurred in BP_{da} (Table 1), pH_v, pH_a, and venous oxygen content ($C_{\bar{v},O_2}$) (Table 3). Calculated venous oxygen saturation ($S_{\bar{v},O_2}$), which reflects differences in Hb, also increased significantly (Table 3). Significant decreases occurred in arterial oxygen partial pressure (P_{a,O_2} , Table 3), plasma lactate, and plasma glucose (Table 2). The decreasing trend in $(a-v)_{O_2}$ for yellowfin having both functional catheters parallels that determined from all fish (Tables 3 and 4).

Effects of prolonged exercise: changes from pre-exercise to U_{max}

Mean swimming velocities and percentage U_{crit} at pre-exercise and at U_{max} samples are reported in Table 1. Swimming performance varied considerably among fish. Calculated U_{crit} ranged from 1.25 to 2.19 FL/s (mean 1.70 ±0.09) and the maximal velocity achieved (U_{max}) ranged from 1.42 to 2.38 FL/s or 98 to 117% U_{crit}. The post-exercise velocity was chosen as the lowest velocity at which the fish would swim stably, and was generally higher than the pre-exercise level (range 0.93 to 1.84 FL/s, or 66 to 98% U_{crit}).

Both heart rate (f_H) and BP_{va} increased from pre-exercise to U_{max} (Table 1). An example of the changes in blood pressure and ECG recordings in one fish is shown in Fig. 1. Individual variability in f_H at the pre-exercise sample was negatively correlated with Hct ($r=-0.71$, $p=0.01$). Although BP_{va} increased with exercise, there was no significant change in BP_{da}, indicating an increased relative R_{branch}. This finding is consistent with results for the tuna with both ventral- and dorsal-aortic catheters; pre-exercise R_{branch} ranged from 24.4 to 40.0% and tended to increase with exercise (Table 4).

Table 2 shows that a decrease occurred in Hct and Hb_a , but not Hb_v during exercise. In addition, plasma lactate increased, while plasma glucose concentration declined.

Blood-gas and pH changes with exercise are reported in Table 3. Both $C_{\bar{v},O_2}$ and $S_{\bar{v},O_2}$ decreased, and S_{a,O_2} increased, although there were no significant changes in arterial or venous P_{O_2}. In addition, pH_v decreased with exercise.

The relationships between blood-gas values are most conveniently described in relation to the hemoglobin-oxygen dissociation curve (ODC). The ODC in Fig. 2 was constructed using *in vivo* values of hemoglobin oxygen saturation (S_{O_2}) and blood P_{O_2}. To control for the effect of blood pH on S_{O_2}, only data within the pH range of 7.74 to 7.84 are shown (this is the range of pre-exercise pH_v values). The ODC curve was estimated by fitting the non-linear, ODC equation described in Bushnell and Brill (1992) to the data, and forcing it through zero and 100% S_{O_2} (r^2=0.85). The estimated P_{50} from this curve is 5.3 kPa.

Figure 3 shows individual exercise effects on both arterial and venous P_{O_2} and S_{O_2} in relation to the ODC. All fish increased arterial S_{O_2} and five of seven fish showed increases in P_{a,O_2}. The two fish in which P_{a,O_2} did not increase also had the lowest pH values (7.69 and 7.63), and showed an increase in pH with exercise (to 7.71 and 7.67, respectively), suggesting they were still recovering from anesthesia.

Although $P_{\bar{v},O_2}$ showed no consistent trend, $S_{\bar{v},O_2}$ decreased in six of the seven fish for which data were obtained (Fig. 3). Figure 4 illustrates the correlation between the decline in venous S_{O_2} and pH during exercise. With one exception, exercise caused a marked drop in venous pH. Individual variability in $S_{\bar{v},O_2}$ was also correlated with differences in pH_v, at both the pre-exercise period (r=-0.77, p=0.02) and at U_{max} (r=-0.93, p=0.001).

Coronary artery measurements

In one fish fitted with a dorsal-aortic catheter the intended ventral-aortic catheter was inadvertently passed through the bulbus arteriosus and entered the coronary

(hypobranchial) artery, a single vessel running along the dorsal surface of the ventral aorta and bulbus arteriosus.

Table 5 reports the cardiorespiratory variables measured in the coronary artery and the dorsal aorta of this fish and Fig. 5 shows an example of the pressure tracings. An example of the blood pressure measurements from this fish is shown in Fig. 5. Compared to the dorsal aorta, coronary artery blood pressure as well as P_{O_2}, C_{O_2}, and S_{O_2} were elevated. Exercise increased the blood pressures, P_{O_2}, and S_{O_2} of both vessels.

Post-mortem examination confirmed that the tip of the catheter was completely within the coronary artery. As this vessel is relatively small compared to the ventral aorta, the catheter may have partially obstructed flow and increased vascular resistance. However, the vessel was not completely blocked, a blood clot did not form, and the observed blood-gases suggest fresh blood was being delivered to the heart.

Discussion

This study is the first to examine changes in hemodynamics and blood-gases with exercise in tuna. As in most fishes, total oxygen consumption in tuna increases exponentially with swimming velocity (Dewar and Graham, 1994a) and oxygen demand during exercise must be met with increased oxygen delivery by the cardiorespiratory system (eq. 1). In the yellowfin, this increase occurs through increases in f_H and BP_{va}, suggesting an increased blood flow (Table 1). The arterial-venous oxygen content difference [$(a-v)_{O_2}$; Table 4] was also increased, through an increased oxygen delivery (increased S_{a,O_2}) and an increased oxygen extraction from the blood (decline in $S_{\bar{v},O_2}$) (Table 3). The following sections will discuss the hemodynamic and blood-gas responses of both the arterial and venous blood to exercise in the yellowfin tuna.

Hemodynamics

The measured heart rate, its variability, and response to swimming velocity for yellowfin tuna in this study are in good agreement with values reported for yellowfin that had not been anesthetized and only instrumented with ECG electrodes (Chapter IV). For example, fish swimming at a the pre-exercise velocity of 1.02 FL/s had a mean heart rate of 61.4 bpm and at 1.83 FL/s (U_{max}) mean heart rate was 84.6 bpm (Table 1). Comparable values for unanesthetized yellowfin are 67.9 and 82.7 bpm, respectively (Chapter IV). In addition, variability in heart rate among individual fish was comparable (Chapter IV) and these similarities suggest that the potential side-effects of the anesthesia and surgery, and the towing of catheter lines on cardiovascular responses were minimal. The individual differences in pre-exercise heart rate in this study were found to be negatively correlated with Hct, which may reflect compensation for differences in oxygen carrying capacity through increased blood flow (Gallaugher *et al.*, 1995).

This study confirms previous findings of a high ventral-aortic blood pressure in tunas (Fig. 1) (Breisch *et al.*, 1983; Lai *et al.*, 1987; White *et al.*, 1988; Bushnell and Brill, 1992; Jones *et al.*, 1993), which is consistent with their large relative ventricular mass, high percentage of compact myocardium, and myocardial fiber arrangements favoring efficient pumping (Poupa *et al.*, 1981; Sanchez-Quintana and Hurle, 1987; Farrell and Jones, 1992). Although already substantial, BP_{va} increased in exercising yellowfin (Table 1). Dorsal aortic pressures in this study were higher than those reported for spinalized yellowfin (4.35 kPa; Bushnell and Brill, 1992), but similar to anesthetized and slowly swimming yellowfin (6.3 to 6.8 kPa; Jones *et al.*, 1993).

The driving pressure needed for a given blood flow will depend on the down-stream vascular resistance. The total vascular resistance (R_{total}) can be divided into two parts associated with the branchial (R_{branch}) and systemic (R_{syst}) vascular beds. The large surface area of tuna gills is thought to pose a higher resistance to blood flow than in other

teleosts, potentially increasing cardiovascular costs (Brill and Bushnell, 1991b). Indeed, R_{branch} has been reported to be 60 to 68% of R_{total} in spinally-blocked tuna (Bushnell and Brill, 1992) compared to 18 to 40% in most fishes (Bushnell *et al.*, 1992). In this study, however, pre-exercise R_{branch} was only 25 to 40% of R_{total}, and is similar to the 42% reported by Jones *et al.* (1993) for slowly swimming yellowfin.

The increased heart rate and BP_{va} measured for yellowfin in this study suggest that cardiac output increased with exercise. However, the absence of a change in BP_{da} (Table 1) means that systemic resistance (R_{syst}) had to decrease, which is similar to findings for other fishes during exercise (Bushnell *et al.*, 1992). Table 4 shows that the relative proportion of R_{branch} to R_{total} increased with exercise, and was highest post-exercise. However, as R_{syst} decreased, absolute R_{branch} may have remained the same, with the increase in pressure drop across the gills due solely to the increase in blood flow.

The coronary artery measurements from the single tuna warrant discussion because of the few *in vivo* data of this type on fishes, and documentation of a strong dependency of the tuna heart on coronary blood flow (Farrell *et al.*, 1992). Coronary artery blood pressure was higher than dorsal-aortic pressure with peaks occurring towards the end of ventricular systole, and before peak dorsal-aortic pressure (Fig. 5). This is similar to that found in the mako shark (*Isurus oxyrinchus*) (Lai *et al.*, in review). These results suggest a lower vascular resistance for the coronary vessels. Coronary artery blood flow has been estimated (0.67 ml/min/g ventricular mass) based on BP_{da} measurements from spinalized tuna and *in vitro* pressure-flow relationships (Farrell *et al.*, 1992). However, the elevated coronary pressures measured in this study suggest that coronary blood flow will be even higher (~1.0 ml/min/g ventricular mass).

Exercise effects on arterial blood-respiratory properties

Pre-exercise status

This study confirms the yellowfin tuna's high blood oxygen carrying capacity (16-18 ml O_2/dl), however, the lower *in vivo* blood oxygen affinity (i.e., a high P_{50}; Fig. 2) determined for fish in this study is different from previous results (Bushnell and Brill, 1992). Also, the findings of a low mean S_{a,O_2} (74.1%) and P_{a,O_2} (9.96 kPa) in pre-exercise yellowfin (Table 3) were unexpected. Although high P_{a,O_2} values (about 13 kPa) have been measured in active species such as rainbow trout (*Oncorhynchus mykiss*) (Kiceniuk and Jones, 1977; Randall and Daxboeck, 1982), the low P_{a,O_2} and S_{a,O_2} of pre-exercise yellowfin suggests that branchial oxygen exchange was limited.

Even though tunas and other scombrids are dependent on ram ventilation, which depends to some degree on swimming velocity, our data enable us to eliminate the possibility that the low pre-exercise P_{a,O_2} for yellowfin was due to a low swimming speed. Table 3 shows that P_{a,O_2} was significantly higher at the beginning of the anesthesia recovery period (i.e., at 0 h, when $\dot{V}_{O_2}$ was likely elevated), but swimming speed was the same as at later times during recovery. In addition, a review of the literature shows that generally low P_{a,O_2} values have been measured in several tunas: kawakawa tuna, *Euthynnus affinis*, 8.36 kPa; albacore, *T. alalunga*, 8.27 kPa; skipjack, *Katsuwonus pelamis*, 9.29 kPa; and yellowfin, 9.91 kPa (Jones *et al.*, 1986; White *et al.*, 1988; Bushnell and Brill, 1992). Finally, another scombrid, the Atlantic mackerel (*Scomber scombrus*) has a low P_{a,O_2} (7.76 kPa) and S_{a,O_2} (75%) while swimming at low speeds (Boutilier *et al.*, 1984). These findings suggest that a reduced P_{a,O_2} may be the normal condition in these fishes.

A limited branchial gas exchange in pre-exercise yellowfin is further suggested by their relatively high P_{a,CO_2} (Table 3), a condition also found in kawakawa and albacore (Jones *et al.*, 1986; White *et al.*, 1988). As blood transits the gill lamellae, carbon dioxide

is excreted resulting in an elevated post-branchial blood pH (Randall and Daxboeck, 1984; Nikinmaa and Jensen, 1986). In yellowfin, however, we found a small, but significant decrease in post-branchial pH (Table 4). Bushnell and Brill (1992) similarly report a small, although not significant, transbranchial decrease in blood pH (-0.01) for spinalized yellowfin and skipjack. Although the reason for slight arterial acidosis is unknown, the co-occurrence of a reduced post-branchial MCHC suggests that ion disequilibria may be occurring, and it has been shown that a drop in blood pH will cause red blood cell swelling (Nikinmaa, 1992).

