

Form and function of the bulbus arteriosus in yellowfin tuna (*Thunnus albacares*): dynamic properties

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Summary

The bulbus arteriosus of the teleost heart possesses a static inflation curve that is r-shaped over the *in vivo* pressure range. To examine the possible significance of this in living animals, we recorded arterial blood pressure from anaesthetized yellowfin tuna and utilized a video dimensional analyser to simultaneously record changes in bulbar diameter. By plotting the changes in pressure against the changes in diameter, it was possible to create dynamic pressure–diameter (P–D) loops as well as calculate the instantaneous volume changes within the

bulbus. The dynamic P–D loops showed the same features exhibited by static inflation. When nearly empty, a small stroke volume caused a large increase in blood pressure, while around systolic pressure large changes in volume resulted in small changes in pressure. We conclude that these features allow the bulbus to maintain ventral aortic flows and pressures over a large range of volumes.

Key words: bulbus arteriosus, P–D loop, r-shaped curve, video dimensional analysis, tuna, *Thunnus*.

Introduction

The smoothness of flow and pressure in the ventral aorta of teleosts is due to the presence of a large central compliance that is the product of elastic and resistive elements downstream of the heart. While the resistance of the gills is only about 30–50% of the total peripheral resistance, the ventral aorta and branchial arteries are short, resulting in a small total compliance of the central arterial circulation. The bulbus arteriosus, the most anterior of the four chambers of the teleost heart, greatly increases central vascular compliance, largely subserving the Windkessel functions of the whole mammalian arterial tree (von Skramlick, 1935; cited in Mott, 1950; Satchell, 1971; Stevens et al., 1972; Licht and Harris, 1973; Jones et al., 1974, 1993; Priede, 1976; Farrell, 1979; Watson and Cobb, 1979; Benjamin et al., 1983, 1984; Santer, 1985; Bushnell et al., 1992; Jones, 1999). In a Windkessel, the arteries expand with each heartbeat and recoil elastically, causing the highly pulsatile inflow to become relatively smooth in the periphery. How a relatively short bulbus mimics these effects of a longer arterial tree has never been explained.

Like an artery, the bulbus is composed of elastin, collagen and smooth muscle; however, it is highly modified, resulting in specialized inflation properties (Braun et al., 2003). Over the *in vivo* pressure range, an artery has a J-shaped P–V (pressure–volume) loop, while the bulbus has an r-shaped P–V loop. The bulbar curve can be broken into distinctive stages: (1) a sharp initial rise in pressure for a relatively small volume

change and (2) a plateau stage where the bulbus is largely unaffected by even large changes in volume. There is even some evidence to suggest that there is a third stage of the bulbar inflation (Braun et al., 2003); when greatly distended, the bulbar material rapidly increases in stiffness.

Stage 1 is due to the relationship between the wall tension, pressure and volume of the bulbus, as described by the Law of Laplace. The bulbar lumen is very small at low pressure and therefore bulbar expansion requires a large initial pressure increment. Stage 2 is a result of the specialized material properties of the bulbus. The bulbar wall has a very high elastin:collagen ratio and is almost entirely composed of novel elastin (low hydrophobicity, high solubility) aligned in a novel manner (loose fibrils, no lamellae). These modifications produce very low wall stiffness and the ability to undergo large strain changes and result in the compliance of the plateau. At large extensions, stiff adventitial collagen is recruited to resist the expansion of the bulbus.

Knowing the causes of the strange bulbar P–V loop is an important first step in understanding how the bulbus works. However, in order to make inferences based on the *in vitro* inflation curve, it is vital that the bulbus shows similar traits *in vivo*. To this end, *in vivo* changes in pressure and bulbar diameter during normal beating in anaesthetised yellowfin tuna were recorded using video dimensional analysis (VDA) and pressure recordings.

Materials and methods

The experiments were performed on the bulbi of five *Thunnus albacares* L. (1.12 ± 0.32 kg) that were held in large outdoor tanks at the National Marine Fisheries Service Kewalo Research Facility in Honolulu, HI, USA. The water temperature in the holding tanks was 25°C.

Yellowfin tuna were anaesthetized using 0.2 g l⁻¹ ethyl p-amino benzoate and equimolar NaHCO₃. Following anaesthesia, the fish were placed supine in a chamois leather cradle. A hose running aerated seawater was placed in the mouth of the anesthetized fish in order to simulate ram ventilation. A midline incision was made along the ventral surface to expose the pericardial cavity. The pericardium was opened and the heart exposed. During the experiment, anesthesia was maintained with Saffan (3 mg kg⁻¹ intra-arterially; Glaxovet, Harefield, UK). To decrease heart rate, water flow over the gills was stopped for several seconds.

Arterial blood pressure was measured through a cannula inserted into the bulbus and connected to a Unonics model P-106 pressure transducer (Wayland, MA, USA) (Fig. 1). Pop tests established the frequency response of the system to be 32 Hz, with damping being 0.12 of critical damping (Jones, 1970). Changes in the diameter of the bulbus during systole and diastole were measured using a video dimensional analyzer (VDA; Instrumentation for Physiology and Medicine, model 303). This system consists of a video camera, a video processor and a monitor. The camera was focused on the bulbus, and the signal fed through the processor. The VDA utilizes the video signal to give a DC voltage that is proportional to the distance between two selected contrast boundaries on the monitor. The VDA 'window' (Fig. 1) was used to track the movement of the outside surface of the bulbus as it expanded and contracted during systole and diastole. By calibrating the voltage generated, dynamic dimensional changes were recorded. The VDA has a 15 Hz low-pass RC filter on the output signal, which introduces 180° of phase delay at 15 Hz. Appropriate corrections were applied to the diameter traces. For a more in-depth explanation of the VDA, see Fung (1981). Voltages and pressures were collected and stored using DASYPALAB (Dasytec USA, Amherst, NH, USA).

