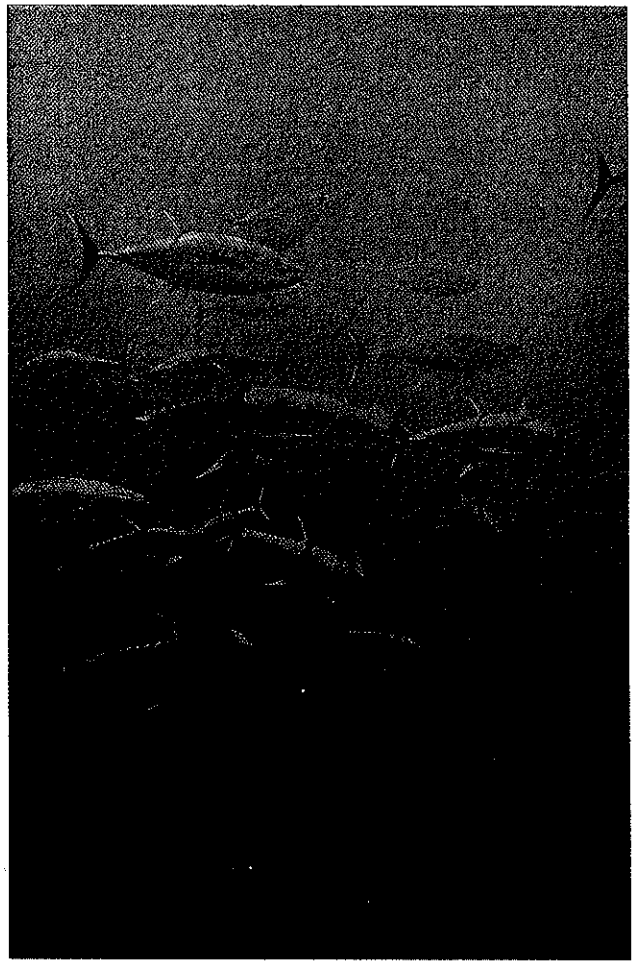

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TUNA: PHYSIOLOGY, ECOLOGY, AND EVOLUTION

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3

THE CARDIOVASCULAR SYSTEM OF TUNAS

RICHARD W. BRILL
PETER G. BUSHNELL

I. INTRODUCTION

Tunas (family *Scombridae,* tribe *Thunnini*) have high metabolic rates (Korsmeyer and Dewar, this volume) and are obligate ram ventilators (Brown and Muir, 1970; Roberts, 1975, 1978). They suffocate rapidly if prevented from swimming so special care must be taken to ensure that ventilatory requirements are met during all stages of an experiment. Tunas also struggle violently during any restraining procedure. It is impossible to use completely intact, unanesthetized, "resting" fish to make even the simplest cardiorespiratory measurements using techniques commonly employed with other species (e.g., Smith and Davie, 1984; Smatresk, 1986). Some data have been obtained on swimming tunas (Jones *et al.,* 1990, 1993; Bushnell and Brill 1991; Korsmeyer *et al.,* 1997a,b) but these procedures are inherently very difficult and fish can carry only minimal instrumentation with-

Table I
Cardiorespiratory Function in Tunas and Other Teleosts

	Yellowfin tuna[a]	Skipjack tuna[a]	Yellowtail[b]	Rainbow trout[c]	
Temperature	25°C	25°C	19–25°C	10°C	10°C
Body mass (kg)	≈1–2	≈1–2	≈1	0.9–1.5	0.9–1.5
Swimming speed (body lengths s^{-1})	1.0–1.3	1.6–2.2	—	—	≈0.5–1.5[g]
Activity level	Routine[b]	Routine	Rest	Rest	Maximum sustainable
Total O_2 consumption (mg O_2 kg^{-1} h^{-1})	**776**	**974**	138	48	372
Gill O_2 consumption (mg O_2 kg^{-1} h^{-1})	**362**	**420**	2	≈0	213
Body O_2 consumption (mg O_2 kg^{-1} h^{-1})	**414**	**554**	136	54	159
Ventilation volume (liter min^{-1} kg^{-1})	**2.4–4.7**	**3.8**	0.46	0.5	1.7
Utilization (%)	**51–59**	**52**	25	33	33
TO_2 (mg O_2 min^{-1} kPa^{-1} kg^{-1})[d]	**0.030**	**0.056**	—	0.00076	0.0038
E_w (%)[d]	**63**	**67**	—	42	37
E_b (%)[d]	89	74	73	100	—
P_aO_2 (kPa)[e]	10–12	9.3	10.6	18.3	16.8
P_vO_2 (kPa)[e]	5.2–5.3	4.9	—	4.4	2.1
C_aO_2 (mg dl^{-1})[e]	18	20	16	15	14
C_vO_2 (mg dl^{-1})[e]	12	13	9.5	10	1.9
O_2 delivery (mg O_2 min^{-1} kg^{-1})[e]	**21**	**26**	5.6	2.7	7.4
Ventilation perfusion capacity-rate ratio[d]	0.73	1.1	0.55	1.2–2.0	2.2
Hematocrit (%)[e]	27–35	34–38	29	23	27
Hemoglobin concentration (g dl^{-1})[e]	11–12	13	11	6.4	—
Mean cell hemoglobin concentration (g dl^{-1})	31–44	34–38	38	29	—
Gill surface area (cm^2/kg body mass)	**14,380**	**18,400**	—	≈2000	—
Gill blood–water barrier thickness (μm)	**0.533**	**0.596**	—	6.37	—
Blood volume (ml kg^{-1})	31–54	≈50	58	41–52	—
Cardiac output (ml min^{-1} kg^{-1})[e]	**115**	**132**	35	18	53
Heart rate (beats min^{-1})[e]	62–97	79	96	38	51
Stroke volume (ml kg^{-1})[e]	**1.1**	**1.3**	0.35	0.46	1.03
Mean VA blood pressure (kPa)[e]	**10.8–11.8**	**11.6**	5.8	5.2	8.3
Mean DA blood pressure (kPa)[e]	**6.7–6.8**	**5.3**	1.4	4.1–4.5	—
Gill resistance (kPa ml^{-1} min^{-1} kg^{-1})[e]	0.035–0.044	0.047	0.12	0.039–0.061	—
Systemic resistance (kPa ml^{-1} min^{-1} kg^{-1})[e]	0.058–0.059	0.040	0.040	0.23–0.25	—
Total resistance (kPa ml^{-1} min^{-1} kg^{-1})[e]	**0.094–0.10**	**0.088**	0.17	0.29	0.16
Ventricle mass (% body mass)	**0.29**	**0.38**	0.11	0.08–0.13	0.08–0.13
Cardiac power output (mW kg^{-1} body mass)[f]	**21–23**	**26**	3.4	1.6	7.7
Cardiac power output (mW g^{-1} ventricle mass)	**7.1–7.8**	**6.7**	3.1	1.2–1.9	5.6–9.1

Note: Those parameters that we consider clearly enhanced in skipjack and yellowfin tunas are printed in bold.

[a]Data for yellowfin (*Thunnus albacares*) and skipjack (*Katsuwonus pelamis*) tunas compiled from Muir and Hughes (1969); Bushnell (1988); Bushnell *et al.* (1990); Jones *et al.* (1986, 1990, 1993); Brill and Bushnell (1991); Bushnell and Brill (1991, 1992); Dewar (1993); Brill and Jones (1994); Dewar and Graham (1994); Korsmeyer *et al.* (1997a,b); Brill *et al.* (1998); Lowe *et al.* (1998); and R. Brill, T. Lowe, and K. Cousins (unpublished data).

[b]Data for yellowtail (*Seriola* spp.) compiled from Yamamoto *et al.* (1981); Itazawa *et al.* (1983); and Ishimatsu *et al.* (1990, 1997).

[c]Data for rainbow trout (*Oncorhynchus mykiss*) compiled from Holeton and Randall (1967); Randall *et al.* (1967); Stevens and Randall (1967); Kiceniuk and Jones (1977); Gngerich *et al.* (1987, 1990); Tetens and Christensen (1987); Palzenberger and Pohola (1992); and Farrell and Jones (1992).

[d]Data for skipjack and yellowfin tunas from spinally blocked (i.e. nonswimming) fish.

[e]Data for skipjack tuna from spinally blocked (i.e., nonswimming) fish.

[f]Cardiac power output is calculated as cardiac output multiplied by mean ventral aortic (VA) pressure.

[g]As a result of the extra hydrodynamic drag induced by the catheters and wires required to measure heart rate, blood pressures, etc., maximum sustainable swimming speeds were below those of uninstrumented fish (~2 body lengths s^{-1}).

impacting hydrodynamics and increasing metabolic energy demand (Kiceniuk l Jones, 1977; Jones *et al.*, 1990). Much of the data on tuna cardiovascular ction has, therefore, been recorded in somewhat unusual circumstances such total neuromuscular blockade with forced ventilation (Stevens, 1972; White *il.*, 1988) or spinal blockade with fish in a water stream (Bushnell and Brill,)2). Tunas are also expensive to acquire, difficult to maintain in captivity, and routinely kept in shore side tanks at only a handful of laboratories (Nakamura, '2; Brill, 1999; Farwell, this volume). The difficulties of working with indiual fish are compounded because relatively few animals are available for study. As a result, our understanding of cardiovascular function in tunas is incomte. This should not be construed to mean that it remains a mystery. On the trary, in spite of the difficulties of working with these fishes, there exists a iificant body of publications that range from the molecular and biochemical to ole-animal physiology. In this chapter we synthesize the more recent of these erse studies and endeavor to show how they are beginning to present a unified ure of these fascinating animals.

We start our discussion by attempting to distinguish which aspects of the carvascular system of tunas are clearly enhanced compared to other teleosts. For following reasons, however, we do so with only a modest degree of certainty. es of virtually all physiological functions are influenced by temperature and le nonlinearly with body mass. Yet correcting for these variables, and makappropriate comparisons, can be problematic because temperature effects and ing relationships are often ill-defined (Packard and Boardman, 1999). This is ecially true when comparing rates of cardiovascular function in tunas to those ther teleosts. Because of their accessibility, most of our knowledge of teleost liovascular physiology comes from measurements made on temperate (i.e., at)°C), small (<1 kg body mass), freshwater fishes (e.g., rainbow trout, *Oncorhchus mykiss*). In contrast, information on tuna cardiovascular function comes tly from measurements made on 1- to 3-kg skipjack tuna (*Katsuwonus pelis*) and yellowfin tuna (*Thunnus albacares*) at 25°C. Data at ≈15°C are avail- for albacore (*Thunnus alalunga*), but are from fish of ≈7–10 kg body mass i *et al.*, 1987; White *et al.*, 1988; Graham *et al.*, 1989). Also, albacore are not inely maintained in captivity and all *in vivo* data have been collected at sea on ntly captured fish when they may have been physiologically stressed and reering from exhaustive exercise. The different methods used to quantify cardioular function in tunas and other teleosts can also generate what appear to be cies-specific differences, but that are artifactual (Metcalf and Butler, 1982; inin *et al.*, 1999). Therefore, as in all experiments involving whole animals, more so with tunas, the anesthesia, surgical, handling, and instrumentation cedures required to make cardiovascular measurements can easily distort the processes being evaluated.

We also attempt to discern the important functional adaptations that set tunas

apart from other teleosts. In theory, this type of analysis should involve comparisons of more than two or three species, include data from "primitive" members of the clade, and employ statistical procedures that take phylogenetic distances into account (Garland and Adolph, 1994). In our case, the application of these principles is problematic as the *in vivo* data on tuna cardiovascular physiology come from only three species: skipjack tuna, yellowfin tuna, and albacore. Moreover, although critical for discerning the evolution of cardiovascular function, the physiology of primitive members of the family *Scombridae* (the so-called "ectothermic scombrids") remains almost unstudied. [The ectothermic scombrids include the bonitos (tribe *Sardini*), seerfishes (tribe *Scomberomorini*), and mackerel (tribe *Scombrini*).]

