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BLOOD VOLUME, PLASMA VOLUME AND CIRCULATION TIME IN A HIGH-ENERGY-DEMAND TELEOST, THE YELLOWFIN TUNA (*THUNNUS ALBACARES*)

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Summary

We measured red cell space with ^{51}Cr-labeled red blood cells, and dextran space with 500 kDa fluorescein-isothiocyanate-labeled dextran (FITC-dextran), in two groups of yellowfin tuna (*Thunnus albacares*). Red cell space was 13.8±0.7 ml kg^{-1} (mean ± S.E.M.) Assuming a whole-body hematocrit equal to the hematocrit measured at the ventral aortic sampling site and no significant sequestering of ^{51}Cr-labeled red blood cells by the spleen, blood volume was 46.7±2.2 ml kg^{-1}. This is within the range reported for most other teleosts (30–70 ml kg^{-1}), but well below that previously reported for albacore (*Thunnus alalunga*, 82–197 ml kg^{-1}). Plasma volume within the primary circulatory system (calculated from the ^{51}Cr-labeled red blood cell data) was 32.9±2.3 ml kg^{-1}. Dextran space was 37.0±3.7 ml kg^{-1}. Because 500 kDa FITC-dextran appeared to remain within the vascular space, these data imply that the volume of the secondary circulatory system of yellowfin tuna is small, and its exact volume is not measurable by our methods. Although blood volume is not exceptional, circulation time (blood volume/cardiac output) is clearly shorter in yellowfin tuna than in other active teleosts. In a 1 kg yellowfin tuna, circulation time is approximately 0.4 min (47 ml kg^{-1}/115 ml min^{-1} kg^{-1}) compared with 1.3 min (46 ml kg^{-1}/35 ml min^{-1} kg^{-1}) in yellowtail (*Seriola quinqueradiata*) and 1.9 min (35 ml kg^{-1}/18 ml min^{-1} kg^{-1}) in rainbow trout (*Oncorhynchus mykiss*). In air-breathing vertebrates, high metabolic rates are necessarily correlated with short circulation times. Our data are the first to imply that a similar relationship occurs in fishes.

Key words: fish, yellowfin tuna, *Thunnus albacares*, pelagic, cardiorespiratory, metabolic rate, cardiac output, red cell space, ^{51}Cr, dextran, primary circulatory system, secondary circulatory system.

Introduction

The relationships between metabolic rate and the respiratory, circulatory and cellular mechanisms governing oxygen transport from the respiratory medium to the tissues in air-breathing vertebrates have been the subject of intensive scrutiny over the last two decades (e.g. Taylor *et al.* 1996 and the related papers in the same volume). Many of the lessons learned from these studies appear to be applicable to fishes (Mathieu-Costello *et al.* 1992, 1995, 1996; Moyes *et al.* 1992), although a complete set of supporting physiological and morphometric data for a given fish species is often wanting. Regardless, it is clear that tunas (family *Scombridae*, tribe *Thunnini*) are high-energy-demand teleosts and that both their standard and maximum rates of oxygen consumption exceed those of other active teleosts (e.g. salmonids) by at least fourfold (Brill, 1987; Boggs and Kitchell, 1991; Dewar and Graham, 1994; Korsmeyer *et al.* 1996*a*). Adaptations supporting the high metabolic rates of tunas include large gill surface area, high cardiac output, elevated hemoglobin concentrations and the ability to maintain muscle temperature significantly above ambient (Bushnell and Jones, 1994; Dickson, 1996). Other adaptations that could potentially allow tunas to achieve high rates of oxygen consumption are either a significantly elevated blood volume (Korzjynew and Nikolskria, 1951) or a short circulation time (i.e. blood volume/cardiac output) (Coulson, 1986). Using radio-iodinated bovine serum albumin, Laurs *et al.* (1978) reported blood volumes of albacore (*Thunnus alalunga*) ranging from 82 to 197 ml kg^{-1}. In contrast, blood volumes for 25 species of elasmobranch, teleost and holostean fishes listed by Tort *et al.* (1991) range from 18 to 80 ml kg^{-1}, although blood volumes of most teleosts lay within a more restricted range (30–70 ml kg^{-1}, Itazawa *et al.* 1983; Olson, 1992). In albacore, elevated blood volumes would extend