Following dynamic recordings of heart beats from tuna, static *in vivo* inflations of the bulbi were performed. The proximal ventral aorta and the bulbo-ventricular junction were ligated, and a T-junction was inserted into the pressure catheter to allow simultaneous bulbar inflation and pressure measurement. Measured volumes of saline (25°C) were injected into the bulbus, and the resultant pressure signal was amplified and recorded using DASYPALAB software. Cycles of inflation and deflation were performed until consistent results were seen. Preconditioning usually required 5–10 cycles. These initial cycles were discarded. Each experiment consisted of 8–15 trials, and results from any trials in which a loss of more than 5% of the injected saline occurred were

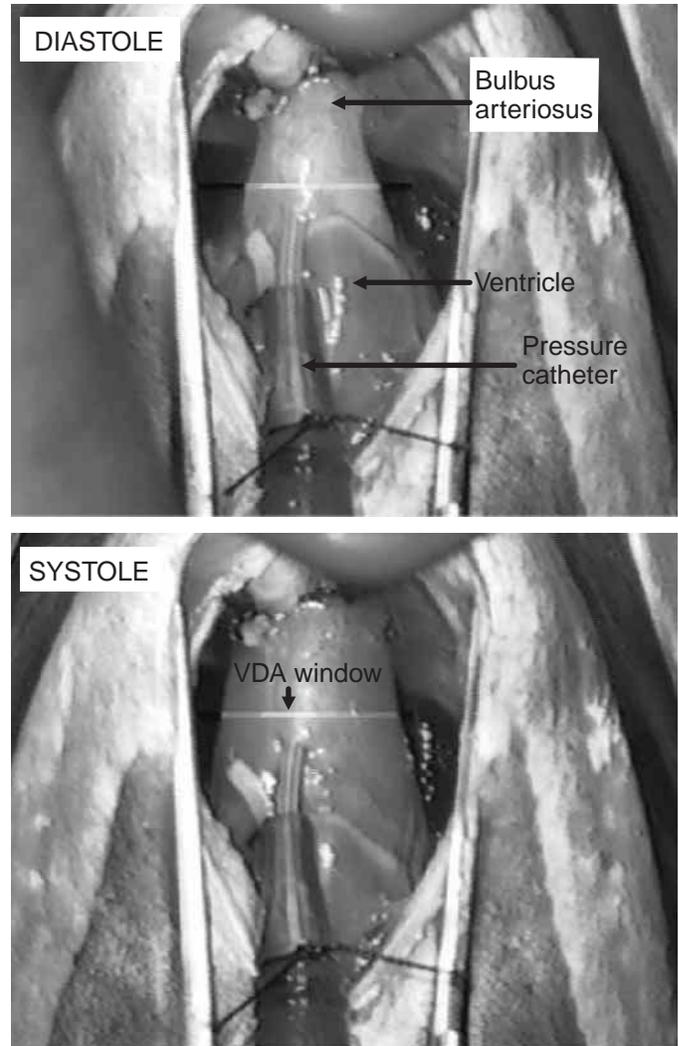


Fig. 1. A yellowfin tuna used in the video dimensional analysis (VDA) study showing the bulbus in relation to the ventricle within the pericardial cavity during diastole and systole. The catheter used to record pressure entered the bulbus ventrally. The VDA window was aligned with the bulbus so that from diastole to systole the black portion of the window followed the edges of the bulbus during a heartbeat. Length, as well as width, changed with each beat of the heart.

not used. After preconditioning, the data were recorded and plotted as pressure (kPa) versus volume (ml or µl). By simultaneously measuring the diameter changes due to each injection of fluid, it was also possible to create a plot of pressure versus diameter. Linear regressions of the curves yielded calibration curves describing the interactions between injected volume and diameter. Due to the differences in dimension along the bulbus, the dynamic and static measurements must be taken from the same locations. This was not the case for all observations, and, therefore, calibration curves could not be calculated for all recordings of pressure and diameter. These recordings of pressure and diameter were analyzed using Microsoft EXCEL.

Results

Longitudinal and circumferential strains

The maximal longitudinal and circumferential strains recorded with the VDA occurred during static inflations of the bulbus. The stroke volume of yellowfin tuna is in the range of 0.65–1.00 ml (Jones et al., 1993) for the mass of animals used in these experiments. The bulbus was inflated with 2.5 ml of fluid. Maximal static circumferential strain was 0.47, while maximal static longitudinal strain was 0.48.

When the heart was beating normally [i.e. heart rate approximately 1 Hz, peak systolic pressure around 9.5–13.5 kPa and pulse pressure in the range of 5–6.5 kPa (Jones et al., 1993)], the range of dynamic circumferential strains was 0.25–0.38. Ventricular movements moved the position of the bulbus within the pericardial cavity so that finding the dynamic longitudinal range of strain was not possible.

Static and dynamic P-V loops

The VDA followed the walls of the yellowfin tuna bulbus during both systole and diastole and allowed mapping of dimensional changes associated with each heartbeat (Fig. 1). Fig. 2A compares the dimensional and pressure changes occurring during a single heartbeat. The rapid increase in pressure resulted in a sharp increase in diameter. Systolic pressure of 9.3 kPa gradually declined to 4 kPa, while diameter initially fell very rapidly, followed by a smoother decline that more closely followed the fall in pressure. By plotting pressure against diameter for the heartbeat in Fig. 2A, a pressure–diameter (P-D) loop was generated (Fig. 2B), showing the inflation behaviour of the bulbus under *in vivo* conditions. When this dynamic P-D loop was compared with a P-D loop produced using the static inflation technique, the dynamic and static behaviours matched well. In both cases, the slope initially rose sharply, followed by a levelling off as the bulbus reached the plateau phase of the inflation.

