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Posterior lymph heart function in two species of anurans: analysis based on both *in vivo* pressure–volume relationships by conductance manometry and ultrasound

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SUMMARY

***Rhinella marina* and *Lithobates catesbeianus* have known differences in the capacity to mobilize lymph to stabilize blood volume following dehydration and hemorrhage. The purpose of these experiments was to assess whether there are interspecific differences in basic lymph heart functions. The end diastolic volumes of posterior lymph hearts averaged 10.8 μl kg^{-1} in *R. marina* and 7.9–10.8 μl kg^{-1} in *L. catesbeianus* by conductance manometry, and 9–32 μl kg^{-1} in *R. marina* by ultasound techniques, which correlated with body mass. Stroke volumes were approximately 20% of end diastolic volumes in both species. Peak systolic pressures and stroke work were correlated with the index of contractility (dP/dt_{max}) in both species. Stroke volume was correlated to stroke work but not peak systolic pressure, end diastolic volume or end diastolic pressure indicating the preload variables do not seem to determine stroke volume as would be predicted from Starling considerations of the blood heart. Renal portal elastance (end systolic pressure/stroke volume) an afterload index did not differ interspecifically, and was equivalent to values for systemic flow indices from mice of equivalent ventricular volume. These data, taken together with predictions derived from mammalian models on the effect of high resistance indicate afterload (renal portal pressure), may be important determinants of posterior lymph heart stroke volume. The shape of the pressure–volume loop is different from an idealized version previously reported, and is influenced by end diastolic volume. Our data indicate that increasing end diastolic pressure and volume can influence the loop shape but not the stroke volume. This indicates that lymph hearts do not behave in a Starling Law manner with increased preload volume.**

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Key words: anuran, lymph hearts, contractility, pressure–volume relationships, power output, stroke volume.

INTRODUCTION

Anuran water turnover rates are approximately an order of magnitude greater than rates in other vertebrate groups in terrestrial environments (Shoemaker and Nagy, 1977). Consequently, dehydration is a constant potential environmental stress. Species have varying tolerance to dehydration, which is generally positively correlated with the degree of terrestriality, indicating selection to compensate for dehydration stress (Thorson and Svihla, 1943; Thorson, 1955; Farrell and MacMahon, 1969). Dehydration ultimately leads to death resulting from a decline in maximal aerobic capacity caused by a compromise of systemic oxygen transport (Shoemaker, 1964; McClanahan, 1967; Hillman, 1978; Hillman, 1987; Hillman, 1991). One hypothesis to explain differential tolerance to dehydration is that anurans have differing abilities to compensate for low blood volume and maintain systemic oxygen transport during dehydration. Blood volumes are greater and better maintained in dehydration-tolerant species (Shoemaker, 1964; Hillman, 1978; Hillman, 1980; Hillman et al., 1987; Hillman and Withers, 1988; Hillman et al., 2010), supporting differential ability to maintain blood volume as a potential mechanism for differential dehydration tolerance. Interstitial fluid can replace dehydrational plasma volume loss in terrestrial species. The mobilization of this fluid reserve into the cardiovascular system is dependent upon a functioning lymphatic system (Zwemer and Foglia, 1943; Baustian, 1988) and appears to be independent of Starling Law-based transcapillary fluid mobilizing mechanisms in anurans (Hillman et al., 1987; Hillman et al., 2010).

In anurans, lymph is generally returned to the venous circulation by two pairs of lymph hearts. The anterior pair is lateral to the third vertebra, under the suprascapular cartilage; the posterior pair, which can be multiple in some anurans, is lateral to the urostyle at the nexus of the subvertebral, lateral, iliac and pubic lymph sacs (Kampmeier, 1969). The fundamental importance of anuran lymph hearts in returning lymph to the circulation has been experimentally documented by both ablation and anesthesia. In toads, if the lymph hearts are destroyed by electrocautery, the toad dies within a few days of hemoconcentration due to lost plasma (Zwemer and Foglia, 1943). Furthermore, cane toads cannot compensate for hemorrhagic stress with cauterized lymph hearts (Baustian, 1988). In frogs, if lymph hearts are stopped by anesthesia, there is also a hemoconcentration resulting from filtered plasma not being returned to the circulation (Baldwin et al., 1993). All lymph flow must pass through the four lymph hearts, so the combined output is the total lymphatic flow. Recent estimates indicate that flow for all four lymph hearts ranges from 0.9 to 5 ml kg^{-1} min^{-1} (Baustian, 1988; Baldwin et al., 1993; Malvin et al., 1995; Jones et al., 1997; Hillman et al., 2010). The magnitude of this flux is remarkable when compared with mammalian values of 0.083 ml kg^{-1} min^{-1} in adult sheep (Brace and Power, 1981).