circulation times even in the face of the elevated cardiac outputs of tuna (Lai *et al.* 1987; White *et al.* 1988; Bushnell and Brill, 1992; Jones *et al.* 1993), implying that the former is more important than the latter for achieving exceptionally high metabolic rates. However, based on the dilution of iodocyanin green dye ('cardio-green'), Bushnell (1988) estimated blood volumes in skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) tunas to be approximately 50 ml kg^{-1}. If correct, the impact of these data on our concepts of circulation time, oxygen and substrate delivery, and their relationship to metabolic rate is considerable because of the interrelationship between these variables (Coulson *et al.* 1977; Coulson and Herbert, 1984; Coulson, 1986; Bushnell and Brill, 1992; Korsmeyer *et al.* 1996*a*,*b*).

Olson (1992), in his excellent and comprehensive review on the blood volumes of fishes, identified two trends. First, Osteichthyes have the lowest blood volumes of any vertebrate and, second, estimated blood volumes depend on the techniques employed. Larger blood volumes are usually measured with plasma volume indicators (e.g. Evans Blue or radio-iodinated albumins) than are obtained with ^{51}Cr-labeled red blood cells. Problems associated with dyes or protein markers arise because they may not remain within the primary circulatory system, instead entering the secondary circulatory system (a parallel 'lymph-like' circulatory system in teleosts; Steffensen and Lomholt, 1992; Olson, 1996), the interstitial space or even be excreted. The use of ^{51}Cr-labeled red blood cells can, however, also result in an overestimate of red cell space and blood volume because labeled red blood cells may be selectively sequestered by the spleen (Duff *et al.* 1987). Moreover, the accuracy of both techniques is dependent on measured hematocrit because these data are needed to calculate blood volume from the measured plasma volume or red cell space (Jones, 1970; Fairbanks *et al.* 1996). It is generally assumed that whole-body hematocrit and hematocrit measured at the sampling site (usually a large blood vessel) are the same, although they can differ considerably (Albert, 1971; Gingerich and Pityer, 1989; Olson, 1992; Fairbanks *et al.* 1996).

Consequently, we decided to quantify red cell space and plasma volume of yellowfin tuna using two independent techniques. The former was measured directly using ^{51}Cr-labeled red blood cells and the latter by dilution of large (500 kDa) fluorescein-isothiocyanate-labeled dextran (FITC-dextran). We chose 500 kDa FITC-dextran as a marker in an attempt specifically to reduce complications associated with non-binding of a dye to plasma proteins and extravasation (Gingerich and Pityer, 1989; Tort *et al.* 1991).

Materials and methods

Yellowfin tuna *Thunnus albacares* (Bonaterre) were purchased from local commercial fishermen and maintained at 25±1 °C in circular outdoor tanks (approximately 8 m in diameter and 1.5 m deep) at the Kewalo Research Facility (National Marine Fisheries Service, Honolulu Laboratory). Fish were fed chopped squid daily, but were not fed for approximately 20 h before use in an experiment to allow sufficient time for gut clearance (Magnuson, 1969).

