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OXYGEN TRANSPORT AND CARDIOVASCULAR RESPONSES TO EXERCISE IN THE YELLOWFIN TUNA *THUNNUS ALBACARES*

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Summary

Yellowfin tuna *Thunnus albacares* (1400–2175 g) instrumented with electrocardiogram electrodes and pre- and post-branchial catheters were subjected to incremental swimming velocity tests. Increasing velocity, from a minimal speed of 1.0 *FL* s^{-1}, where *FL* is fork length, resulted in a 1.4-fold increase in heart rate (from 61.4 to 84.6 beats min^{-1}), an elevated ventral-aortic blood pressure (from 10.8 to 12.2 kPa) and a decreased systemic vascular resistance. Relative branchial vascular resistance at minimal speed ranged from 24.4 to 40.0 % of total vascular resistance and tended to increase with velocity. Yellowfin blood has a high oxygen-carrying capacity (16–18 ml O_2 dl^{-1}), and a low *in vivo* oxygen affinity (P_{50}=5.3 kPa). Exercise caused a rise in arterial saturation (from 74 to 88 %) and a decline in venous saturation (from 48 to 44 %), resulting in a 1.3-fold increase in tissue oxygen extraction from the blood (arterial–venous oxygen content difference). Whereas arterial oxygen partial pressure (P_{O_2}) tended to increase with exercise, venous P_{O_2} remained unchanged (approximately 5.3 kPa). The observed decrease in venous oxygen content was brought about by a lowered blood pH (from 7.80 to 7.76) and a large Bohr shift. Cardiac output and the increased blood oxygen extraction are estimated to have contributed nearly equally to the increased oxygen consumption during exercise. The large venous oxygen reserve still available to yellowfin tuna at maximal prolonged velocities suggests that the maximal oxygen delivery potential of the cardiovascular system in this species is not fully utilized during aerobic swimming. This reserve may serve other aerobic metabolic processes in addition to continuous swimming.

Key words: oxygen, cardiovascular physiology, swimming, exercise, blood gases, tuna, *Thunnus albacares.*

Introduction

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

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

$$\dot{V}_{O_2} = \dot{V}\text{b} \times (\text{a–v})_{O_2}, \quad (1)$$

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

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

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Bushnell and Jones, 1994). Brill and Bushnell (1991*b*), for example, estimated that the maximal aerobic metabolic rate of tunas is 8–9 times higher than their standard metabolic rate and suggested that this increase is achieved by a three- to fourfold increase in oxygen extraction [$(a-v)_{O_2}$]. Thus, the elevated oxygen demands during exercise could be met by utilizing the high oxygen-carrying capacity of the blood, with only a 1.5- to twofold increase in $\dot{V}$b. More recently, however, it has been hypothesized that maximal oxygen consumption in tunas is reached through a fourfold increase in $\dot{V}$b and only a twofold increase in $(a-v)_{O_2}$ (Bushnell and Jones, 1994).

The development of a water tunnel for tunas has enabled *in vivo* physiological measurements under controlled swimming conditions (Dewar and Graham, 1994*a*; Dewar *et al.* 1994; Korsmeyer *et al.* 1996*b*, 1997). The objectives of this paper are to report measurements of both pre-branchial (venous) and post-branchial (arterial) hemodynamic, blood gas and hematological variables in unrestrained and unanesthetized yellowfin tuna, and to examine the relationship between exercise (i.e. swimming velocity) and tissue oxygen delivery.

Materials and methods

Experimental animals and equipment

Animal care, the water tunnel and the associated equipment are described in Korsmeyer *et al.* (1997). The yellowfin tuna used in this study ranged in mass from 1400 to 2175 g (1850±71 g) and in fork length (*FL*) from 43 to 50 cm (47.6±0.6 cm) (mean ± S.E.M., N=11).

Anesthesia and instrumentation

Fish handling and anesthesia procedures are described in Korsmeyer *et al.* (1997). The dorsal and ventral aortae were catheterized using 18 gauge thin-walled needles advanced percutaneously under manometric guidance. The dorsal vessel was entered through the roof of the mouth, and the ventral aorta was accessed through the ventral body wall. Once access to the vessel was confirmed by pulsatile pressure recordings, heparinized (100 i.u.), saline-filled PE 50 tubing was advanced through the hub and beyond the tip of the needle into the vessel. The dorsal aorta was catheterized beyond the confluence (1–2 cm) of the efferent branchial arteries of the first and second gill arches, and the catheter was fed 2–3 cm down the vessel so that its tip was posterior to the union of the third and fourth branchial arteries. The ventral-aortic catheter entered the bulbus arteriosus and was fed 1.5–2 cm anteriorly into the ventral aorta. After correct placement had been confirmed by checking blood pressure and the ease of blood aspiration, the needle was carefully withdrawn and passed over the end of the tubing, leaving the catheter in place. The dorsal-aortic catheter was sutured to the roof of the mouth and passed out through the snout *via* a hole formed by an 18 gauge needle.

Electrocardiogram (ECG) electrodes (30 gauge insulated wire) were inserted subcutaneously adjacent to the ventricle. The two catheters and ECG wires were secured with sutures along the body of the fish leading to the second dorsal fin, and the free ends were tied together at regular intervals to prevent entanglement once the fish was swimming. The catheters and ECG leads were long enough to extend from the fish to outside the water tunnel (approximately 150 cm). The entire procedure was completed within 30 min, as it was found that longer times under anesthesia resulted in poor recovery (Korsmeyer, 1996).

Experimental protocol

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

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

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

Following the experiment, blood samples were taken from each fish to determine the oxygen-carrying capacity, as described in Lai *et al.* (1990). The fish were killed by a blow to the head, and the locations of the catheters were verified.

