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VENTRICULAR AND ARTERIAL DYNAMICS OF ANAESTHETISED AND SWIMMING TUNA

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Summary

Cardiovascular dynamics of tuna have been investigated by recording blood pressures and flows in the central circulation of both anaesthetised and swimming individuals. In anaesthetised fish (*N*=5), heart rate averaged 112±21 beats min^{-1} (mean ± S.E.) and stroke volume was 0.67±0.24 ml kg^{-1} when normoxic water flowed over the gills. Ventricular diastolic pressure was zero until atrial contraction filled the ventricle. Ventral aortic pressures were high (mean 12.08±1.15kPa), and blood flow was continuous in the ventral aorta throughout diastole. Dorsal aortic pressure (mean 6.3±1.28kPa; *N*=4) and flow were both pulsatile. Pressure pulsatility (pulse pressure as a proportion of mean pressure) was about one-quarter of flow pulsatility, indicating considerable compliance in the dorsal aortic circulation. Total peripheral resistance averaged 0.17±0.4 kPa ml^{-1} kg^{-1} min^{-1} of which gill resistance averaged 48±15% (*N*=4). For the ventral aorta, impedance modulus fell markedly from the mean value and then declined more gradually towards zero with increasing harmonic frequencies. Impedance phase was negative (−0.8 to −1.1rad) meaning that flow leads pressure at all harmonics. In swimming yellowfin tuna (*N*=5), heart rate averaged 108.8±12.1 beats min^{-1} and mean ventral and dorsal aortic pressures were 11.6±0.5 and 6.8±1.2kPa, respectively, so gill resistance was 42% of total peripheral resistance. Average stroke volume in three swimming kawakawa was 0.54±0.2ml kg^{-1} at a mean heart rate of 128±48 beats min^{-1}. Data from swimming fish were within the range obtained from anaesthetised tuna. A simple model of the fish circulation consisting of two sets of compliant and resistive elements coupled in series (a second-order *RC* network) gave reasonable predictions of arterial pressure–flow relationships. Hence, we conclude that a ‘Windkessel’ dominates central cardiovascular dynamics of tuna despite heart rates and blood pressures that fall in the mammalian range.

Introduction

A Windkessel model describes the arterial system in terms of a simple elastic reservoir

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(the lumped compliance of the major arteries) which feeds the terminal resistance vessels (Burkhoff *et al.* 1988). The electrical analogue of the Windkessel is an *RC* network (where *R* is resistance and *C* is capacitance). The cardiovascular system of fish is usually described as two '*RC* networks' in series (a second-order *RC* network), supplied by a single signal generator (the heart) (Satchell, 1971). The lumped, but separate, compliances of the ventral and dorsal aortae feed resistance vessels of the gills and systemic vasculature, respectively. The ventral aorta is short, and gill resistance is usually about one-quarter of total peripheral resistance (Holeton and Randall, 1967; Satchell, 1971; Kiceniuk and Jones, 1977; Lai *et al.* 1987). However, compliance of the ventricular outflow tract is enhanced by a highly elastic segment, the bulbus arteriosus, contained within the pericardium (Satchell, 1971; Licht and Harris, 1973; Priede, 1976; Farrell, 1979; Bushnell *et al.* 1992).

Pressure and flow relationships are usually analysed using a hydraulic equivalent (pressure=flow×resistance) of Ohm's law for direct current (d.c.) electrical circuits. However, both pressure and flow are pulsatile, and haemodynamic analysis is better served by considering not only the mean values of pressure and flow but also the sine-wave harmonics of the pulsatile waveforms. The harmonics are obtained from a Fourier analysis of the original pressure and flow waveforms (McDonald, 1974; Jones, 1991). The quotient obtained by dividing oscillatory pressure by oscillatory flow is termed impedance modulus, and the phase angle between any pair of harmonics of pressure and flow of the same frequency is termed impedance phase. For a Windkessel, impedance modulus falls markedly from the d.c. value (peripheral resistance) at the first harmonic and then decreases successively towards zero with increasing harmonics. Peak flow occurs before peak pressure so the phase angle between pressure and flow is negative at all harmonics. In birds and mammals, however, oscillations in both modulus and phase occur with increasing harmonic frequencies (Langille and Jones, 1975; Milnor, 1979). The oscillations are caused by interactions between incident waves, generated by cardiac contraction, and reflected waves from the peripheral vasculature. These wave transmission effects are obviously not observed in a Windkessel formulation because one of the fundamental tenets of the Windkessel theory is that changes generated by cardiac contraction occur simultaneously throughout the arterial system (McDonald, 1974; Jones, 1991).

Heart rates in most fish are low, so wave transmission time from the bulbus to the gills (the first Windkessel) will be a negligible fraction of the cardiac cycle. Hence, wave transmission effects in this part of the circulation should be negligible. However, heart rates of tuna in normoxic water and at high temperatures (13–25°C) may reach $260\,\mathrm{beats\,min^{-1}}$ (Kanwisher *et al.* 1974; Brill, 1987), which are far higher than those achieved by other active teleosts such as salmon and trout (Smith *et al.* 1967; Kiceniuk and Jones, 1977). It is possible that, at high repetition rates, wave transmission phenomena, such as the oscillating pattern of arterial impedance seen in birds and mammals, could influence arterial haemodynamics in fish (O'Rourke, 1967; O'Rourke and Taylor, 1967; McDonald, 1974; Milnor, 1979).

Consequently, in order to investigate arterial haemodynamics we have made pressure recordings in the heart, and pressure and flow recordings in the ventral and dorsal aortae,

of anaesthetised tuna to examine pressure–flow relationships. We also wanted to know how well our data, obtained from anaesthetised fish on an operating table, would relate to fish swimming in the holding tanks (Jones *et al.* 1986). Our equipment did not allow high-fidelity recordings of pressure and flow from swimming fish. Nevertheless, we felt that these data would be useful, even if only to put our haemodynamic study in a more realistic context. Finally, a Windkessel model consisting of two sets of compliant and resistive elements coupled in series (Satchell, 1971; Jones *et al.* 1974; Langille *et al.* 1983) has been used to predict pressure and flow relationships in the ventral aorta, which were compared with measured values.