II. CARDIOVASCULAR FUNCTION AT ROUTINE ACTIVITY LEVELS

Besides being obligate ram ventilators, tunas also depend on constant forward motion to produce lift from their pectoral fins for hydrostatic equilibrium (Magnuson, 1978; Magnuson and Weininger, 1978). If tunas stop swimming they sink as well as suffocate. As a result, throughout this review we refer to "routine" rates of cardiovascular function. By this we mean those associated with swimming speeds ranging from 1 to 2 body lengths s^{-1}. This level of activity is commonly recorded when skipjack and yellowfin tunas are able to set their swimming speeds either in the laboratory, or when fish carrying ultrasonic transmitters are followed in the open ocean (e.g., Dizon *et al.*, 1978; Dizon and Brill, 1979; Gooding *et al.*, 1981; Holland *et al.*, 1990b; Block *et al.*, 1997; Brill *et al.*, 1999; Freund, 1999).

Table I summarizes the anatomical and physiological descriptors of cardiovascular function in tunas that we regard as the most reflective of unstressed animals. We have highlighted in bold those values we consider unusual in tunas, and on which we will focus. For comparison, we have included data from yellowtail (*Seriola* spp.) and rainbow trout. We selected the former because they are active, pelagic, warm-water, marine fishes, and because data are available from a range of body masses similar to those of skipjack and yellowfin tunas commonly used in physiological experiments. We chose the latter because it is the most well-studied teleost species in terms of its physiology, and because data are available on minimal as well as maximal rates of cardiovascular function.

The standard metabolic rates (i.e., those measured in fish paralyzed with neuromuscular blocking agents) of skipjack and yellowfin tuna are 412 and 286 mg O_2 kg^{-1} h^{-1}, respectively (Brill, 1987), values several times those of yellowtail at "rest." At routine swimming speeds (≈1–2 body lengths s^{-1}), the metabolic rates of skipjack and yellowfin tunas are 974 and 776 mg O_2 kg^{-1} h^{-1}, which are approximately six to eight times that of yellowtail, a marine species living at a

similar temperature (Table I). Clearly, the cardiovascular and respiratory systems of skipjack and yellowfin tunas must supply oxygen and metabolic substrates to the tissues at exceptional rates, and hence we often use the term "high-energy-demand fishes" as a general descriptor (e.g., Brill and Bushnell, 1991; Brill *et al.*, 1998). Interestingly, the metabolic rates of resting yellowtail and rainbow trout are comparable when corrected for temperature (assuming a Q_{10} of 2; Boehlert, 1978; Moffitt and Crawshaw, 1983).

A. Oxygen Transport from Water to Tissues

The rate of oxygen movement across the gill respiratory epithelium is directly proportional to functional surface area and inversely proportional to the thickness of the diffusion barrier (Wood and Perry, 1985). Tunas have gill surface areas approximately seven to nine times larger, and gill blood–water barrier thicknesses approximately an order of magnitude less, than those of rainbow trout (Table I). These anatomical adaptations appear to be required for the relatively elevated rate of gas transfer across tuna gills occurring even at routine activity levels (Hughes, 1984; Perry, 1992). Skipjack and yellowfin tunas have ventilation volumes approximately 5 to 10 times those of other teleosts (Table I), yet because of their large thin gills, both are able to extract approximately 50% of the oxygen from the ventilatory water stream (utilization, Table I). The effectiveness of oxygen transfer from water (E_w) is the ratio of the actual rate of removal of oxygen from the ventilatory water stream to the theoretically maximum possible rate. E_w in tunas (>60%) is substantially higher than that in trout (33%), indicating that a larger fraction of the ventilatory water stream is in effective contact with the gas exchange surface in tunas.

Oxygen diffusing across the lamella is carried away from the gill by red blood cells. Contrary to much of the speculation in the early literature, the oxygen content of arterial blood (C_aO_2) in tunas is not unusual (Bushnell and Brill, 1992; Korsmeyer *et al.*, 1997b). This is because the elevated hematocrit and hemoglobin levels reported originally were based on blood samples taken from highly stressed, recently boated fish (Klawe *et al.*, 1963). Neither are seen in catheterized tunas that are fully recovered from handling and surgery (Bushnell and Brill, 1992; Brill and Jones, 1994; Lowe *et al.*, 1998). High rates of oxygen delivery to the tissues (O_2 delivery, Table I) at routine activity levels are, therefore, sustained by high cardiac output (approximately three times those of other teleosts). This, in turn, is made possible by large stroke volumes, rather than elevated heart rates (when comparisons are made at equivalent body temperatures). It should be noted, however, that the cardiac output of rainbow trout has been shown to be linearly related to acclimation temperature, with a Q_{10} of ≈4 (Barron *et al.*, 1987). When extrapolated to an acclimation temperature of 25°C (skipjack and yellowfin tunas' normal water temperature), the cardiac output of rainbow trout (80–90 ml min^{-1} kg^{-1}) could theoretically approach those of tunas. This cardiac output, however, more likely reflects maximum, rather than routine, outputs in trout (Farrell *et al.*, 1996).

Elevated cardiac outputs increase the transfer factors (TO_2; i.e., the rate of oxygen transfer from the water to the blood per unit O_2 partial pressure difference between the water and venous blood) of skipjack and yellowfin tunas to approximately two orders of magnitude above those measured in other fishes (Bushnell and Brill, 1992). High cardiac outputs also ensure that ventilation and perfusion capacities in tunas are well matched, since the rate at which oxygen is delivered to the gills by the ventilatory water stream will be equivalent to the rate oxygen can be transported away by the blood. To express this more formally, the ventilation perfusion capacity–rate ratio (i.e., the ratio of the oxygen content of the inhalant water multiplied by the ventilation volume to the oxygen content of arterial blood multiplied by the cardiac output) is close to the theoretically ideal value of one (Piiper and Scheid, 1984).

B. High Blood Pressures and the Maintenance of Tissue Fluid Balance

Skipjack and yellowfin tunas' high cardiac outputs also generate unusually high (for a teleost) arterial blood pressures (Table I). Although blood pressure is the product of cardiac output and total peripheral resistance, the elevated dorsal and ventral aortic blood pressures of tunas are solely a result of the former, as the total vascular resistance is actually below that of other fishes (Bushnell *et al.*, 1992). The tunas' large gills and vascular countercurrent heat exchangers both contain numerous parallel blood flow channels (Kishinouye, 1923; Muir and Hughes, 1969; Muir, 1970; Muir and Brown, 1971; Stevens *et al.*, 1974; Graham and Diener, 1978) and have large total cross-sectional areas. Therefore, they do not add significantly to total vascular resistance.

The high arterial blood pressures may, however, have consequences with respect to tissue fluid balance. Although our understanding of this aspect of tuna cardiovascular function remains incomplete, recent progress has been made. Plasma colloid osmotic pressure (COP) works in opposition to capillary hydrostatic pressure to maintain tissue fluid balance. At the arterial end of the capillary, where capillary hydrostatic pressure exceeds COP, fluid filtration results and there is transfer of extracellular fluid from the plasma to interstitium. At the venous end of the capillary, when COP exceeds capillary hydrostatic pressure, fluid reabsorption occurs (Friedman, 1976). The net fluid flux during the passage of blood through capillaries, therefore, depends on arterial blood pressure (the primary determinant of capillary hydrostatic pressure) and permeability of the capillaries to blood proteins and their concentration (the primary contributors to plasma COP). Hargens *et al.* (1974) found protein concentrations in cod (*Gadus morhua*) and

flounder (*Pleu.. .ectes platessa*) plasma, interstitial, and peritoneal fluid to be essentially identical and concluded that capillary permeability of fishes was high, and therefore, effective COP across the capillaries was low. In general, it appears that if capillary hydrostatic pressure is low, a high COP may not be needed to ensure adequate fluid reabsorption.

Following this chain of logic, high blood pressure (i.e., capillary hydrostatic pressure) in tunas could result in elevated rates of net fluid transfer from the capillaries to the interstitium. In mammals, fluid not reabsorbed by the capillaries is drained from the interstitial space by the lymph system. Fish do not have a lymph system anatomically identical to that of higher vertebrates, but rather a "secondary circulatory system" which appears to play a similar role (Steffensen and Lomholt, 1992; Olson, 1996). The vessels of the secondary circulation arise from the walls of various arteries as arteriolar-sized vessels that coalesce into larger trunks. Dewar *et al.* (1994) described vessels of a secondary circulation in the central vascular heat exchangers of skipjack tuna similar to those seen in other teleosts (e.g., Chopin *et al.*, 1998). There is no evidence, however, that the secondary circulation is more developed in tunas than in other teleosts (Brill *et al.*, 1998), as would be expected if tunas had significantly high rates of net fluid transfer out of the capillaries.

We have attempted to measure capillary pressures in skipjack and yellowfin tunas (along with coinvestigators D. R. Jones and J. F. Steffensen), but have not yet succeeded. Our recent work on the relative capillary permeability and protein concentrations of plasma and various interstitial fluids of yellowfin tuna, cod, and rainbow trout, however, suggests tuna capillaries have low protein permeability (Jones *et al.*, 2000). Figure 1 shows that capillaries of yellowfin tuna are far less permeable to large molecules (e.g., 40-kDa dextran) than those of the other two species. In yellowfin tuna, plasma COP is thus maintained during passage through the capillaries, and net fluid transfer out of the vascular system is not excessive. As might be expected, Figure 1 also suggests that capillary permeability is inversely related to dorsal aorta blood pressure (≈7, ≈5, and ≈4 kPa in yellowfin tuna, rainbow trout, and cod, respectively), and presumably capillary pressure. Bluefish (*Pomatomus saltatrix*) have mean ventral aortic blood pressures approaching those of tunas (≈10 kPa; Ogilvy *et al.*, 1988), and a study of their capillary permeability could be useful to test the hypothesis that the capillary permeability and blood pressures are indeed inversely related.