Analytical procedures

Blood pressure was recorded with calibrated Statham pressure transducers (Gould model P231D), with zero determined through a PE tubing connection to the sea water in the tunnel. Pressure signals were amplified (Gould model 13-4615-50) and recorded on a strip chart recorder (Gould model 2400), as was the ECG signal (Grass model P15D and Gould model 13-4615-58). The pressure and ECG signals were simultaneously digitized (200 Hz) and saved to computer hard disk (486 PC-compatible, Axotape data acquisition system, Axon Instruments).

Venous and arterial blood samples (0.8 ml) were drawn in sequence for immediate analysis of hematological and blood gas parameters. An additional 1 ml sample was taken less frequently for a separate study on catecholamine levels (Lai *et al.* 1994). The size and large blood volume of these fish resulted in a total

blood loss of less than 10 % (based on a blood volume of 8 % of body mass, Laurs *et al.* 1978). Blood oxygen and carbon dioxide partial pressures (P_{O_2} and P_{CO_2}) and pH were determined using a Radiometer blood-gas microsystem (BMS-3MK2) thermostatted at 25 °C. The P_{O_2} electrode was calibrated with a standard zero-P_{O_2} solution and air-saturated water. P_{CO_2} measurements were made in only a few experiments when precision-mixed gases for calibration of the P_{CO_2} electrode were available. The pH electrode was calibrated with Radiometer precision buffer solutions. Total O_2 content (C_{O_2}) was determined as described by Tucker (1967). Hematocrit (Hct) was determined by centrifugation, and hemoglobin concentration ([Hb]) was measured using a Radiometer OSM2b Hemoximeter. The remaining blood was centrifuged and the plasma immediately frozen on dry ice for transfer to Scripps Institution of Oceanography, La Jolla, CA, USA (within 4 weeks), where it was stored at −80 °C until analyses were made of lactate and glucose concentrations (YSI Stat2000).

Data analysis and statistics

Mean ventral- and dorsal-aortic blood pressures (P_{VA} and P_{DA}) were calculated by averaging the digitized pulsatile recording (DADiSP Worksheet, DSP Development Corporation). The typical frequency response for the system was approximately 25 Hz, with damping between 10 and 25 % of critical damping. However, only mean pressures were analyzed because of apparent changes in the frequency response during the course of the experiments from partial blood clotting or movement of the catheters. In addition, the fidelity of the signals decreased at high swimming speeds owing to catheter vibration. Heart rates were determined using a Turbo Pascal program that counted the number of QRS complexes over 1 min intervals. Blood pressure and heart rates measured during the last 30 min of each 1 h measurement period were averaged for each fish. Branchial vascular resistance (R_{branch}) was calculated relative to total peripheral resistance (R_{total}) as $[(P_{VA}-P_{DA})/P_{VA}]\times 100$ %, with the assumption that venous pressure was negligible.

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

All swimming velocities were corrected for the solid-blocking effect of the fish, as described by Bell and Terhune (1970). To normalize for differences in swimming performance, swimming velocities were converted to a percentage of the critical swimming velocity (U_{crit}, $FL\,s^{-1}$) (Beamish, 1978):

$$U_{crit} = U_i + [(t_i/t_{ii}) \times U_{ii}], \quad (2)$$

where U_i is the highest velocity maintained for the entire prescribed time, U_{ii} is the velocity increment (approximately $0.25\,FL\,s^{-1}$), t_i is the length of time at the velocity that produced fatigue (U_{max}), and t_{ii} is the prescribed time between velocity increments (60 min).

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

Results

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

Of 22 attempted studies, data for only 11 yellowfin were of sufficient quality to warrant reporting owing to the difficulty in handling tuna and their sensitivity to respiratory stress. Data were eliminated if fish were not swimming well at the end of the 2 h recovery period. In general, these fish had higher plasma lactate levels (some greater than 15 $mmol\,l^{-1}$) and a lower blood pH (<7.6) than those that survived and swam in the experiment (see below). Results for one fish were eliminated because of a dramatic drop in Hct (from 40 to 12 %) during the experiment.

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

Arterial–venous blood differences

To determine whether there were significant changes in blood variables between venous and arterial samples, irrespective of changes between experimental periods, comparisons were made using only paired data from yellowfin that had both catheters functional. These comparisons, combining data from all experimental sampling periods, revealed no significant arterial–venous differences in Hct, lactate levels or glucose levels. However, significant differences were found between arterial and venous pH, [Hb]

Table 1. *Mean swimming velocity, heart rate and blood pressure measurements in swimming yellowfin tuna following anesthesia, at maximum swimming velocity and post-exercise*

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

*Significantly different from 0 h (P<0.05).
†Significantly different from pre-exercise value (P<0.05).
‡Significantly different from U_{max} (P<0.05).
U, swimming velocity; U_{max}, maximal swimming velocity; FL, fork length; U_{crit}, critical swimming speed; f_H, heart rate; P_{VA}, blood pressure in the ventral aorta; P_{DA}, blood pressure in the dorsal aorta.
The mean size of the tuna was 47.6±0.6 cm FL, 1850±70 g, N=11.
Values are means ± S.E.M. (N).