The difference between the areas under the inflation and deflation curves is the amount of energy lost as heat. When this loss is normalized to the area under the inflation curve, the resulting percentage is known as hysteresis. There was significant hysteresis in both loops, indicative of a viscous element in the bulbar wall. In the dynamic loop, the larger hysteresis was due to the increased rate at which the bulbus was inflated. The faster the changes in the dimensions of a viscous element, the stiffer it becomes, and more energy is lost executing the changes.

During the initial rise of the *r*-curve, the inflation and deflation curves crossed over, indicating energy added. The bulbus lacks cardiac muscle and cannot contract beat-to-beat; therefore, the positive work loop was due to the changing length of the bulbus. The change in the length of the bulbus could be clearly seen at the end of the deflation (Fig. 2B). While pressure continued to drop, the diameter of the bulbus increased, indicating wider, upstream segments entering the field of view of the video camera.

The dynamic P-D loop in Fig. 2B demonstrates bulbar

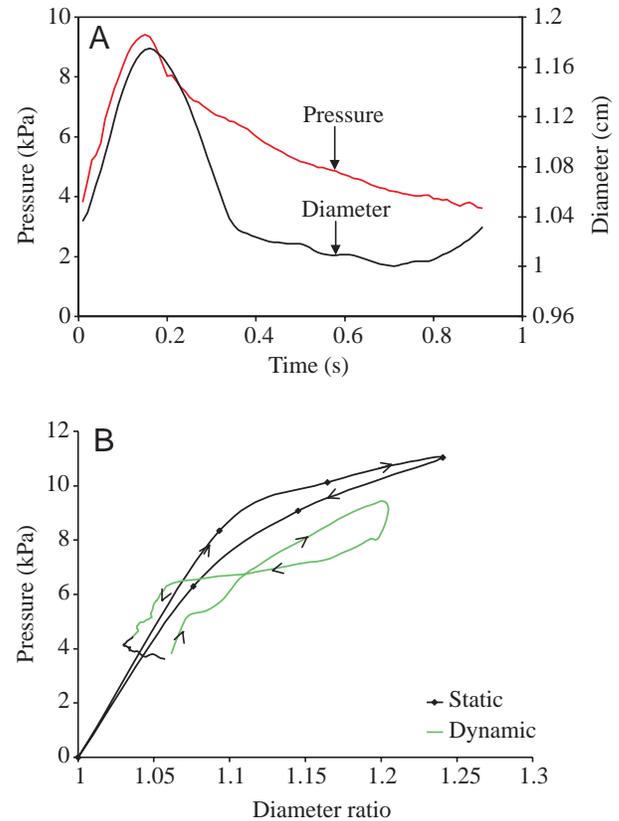


Fig. 2. (A) Recordings of bulbar blood pressure and diameter from a yellowfin tuna. (B) Comparison of static and dynamic pressure–diameter (P-D) loops. The dynamic trace (green) was created by plotting pressure against diameter for the heartbeat in A and is superimposed on a P-D curve (black) created by a bulbar inflation from a syringe, *post mortem*. Arrows indicate the clockwise or anticlockwise cycling of the loop. (Diameter ratio is the diameter divided by the initial diameter.)

behaviour over the pressure range of 4–9.5 kPa. However, by looking at beats covering the pressure range of 2.5–21.5 kPa, the features of the static bulbar inflation curve (Fig. 3A) were recreated: the initial steep rise, the plateau and the final steep rise at large inflations and high pressures (Braun et al., 2003). At the low end of the pressure range, the bulbus was operating on the steep part of the inflation curve and small changes in volume resulted in large, rapid changes in pressure (Fig. 3B). A small, low-pressure heart beat generated a very steep P-D loop. For a heart beat covering the pressure range 11.3–12.6 kPa, the P-D loop showed that the bulbus was inflating entirely on the plateau (Fig. 3C); the loop was horizontal, with very little vertical component. The P-D loop from a heart beat over the range of 15.3–22.6 kPa showed that, while the bulbus operated on the plateau for much of the beat, at very high blood pressures bulbus stiffness rapidly increased (Fig. 3D).

In Fig. 4A, following a prolonged cardiac interval (marked with an asterisk), the smallest increase in diameter resulted in the generation of the largest pressure pulse. As peak pressure

