Naturwissenschaften (2005) 92: 582–585
DOI 10.1007/s00114-005-0046-1

SHORT COMMUNICATION

David R. Jones · Kevina Perbhoo · Marvin H. Braun

Necrophysiological determination of blood pressure in fishes

Received: 10 June 2005 / Accepted: 7 August 2005 / Published online: 17 September 2005

Abstract Bony fishes have an elastic chamber between the heart and aorta, the *bulbus arteriosus*, which has unique mechanical properties. On inflation, the isolated bulbus is initially very stiff but soon becomes extremely compliant yielding a steady (plateau) pressure upon further inflation, which appears to be similar in any given species. Here we show that the plateau pressure correlates with mean blood pressure determined in vivo. Consequently, inflation of the bulbus can be used to determine blood pressure in the living animal from recordings made after it is dead.

Introduction

Blood pressure recorded in adult bony fishes (teleosts) varies by over an order of magnitude from 0.2 kPa in the zebrafish (Hu et al. 2001) to nearly 12 kPa in the yellowfin tuna (Korsmeyer et al. 1997). Hence fishes should present a unique opportunity for phylogenetically based studies on the relation of blood pressure to evolutionary forces and constraints as evinced in niche selection, morphology, physical or metabolic activity levels, stress and behaviour. Bony fishes contribute about half of the species (>28,000) to the total vertebrate radiation, yet blood pressure is known only for a handful. Part of the problem for a lack of data about this undeniably important variable is that half of the teleost fish species are less than 18 cm in length as adults (Binohlan and Pauly 1998). Direct recording of blood pressure in live fish requires both surgical and anaesthetic skills while the necessary equipment increases in complexity and cost in inverse proportion to body length. Consequently, the major factors inhibiting studies in phylogeny and evolution of a physiological character in fishes are access to a suitable range of species and obtaining the appropriate physiological data.

Fortunately, access to a wide range of fish species does not present a problem in view of fisheries activity now occurring in all the world's oceans, including many artisanal fisheries that are often restricted to a specific ecological niche. From the physiologists' perspective, however, the advantage of widespread access to species is offset by the fact that virtually all fish landed by the commercial fishery are dead. Hence the big question for research in evolutionary physiology of fishes is how to record the requisite variable in the dead animal.

Recording physiology of the living animal after death defines "necrophysiology," which was the only approach to circulatory physiology before the advent of the scientific revolution and William Harvey's magnum opus on the circulation of the blood published in 1628 (Harvey 1628). Nevertheless, necrophysiology had some great successes. The Arab scholar Ibn al-Nafis (~1,208–1,288) discovered the pulmonary and coronary circulations (Haddad and Khairallah 1936), and now, three quarters of a Millennium later, we present and validate a method that allows determination of blood pressure that is quick to perform, relatively simple, has a high success rate and is applicable to virtually all the world's fishes.

Methods

Dead fish were obtained from the sport and commercial fishery as well as from the aquarium trade. Only fish that had died in the previous 6 h were used for measurements. Live fish were killed by immersion in MS 222 (0.5 to 2 g/l, increasing with the mass of the fish) just before use. The ventricle, *bulbus arteriosus* and ventral aorta, were removed through an incision in the ventral wall of the opercular cavity except in small fish (<5 g) when a ventral midline incision was made to expose the bulbus that was cannulated in situ.

D. R. Jones (✉) · K. Perbhoo
Zoology Animal Care, University of British Columbia,
6199 South Campus Road,
Vancouver, BC V6T 1W5, Canada
e-mail: jones@zoology.ubc.ca
Tel.: +1-604-8222180
Fax: +1-604-8228121