Table 2. *Hematological measurements in swimming yellowfin tuna following anesthesia, at maximum velocity and post-exercise*

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

*Significantly different from 0 h (P<0.05).
†Significantly different from pre-exercise value (P<0.05).
‡Significantly different from U_{max} (P<0.05).
Hct, hematocrit; $[Hb]_a$, $[Hb]_v$, arterial and venous hemoglobin concentration; MCHC, mean cellular hemoglobin concentration; U_{max}, maximal swimming velocity; FL, fork length.
The mean size of the tuna was 47.6±0.6 cm FL, 1850±70 g, N=11.
Values are means ± S.E.M. (N).

and MCHC, and therefore these values are reported separately for the larger data set including all fish in Tables 2 and 3. The mean arterial–venous differences for these parameters at each experimental sample in the four tuna are shown in Table 4. In addition, Table 4 reports the relative branchial vascular resistances determined from paired P_{VA} and P_{DA} measurements and the calculated $(a–v)_{O_2}$ values from the four tuna. There were no significant changes between experimental periods for the arterial–venous differences, but this may be a result of the small sample sizes (N=3–4).

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

Over the 2 h period from the end of anesthesia to the pre-exercise sample, swimming velocity was relatively constant (Table 1). The observed changes in cardiorespiratory parameters therefore reflect recovery from the effects of the brief (<1 min) struggle before the fish was subdued by the anesthetic and from potential respiratory distress caused by anesthesia and surgery. During this recovery period, a significant decrease occurred in P_{DA} (Table 1), and venous pH (pHv), arterial pH (pHa) and venous oxygen content (Cv_{O_2}) increased significantly (Table 3). Calculated venous oxygen saturation (Sv_{O_2}), which reflects differences in [Hb], also increased significantly (Table 3). Significant decreases occurred in arterial oxygen partial pressure (Pa_{O_2}, Table 3), plasma [lactate] and plasma [glucose] (Table 2). The decreasing trend (P>0.05) in $(a–v)_{O_2}$ for yellowfin with both catheters functional parallels that determined from all fish (Tables 3, 4).

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

Mean swimming velocities and percentage U_{crit} at pre-exercise and at U_{max} samples are reported in Table 1.

Table 3. *Measurements of blood gases and pH in swimming yellowfin tuna following anesthesia, at maximum velocity and post-exercise*

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

*Significantly different from 0 h (P<0.05).
†Significantly different from pre-exercise value (P<0.05).
U_{max}, maximal swimming velocity; see text for an explanation of abbreviations for respiratory variables.
The mean size of the tuna was 47.6±0.6 cm *FL*, 1850±70 g, N=11.
Values are means ± S.E.M. (N).

Table 4. *Differences in arterial and venous blood-gas and hematological variables and relative branchial vascular resistance in four swimming yellowfin tuna following anesthesia, at maximum velocity and post-exercise*

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

R_{branch}, relative branchial vascular resistance; U_{max}, maximal swimming velocity.
Values are means ± S.E.M. (N).

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

Both heart rate (f_H) and P_{VA} increased significantly from pre-exercise to U_{max} (Table 1). An example of the changes in blood pressure and ECG recordings in one fish is shown in Fig. 1. Although P_{VA} increased with exercise, there was no significant change in P_{DA}, indicating an increased relative R_{branch}. This finding is consistent with results for the tuna with both ventral- and dorsal-aortic catheters functional; pre-exercise R_{branch} ranged from 24.4 to 40.0 % and tended to increase with exercise (P>0.05; Table 4).

Table 2 shows that a decrease occurred in Hct and $[Hb]_a$, but not in $[Hb]_v$, during exercise. In addition, plasma [lactate] increased, while plasma glucose concentration declined.

Blood-gas and pH changes with exercise are reported in Table 3. Both Cv_{O_2} and Sv_{O_2} decreased, and Sa_{O_2} increased, although there were no significant changes in arterial or venous P_{O_2}. In addition, pHv decreased with exercise.

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

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

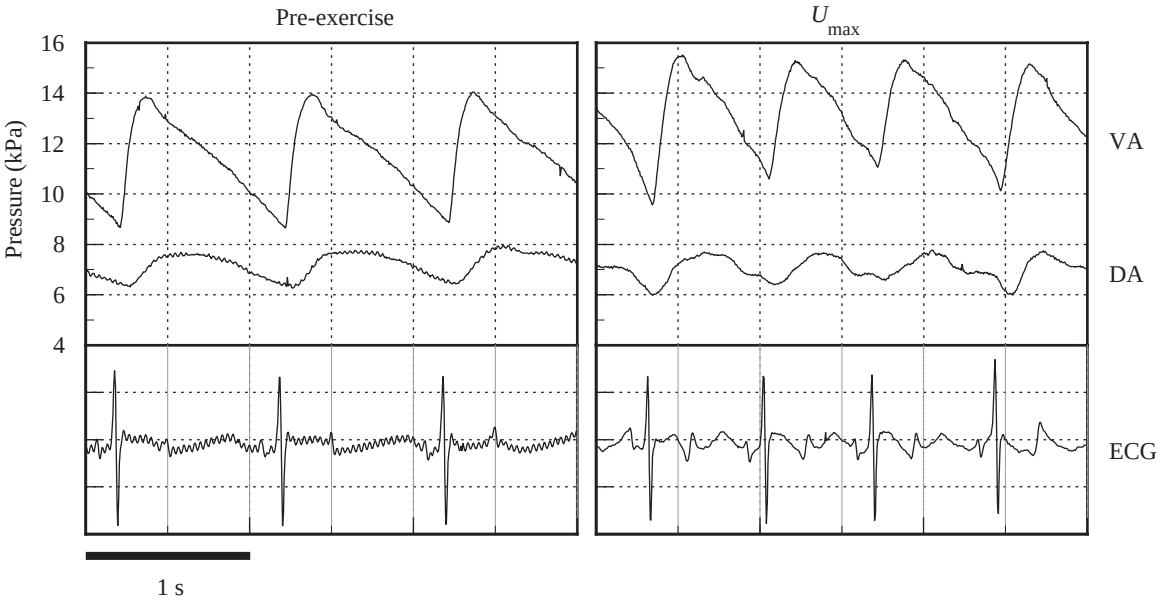

Fig. 1. Simultaneous recordings of ventral- and dorsal-aortic (VA and DA) blood pressure and the electrocardiogram (ECG) during pre-exercise and at maximal velocity (U_{max}) in a yellowfin tuna (46 cm fork length, 1.7 kg).