III. MAXIMAL RATES OF CARDIOVASCULAR FUNCTION IN TUNAS

To take advantage of the resources of the pelagic environment, skipjack, yellowfin, and bigeye tunas are highly aggregated and highly mobile. Although

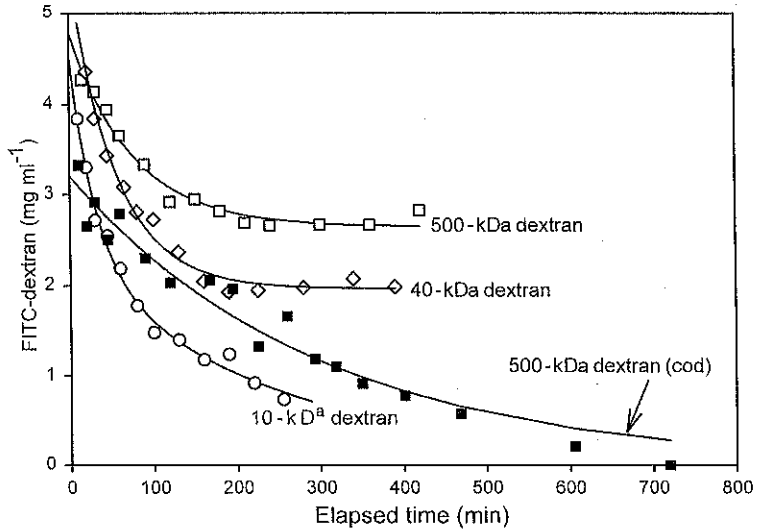

Fig. 1. Plasma concentration of fluorescein-isothiocyanate-labeled dextran (FITC-dextran) following a bolus injection of the marker into the ventral aorta. The initial rapid drop is most likely a result of the marker equilibrating within the circulatory system. In yellowfin tuna (open symbols), dextran molecules as small as 40 kilo-Daltons (kDa) remain within the vascular compartment, as demonstrated by constant plasma concentrations after 200 min. In cod (filled symbols), even with molecules as large as 500 kDa, plasma concentration continues to decline, indicating a leak from the vascular compartment, most likely into the interstitial space. Plasma decay curves for rainbow trout (data not shown) injected with 500-kDa dextran were similar to those for cod. The data clearly show the capillaries of yellowfin tuna are less permeable than are those of other teleosts, a characteristic which is important for maintaining tissue fluid balance in the face of elevated blood pressures (data from Jones *et al.*, 2000).

the former makes them an exploitable, economically important resource (Sharp, 1978), it is the latter that has shaped much of the thinking about tuna biology. As a result, there has been a general focus on what initially appeared to be their exceptional locomotor abilities (e.g., Walters, 1962; Carey, 1973; Graham, 1975; Magnuson, 1978; Stevens and Carey, 1981; Sharp, 1983; Bushnell and Holland, 1989; Block, 1991). While they are certainly capable of high burst and cruising speeds, there is little evidence that maximum burst and maximum sustainable swimming speeds of skipjack and yellowfin tunas are significantly above those of other active teleosts living at similar temperatures (Brill, 1996). Rather, as first recognized by Bushnell and Brill (1991) and later reinforced by Korsmeyer *et al.* (1996a), the cardiovascular systems of skipjack and yellowfin tunas appear capable of delivering oxygen and metabolic substrates at exceptionally high rates to meet multiple metabolic demands simultaneously (e.g., rapid lactate metabolism,

rapid digestion, and high growth rates) rather than to allow exceptionally high sustained swimming speeds. For example, based on data presented in Guppy *et al.* (1979), Arthur *et al.* (1992), Dewar (1993), Brill (1994), and Bushnell and Jones (1994), during recovery from exhaustive exercise, skipjack tuna swimming at about 1.5 body lengths s^{-1} would have to reach a total metabolic rate of 2276 mg O_2 kg^{-1} h^{-1} to metabolize white muscle lactate over the observed 2-h time course. This prediction closely approaches the highest metabolic rate thus far measured in tunas—≈2500 mg O_2 kg^{-1} h^{-1} in skipjack tuna immediately after capture at sea and which were presumably recovering from exhaustive exercise (Gooding *et al.*, 1981). In contrast, the highest metabolic rate of which other teleosts of approximately equivalent body mass are capable is approximately 1000 mg O_2 kg^{-1} h^{-1} (Brett, 1972).

A. How Skipjack and Yellowfin Tunas Achieve High Maximum Metabolic Rates

Although routine rates of cardiovascular function (Table I) have been quantified in skipjack and yellowfin tunas with at least some degree of certainty, maximal rates have not. Rather, they are based on a limited number of observations (Korsmeyer, 1996; Korsmeyer *et al.*, 1997a,b) and extrapolations (Brill and Bushnell, 1991; Brill, 1994; Bushnell and Jones, 1994; Korsmeyer *et al.*, 1996a,b). To date, Graham *et al.* (1989), Dewar and Graham (1994), and Korsmeyer *et al.* (1997a,b) are the only investigators to have successfully measured changes in metabolic rate and cardiovascular function in tunas swimming at various velocities in a water tunnel. Unfortunately, the range of speeds at which the instrumented fish would swim was limited (≈0.8–2.4 body lengths s^{-1}), and it remains unclear if metabolic demand and associated rates of cardiovascular function were indeed maximal and representative of rates in free-swimming tunas in the wild. Regardless, the experimental observations and mathematical extrapolations are now beginning to form a reasonably consistent picture of tunas' physiological capabilities.

The relationship between metabolic rate, cardiac output, and blood oxygen-carrying capacity can be described by three equations,

$$\dot{V}O_2 = Q \cdot ([O_2]_{\text{arterial blood}} - [O_2]_{\text{venous blood}}), \quad (1)$$

$$Q = HR \cdot SV, \quad (2)$$

$$[O_2]_{max} = MCHC \cdot HCT \cdot [O_{2\ Hb}], \quad (3)$$

where:

$\dot{V}O_2$ is metabolic rate;
Q, cardiac output;

$[O_2]_{\text{arterial blood}}$, oxygen content of the arterial blood;
$[O_2]_{\text{venous blood}}$, oxygen content of the venous blood;
HR, heart rate;
SV, stroke volume;
$[O_2]_{max}$, maximum oxygen-carrying capacity of the arterial blood;
MCHC, mean red blood cell hemoglobin concentration;
HCT, hematocrit; and
$[O_{2\ Hb}]$, oxygen-carrying capacity per unit mass of hemoglobin.

Although tunas could increase any or all of the variables in Equations (1)–(3) to reach their maximum metabolic rates, they appear to attain high rates of oxygen and substrate delivery as a result of (a) three anatomical adaptations (large gill surface area, thin blood–water barrier in the gills, and enlarged ventricle mass; Table I), (b) the ability to achieve high arterial–venous content difference (Equations 1 and 3), and (c) the ability to reach exceptionally high maximum cardiac outputs through high heart rates and large stroke volumes (Equation 2; Brill and Bushnell, 1991; Bushnell and Jones, 1994; Farrell, 1991, 1996).

The cardiac outputs required to meet tunas' estimated maximum metabolic rates, and how these are achieved, are relevant to several other aspects of tuna biology and therefore we examine these subjects in some detail. Unfortunately, the maximum cardiac output of any tuna species has never been measured. By combining available data from a number of sources, however, we can use Equation (1) to model an upper, physiologically reasonable limit that can be tested in future experiments. As is clear from Equation (1), in addition to knowing maximal $\dot{V}O_2$, an estimate of the maximal $([O_2]_{\text{arterial blood}} - [O_2]_{\text{venous blood}})$ is also required. While this too has never been measured, previous studies give us some insight into tuna blood oxygen-carrying capacity as well as oxygen extraction potential at the tissues.

The maximal $([O_2]_{\text{arterial blood}} - [O_2]_{\text{venous blood}})$ will be determined in part by $[O_2]_{max}$, which, in turn, is determined by hematocrit (Equation 3). The normal hematocrits of skipjack and yellowfin tuna range from 27 to 34% (Bushnell and Brill, 1992; Korsmeyer *et al.*, 1997b). An hematocrit of ≈50% is seen during recovery from exhaustive exercise (White *et al.*, 1988) due to red cell ejection from the spleen, red cell swelling, loss of plasma volume, or (more likely) a combination of all three (Yamamoto and Itazawa, 1989). A HCT of 50% and a MCHC of 370 g $liter^{-1}$ (Korsmeyer *et al.*, 1997b; Lowe *et al.*, 1998) would result in a blood hemoglobin concentration of 185 g $liter^{-1}$, a value within the range of those measured in tunas recently boated at sea (141–207 g $liter^{-1}$; Klawe *et al.*, 1963). At 25°C, the oxygen-carrying capacity of hemoglobin is 1.25 ml O_2 g^{-1} (Ganong, 1973), resulting in a maximum predicted $[O_2]_{\text{arterial blood}}$ of 231 ml O_2 $liter^{-1}$. It is unlikely that tuna could produce a $[O_2]_{\text{venous blood}}$ of zero. The lowest values for skipjack and yellowfin tuna reported in the literature are ≈70–80 ml O_2 $liter^{-1}$—

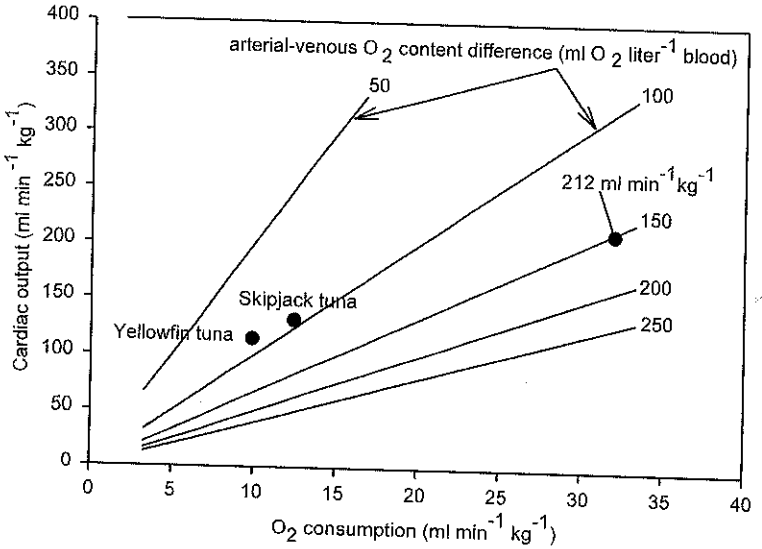

Fig. 2. Predicted cardiac outputs required to reach various rates of oxygen consumption in skipjack and yellowfin tuna as a function of the arterial–venous (A-V) blood oxygen content difference. At a reasonable maximum A-V blood oxygen content difference (150 ml O_2 liter^{-1} blood), estimated maximum metabolic rates could be achieved by doubling routine cardiac output. Figure is adapted from Bushnell and Jones (1994); reprinted with permission.

values recorded during severe hypoxia (Bushnell and Brill, 1992) and recovery from anesthesia (Korsmeyer *et al.*, 1997b). Based on these estimates, maximum ($[O_2]_{\text{arterial blood}} - [O_2]_{\text{venous blood}}$) in tunas is most likely about 151–161 ml O_2 liter^{-1}.

By applying these data to Figure 2, in order to generate their maximum documented metabolic rate (32 ml O_2 min^{-1} at ≈25°C; Gooding *et al.*, 1981), skipjack tuna would have to attain a cardiac output of 212 ml min^{-1} kg^{-1}, or an increase of approximately 1.6 times their routine cardiac output (Table I). The predicted increase in cardiac output is less than that measured by Kiceniuk and Jones (1977) in freshwater rainbow trout going from rest to maximum sustainable swimming speed (3× increase), but it is similar to that observed by Thorarensen *et al.*, (1996) in seawater-acclimated rainbow trout (1.8× increase).

The limited amount of data collected *in vivo* supports the model's predictions. For instance, the maximum increases in cardiac output and heart rate in yellowfin tuna observed by Korsmeyer *et al.* (1997a) during exercise was approximately about 1.3 and 1.5 times above those seen in slowly swimming fish. Moreover, the maximum increase in heart rate observed in swimming yellowfin tuna matches that seen in nonswimming fish whose vagal control of heart rate has been blocked by injection of atropine (Keen *et al.*, 1995). Since stroke volume appears to be

fixed in skipjack and yellowfin tunas (discussed in detail in next section), cardiac output mirrors changes in heart rate. Taken together, both the model and data suggest that the ratio of maximal to routine cardiac output in tunas is similar to that of other active teleosts. It should be noted, however, that the predicted maximum cardiac output of skipjack tuna (>200 ml min^{-1} kg^{-1} at 25°C) is still substantially higher (≈3 to 4 times) than those of other fishes (Table I).

B. Elevated Hematocrit, Oxygen Transport, and Blood Viscosity

The positive impact of high maximum hematocrit (≈50% in tunas) on maximum blood oxygen-carrying capacity can potentially be offset by the resulting increase in blood viscosity which raises blood pressure, a primary determinant of cardiac work and maximum cardiac output. As a result, increases in blood viscosity could reduce maximum cardiac output and actually impair blood flow and oxygen delivery (Egginton, 1998). In tuna (as well as all vertebrates studied to date), blood viscosity increases exponentially with hematocrit (Figure 3A), while maximum blood oxygen content increases linearly (Fletcher and Hedrich, 1987; Wells and Baldwin, 1990). The relationship between the two is reflected in the blood oxygen transport capacity, which is the quotient of maximum blood oxygen capacity divided by viscosity. When plotted against hematocrit (Figure 3B), blood oxygen transport capacity is highly nonlinear, with maximal blood oxygen transport capacity representing the theoretically "optimal" hematocrit.