The limited branchial gas-transfer indicated for pre-exercise yellowfin seems paradoxical in light of the correlation between tuna metabolic capacity and a large gill surface area (Hughes, 1984). Nevertheless, at respiration levels not requiring the entire gill area, there are osmoregulatory advantages for reducing branchial perfusion which could be done by shunting blood through non-respiratory lamellar channels (Tuurala *et al.*, 1984) or reducing ventilatory volume. Bushnell and Brill (1992) reported a ventilation-perfusion conductance ratio of 0.73 for spinalized yellowfin, indicating that the gills were under-ventilated relative to blood perfusion.

The tuna heart has a high aerobic demand (Farrell *et al.*, 1992) and the higher P_{a,O_2} and C_{a,O_2} in the coronary artery relative to the dorsal aorta indicate the presence of a perfusion pathway that ensures a high rate of oxygen delivery (Table 5). In the yellowfin, the coronary circulation arises from the efferent arteries of the first, second, and third branchial arches (D. Bernal and K. E. Korsmeyer, unpublished observations), suggesting that the extent of blood oxygenation is affected by differences in branchial arch blood flow and perfusion pathways.

Exercise

With increases in swimming velocity, yellowfin dorsal-aortic P_{a,O_2} increased as did blood-oxygen saturation which, in several fish, rose to greater than 95% (Fig. 3). Although S_{a,O_2} increased in all of the yellowfin in this study, C_{a,O_2} did not change significantly (Table 3). This is likely a result of sequential blood sampling and the loss of hemoglobin containing erythrocytes during the experiment (Table 2). In the absence of blood loss, the increases in P_{a,O_2} and S_{a,O_2} suggest that C_{a,O_2} would have increased with exercise.

The increased P_{a,O_2} and S_{a,O_2} during exercise indicates that the ventilation-perfusion conductance ratio was elevated. Exercise likely increased both cardiac output and ventilation volume. In addition, the higher BP_{va} of exercising yellowfin would lead to the recruitment of additional lamellar surface area and a decreased diffusion distance, thus increasing both P_{a,O_2} and S_{a,O_2} (Booth, 1979; Farrell *et al.*, 1980; Tuurala *et al.*, 1984).

The finding that exercising yellowfin increased both its P_{a,O_2} and S_{a,O_2} is similar to results obtained for the lemon shark, *Negaprion brevirostris*, during swimming (Bushnell *et al.*, 1982). By contrast, no other species of fish, including the mackerel, has been shown to elevate both P_{a,O_2} and S_{a,O_2} during prolonged swimming (Stevens and Randall, 1967; Kiceniuk and Jones, 1977; Piiper *et al.*, 1977; Boutilier *et al.*, 1984; Thomas *et al.*, 1987; Lai *et al.*, 1990; Gallaugher *et al.*, 1992; Thorarensen *et al.*, 1993; Wilson and Egginton, 1994).

Exercise effects on venous blood-respiratory properties

Pre-exercise status

Pre-exercise $P_{\bar{v},O_2}$ in swimming yellowfin (~5.3 kPa) was slightly higher than in spinalized yellowfin and skipjack (4.31 and 4.89 kPa, respectively; Bushnell and Brill, 1992) reflecting the relatively high P_{50} (~5.3 kPa), or low oxygen affinity, of the blood

(Fig. 2). Although this *in vivo* P_{50} is higher than determined for yellowfin blood *in vitro* (2.73 to 4.30 kPa; Brill and Bushnell, 1991a), a high P_{50} has been predicted for active, pelagic fish that live in well oxygenated waters (Riggs, 1970; Powers, 1980). In addition, the *in vitro* ODC determined for mackerel blood (Boutilier *et al.*, 1984) suggests a similar P_{50} at the P_{CO_2} levels measured in this study for tuna. A high P_{50} will aid unloading of oxygen in the tissues, by maintaining a high P_{O_2} gradient from the capillaries to the mitochondria. Because tuna elevate red (aerobic) muscle temperature (Dewar *et al.*, 1994), the P_{O_2} gradient in this muscle will be affected by the rapid increase in temperature as the blood moves through the heat exchangers (*retia mirabilia*). Oxygen affinity in yellowfin decreases with increases in temperature in a closed system (i.e., gas content is constant), which is the condition in the *retial* vessels (Brill and Bushnell, 1991a). This means that the P_{50} of the blood in the warmed red-muscle will be even higher.

Although pre-exercise $C_{\bar{v},O_2}$ (9.5 ml O_2/dl) was similar to that in spinalized tuna (9.0 to 10.0 ml O_2/dl; Bushnell and Brill, 1992), this value is remarkably high compared to other fishes, especially considering the swimming velocity of 1.0 FL/s. This is a result of the yellowfin's high blood oxygen carrying capacity as this $C_{\bar{v},O_2}$ corresponds to a $S_{\bar{v},O_2}$ of only 48%. By comparison, $C_{\bar{v},O_2}$ in resting rainbow trout was 7.1 ml O_2/dl (Kiceniuk and Jones, 1977), but at this content trout blood is 70% saturated (Jones and Randall, 1978).

Exercise

With exercise, both $S_{\bar{v},O_2}$ and $C_{\bar{v},O_2}$ decreased but there was little or no change in $P_{\bar{v},O_2}$ (Fig. 3, Table 3). With the exception of the lemon shark (Bushnell *et al.*, 1982), a decrease in $S_{\bar{v},O_2}$ is the usual response to increased swimming velocity in fishes (Kiceniuk and Jones, 1977; Piiper *et al.*, 1977; Lai *et al.*, 1990). This decrease is normally accompanied by declines in both pH_v and $P_{\bar{v},O_2}$. However in the yellowfin, $P_{\bar{v},O_2}$ was

essentially unchanged throughout the experiment (Table 3). This may be a consequence of $P_{\bar{v},O_2}$ occurring close to the P_{50} for oxygen saturation (Figs 2 and 3), and therefore lying on the steepest part of the ODC. As a result, large changes in $S_{\bar{v},O_2}$ can occur with only small changes in $P_{\bar{v},O_2}$.

The decline in $S_{\bar{v},O_2}$ was brought about almost entirely through the decreases in oxygen affinity associated with a reduction in pH (i.e., a Bohr shift, or rightward shift of the ODC, Fig. 4). Even though the decline in pH_v was relatively small (-0.05 pH units) it was sufficient to cause the observed decline in $S_{\bar{v},O_2}$ because of the large Bohr shift in yellowfin (-0.865 $\Delta \log P_{50}/\Delta pH$; Brill and Bushnell, 1991a). The observed acidosis appears to have both respiratory and metabolic components, as indicated by increases in plasma lactate and the rise in P_{CO_2} (Tables 2 and 3).

Tuna blood has a high buffering capacity, and a large Bohr effect may be necessary to ensure maximum oxygen dissociation in the exercising tissues which are not undergoing marked changes in pH (Bushnell and Jones, 1994). The large Bohr shift also accounts for the correlation of the interindividual variability in pH_v and $C_{\bar{v},O_2}$ (Fig. 4). Finally it is emphasized that the venous pH reduction measured for yellowfin in this study is much less than that recorded for skipjack (-0.45 pH units) following exhaustive exercise (Perry *et al.*, 1985).

Swimming performance

The calculated U_{crit} values in this study (1.3 to 2.2 FL/s) are low compared to the predicted maximal sustainable speed of yellowfin (3 to 6 FL/s) based on models of oxygen uptake and delivery (Bushnell and Brill, 1991; Brill, 1996; Korsmeyer *et al.*, 1996b). In the present study, the protocol for determining fatigue minimized stress and damage to the fish, and therefore may have underestimated U_{crit}. Although incremental velocity tests with most other fishes may proceed to the point where they are exhausted and unable to

swim (Beamish, 1978), the continuous swimming requirements of tuna make this impractical.

In addition, U_{max} determined in this study was likely affected by the recent anesthesia and surgery, as well as the towed catheters and electrode wires. Maximal velocities (up to 2.4 FL/s) were less than those reported for yellowfin (up to 3.0 FL/s) that were uninstrumented or towing only a pair of ECG wires (Dewar and Graham, 1994a; Chapter IV). The maximal velocities of other fishes have been shown to decrease following surgery and instrumentation by 25 to 75%, although maximal oxygen consumption was unaffected (Kiceniuk and Jones, 1977; Thorarensen *et al.*, 1993).

Despite lower than expected velocities, the yellowfin in this study appear to have been working near their maximal aerobic limits at their respective U_{max}. Shortly before velocity was reduced, the fish would begin an unsteady swimming pattern, characterized by intermittent bursts forward in order to maintain position in the swim tunnel. This "burst and glide" swimming behavior is associated with white muscle activity (based on EMG recordings; personal observations), and is likely anaerobically powered. The increased lactate levels at U_{max} and post-exercise (Table 2) also suggest the beginnings of recruitment of fast-fatiguable white muscle. However, post-exercise lactate values observed for yellowfin in this study were considerably less than those following exhaustive exercise in skipjack (30 mM; Perry *et al.*, 1985), suggesting that swimming had remained primarily aerobic.

Oxygen transport during exercise

Table 6 reports the contributions of heart rate and oxygen extraction from the blood [$(a - v)_{O_2}$] to increased oxygen delivery, along with estimates of total oxygen consumption ($\dot{V}_{O_2}$), stroke volume, and cardiac output. Although not measured in this study, $\dot{V}_{O_2}$ was estimated from data reported by Dewar and Graham (1994a) for

uninstrumented, swimming yellowfin tuna. Cardiac output and stroke volume were calculated using the Fick equation (eq. 1). Although the possible errors associated with the Fick calculation in fishes may cancel out (Metcalfe and Butler, 1982; Neumann *et al.*, 1983; Randall, 1985; Thorarensen *et al.*, 1996), the potentially high gill metabolism of tuna (Brill, 1996) suggests that Fick estimates will result in an overestimation of cardiac output. Data from spinalized yellowfin suggest that gill metabolism may account for as much as 50% of total $\dot{V}_{O_2}$ (Bushnell and Brill, 1992), and we have included a correction for this in Table 6.

The response of yellowfin tuna to prolonged exercise is different from all other fishes studied in that $(a-v)_{O_2}$ is elevated both through an increased arterial oxygen saturation, and an increased extraction from a large venous oxygen reserve (Table 3). As a result of these changes, $(a-v)_{O_2}$ increased by a factor of 1.29 and, in addition, heart rate increased by a factor of 1.38 (Table 6). Together, these two variables can account for all of the estimated increase in oxygen demand (x 1.69), with no change, or even a slight decrease in stroke volume (Table 6). This result agrees with blood-flow measurements on swimming yellowfin during exercise, which showed an average decrease in stroke volume of 3.9% (Chapter V).

How does oxygen transport in yellowfin compare with that of other fishes? Kiceniuk and Jones (1977) have provided the most complete analysis of exercise effects on oxygen delivery in a teleost. Rainbow trout increased $(a-v)_{O_2}$ by 2.5 times, heart rate by 1.36 times, and stroke volume 2.24 times, to achieve a 7.6 fold increase in oxygen transport from rest to U_{max}. Although this relative increase in oxygen transport is much higher than in the yellowfin, it is important to note that the yellowfin increased velocity, not from rest, but from a minimal swimming velocity of 1.0 FL/s (60.7 % U_{crit}, Table 1), and, in tuna, the rate of increase in $\dot{V}_{O_2}$ with velocity is less than in trout (Dewar and Graham,

1994a). Moreover, as in most vertebrates, cardiac output and $(a-v)_{O_2}$ contribute nearly equally to increased oxygen delivery in the yellowfin (Table 6) (Farrell, 1991).