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

Discussion

As in most fishes, total oxygen consumption in tuna increases exponentially with swimming velocity (Dewar and Graham, 1994*a*) and oxygen demand during exercise must be met by increased oxygen delivery through the cardiorespiratory system (equation 1). In the yellowfin, this increase occurs through increases in f_H and P_{VA}, suggesting an increased blood flow (Table 1). The arterial–venous oxygen content difference [$(a–v)_{O_2}$; Table 4] also increased, through an increased arterial oxygen saturation and an increased oxygen extraction from the blood (decline in $S\text{v}_{O_2}$) (Table 3). The following sections will discuss recovery from anesthesia and the hemodynamic and blood-gas responses of both the arterial and venous blood to exercise in the yellowfin tuna.

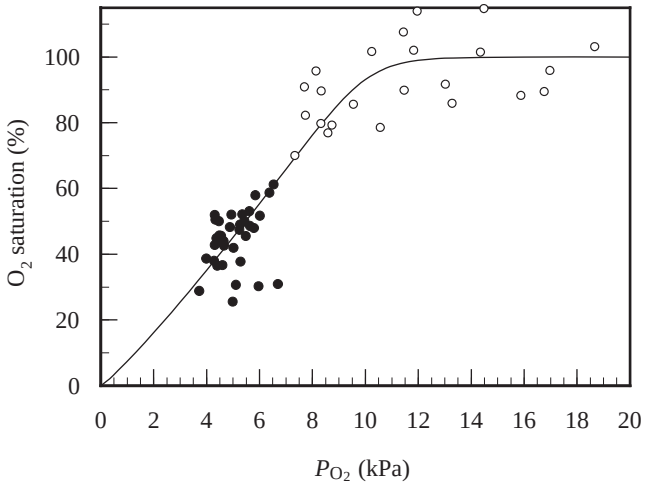

Fig. 2. Functional *in vivo* oxygen dissociation curve for arterial (open circles) and venous (filled circles) blood in yellowfin tuna for blood pH 7.74–7.84 (r^2=0.85).

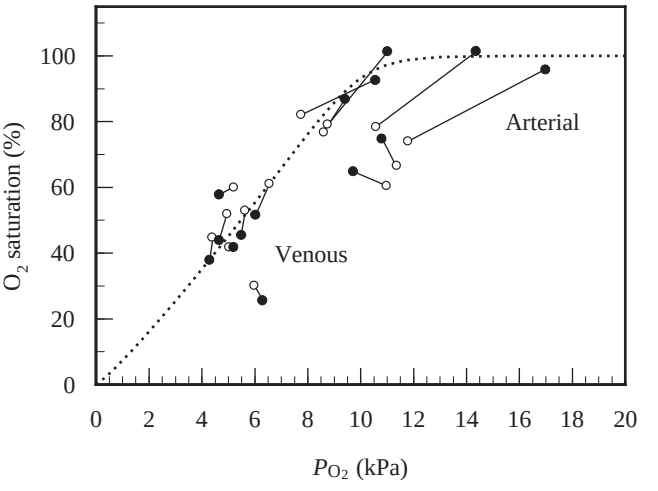

Fig. 3. Individual changes in percentage O_2 saturation and P_{O_2} from pre-exercise (open circles) to maximal velocity (filled circles) for both arterial and venous blood in yellowfin tuna. Paired values for individual fish are connected by a line. The dotted line is the oxygen dissociation curve from Fig. 2.

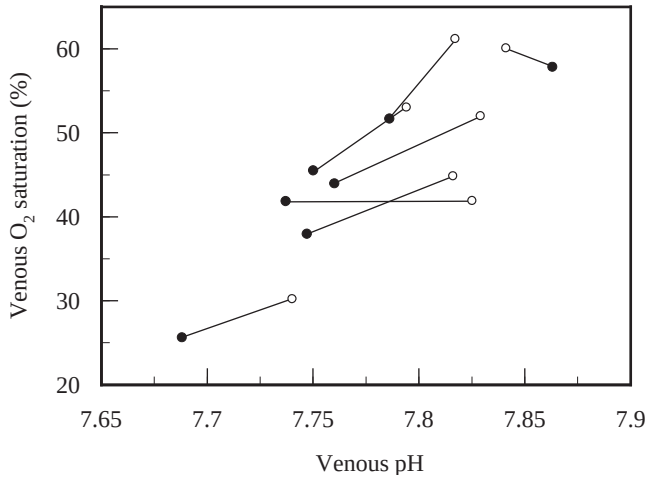

Fig. 4. Individual changes in venous oxygen saturation and venous pH from pre-exercise (open circles) to maximal velocity (closed circles) in yellowfin tuna. Paired values for individual fish are connected by a line.