Anesthesia and surgical procedures were as described previously (Bushnell and Brill, 1992). Briefly, fish were dip-netted from their holding tank and immediately placed in a plastic bag containing 1 g l^{-1} of tricaine methanosulfonate (MS222) buffered with an equal molar concentration of sodium bicarbonate. After initial anesthesia, fish were rushed into the laboratory, placed ventral-side up in a soft chamois sling and ventilated with oxygenated sea water (temporarily chilled to 21–22 °C) containing 0.1 g l^{-1} of buffered tricaine methanosulfonate. A 20 gauge, 3.2 cm Instyle Vialon intravenous catheter (Becton Dickinson Vascular Access, Sandy, Utah, USA) was introduced into the ventral aorta under manometric guide and connected to a 20 cm length of polyethylene tubing (PE 160). Fish were turned upright, a 20 gauge hypodermic needle was placed into the neural canal immediately posterior to the skull and used to inject 0.1–0.2 ml doses of 4 % lidocaine. This procedure blocks spinal motor nerves and prevents excessive tail movements, but leaves all cranial nerves and cardiorespiratory function intact (Bushnell and Brill, 1991). Fish were then placed in front of a pipe delivering approximately 30–35 l min^{-1} of oxygenated sea water and were thus able to set their own ventilation volume by adjusting mouth gape. Throughout the duration of the experiment, fish were kept sedated by repeated 0.1–0.3 ml intramuscular injections of the steroid anesthetic Saffan (alphaxalone, Glaxovet, Harefield, Uxbridge, UK), a highly effective anesthetic in fish (Oswald, 1978). At the conclusion of an experiment, animals were killed with an overdose of sodium pentobarbital, injected *via* the indwelling ventral aortic catheter, and weighed. Data were obtained from seven fish using ^{51}Cr-labeled red blood cells (body mass 0.750–2.420 kg) and from seven fish using 500 kDa FITC-dextran (body mass 0.865–3.325 kg).

Measurement of red cell space

Red blood cells labeled with ^{51}Cr (Amersham Canada Limited, Oakville, Ontario, Canada) were used to measure red cell space. To avoid any complications due to blood clotting, fish were given 1000 i.u. of sodium heparin (0.1 ml of 10 000 i.u. ml^{-1}) *via* the ventral aortic catheter approximately 1 h after catheterization. Ten minutes later, 2 ml of blood was removed. The blood was mixed with 10 ml of tuna saline (1.17 % NaCl), centrifuged (approximately 50 g for 2 min), and the supernatant discarded. The red blood cells were resuspended in 2 ml of saline, 1.85×10^{6} Bq (50 μCi) of ^{51}Cr was added, and the cells were then gently swirled for 2 h at room temperature (approximately 25 °C). The labeled red blood cells were centrifuged (approximately 50 g for 2 min), washed three times with 14 ml of saline each time, and finally resuspended in 1.5 ml of saline for injection into the same fish from which they were originally withdrawn. The precise volumes injected (mean ± S.E.M., 1.57±0.14 ml) were determined by mass. A sample of the final rinse solution was taken to determine whether any unincorporated ^{51}Cr remained

in the red blood cell suspension. The hematocrit of the final injectate was measured, and a 300 μl sample was taken for counting. Because of the difficulties in placing and maintaining a second patent catheter in the dorsal aorta of yellowfin tuna (Brill *et al.* 1987; Jones *et al.* 1986; Bushnell, 1988), the ventral aortic catheter was used for both injection of labeled red blood cells and blood sampling. To minimize contamination of blood samples with labeled red blood cells from the initial injectate, approximately 2–3 ml of blood was withdrawn prior to injection of the labeled cells, and this blood used to flush the catheter immediately following injection. Blood samples (500 μl) were withdrawn at 30, 60 and 300 min post-injection and hematocrits were measured. Subsamples were centrifuged (8000 ***g*** for approximately 1 min) and the plasma was retained to test for hemolysis of labeled red blood cells. Duplicate 50 or 100 μl whole-blood and plasma samples were mixed with 500 μl of distilled water, and their radioactivity was measured in a Beckman 5500 gamma counter (Beckman Instruments, Fullerton, California, USA).

Measurement of FITC-dextran space

500 kDa FITC-dextran (Sigma Chemical Co., St Louis, Missouri, USA) was used to determine dextran space. Approximately 60–75 mg ($12.9\pm0.9\,mg\,kg^{-1}$ body mass) was dissolved in 2–4 ml of saline, and a weighed volume (2–3 ml) was injected *via* the ventral aortic catheter. To minimize any increase in circulatory system volume caused by the injectate, an equal volume of blood was removed and centrifuged (as above), and the red blood cells were reinjected after the dye. Blood samples (200 μl) were taken at timed intervals (nominally 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360 and 420 min post-injection). Samples were centrifuged in micro-hematocrit tubes, 40 μl subsamples of plasma were diluted with 960 μl of saline, and the dye concentration was measured spectrophotometrically (Coleman Junior 6/20A, Perkin Elmer, Maywood, Illinois, USA) at 496 nm.