Interestingly, the increase in viscosity with hematocrit in yellowfin tuna blood (Figure 3A) is clearly less than that in blood from other fishes (Brill and Jones, 1994). As a result, increases in hematocrit above ≈30% have little effect on blood oxygen transport capacity (Figure 3B). The hematocrit likely occurring in yellowfin tuna during periods of maximal metabolic rates (≈50%) would still, therefore, be considered "optimal." Mention should be made, however, that contrary to the predicted detrimental effects of high viscosity, *in vivo* studies of rainbow trout show that increases in hematocrit from ≈30% to over 50% had no effect on maximum rates of oxygen uptake. The expected decrease in maximum cardiac output with increase in hematocrit did not occur, and increased blood viscosity actually had a slight positive effect on maximum sustainable swimming speeds (Gallaugher *et al.*, 1995). It is, therefore, an open question if the concept of "optimal hematocrit" is applicable *in vivo*, and the reason(s) for the unusual behavior of yellowfin tuna blood remains unexplained.

IV. TUNA CARDIAC FUNCTION

Tuna cardiac anatomy and physiology have been the subject of comprehensive reviews (Brill and Bushnell, 1991; Farrell, 1991, 1996; Bushnell *et al.*, 1992;

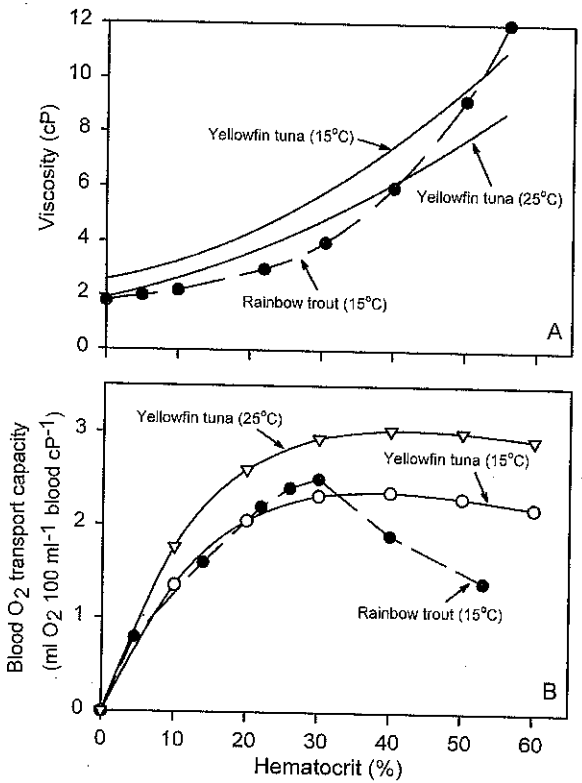

Fig. 3. (A) Effects of hematocrit on viscosity of yellowfin tuna blood at 15 and 25°C, and equivalent data for rainbow trout (at 15°C). (The shear rate in both instances was 90 s^{-1}.) (B) Effects of hematocrit on blood oxygen transport capacity in yellowfin tuna at 15 and 25°C and rainbow trout (at 15°C). The increase in blood viscosity with increasing hematocrit is less steep in yellowfin tuna blood than in rainbow trout blood. As a result, in yellowfin tuna, increases in hematocrit above 30% have almost no influence on blood oxygen transport capacity. Figure redrawn from data present in Wells and Weber (1991) and Brill and Jones (1994).

Bushnell and Jones, 1994; Agnisola and Tota, 1994; Moyes, 1996; Tibbits, 1996). We have, however, chosen to examine this subject in some detail because cardiac function is central to understanding of cardiovascular physiology and perhaps even the physiological ecology of tunas (Brill *et al.*, 1999).

As described in Sanchez-Quintana and Hurle (1987) and Farrell and Jones (1992), tuna ventricles are large (Table I), thick walled, and pyramidal, characteristics required to produce high ventricular pressures. The inner compact muscle

fibers surrounding the vertices have a coil-like arrangement. The more superficial compact layer muscle fibers form loops around the ventricle, but with a transverse fiber orientation on the caudal face. This fiber morphology is thought to provide the necessary mechanical advantage for rapid ejection of stroke volume, since the ventricle can contract simultaneously in the longitudinal and transverse directions.

According to the classification scheme developed by Tota *et al.* (1983) and Tota (1983, 1989), tunas have type IV hearts typical of high-energy-demand teleosts—hearts with more than 30% compact myocardium and coronary arteries in the compact and spongy myocardium. The fraction of ventricular mass composed of compact myocardium is, however, clearly elevated only in bigeye tuna (>70%) compared to other fishes (≈15–50%; Brill and Bushnell, 1991). The tuna myocardium is very well vascularized, with highly branched arterioles and venules, and extensive capillarization which extends even into the spongy myocardium (Tota, 1983). Not surprisingly, tuna hearts may be more dependent on coronary circulation than other teleosts (Farrell *et al.*, 1992). Whereas in other active fishes an intact coronary circulation is important only during strenuous exercise or severe hypoxia (Farrell and Steffensen, 1987), *in vitro* experiments have shown that skipjack tuna hearts depend on an intact coronary circulation for normal function (Farrell *et al.*, 1992). This presumably is also the case *in vivo*. As expected, the estimated coronary blood flow rate (ml blood g^{-1} ventricle min^{-1}) is approximately twice as great in skipjack tuna hearts as in those of rainbow trout (Farrell, 1996). No work has been conducted on factors controlling coronary blood flow in tunas.

A. Stroke Volume

The routine stroke volume of skipjack and yellowfin tuna hearts (≈1 ml kg^{-1}) approaches the maximum stroke volume of other fishes (Table I). More importantly, however, both *in vivo* and *in vitro* data suggest that tuna hearts normally function at close to their maximum stroke volumes. In other words, unlike hearts of other teleosts, tuna ventricles appear to have a very limited ability to increase stroke volume. This was first noted during experiments to quantify hypoxia tolerance. Bushnell *et al.* (1990) and Bushnell and Brill (1992) found hypoxic bradycardia occurring in skipjack and yellowfin tunas to be accompanied by nearly equivalent reductions in cardiac output. In other fishes (e.g., cod and lingcod *Ophiodon elongatus*) hypoxic bradycardia is accompanied by compensatory increases in stroke volume sufficient to maintain cardiac output (Farrell, 1982; Fritsche and Nilsson, 1989). Yellowfin and skipjack tunas' limited ability to increase stroke volume is also observed in fish subjected to acute reductions in ambient temperature (from 25 to ≈18°C; Korsmeyer *et al.*, 1997a; R. Brill, K. Cousins, and T. Lowe, unpublished observations). Under these conditions too, decreases in

heart rate result in nearly parallel reductions in cardiac output because of only modest (≈15%) increases in stroke volume. In contrast, when porgy (*Pagrus major*) are subjected to a similar acute reduction in ambient temperature, the fall in heart rate is accompanied by an increase in stroke volume sufficient to maintain cardiac output (Azuma *et al.*, 1998). This compensatory response to acute temperature changes is not universal, however. Although hypoxic bradycardia in lingcod results in increased stroke volume, bradycardia accompanying acute reductions in ambient temperatures does not, and cardiac output decreases in almost direct proportion to heart rate (Stevens *et al.*, 1972).

The limited ability of tunas to elevate stroke volume *in vivo* is also apparent during forced activity. While elevated activity levels in yellowfin tuna result in increases in cardiac output (Korsmeyer *et al.*, 1997a,b), as they do in other teleosts (Farrell and Jones, 1992), increases in cardiac output are accomplished solely by increases in heart rate with no measurable change in stroke volume (Figure 4A). In contrast, rainbow trout increase cardiac output during exercise by doubling stroke volume and only fractionally changing heart rate (Figure 4B). In general, in teleosts other than tunas, ≈40–60% of the increase in cardiac output during exercise is due to a rise in stroke volume, with the remainder due to elevations in heart rate (Farrell, 1991).

The tuna heart's limited ability to increase stroke volume can also be demonstrated *in vitro*. In fish hearts, as in mammalian hearts, there is a positive correlation between end diastolic ventricular volume (preload) and stroke volume (Farrell, 1984). The phenomenon is intrinsic to the myocardium (Harwood *et al.*, 1999) and is referred to as the "Frank–Starling mechanism," after its discoverers. *In vitro*, end diastolic ventricular volume is altered by changing ventricular filling pressure, and the resultant relationship of ventricular filling pressure to stroke volume is referred to as a "Starling curve." Examples from several teleosts are shown in Figure 5. Note that the Starling curve for skipjack tuna ventricles has the same shape as those for other fishes, but only at subambient filling pressures. Unfortunately, with skipjack tuna hearts *in vitro*, raising filling pressures above ambient results in ventricular failure, possibly due to the inability to adequately perfuse the coronary circulation in these circumstances (Farrell *et al.*, 1992). Lai *et al.* (1987) have reported subambient pericardial pressures in albacore, although these were subsequently not observed in yellowfin tuna (Jones *et al.*, 1993). Regardless of these inconsistencies, it is important to appreciate the fact that over the range of input pressures needed to produce near-routine stroke volume (≈1 ml kg^{-1}), stroke volume appears almost insensitive to filling pressure (i.e., to end diastolic ventricular volume) in skipjack tuna hearts. We surmise that, in general, both skipjack and yellowfin tuna hearts normally function on the upper flat portion of their Starling curve where stroke volume is insensitive to preload filling pressure or filling time (i.e., cardiac interval). Other teleost hearts function on the "steeper" parts of their Starling curves (Figure 5).

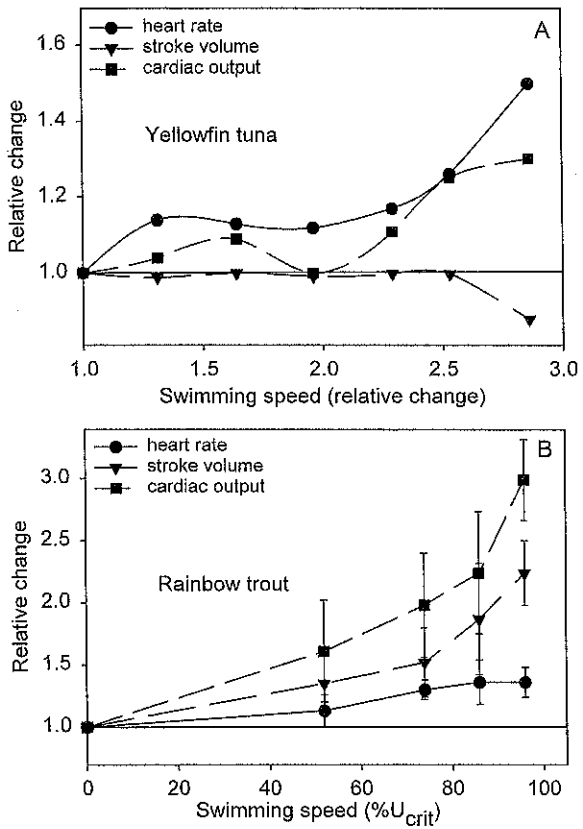

Fig. 4. Effect of swimming speed on the relative change in cardiac output, heart rate, and stoke volume in a yellowfin tuna (A) and rainbow trout (B). Note that in yellowfin tuna increases in cardiac output are accomplished by increases in heart rate alone, whereas in rainbow trout both heart rate and stoke volume increase with increases in swimming speed. Data for yellowfin tuna are from Korsmeyer *et al.* (1997a), and those for rainbow trout from Kiceniuk and Jones (1977).