Recovery from anesthesia

Because of the difficulty in handling and maintaining tunas, this and previous studies of these fishes have allowed a comparatively short recovery time following anesthesia before experimental manipulation. The anesthetic, tricaine methanesulfonate, was chosen because of its minimal disturbance and the rapid recovery (<60 min) of cardiorespiratory variables (Soivio *et al.* 1977; Fredricks *et al.* 1993). In addition, a 2 h recovery period has been shown to be sufficient for tunas to restore acid–base equilibrium, clear lactate build-up, and stabilize $\dot{V}_{O_2}$ and cardiorespiratory parameters following handling and anesthesia (Perry *et al.* 1985; Arthur *et al.* 1992; Bushnell and Brill, 1992; Dewar and Graham, 1994*a*). During the recovery period in this study, plasma lactate concentrations (Table 2) decreased to levels similar to those in free-swimming rainbow trout, *Oncorhynchus mykiss*, and healthy swimming Atlantic mackerel, *Scomber scombrus* (0.5–1.7 mmol l^{-1}; Driedzic and Kiceniuk, 1976; Boutilier *et al.* 1984). Plasma glucose levels (Table 2) also declined to within levels reported for resting rainbow trout (2–5 mmol l^{-1}; West *et al.* 1993).

Hemodynamics

The measured f_H, its variability and the response to swimming velocity for yellowfin tuna in this study are in good agreement with values reported for yellowfin that had not been anesthetized and were only instrumented with ECG electrodes (Korsmeyer *et al.* 1997). For example, fish swimming at a the pre-exercise velocity of 1.02 $FL\,s^{-1}$ had a mean f_H of 61.4 beats min^{-1}, and at 1.83 $FL\,s^{-1}$ (U_{max}) mean f_H was 84.6 beats min^{-1} (Table 1). Comparable values for unoperated yellowfin are 67.9 and 82.7 beats min^{-1}, respectively (Korsmeyer *et al.* 1997). These similarities suggest that the potential side-effects of the anesthesia and surgery, and the towing of catheter lines, on cardiovascular responses were minimal. The present study confirms previous findings of a high ventral-aortic blood pressure in tunas (Fig. 1) (reviewed in Bushnell and Jones, 1994). Although already substantial, P_{VA} increased in exercising yellowfin (Table 1). Dorsal aortic pressures in this study were higher than those reported for spinalized yellowfin (4.35 kPa, Bushnell and Brill, 1992), but similar to those of anesthetized and slowly swimming yellowfin (6.3–6.8 kPa, Jones *et al.* 1993).

The large surface area of tuna gills is thought to pose a higher resistance to blood flow than in other teleosts, potentially increasing cardiovascular costs (Brill and Bushnell, 1991*b*). Indeed, branchial resistance (R_{branch}) has been reported to account for 60–68 % of the total vascular resistance (R_{total}) in spinally blocked tuna (Bushnell and Brill, 1992) compared with 18–40 % in most fishes (Bushnell *et al.* 1992). In the present study, however, pre-exercise R_{branch} was only 25–40 % of R_{total} and is similar to the 42 % reported by Jones *et al.* (1993) for slowly swimming yellowfin.

The increased f_H and P_{VA} measured for yellowfin in this study suggest that cardiac output increased with exercise, which is consistent with ventral-aortic blood flow measurements from a separate study (Korsmeyer *et al.* 1997). However, the absence of a change in P_{DA} (Table 1) means that systemic resistance (R_{syst}) had to decrease, which is similar to findings for other fishes during exercise (Bushnell *et al.* 1992).

Effects of exercise on arterial blood-respiratory properties

Pre-exercise status

This study confirms the yellowfin tuna's high blood oxygen-carrying capacity (16–18 ml O_2 dl^{-1}); however, the findings of a low mean S_{aO_2} (74.1 %) and P_{aO_2} (9.96 kPa) in pre-exercise yellowfin (Table 3) were unexpected. Although P_{aO_2} values of approximately 13 kPa have been measured in active species, such as rainbow trout (Kiceniuk and Jones, 1977; Randall and Daxboeck, 1982), the low P_{aO_2} and S_{aO_2} of pre-exercise yellowfin suggest that branchial oxygen exchange was limited.

Even though tunas and other scombrids are dependent on ram ventilation, which depends to some degree on swimming velocity, our data enable us to eliminate the possibility that the low pre-exercise P_{aO_2} for yellowfin was due to a low swimming speed. Table 3 shows that P_{aO_2} was significantly higher at the beginning of the anesthesia recovery period (i.e. at 0 h, when $\dot{V}_{O_2}$ was probably elevated), but swimming speed was the same as at later times during recovery. In addition, a review of the literature shows that generally low P_{aO_2} values (7.76–9.91 kPa) have been measured in several tunas, including kawakawa (*Euthynnus affinis*), albacore (*T. alalunga*) and skipjack (*Katsuwonus pelamis*), as well as another scombrid, the Atlantic mackerel *Scomber scombrus* (Boutilier *et al.* 1984; Jones *et al.* 1986; White *et al.* 1988; Bushnell and Brill, 1992). These findings suggest that a reduced P_{aO_2} may be the normal condition in these fishes.

The limited branchial gas-transfer indicated for pre-exercise yellowfin seems paradoxical in the light of the correlation between tuna metabolic capacity and a large gill surface area (Hughes, 1984). Nevertheless, at respiration levels not

requiring the entire gill area, there are osmoregulatory advantages for reducing branchial perfusion which could be achieved by shunting blood through non-respiratory lamellar channels (Tuurala *et al.* 1984) or by reducing ventilatory volume.

The small, but significant, decrease in post-branchial pH (Table 4) is probably a result of the disproportionate release of Bohr protons from hemoglobin (the Haldane effect) at high oxygen saturations, as has been found for rainbow trout (Brauner, 1995). During hemoglobin oxygenation from $C\mathrm{v}_{\mathrm{O}_2}$ values close to 50 % (see below), there is a greater release of protons than there is consumption during the dehydration of HCO_3^- to CO_2, causing a net arterial acidosis (for a discussion, see Brauner and Randall, 1996). The large Bohr effect in yellowfin blood (Brill and Bushnell, 1991*a*) will increase this effect. Bushnell and Brill (1992) similarly report a small, although not significant, transbranchial decrease in blood pH (–0.01) for spinalized yellowfin and skipjack.