M. H. Braun
Department of Physiology,
Downing Street,
Cambridge, CB2 3EG, UK

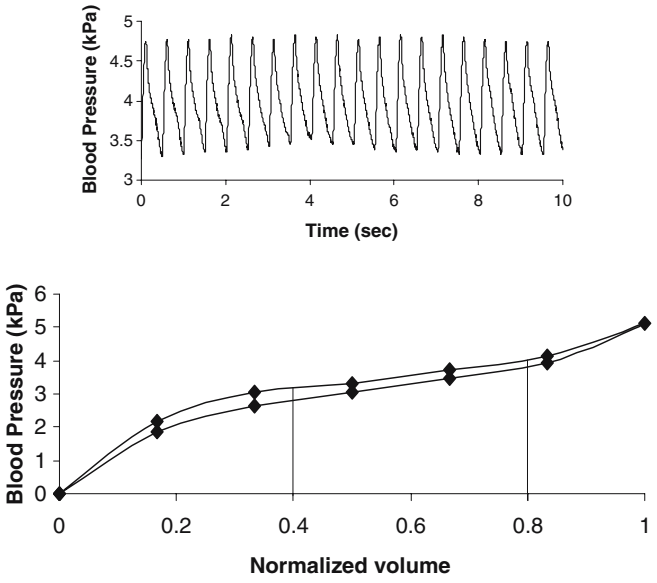

Fig. 1 Pressure–volume loop for a 2.17 g stickleback showing the region integrated (*vertical bars*) to establish mean blood pressure during inflation (*upper curve*) and deflation (*lower curve*). *Inset*, blood pressure recorded from an anaesthetized 5.5 g stickleback by bulbar puncture

The *bulbus arteriosus* was cannulated, using appropriately sized polyethylene tubing, through an incision in the ventricle. The cannula was advanced and tied into the ventricular–bulbar junction. The ventral aorta was ligated at the bulbar–aortic junction, and a saline-filled syringe was connected to the cannula and, via a side arm, to an electromanometer (Deltran DPT-100, Utah Medical Products, Midvale, Utah, USA). Pressures generated with inflation/deflation of the bulbus were recorded on a computer using Labtech Notebook 7.2.0 (Labtech Wilmington, MA, USA), and data analysis was done using AcqKnowledge 3.7.3 (Biopac Systems Inc., Goleta, CA, USA). With large fish it was possible to dispense with the electromanometer and computer, connecting the side arm to a vertical tube. The height of the column of saline in the tube was measured after each injection step. Both measurement systems gave identical results. Unitary volumes were injected in 0.1 μl to 1 ml steps, depending on the size of the bulbus, using Hamilton calibrated syringes (Reno, Nevada, USA) until pressure in the bulbus changed little over a series of injections. The injectate was then removed in steps. If the volume removed was within ±5% of that injected, then the system was assumed to be leak free. The total volume injected depended on the size of the bulbus, but, in presentation of data, volume was normalised to the maximum volume injected.

Blood pressures of living fish were obtained from the published literature, but due to the dearth of these recordings from teleost fish, blood pressure was also measured directly in coho salmon, giant danios and sticklebacks by bulbar puncture. Animals were surgically anaesthetized by immersion in MS 222 (0.4 g/l<10 g body mass, 1 g/l for large coho) and maintained by mixing anaesthetic and oxygenated freshwater as it flowed over the gills. A level of maintenance anaesthesia was judged suitable if the fish displayed bradycardia when flow over the gills was briefly interrupted. In coho, the bulbus was penetrated, blindly, through the wall of the opercular cavity. In giant danios and sticklebacks, the bulbus was exposed ventrally and punctured by a 30-G needle directly attached to the manometer. Frequency response of the manometric system to a square-wave pressure change (Hansen "pop-test") was 80 Hz, with damping of 30% of critical for the coho. The manometers were over-damped for danios and sticklebacks, with 90% response taking 80 msec. Nevertheless, the manometers were adequate to follow pulse pressures at the low cardiac frequencies encountered in these experiments (1 to 1.5 Hz, Fig. 1). The manometers were filled with heparinised saline that contained a few drops of wetting agent per liter. All experiments complied with University of British Columbia Animal Care, certificate # AO3-0250.

Mean pressures were obtained, necrophysiologically, by integrating the area under the plateau of the inflation or deflation portion of the pressure–volume curve of the bulbus (between 40 and 80% of maximum inflation, Fig 1) and compared with mean blood pressures given in the