A surprising feature of this study's findings is that although the yellowfin tuna appear to have reached their maximal aerobic swimming capacity, the blood-gas data indicate that these fish still had a sizable venous oxygen reserve (8.5 ml O_2/dl, or >40% saturation). Consequently, the quantity of oxygen extracted at U_{max} [$(a-v)_{O_2}$ = 7.2 ml O_2/dl] is much less than values predicted (15-20 ml O_2/dl) to achieve the estimated maximal $\dot{V}_{O_2}$ for yellowfin (Brill and Bushnell, 1991b). One possible explanation for this finding is that the fish had not, in fact, reached their maximal aerobic swimming limits. Although tunas are difficult to study in a water tunnel and many variables could have affected their swimming performance, observations (intermittent burst swimming and increased plasma lactate [see above]) suggest that these fish had approached their maximal capacity for aerobic swimming. Another explanation is that our measurement of the average (mixed) venous oxygen content in the ventral aorta did not reflect the extent of oxygen extraction occurring in the red (aerobic) muscle. The untapped venous reserve of other parts of the body during aerobic swimming (i.e., visceral organs and white muscle) may have masked greater oxygen extraction by the red muscle during exercise, with the change in mixed venous oxygen content reflecting only the partial contribution of red muscle blood flow to total $(a-v)_{O_2}$.

This does not mean that the high blood oxygen carrying capacity of the yellowfin is not utilized. The large venous oxygen reserve may permit maintenance of red muscle oxygen delivery during oxygen limiting environmental conditions. Yellowfin make rapid vertical migrations in search of prey, potentially encountering deep, cooler waters that may be oxygen limiting (Dizon *et al.*, 1978; Holland *et al.*, 1990; Korsmeyer *et al.*, 1996b). In response to hypoxia, spinalized tuna effectively draw on venous oxygen reserves in order to maintain aerobic metabolism (Bushnell and Brill, 1992).

The high blood carrying capacity of tuna may serve other aerobic metabolic processes in addition to the continuous requirement of sustained swimming, for example during repayment of an oxygen debt following the intense anaerobic activity of burst swimming. Maximal $\dot{V}_{O_2}$ in fishes may not be reached using the critical swimming speed protocol, due to higher rates of oxygen consumption during recovery from exhaustive exercise (Soofiani and Priede, 1985; Goolish, 1991; Reidy *et al.*, 1995). Similarly, it has been suggested that maximal $\dot{V}_{O_2}$ in yellowfin does not occur during maximal aerobic swimming, but instead may involve additional metabolic processes (Korsmeyer *et al.*, 1996a). This hypothesis is substantiated by the blood-gas values during the recovery from anesthesia (Table 3 and 4), when repayment for an oxygen debt was likely occurring. The lowest $C_{\bar{v},O_2}$ and largest $(a-v)_{O_2}$ values were seen shortly after anesthesia and surgery, and not at U_{max}. Thus, the high oxygen carrying capacity of the yellowfin's blood results in a large venous oxygen reserve which can supply potentially competing metabolic processes, in addition to effectively meeting the oxygen demands of aerobic swimming.

Table VI-1: Mean swimming velocity (U in FL/s, and as a percentage U_{crit}), heart rate (f_H), and blood pressure measurements in swimming yellowfin tuna (47.6 ±0.6 FL/s, 1850 ±70 g, n=11) following anesthesia, at maximum velocity (U_{max}), and post-exercise.

Variable	Post-anesthesia		Pre-exercise	U_{max}	Post-exercise
	0 h	1 h	(2 h)		
U (FL/s)	0.97 ±0.02 (11)	1.00 ±0.04 (11)	1.02 ±0.04 (11)	1.83 ±0.10 (11)†	1.38 ±0.07 (11)†‡
U_{crit} (%)	58.2 ±3.0 (11)	59.5 ±2.5 (11)	60.7 ±2.1 (11)	107.6 ±1.8 (11)†	81.5 ±2.5 (11)†‡
f_H (bpm)	60.7 ±3.2 (11)	63 ±4.5 (11)	61.4 ±4.2 (11)	84.6 ±5.1 (11)†	83.0 ±4.1 (11)†
BP_{va} (kPa)	11.31 ±0.36 (8)	11.06 ±0.27 (8)	10.80 ±0.43 (8)	12.16 ±0.68 (7)†	12.15 ±0.61 (6)
BP_{da} (kPa)	7.58 ±0.30 (7)	7.12 ±0.29 (7)	6.71 ±0.41 (7)*	7.12 ±0.25 (7)	6.86 ±0.27 (7)

*significantly different from 0 h ($p<0.05$)
†significantly different from Pre-exercise ($p<0.05$)
‡significantly different from U_{max} ($p<0.05$)

Table VI-2: Hematological measurements in swimming yellowfin tuna (47.6 ±0.6 FL/s, 1850 ±70 g, n=11) following anesthesia, at maximum velocity (U_{max}), and post-exercise.

Variable	Post-anesthesia		Pre-exercise	U_{max}	Post-exercise
	0 h	1 h	(2 h)		
Hct (%)	33.6 ±0.8 (11)	32.3 ±1.0 (11)	30.7 ±1.3 (11)	28.3 ±1.0 (11)†	27.3 ±1.1 (10)
Hb_a (g/dl)	12.2 ±0.4 (6)	11.6 ±0.7 (6)	11.3 ±0.7 (6)	10.0 ±0.6 (6)†	9.1 ±0.6 (5)
Hb_v (g/dl)	12.8 ±0.4 (7)	11.7 ±0.6 (7)	11.4 ±0.7 (7)	10.9 ±0.4 (6)	10.8 ±0.5 (6)
$MCHC_a$ (g/l)	366 ±5 (6)	356 ±8 (6)	366 ±4 (6)	357 ±2 (6)	360 ±4 (5)
$MCHC_v$ (g/l)	373 ±6 (7)	370 ±2 (7)	373 ±3 (7)	384 ±6 (6)	385 ±6 (6)†
Lactate (mM)	2.2 ±0.5 (11)	1.1 ±0.3 (11)*	0.8 ±0.1 (11)*	2.5 ±0.4 (11)†	3.6 ±0.9 (10)†
Glucose (mM)	5.6 ±0.6 (11)	4.2 ±0.5 (11)	3.3 ±0.5 (11)*	2.0 ±0.2 (11)†	1.9 ±0.2 (10)†‡

*significantly different from 0 h ($p<0.05$)
†significantly different from Pre-exercise ($p<0.05$)
‡significantly different from U_{max} ($p<0.05$)

Table VI-3: Measurements of blood gasses and pH in swimming yellowfin tuna (47.6 ±0.6 FL/s, 1850 ±70 g, n=11) following anesthesia, at maximum velocity (U_{max}), and post-exercise.

Variable	Post-anesthesia		Pre-exercise	U_{max}	Post-exercise
	0 h	1 h	(2 h)		
P_{a,O_2} (kPa)	12.13 ±0.90 (7)	10.62 ±0.98 (7)	9.96 ±0.60 (7)*	11.83 ±1.05 (7)	12.25 ±1.62 (6)
$P_{\bar{v},O_2}$ (kPa)	5.12 ±0.36 (8)	5.23 ±0.19 (8)	5.36 ±0.24 (8)	5.21 ±0.28 (7)	5.25 ±0.29 (7)
C_{a,O_2} (ml O_2/dl)	13.7 ±1.0 (7)	14.3 ±0.6(7)	14.0 ±0.8 (7)	14.8 ±1.0 (7)	14.8 ±1.3 (6)
$C_{\bar{v},O_2}$ (ml O_2/dl)	7.3 ±0.9 (8)	8.9 ±0.7 (8)*	9.5 ±0.7 (8)*	8.5 ±0.7 (7)†	8.3 ±0.8 (7)†
S_{a,O_2} (%)	68.1 ±4.8 (7)	73.3 ±4.6 (7)	74.1 ±2.9 (7)	88.3 ±5.2 (7)†	93.1 ±8.2 (6)†
$S_{\bar{v},O_2}$ (%)	32.0 ±3.9 (8)	43.4 ±3.0 (8)*	47.7 ±3.8 (8)*	43.5 ±3.9 (7)†	43.4 ±3.9 (7)
pH_a	7.64 ±0.03 (7)	7.70 ±0.03 (7)	7.74 ±0.03 (7)*	7.73 ±0.01 (7)	7.73 ±0.03 (6)
pH_v	7.73 ±0.02 (8)	7.78 ±0.02 (8)	7.80 ±0.01 (8)*	7.76 ±0.02 (7)†	7.74 ±0.02 (7)†
P_{a,CO_2} (kPa)	0.80 ±0.09 (3)	0.76 ±0.10 (3)	0.81 ±0.05 (3)	0.89 ±0.14 (3)	0.78 ±0.26 (2)
$P_{\bar{v},CO_2}$ (kPa)	0.96 ±0.07 (3)	0.92 ±0.12 (3)	0.91 ±0.08 (3)	1.10 ±0.07 (2)	1.16 ±0.25 (2)

*significantly different from 0 h ($p<0.05$)
†significantly different from Pre-exercise ($p<0.05$)
‡significantly different from U_{max} ($p<0.05$)

Table VI-4: Differences in arterial and venous blood-gas and hematological variables, and relative branchial vascular resistance (R_{branch}) in swimming yellowfin tuna (n=4) following anesthesia, at maximum velocity (U_{max}), and post-exercise.

Variable	Post-anesthesia		Pre-exercise	U_{max}	Post-exercise
	0 h	1 h	(2 h)		
$(a-v)_{O_2}$ (ml O_2/dl)	8.7 ±1.2 (4)	6.4 ±0.4 (4)	5.6 ±1.1 (4)	7.2 ±0.8 (3)	8.2 ±1.7 (3)
pH_a-pH_v	-0.05 ±0.01 (4)	-0.03 ±0.01 (4)	-0.03 ±0.01 (4)	-0.04 ±0.03 (3)	+0.03 ±0.04 (3)
Hb_a-Hb_v (g/dl)	-0.8 ±0.8 (4)	0.0 ±0.1 (4)	-0.4 ±0.1 (4)	-0.9 ±0.2 (3)	-0.8 ±0.4 (3)
$MCHC_a$-$MCHC_v$ (g/l)	-6 ±14 (4)	-4 ±8 (4)	-14 ±3 (4)	-32 ±8 (3)	-25 ±7 (3)
R_{branch} (%)	32.4 ±1.2 (4)	33.8 ±1.8 (4)	32.4 ±3.6 (4)	39.5 ±3.9 (3)	48.3 ±1.3 (2)

Table VI-5: Cardio-respiratory variables measured in the coronary artery and dorsal aorta of a yellowfin tuna (48.5 cm fork length, 1913 g) at pre-exercise and at maximal velocity (U_{max})

Variable	62 % U_{crit} (Pre-exercise)		117 % U_{crit} (U_{max})	
	Coronary	Dorsal aorta	Coronary	Dorsal aorta
P_{O_2} (kPa)	10.16	8.73	11.96	11.00
C_{O_2} (ml O_2/dl)	16.4	14.6	19.5	17.5
S_{O_2} (%)	92.0	79.2	114.0	101.4
pH	7.73	7.76	7.75	7.73
Hct (%)	35.0	34.0	32.0	33.0
Hb (g/dl)	12.3	12.7	11.8	11.9
f_H (bpm)	48.6	48.6	72.4	72.4
BP (kPa)	6.48	4.41	7.24	6.97

Table VI-6: Oxygen transport variables in the yellowfin tuna during prolonged swimming

	Pre-exercise (1.02 FL/s)	Exercise (U_{max})	Increase factor
Total O_2 consumption (ml O_2/kg/min)	4.96*	8.39**	x 1.69
$(a-v)_{O_2}$ (ml O_2/dl)	5.6	7.2	x 1.29
Heart rate (bpm)	61.4	84.6	x 1.38
Stroke volume (ml/kg)	1.44† (0.72†‡)	1.38† (0.69†‡)	x 0.96
Cardiac output (ml/kg/min)	88.5† (44.3†‡)	116.6† (58.3†‡)	x 1.32

*estimated from O_2 consumption-velocity regression reported in Dewar and Graham (1994a)

**mean O_2 consumption of 10 yellowfin at maximal velocities (mean velocity = 1.84 FL/s) during energetics experiments (Dewar and Graham, 1994a; H. Dewar, pers. comm.)