Exercise

With increases in swimming velocity, yellowfin $P\mathrm{a}_{\mathrm{O}_2}$ tended to increase, as did blood oxygen-saturation which, in several fish, rose to greater than 95 % (Fig. 3). The higher P_{VA} and blood flow of exercising yellowfin would lead to recruitment of additional lamellar surface area and a decreased diffusion distance, aiding gas transfer (Farrell *et al.* 1980; Tuurala *et al.* 1984). Although $S\mathrm{a}_{\mathrm{O}_2}$ increased in all the yellowfin in this study, $C\mathrm{a}_{\mathrm{O}_2}$ did not change significantly (Table 3). This is probably a result of sequential blood sampling and the loss of hemoglobin-containing erythrocytes during the experiment (Table 2). In the absence of blood loss, the increases in $P\mathrm{a}_{\mathrm{O}_2}$ and $S\mathrm{a}_{\mathrm{O}_2}$ suggest that $C\mathrm{a}_{\mathrm{O}_2}$ would have increased with exercise.

The finding that exercising yellowfin can increase both $P\mathrm{a}_{\mathrm{O}_2}$ and $S\mathrm{a}_{\mathrm{O}_2}$ is similar to results obtained for the lemon shark *Negaprion brevirostris* during swimming (Bushnell *et al.* 1982). In contrast, no other species of fish, including the mackerel, has been shown to elevate both $P\mathrm{a}_{\mathrm{O}_2}$ and $S\mathrm{a}_{\mathrm{O}_2}$ during prolonged swimming (Kiceniuk and Jones, 1977; Piiper *et al.* 1977; Boutilier *et al.* 1984; Lai *et al.* 1990; Thorarensen *et al.* 1993).

Effects of exercise on venous blood-respiratory properties

Pre-exercise status

Pre-exercise $P\mathrm{v}_{\mathrm{O}_2}$ in swimming yellowfin (approximately 5.3 kPa) was slightly higher than in spinalized yellowfin and skipjack (4.31 and 4.89 kPa, respectively; Bushnell and Brill, 1992), reflecting the relatively high P_{50} (approximately 5.3 kPa), or low oxygen-affinity, of the blood (Fig. 2). Although this *in vivo* P_{50} is higher than that determined for yellowfin blood *in vitro* (2.73–4.30 kPa, Brill and Bushnell, 1991*a*), a high P_{50} has been predicted for active, pelagic fish that live in well-oxygenated waters (Riggs, 1970; Powers, 1980). In addition, the *in vitro* ODC determined for mackerel blood (Boutilier *et al.* 1984) suggests a similar P_{50} at the P_{CO_2} levels measured in this study for tuna. A high P_{50} will aid unloading of oxygen in the tissues, by maintaining a high P_{O_2} gradient from the capillaries to the mitochondria. Because tuna elevate red (aerobic) muscle temperature (Dewar *et al.* 1994), the P_{O_2} gradient in this muscle will be affected by the rapid increase in temperature as the blood moves through the heat exchangers (retia mirabilia). Oxygen affinity in yellowfin blood decreases with increases in temperature in a closed system (i.e. the gas content is constant), which is the condition in the retial vessels (Brill and Bushnell, 1991*a*). This means that the P_{50} of the blood in the warmed red muscle will be even higher.

Although pre-exercise $C\mathrm{v}_{\mathrm{O}_2}$ (9.5 ml O_2 dl^{-1}) was similar to that in spinalized tuna (9.0–10.0 ml O_2 dl^{-1}, Bushnell and Brill, 1992), this value is remarkably high compared with values for other fishes, especially considering the swimming velocity of 1.0 *FL* s^{-1}. This is a result of the yellowfin's high blood oxygen-carrying capacity as this $C\mathrm{v}_{\mathrm{O}_2}$ corresponds to an $S\mathrm{v}_{\mathrm{O}_2}$ of only 48 %. In contrast, $C\mathrm{v}_{\mathrm{O}_2}$ in resting rainbow trout was only 7.1 ml O_2 dl^{-1} (Kiceniuk and Jones, 1977), but at this $C\mathrm{v}_{\mathrm{O}_2}$ trout blood is 70 % saturated (Jones and Randall, 1978).

Exercise

With exercise, both $S\mathrm{v}_{\mathrm{O}_2}$ and $C\mathrm{v}_{\mathrm{O}_2}$ decreased, but there was little or no change in $P\mathrm{v}_{\mathrm{O}_2}$ (Fig. 3; Table 3). With the exception of the lemon shark (Bushnell *et al.* 1982), a decrease in $S\mathrm{v}_{\mathrm{O}_2}$ is the usual response to increased swimming velocity in fishes (Kiceniuk and Jones, 1977; Piiper *et al.* 1977; Butler *et al.* 1989; Lai *et al.* 1990). This decrease is normally accompanied by declines in both pHv and $P\mathrm{v}_{\mathrm{O}_2}$. However, in the yellowfin, $P\mathrm{v}_{\mathrm{O}_2}$ was essentially unchanged throughout the experiment (Table 3). This may be a consequence of $P\mathrm{v}_{\mathrm{O}_2}$ occurring close to the P_{50} for oxygen saturation (Figs 2, 3) and therefore lying on the steep part of the ODC. As a result, large changes in $S\mathrm{v}_{\mathrm{O}_2}$ can occur with only small changes in $P\mathrm{v}_{\mathrm{O}_2}$.