The ability to compensate for decreases in blood volume by mobilizing lymph is an important regulatory mechanism involved in stabilizing cardiovascular function during dehydration in anurans (Hillman et al., 1987). This suggests the question: Do species with greater lymph-mobilizing capacity have lymph hearts with greater

work capacity? In a two-species comparison, the more terrestrial cane toad, *Rhinella marina*, generates greater lymph heart pressures than does the more aquatic bullfrog, *Lithobates catesbeianus* (DeGrauw and Hillman, 2004). There is some consensus that lymph heart muscle fibers are modified skeletal muscle fibers, based on their embryogenesis (Greber and Schipp, 1990), polynucleation and absence of intercalated discs (Kawaguti, 1967; Rumyantsev and Shmantzar, 1967; Schipp and Flindt, 1968) and the occurrence of satellite-like cells (Rumyantsev and Shmantzar, 1967). Interestingly, the myofibrils do not all run in the same direction along the long axis of the cell, as in skeletal muscle fiber (Lindner and Schaumburg, 1968; Schipp and Flindt, 1968). Given the anatomical similarity to skeletal muscle, it is unlikely that the lymph hearts will behave in a Starling Law manner when stretched like cardiac muscle fibers. Another anatomic feature that differentiates lymph hearts from blood hearts is the external wall, which, in a lymph heart, is anchored to the surrounding tissue by elastic fibers that presumably stretch during systole and shorten during diastole. These external elastic fibers that influence filling characteristics and volume are not present in blood hearts.

The bullfrog has a more limited capacity to compensate for volume stress elicited by dehydration (Hillman et al., 1987) or hemorrhage (Hillman and Withers, 1988), compared to the cane toad. The objective of our study was to characterize the pressure–volume characteristics of the posterior lymph hearts of the semi-aquatic bullfrog *L. catesbeianus* and the terrestrio-fossorial cane toad *R. marina*, and to determine whether differences in lymph-mobilizing capacity are the result of differences in heart performance characteristics. In addition, we have simultaneously quantified pressure–volume characteristics in anuran posterior lymph hearts using both conductance and ultrasound technologies, for the first time.

MATERIALS AND METHODS

Six *Rhinella marina* (Chaparro et al., 2007), ranging in mass between 307 and 639 g (mean 448 g), were used in the conductance experiments, and five individuals of 127–239 g (mean 160 g) were used in the ultrasound experiments. Five *Lithobates catesbeianus* (Frost et al., 2006) of 400–670 g (mean 524 g) were used in the conductance experiments. Ultrasound measurements could not be performed on *L. catesbeianus*, because the posterior lymph hearts were effectively shielded by dense connective tissue that prevented ultrasound images. All animals were purchased from commercial suppliers (Strictly Reptiles, Hollywood, FL, USA or Charles D. Sullivan Co. Inc. Nashville, TN, USA). They were maintained and experiments were conducted at 20–22°C. Animals had access to water at all times.

Surgery, pressure and conductance measurement of volume

Animals were anesthetized by immersion of the ventral surface in buffered 0.3% MS-222. The posterior lymph heart was exposed and flared PE 10 was inserted through an ostium and secured to the lymph heart wall by purse-string ligatures using 6-0 silk. A Millar mikro-tip pressure–volume combination catheter transducer catheter (1.4 Fr) model SPR-719 (Millar Instruments Inc., Houston, TX, USA) was inserted along the long axis of the heart *via* a 2 mm incision in the posterior border and secured again by purse-string ligatures using 6-0 silk. In some animals, a PE 10 infusion catheter and Millar pressure probe (model no. SPR 524) were inserted into the iliofibular lymphatic sinus, which connects to the iliac sac and then the posterior lymph heart. This allowed the infusion of 0.8% saline into the lymphatic compartment around the lymph heart to experimentally raise its diastolic pressures. The animals were then allowed to recover from the anesthesia. Because animal movement led to the probe pulling out of the lymph heart in some cases, we were only able to record one pressure–volume loop from some animals.

At the completion of the study, in five *R. marina* and four *L. catesbeianus,* 50 µl of 0.8% saline was infused *via* the lymph heart pressure catheter to determine the conductance volume change associated with this infusion.