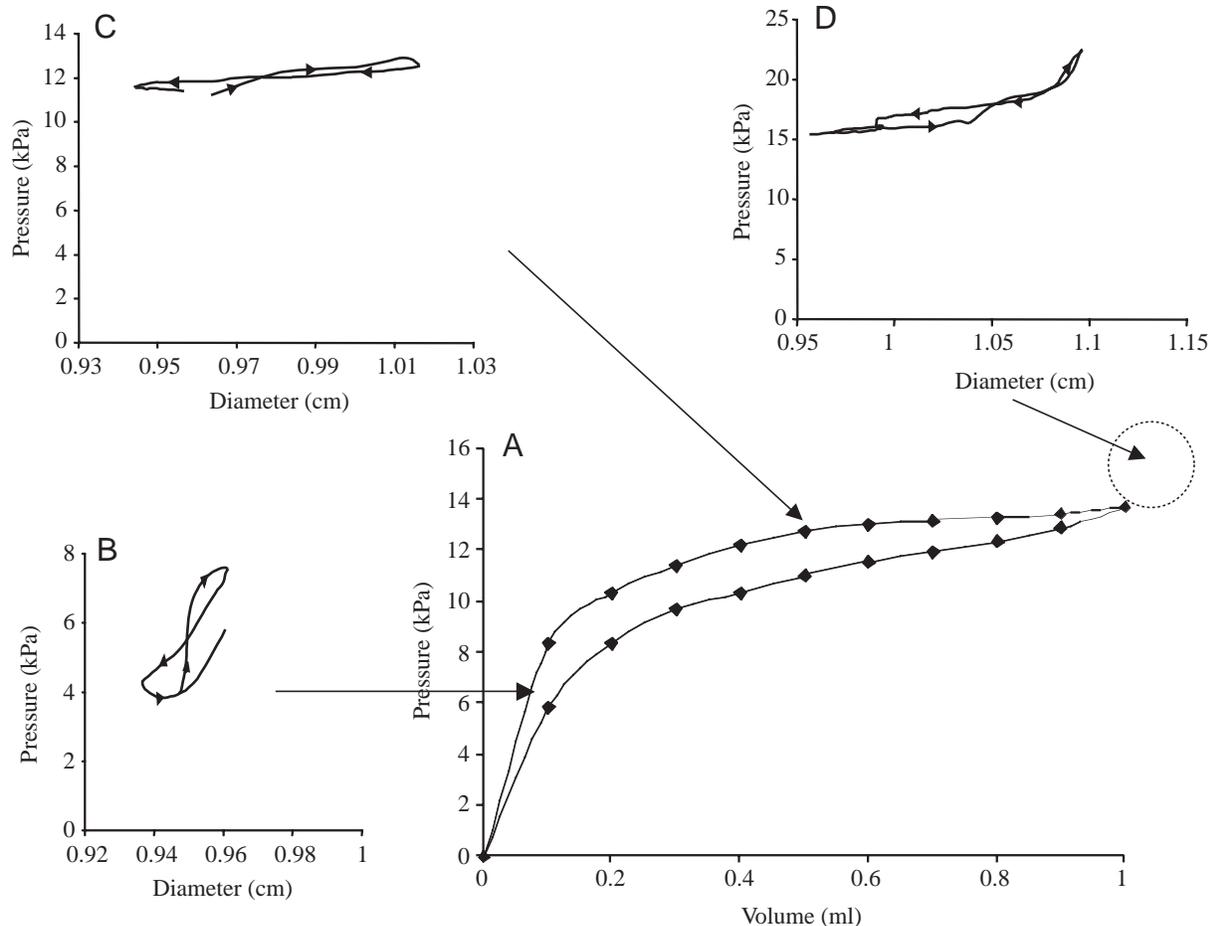


Fig. 3. Comparison of dynamic and static inflations over a wide pressure range. (A) Pressure–volume (P–V) loop from static inflation of a yellowfin tuna bulbus arteriosus. (B) A dynamic pressure–diameter (P–D) loop for the pressure range 4–8 kPa. (C) A dynamic P–D loop for the pressure range 10.6–12.6 kPa. (D) A dynamic P–D loop for the pressure range 16–22 kPa. Arrows link the pressure ranges in B, C and D to the static loop in A.

increased, subsequent pressure pulses became smaller while diameter changes increased. The first fluid injection after the long cardiac interval had a larger impact on the pressure than those that followed. The highlighted beat (marked with an asterisk) is equivalent to the sharp initial rise of the static inflation tests.

Immediately after Saffan injection, the tuna hearts often deviated from normal beating patterns by speeding up and/or increasing pressure. In a fish recently injected with Saffan (Fig. 4B), the lowest diastolic pressure was 6.7 kPa, while the highest pressure was 20.6 kPa. During a period of declining pressure, there was an occasional small pressure ‘blip’ (arrows in Fig. 4B). The small increases in pressure (0.13–0.67 kPa) were associated with large changes in the diameter of the bulbus. These diameter changes were frequently as large as those associated with pressure changes that were ≥ 20 times larger (diamond in Fig. 4B). In the high-pressure range (13.3–16 kPa), large volume changes (as evidenced by large changes in bulbar diameter) result in relatively small changes in pressure, indicating that the bulbus was on the plateau stage of static inflations.

At pressures greater than 17.3 kPa, however, small volume changes resulted in large changes in pressure. In Fig. 4C, the highlighted beats (triangles) had peak systolic pressures of 17.3 kPa, 22.7 kPa and 22.2 kPa. However, the resultant systolic diameters only varied slightly (1.077–1.095 cm) and the systolic–diastolic diameter changes were 0.18–0.23 cm. The insensitivity of the bulbar pressure to volume injections disappeared at very high pressures due to a rapid increase in the stiffness of the bulbar wall.

Dynamic P–D loops (Figs 2, 3) had the same features as the static P–V loops, and bulbar diameter seemed to be an accurate indication of bulbar volume. The validity of this assumption was checked by static inflations. Static r-shaped P–V loops were generated using the *in situ* VDA preparations after dynamic experiments (Fig. 5). The relationship between diameter and volume was linear for examples from both the anterior (Fig. 5C) and posterior (Fig. 5D) portions of the bulbus.

Fig. 6 is an example of a typical beating pattern for an anaesthetized yellowfin tuna. The diameter and pressure were measured at a point near the middle of the bulbus. At a heart