Calculations

^{51}Cr-labeled red cells

Red cell space was calculated from the volume of cells injected multiplied by the ratio of ^{51}Cr activity in the injected red blood cells to ^{51}Cr activity in the red blood cells sampled from the fish. ^{51}Cr activity in the red blood cells was calculated by dividing the activity measured in the whole blood by the hematocrit of that sample (expressed as a decimal fraction). Blood volume was obtained by dividing red cell space by the hematocrit (expressed as a decimal fraction). Plasma volume was calculated as the differences between blood volume and red cell space.

FITC-dextran

In teleosts, the concentration of plasma-borne indicators decays in a two-step fashion; an initial rapid fall (1–2 h) followed by a prolonged gradual decline (4–24 h). Plasma concentrations decaying in this fashion are assumed to represent movement of the indicator between two connected compartments (herein called ‘fast-mixing’ and ‘slow-mixing’), with a simultaneous leak out of the compartments (Nichols, 1987; Steffensen and Lomholt, 1992). This situation is best modeled by fitting the data to a double-exponential decay equation (Riggs, 1963; Shipley and Clark, 1972):

$$[\mathrm{dye}]_t = c_{f\rightarrow s}e^{-R_{f\rightarrow s}t} + c_{out}e^{-R_{out}t}\,, \quad (1)$$

where t is elapsed time since dye injection (min), $[\mathrm{dye}]_t$ is plasma dye concentration measured at time t, $c_{f\rightarrow s}$ is the fitted parameter for FITC-dextran moving between the ‘fast-mixing’ and ‘slow-mixing’ vascular compartments (in units of dye concentration), c_{out} is the fitted parameter for the dye leaving the vascular compartments (in units of dye concentration), $R_{f\rightarrow s}$ is the rate constant (min^{-1}) for FITC-dextran movement between the ‘fast-mixing’ and ‘slow-mixing’ vascular compartments, and R_{out} is the rate constant (min^{-1}) for FITC-dextran movement out of the ‘fast-mixing’ and ‘slow-mixing’ vascular compartments.

The first exponential decay term describes the movement of the marker between the compartments, and the second exponential decay term describes movement of the marker out of the vascular space. Because of the high protein permeability of fish capillaries (Wardle, 1971; Hargens *et al.* 1974), and lacking any data to the contrary, we initially assumed that 500 kDa FITC-dextran would mix into and leak from the circulatory system of yellowfin tuna in a manner similar to the behavior of plasma-borne markers used in other fishes. Consequently, we initially fitted plasma dye concentration data to the double-exponential decay equation (equation 1).

When our data were fitted to equation 1, the term representing the rate of loss of FITC-dextran from the vascular space (R_{out}) was not significantly different from zero (see below). As a result, the equation best describing the behavior of 500 kDa FITC-dextran in yellowfin tuna (for the purposes of estimating compartment volumes) reduces to a single-exponential decay equation with a constant:

$$[\mathrm{dye}]_t = c_{f\rightarrow s}e^{-R_{f\rightarrow s}t} + c_{out}\,. \quad (2)$$

We therefore refitted our data using equation 2. For fitting the data to both equations, we employed an iterative least-squares curve-fitting technique (Curvefit, Jandel Scientific, San Rafael, California, USA).

The dextran space of the ‘fast-mixing’ compartment was calculated by dividing the quantity of FITC-dextran injected by the concentration of FITC-dextran in the plasma at t=0 (i.e. $c_{f\rightarrow s}+c_{out}$, see Fig. 1). The combined dextran space of the ‘fast-mixing’ plus ‘slow-mixing’ compartments was calculated by dividing the quantity of dye injected by the dye concentration at $t=\infty$ (i.e. c_{out}, see Fig. 1). The dextran space of the ‘slow-mixing’ compartment is the mean of the difference between the two.