Signal recording and calibration

The lymph heart catheter was attached to a pressure transducer (P23 Statham, Oxnard, CA, USA), which in turn was connected to a bridge amplifier (CB Sciences, Dover, NH, USA; model ETH-400), and the pressure trace was stored in a computer using a PowerLab data acquisition system. Lymph heart rate was continuously calculated from the pressure signal *via* an acquisition software tachograph. The Millar conductance catheter was connected to an Aria pressure–volume system, and the output was collected and stored in a computer using a PowerLab data acquisition system. The Aria system was operated at an excitation frequency of 5 kHz with an output filter of 500 Hz.

The Millar 1.4 Fr catheter (SPR-719) is a composite of four conductance electrodes separated by 4.5 mm, which is the distance from the apex to the aortic valve of a mouse heart, the experimental model system for which the device was developed. This device was well suited to our study, because 4.5 mm is the distance of the long axis of the posterior lymph hearts of the toads and frogs we used. The principle of this system is that volume is proportionate to the measured conductance according to the following formula: volume = raw conductance signal$\times(4.5)^2$/conductivity of lymph. The volume–conductance relationship was calibrated after the method of Yang et al. (Yang et al., 1999), using 0.8% saline to mimic lymph and tubing of a variety of diameters filled to a depth of 6 mm to vary volume (see Fig. 1). Volumes are expressed on a mass-specific basis (kg^{-1}) assuming an isometric relationship to body mass.

Individual pressure–volume loops were analyzed for index of contractility (dP/dt), volume change, etc., using the PowerLab software, and graphical loops were produced using the digitized PowerLab recordings exported into Prism 4. These loops were saved as an image for graphical analysis of the loop area using NIH Image 1.3. Stroke work (SW) was calculated as $mJ\,kg^{-1}$ (= loop area in $ml\,kPa^{-1}\,kg^{-1}$ body mass). Power output was calculated as $mW\,kg^{-1}$ [= SW lymph heart rate (beats min^{-1}) 60 s^{-1}].

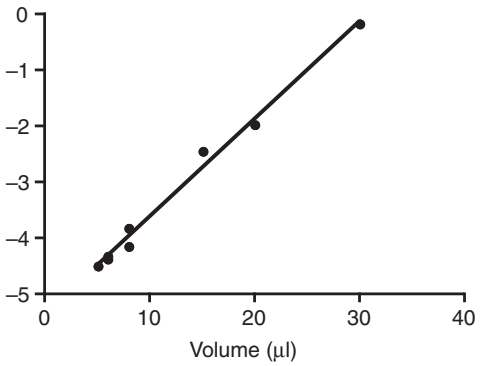

Fig. 1. Calibration curve for Millar conductance catheter placed in tubing of differing diameters filled to a depth of 6 mm with 0.8% saline. Conductance in expressed as arbitrary units.

We used linear regression to assess the relationship between two variables, and two-way *t*-tests to compare species differences. We considered $P<0.05$ significant.

Ultrasonic volume determination

In five anesthetized *R. marina*, we used a high-resolution ultrasonic imaging system (Visual Sonics Vevo 660, Visual Sonic Inc., Toronto, ON, Canada) to determine the volume of a posterior lymph heart. The ultrasonic transducer (RMV707) was set to a frequency of 30 MHz with an image depth of 14 mm. For the purposes of the measurement, the anesthetized animal was placed on a 6 cm^2 stage under the ultrasonic transducer. Ultrasonic gel was placed on the animal above the estimated location of the posterior lymph heart. The transducer was then lowered into position in contact with the gel interface. Once a clear image was obtained along both the long axis and cross section of the lymph heart, the image was recorded and stored on computer at a frame rate of 3 Hz (see supplementary Movie 1). The heart volume was calculated using two estimates of shape: a cylinder ($\text{length}\times\pi r^2$) and a prolate spheroid ($[(\text{length}/2\times\text{height}/2)^2\times 4/3\pi$). Representative ultrasound images of a posterior lymph heart in diastole and systole are presented in Fig. 2A,B.

At the conclusion of each experiment all surgically instrumented animals were killed with an overdose of buffered 0.3% MS-222.

RESULTS

Conductance pressure–volume loops

There was no typical pattern to the pressure–volume loop (Fig. 3). There was no isovolumetric relaxation phase to the cycles. The diastolic filling phase was characterized by increasing volumes that could be broken into two categories: (1) volume increased during periods of decreasing pressures; (2) volume increased during periods of increasing pressures. The first category could be attributed to the shortening of elastic fibers lengthened during lymph heart muscle contraction, and the second category to the compliance characteristics of the lymph heart. There was generally no 'isovolumetric contraction period'. Instead, the general pattern was that volume decreased during both the rising and declining systolic pressure phase. The end of relaxation was frequently associated with an increase in volume, indicating lymph entry. The end diastolic volume was correlated with body mass (r^2=0.65) in *R. marina* but not *L. catesbeianus* (r^2=–0.26). End diastolic volume was correlated with the peak systolic pressure in *R. marina* (r^2=0.81) but not in *L. catesbeianus* (r^2=0.2).