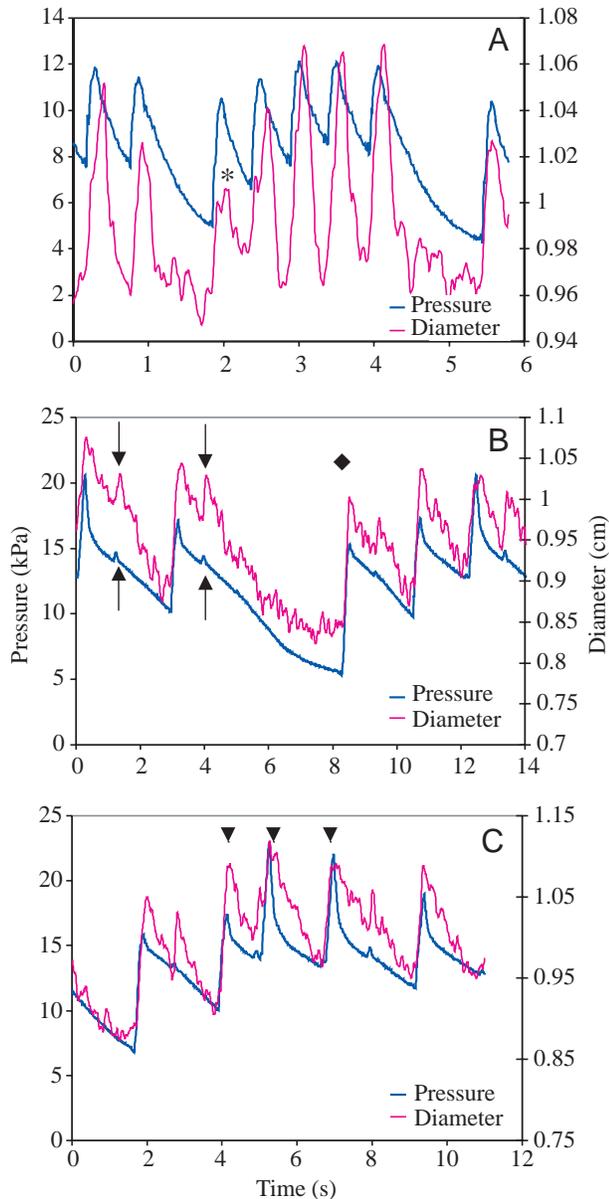


Fig. 4. Recordings of bulbar blood pressure and diameter from yellowfin tuna. (A) Following a long cardiac interval, the smallest stroke volume (*) generated the largest pulse pressure. (B) At mean systolic pressure, large changes in diameter result in small changes in pressure (arrows). See text for explanation of diamond. (C) At very high pressures, small changes in diameter result in large fluctuations in pressure (triangles).

rate of 1 Hz, pulse pressure was approximately 6 kPa with a peak systolic pressure of 10 kPa. Diameter changes were about 0.1 cm, from 0.7 cm to 0.8 cm. Fig. 6B illustrates how these diameter changes translated into volume within the bulbus. The 0.1 cm-diameter change resulted in bulbar volume varying from 0.2 ml to 0.8 ml.

Bulbar volume fell when blood pressure and heart rate decreased. In Fig. 7A,B, the heart was beating normally during the first 12 s, after which it began to slow from a rate of 1.2 Hz

to 0.8 Hz. Bulbus diameter and internal volume began to fall, and, between 20 s and 25 s, the heart appeared to miss several beats, resulting in long diastolic periods. During these periods, bulbar volume fell close to zero. However, even at these low internal volumes, the pressure remained at 2.7 kPa (Fig. 7A,B).

Fig. 7C shows a VDA recording of the ventricular end of the bulbus from a fish shortly after an inter-arterial injection of Saffan. The heart was beating extremely fast (4 Hz) and pulse pressure was approximately 2 kPa (10.6–12.6 kPa). Despite the small pulse pressure, the changes in diameter (0.08 cm) were nearly as large as in Figs 6B, 7B. This suggests that the bulbus was on the plateau phase of the r-shaped curve; large changes in volume generated small changes in pressure. Indeed, the volume changes (0.6–1 ml) seen in Fig. 7D were similar to those in Fig. 6.

Discussion

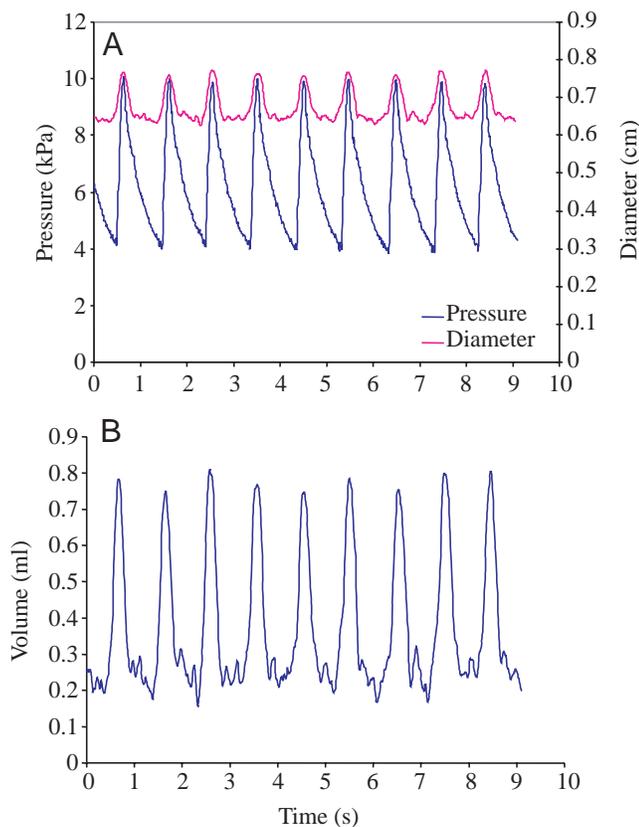
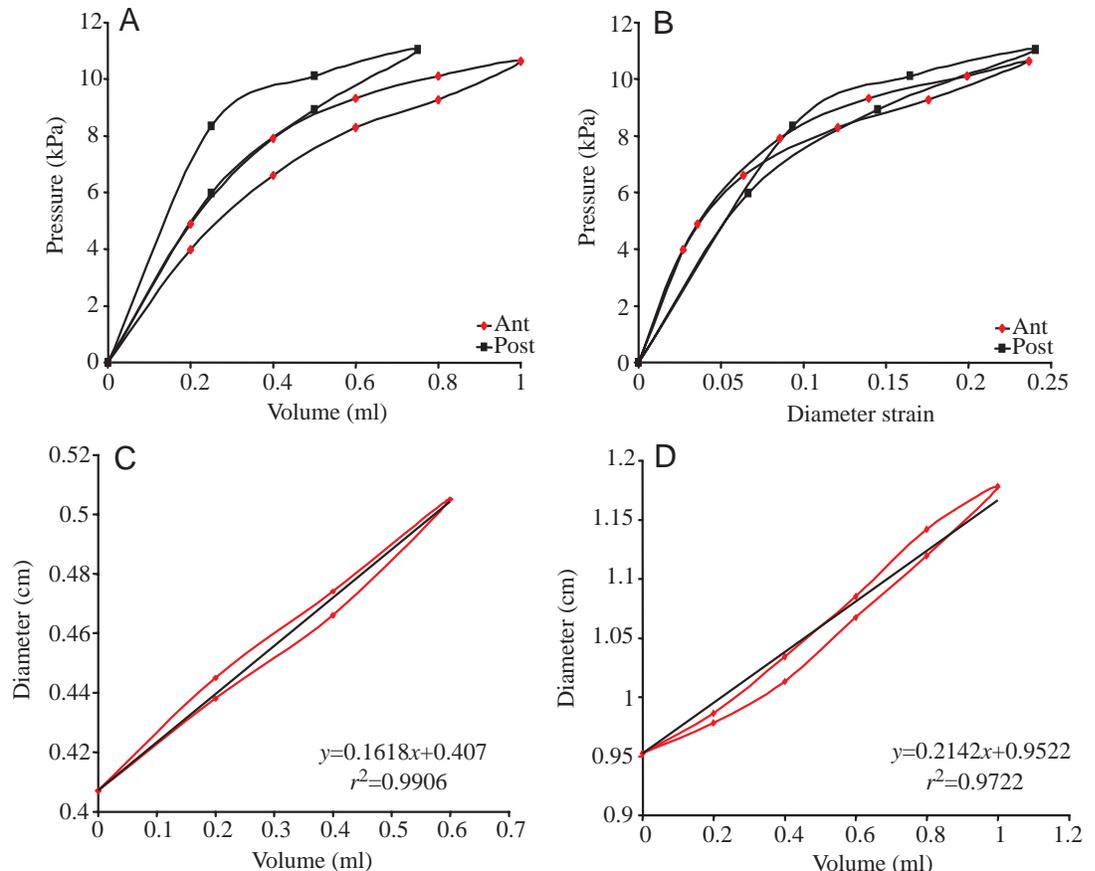
In simple geometrical figures like cones or spheres, the relationship between diameter and volume is not linear. Bulbus geometry, however, is not simple and the analysis was further complicated because the bulbus lengthened as it inflated (Fig. 1). Since the VDA windows were fixed, the point that was followed at the start of the inflation was not the point being followed at the end. The result of this was a consistent underestimation of the actual diameter changes as narrower, more-distal portions of the bulbus were being 'pushed' into the field of view. The unexpected linear relationship between diameter and volume in the bulbus is a coincidence between the interactions of the linear and radial expansions and how they were interpreted by the VDA. This was shown by modelling volume and diameter changes in cones undergoing hypothetical inflations (Braun, 2001). Cones modelled with longitudinal and circumferential strains equivalent to those that the bulbus experienced also showed a linear relationship between diameter and volume.