Statistical treatment

Data were analyzed using a one-way analysis of variance (ANOVA) for treatment groups (red cell space and blood

volume calculated from samples taken 30, 60 and 300 min post-injection) or by pairwise comparisons (plasma volume, red cell space and blood volume measured with ^{51}Cr-labeled red blood cells and their equivalents measured with 500 kDa FITC-dextran) (Sigma Stat, Jandel Scientific, San Rafael, California, USA), and 5 % was taken as the fiducial limit for a significant difference. All data presented in the text and tables are means ± standard error of the mean (S.E.M.).

Results

^{51}Cr-labeled red cells

Body mass, hematocrit, red cell space, blood volume and plasma volume data are summarized in Table 1. Mean ^{51}Cr activity in the final rinse solution was less than 1 % of the activity in the injectate, showing that no significant amount of unincorporated label was given to the fish. Plasma samples also showed no significant amount of radioactivity at any sampling time, indicating no hemolysis of the labeled red blood cells within the fish. Other investigators have observed hemolysis of labeled red blood cells, presumably because blood from one fish was injected into a another fish (Gingerich *et al.* 1987). We avoided this problem by re-injecting ^{51}Cr-labeled red blood cells into the same fish from which they were withdrawn.

There were no significant differences between mean red cell spaces calculated from samples taken 30, 60 and 300 min post-injection when the data were averaged across fish (see Fig. 1). The 30, 60 and 300 min data were therefore combined, and a single value of red cell space, blood volume and plasma volume was calculated for each fish.

FITC-dextran

When the FITC-dextran data were fitted to the double-exponential decay equation (equation 1), R_{out} (i.e. the term representing the rate of loss from the vascular space) was not significantly different from zero ($6.24\times10^{-5}\pm5.03\times10^{-3}$ min^{-1}). The half-time for loss from the vascular space ($\ln2\times R_{out}^{-1}$) was $5.27\times10^{18}\pm3.49\times10^{18}$ min. These data imply that the marker did not leave the vascular compartment even when plasma concentrations were followed for more than 400 min.

The data from the FITC-dextran experiments are summarized in Table 2. A representative trace of the mixing-in kinetics for 500 kDa FITC-dextran is shown in Fig. 1. In contrast to the ^{51}Cr-labeled red blood cells, which were completely mixed some time before the first sample (30 min post-injection), the half-time (calculated as $\ln2\times R_{f\rightarrow s}^{-1}$; Riggs, 1963) for the mixing-in process of FITC-dextran was 75±23 min.

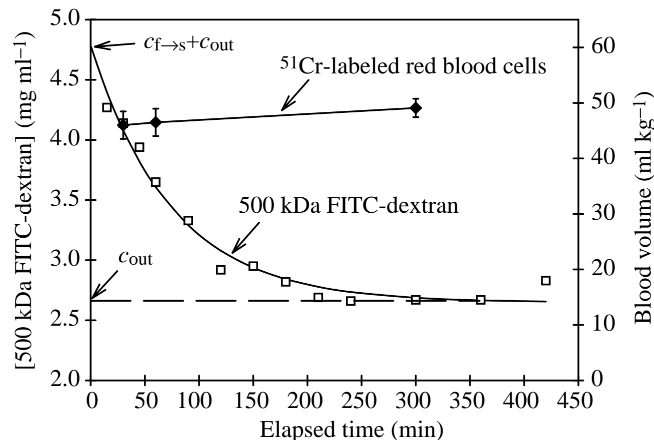

Fig. 1. Mean (± S.E.M.) blood volumes calculated from samples taken 30 min (N=7), 60 min (N=7) and 300 min (N=5) after injection of ^{51}Cr-labeled red blood cells (filled symbols), and a representative data set showing the change in plasma concentrations of 500 kDa FITC-dextran over time in a single yellowfin tuna (open symbols). The parameters of the exponential decay equation used to calculate the dextran spaces of the 'fast-mixing' and 'slow-mixing' compartments ($c_{f\rightarrow s}+c_{out}$ and c_{out}, respectively) are also shown.