†calculated using Fick principle

‡adjusted value assuming 50% O_2 uptake directly by the gills (Bushnell and Brill, 1992)

Figure VI-1

Simultaneous recordings of ventral and dorsal-aortic (VA and DA) blood pressure and electrocardiogram (ECG) during pre-exercise (68% U_{crit}) and at maximal velocity (108% U_{crit}) in a yellowfin tuna (46 cm fork length, 1.7 kg).

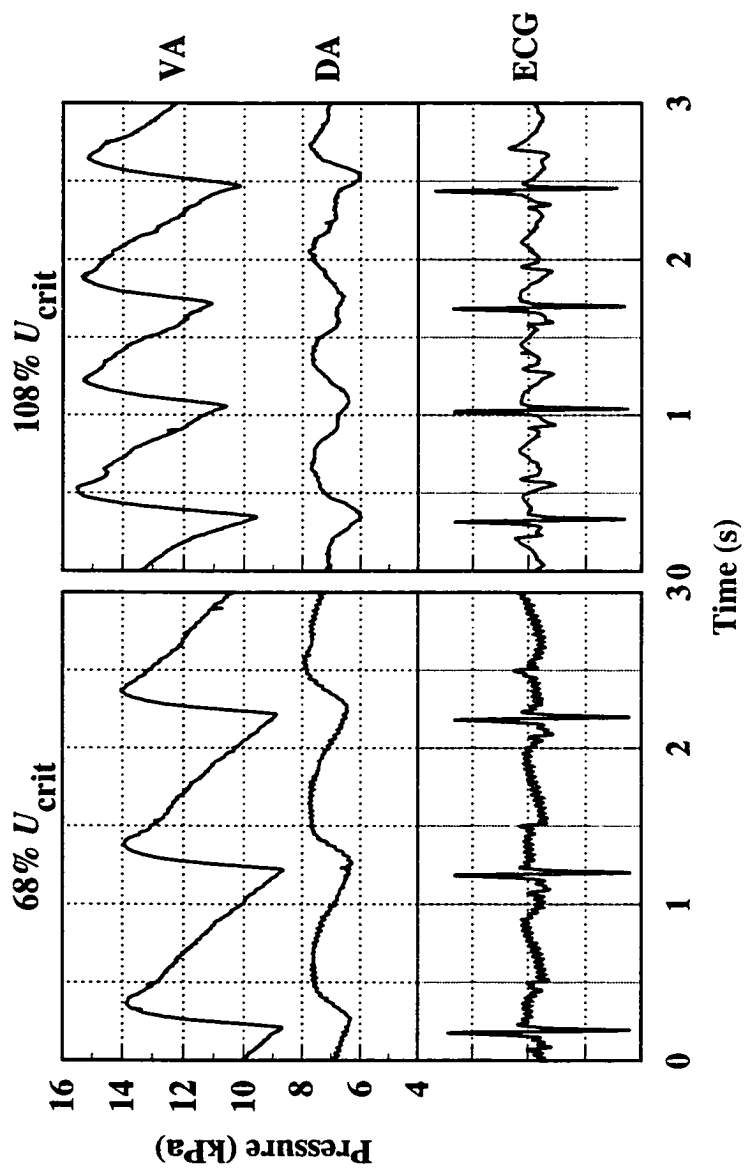


Figure VI-2

Functional *in vivo* oxygen dissociation curve for arterial (open circles) and venous (closed circles) blood in yellowfin tuna for blood pH 7.74 to 7.84.

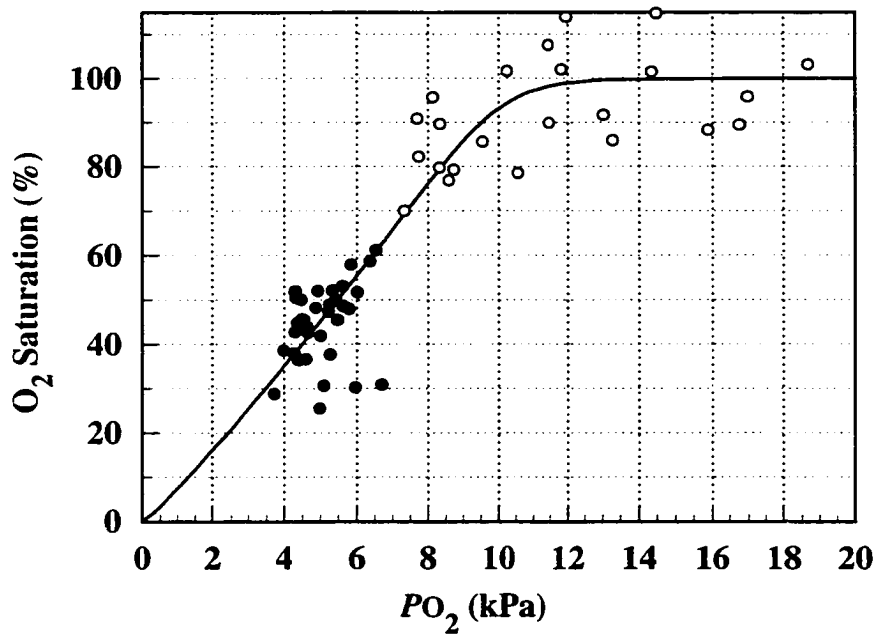


Figure VI-3

Individual changes in percentage saturation and P_{O_2} from pre-exercise (open circles) to maximal velocity (closed circles) for both arterial and venous blood in yellowfin tuna. Paired values for individual fish are connected by a line. Dashed line is oxygen dissociation curve from Fig. 2.

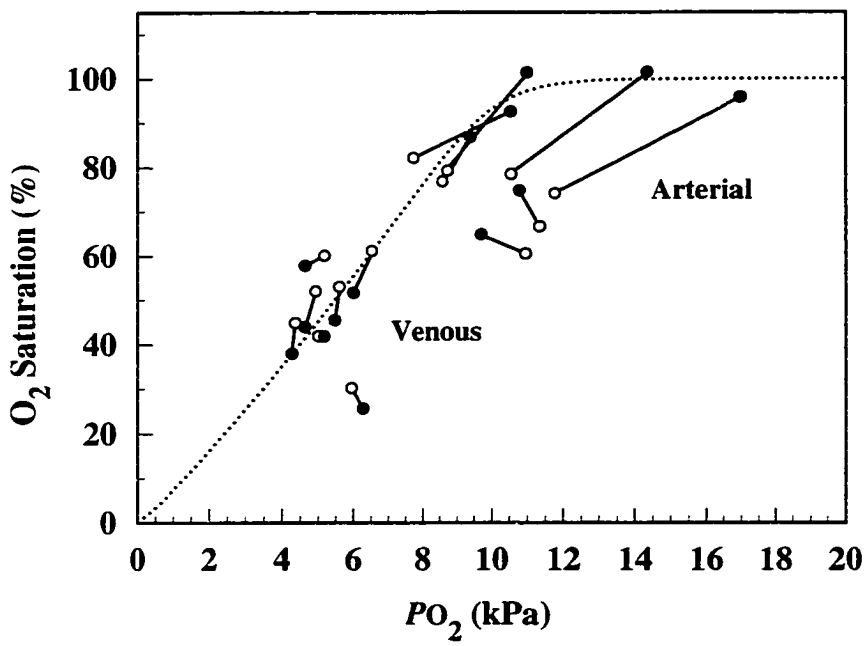


Figure VI-4

Individual changes in venous oxygen saturation and venous pH from pre-exercise (open circles) to maximal velocity (closed circles) in yellowfin tuna. Paired values for individual fish are connected by a line.

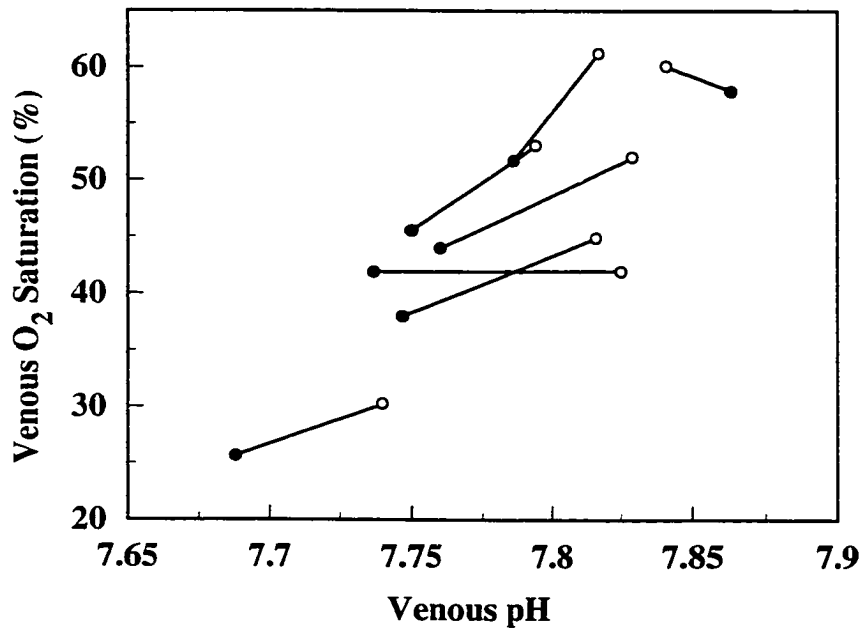
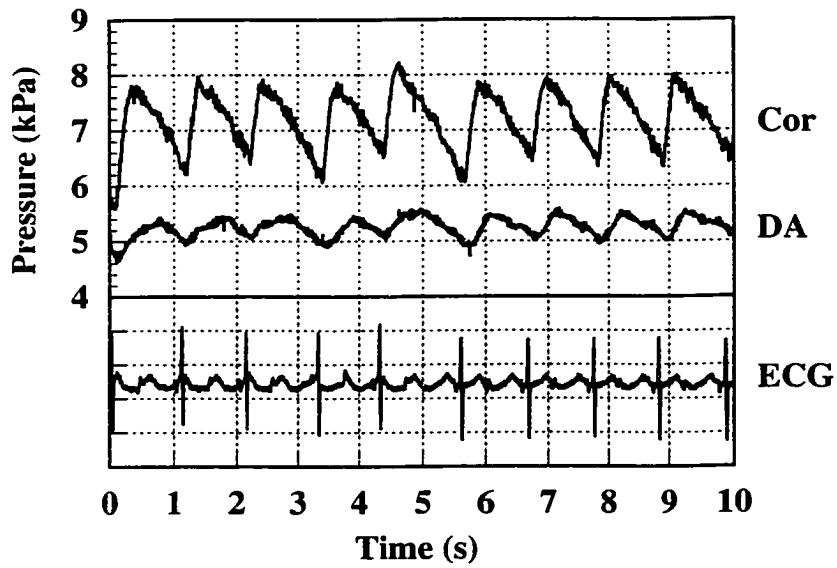


Figure VI-5

Example of simultaneous recordings of coronary (Cor) and dorsal-aortic (DA) blood pressure, along with the ECG in a swimming yellowfin tuna (48.5 cm fork length, 1.9 kg).



CHAPTER VII: SUMMARY

Tunas are highly specialized for life as active, pelagic predators. They have numerous adaptations for efficient, continuous swimming and for sustaining high levels of aerobic metabolism. These adaptations, in turn, permit tunas to find, capture, and process prey at the rates necessary to maintain their high metabolic rates. Consonant with the tuna's ecophysiology is a cardio-respiratory system capable of high rates of oxygen uptake and delivery.

This study is the first to examine tuna cardiovascular function in response to increased levels of exercise. The results indicate that while many of the swimming cardio-respiratory variables of tuna are enhanced compared to other fish species, the differences are not as "extraordinary" as has been previously implied. The heart rate of a swimming yellowfin tuna is, for example, comparable to that of other active teleosts, such as salmon, once correction for temperature differences is made. This overestimation of tuna cardiovascular function stems from data taken on anesthetized and spinally-blocked, non-swimming tuna; these data were regarded as "resting" values and therefore indicative of basal, or minimal levels. It was assumed therefore, that during swimming, cardiovascular function would necessarily increase above these levels in order to meet the increased oxygen demand. However, it is now clear that heart rate, cardiac output, and $(a-v)_{O_2}$ values in non-swimming tuna match or actually exceed those in swimming fish.