In addition, the decline in $S\mathrm{v}_{\mathrm{O}_2}$ can be accounted for by the decreases in oxygen affinity associated with a reduction in pH (i.e. a Bohr shift, or rightward shift of the ODC, Fig. 4). Even though the decline in pHv was relatively small (–0.04 pH units), it was sufficient to cause the observed decline in $S\mathrm{v}_{\mathrm{O}_2}$ because of the large Bohr shift in yellowfin ($-0.865\Delta\log P_{50}/\Delta\mathrm{pH}$, Brill and Bushnell, 1991*a*). This large Bohr shift also accounts for the correlation between the individual variability in pHv and $S\mathrm{v}_{\mathrm{O}_2}$ (Fig. 4). The observed acidosis appears to have both respiratory and metabolic components, as indicated by the rise in plasma [lactate] and P_{CO_2} (Tables 2, 3), although the reduction in venous pH was much less than that recorded for skipjack tuna (–0.45 pH units) following exhaustive exercise (Perry *et al.* 1985).

Swimming performance

The calculated U_{crit} values in this study (1.3–2.2 *FL* s^{-1}) are low compared with the predicted maximal sustainable speed of yellowfin (3–6 *FL* s^{-1}) based on models of oxygen uptake and delivery (Bushnell and Brill, 1991; Brill, 1996; Korsmeyer *et al.* 1996*b*). In the present study, the protocol for determining fatigue minimized stress and damage to the fish and therefore

may have underestimated U_{crit}. Although incremental velocity tests with most other fishes may proceed to the point of exhaustion and inability to swim (Beamish, 1978), the continuous swimming requirements of tuna make this impractical.

In addition, U_{max} determined in this study was probably affected by the recent anesthesia and surgery, as well as by the towed catheters and electrode wires. Maximal swimming velocities (up to 2.4 $FL\,s^{-1}$) were lower than those reported for yellowfin (up to 3.0 $FL\,s^{-1}$) that were uninstrumented or towing only a pair of ECG wires (Dewar and Graham, 1994*a*; Korsmeyer *et al.* 1997). The maximal velocities of other fishes have been shown to decrease following surgery and instrumentation by 13–75 %, although maximal oxygen consumption was unaffected (Kiceniuk and Jones, 1977; Butler *et al.* 1989; Thorarensen *et al.* 1993).

Despite lower than expected velocities, the yellowfin in this study appear to have been working near their maximal aerobic limits at their respective U_{max}. Shortly before fatigue, the fish began an unsteady swimming pattern, characterized by intermittent bursts forward in order to maintain position in the swim tunnel. This unsteady swimming behavior is associated with white muscle activity (based on EMG recordings; T. Knower and R. E. Shadwick, unpublished observations) and is probably powered anaerobically. The increased lactate levels at U_{max} and post-exercise (Table 2) also suggest the beginnings of recruitment of fast-fatiguable white muscle. However, post-exercise lactate values observed for yellowfin in this study were considerably lower than those following exhaustive exercise in skipjack (30 $mmol\,l^{-1}$, Perry *et al.* 1985), suggesting that swimming had remained primarily aerobic.

Oxygen transport during exercise

Fig. 5 summarizes the contributions of each of the parameters of the Fick equation (equation 1) to increased oxygen uptake during swimming in the yellowfin tuna. For comparison, values from non-swimming, spinalized yellowfin (Bushnell and Brill, 1992) are shown, along with values from rainbow trout *Oncorhynchus mykiss* (Kiceniuk and Jones, 1977) at similar relative levels of exercise. Although not measured in this study, $\dot{V}_{O_2}$ was estimated from data reported by Dewar and Graham (1994*a*) for uninstrumented, swimming yellowfin tuna. Using the Fick equation, cardiac output is calculated to have increased from 88.5 $ml\,kg^{-1}\,min^{-1}$ at slow swimming velocities (pre-exercise) to 116.6 $ml\,kg^{-1}\,min^{-1}$ at U_{max}; an increase of 1.32 times. This is similar to the observed 1.29-fold increase in $(a–v)_{O_2}$ (Table 4; Fig. 5), indicating that these two variables account nearly equally for the increase in $\dot{V}_{O_2}$. Stroke volume is calculated to have decreased by 4 %, from 1.44 to 1.38 $ml\,kg^{-1}$ (Fig. 5), which agrees with the average decrease determined from relative blood-flow measurements (Korsmeyer *et al.* 1997).