The dextran spaces of the 'fast-mixing' and 'slow-mixing' compartments were 21.6±2.2 ml kg^{-1} and 15.4±2.4 ml kg^{-1}, respectively. If the dextran space of the 'fast-mixing' compartment is assumed to be equivalent to plasma volume within the primary circulatory system, then mean blood volume is the dextran space divided by one minus the hematocrit (expressed as a decimal fraction) or 31.2±3.1 ml kg^{-1}, a value significantly less than the blood volume calculated using the ^{51}Cr-labeled red blood cells (Table 1). If, however, the dextran space of the 'fast-mixing' and 'slow-mixing' compartments is assumed to be equivalent to plasma volume within the primary circulatory system, then blood volume is 53.7±5.4 ml kg^{-1}, a value not significantly different from the blood volume calculated using the ^{51}Cr-labeled red blood cells.

Table 1. *Summary data from experiments using ^{51}Cr-labeled red blood cells to measure red cell space in yellowfin tuna* Thunnus albacares

Parameter	Mean ± S.E.M.
Body mass (kg)	1.071±0.600
Hematocrit (%)	30±2
Red cell space (ml kg^{-1})	13.8±0.7
Blood volume (ml kg^{-1})	46.7±2.2
Plasma volume (ml kg^{-1})	32.9±2.3
Number of animals (N)	7

Discussion

^{51}Cr-labeled red blood cells

Mean red cell space and blood volumes were not significantly different at 30, 60 or 300 min post-injection (Fig. 1). These data therefore imply either that the spleen of yellowfin tuna did not selectively remove any ^{51}Cr-labeled red blood cells or that removal of labeled cells ceased prior to our first sample. Duff *et al.* (1987) found that removal of ^{51}Cr-labeled red blood cells by the spleen of rainbow trout (*Oncorhynchus mykiss*) continued for up to 240 min post-

Table 2. *Summary data from experiments using a plasma-borne marker (500 kDa FITC-dextran) to measure dextran space and blood volume in yellowfin tuna* Thunnus albacares

Parameter	Mean ± S.E.M.
Body mass (kg)	1.410±0.339
Hematocrit (%)	31±1
$c_{f\rightarrow s}$ (mg ml^{-1})	3.56±0.21
$R_{f\rightarrow s}$ (min^{-1})	$1.23\times10^{-2}\pm0.18\times10^{-2}$
c_{out} (mg ml^{-1})	2.61±0.47
r^2	0.914±0.015
Dextran space of the ‘fast-mixing’ compartment (ml kg^{-1})	21.6±2.2
Dextran space of the ‘fast-mixing’+‘slow-mixing’ compartments (ml kg^{-1})	37.0±3.7
Dextran space of the ‘slow-mixing’ compartment (ml kg^{-1})	15.4±2.4
Blood volume based on dextran space of the ‘fast-mixing’ compartment (ml kg^{-1})	31.2±3.1
Blood volume based on dextran space of the ‘slow-mixing’+‘fast-mixing’ compartment (ml kg^{-1})	53.7±5.4
Number of animals (*N*)	7

$c_{f\rightarrow s}$, fitted parameter for FITC-dextran moving between the ‘fast-mixing’ and ‘slow-mixing’ compartments (in units of dye concentration); $R_{f\rightarrow s}$, rate constant for FITC-dextran movement between the ‘fast-mixing’ and ‘slow-mixing’ vascular compartments; c_{out}, fitted parameter for the dye remaining in the vascular compartments equilibrium (in units of dye concentration).

r^2 is the Pearson product moment correlation coefficient obtained from fitting data to equation 2.

injection, something we did not observe. The relatively short circulation time of the yellowfin tuna (see below) might, however, allow the splenic sequestration of ^{51}Cr-labeled red blood cells to be completed rapidly. Why the spleen selectively accumulates labeled red blood cells is not clear, but it is possible that the incubation, centrifugation and resuspension procedures used during labeling of red blood cells with ^{51}Cr slightly damages a fraction of the cells. It may be these ‘damaged’ cells that the spleen selectively removes from the circulation. More importantly, Duff *et al.* (1987) calculated that in rainbow trout blood volume would be overestimated by only approximately 15 % as a result of splenic accumulation of ^{51}Cr-labeled red blood cells.