However, several important differences exist between cardio-respiratory function during exercise in yellowfin and other studied fishes. Unlike the response in most fishes, cardiac stroke volume does not increase with swimming velocity. This supports the hypothesis that heart rate accounts for nearly all of the modulation of cardiac output in tuna (Farrell *et al.*, 1992). However, this hypothesis was based on the supposition that the tuna heart always operated at its maximal stroke volume. The data from acute temperature

change experiments indicate that this in not the case, as stroke volume increased during a drop in ambient temperature. The reason for the lack of a stroke volume increase during aerobic exercise, and the ranges of absolute stroke volumes in tunas, remain to be determined.

The oxygen carrying capacity of yellowfin blood is much higher than that of non-tunas and is thus sufficient to permit oxygen delivery during slow swimming despite sub-maximal arterial oxygen saturations. A reduced arterial saturation at slow speed reflects a lower metabolic demand and implies that the large gill surface area of the tuna is not fully perfused, which would in turn reduce osmoregulatory problems. With exercise, however, arterial saturation increases, aiding oxygen delivery to the tissues.

The high blood-oxygen carrying capacity also results in a large venous oxygen reserve in the yellowfin, even at maximal sustained swimming velocities. This venous reserve suggests that maximal oxygen consumption had not been reached. A model of aerobic swimming performance in tuna predicts that the total oxygen uptake rate at the maximal sustained swimming speed is much less than the estimated maximal aerobic capacity and that the residual aerobic scope not required for swimming may serve to sustain other metabolic functions. This is particularly important for tunas, which, in addition to the requirement of continuous swimming, require high, and simultaneous metabolic performance in several physiological functions, such as digestion, oxygen debt recovery, and growth.

Further research is needed to quantify the aerobic scope of tunas. In addition, because the full oxygen delivery potential of the tuna cardio-respiratory system was not achieved during sustained swimming, further studies examining increases in oxygen demand associated with other metabolic functions, particularly recovery from oxygen debt, are needed. Tunas have a high anaerobic potential, which is linked to a high aerobic scope.

Rapid recovery from intense anaerobic activity is likely one of the most important aerobic costs in tuna.

Understanding the significance of the tunas' morphological, anatomical, biochemical, and physiological features is hindered by the lack of appropriate comparable data on other active tropical or pelagic fish species. The results of other workers (Block, 1991; Dickson, 1995; Brill, 1996) are beginning to show that the high-performance physiology of tunas may not be unique. Hopefully, further improvements in our ability to work with these species, both in the laboratory and in the field, will increase our understanding of other scombrids, endothermic sharks, and other active pelagic fishes. In addition, there is considerable variability in the ecology, morphology, and anatomy among the tunas themselves, and physiological comparisons will likely reveal similar differences. These types of investigations are necessary to define the selective pressures leading to endothermy and high aerobic metabolic capacities in tunas, and perhaps other fishes.

REFERENCES

Agnisola, C. and Tota, B. (1994). Structure and function of the fish cardiac ventricle: Flexibility and limitations. *Cardioscience* **5**, 145-153.

Arthur, P. G., West, T. G., Brill, R. W., Schulte, P. M. and Hochachka, P. W. (1992). Recovery metabolism of skipjack tuna (*Katsuwonus pelamis*) white muscle: rapid and parallel changes in lactate and phosphocreatine after exercise. *Can. J. Zool.* **70**, 1230-1239.

Axelsson, M., Davison, W., Forster, M. E. and Farrell, A. P. (1992). Cardiovascular responses of the red-blooded Antarctic fishes *Pagothenia bernachii* and *P. borchgrevinki*. *J. exp. Biol.* **167**, 179-201.

Barkley, R. A., Neill, W. H. and Gooding, R. M. (1978). Skipjack tuna, *Katsuwonus pelamis*, habitat based on temperature and oxygen requirements. *Fish. Bull.* **76**, 653-662.

Barron, M. G., Tarr, B. D. and Hayton, W. L. (1987). Temperature-dependance of cardiac output and regional blood flow in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* **31**, 735-744.

Batty, R. S. and Wardle, C. S. (1979). Restoration of glycogen from lactic acid in the anaerobic swimming muscle of plaice, *Pleuronectes platessa* L. *J. Fish Biol.* **15**, 509-519.

Beamish, F. W. H. (1974). Apparent specific dynamic action of largemouth bass, *Micropterus salmoides*. *J. Fish. Res. Bd Can.* **31**, 1763-1769.

Beamish, F. W. H. (1978). Swimming capacity. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar and D. J. Randall), pp. 101-189. New York: Academic Press.

Bell, W. H. and Terhune, L. D. B. (1970). Water tunnel design for fisheries research. *Fisheries Research Board of Canada Technical Report* **No. 195**, 1-69.

Bennion, G. R. (1968). The control of the function of the heart in teleost fish. M.S. thesis, University of British Columbia.

Blackburn, M. (1968). Micronecton of the eastern tropical pacific ocean: family composition, distribution, abundance, and relations to tuna. *Fish. Bull.* **67**, 71-115.

Block, B. A. (1991). Endothermy in fish: thermogenesis, ecology and evolution. In *Biochemistry and Molecular Biology of Fishes*, vol. 1 (ed. P. W. Hochachka and T. P. Mommsen), pp. 269-311. New York: Elsevier Science Publishers.

Boggs, C. H. and Kitchell, J. F. (1991). Tuna metabolic rates estimated from energy losses during starvation. *Physiol. Zool.* **64**, 502-524.

Bone, Q., Kiceniuk, J. and Jones, D. R. (1978). On the role of the different fibre types in fish myotomes at intermediate swimming speeds. *Fish. Bull.* **76**, 691-699.

Booth, J. H. (1979). The effects of oxygen supply, epinephrine, and acetylcholine on the distribution of blood flow in trout gills. *J. exp. Biol.* **83**, 31-39.

Boutilier, R. G., Aughton, P. and Shelton, G. (1984). O_2 and CO_2 transport in relation to ventilation in the Atlantic mackerel, *Scomber scombrus*. *Can. J. Zool.* **62**, 546-554.

Boutilier, R. G., Dobson, G., Hoeger, U. and Randall, D. J. (1988). Acute exposure to graded levels of hypoxia in rainbow trout (*Salmo gairdneri*): metabolic and respiratory adaptations. *Resp. Physiol.* **71**, 69-82.

Breisch, E. A., White, F., Jones, J. and Laurs, R. M. (1983). Ultrastructural morphometry of the myocardium of *Thunnus alalunga*. *Cell Tissue Res.* **233**, 427-438.

Brett, J. R. and Glass, N. R. (1973). Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. *J. Fish. Res. Bd Can.* **30**, 379-387.

Brett, J. R. and Groves, T. D. D. (1979). Physiological energetics. In *Fish Physiology*, vol. 8 (ed. W. S. Hoar, D. J. Randall and J. R. Brett), pp. 279-352. New York: Academic Press.

Brill, R. W. (1987). On the standard metabolic rates of tropical tunas, including the effect of body size and acute temperature change. *Fish. Bull.* **85**, 25-35.

Brill, R. W. (1992). *The Kewalo Research Facility, 1958-92: Over 30 years of progress.* NOAA Technical Memorandum NMFS No. NOAA-TM-NMFS-SWFSC-171, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center.

Brill, R. W. (1996). Selective advantages conferred by the high performance physiology of tunas, billfishes, and dolphin fish. *Comp. Biochem. Physiol.* **113A**, 3-15.

Brill, R. W., Bourke, R., Brock, J. A. and Dailey, M. D. (1987). Prevalence and effects of infection of the dorsal aorta in yellowfin tuna, *Thunnus albacares*, by the larval cestode, *Dasyrhynchus talismani*. *Fish. Bull.* **85**, 767-776.

Brill, R. W. and Bushnell, P. G. (1991a). Effects of open- and closed-system temperature changes on blood oxygen dissociation curves of skipjack tuna, *Katsuwonus pelamis*, and yellowfin tuna, *Thunnus albacares*. *Can. J. Zool.* **69**, 1814-1821.

Brill, R. W. and Bushnell, P. G. (1991b). Metabolic and cardiac scope of high energy demand teleosts, the tunas. *Can. J. Zool.* **69**, 2002-2009.

Brill, R. W., Dewar, H. and Graham, J. B. (1994). Basic concepts relevant to heat transfer in fishes, and their use in measuring the physiological thermoregulatory abilities of tunas. *Env. Biol. Fish.* **40**, 109-124.

Brill, R. W. and Dizon, A. E. (1979a). Effect of temperature on isotonic twitch of white muscle and predicted maximum swimming speeds of skipjack tuna, *Katsuwonus pelamis*. *Env. Biol. Fish.* **4**, 199-205.

Brill, R. W. and Dizon, A. E. (1979b). Red and white muscle fibre activity in swimming skipjack tuna, *Katsuwonus pelamis* (L.). *J. Fish Biol.* **15**, 679-685.

Brown, C. R. and Cameron, J. N. (1991). The induction of specific dynamic action in channel catfish by infusion of essential amino acids. *Physiol. Zool.* **64**, 276-297.

Buck, L. T., Brill, R. W. and Hochachka, P. W. (1992). Gluconeogenesis in hepatocytes isolated from the skipjack tuna (*Katsuwonus pelamis*). *Can. J. Zool.* **70**, 1254-1257.

Burleson, M. L., Smatresk, N. J. and Milsom, W. K. (1992). Afferent inputs associated with cardioventilatory control in fish. In *Fish Physiology*, vol. 12B (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 389-426. San Diego: Academic Press.

Bushnell, P. G. (1988). Cardiovascular and respiratory responses to hypoxia in three species of obligate ram ventilating fishes, skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), and bigeye tuna (*T. obesus*). Ph.D. dissertation, University of Hawaii.

Bushnell, P. G. and Brill, R. W. (1991). Responses of swimming skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) tunas to acute hypoxia, and a model of their cardiorespiratory function. *Physiol. Zool.* **64**, 787-811.

Bushnell, P. G. and Brill, R. W. (1992). Oxygen transport and cardiovascular responses in skipjack (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) exposed to acute hypoxia. *J. comp. Physiol. B* **162**, 131-143.

Bushnell, P. G., Brill, R. W. and Bourke, R. E. (1990). Cardiorespiratory responses of skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), and bigeye tuna (*Thunnus obesus*) to acute reductions of ambient oxygen. *Can. J. Zool.* **68**, 1857-1865.

Bushnell, P. G. and Jones, D. R. (1994). Cardiovascular and respiratory physiology of tuna: adaptations for support of exceptionally high metabolic rates. *Env. Biol. Fish.* **40**, 303-318.

Bushnell, P. G., Jones, D. R. and Farrell, A. P. (1992). The arterial system. In *Fish Physiology*, vol. 12A (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 89-139. San Diego: Academic Press.

Bushnell, P. G., Lutz, P. L., Steffensen, J. F., Oikari, A. and Gruber, S. H. (1982). Increases in arterial blood oxygen during exercise in the lemon shark (*Negaprion brevirostris*). *Journal of Comparative Physiology* **147**, 41-47.

Butler, P. J. and Taylor, E. W. (1971). Response of the dogfish (*Scyliorhinus canicula* L.) to slowly induced and rapidly induced hypoxia. *Comp. Biochem. Physiol.* **39A**, 307-323.

Carey, F. G., Kanwisher, J. W. and Stevens, E. D. (1984). Bluefin tuna warm their vicera during digestion. *J. exp. Biol.* **109**, 1-20.

Carey, F. G. and Olson, R. J. (1982). Sonic tracking experiments with tunas. *ICCAT Collective Volume of Scientific Papers* **17**, 458-466.

Carey, F. G. and Teal, J. M. (1966). Heat conservation in tuna fish muscle. *Proc. Nat. Acad. Sci.* **56**, 1464-1469.

Carey, F. G. and Teal, J. M. (1969). Regulation of body temperature by the bluefin tuna. *Comp. Biochem. Physiol.* **28**, 205-213.

Carey, F. G., Teal, J. M., Kanwisher, J. W. and Lawson, K. D. (1971). Warm-bodied fish. *Am. Zool.* **11**, 135-145.