Qualitatively, the linear relationship between diameter and volume allowed the inference that a change in diameter was due to an equivalent change in volume: if one heart beat resulted in a bulbus diameter change twice as large as another, then twice as much fluid entered the bulbus during that beat. Quantitatively, the fact that a linear regression closely described the interaction between diameter and volume (Fig. 5) allowed an analysis of the volume into and out of the bulbus with each beat.

The features of the static bulbar inflation curve (Braun et al., 2003) occur *in vivo*. Blood initially entering the bulbus caused a large jump in pressure, followed by a stage in which large volume changes result in small pressure differences. At very high pressures, bulbar stiffness rapidly rose, and the ability to expand further was limited.

Both the sharp initial rise in pressure and the compliant plateau phase are important in the bulbus' function as a pressure reservoir. Due to the Law of Laplace (tension = pressure \times radius), the relatively small internal lumen of the bulbus results in a negligible tension in the bulbar wall at low pressures (Braun

Fig. 5. (A) Pressure–volume (P–V) loops from static, *in situ* inflations of bulbi from yellowfin tuna. Anterior and posterior refer to where on the bulbus the video dimensional analysis (VDA) window was centred. (B) Pressure–diameter strain loop for the same bulbi as in A. The diameter strain was calculated using diameter data from the VDA. (C) Diameter plotted against volume for the bulbus measured at the anterior end. A linear regression was run on this plot and the solution is shown. (D) Diameter plotted against volume for the bulbus measured at the posterior end. A linear regression was run on this plot and the solution is shown.



et al., 2003). This small internal radius necessitates a large pressure in order for expansion to occur and results in the large initial jump in the bulbar P–V loop (Fig. 8A). The larger lumen radius of an artery allows much larger changes in volume at low pressures due to the larger tension generated (Fig. 8A). For the example in Fig. 8B, the tension initially created in the artery is over four times larger than in the bulbus. Arteries generally expand 40–50% when pressurized to physiological ranges (McDonald, 1974). In the yellowfin tuna bulbus, going from zero to physiological pressure requires a strain of around 10%. The bulbus can reach the same pressure as an artery at a fraction of the volume (Fig. 8A, broken arrow), which allows the bulbus to become ‘primed’ to a high pressure with a single heartbeat, regardless of cardiac output.

When stroke volume is high, the compliance of the bulbus allows it to expand and ‘absorb’ excess fluid while preserving a relatively constant pressure head. Even when stroke volume is low, the bulbus will maintain blood flow through the gills at a high pressure. Following a long diastolic period, the first heartbeat will have a larger effect on pressure than any following beats (Fig. 4A). The benefit of the bulbar design is that it allows the bulbus to behave similarly under both high and low cardiac outputs.

Fig. 6. (A) Recordings of bulbar blood pressure and diameter from a yellowfin tuna during normal beating. (B) The volume changes within the bulbus during the beating in A.