Materials and methods

The experiments were performed on six kawakawa (*Euthynnus affinis* Cantor) and twelve yellowfin tuna (*Thunnus albacares* Bonnaterre). The fish were held in large outdoor tanks at the National Marine Fisheries Service Kewalo Research Facility in Honolulu, HI, USA. Water temperature in the holding and working tanks was 25˚C. Fish were trapped and anaesthetised in the holding tanks as described previously (Jones *et al.* 1986) and then quickly moved to an operating table, where they were suspended in a chamois-leather-lined cradle. Anaesthesia was maintained by pumping aerated sea water containing 1:1200 w/v MS-222 over the gills. After instrumentation, the fish was removed from the operating cradle and held upright in a large tank under light anaesthesia, maintained by periodic injections of Saffan ($3 mg kg^{-1}$ intra-arterially; Glaxovet, Montreal, PQ, Canada). At this level of anaesthesia, the fish made slow swimming movements. Gill irrigation was achieved by pumping water into the mouth through a soft rubber hose.

Studies on cardiovascular dynamics

Restrained fish

An anaesthetised fish was suspended in the chamois-leather-lined cradle ventral side up. An incision was made in the midline, in front of the bulbus arteriosus. Muscles and blood vessels were parted by blunt dissection and one of two types of flow transducer was placed around the ventral aorta. The transducer was connected to a Biotronix BL 610 pulsed logic electromagnetic flowmeter (Biotronix Inc, Silver Spring, MD) or a Crystal Biotech pulsed Doppler flowmeter (Crystal Biotech, Holliston, MA, USA) as appropriate. Large venous sinuses surrounding the dorsal aorta prevented a direct approach to that vessel. Instead, flow was monitored through the roof of the buccal cavity using a specially modified surface probe (paediatric probe) connected to a portable, battery-operated, Parks Medical Systems continuous Doppler flowmeter (Portland, OR, USA). The probe was sutured to the roof of the mouth, over the dorsal vessel, just in front of the oesophageal sphincter. Sutures were passed through holes bored in each corner of the probe. To restrict any movement, the probe was also glued to the roof of the mouth with Histoacryl tissue cement (B. Braun, Germany). At the conclusion of measurements the animal was killed by anaesthetic overdose (Pentothal, intra-arterially; Abbott Labs, IL, USA) and, after exposure and cannulation of the ventral and dorsal aortae, the flow

probes were calibrated *in situ* using tuna blood pumped at three or more different flow rates through the vessels by a Harvard infusion pump (Harvard Apparatus Company Inc, Millis, MA, USA). A regression line constrained to go through zero was fitted to the data on a plot of flow ($ml min^{-1}$) *versus* flowmeter output. The flow recording was deemed acceptable if the three calibration values were within ±5% of the regression line. This stricture led to the rejection of two dorsal aortic flow recordings made in anaesthetised fish and three cardiac output recordings made in swimming fish. However, in data used for harmonic analysis, the pulsed Doppler flow probe was calibrated by injecting a known volume of blood into the ventral aorta during diastole when the heart rate was slowed by passing deoxygenated water over the gills. Volumes injected varied from 0.5 to 2ml. The flowmeter signal was integrated over time to give volume flow. Up to 10 injections were made in an animal and regression lines were fitted to the data on a plot of volume flow *versus* flowmeter output.

Angiocaths (nos 18, 20 or 22; 38 or 50mm long) were inserted into the ventricle, bulbus arteriosus, ventral aorta and dorsal aorta under manometric guide, as described previously (Jones *et al.* 1986). Each angiocath was connected to a Bio-Tek BT 70 pressure transducer (Bio-Tec, Pasadena, CA, USA) using polyethylene tubing of various lengths and bores filled with heparinized saline ($50 i.u. ml^{-1}$). The frequency response of the manometric system was between 35 and 60Hz, with damping between 0.12 and 0.4 of critical damping when subjected to pop-tests before and after recordings (Jones, 1970). Manometers with high-frequency responses (60Hz) and low damping (0.12–0.2 of critical) were used to obtain pressure data for Fourier analysis. The polyethylene tubing was sewn into position close to the incision and near the dorsal fin.

The fish was turned upright, removed from the cradle and suspended in a large tank. Pressures and flows were recorded at resting heart rates, at reduced heart rates, caused either by stopping water flow or by passing deoxygenated water over the gills, and after stimulation of cardiac function by injection of $0.3 ml kg^{-1}$ of phenylephrine (intra-arterially).

Swimming fish

The dorsal and ventral aortae of five yellowfin tuna were cannulated as described previously (Jones *et al.* 1986) and, after recovery from anaesthesia, fish were allowed to swim laps in a doughnut-shaped tank (17.1m internal and 17.9m external diameter). Dorsal and ventral aortic pressures were measured using pressure manometers connected to hand-held strain gauge bridge amplifiers. These amplifiers were connected by long cables to a two-channel pen recorder. One of us, holding the manometers and amplifiers, ran around the tank keeping pace with the fish while the others endeavoured to keep the electrical connections to the chart recorder untwisted and out of the water.

Ventral aortic flow was recorded in six kawakawa using the modified surface probe (described above) connected to the Parks Medical Systems continuous Doppler flowmeter. The anaesthetised fish was placed in the operating cradle and a flexible plastic spatula was used to reflect the gills and expose the ventral aorta. The surface probe was placed on the skin over the aorta, sutured at each corner, and fixed in position with tissue cement. The

fish was placed in the doughnut-shaped tank and artificially ventilated until recovery was complete. Zero flow was defined as diastolic flowmeter output during extreme bradycardia induced by stopping artificial ventilation. One of us, carrying the flowmeter, accompanied the fish while it swam around the tank. At the end of the recording period the flowmeter was calibrated by passing blood at three or more known rates of flow through the ventral aorta using the Harvard pump attached to a cannula tied into the bulbus arteriosus. The flowmeter was calibrated successfully in three of the six tuna.