B. Maximal Heart Rates in Tunas and Other Teleosts

Because maximum heart rates in fishes are temperature labile, comparisons of maximum heart rates in tunas to those of other species are somewhat difficult. In general, the maximum heart rate of teleosts has been reported to be 120 beats min^{-1} (Farrell, 1991), even at temperatures as high as 35°C (Gehrke and Fielder, 1988). The maximum heart rates of skipjack tuna (≈200 beats min^{-1}; Kanwisher *et al.*, 1974; Keen *et al.*, 1995) have, therefore, been considered to be the highest

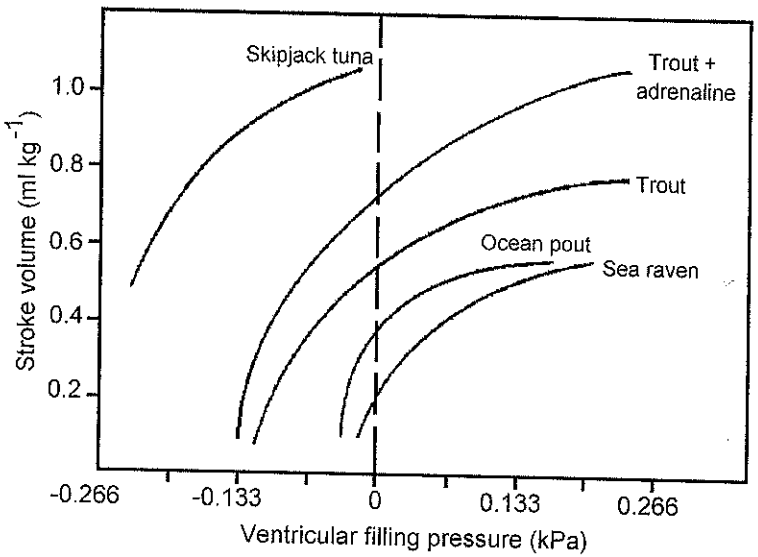

Fig. 5. Effects of filling pressure on cardiac stroke volume (i.e., Starling curves) of isolated perfused hearts from several species of teleosts. With the exception of tunas, teleost hearts normally operate over the steep portion of their Starling curves. Tuna hearts, however, normally operate at nearly maximum stroke volumes (i.e., at positive ventricular filling pressures where the Starling curves are relatively flat). Figure is from Farrell (1991); reprinted with permission.

among teleosts. This may no longer strictly be the case since a tropical tide pool goby, *Bathygobius soporator,* has now been reported to have maximum heart rates of 225 beats min^{-1} (at 35°C; Rantin *et al.,* 1998).

In addition to having heart rates that are generally higher than most other teleosts, there are also clear differences in the neural control of heart rate in tunas. The intrinsic activity of the teleost heart's pacemaker cells can be influenced by several factors, including temperature, adrenergic stimulation, and cholinergic stimulation (Farrell and Jones, 1992). Tunas are unusual among teleosts in that cholinergic inhibitory tone (via the vagus nerve) is the predominate controller of heart rate. Blocking vagal activity with atropine causes a 58% (75 to 120 beats min^{-1}) and 143% (79 to 193 beats min^{-1}) increase in heart rate in yellowfin and skipjack tunas, respectively (Keen *et al.,* 1995). In other teleosts (excluding the antarctic icefishes, *Pagothenia* spp.), the same treatment generally results in less than a 30% increase in heart rate (Farrell and Jones, 1992). Adrenergic blocking agents in tunas have minimal influence on resting heart rates (Keen *et al.,* 1995), and clearly less than that seen in other teleosts (Farrell and Jones, 1992). The

predominant vagal control of heart rate in tunas begs the question, "What (if any) is the role of sympathetic, adrenergic stimulation on routine or maximum heart rates of tunas?" Injection of a sympathomimetic, epinephrine, is vasoactive and can increase ventral aortic blood pressure (to >25 kPa), heart rate, and cardiac output in skipjack and yellowfin tunas, although none of these effects have been quantitatively investigated (Bushnell and Brill, unpublished observations). To our knowledge the sympathetic system's influence on blood flow distribution to various vascular beds in tunas remains unstudied.

In spite of a fixed stroke volume, the skipjack tuna's high heart rate can easily accommodate its documented maximum metabolic demand. As shown in Figure 6, assuming no increase in stroke volume, maximum heart rate (≈200 beats min^{-1}) is more than sufficient to account for the cardiac output (212 ml min^{-1} kg^{-1}; Figure 2) accompanying the estimated maximum metabolic rate. Also as shown in Figure 6, this is in direct contrast to observed changes with exercise occurring in trout, where increased cardiac output is accomplished by increases in both stroke volume and heart rate.

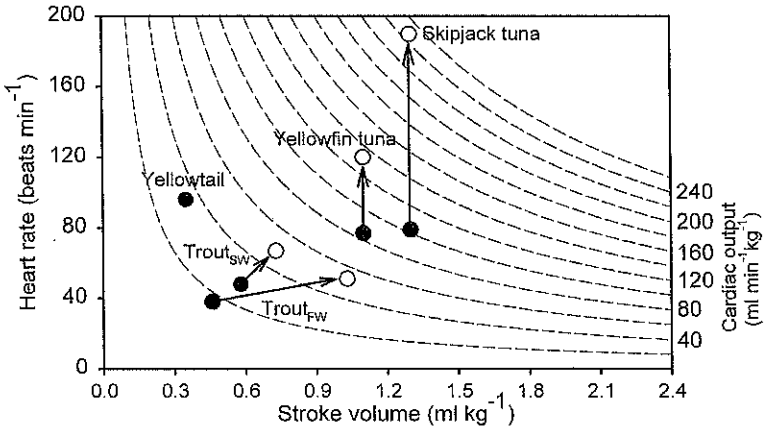

Fig. 6. Relationship of heart rate, stroke volume, and cardiac output in freshwater ($Trout_{FW}$)- and seawater ($Trout_{SW}$)-adapted rainbow trout, yellowtail, yellowfin tuna, and skipjack tuna. Filled circles show minimal values, and open circles maximal values. In fish other than tunas, increases in cardiac output accompanying increases in activity are accomplished primarily by increases in stoke volume ($Trout_{FW}$) or approximately equal increases in stoke volume and heart rate ($Trout_{SW}$). In contrast, the predicted cardiac output (>200 ml min^{-1} kg^{-1}) required to meet skipjack tuna's observed maximum metabolic rates can be met by maximum observed heart rates, with no increase in stroke volume. Data for freshwater-adapted trout are from Kiceniuk and Jones (1977), seawater-adapted trout from Thorarensen *et al.* (1996), yellowtail from Ishimatsu *et al.* (1997), and skipjack and yellowfin tuna from Bushnell and Brill (1992).

C. Sarcoplasmic Reticulum

A key factor influencing rates of ventricular contraction and relaxation is the cycling of calcium ions (Ca^{2+}) into and out of the cardiac muscle cells (myocytes). In mammalian myocardium, Ca^{2+} influx is initiated through sarcolemmal voltage-gated Ca^{2+} channels which serve to trigger a larger Ca^{2+} release from the sarcoplasmic reticulum (SR). This process is referred to as "Ca^{2+}-induced Ca^{2+} release" (Fabiato, 1983). Since SR-Ca^{2+} stores are intracellular, diffusion distances are relatively short, and the time course of Ca^{2+} release and resequestering can be rapid. When myocyte volumes are small relative to their surface areas, however, a well-developed SR may not be necessary. As a consequence of a high ratio of sarcolemmal (i.e., cell surface) area to myocyte volume, extracellular Ca^{2+} alone can be sufficient to initiate myofibrillar contraction (Tibbits *et al.*, 1992b). Anatomical studies tend to support this idea. Fish myocyte diameters range from approximately 2 to 10 μm, compared with 10 to 25 μm in mammals (Santer, 1985; Satchell, 1991), and the SR in teleost hearts is generally considered to be not well developed (Yamamoto, 1967; Santer, 1974; Breisch *et al.*, 1983; Satchell, 1991). Myocyte diameters in albacore, the only tuna species from which we currently have data, range from 2.5 to 6 μm and therefore are not unique in this regard (Breisch *et al.*, 1983).

Although there are some species-specific differences in the extent of SR development among the teleosts, it is unclear if SR volume is related to general activity level as might be expected. For example, electron micrograph studies by Yamamoto (1967) and Santer (1974) indicate that the SR is less extensive in the ventricle of goldfish (*Carassius auratus*) than in that of the more active rainbow trout. In contrast, Breisch *et al.* (1983) stated that in albacore, "the sarcoplasmic reticulum, although present, is very poorly developed." More recently, preliminary studies (K. L. Cousins, unpublished observations) suggest that the yellowfin tuna ventricle (Figure 7) has a SR at least as well developed as that of rainbow trout or carp (*Cyprinus carpio*), but all are clearly less developed than the rat's (Santer, 1974; Chugun *et al.*, 1999). Electron microscopy quantitatively comparing the development of the SR in skipjack tuna, yellowfin tuna, the ectothermic scombrids, and several other fish (including those of different activity levels) seems clearly warranted.

While anatomical studies can be used to establish the distribution and development of SR, accompanying physiological studies on the importance of SR-derived Ca^{2+} in initiating contraction are required. The latter involve the use of ryanodine, which, at high concentrations, locks the SR Ca^{2+}-gated Ca^{2+}-release channel in a subconducting state, making the SR ineffective with respect to Ca^{2+} release or sequestering (Rousseau *et al.*, 1987). Decreases in peak tension developed by isolated atrial or ventricular strips exposed to ryanodine can be used to assess the relative contribution of extracellular (sarcolemmal)- and SR-derived

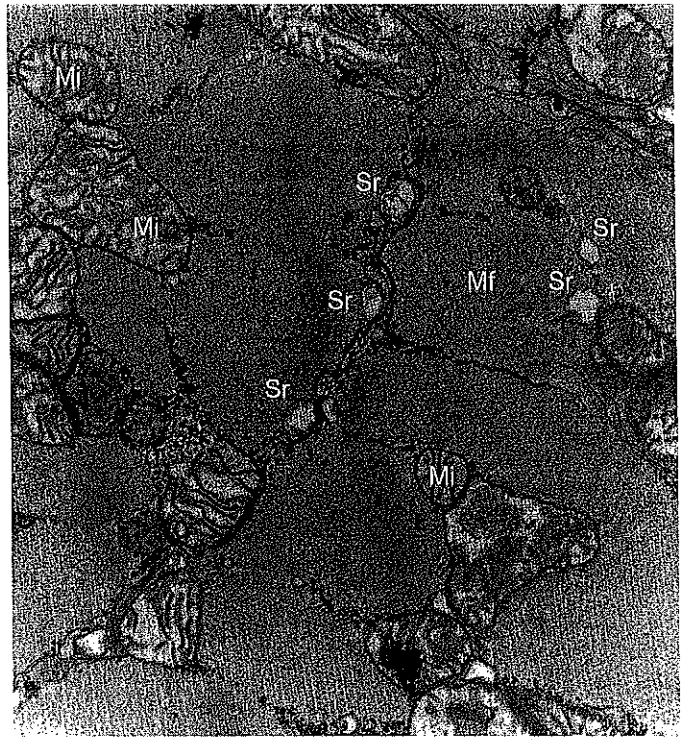

Fig. 7. Transmission electron micrograph of yellowfin tuna ventricle (compact myocardium) with myofibers cut in transverse section (original magnification 15,900×). Although sarcoplasmic reticulum (SR) is clearly evident, pending a careful quantitative study, it remains an open question if the SR is more developed in tuna myocardium than in the hearts of other active teleosts (K. Cousins, unpublished observations). Mi indicates mitochondria, Mf myofilaments.