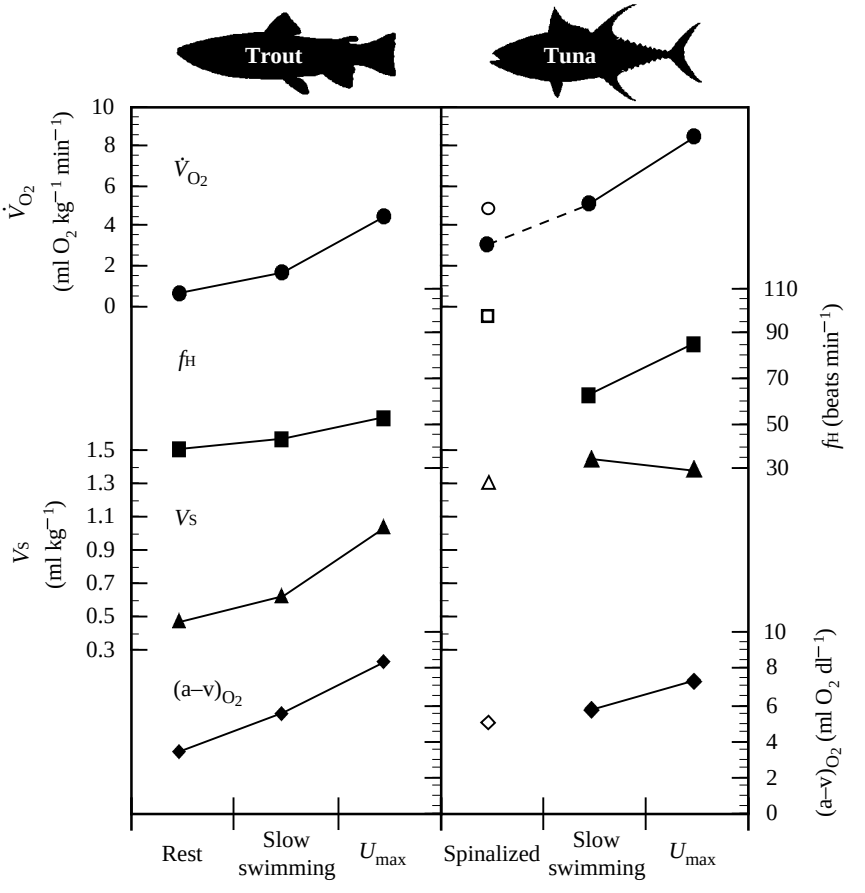

Fig. 5. Oxygen transport variables in the rainbow trout *Oncorhynchus mykiss* and yellowfin tuna *Thunnus albacares* during prolonged swimming. Tuna (25 °C) heart rate (f_H) and $(a–v)_{O_2}$ values during slow swimming (pre-exercise, 1.0 $FL\,s^{-1}$, 61 % U_{crit}) and at U_{max} (1.83 $FL\,s^{-1}$, 108 % U_{crit}) are taken from Tables 1 and 4. The rates of oxygen consumption ($\dot{V}_{O_2}$) during slow swimming and extrapolated to zero velocity are taken from the O_2 consumption–velocity regression reported in Dewar and Graham (1994*a*). At U_{max}, the rate of O_2 consumption is the mean value from 10 yellowfin at maximal velocities (mean velocity 1.84 $FL\,s^{-1}$) during energetics experiments (Dewar and Graham, 1994*a*; H. Dewar, personal communication). Stroke volume (V_S) was calculated from the Fick equation (see text). Spinalized yellowfin data (open symbols) are taken from Bushnell and Brill (1992). Trout data (10 °C) are taken from Kiceniuk and Jones (1977) (slow swimming at 41–63 % U_{crit}).

Although the possible errors associated with the Fick calculation in fishes may cancel out (Metcalfe and Butler, 1982; Thorarensen *et al.* 1996), the potentially high gill metabolic rate of tuna (Brill, 1996) suggests that Fick estimates will result in an overestimation of cardiac output. If, as data from spinalized yellowfin suggest, gill metabolism accounts for up to 50 % of total $\dot{V}_{O_2}$ (Bushnell and Brill, 1992), the values of cardiac output and stroke volume calculated above may be as much as two times too high.

The most complete analysis of the effects of exercise on oxygen delivery in a teleost is for the rainbow trout *Oncorhynchus mykiss* (Kiceniuk and Jones, 1977), which can be compared with data for the yellowfin tuna (Fig. 5). It should be noted, however, that tunas are continuous swimmers and therefore there is no non-swimming, or resting, level as in the trout. From Fig. 5, it can be seen that, although within the range of our measurements, the data from spinalized yellowfin do not appear to represent the equivalent of a resting state. In addition, differences in measurement temperature between the trout (10 °C) and tuna (25 °C) data should be taken into account. Although this difference does not explain all of the disparity in $\dot{V}_{O_2}$ values (Dewar and Graham, 1994*a*), it can account for differences in f_H (Korsmeyer *et al.* 1997). A clear distinction between the two species is that stroke volume does not increase with sustained exercise in the yellowfin (for a discussion, see Korsmeyer *et al.* 1997).

Perhaps the most surprising finding of this comparison, however, is that, despite a blood oxygen-carrying capacity that is nearly twice as great, $(a–v)_{O_2}$ values in the yellowfin are similar to those in the trout (Fig. 5). Although the yellowfin appear to have reached their maximal aerobic swimming capacity, $(a–v)_{O_2}$ at U_{max} is achieved with an extraordinary venous oxygen reserve of greater than 40 % saturation (Fig. 3; Table 3), compared with only 10 % in the trout (Kiceniuk and Jones, 1977). Consequently, the quantity of oxygen extracted at U_{max} [$(a–v)_{O_2}$=7.2 ml O_2 dl^{-1}] is much less than values predicted (15–20 ml O_2 dl^{-1}) to achieve the estimated maximal $\dot{V}_{O_2}$ for yellowfin (Brill and Bushnell, 1991*b*). One possible explanation for this finding is that the fish had not, in fact, reached their maximal aerobic swimming limits. Although tunas are difficult to study in a water tunnel and many variables could have affected their swimming performance, observations [intermittent burst swimming and increased plasma lactate levels (see above)] suggest that these fish had approached their maximal capacity for aerobic swimming. Another explanation is that our measurement of the average (mixed) venous oxygen content in the ventral aorta did not reflect the extent of oxygen extraction occurring in the red (aerobic) muscle. The untapped venous reserve of other parts of the body during aerobic swimming (i.e. visceral organs and white muscle) may have masked greater oxygen extraction by the red muscle during exercise, with the change in mixed venous oxygen content reflecting only the partial contribution of red muscle blood flow to total $(a–v)_{O_2}$. The high blood oxygen-carrying capacity may serve other aerobic metabolic processes, for example the repayment of an oxygen debt following the intense anaerobic activity of burst swimming (Brill, 1996; Korsmeyer *et al.* 1996*a*), in addition to the continuous requirement of sustained swimming.

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