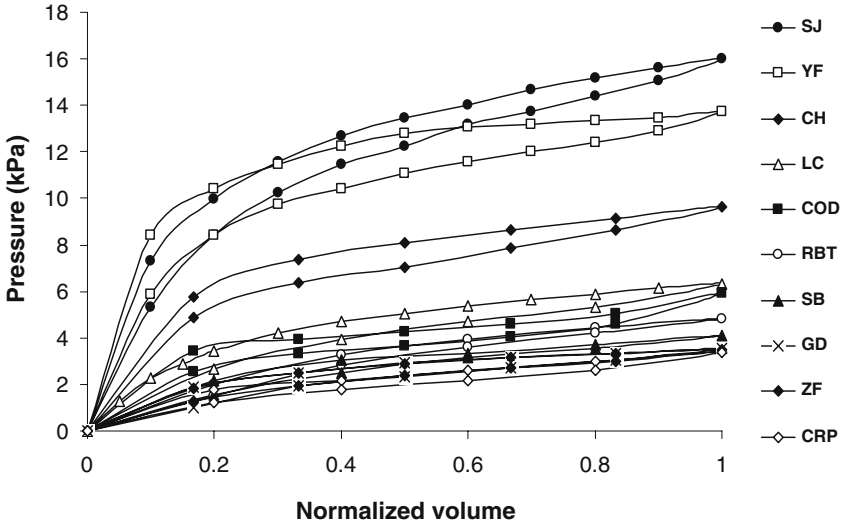

Fig. 2 Pressure–volume loops for the *bulbus arteriosus* of ten species of teleost fish. Species are as follows: *SJ*, skipjack tuna, *Katsuwonus pelamis* (<5 kg); *YF*, yellowfin tuna, *Thunnus albacares* (1.9±0.1 kg); *CH*, coho salmon, *Oncorhynchus kisutch* (3.1±0.4 kg); *LC*, lingcod, *Ophiodon elongates* (<2 kg); *COD*, cod, *Gadus morhua* (>0.5 kg); *RBT*, rainbow trout, *Oncorhynchus mykiss* (0.6 kg); *SB*, stickleback, *Gasterosteus aculeatus* (0.003±0.001 kg); *GD*, giant danio, *D. aequipinnatus* (0.0037±0.0003 kg); *ZF*, zebrafish, *Danio rerio* (0.0006± 0.0001 kg); *CRP*, carp, *Cyprinus carpio carpio* (0.5±0.1 kg)

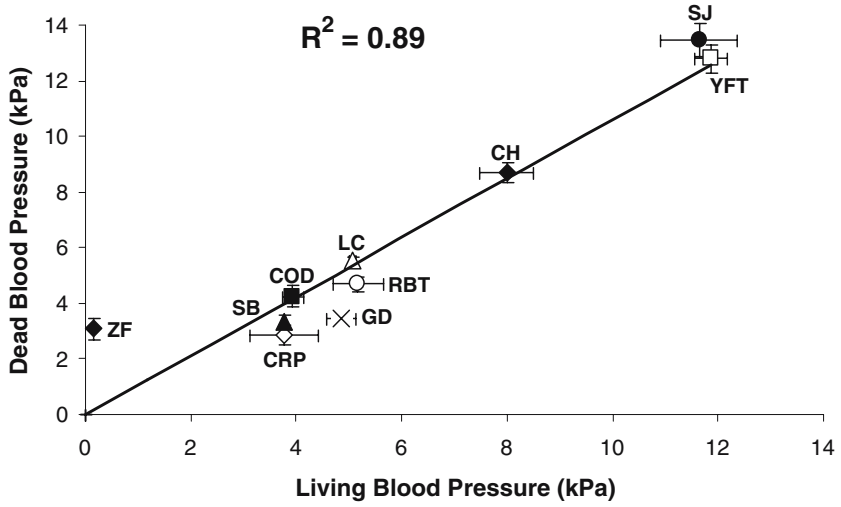

Fig. 3 The relationship between blood pressure of ten species of fish determined in vivo and by integration of the area between 40 and 80% bulbar inflation. Standard error of the mean for in vivo determinations and bulbar inflations are shown for each species. R^2=coefficient of determination. Status and number (N, iv=in vivo; d=dead) of animals used are as follows: *SJ* (1.6±0.3 kg; spinal block), N_{iv}=8, N_d=5 (Bushnell and Brill 1992); *YF* (1.9±0.07 kg; swimming), N_{iv}=8, N_d=8; *CH* (2.6±0.3 kg; anaesthetized), N_{iv}=3, N_d=5; *LC* (>3.8 kg; unanaesthetized), N_{iv}=6, N_d=4 (Farrell 1982); *COD* (0.7±0.15 kg; unanaesthetized), N_{iv}=8, N_d=3 (Platzack et al. 1993); *RBT* (1.2±0.3 kg; swimming), N_{iv}=7, N_d=14; *SB* (0.005±0.0004 kg; anaesthetized), N_{iv}=3, N_d=7; *GD* (0.0037±0.0003 kg; anaesthetized), N_{iv}=4, N_d=2; *ZF* (mass not available; anaesthetized), N_{iv}>5, N_d=5; *CRP* (0.9±0.2 kg; unanaesthetized), N_{iv}=3, N_d=5 (Ngan et al. 1974)

literature or obtained in the present study by integration of the area under the blood pressure traces. Pressures in the living and dead fish were compared using regression techniques after removal of the intercept. The intercept was removed based on the assumption that the line should go through 0 (Neter et al. 1996). Different models were compared by Fisher *z*-transforming correlation coefficients followed by a *z* test (Neter et al. 1996).