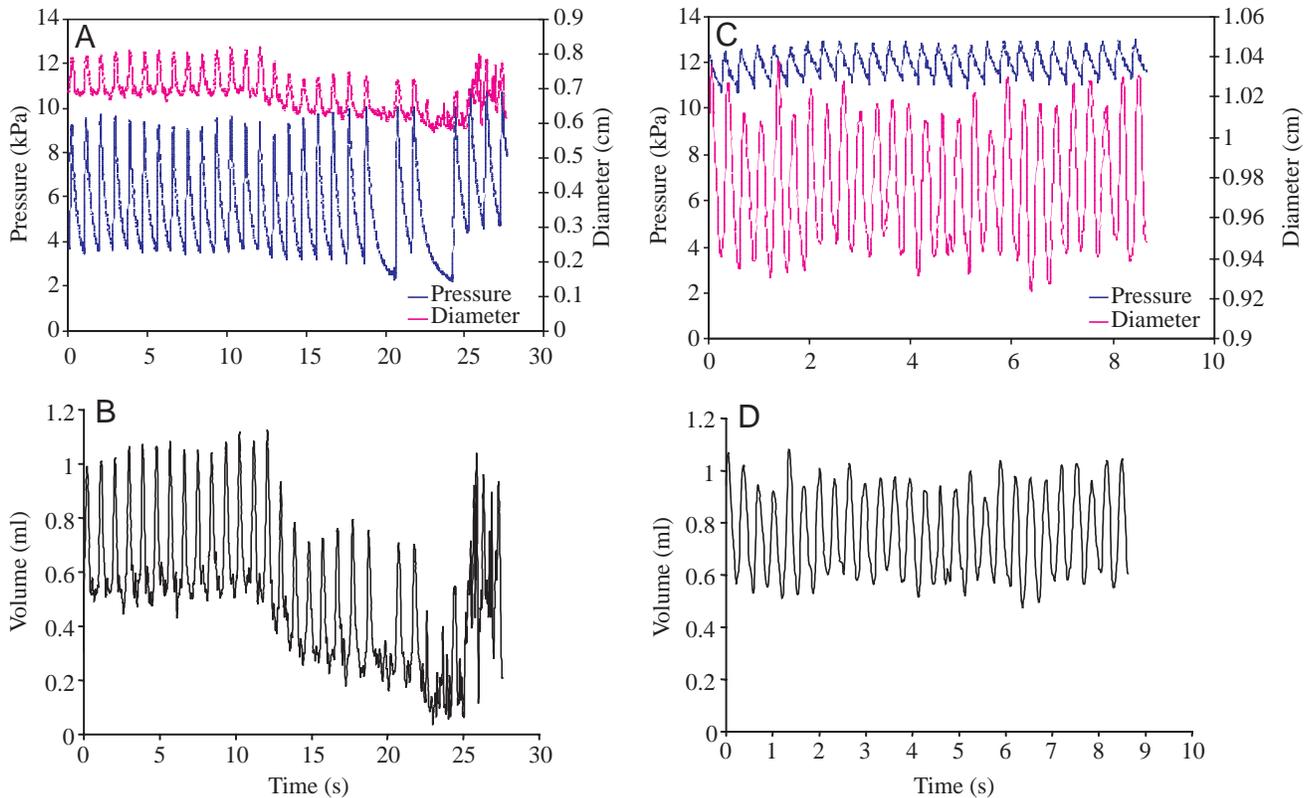


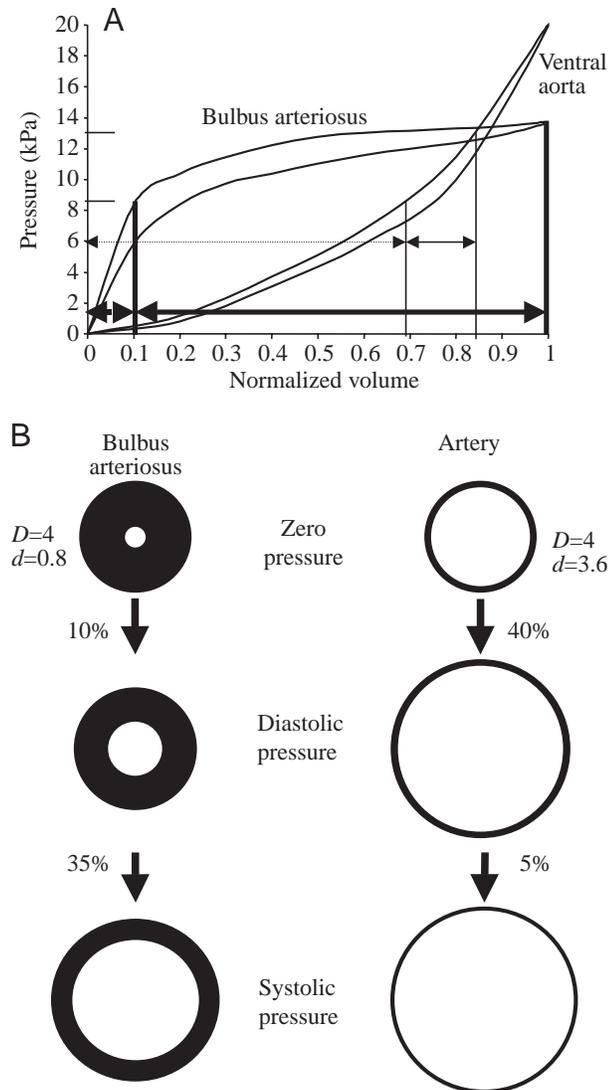
Fig. 7. (A) Recordings of bulbar blood pressure and diameter from a yellowfin tuna during a marked fall in pressure and heart rate. (B) Volume changes within the bulbus from A. (C) Pressure and diameter from a tuna after Saffan injection. (D) Volume changes within the bulbus from C.

In rainbow trout (*Oncorhynchus mykiss*), the bulbus is most compliant near the systolic pressure (Clark and Rodnick, 1999), and the same phenomenon occurs in yellowfin tuna. During systole, increasingly large changes in volume result in relatively small pressure increases. Once systolic pressure has been reached, the compliant plateau of the bulbus allows it to effectively 'store' pressure, despite large increases in volume. During diastole, the plateau allows the bulbus to maintain a high pressure while internal volume is decreasing. In fact, the bulbus can lose most of its volume, and pressure only falls by a small amount (Fig. 8A, solid arrows). In this manner, the bulbus extends the proportion of the cardiac cycle during which blood flows into the gills (Randall, 1968; Stevens et al., 1972).