Can the ^{51}Cr-labeled red blood cell method, therefore, be regarded as the ‘gold-standard’ for blood volume determination? Perhaps only up to a point, because any assessment of blood volume is also at the mercy of the measured hematocrit (Fairbanks *et al.* 1996). Our calculations assume that the hematocrit was the same throughout the vascular space as at our sampling point in the ventral aorta (i.e. whole-body hematocrit = large-vessel hematocrit). In mammals, the hematocrit in the large vessels is greater than that within the remainder of the circulatory system (whole-body hematocrit/large-vessel hematocrit ≈ 0.9) and the difference may be even larger in fish (whole-body hematocrit/large-vessel hematocrit ≈ 0.8) (Albert, 1971; Gingerich and Pityer, 1989; Olson, 1992). If this is also true in yellowfin tuna, then we may have underestimated the actual blood volume by as much as approximately 25 %.

FITC-dextran

There is good evidence that commonly used plasma-borne indicators (e.g. Evans Blue, radio-iodinated albumin) are lost from the vascular compartments of fishes (Laurs *et al.* 1978; Duff and Olson 1989; Gingerich and Pityer, 1989; Olson, 1992). Indeed, Nichols (1987) found that in rainbow trout albumin exchanged with the extravascular compartment at a rate of up to 48 % h^{-1}. Extravasation of the marker, dilution into vascular compartments not accessed by labeled red blood cells (e.g. owing to plasma skimming or plasma movement into the secondary circulatory system) and the necessity of extrapolating plasma concentration decay curves back to time zero increase inaccuracies in the calculation of plasma volume and usually result in estimated blood volumes larger than those measured using ^{51}Cr-labeled red blood cells (Olson, 1992). The difference in measured plasma volumes has been used as an estimate of the volume of the secondary circulatory system, a volume to which plasma-borne markers have access but red blood cells do not (Steffensen and Lomholt, 1992).

We therefore expected the dextran space of the ‘fast-mixing’ compartment (Table 2) to have been at least as large as, if not larger than, the plasma volume calculated from the ^{51}Cr-labeled red blood cell data (Table 1) and were surprised when we found just the opposite. What are the anatomical counterparts of the ‘fast-mixing’ and ‘slow-mixing’ compartments in tuna? Given that our data show that 500 kDa FITC-dextran remains within the vascular space, there are two plausible answers. First, the former is equivalent to the plasma volume within the primary circulatory system and the latter to the plasma volume within the secondary circulatory system; second, both compartments are within the primary circulatory system.

Analysis of similarly shaped plasma concentration decay curves of radio-iodinated albumin in rainbow trout obtained by Steffensen and Lomholt (1992) suggests that the dextran spaces of the ‘fast-mixing’ and ‘slow-mixing’ compartments are indeed equivalent to the plasma volumes within the primary and secondary circulatory systems in tuna. Therefore, our FITC-dextran data show that blood volume in yellowfin tuna

is 31.2±3.1 ml kg^{-1} and that the volume of the secondary circulatory system is 15.4±2.4 ml kg^{-1}, or approximately 50 % of blood volume. In contrast, the volume of the secondary circulatory system in rainbow trout has been estimated to be 150 % of blood volume (Steffensen and Lomholt, 1992). Dewar *et al.* (1994) have described a secondary circulatory system associated with the central vascular heat exchangers in skipjack tuna. Although this system has been well described anatomically in other fishes (e.g. Vogel and Claviez, 1981; Steffensen *et al.* 1986), its volume and function(s) remain uncertain (Wardle, 1971; Steffensen and Lomholt, 1992; Olson, 1996).