Ca^{2+}. These types of studies have shown that SR-Ca^{2+} release in teleosts myocardium is negligible except in nonphysiological circumstances such as supraphysiological temperatures or subphysiological pacing frequencies (Tibbits *et al.*, 1992b; Keen *et al.*, 1994; Gesser, 1996; Shiels and Farrell, 1997). In contrast, Keen *et al.* (1992) and Shiels *et al.* (1999) demonstrated that in skipjack and yellowfin tuna atrial strips, SR-Ca^{2+} release contributes ≈30% and ≈60% (respectively) of activator Ca^{2+} under normal operating temperatures (15–25°C) and pacing frequencies (up to 2.5 Hz).

Interpretation of these findings is somewhat obfuscated by the apparent temperature sensitivity of the ryanodine response. Hove-Madsen (1992) found that the ryanodine sensitivity of rainbow trout myocardium was negligible at 15°C,

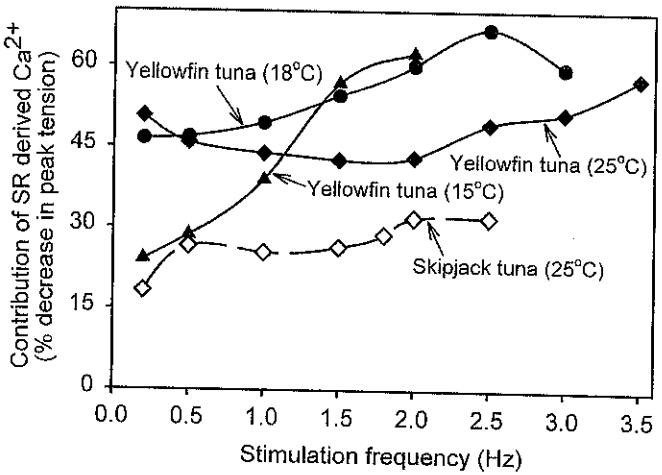

Fig. 8. Contribution of SR-Ca^{2+} release to force development in isolated myocardial strips from yellowfin tuna at 15, 18, and 25°C (from Shiels *et al.*, 1999) and from skipjack tuna at 25°C (from Keen *et al.*, 1992). Data are presented as the fractional reductions in force resulting from exposure to ryanodine. Error bars have been omitted for clarity. Note that contribution of SR-derived Ca^{2+} is less in skipjack tuna than in yellowfin tuna myocardial strips. The contribution of SR-derived Ca^{2+} is greater at lower temperatures in yellowfin tuna, which is in direct contrast to results from other teleosts. In rainbow trout myocardium, the importance of SR-derived Ca^{2+} is greater at higher temperatures.

while at 25°C, SR-Ca^{2+} release accounted for 40% of activator Ca^{2+}. The lack of ryanodine sensitivity at 15°C most likely results from locking the SR-Ca^{2+} release channel in the open position (Tibbits, 1996). In contrast, the temperature sensitivity of ryanodine response in tuna hearts appears to be exactly the opposite, as Shiels *et al.* (1999) found yellowfin tuna atrial strips to have a greater ryanodine sensitivity at 15°C than at 25°C (Figure 8). The implied fundamental differences between SR-Ca^{2+} release channels in tuna and those in other teleosts are obviously another avenue that needs to be explored, perhaps at the molecular level (e.g., Tibbits *et al.*, 1992a).

Temperature effects not withstanding, there appear to be substantial differences in the importance of SR-Ca^{2+} release just within tuna species. Shiels *et al.* (1999) found a greater importance of SR-Ca^{2+} release in atrial strips from yellowfin tuna (≈60%; Figure 8) than in those from skipjack tuna (≈30%) studied by Keen *et al.* (1995). Given the differences in maximum heart rates of yellowfin (120–140 beats min^{-1}) and skipjack (≈200 beats min^{-1}) tunas, the opposite relative SR-Ca^{2+} contributions would be predicted as higher maximum heart rates should be more dependent on SR-Ca^{2+} release and resequestering (Farrell, 1996). It is possible that these findings may result from different techniques; Shiels *et al.*

(1999) used 1 nM adrenaline in their tissue bathing medium whereas Keen *et al.* (1995) did not. If in tuna myocardium adrenaline is required to enhance extracellular Ca^{2+} release enough so as to fully stimulate SR-Ca^{2+} release, then its absence may cause the contribution SR-Ca^{2+} release to be underestimated. Freund (1999), however, also found that increasing extracellular Ca^{2+} has no effect on force of contraction of ventricular strips from yellowfin tuna at high pacing frequencies, as it does in atrial strips from skipjack tuna (Keen *et al.*, 1992) and cod (Driedzic and Gesser, 1985). These data also indicate a larger dependence on SR-Ca^{2+} release in yellowfin tuna than in skipjack tuna myocardium. A dependence on SR-Ca^{2+} may, however, not be strictly necessary for high maximum heart rates. SR-Ca^{2+} release and resequestering do not appear to be important in the tide pool goby (*Bathygobius soporator*) even though maximum heart rates in this species exceed 225 beats min^{-1}. The functioning of ventricular strips isolated from this species are nearly insensitive to ryanodine (Rantin *et al.*, 1998).

A second method of evaluating SR-Ca^{2+} release is postrest potentiation, a technique that monitors the increase in twitch tension of isolated myocardial strips following a single 20-s "rest" period between twitches (Driedzic and Gesser, 1988). Freund (1999) has recently used this procedure to demonstrate that ventricular strips of Pacific mackerel (*Scomber japonicus*) are equally dependent on SR-Ca^{2+} release as those of yellowfin tuna. These data imply that some of the unusual characteristics of SR-Ca^{2+} release channels may have developed early in scombrid evolution, as they are appear to be present in even primitive members of the family *Scombridae*. Shiels and Farrell (2000), however, also using ventricular trabeculae from Pacific mackerel, found ryanodine induced significantly smaller (20%) reductions in peak tension than those observed in similarly treated tuna myocardium. To the best of our knowledge, there are no data on routine or maximal cardiac function in mackerel, so these observations are yet to be put in an *in vivo* physiological context. Moreover, mammalian atrial muscle shows a greater ryanodine sensitivity than ventricular muscle (Bers, 1991). If the same occurs in teleosts, conclusions based on the various studies could be compromised, as some investigators have used atrial muscle and others ventricular muscle. A comparative study of ryanodine sensitivity of tunas, ectothermic scombrids, and other teleosts using the same methodology throughout is clearly needed.

D. Mechanical and Electrical Coupling of Myocytes

The high maximum heart rates of skipjack and yellowfin tunas require that all links in the chain of excitation–contraction coupling be enhanced (Satchell, 1991). For example, the short Ca^{2+} diffusion distances from the SR to the myofibrils require the depolarization triggering Ca^{2+} release from the pacemaker tissue to spread around the ventricle with equal rapidity. Small myocyte diameters, however, can result in high electrical resistance and slow conduction velocities (Farrell

and Jones, 1992). Since the rate of increase in ventricular pressure (dP/dt) in yellowfin tuna hearts *in vivo* is rapid (up to 716 kPa s^{-1}) and in the mammalian range (Jones *et al.*, 1993), we conclude that there is indeed near-synchronous contraction of the myocytes. This, in turn, implies the presence of fast conducting fibers analogous to the bundle of His and Purkinje fibers of mammalian hearts. Although such fibers have not been described histologically in any fish species, based on the pathways over which depolarization is known to spread across teleost ventricles, Satchell (1991) concluded that fast conducting fibers "certainly occur." We would not be surprised to find that specialized fast-conducting fibers are especially well developed in tuna cardiac muscle, and encourage investigation in this area.

In addition to rapid electrical conduction from the pacemaker, cells of the cardiac tissue require both mechanical and electrical coupling. Mechanical coupling of myocytes is accomplished via intercalated disks consisting of fasciae adherentes (where actin filaments of adjacent myocytes insert onto a dense fibrous mat) separated by desmosomes (regions of the cell surface specialized for maintaining cell-to-cell cohesion; Bloom and Fawcett, 1975). Intercalated disks are present in the hearts of all vertebrates and are clearly present in albacore (Breisch *et al.*, 1983) and yellowfin tuna (Figure 9). Electrical coupling between myocytes

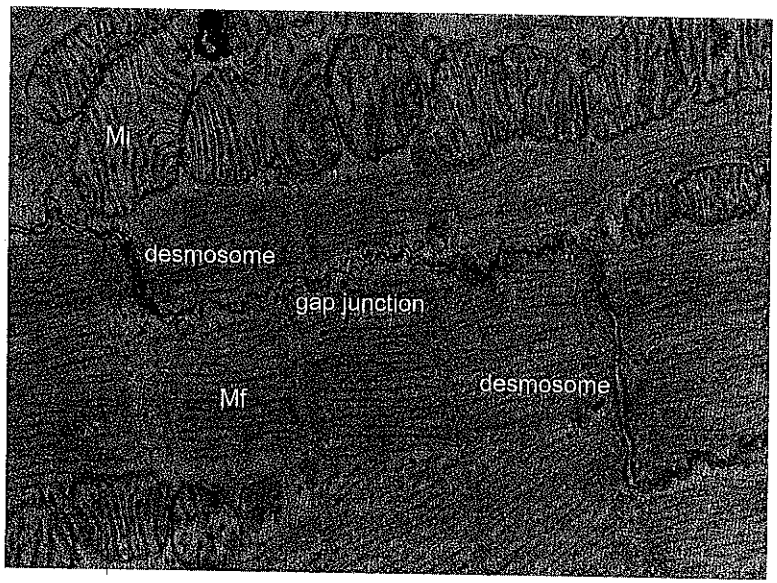

Fig. 9. Transmission electron micrograph of yellowfin tuna ventricle (compact myocardium) with myofibers cut in longitudinal section (original magnification 12,500×). Desmosomes, intercalated disk, and gap junction are shown. Note that, as in mammalian myocardium, gap junctions appear to be part of the intercalated disk. Mi indicates mitochondria, Mf myofilaments (K. Cousins, unpublished observations).

occurs through gap junctions (also referred to as nexus junctions) which have been reported to occur on the lateral walls of adjacent myocytes in several fish species, including albacore (Shibiata and Yamamoto, 1977; Breisch *et al.*, 1983). As can be seen in Figure 9, gap junctions are also present in the compact myocardium of yellowfin tuna and appear to be part of the intercalated disk, as they are in mammalian hearts (Bloom and Fawcett, 1975; Satchell, 1991). Cobb (1974) has reported that in fishes, gap junctions are not associated with the fasciae adherentes of the intercalated disks, but are only on the lateral wall of the myocytes. The physiological implications of the unusual gap junction distribution in yellowfin tuna await further investigation. As with the SR, a quantitative morphological study of gap junctions and intercalated disks involving several species of tunas, ectothermic scombrids, and other teleosts is clearly needed to discern if the ability of tunas to reach high maximum heart rates or cardiac outputs is correlated with more extensive development of these structures.

E. Biochemical Adaptations of Tuna Hearts for High Power Output

Skipjack and yellowfin tunas' high cardiac outputs and ventral aortic blood pressures result in routine cardiac power outputs (per unit body mass) that are approximately seven times those of other teleosts (Table I). Due to their enlarged ventricles, however, power output per gram ventricular mass is only twice that seen in other fishes (Table I). In other words, tunas' elevated cardiac power outputs are achieved primarily through ventricular hyperplasia (Moyes *et al.*, 1992; Farrell, 1996; Tibbits, 1996).