Over a physiological pressure range of 4 kPa, the bulbus can hold and return 90% of its volume (Fig. 8A, thick, solid arrows), compared with only 15% in an artery. Bulbar volume changes of 0.2–0.8 ml yield circumferential strain changes of 30–40% (Fig. 8B). This behaviour is in stark contrast to arteries, which typically experience circumferential strains of 2–7% during an inflation cycle (McDonald, 1974). These large differences between the behaviour of arteries and bulbi illustrate two different means to the same end. Both bulbi and arteries are designed to increase the capacitance in the circulatory system in order to depulsate and attenuate flows and pressures. Capacitance in arteries is achieved through length.

Even a relatively inextensible tube can provide significant capacitance if it is of sufficient length. Teleosts lack the luxury of a long arterial tree separating the heart from the gills. Instead, capacitance is increased by the bulbus and its r-shaped inflation. The tremendous compliance of the bulbus on the plateau of its P-V loop results in a large volume change (ΔV) over the physiological pressure range and allows a relatively short bulbus to greatly increase the capacitance of the teleost arterial system. Furthermore, an artery needs to be almost completely filled in order to reach a high pressure, at which point a rapid increase in stiffness occurs. Working against the very rigid walls of an artery-like bulbus would increase the work of the heart. During diastole, a small amount of fluid loss in an artery results in a rapid fall in pressure. The bulbus ordinarily experiences large volume changes; in an artery-like bulbus, much of the diastolic period would occur at low pressure, reducing the flow of blood through the gills.

The bulbus is capable of both expanding to store cardiac output and recoiling elastically to return the stored fluid to the circulation. When contracted, bulbar volume is smaller than a single stroke volume. However, the bulbus is capable of holding a very large blood volume: 200–300% of stroke volume (Bushnell et al., 1992). During diastole, it is important to have some way of maintaining blood flow through the gills. Observation of the bulbus' *in vivo* functioning shows that it maintains a reservoir and never completely empties during



diastole (Figs 6, 7). Following a bradycardia, the bulbus ‘pumps’ up until the reserves are replenished (Fig. 7A,B). The bulbar reservoir allows positive flow to occur during long diastolic periods. In ling cod (*Ophiodon elongatus*), blood flow in the ventral aorta due to the elastic rebound of the bulbus arteriosus represents about 29% of total cardiac output (Randall, 1968).

The central location of the bulbus has benefits to the teleost circulatory system. A model study performed by Campbell et al. (1981) showed that a large compliance located far from the heart is equally good at raising diastolic pressure as one located proximally, but only a compliance located directly outside the heart effectively decreases peak systolic pressure. An elevated diastolic pressure ensures continuing flow through peripheral vascular beds during diastole, while decreasing the peak systolic pressure translates into large cardiac energy savings by lowering the tension–time integral during cardiac contraction. The majority of the heart’s work is involved in generating tension rather than in ejecting blood from the heart. (Jones, 1991). Therefore, the position of the bulbus in the teleost

Fig. 8. (A) Comparison between pressure–volume (P–V) loops from a yellowfin tuna bulbus arteriosus and ventral aorta. The broken arrows indicate the normalized volume required to reach the indicated pressure. The solid arrows indicate the volume change (ΔV) experienced by the bulbus arteriosus and ventral aorta over a physiological pressure range. The thick vertical lines indicate the bulbar volume changes, and the thin vertical lines indicate the ventral aortic changes. Volume was normalized to maximal internal volume. (B) Dimensional changes that a bulbus and a ventral aorta of the same external diameter would undergo from zero to diastolic and then to systolic pressure. *Zero to diastolic pressure.* At zero pressure, the bulbus’ lumen is smaller than the artery’s lumen, resulting in a much lower tension during inflation. The low tension in the bulbar wall results in a requirement for a large pressure to allow expansion. As shown in A, the small strain change in the bulbus generates a large pressure for a small volume. *Diastolic to systolic pressure.* During inflation from diastole to systole, the bulbus undergoes a large strain (35%) due to the large volume changes seen in A at systolic pressure. The artery undergoes a strain of 5%, due to the low compliance seen in A at systolic pressure. D , external diameter; d , lumen diameter. Values of strain for the bulbus were calculated using video dimensional analysis (VDA). Values of strain for the proximal aorta came from McDonald (1974).

circulation, just distal to the heart, makes it of great importance for increasing the overall efficiency of the piscine cardiovascular system.

At large volumes, the bulbus increases in stiffness (Braun et al., 2003). As in arteries, this feature may serve a similar strain-limitation function in the bulbus. However, the pressures at which this rise in stiffness occurs are extreme. In yellowfin tuna, pressures in excess of 20 kPa were required for the final increase in stiffness to occur. Normal static inflations were never taken to this level for two reasons: (1) the preparations would begin to leak and (2) the very high pressures are far above the normal *in vivo* pressure range (Jones et al., 1993). Clues suggesting that the bulbus did indeed possess a final rise in stiffness came from several sources. Braun et al. (2003), after dissecting out the bulbar media, demonstrated bulbar inflations with a rapid rise in stiffness at large volumes. In the present study, the analysis of very high (>20 kPa) blood pressure traces showed that, in contrast to what ordinarily occurs on the plateau, large jumps in pressure were occurring for very small changes in dimension (Figs 3, 4C), indicating an increased stiffness. In reality, however, the bulbar location within the pericardium means that the third phase may never be attained in an intact animal. An extremely full and swollen bulbus could interfere with the functioning of the atrium, reducing cardiac output and causing the bulbus to empty and shrink, while the very rigid pericardium found in these fish would also limit the size to which the bulbus could expand.

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