Cech, J. J. (1976). Cardiovascular responses of winter flounder, *Pseudopleuronectes americanus* (Walbaum), to acute temperature increase. *Can. J. Zool.* **54**, 1383-1388.

Cech, J. J., Laurs, R. M. and Graham, J. B. (1984). Temperature-induced changes in blood gas equilibria in the albacore, *Thunnus alalunga*, a warm-bodied tuna. *J. exp. Biol.* **109**, 21-34.

Collette, B. B. (1978). Adaptations and systematics of the mackerels and tunas. In *The Physiological Ecology of Tunas*, (ed. G. D. Sharp and A. E. Dizon), pp. 7-39. New York: Academic Press.

Collette, B. B. and Nauen, C. E. (1983). FAO species catalogue. Vol. 2. Scombrids of the world. *FAO Fish. Synop.* **2**, 1-137.

Collette, B. B., Potthoff, T., Richards, W. J., Ueyanagi, S., Russo, J. L. and Nishikawa, Y. (1984). Scombroidei: development and relationships. In *Ontogeny and Systematics of Fishes*, (ed. H. G. Moser), pp. 591-620. Lawrence, KS: Allen Press.

Davis, J. C. (1975). Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. *J. Fish. Res. Bd Can.* **32**, 2295-2332.

Daxboeck, C., Davie, P. S., Perry, S. F. and Randall, D. J. (1982). Oxygen uptake in a spontaneously ventilating, blood-perfused trout preparation. *J. exp. Biol.* **101**, 35-45.

Dewar, H. (1993). Studies of tropical tuna swimming performance: thermoregulation, swimming mechanics and energetics. Ph.D. dissertation, Scripps Institution of Oceanography, University of California, San Diego.

Dewar, H. and Graham, J. B. (1994a). Studies of tropical tuna swimming performance in a large water tunnel. I. Energetics. *J. exp. Biol.* **192**, 13-31.

Dewar, H. and Graham, J. B. (1994b). Studies of tropical tuna swimming performance in a large water tunnel. III. Kinematics. *J. exp. Biol.* **192**, 45-59.

Dewar, H., Graham, J. B. and Brill, R. W. (1994). Studies of tropical tuna swimming performance in a large water tunnel. II. Thermoregulation. *J. exp. Biol.* **192**, 33-44.

Dickson, K. A. (1988). Why are some fishes endothermic? Interspecific comparisons of aerobic and anaerobic metabolic capacities in endothermic and ectothermic scombrids. Ph.D. dissertation, Scripps Institution of Oceanography, University of California, San Diego.

Dickson, K. A. (1994). Tunas as small as 207mm fork length can elevate muscle temperatures significantly above ambient water temperature. *J. exp. Biol.* **190**, 79-93.

Dickson, K. A. (1995). Unique adaptations of the metabolic biochemistry of tunas and billfishes for life in the pelagic environment. *Env. Biol. Fish.* **42**, 65-97.

Dickson, K. A. and Somero, G. N. (1987). Partial characterization of the buffering components of the red and white myotomal muscle of marine teleosts, with special emphasis on scombrid fishes. *Physiol. Zool.* **60**, 699-706.

Dizon, A. E. (1977). Effect of dissolved oxygen concentration and salinity on swimming speed of two species of tunas. *Fish. Bull.* **75**, 649-653.

Dizon, A. E. and Brill, R. W. (1979). Thermoregulation in tunas. *Am. Zool.* **19**, 249-265.

Dizon, A. E., Brill, R. W. and Yuen, H. S. H. (1978). Correlations between environment, physiology and activity and the effects on thermoregulation in skipjack tuna. In *The Physiological Ecology of Tunas*, (ed. G. D. Sharp and A. E. Dizon), pp. 233-259. New York: Academic Press.

Dizon, A. E., Neill, W. H. and Magnuson, J. J. (1977). Rapid temperature compensation of volitional swimming speeds and lethal temperatures in tropical tunas (Scombridae). *Env. Biol. Fish.* **2**, 83-92.

Driedzic, W. R. and Gesser, H. (1994). Energy metabolism and contractility in ectothermic vertebrate hearts: hypoxia, acidosis, and low temperature. *Physiological Reviews* **74**, 221-258.

Farrell, A. P. (1984). A review of cardiac performance in the teleost heart: intrinsic and humoral regulation. *Can. J. Zool.* **62**, 523-536.

Farrell, A. P. (1991). From hagfish to tuna: a perspective on cardiac function in fish. *Physiol. Zool.* **64**, 1137-1164.

Farrell, A. P., Davie, P. S., Franklin, C. E., Johansen, J. A. and Brill, R. W. (1992). Cardiac physiology in tunas. I. *In vitro* perfused heart preparations from yellowfin and skipjack tunas. *Can. J. Zool.* **70**, 1200-1210.

Farrell, A. P. and Jones, D. R. (1992). The heart. In *Fish Physiology*, vol. 12A (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 1-88. San Diego: Academic Press.

Farrell, A. P., Sobin, S. S., Randall, D. J. and Crosby, S. (1980). Intralamellar blood flow patterns in fish gills. *Am. J. Physiol.* **239**, R428-R436.

Farrell, A. P. and Steffensen, J. F. (1987). An analysis of the energetic cost of the branchial and cardiac pumps during sustained swimming in trout. *Fish Physiology and Biochemistry* **4**, 73-79.

Fierstine, H. L. and Walters, V. (1968). Studies in locomotion and anatomy of scombroid fishes. *Memoirs of the Southern California Academy of Sciences* **6**, 1-31.

Forsbergh, E. D. (1980). Synopsis of biological data on the skipjack tuna, *Katsuwonus pelamis* (Linnaeus, 1758), in the Pacific ocean. *Inter-American Tropical Tuna Commision, Special Report* **2**, 295-360.

Forster, M. E. and Farrell, A. P. (1994). The volumes of the chambers of the trout heart. *Comp. Biochem. Physiol.* **109A**, 127-132.

Franklin, C. E. and Davie, P. S. (1992). Dimensional analysis of the ventricle of an in situ perfused trout heart using echocardiography. *J. exp. Biol.* **166**, 47-60.

Fritsche, R. and Nilsson, S. (1990). Autonomic nervous control of blood pressure and heart rate during hypoxia in the cod, *Gadus morhua*. *J. comp. Physiol. B* **160**, 287-292.

Fry, F. E. J. (1947). Effects of environment on animal activity. *Univ. Toronto Stud. Biol. Ser.* **55**, 1-62.

Gallaugher, P., Axelsson, M. and Farrell, A. P. (1992). Swimming performance and haematological variables in splenectomized rainbow trout, *Oncorhynchus mykiss*. *J. exp. Biol.* **171**, 301-314.

Gallaugher, P., Thorarensen, H. and Farrell, A. P. (1995). Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Resp. Physiol.* **102**, 279-292.

Gayeski, T. E. J. and Honig, C. R. (1988). Intracellular PO_2 in long axis of individual fibers in working dog gracilis muscle. *Am. J. Physiol.* **254**, H1179-H1186.

George, J. C. and Stevens, E. D. (1978). Fine structure and metabolic adaptations of red and white muscles in tuna. *Env. Biol. Fish.* **3**, 185-191.

Giovane, A., Greco, G., Maresca, A. and Tota, B. (1980). Myoglobin in the heart ventricle of tuna and other fishes. *Experientia* **36**, 219-220.

Gooding, R. M., Neill, W. H. and Dizon, A. E. (1981). Respiration rates and low-oxygen tolerance limits in skipjack tuna, *Katsuwonus pelamis*. *Fish. Bull.* **79**, 31-48.

Goolish, E. M. (1991). Aerobic and anaerobic scaling in fish. *Biol. Rev.* **66**, 33-56.

Gordon, M. S. (1968). Oxygen consumption of red and white muscles from tuna fishes. *Science* **159**, 87-90.

Graham, J. B. (1973). Heat exchange in the black skipjack, and the blood-gas relationship of warm-bodied fishes. *Proc. Nat. Acad. Sci. USA* **70**, 1964-1967.

Graham, J. B. (1975). Heat exchange in the yellowfin tuna, *Thunnus albacares*, and skipjack tuna, *Katsuwonus pelamis*, and the adaptive significance of elevated body temperatures in scombrid fishes. *Fish. Bull.* **73**, 219-229.

Graham, J. B., Dewar, H., Lai, N. C., Korsmeyer, K. E., Fields, P. A., Knower, T., Shadwick, R. E., Shabetai, R. and Brill, R. W. (1994). Swimming physiology of pelagic fishes. In *Mechanics and Physiology of Animal Swimming*, (ed. L. Maddock, Q. Bone and J. M. V. Rayner), pp. 63-74. Cambridge: Cambridge University Press.

Graham, J. B., Dewar, H., Lai, N. C., Lowell, W. R. and Arce, S. M. (1990). Aspects of shark swimming performance determined using a large water tunnel. *J. exp. Biol.* **151**, 175-192.

Graham, J. B., Koehrn, F. J. and Dickson, K. A. (1983). Distribution and relative proportions of red muscle in scombrid fishes: consequences of body size and relationships to locomotion and endothermy. *Can. J. Zool.* **61**, 2087-2096.

Graham, J. B. and Laurs, R. M. (1982). Metabolic rate of the albacore tuna *Thunnus alalunga*. *Mar. Biol.* **72**, 1-6.

Graham, J. B., Lowell, W. R., Lai, N. C. and Laurs, R. M. (1989). O_2 tension, swimming-velocity, and thermal effects on the metabolic rate of the Pacific albacore *Thunnus alalunga*. *Exp. Biol.* **48**, 89-94.

Graham, M. and Farrell, A. (1985). The seasonal intrinsic cardiac performance of a marine teleost. *J. exp. Biol.* **118**, 173-183.

Groebe, K. and Thews, G. (1990). Calculated intra- and extracellular PO_2 gradients in heavily working red muscle. *Am. J. Physiol.* **259**, H84-H92.

Guppy, M. and Hochachka, P. W. (1978). Skipjack tuna white muscle: a blueprint for the integration of aerobic and anaerobic carbohydrate metabolism. In *The Physiological Ecology of Tunas*, (ed. G. D. Sharp and A. E. Dizon), pp. 175-181. New York: Academic Press.

Guppy, M., Hulbert, W. C. and Hochachka, P. W. (1979). Metabolic sources of heat and power in tuna muscles. II. Enzyme and metabolite profiles. *J. exp. Biol.* **82**, 303-320.

Hanyu, I., Tsukamoto, K., Yamamori, K., Ngan, P. V., Furukawa, K. and Hibiya, T. (1979). Simultaneous recording of physiological information from swimming fish. *Bull. Japan. Soc. Sci. Fish.* **45**, 1261-1265.

Heisler, N. (1989). Interactions between gas exchange, metabolism, and ion transport in animals: an overview. *Can. J. Zool.* **67**, 2923-2935.

Holland, K. N., Brill, R. W. and Chang, R. K. C. (1990). Horizontal and vertical movements of yellowfin and bigeye tuna associated with fish aggregating devices. *Fish. Bull.* **88**, 493-507.

Holland, K. N., Brill, R. W., Chang, R. K. C., Sibert, J. R. and Fournier, D. A. (1992). Physiological and behavioral thermoregulation in bigeye tuna (*Thunnus obesus*). *Nature* **358**, 410-412.

Honig, C. R., Gayeski, T. E. J., Clark, A., Jr. and Clark, P. A. A. (1991). Arteriovenous oxygen diffusion shunt is negligible in resting and working gracilis muscles. *Am. J. Physiol.* **261**, H2031-H2043.

Hughes, G. M. (1984). General anatomy of the gills. In *Fish Physiology*, vol. 10 (ed. W. S. Hoar and D. J. Randall), pp. 1-72. New York: Academic Press.

Hulbert, W. C., Guppy, M., Murphy, B. and Hochachka, P. W. (1979). Metabolic sources of heat and power in tuna muscles. I. Muscle fine structure. *J. exp. Biol.* **82**, 289-301.

Hunter, J. R., Macewicz, B. J. and Sibert, J. R. (1986). The spawning frequency of skipjack tuna, *Katsuwonus pelamis*, from the South Pacific. *Fish. Bull.* **84**, 895-903.