Results

Inflation of the freshly isolated bulbus with saline from a syringe and recording pressures generated yielded an r-shaped pressure–volume relation (Fig. 2) unlike the J-shaped curve obtained upon inflation of arteries from all other vertebrates (Braun et al. 2003a,b). The inflation limb of the P–V loop was at a higher pressure than the deflation limb. Hysteresis was low, meaning that 81–94% of the energy expended for a complete cycle of inflation and deflation was conserved (Fig 2).

The question we wished to answer was whether the bulbar plateau pressure corresponded with ventral aortic blood pressure measured in representatives of these species while alive. In Fig. 3, mean pressures obtained on bulbar inflation are plotted against mean ventral aortic pressures determined in vivo. The small number of teleost ventral aortic pressures that exist in the literature (plus data for three species done for this study) showed a strong correlation with those determined necrophysiologically after integrating the area under the inflation portion of the P–V curve (R^2=0.89). The correlation was reduced slightly when plotting mean pressure over 80 to 40% deflation (R^2=0.88), but the difference was not significant.

For the zebrafish, necrophysiological determinations indicated a blood pressure of 3.1±0.4 kPa, which was 15.5 times the only in vivo value for adults (Hu et al. 2001). A plot of residuals strongly indicated that the zebrafish was an outlier, the value falling outside twice the confidence limits of the regression. Eliminating the zebrafish from the regression analysis significantly increased R^2 to 0.93 for integrating under the inflation curve. Plotting the necrophysiologically determined blood pressures from the inflation part of the curve against those in living animals gave a slope not significantly different from 1, and the partial residuals were randomly distributed, indicating no departures from normality (Neter et al. 1996).

Discussion

The exceptionally good correlation between blood pressures determined from living and dead animals means necrophysiological measurement of mean blood pressure can be made in virtually all species of teleosts, with confidence that the pressures reflect those in the living animal. Although there is considerable variation in gross morphology among bulbi from different teleosts, we found it made no difference whether the bulbus was pear shaped or tubiform, elongated or compact or thick or thin walled for all bulbi-yielded r-shaped pressure–volume loops. On the other hand, the plateau pressure varied by more than five times between the species shown in Fig. 2. The plateau of the pressure–volume loop was consistent within a species, however, confirming that the bulbus is tuned to operate at a specific pressure range.

A notable exception was the large difference between living and dead blood pressures in zebrafish. It seems

unlikely that the zebrafish has a bulbus that is not tuned to ventral aortic pressure, but, unfortunately, the zebrafish defied our attempts to record blood pressure in vivo. Giant danios (*Danio aequipinnatus*) are larger (two to three times) than zebrafish and yielded a blood pressure of 3.5± 0.3 kPa on bulbar inflation and 4.9±0.3 kPa by direct bulbar puncture in the anaesthetized animal. Giant danios are phylogenetically distant from zebrafish (Meyer et al. 1995), but do indicate that the genus contains members in which blood pressure seems not only in line with the necrophysiologically determined pressure but also with the activity levels of this group of fishes.

After removing the zebrafish from our deliberations, the coefficient of determination for the relation between blood pressure determined in living and dead fish approached unity. This is surprising since there was considerable variability in the techniques for obtaining living blood pressures, and not all bulbi yielded a flat plateau on inflation. The high R^2 suggests that blood pressure in fishes must be set morphologically, yet, in reality, we know that there is not a unitary pressure in any given fish. During exercise, for example, mean blood pressure of rainbow trout and yellowfin tuna increases by 50% (Kiceniuk and Jones 1977) and 13% (Korsmeyer et al. 1997), respectively. Therefore, the bulbus has to be tuned to a new pressure during exercise, which must be achieved neurally or by circulating vasoactive compounds. Vasoactive compounds, for example, excite or relax smooth muscle within the bulbar wall changing the stiffness of the bulbus and resetting the pressure–volume plateau to a new level (Farrell 1979). Certainly, blockade of smooth muscle in the bulbus with verapamil reduces plateau pressure by 38% in yellowfin tuna and by 25% in rainbow trout (Braun 2001).