Data collection and analysis

All pressures and flows were recorded on Gould two- or six-channel ink recorders (Gould Instruments, Cleveland, OH, USA) writing on rectilinear coordinates. Vascular impedance at the input to the ventral aorta was determined in three yellowfin tuna. Approximately 40 pairs of ventral aortic pressures and flows were digitized into 256 or 512 points, using a data collection system (Dianechart, Rockaway, NJ, USA), and the data were stored directly on disk. Fourier analysis of the pressure and flow signals was carried out using a computer running SYSTAT software (Systat, Inc; Evanston, IL, USA). The quotient of pressure divided by flow (impedance modulus) and the phase difference between pressure and flow were obtained for the first five harmonics. The frequency response of the flowmeter was 66kHz. The frequency response of the manometers was 4–5 times the highest harmonic analysed. We made no corrections for amplitude or phase distortions introduced by the manometer. Peak, mean and minimum values for pressure and flow, as well as heart rate and temporal events of the cardiac cycle (i.e. time of atrial contraction, diastolic period, etc.) were obtained from recorder charts digitized using SIGMASCAN (Jandel Scientific, Corte Madera, CA, USA). Maximum rate of pressure change (dP/dt) during ventricular systole was obtained manually by drawing a tangent to the rising phase of the pressure curve on the recorder chart. Pressure and flow traces were superimposed, with respect to time, using SIGMAPLOT.

Stroke volume (Q_S) was obtained by dividing heart rate (f_H) into cardiac output ($\dot{Q}b$). Total peripheral resistance (R_{TOT}) was obtained by dividing $\dot{Q}b$ into mean ventral aortic pressure (P_{VA}). Systemic resistance R_S was obtained by dividing $\dot{Q}b$ (or in one case dorsal aortic flow, $\dot{Q}_{DA}$, into mean dorsal aortic pressure (P_{DA}). Gill resistance (R_G) was obtained by subtracting R_S from R_{TOT}. In the text, mean values are given accompanied by the standard error of the mean.

Results

Cardiovascular dynamics

Restrained fish

In normoxic yellowfin tuna, f_H varied from 86 to 139beatsmin^{-1} (mean 112±21beatsmin^{-1}) (Table 1). During ventilation with deoxygentated water, f_H could fall as low as 10beatsmin^{-1}, although measurements were not made at heart rates of less than 60beatsmin^{-1} because cardiovascular function did not appear to be stable at very low f_H. Changes in f_H induced by hypoxia were not accompanied by large variations in

Table 1. *Cardiovascular variables recorded from anaesthetised yellowfin tuna*

	Fish 1	Fish 2		Fish 3	Fish 4	Fish 5	
	Normoxia	Hypoxia	Normoxia	Normoxia	Normoxia	Hypoxia	Normoxia
Mass (kg)	0.93	1.68	1.68	1.32	1.01	2.08	2.08
Heart rate (beatsmin^{-1})	139	71	107	101	86	60	127
Cardiac interval (ms)	431	844	560	594	694	1009	472
Ventricle							
Systolic pressure (kPa)	12.8	15.2	18.5	–	18.9	–	–
Systolic interval (ms)	242	266	270	–	273	–	–
Diastolic pressure after atrial contraction (kPa)	0.24	0.15	0.6	–	0.76	–	–
dP/dt (kPa s^{-1})	344	464	716	405	–	–	–
Cardiac output (mlmin^{-1} kg^{-1})	79.6	47.5	95.0	49.1	81.8	44.0	53.8
Stroke volume (mlkg^{-1})	0.57	0.67	0.89	0.49	0.95	0.75	0.43
Ventral aorta							
Systolic pressure (kPa)	12.4	14.8	19.4	14.8	17.9	10.8	10.5
Diastolic pressure (kPa)	9.4	7.9	13.1	10.0	11.8	5.4	7.6
Mean pressure (kPa)	10.4	9.8	15.5	11.6	13.8	7.7	9.1
% Pressure pulsatility (pulse/mean)	29	70	41	42	44	70	32
Peak flow (mlmin^{-1})	142	226	232	189	156	377	280
Minimum flow (mlmin^{-1})	50.0	13.9	151.0	18.5	47.2	5.9	28.5
% Flow pulsatility (pulse/mean)	125	265	50	263	132	405	224
Dorsal aorta							
Systolic pressure (kPa)	–	8.9	11.3	6.1	–	4.2	4.3
Diastolic pressure (kPa)	–	6.6	9.3	5.0	–	2.9	3.8
Mean pressure (kPa)	–	7.4	10.0	5.4	5.7	3.3	4.1
% Pressure pulsatility	–	31	20	20	–	40	12
Peak flow (mlmin^{-1})	–	–	–	–	–	81.6	92.5
Minimum flow (mlmin^{-1})	–	–	–	–	–	10.2	44.2
Mean flow (mlmin^{-1} kg^{-1})	–	–	–	–	–	23.6	36.7
% Flow pulsatility	–	–	–	–	–	146	63
Resistance							
Total peripheral resistance (kPaml^{-1} kg^{-1} min^{-1})	0.13	0.21	0.16	0.24	0.17	0.17	0.17
Gill resistance (kPaml^{-1} kg^{-1} min^{-1})	–	0.05	0.04	0.13	0.10	0.08	0.09
Systemic resistance (kPaml^{-1} kg^{-1} min^{-1})	–	0.16	0.11	0.11	0.07	0.07 (0.14)*	0.08 (0.11)*

During normoxia, water P_{O_2} was 11–11.5kPa and during hypoxia it was 3.75–5.25kPa.
Ventilation volume was 6l min^{-1}.
*Figures for systemic resistance in parentheses were calculated using dorsal aortic flow measured behind the gills, and therefore they do not include the vascular resistance of the head.