As expected, the levels of aerobic enzyme activity (citrate synthase and 3-hydroxy-o-acyl-CoA dehydrogenase) per gram ventricle mass are likewise all roughly double in tuna hearts compared with hearts of other teleosts (Driedzic, 1992; Moyes *et al.*, 1992; Driedzic and Gesser, 1994; Dickson, 1995). The exception is carnitine-palmitoyl transferase, an enzyme involved in fatty acid metabolism, whose activity is approximately three to seven times higher (Moyes *et al.*, 1992; Dickson, 1995). Tuna hearts, therefore, appear to rely more on fatty acids to power aerobic metabolism than do other teleost hearts (Moyes, 1996; Weber and Haman, 1996). The advantages of fatty acids over glucose as a fuel source are their higher energy content (i.e., moles of ATP derived per gram of substrate) and their ability to be stored in the tissues at high levels. It is also possible that tuna hearts may use lactate as a fuel source, as mitochondria isolated from skipjack tuna ventricle have a higher capacity for lactate oxidation than other metabolic fuels (Moyes *et al.*, 1992). This finding makes intuitive sense since the highest heart rates and cardiac outputs (i.e., highest rates of cardiac energy demand) are likely to occur in tunas during recovery from exhaustive exercise, when plasma lactate levels are elevated (Arthur *et al.*, 1992).

The use of lactate as a metabolic energy source in the heart is also supported

by microanatomical differences which reflect metabolic zonation of compact and spongy myocardium. In albacore, the volume percentage of mitochondria is higher in the spongy layer (31.9%) than in the outer compact myocardium (21.3%; Breisch *et al.*, 1983). While Basile *et al.* (1976) found mitochondrial volume densities (28–32%) in bluefin tuna (*Thunnus thynnus*) hearts to be similar to those of albacore hearts, they did not find differences in mitochondrial volume density between myocardial layers. They did note, however, that the mitochondria from the spongy myocardium had higher cristae density. These anatomical findings support the biochemical observations that the spongy layer has a substantial capacity to oxidize lactate and incorporate it into lipids for possible use by the compact myocardium (Maresca *et al.*, 1976; Gemelli *et al.*, 1980). This implies the possibility of metabolic interactions between the two myocardial layers

Cardiac mitochondrial ATP production capacity can serve as another estimate of cardiac energy demand. As stated earlier, maximum oxygen consumption in skipjack tunas appears to be met by a doubling of cardiac output (Figure 2). In yellowfin tuna (the only species in which this has been measured), there is only a relatively small (>20%) increase in ventral aortic pressure with increasing activity (Korsmeyer *et al.*, 1997a,b). If the same occurs in skipjack tuna, then maximum cardiac power output (per gram ventricle) would be ≈2 times routine levels, and ≈1.5–2.5 times those of other teleosts (Table I). It is not unreasonable to expect that the increase in power output would have to be generated by increases in mitochondrial (i.e., aerobic) ATP production. Studies by Moyes *et al.* (1992) provide evidence that the metabolic support does indeed exist, as the maximal mitochondrial oxygen consumption of skipjack tuna hearts (7 μM O_2 g^{-1} ventricle min^{-1}) is approximately twice the estimated routine consumption rate (3.3 μM O_2 g^{-1} ventricle min^{-1}). This level of oxygen consumption is also about twice that of mitochondria isolated from carp ventricle (per milligram of mitochondrial protein), when corrected for differences in measurement temperature. These data are thus reasonably consistent.

The high maximum power output of tuna hearts may also be supported by the high levels of myoglobin found in tuna ventricles, although exact mechanisms remain unknown. Giovane *et al.* (1980) reported ventricular myoglobin levels in bluefin tuna to be 6–20 mg g^{-1}, a level whose upper range is several times those of other active teleosts (≈1–6 mg g^{-1}; Driedzic, 1983). Driedzic (1983, 1988) has suggested that high ventricular myoglobin levels serve to maintain contractility during episodes of low ambient oxygen. Stevens (1982), however, found that in fish skeletal muscle, elevated myoglobin levels function to facilitate oxygen diffusion from the capillaries to the mitochondria, rather than as an oxygen store utilized during hypoxia. A third possibility, put forth by Tota (1983), is that myoglobin may function as a temporary oxygen store to offset the effects of the brief periods of ischemia occurring during ventricular contraction when coronary blood flow is likely to be diminished.

F. Ventricular and Arterial Dynamics and Functions of the Bulbus Arteriosus

While most of the foregoing discussion has concentrated on how the cardiorespiratory system of tunas achieves high levels of performance, it also important to note that on a second-to-second basis, cardiovascular function involves highly dynamic processes. In this regard, based on tunas' high maximum heart rate it is possible that the complex hemodynamic relationships seen in birds and mammals could be present in the circulatory system of tunas. High heart rates result in short pressure wavelengths, such that length of the arterial tree becomes a significant proportion of the pressure wavelength (Langille *et al.*, 1983; Jones, 1991). As a result, the transmission time of the pressure pulse through the arteries can span a significant portion of the cardiac cycle. In these circumstances, the arterial tree of tunas would not act as a simple elastic reservoir as it does in other fishes (the so-called "Windkessel model"). Rather it would behave as it does in mammals as a wave transmission system with complex wave propagation effects such as pulse amplification, distortion, and wave reflections. Jones *et al.* (1993), however, found no evidence of wave transmission in yellowfin tuna. Rather, pressure and flow waveforms agreed well with theoretical and mathematical Windkessel models. This interesting finding probably results from the functional properties of the bulbus arteriosus, the highly distensible fourth chamber of the teleost heart. In tunas, as in other teleosts, the bulbus arteriosus is capable of stretching to accept an entire stroke volume and recoiling elastically. It thus smooths blood flow pulsatility and maintains blood flow through the gills during diastole (see Figure 5 in Bushnell *et al.*, 1992), which presumably improves gas exchange (Malte, 1992). In contrast, blood flow in the mammalian proximal aorta is highly pulsatile, and often reverses (i.e., momentarily flows backward into the heart) during diastole.

The extreme extensibility of the bulbus arteriosus of yellowfin tuna and albacore is shown in Figure 10A as quasi-static pressure–volume loops and demonstrates how the bulbus arteriosus in tuna is able to maintain blood flow through the gills during diastole (Jones *et al.*, 1993). The initial portion of the compliance curve is very steep (i.e., the bulbus arteriosus is very "stiff" or not very compliant) and pressures remain high even at volumes as low as 10% of maximum injected volume. *In vivo*, this means that during diastole, when the bulbus arteriosus is recoiling, ventral aortic pressure remains elevated until nearly the entire stroke volume has been ejected. This behavior, in turn, provides the motive force to maintain continuous blood flow through the gills between heartbeats. As is the case with most teleosts (Bushnell *et al.*, 1992), the yellowfin tuna bulbus arteriosus is most compliant (i.e., shows relative small increases in pressure with equal increments of inflation) over the range of routine ventral aortic blood pressures (10.8–11.8 kPa, Table I). The plateau phase of the quasi-static pressure–volume loop, therefore, corresponds to ventral aortic systolic pressure. In albacore, how-

ever, the bulbus arteriosus appears to be most compliant at pressures below ventral aortic blood pressure (10–11 kPa; Lai *et al.*, 1987). This result may be somewhat misleading, as blood pressures in albacore were measured on recently boated fish and may have been elevated due to stress. If the compliance characteristics of the bulbus arteriosus are indeed matched to cardiovascular pressures, we would predict that the ventral aortic pressure of unstressed albacore probably lies in the range of 6–8 kPa. We believe the bulbus arteriosus compliance and ventral blood pressure relationship may provide researchers with an *in vitro* experimental tool that can be used to estimate ventral pressures in species which are not amenable to laboratory manipulations because of their size or availability We therefore also present in Figure 10B a series of pressure–volume loops for the bulbi arteriosi from a number of pelagic fishes whose blood pressures have yet to be measured. The data imply that striped marlin (*Tetrapturus audax*), blue marlin (*Makaira nigricans*), and sailfish (*Istiophorus platypterus*) all have blood pressures in the ventral aorta between those of yellowfin tuna and albacore, whereas those of mahimahi (dolphinfish, *Coryphaena hippurus*) and pomfret (*Taractichthys* spp.) are clearly lower.

The highly specialized bulbus arteriosus of tunas most likely performs another very important function: it increases the efficiency of the circulatory system (i.e., the ratio of external cardiac work to cardiac energy demand). The majority of cardiac energy is used to develop systolic blood pressures in the ventricle (i.e., to develop wall tension; Jones, 1991). Therefore, anything that reduces peak systolic pressures, or the time over which they must be generated (i.e., the time–tension index), will reduce cardiac energy demand and thus improve efficiency. Because of the high heart rates, cardiac outputs, and ventral aortic pressures in tunas, adaptations which reduce the myocardial time-tension index should be expected. Mathematical modeling studies have shown that a large, central (rather than peripheral) compliance in the circulatory system is of hemodynamic value as it raises diastolic pressure while effectively lowering peak systole pressures and, thus, the time–tension index of the heart (Campbell *et al.*, 1981). For tunas, which put exceptional demands on their cardiovascular systems (Table I), the energy savings due to the presence of a highly compliant bulbus arteriosus are most likely substantial.

There is, however, a potential problem with having a compliant structure capable of large changes in volume immediately outside the heart. It would remove the ability to regulate blood pressure (beat-to-beat) in an effective manner because any increases in peripheral resistance (or cardiac output), which would normally cause blood pressure to rise, would be damped by the plateau phase of the bulbus arteriosus pressure–volume curve (Figure 10). The solution to the problem lies in having a structure with variable and regulated mechanical properties. This is accomplished by having smooth muscle in the walls of the bulbus arteriosus that is capable of contracting in certain circumstances (Farrell, 1979; Watson and Cobb,

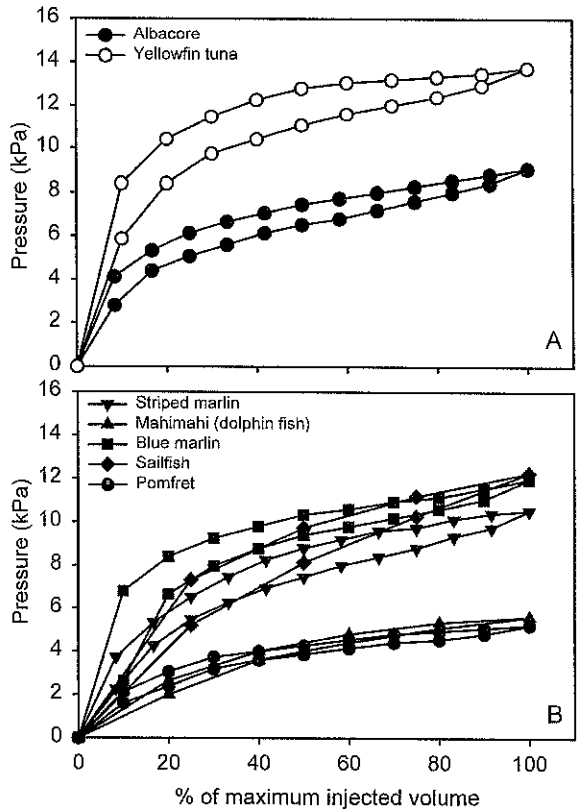

Fig. 10. (A) Pressure–volume loops recorded in isolated bulbi arteriosi taken from yellowfin tuna and albacore. The curves were generated by stepwise injection of measured volumes of saline into isolated sealed vessels, followed by stepwise removal. The pressure–volume curves created during inflation are below those created during deflation. (B) Pressure–volume loops recorded in isolated bulbi arteriosi taken from various pelagic fishes. Based on the tuna data, it is possible that the plateau region of curves shows the normal ventral aortic blood pressures of these fishes. Data from M. Braun, University of British Columbia; reprinted with permission.