If, however, the dextran spaces of the 'fast-mixing' and 'slow-mixing' compartments are equivalent to the total plasma volume within the primary circulatory system, then plasma volume and blood volume measured using ^{51}Cr-labeled red blood cells (Table 1) and using FITC-dextran (Table 2) are not significantly different. When analyzed under this set of assumptions, our data imply that the secondary circulatory system in yellowfin tuna is so small that its volume is unmeasurable by our methods and that there is no significant loss of 500 kDa FITC-dextran from the vascular system of yellowfin tuna. If there were loss of the indicator into the interstitial space, then the dextran space would have significantly exceeded the plasma volume calculated from the ^{51}Cr-labeled red blood cell data. In contrast, when 500 kDa FITC-dextran was used in an attempt to measure plasma space in rainbow trout and cod (*Gadus morhua*), plasma concentrations continued to decline over the 12 h for which they were followed and never reached equilibrium (Bushnell *et al.* 1997). It must be noted, however, that the mixing-in of ^{51}Cr-labeled cells was complete some time prior to the first sample (30 min post-injection), whereas the mixing-in of the 500 kDa FITC-dextran required significantly longer (half-time 75±23 min). The difference in equilibration kinetics between the ^{51}Cr-labeled red blood cells and FITC-dextran and yet the agreement between plasma volumes measured with the two indicators are unexpected if both the 'fast-mixing' and 'slow-mixing' compartments are indeed within the primary circulatory system. At present, we have no explanation for this apparent paradox.

Nevertheless, our estimates of yellowfin tuna blood volume (31–47 ml kg^{-1}) are at the middle to lower end of the range reported for the majority of teleosts (30–70 ml kg^{-1}, Itazawa *et al.* 1983; Olson, 1992) and well below the blood volumes reported for albacore (mean 132 ml kg^{-1}, range 82–197 ml kg^{-1}; Laurs *et al.* 1978). The reasons for the differences in the blood volumes of the two tuna species are unclear but may, in part, be due to species differences, methodological differences or both. Laurs *et al.* (1978) employed radio-iodinated bovine serum albumin and thus had to extrapolate indicator dilution curves back to time zero. They were also forced to work on board ship using animals immediately after they had been landed by hook and line. The elevated hematocrit (53–58 %) compared with normal values in rested tunas (30–41 %, Bushnell and Jones, 1994) imply the fish were still recovering from the stress of capture.

Relationship between blood volume and metabolic rates

After measuring blood volumes in albacore ranging from 82 to 197 ml kg^{-1}, Laurs *et al.* (1978) concluded that large blood volumes are characteristic of high-energy-demand teleosts. Sleet and Weber (1983), however, found that the blood volume of buffalo sculpin (*Enophrys bison*) was 70–90 ml kg^{-1}, which is at the upper end of the range of blood volumes recorded in fishes, except albacore. Because the buffalo sculpin is a relatively inactive benthic species, they concluded that blood volumes in teleosts are not necessarily correlated with activity. Moreover, Coulson *et al.* (1977), Coulson and Herbert (1984) and Coulson (1986) present data showing that metabolic rates in air-breathing vertebrates are directly correlated with short circulation times (i.e. blood volume/cardiac output), rather than with elevated blood volumes or cardiac outputs *per se*. In other words, rates of energy production are set by rates of metabolic substrate delivery which are, in turn, set by the number of circulatory cycles occurring per unit time (Coulson, 1986).

Our estimate of blood volume in yellowfin tuna, therefore, allows us to determine circulation time and to compare this with estimates for other active teleosts. In 1 kg fish and measured under similar circumstances, circulation time is approximately 0.3–0.4 min (31–47 ml kg^{-1}/115 ml min^{-1} kg^{-1}) in yellowfin tuna compared with 1.3 min (46 ml kg^{-1}/35 ml min^{-1} kg^{-1}) in yellowtail (*Seriola quinqueradiata*) (Yamamoto *et al.* 1980, 1981) and 1.9 min (35 ml kg^{-1}/18 ml min^{-1} kg^{-1}) in rainbow trout (Kiceniuk and Jones, 1977). The circulation time of yellowfin tuna is, therefore, approximately one-third to one-sixth that seen in these other active teleosts, a ratio similar to that seen for their metabolic rates (Yamamoto *et al.* 1981; Bushnell and Jones, 1994). These data imply that, when examined over reasonable ranges of temperature and blood oxygen-carrying capacities (i.e. excluding species such as the Antarctic hemoglobinless icefish *Chaenocephalus aceratus*), short circulation times are correlated with high metabolic rates in teleosts as they are in air-breathing vertebrates.

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