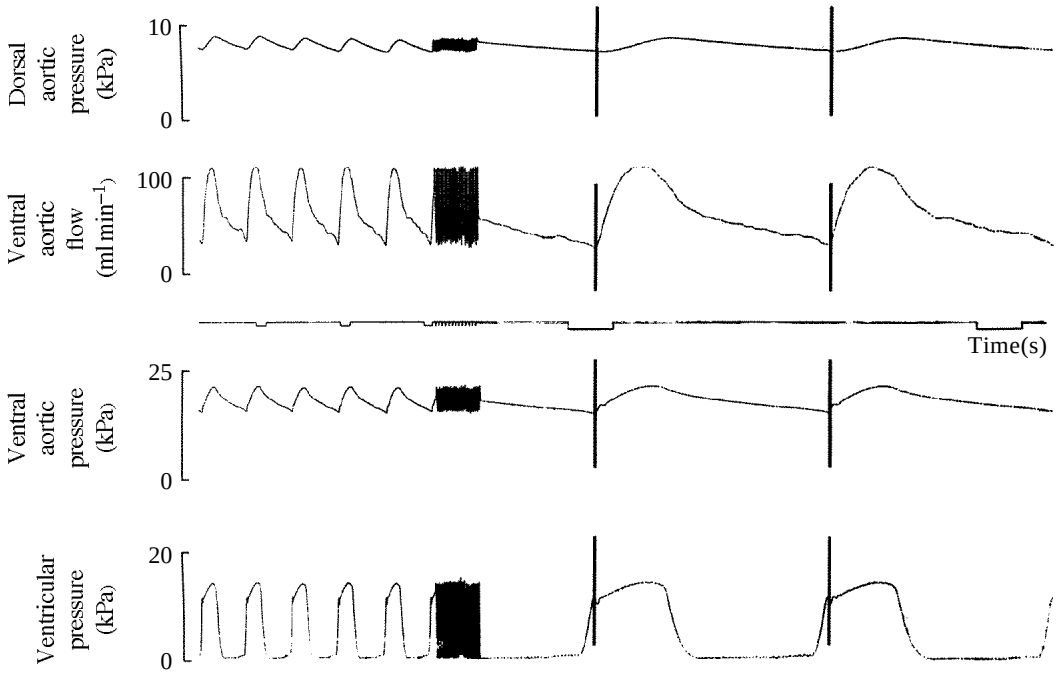

Fig. 1. Pressures and flow in the central circulation of a yellowfin tuna. Traces from top to bottom: dorsal aortic pressure, ventral aortic flow, time, ventral aortic pressure and ventricular pressure. The vertical lines link coincident points in time on pressure and flow traces. Note the changes in time base during the record.

either P_{VA} or P_{DA}. Q_S averaged 0.67±0.24mlkg^{-1} in normoxic tuna and did not change consistently when heart rate changed. Q_S was reduced at low f_H in one fish but increased, compared with normoxic values, in another (Table 1).

Ventricular diastolic pressures were close to zero. Atrial contraction, which occurred some 70–90ms before ventricular systole, increased ventricular pressure by between 0.15 and 0.76kPa. During systole, ventricular pressure generation (dP/dt) attained remarkable rates of 344–716kPa s^{-1} (mean 482±163kPa s^{-1}, N=4; Table 1). The rapid phase of pressure generation ceased when the bulbo-ventricular valves opened and both pressure and flow started in the ventral aorta (Fig. 1). P_{VA} was high (mean 12.08±1.15kPa) and followed ventricular pressure during the ejection phase, but diverged soon after peak systole (see Fig. 3).

The length of the ejection phase averaged 263±14ms (N=4) and bore a positive relationship to Q_S. This relationship was most clearly seen after arterial phenylephrine injection: the ejection phase increased by 50% and Q_S increased by 30%. Peak ventral aortic flow occurred before peak pressure (Figs 1 and 2). The shape of the ventral aortic flow pulse was variable (Figs 1, 2, 3). Flow declined slowly in the ventral aorta after systole, although even at heart rates of 60beatsmin^{-1}, zero flow was sometimes not reached by the end of diastole (Fig. 2). The flow pulsatility (pulse flow as a proportion of mean flow) in the ventral aorta was 4.3±1.0 times the pressure pulsatility (pulse pressure

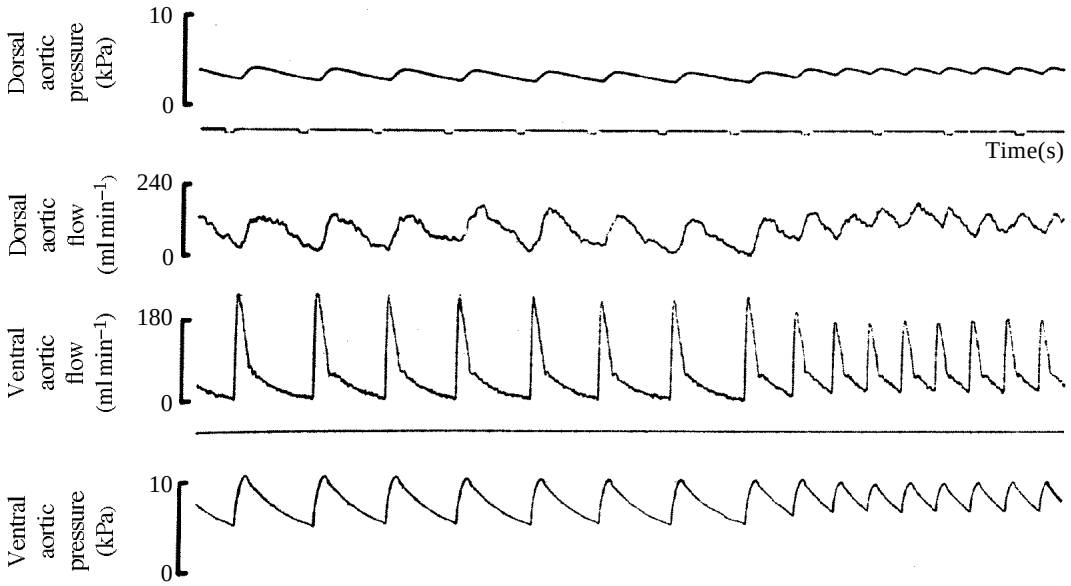

Fig. 2. Pressures and flows in the ventral and dorsal aortae of a yellowfin tuna. The low heart rate during the first 7s was caused by passing hypoxic water over the gills.

as a proportion of mean pressure) during normoxia (Table 1). Mean P_{DA} (6.3±1.28kPa) was about half mean P_{VA}.