Ingham, M. C., Cook, S. K. and Hausknecht, K. A. (1977). Oxycline characteristics and skipjack tuna distribution in the southeastern tropical Atlantic. *Fish. Bull.* **75**, 857-865.

Jobling, M. (1981). The influences of feeding on the metabolic rate of fishes: a short review. *J. Fish Biol.* **18**, 385-400.

Johnston, I. A. (1981). Structure and function of fish muscles. *Symp. zool. Soc. Lond.* **48**, 71-113.

Johnston, I. A. and Brill, R. (1984). Thermal dependence of contractile properties of single skinned muscle fibres from Antarctic and various warm water marine fishes including skipjack tuna (*Katsuwonus pelamis*) and kawakawa (*Euthynnus affinis*). *J. comp. Physiol. B* **155**, 63-70.

Jones, D. R. (1971). Theoretical analysis of factors which may limit the maximum oxygen uptake of fish: the oxygen cost of the cardiac and branchial pumps. *J. ther. Biol.* **32**, 341-349.

Jones, D. R., Brill, R. W. and Bushnell, P. G. (1993). Ventricular and arterial dynamics of anaesthetised and swimming tuna. *J. exp. Biol.* **182**, 97-112.

Jones, D. R., Brill, R. W., Butler, P. J., Bushnell, P. G. and Heieis, M. R. A. (1990). Measurement of ventilation volume in swimming tunas. *J. exp. Biol.* **149**, 491-498.

Jones, D. R., Brill, R. W. and Mense, D. C. (1986). The influence of blood gas properties on gas tensions and pH of ventral and dorsal aortic blood in free-swimming tuna, *Euthynnus affinis*. *J. exp. Biol.* **120**, 201-213.

Jones, D. R. and Randall, D. J. (1978). The respiratory and circulatory systems during exercise. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar and D. J. Randall), pp. 425-501. New York: Academic Press.

Kanwisher, J., Lawson, K. and Sundnes, G. (1974). Acoustic telemetry from fish. *Fish. Bull.* **72**, 251-255.

Kayar, S. R., Hoppeler, H., Jones, J. H., Longworth, K., Armstrong, R. B., Laughlin, M. H., Lindstedt, S. L., Bicudo, J. E. P. W., Groebe, K., Taylor, C. R. and Weibel, E. R. (1994). Capillary blood transit time in muscles in relation to body size and aerobic capacity. *J. exp. Biol.* **194**, 69-81.

Keen, J. E., Aota, S., Brill, R. W., Farrell, A. P. and Randall, D. J. (1995). Cholinergic and adrenergic regulation of heart rate and ventral aortic pressure in two species of tropical tunas, *Katsuwonus pelamis* and *Thunnus albacares*. *Can. J. Zool.* **73**, 1681-1688.

Keen, J. E., Farrell, A. P., Tibbits, G. F. and Brill, R. W. (1992). Cardiac physiology in tunas. II. Effect of ryanodine, calcium, and adrenaline on force-frequency relationships in atrial strips from skipjack tuna, *Katsuwonus pelamis*. *Can. J. Zool.* **70**, 1211-1217.

Keen, J. E. and Farrell, A. P. (1994). Maximum prolonged swimming speed and maximum cardiac performance of rainbow trout, *Oncorhynchus mykiss*, acclimated to two different water temperatures. *Comp. Biochem. Physiol.* **108A**, 287-295.

Kiceniuk, J. W. and Jones, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247-260.

Kitchell, J. F., Neill, W. H., Dizon, A. E. and Magnuson, J. J. (1978). Bioenergetic spectra of skipjack and yellowfin tunas. In *The Physiological Ecology of Tunas*, (ed. G. D. Sharp and A. E. Dizon), pp. 357-368. New York: Academic Press.

Kolok, A. S. and Farrell, A. P. (1994). Individual variation in the swimming performance and cardiac performance of northern squawfish, *Ptycholchellus oregonensis*. *Physiol. Zool.* **67**, 706-722.

Kolok, A. S., Spooner, R. M. and Farrell, A. P. (1993). The effect of exercise on the cardiac output and blood flow distribution of the largescale sucker *Catostomus macrocheilus*. *J. exp. Biol.* **183**, 301-321.

Korsmeyer, K. E., Dewar, H., Lai, N. C. and Graham, J. B. (1996a). The aerobic capacity of tunas: adaptation for multiple metabolic demands. *Comp. Biochem. Physiol.* **113A**, 17-24.

Korsmeyer, K. E., Dewar, H., Lai, N. C. and Graham, J. B. (1996b). Tuna aerobic swimming performance: physiological and environmental limits based on oxygen supply and demand. *Comp. Biochem. Physiol.* **113B**, 45-56.

Korsmeyer, K. E., Graham, J. B. and Shadwick, R. E. (1992). Cardiac performance in swimming yellowfin tuna. *Am. Zool.* **32**, 57A.

Korsmeyer, K. E., Lai, N. C., Dewar, H., Knower, T., Shadwick, R. E. and Graham, J. B. (1994). Cardiovascular responses to exercise in yellowfin tuna. *The Physiologist* **37**, A-93.

Korsmeyer, K. E., Lai, N. C., Knower, T., Dewar, H., Shadwick, R. E. and Graham, J. B. (1993). Cardiovascular function in swimming yellowfin tuna (*Thunnus albacares*). *Am. Zool.* **33**, 44A.

Lai, N. C., Graham, J. B. and Burnett, L. (1990). Blood respiratory properties and the effect of swimming on blood gas transport in the leopard shark *Triakis semifasciata*. *J. exp. Biol.* **151**, 161-173.

Lai, N. C., Graham, J. B., Lowell, W. R. and Laurs, R. M. (1987). Pericardial and vascular pressures and blood flow in the albacore tuna, *Thunnus alalunga*. *Exp. Biol.* **46**, 189-192.

Lai, N. C., Korsmeyer, K. E., Graham, J. B., Shabetai, R. and Ziegler, M. G. (1994). Catecholamine and potassium ion concentrations in swimming yellowfin tuna. *The Physiologist* **37**, A-93.

Lai, N. C., Korsmeyer, K. E., Katz, S., Holts, D. B., Laughlin, L. M. and Graham, J. B. (in review). Hemodynamics and blood properties of the shortfin mako shark, *Isurus oxyrinchus*. *Copeia*

Lai, N. C., Shabetai, R., Graham, J. B., Hoit, B. D., Sunnerhagen, K. S. and Bhargava, V. (1990). Cardiac function of the leopard shark, *Triakis semifasciata*. *Journal of Comparative Physiology* **160B**, 259-268.

Laurs, R. M., Ulevitch, R. and Morrison, D. C. (1978). Estimates of blood volume in the albacore tuna. In *The Physiological Ecology of Tunas*, (ed. G. D. Sharp and A. E. Dizon), pp. 135-139. New York: Academic Press.

Linthicum, D. S. and Carey, F. G. (1972). Regulation of brain and eye temperatures by the bluefin tuna. *Comp. Biochem. Physiol. A* **43**, 425-433.

Lucas, M. C. (1994). Heart rate as an indicator of metabolic rate and activity in adult Atlantic salmon, *Salmo salar*. *J. Fish Biol.* **44**, 889-903.

Lucas, M. C., Johnstone, A. D. F. and Tang, J. (1993). An annular respirometer for measuring aerobic metabolic rates of large, schooling fishes. *J. exp. Biol.* **175**, 325-331.

Magnuson, J. J. (1969). Digestion and food consumption by skipjack tuna (*Katsuwonus pelamis*). *Trans. Am. Fish. Soc.* **98**, 379-392.

Magnuson, J. J. (1978). Locomotion by scombrid fishes: hydromechanics, morphology, and behavior. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar and D. J. Randall), pp. 239-313. New York: Academic Press.

Mathieu-Costello, O., Agey, P. J., Logemann, R. B., Brill, R. W. and Hochachka, P. W. (1992). Capillary-fiber geometrical relationships in tuna red muscle. *Can. J. Zool.* **70**, 1218-1229.

Metcalfe, J. D. and Butler, P. J. (1982). Differences between directly measured and calculated values for cardiac output in the dogfish: a criticism of the Fick method. *J. exp. Biol.* **99**, 255-268.

Milligan, C. L. and McDonald, D. G. (1988). *In vivo* lactate kinetics at rest and during recovery from exhaustive exercise in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **135**, 119-131.

Milsom, W. K. and Brill, R. W. (1986). Oxygen sensitive afferent information arising from the first gill arch of yellowfin tuna. *Resp. Physiol.* **66**, 193-203.

Moyes, C. D., Mathieu-Costello, O. A., Brill, R. W. and Hochachka, P. W. (1992a). Mitochondrial metabolism of cardiac and skeletal muscles from a fast (*Katsuwonus pelamis*) and a slow (*Cyprinus carpio*) fish. *Can. J. Zool.* **70**, 1246-1253.

Moyes, C. D., Schulte, P. M. and Hochachka, P. W. (1992b). Recovery metabolism of trout white muscle: role of mitochondria. *Am. J. Physiol.* **262**, R295-R304.

Moyes, C. D., Schulte, P. M. and West, T. G. (1993). Burst exercise recovery metabolism in fish white muscle. In *Surviving Hypoxia*, (ed. P. W. Hochachka, P. L. Lutz, T. Sick, M. Rosenthal and G. van den Thillart), pp. 527-539. Boca Raton: CRC Press.

Muir, B. S. and Hughes, G. M. (1969). Gill dimensions for three species of tunny. *J. exp. Biol.* **51**, 271-285.

Nakamura, E. L. (1972). Development and uses of facilities for studying tuna behavior. In *Behavior of Marine Animals*, vol. 2 (ed. H. E. Winn and B. L. Olla), pp. 245-277. New York: Plenum Press.

Neill, W. H., Chang, R. K. C. and Dizon, A. E. (1976). Magnitude and ecological implications of thermal inertia in skipjack tuna, *Katsuwonus pelamis* (Linnaeus). *Env. Biol. Fish.* **1**, 61-80.

Neumann, P., Holeton, G. F. and Heisler, N. (1983). Cardiac output and regional blood flow in gills and muscles after exhaustive exercise in rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **105**, 1-14.

Nikinmaa, M. (1992). Membrane transport and control of hemoglobin-oxygen affinity in nucleated erythrocytes. *Physiological Reviews* **72**, 301-321.

Nikinmaa, M. and Jensen, F. B. (1986). Blood oxygen transport and acid-base status of stressed trout (*Salmo gairdneri*): pre- and postbranchial values in winter fish. *Comp. Biochem. Physiol.* **84A**, 391-396.

Olson, R. J. (1982). Feeding and energetics studies of yellowfin tuna, food for ecological thought. *ICCAT Collective Volume of Scientific Papers* **17**, 444-457.

Olson, R. J. and Boggs, C. H. (1986). Apex predation by yellowfin tuna (*Thunnus albacares*): independent estimates from gastric evacuation and stomach contents, bioenergetics, and cesium concentrations. *Can. J. Fish. Aquat. Sci.* **43**, 1760-1775.

Perry, S. F., Daxboeck, C., Emmett, B., Hochachka, P. W. and Brill, R. W. (1985). Effects of exhausting exercise on acid-base regulation in skipjack tuna (*Katsuwonus pelamis*) blood. *Physiol. Zool.* **58**, 421-429.

Piiper, J., Meyer, M., Worth, H. and Willmer, H. (1977). Respiration and circulation during swimming activity in the dogfish *Scyliorhinus stellaris*. *Resp. Physiol.* **30**, 221-239.

Portner, H. O., Branco, L. G. S., Malvin, G. M. and Wood, S. C. (1994). A new function for lactate in the toad *Bufo marinus*. *J. Appl. Physiol.* **76**, 2405-2410.

Poupa, O., Lindstrom, L., Maresca, A. and Tota, B. (1981). Cardiac growth, myoglobin, proteins and DNA in developing tuna (*Thunnus thynnus thynnus* L.). *Comp. Biochem. Physiol.* **70A**, 217-222.

Powers, D. A. (1980). Molecular ecology of teleost fish hemoglobins: strategies for adapting to changing environments. *Am. Zool.* **20**, 139-162.