1979). Not surprisingly, the bulbus arteriosus of tunas is highly vascular, is well enervated, and possesses significant amounts of smooth muscle (Braun *et al.*, 2000). Presumably, contracting or relaxing this smooth muscle allows subtle manipulation of wall properties and the short-term blood pressure changes necessary to maintain proper function of the cardiovascular system. The regulation of the compliance of the tuna bulbus arteriosus is unstudied, but clearly deserves further investigation.

V. CARDIAC FUNCTION AND THE PHYSIOLOGICAL ECOLOGY OF TUNAS—A POSSIBLE CONNECTION

In the following section we consider how the cardiorespiratory physiology of tunas might impact their behavior and distribution in the open ocean. The ability to apply what we have learned in the laboratory to what we believe is happening in the field is possible because of ultrasonic telemetry and new archival tags (i.e., electronic data recording devices carried by the fish). These allow detailed records to be obtained of the horizontal and vertical movements of tunas and other pelagic fishes in the open sea.

The vertical movements of large adult (≈50 kg) and even juvenile (≈4–6 kg) bigeye tuna are much deeper, and into much colder water, than those of yellowfin tuna (Figure 11). Adult bigeye tuna routinely reach maximum depths of 450–500 m during the day while juvenile fish are commonly found at 350 m (Holland *et al.*, 1990b; Boggs, 1992; R. Brill, M. Musyl, D. Curran, and C. Boggs, unpublished observations). The water temperatures at these depths are ≈5–7°C and ≈15°C, respectively. Ultrasonic tracking studies of large (>25 kg) bigeye tuna near Tahiti show that they follow the daily vertical migrations of the small nektonic organisms (crustaceans, cephalopods, and fishes) of the deep sound-scattering layer even as they descend into deeper and colder water (Josse *et al.*, 1998; Dagorn *et al.*, 2000). Bigeye tuna thus have the ability to exploit a prey source which, during daylight hours, apparently is not normally available to yellowfin tuna (Grundin, 1989; Roger, 1994; Marchal and Lebourges, 1996).

While there are clear instances where the depth distribution of tunas is set by the depth distribution of their prey (e.g., Block *et al.*, 1997; Marcinek *et al.*, 2001), the dichotomous depth distributions of yellowfin and bigeye tunas in exactly the same area near the main Hawaiian Islands (Figure 11) implies that one or more abiotic factors are having an impact on their vertical movements. It is possible that changes in oxygen levels in the water column may be limiting their distribution, but near the main Hawaiian Islands significant decreases in ambient oxygen do not occur in the portion of the water column occupied by yellowfin and juvenile bigeye tunas (Brill *et al.*, 1999). We suggest, as first noted by Brill *et al.* (1993, 1999), that a major limiting factor is the relative change in water temperature occurring between the surface layer and the waters below the thermocline. We believe this to be the case because of the documented effect of acute reductions in ambient temperature on cardiac function. Since tunas' hearts are near the ventral body wall and outside the area warmed by tunas' vascular countercurrent heat exchangers, the temperature of cardiac muscle will almost immediately reflect changes in ambient temperature. This occurs regardless of body size or regional endothermy (Brill *et al.*, 1994).

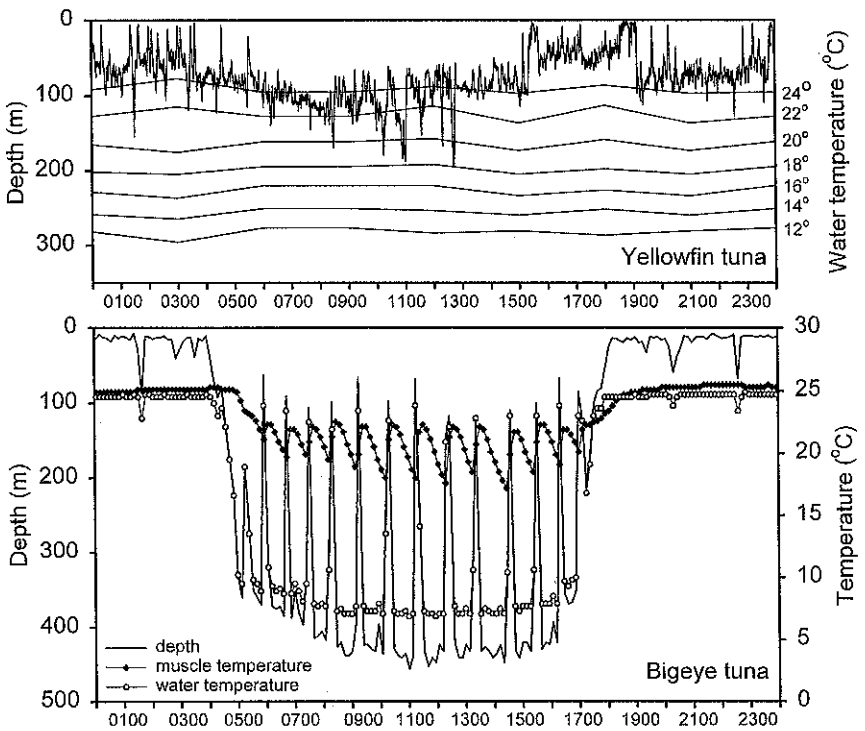

Fig. 11. Representative 24-h records (midnight to midnight) of the vertical movements of adult yellowfin tuna (64 kg estimated body mass) and bigeye tuna (50 kg estimated body mass) in areas near the main Hawaiian Islands. The former was carrying an ultrasonic depth-sensitive transmitter, and swimming depth data were recorded approximately every 10 s onboard a ship that was following the fish. Temperature changes with depth were measured by lowering a temperature probe through the water column and are plotted as 1°C isotherms. (Data replotted from Brill *et al.*, 1999.) The bigeye tuna was implanted with an archival (i.e., electronic data recording) tag that recorded swimming depth, water temperature, and deep red muscle temperature every 8.5 min (R. Brill, M. Musyl, D. Curran, and C. Boggs, unpublished observations). Note that the yellowfin tuna did not descend into water more than ≈8°C below surface layer temperature (≈25°C). In contrast, the adult bigeye tuna repeatedly descended to water as cold as ≈7°C during the day (≈0500–1800 h) and thus repeatedly subjected its heart to ≈18°C temperature changes.

As noted earlier, acute reductions in ambient temperature from 25 to 15°C result in immediate and parallel decreases in heart rate and cardiac output (Q_{10} of ≈2) in both yellowfin and skipjack tunas (Korsmeyer *et al.*, 1997a; R. Brill, K. Cousins, and T. Lowe, unpublished observations). Moreover, these do not appear to be neurally mediated effects, as the decline in heart rate with reductions in

ambient temperature is not counteracted by injection of atropine, a vagolytic drug (Brill, Cousins, and Lowe, unpublished observations). These studies imply, at low ambient temperatures, that skipjack and yellowfin tunas have little or no ability to increase cardiac output. Although they would be able to meet oxygen delivery requirements at low swimming speeds, it seems unlikely that they would be able to increase cardiac output enough to accommodate the doubling (or more) required during high-speed swimming, recovery from exhaustive exercise, and so on. It is interesting to note that the apparent reduction in scope for activity results from two of the facets that make tuna unique: a countercurrent heat exchange system that will maintain metabolism of tissues at higher rates despite the colder ambient temperature, and a heart that operates at maximal stroke volume. In others words, since tuna hearts function on the upper (flat) end of their Starling curves (Figure 5) and heart rate declines immediately with decreases in ambient temperature, then maximal cardiac output declines in direct proportion to ambient temperature. Unfortunately, thanks to the heat exchange system, metabolic demand will not.

The next obvious question is how are bigeye tuna, and possibly other large pelagic species such as bluefin tuna (*Thunnus thynnus;* Lutcavage *et al.,* 2000; Brill *et al.,* 2000) and swordfish (*Xiphias gladius;* Carey, 1990), apparently able to maintain cardiac function in the face of rapid ambient temperature change, and thus achieve their extensive vertical mobility and apparent ability to exploit food resources below the thermocline? There are several possible answers. Heart rates may be less affected by temperature, or like other teleosts, they may be able compensate for reductions in heart rate by increasing stroke volume. In the only *in vivo* study of bigeye tuna physiology, fish exposed to hypoxia responded with a bradycardia that was unaccompanied by increases in stroke volume, and cardiac output fell with heart rate (Bushnell *et al.,* 1990). It should be noted, however, that both Freund (1999) and Shiels *et al.* (1999) found that acute temperature reductions increase Ca^{2+} release from the SR in isolated myocardial strips from yellowfin tuna. The Ca^{2+} sensitivity of isolated myofilaments from catfish (*Pterygoplichthys* spp.) is, however, temperature independent (Meadows *et al.,* 1998). If the same is true in tuna hearts, increased SR-Ca^{2+} release could step up cardiac contractility *in vivo* and potentially offset the decrease in cardiac output resulting from reduced heart rates. Isolated hearts from other teleosts (e.g., pickerel, *Esox niger*) can maintain power output in spite of acute reductions in temperature from 15 to 5°C (Bailey *et al.,* 1991). As stated, however, *in vivo* data suggest that any temperature-induced increases in contractility occurring in yellowfin and skipjack tunas are ineffective in maintaining cardiac output when these fish are subjected to acute temperature changes. Clearly, the influence of environmental temperature changes on cardiac function, both *in vivo* and *in vitro,* in a range of tuna species is a subject that warrants further study.

VI. SUMMARY AND CONCLUSIONS

While significant gaps in our understanding of cardiovascular function in tunas remain, available data, and the extrapolations constructed from them, are now becoming consistent. For example, maximum measured heart rates, stroke volumes, and reasonable arterial–venous oxygen content differences appear sufficient to explain the (admittedly limited) data on maximum metabolic rates.

Several important anatomical adaptations allow tunas to achieve their exceptional rates of oxygen extraction from the ventilatory water stream and delivery to the tissues. Tunas have gill surface areas approximately an order of magnitude larger, and a blood–water barrier thickness up to an order of magnitude less, than those of other teleosts. Tunas also have exceptionally large hearts. As a result, even during periods of routine energy demand, high cardiac outputs are achieved by large stroke volumes. To reach the cardiac outputs necessary to achieve their exceptional maximum metabolic rates, tunas increase heart rate almost exclusively, whereas other teleosts increase stroke volume almost exclusively or stroke volume and heart rate about equally. The hearts of skipjack and yellowfin tunas appear to rely more on sarcoplasmic Ca^{2+} release and resequestering during routine functioning than the myocardium of other fishes, and probably on well-developed specialized electrical conducting pathways as well.

For reasons that are not yet fully understood, tuna hearts normally function on the flat upper limb of their Startling curve (i.e., they have a more limited ability to increase stroke volume than do hearts of other teleosts). This characteristic may, in turn, limit the vertical mobility of some tuna species (e.g., skipjack and yellowfin tunas). As water temperature decreases with depth, decreases in heart rate are necessarily accompanied by decreases in cardiac output. Some tuna species (e.g., bigeye and bluefin tuna) appear to have evolved additional (e.g., Lowe *et al.,* 2000), but as yet mostly unidentified, physiological abilities that allow them to expand their vertical range and thus exploit food resources in ways not available to other tuna species. Due to the paucity of data gathered from members of the family *Scombridae,* a careful comparative study on the physiological/biochemical adaptations of hearts from various tuna and ectothermic scombrids will be fruitful from both an evolutionary and a physiological perspective.

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ANATOMICAL AND PHYSIOLOGICAL SPECIALIZATIONS FOR ENDOTHERMY

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I. INTRODUCTION

Tunas are endothermic, which means that they utilize metabolic heat to elevate and maintain regional body temperatures (T_b) that are warmer than the ambient seawater temperature (T_w). The objective of this chapter is to review the contributions made by laboratory investigations to our current understanding of endothermy and its biological importance for these fishes. This will complement this volume's chapter by Gunn and Block, which reports on how information obtained from electronic tagging studies has contributed to our knowledge of tuna endothermy.