$\dot{Q}_{DA}$ was pulsatile, but at normoxic values of f_H flow was maintained throughout diastole (Fig. 2). Pulsatile dorsal aortic flow was monitored in three animals. Flow characteristics were similar in all three but a successful calibration was obtained only in one. Dorsal aortic peak pressure and flow seemed to be more nearly in phase compared with the situation in the ventral aorta (Fig. 2). The ratio of pressure and flow pulsatilities in the dorsal aorta was similar to the ratio in the ventral aorta (Table 1). The appearance of the pulse in the dorsal aorta lagged behind that in the ventral aorta by some 30–40ms (Fig. 3). Interestingly, two of the five fish we used had a massive parasitic infection of the dorsal aorta, which made pressure recording extremely difficult (see Brill *et al.* 1987, for a complete discussion of this phenomenon).

R_{TOT} was between 0.13 and 0.24 kPa ml^{-1} kg^{-1} min^{-1} (mean 0.17±0.04 kPa ml^{-1} kg^{-1} min^{-1}) of which R_G accounted for about 25% in one fish and over 50% in the others (Table 1). On average, R_G was 48±15% of R_{TOT} (N=4). The impedance modulus for the ventral aorta displayed a sharp initial fall and then declined gradually over the next four harmonics (Fig. 4). Impedance phase was negative (pressure lagging behind flow oscillations), ranging between −0.8 and −1.1 rad at all pulsatile harmonics except for the first (Fig. 4). These relationships were similar for both fast (2.08Hz) and slow (0.96 Hz) heartbeats. The analysis was limited to five harmonics because, for pressure, the fifth harmonic was only 1–2% of the mean value and was close to the resolving power of the digitizer. In contrast, the fifth harmonic of flow was typically 5–10% of the value at zero frequency. Hence, reconstitution of the original traces by summing the first five harmonics gave exceptional agreement for pressure but not for flow.

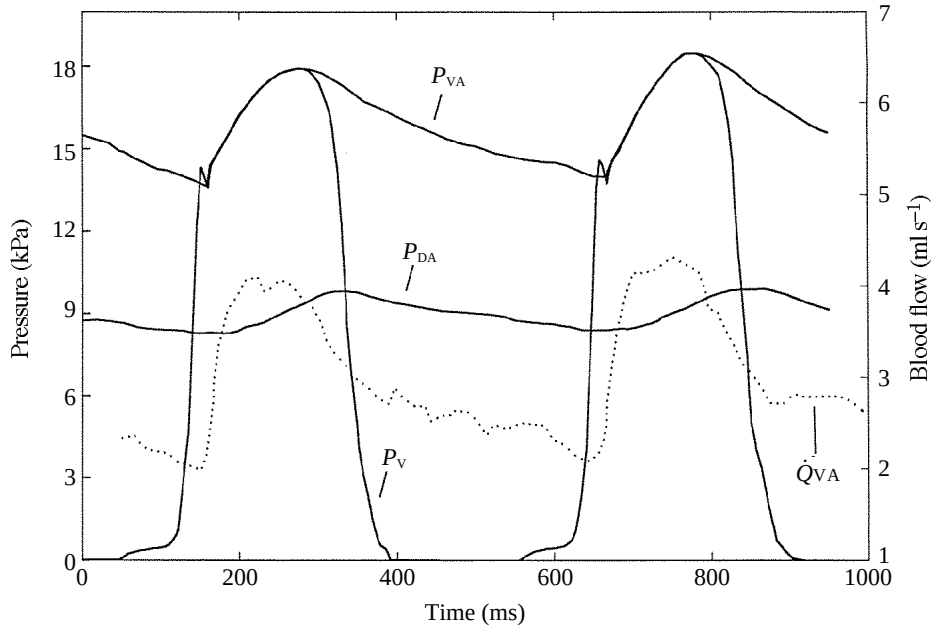

Fig. 3. Superimposed traces of ventral aortic pressure (P_{VA}), dorsal aortic pressure (P_{DA}), ventral aortic flow ($\dot{Q}_{VA}$) and ventricular pressure (P_V) in yellowfin tuna. Note that P_V and P_{VA} diverge at, or soon after, peak systole.

Table 2. *Heart rate and blood pressure of swimming yellowfin tuna*

	Ventral aorta			Dorsal aorta		
Heart rate (beatsmin^{-1})	Systole (kPa)	Diastole (kPa)	Mean (kPa)	Systole (kPa)	Diastole (kPa)	Mean (kPa)
130	14.3	9.5	11.1	–	–	6.8
102	12.4	10.4	11.1	6.8	4.9	5.6
108	13.1	11.3	11.9	6.5	5.2	5.6
102	15.1	10	11.7	–	–	8.4
102	15.2	10.5	12.1	–	–	7.6
(108.8±12.1)	(14.0±1.2)	(10.3±0.7)	(11.6±0.5)	(6.7)	(5.1)	(6.8±1.2)

Mean values ± S.E. are given in parentheses.

Swimming fish

P_{VA} and P_{DA} were recorded in five yellowfin tuna swimming laps of the doughnut-shaped tank. Lap times were not recorded. Heart rate averaged 108.8±12.1beatsmin^{-1}. Mean ventral aortic pressure was 11.6±0.5kPa and mean dorsal aortic pressure was 6.8±1.2kPa (Fig. 5A; Table 2). Hence, mean R_G in these swimming fish was 42% of R_{TOT}. $\dot{Q}_{VA}$ was measured in three kawakawa. Zero flow was established by stopping water flow over the gills while the fish was being mechanically ventilated in the doughnut-shaped tank during recovery from anaesthesia. The flow profile recorded from a swimming kawakawa is shown in Fig. 5B. $\dot{Q}$b averaged 67±40mlmin^{-1} kg^{-1} (N=3) at