Priede, I. G. (1974). The effect of swimming activity and section of the vagus nerves on heart rate in rainbow trout. *J. exp. Biol.* **60**, 305-319.

Priede, I. G. (1985). Metabolic scope in fishes. In *Fish Energetics: New Perspectives*, (ed. P. Tyler and P. Calow), pp. 33-64. Baltimore: John Hopkins University Press.

Priede, I. G. and Tytler, P. (1977). Heart rate as a measure of metabolic rate in teleost fishes; *Salmo gairdneri*, *Salmo trutta*, and *Gadus morhua*. *J. Fish Biol.* **10**, 231-242.

Randall, D. (1985). Shunts in fish gills. In *Cardiovascular Shunts*, (ed. K. Johansen and W. W. Burggren), pp. 71-87. Copenhagen: Munksgaard.

Randall, D. and Daxboeck, C. (1984). Oxygen and carbon dioxide transfer across fish gills. In *Fish Physiology*, vol. 10 (ed. W. S. Hoar and D. J. Randall), pp. 263-314. San Diego: Academic Press.

Randall, D. J. (1970). The circulatory system. In *Fish Physiology*, vol. 4 (ed. W. S. Hoar and D. J. Randall), pp. 132-172. New York: Academic Press.

Randall, D. J. and Daxboeck, C. (1982). Cardiovascular changes in the rainbow trout (*Salmo gairdneri* Richardson) during exercise. *Can. J. Zool.* **60**, 1135-1140.

Randall, D. J. and Smith, J. C. (1967). The regulation of cardiac activity in fish in a hypoxic environment. *Physiol. Zool.* **40**, 104-113.

Rao, G. M. M. (1968). Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. *Can. J. Zool.* **46**, 781-786.

Rayner, M. D. and Keenan, M. J. (1967). Role of red and white muscles in the swimming of the skipjack tuna. *Nature* **214**, 392-393.

Reidy, S. P., Nelson, J. A., Tang, Y. and Kerr, S. R. (1995). Post-exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. *J. Fish Biol.* **47**, 377-386.

Riggs, A. (1970). Properties of fish hemoglobins. In *Fish Physiology*, vol. 4 (ed. W. S. Hoar and D. J. Randall), pp. 209-252. New York: Academic Press.

Ristori, M.-T. and Laurent, P. (1989). Plasma catecholamines in rainbow trout (*Salmo gairdneri*) during hypoxia. *Exp. Biol.* **48**, 285-290.

Roberts, J. L. (1978). Active branchial and ram ventilation in fishes. *Biol. Bull.* **148**, 85-105.

Sanchez-Quintana, D. and Hurle, J. M. (1987). Ventricular myocardial architecture in marine fishes. *The Anatomical Record* **217**, 263-273.

Santer, R. M., Greer Walker, M., Emerson, L. and Witthames, P. R. (1983). On the morphology of the heart ventricle in marine teleost fish (Teleostei). *Comp. Biochem. Physiol.* **76A**, 453-457.

Satchell, G. H. (1991). *Physiology and Form of Fish Circulation*. New York: Cambridge University Press.

Scharold, J. and Gruber, S. H. (1991). Telemetered heart rate as a measure of metabolic rate in the lemon shark, *Negaprion brevirostris*. *Copeia* **1991**, 942-953.

Scharold, J., Lai, N. C., Lowell, W. R. and Graham, J. B. (1989). Metabolic rate, heart rate, and tailbeat frequency during sustained swimming in the leopard shark *Triakis semifasciata*. *Exp. Biol.* **48**, 223-230.

Schulte, P. M., Moyes, C. D. and Hochachka, P. W. (1992). Integrating metabolic pathways in post-exercise recovery of white muscle. *J. exp. Biol.* **166**, 181-195.

Schwerzmann, K., Hoppeler, H., Kayar, S. R. and Weibel, E. R. (1989). Oxidative capacity of muscle and mitochondria: Correlation of physiological, biochemical, and morphometric characteristics. *Proc. Natl. Acad. Sci. USA* **86**, 1583-1587.

Sharp, G. D. (1978). Behavioral and physiological properties of tunas and their effects on vulnerability to fishing gear. In *The Physiological Ecology of Tunas*, (ed. G. D. Sharp and A. E. Dizon), pp. 397-449. New York: Academic Press.

Sharp, G. D. and Francis, R. C. (1976). An energetics model for the exploited yellowfin tuna, *Thunnus albacares*, population in the eastern Pacific Ocean. *Fish. Bull.* **74**, 36-51.

Somero, G. N. and Childress, J. J. (1985). Scaling of oxidative and glycolytic enzyme activities in fish muscle. In *Circulation, Respiration, and Metabolism*, (ed. R. Gilles), pp. 251-262. Berlin: Springer-Verlag.

Soofiani, N. M. and Hawkins, A. D. (1982). Energetic costs at different levels of feeding in juvenile cod, *Gadus morhua* L. *J. Fish Biol.* **21**, 577-592.

Soofiani, N. M. and Priede, I. G. (1985). Aerobic metabolic scope and swimming performance in juvenile cod, *Gadus morhua* L. *J. Fish Biol.* **26**, 127-138.

Stevens, E. D. (1972). Some aspects of gas exchange in tuna. *J. exp. Biol.* **56**, 809-823.

Stevens, E. D. (1982). The effect of temperature on facilitated oxygen diffusion and its relation to warm tuna muscle. *Can. J. Zool.* **60**, 1148-1152.

Stevens, E. D., Bennion, G. R., Randall, D. J. and Shelton, G. (1972). Factors affecting arterial pressures and blood flow from the heart in intact, unrestrained lincod, *Ophiodon elongatus*. *Comp. Biochem. Physiol.* **43A**, 681-695.

Stevens, E. D. and Carey, F. G. (1981). One why of the warmth of warm-bodied fish. *Am. J. Physiol.* **240**, R151-R155.

Stevens, E. D. and Dizon, A. E. (1982). Energetics of locomotion in warm-bodied fish. *Annual Review of Physiology* **44**, 121-131.

Stevens, E. D. and McLeese, J. M. (1984). Why bluefin tuna have warm tummies: temperature effect on trypsin and chymotrypsin. *Am. J. Physiol.* **246**, R487-R494.

Stevens, E. D. and Neill, W. H. (1978). Body temperature relations of tunas, especially skipjack. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar and D. J. Randall), pp. 315-359. New York: Academic Press.

Stevens, E. D. and Randall, D. J. (1967). Changes of gas concentrations in blood and water during moderate swimming activity in rainbow trout. *J. exp. Biol.* **46**, 329-337.

Suarez, R. K., Lighton, J. R. B., Brown, G. S. and Mathieu-Costello, O. (1991). Mitochondrial respiration in hummingbird flight muscles. *Proc. Natl. Acad. Sci. USA* **88**, 4870-4873.

Sund, P. N., Blackburn, M. and Williams, F. (1981). Tunas and their environment in the Pacific ocean: a review. *Oceanogr. Mar. Biol. Ann. Rev.* **19**, 443-512.

Thomas, S., Poupin, J., Lykkeboe, G. and Johansen, K. (1987). Effects of graded exercise on blood gas tensions and acid-base characteristics of rainbow trout. *Resp. Physiol.* **68**, 85-97.

Thorarensen, H., Gallaugher, P. and Farrell, A. P. (1996). Cardiac output in swimming rainbow trout, *Oncorhynchus mykiss*, acclimated to seawater. *Physiol. Zool.* **69**, 139-153.

Thorarensen, H., Gallaugher, P. E., Kiessling, A. K. and Farrell, A. P. (1993). Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of haematocrit on blood flow distribution. *J. exp. Biol.* **179**, 115-139.

Tibbits, G. F. and Kashihara, H. (1992). Myocardial sarcolemma isolated from skipjack tuna, *Katsuwonus pelamis*. *Can. J. Zool.* **70**, 1240-1245.

Tota, B. (1978). Functional cardiac morphology and biochemistry in Atlantic bluefin tuna. In *The Physiological Ecology of Tunas*, (ed. G. D. Sharp and A. E. Dizon), pp. 89-112. New York: Academic Press.

Tucker, V. A. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* **23**, 410-414.

Tuurala, H.. Part, P., Nikinmaa, M. and Soivio, A. (1984). The basal channels of secondary lamellae in *Salmo gairdneri* gills -- a non-respiratory shunt. *Comp. Biochem. Physiol.* **79A**, 35-39.

Videler, J. J. (1993). *Fish Swimming*. New York: Chapman and Hall.

Walters, V. and Fierstine, H. L. (1964). Measurements of swimming speeds of yellowfin tuna and wahoo. *Nature* **202**, 208-209.

Wardle, C. S. (1977). Effects of size on the swimming speeds of fish. In *Scale Effects in Animal Locomotion*, (ed. T. J. Pedley), pp. 299-313. New York: Academic Press.

Wardle, C. S., Videler, J. J., Arimoto, T., Franco, J. M. and He, P. (1989). The muscle twitch and the maximum swimming speed of giant bluefin tuna, *Thunnus thynnus* L. *J. Fish Biol.* **35**, 129-137.

Webb, P. W. (1975). Hydrodynamics and energetics of fish propulsion. *Bull. Fish. Res. Bd. Can.* **190**, 109-119.

Webb, P. W. (1977). Effects of size on performance and energetics of fish. In *Scale Effects in Animal Locomotion*, (ed. T. J. Pedley), pp. 315-331. New York: Academic Press.

Weber, J., Brill, R. W. and Hochachka, P. W. (1986). Mammalian metabolite flux rates in a teleost: lactate and glucose turnover in tuna. *Am. J. Physiol.* **250**, R452-R458.

Weber, J. M. (1992). Pathways for oxidative fuel provision to working muscles: Ecological consequences of maximal supply limitations. *Experientia* **48**, 557-564.

Westneat, M. W., Hoese, W., Pell, C. A. and Wainwright, S. A. (1993). The horizontal septum: mechanisms of force transfer in locomotion of Scombrid fishes (Scombridae, Perciformes). *Journal of Morphology* **217**, 183-204.

White, F. C., Kelly, R., Kemper, S., Schumacker, P. T., Gallagher, K. R. and Laurs, R. M. (1988). Organ blood flow haemodynamics and metabolism of the albacore tuna *Thunnus alalunga* (Bonnaterre). *Exp. Biol.* **47**, 161-169.

Wild, A. (1986). Growth of yellowfin tuna, *Thunnus albacares*, in the eastern tropical Pacific Ocean based on otolith increments. *Int.-Am. Trop. Tuna Comm. Bull.* **18**, 422-479.

Wild, A. (1994). A review of the biology and fisheries for yellowfin tuna, *Thunnus albacares*, in the eastern Pacific ocean. In *Interactions of Pacific Tuna Fisheries. Proceedings of the First FAO Expert Consultation on Interactions of Pacific Tuna Fisheries. 3-11 December 1991. Noumea, New Caldonia. Volume 2: Papers on*

Biology and Fisheries, (ed. R. S. Shomura, J. Majkowski and S. Langi), pp. 52-108. Rome: FAO.

Wilson, R. W. and Egginton, S. (1994). Assessment of maximum sustainable swimming performance in rainbow trout (*Oncorhynchus mykiss*). *J. exp. Biol.* **192**, 299-305.

Wood, C. M. (1991). Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. *J. exp. Biol.* **160**, 285-308.

Wood, C. M. and Shelton, G. (1980). The reflex control of heart rate and cardiac output in the rainbow trout: interactive influences of hypoxia, haemorrhage, and systemic vasomotor tone. *J. exp. Biol.* **87**, 271-284.

Yamamitsu, S. and Itazawa, Y. (1990). Effects of preload, afterload and temperature on the cardiac function and ECG in the isolated perfused heart of carp. *Nippon Suisan Gakkaishi* **56**, 229-237.

Yuen, H. S. H. (1970). Behavior of skipjack tuna, *Katsuwonus pelamis*, as determined by tracking with ultrasonic devices. *J. Fish. Res. Bd Can.* **27**, 2071-2079.

Zar, J. H. (1984). *Biostatistical Analysis*. Englewood Cliffs, New Jersey: Prentice Hall.