Over the course of 1 to 2 days, replicate necrophysiological determinations of blood pressure declined by more than 25% in fishes with moderate to high blood pressures (>7 kPa) and by about half that in fishes with low blood pressures (<4 kPa) despite being refrigerated at 4°C. We attribute this decline in pressure over time to the death of smooth muscle fibers in the bulbus (Braun 2001). Therefore, determinations of blood pressure must be made on freshly killed animals, or the decline in the blood pressure plateau over time must be obtained for the species at hand. Alternatively, we looked at preservation as a way of obviating this problem. There was no significant difference between blood pressure in six juvenile rainbow trout hearts before (2.72±0.42 kPa) and after (2.75±0.31 kPa) preserving them in refrigerated (4°C) 36% isopropyl alcohol for 3 days, indicating that this level of preservation retards the disruption of the smooth muscle and may indeed be useful in any approach to population studies.

Necrophysiological determination of blood pressure promises to open new avenues of research both at the population and individual level in addition to those with an evolutionary perspective. For example, farmed coho salmon develop ventral aortic lesions that may adversely affect blood pressure and perhaps growth potential (Farrell 2002). In addition, fish offer what may be a unique opportunity to study the relation between blood pressure and body mass over many orders of magnitude because all neonatal fishes are small. Finally, at the individual level, necrophysiology allows determination of blood pressure in abyssal fishes as well as in very small or exceptionally large animals in which direct recordings by standard techniques are impractical if not impossible.

Acknowledgements We are grateful to Manuela Gardner for invaluable help with data analysis and preparation of the manuscript and Andreas Fahlman for help with the regression analysis. We are grateful to many people who helped in procuring fish for this study including Richard Brill, Amanda Southwood, Bob Devlin, Mervin Hastings and Brian Bostrom. Financial assistance was provided by a Discovery grant from NSERC (DRJ) and a Cambridge University Gates scholarship (MHB).

References

Binohlan C, Pauly D (1998) The POPCHAR table. In: Froese R, Pauly D (eds) FishBase 98: concepts, design and data sources. ICLARM, Manila pp 120–121

Braun MH (2001) The *bulbus arteriosus* of tuna; form and function. M.Sc. thesis. University of British Columbia, Vancouver

Braun MH, Brill R, Gosline J, Jones DR (2003a) Form and function of the *bulbus arteriosus* in yellowfin tuna (*Thunnus albacares*), bigeye tuna (*Thunnus obesus*) and blue marlin (*Makaira nigricans*): static properties. J Exp Biol 206:3311–3326

Braun MH, Brill R, Gosline J, Jones DR (2003b) Form and function of the *bulbus arteriosus* in yellowfin tuna (*Thunnus albacares*): dynamic properties. J Exp Biol 206:3327–3335

Bushnell PG, Brill RW (1992) Oxygen transport and cardiovascular responses in skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) exposed to acute hypoxia. J Comp Physiol B 162:131–143

Farrell AP (1979) The windkessel effect of the *bulbus arteriosus* in trout. J Exp Zool 209:169–173

Farrell AP (1982) Cardiovascular changes in the unanaesthetized lingcod (*Ophiodon elongates*) during short-term, progressive hypoxia and spontaneous activity. Can J Zool 60:933–941

Farrell AP (2002) Coronary arteriosclerosis in salmon: growing old or growing fast? Comp Biochem Physiol A 132:723–735

Haddad TE, Khairallah AA (1936) A forgotten chapter in the circulation of the blood. Ann Surg 104:1–8

Harvey W (1628) Movement of the heart and blood in animals. An anatomical essay. Trans. by Kenneth J. Franklin (1957). Blackwell, Oxford

Hu N, Yost H, Clark E (2001) Cardiac morphology and blood pressure in the adult zebrafish. Anat Rec 264:1–12

Kiceniuk J, Jones DR (1977) The oxygen transport system in trout (*Salmo gairdneri*) during exercise. J Exp Biol 69:247–260

Korsmeyer KE, Chin Lai N, Shadwick, RE, Graham JB (1997) Oxygen transport and cardiovascular responses to exercise in the yellowfin tuna *Thunnus albacares*. J Exp Biol 200:1987–1997

Meyer A, Ritchie P, Witte K (1995) Predicting developmental processes from evolutionary patterns: a molecular phylogeny of the zebrafish (*Danio rerio*) and its relatives. Philos Trans R Soc Lond B 349:103–111

Neter J, Kutner MH, Nachtsheim CJ, Wasserman W (1996) Applied linear regression models. Irwin Book Team, Chicago

Ngan PV, Yomamori K, Hayu I, Hibiya T (1974) Measurement of blood pressure of carp. Jpn J Ichthiol 21(1):1–8

Platzack B, Axelsson M, Nilsson S (1993) The rennin–angiotensin system in blood pressure control during exercise in the cod *Gadus morhua*. J Exp Biol 180:253–262