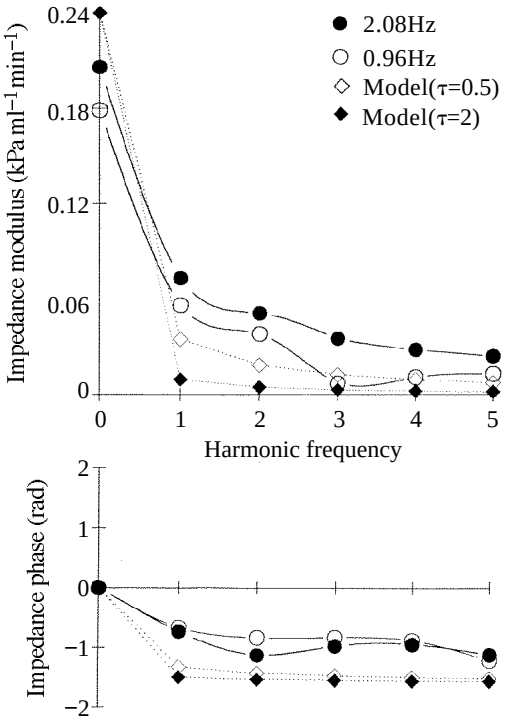

Fig. 4. Input impedance modulus and phase of the ventral aortic circulation of yellowfin tuna at normal (2.08Hz, ●) and slow (0.96Hz, ○) heart rates. The output of a Windkessel model at two different time constants (τ) is shown for comparison. See text for further details.

a mean f_H of 128±48beatsmin^{-1}. Hence, Q_S (0.54±0.2mlkg^{-1}) was within the range of values recorded from anaesthetised yellowfin (Table 1). The kawakawa swam at a mean speed of 0.6 ms^{-1}, which was equivalent to 1.3bodylengths s^{-1}. An interesting feature of these recordings was that there was a bradycardia whenever this fish turned around to change its direction of swimming (Fig. 5B).

Analysis of a model circulation

Experimentally determined impedance modulus (Z) and impedance phase (ϕ) can be compared with impedance values obtained for a second-order *RC* network (Satchell, 1971):

$$|Z| = \sqrt{\frac{(R_G + R_S)^2 + \omega^2 (C_{DA} \times R_S \times R_G)^2}{(1 - \omega^2 R_S \times R_G \times C_{DA} \times C_{VA})^2 + \omega^2 [(R_G \times C_{VA}) + (R_S \times C_{DA}) + (C_{VA} \times R_S)]^2}}, \quad (1)$$

where C_{VA} is ventral aortic compliance, C_{DA} is dorsal aortic compliance and $\omega = 2\pi f$, where f is harmonic frequency.

$$\phi = \tan^{-1}\left(\frac{\omega \times R_S \times C_{DA} \times R_G}{R_G + R_S}\right) - \pi + \tan^{-1}\left(\frac{\omega[(R_G \times C_{VA}) + (R_S \times C_{DA}) + (R_S \times C_{VA})]}{-1 + \omega^2 C_{DA} \times R_S \times C_{VA} \times R_G}\right), \quad (2)$$

impedance phase is expressed for the case when $\omega^2 C_{DA} \times R_S \times C_{VA} \times R_G > 1$.

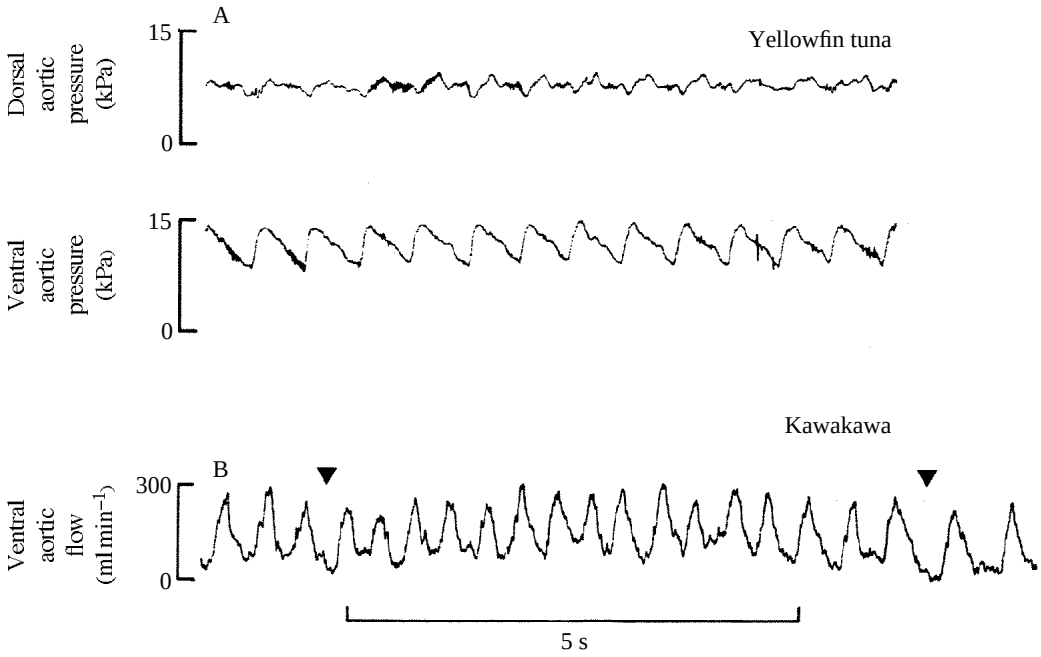

Fig. 5. (A) Dorsal and ventral aortic pressures in swimming yellowfin tuna. (B) Ventral aortic flow in a kawakawa. Note that flow fell to zero when the animal changed swimming direction (arrowheads). The time bar of 5s applies to both A and B.

In the one tuna in which determinations were made, the ratio between pressure and flow pulsatilities was similar in the ventral and dorsal aortae, and R_G=R_S, so it is a reasonable assumption that compliance of the ventral (C_{VA}) and dorsal (C_{DA}) aortic systems were similar. Therefore, the time constant for the ventral aortic/gill circulation and dorsal aortic/systemic circulation will be the same. Unfortunately, previous model analyses have shown that the diastolic declines in pressure in both the ventral and dorsal circulations (the time constants, τ) are not independent of one another (Jones *et al.* 1974; Langille *et al.* 1983). However, the time constant (τ) for the dorsal circulation can be calculated by rearranging equation 8 of Langille *et al.* (1983):

$$(2\tau)^2 = \left(\frac{PP_{VA}}{PP_{DA}}\right)^2 - \left(1+\frac{R_S}{R_G}\right)^2, \qquad (3)$$

where *PP* is the difference between systolic and diastolic pressure in the ventral and dorsal aortae and

$$\left(1+\frac{R_S}{R_G}\right)^2 < \left(1+\frac{PP_{VA}}{PP_{DA}}\right)^2.$$

Hence, if: $C_{VA} \times R_G = C_{DA} \times R_S = \tau$, then equation 1 reduces to:

$$|Z| = \sqrt{\frac{(R_G^2)[4+(\omega\tau)^2]}{[I-(\omega\tau)^2]^2+9(\omega\tau)^2}}, \qquad (1')$$

and equation 2 to:

$$\phi = \tan^{-1}\left(\frac{\omega\tau}{2}\right) - \pi + \tan^{-1}\left(\frac{3\omega\tau}{-1+(\omega\tau)^2}\right). \quad (2')$$

Curves describing impedance modulus and phase, obtained from the model analysis, are shown on Fig. 4 for two values of τ (0.5 and 2). For some of the anaesthetised tuna, τ averaged 2.06 (N=3). The curves show reasonably good agreement with those obtained from harmonic analyses of ventral aortic pressure and flow in anaesthetised yellowfin (Fig. 4).

Discussion

The present data suggest that a simple, four-element 'lumped-parameter' model yields a reasonable estimate of pressure–flow relationships in the ventral aortic circulation of tuna as measured by arterial impedance. Although predicted values underestimate and overestimate measured values for impedance modulus and phase, respectively, the curves for both predicted and measured modulus and phase are of the same shape (Fig. 4). Unfortunately, our analysis of vascular impedance was limited by being taken to only five harmonics but, even at heart rates in excess of 2Hz, there were no signs of the oscillations in modulus and phase which are so apparent in analyses of mammalian circulations. Pulse wave velocities will be slow, but will accelerate towards the gills because of the increased stiffness of the distal ventral aorta. Nevertheless, it is unlikely that pulse transmission will occupy a considerable portion of the cardiac cycle in fish of the size range used in these observations. Reflections make major contributions to wave transmission effects in mammals (O'Rourke, 1967; McDonald, 1974; Milnor, 1979; Jones, 1991) and are more pronounced for higher terminal resistances (van den Bos *et al.* 1982). The terminal resistance for the ventral aorta is the gill resistance which, in tuna, is similar to or less than the systemic resistance. Hence, tuna are like other fish in that a Windkessel dominates central haemodynamics despite heart rates that fall in the mammalian range. However, several factors contribute to the lack of wave transmission effects in our animals and it is entirely possible that some circumstances (i.e. large body mass, extremely high heart rate, low pulse wave velocity and high gill resistance) may promote considerable deviation from the Windkessel model.

Both pressure and flow in the dorsal aorta were pulsatile, although damped compared with ventral aortic waves. Pulsatile pressures and flows, with a loss of high-frequency components, have been recorded previously in the dorsal aorta of the cod (Jones *et al.* 1974). The ratio of pressure to flow pulsatilities in the one fish in which dorsal aortic flow was calibrated was similar in both ventral and dorsal aortic circulations, indicating similar compliance. The elasticity of the dorsal aorta is similar to that of the ventral aorta (D. R. Jones, R. W. Brill and C. L. Watson, unpublished observations) but the length of the dorsal circulation is much greater, thus illustrating the effectiveness of the contribution of the bulbus arteriosus to ventral aortic compliance.

Blood pressures in ventral and dorsal aortae in anaesthetised fish were similar to those in swimming fish. Pressures measured by us were close to those obtained by others (Lai *et al.* 1987; White *et al.* 1988; Bushnell and Brill, 1992). Mass-specific cardiac output ($75 ml min^{-1} kg^{-1}$) was substantially lower than $\dot{Q}$b measured in spinalized yellowfin

(115ml$\text{min}^{-1}\,\text{kg}^{-1}$) and skipjack tuna (132ml$\text{min}^{-1}\,\text{kg}^{-1}$) (Bushnell and Brill, 1992), but was 2–3 times greater than that recorded by Lai *et al.* (1987), who used considerably bigger fish. Lai *et al.* (1987) suggest that a scaling factor for cardiac output of (body mass, kg)$^{0.81}$ might apply to albacore tuna which would give a Q_S of 0.5ml in a 1kg albacore. This seems to be at the lower end of the range for tuna of this size, although all other determinations of $\dot{Q}$b in tuna have been made at a somewhat higher temperature which might, in itself, elevate $\dot{Q}$b. At heart rates in excess of 90$\text{beats}\,\text{min}^{-1}$, flow in the ventral aorta was continuous. This made determination of zero flow difficult except when heart rate slowed in the absence of large changes in ventral aortic blood pressure. Lai *et al.* (1987) make no mention of how they established zero flow in their fish, and it is possible that they underestimated the continuous flow component.

Tuna have exceptionally large hearts which beat at a higher frequency and generate greater pressures than those of most teleosts (Kanwisher *et al.* 1974; Poupa *et al.* 1981; Breisch *et al.* 1983; Lai *et al.* 1987). Maximum heart rates reported for swimming tuna are around 260$\text{beats}\,\text{min}^{-1}$ (Kanwisher *et al.* 1974). In comparison, heart rates in kawakawa, swimming at low speed, averaged 128$\text{beats}\,\text{min}^{-1}$, somewhat higher than the range of 40–85$\text{beats}\,\text{min}^{-1}$ reported for slowly swimming yellowfin and skipjack tuna (Bushnell and Brill, 1991). However, heart rates in excess of 250$\text{beats}\,\text{min}^{-1}$ have been observed in paralyzed, artificially ventilated tuna (Brill, 1987) and heart rates obtained from our swimming kawakawa were similar to those obtained from anaesthetized or spinalized tuna (Breisch *et al.* 1983; Bushnell *et al.* 1990). Obviously, heart rate does not appear to correlate well with the fish's level of activity.

In fishes, unlike many higher vertebrates, the ventricle is filled by atrial contraction and the atrium, in turn, is filled by the sinus venosus or by the kinetic and potential energy of the flowing venous blood (Farrell and Jones, 1992). Atrial contraction lasts about 70–100ms whereas the systolic ejection phase takes 250–300ms (Table 1). Hence, if there is no diastolic period (i.e. the sinus venosus fills, and then fills the atrium during ventricular ejection), the theoretical maximum heart rate in tuna will be approximately 250–300$\text{beats}\,\text{min}^{-1}$, which agrees with maximum recorded heart rates (Kanwisher *et al.* 1974; Brill, 1987).

At these high heart rates there could be a problem of filling and emptying the ventricle. Lai *et al.* (1987) reported that tuna have large negative intrapericardial pressures, which assist in cardiac filling. We feel, however, that the existence of large negative intrapericardial pressures are unlikely in an animal that exercises continuously. Tuna should not need much help with venous return other than that provided by muscular activity. Given that the heart can fill, there is still a problem with ejecting a large stroke volume. Stroke volume can be increased by phenylephrine injection. In the present experiments, Q_S increased by 30% but the systolic ejection phase increased by 50% compared with that before inotropic stimulation. Obviously, there seems to be an antagonistic rather than synergistic interplay between increasing inotropic and chronotropic forces in tuna.

The heart is pyramidal with a compact myocardium surrounding an inner trabeculate (spongy) myocardium (Sanchez-Quintana and Hurle, 1987). The muscle fibres in the compact layer are arranged in longitudinal and circular bundles (Sanchez-Quintana and

Hurle, 1987). On the ventral faces of the ventricle, the superficial fibres are arranged longitudinally but on the caudal face they are predominantly transverse. An inner layer of fibres is arranged circularly around the ventricles. Hence, during systole there is probably a reduction in both longitudinal and transverse diameters of the ventricle. Further, many fibres are inserted into a bulbo-ventricular fibrous ring and could actively open the bulbo-ventricular orifice (Sanchez-Quintana and Hurle, 1987). The observation that ventricular and ventral aortic pressures begin to diverge soon after peak ventricular systole indicates that active tension on the bulbo-ventricular valve may be released at this time.

The contribution of compact and spongy myocardium to the generation of high systolic pressures is equivocal. Johansen (1965) envisaged the trabeculate hearts of ectotherms as a group of pumps in parallel with one another. It has been suggested that Laplacian relationships might not govern trabeculate hearts in the same way that they govern endotherm hearts, in which the ventricles are hollow and have compact walls (Seymour, 1987). However, each individual lacuna in the trabeculate portion of the heart will be governed by Laplacian relationships. Furthermore, because the radius of each lacuna is small, pressure will be generated at a considerable mechanical advantage compared with hearts consisting of solely compact myocardium. The rate of pressure generation seems to be very fast in compact/spongy hearts, although the values we obtained are probably too high by 50–100% because of the low-frequency response of our recordings (McDonald, 1974). This artefact is confirmed by the presence of 'spikes' and after-vibrations on some of our pressure records. Nevertheless, a dP/dt of half our measured rate would still compare favourably with dP/dt in most mammals. There must be costs associated with trabeculate hearts because not all animals use this design. The ability to increase stroke volume by reducing end-diastolic volume is a major factor contributing to increased cardiac output in mammals, and this may be seriously restricted in trabeculate hearts.

The above limitation on our estimation of dP/dt notwithstanding, our recordings are of sufficiently high fidelity to be compared critically with those of others. For instance, ventricular diastolic pressures were usually zero, except after atrial contraction, when pressures rose to between 0.15 and 0.76kPa. However, others have reported ventricular diastolic pressures ranging from −0.5 to +6.2kPa (Breisch *et al.*1983; Lai *et al.* 1987; White *et al.* 1988). Negative ventricular pressure could result from a negative intra-pericardial pressure (Lai *et al.* 1987), but negative venticular pressures were not seen in our fish. Surprisingly, two studies have reported that dorsal aortic diastolic pressures can be above those in the bulbus (Breisch *et al.* 1983; White *et al.* 1988). This would reverse the pressure gradient for flow through the gills. However, neither our pressure nor our flow recordings suggested such a situation. Finally, R_G in both anaesthetised and swimming fish was usually between 40% and 50% of R_{TOT}, which was not as high as R_G values calculated for spinalized skipjack (60%) and yellowfin (68%) tuna (Bushnell and Brill, 1992), but was much higher than the mean value of 26% recorded by Lai *et al.* (1987) or the 6% that can be estimated from the data of White *et al.* (1988). Nevertheless, the massive increase in surface area of the gills in tuna is achieved without much cardiovascular cost for the resistance presented to blood flow by tuna gills is similar to that of trout gills (Kiceniuk and Jones, 1977).

Although massive parasitic infections of the dorsal aorta made pressure recording difficult, they did not seem to be having any deleterious effect on the tuna. Brill *et al.* (1987) suggested that these infections cause a large increase in resistance in the dorsal aorta, although this conclusion was confounded by the significant resistance of the tubing in their perfusion apparatus and also by the extreme pressure required to open the parasitized vessel for flow. However, on a plot of flow *versus* pressure, resistance is given by the slope of the line. The data of Brill *et al.* (1987) indicate that resistance of the parasitized segment was no more than three times that of the unparasitized vessel. Because the flow resistance in a large vessel is small, it can be concluded that the parasites have a negligible effect on either pressure or flow.

Maximum $\dot{V}_{O_2}$ in tuna is close to $30 \mathrm{ml min^{-1} kg^{-1}}$ (Gooding *et al.* 1981), some 10 times the 'resting' rate, yet maximum $\dot{Q}$b appears to be only two or three times that in anaesthetised fish, largely because of the antagonistic interference between f_H and Q_S. Hence, arteriovenous oxygen difference must increase markedly during exercise. Tuna differ from most teleosts by having a high blood oxygen-carrying capacity (Jones *et al.* 1986). The potential to increase the arteriovenous oxygen difference must be used to good effect because, during swimming, $\dot{Q}$b was less than in anaesthetised preparations. In fact, the arteriovenous oxygen difference in anaesthetised or spinalized tuna is extremely low (Bushnell and Brill, 1991), indicating that $\dot{Q}$b in these fish should not be regarded as a 'resting' value.

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