The effect of variation in end diastolic pressure on pressure–volume loops during infusion of saline into the iliac sac is shown in Fig. 4. Increased end diastolic pressure increased end systolic pressure with little change in stroke volume. The shape of the loop changed to create a 'tail' indicating that the initial period of contraction leads immediately to a decrease in volume.

Lymphodynamics

Comparisons of the lymphodynamic function from this study are presented in Table 1. *R. marina* had a significantly increased ($P<0.001$) index of contractility [i.e. maximum derivative of change in systolic pressure over time (dP/dt_{max})] compared with *L. catesbeianus*. The index of contractility was significantly correlated ($P<0.0001$; Fig. 5) with peak systolic pressure in *R. marina* (r^2=0.87) and *L. catesbeianus* (r^2=0.85) as well as with stroke work ($P<0.0001$; Fig. 6) for both *R. marina* (r^2=0.33) and *L. catesbeianus* (r^2=0.67). The index of contractility indicates that lymph hearts of *R. marina* are capable of greater indexed work than those of *L. catesbeianus*. Stroke volume was also correlated to stroke work ($P<0.0001$; Fig. 7) in both *R. marina* (r^2=0.3) and *L. catesbeianus* (r^2=0.82). Stroke volume was not correlated with either end diastolic volume or end diastolic pressure.

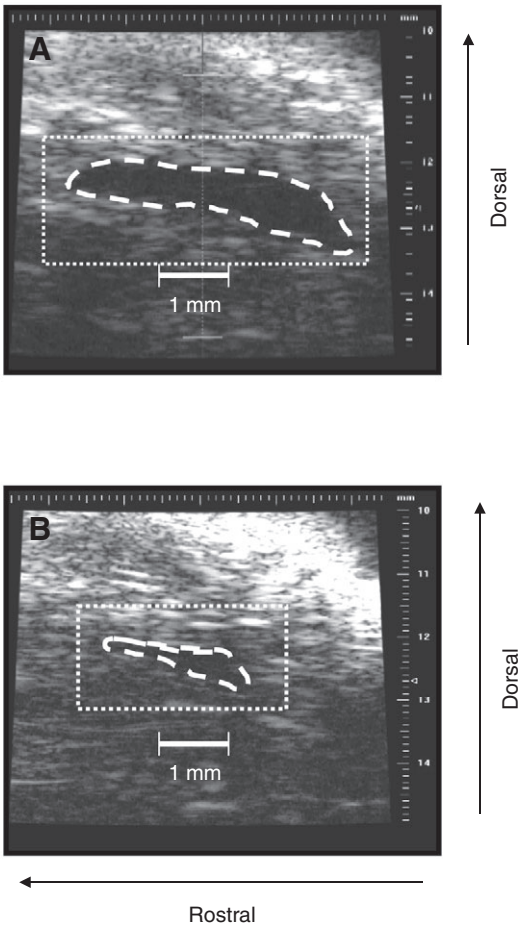

Fig. 2. Captured ultrasound images of a single posterior lymph heart in one *R. marina* at end diastole (A) and end systole (B). Dashed lines outline the interior margins of the lymph heart. Scale bar, 1 mm.

Ultrasonic volume measurements

The end diastolic volume estimated by the volume equation for a cylinder was 3.6±1.0 µl. The end diastolic volume estimated approximating the shape of the lymph heart as a prolate spheroid was 2.4±0.7 µl. The volume of the lymph hearts was significantly related to body mass ($P<0.005$, r^2=0.95).

In a lymph heart contracting under anesthesia (animal mass 136 g), the end diastolic volume was 0.40 µl whereas the end systolic volume was 0.27 µl for a stroke flow of 0.13 µl. Ejection fraction was in the 20–30% range, similar to the conductance measurements.

DISCUSSION

R. marina and *L. catesbeianus* have known differences in their capacity to mobilize lymph to stabilize blood volume following dehydration and hemorrhage (Hillman et al., 1987; Hillman and Withers, 1988). The purpose of these experiments was to assess whether interspecific differences in basic lymph heart function exist. Lymph hearts and blood hearts fill differently: lymph hearts

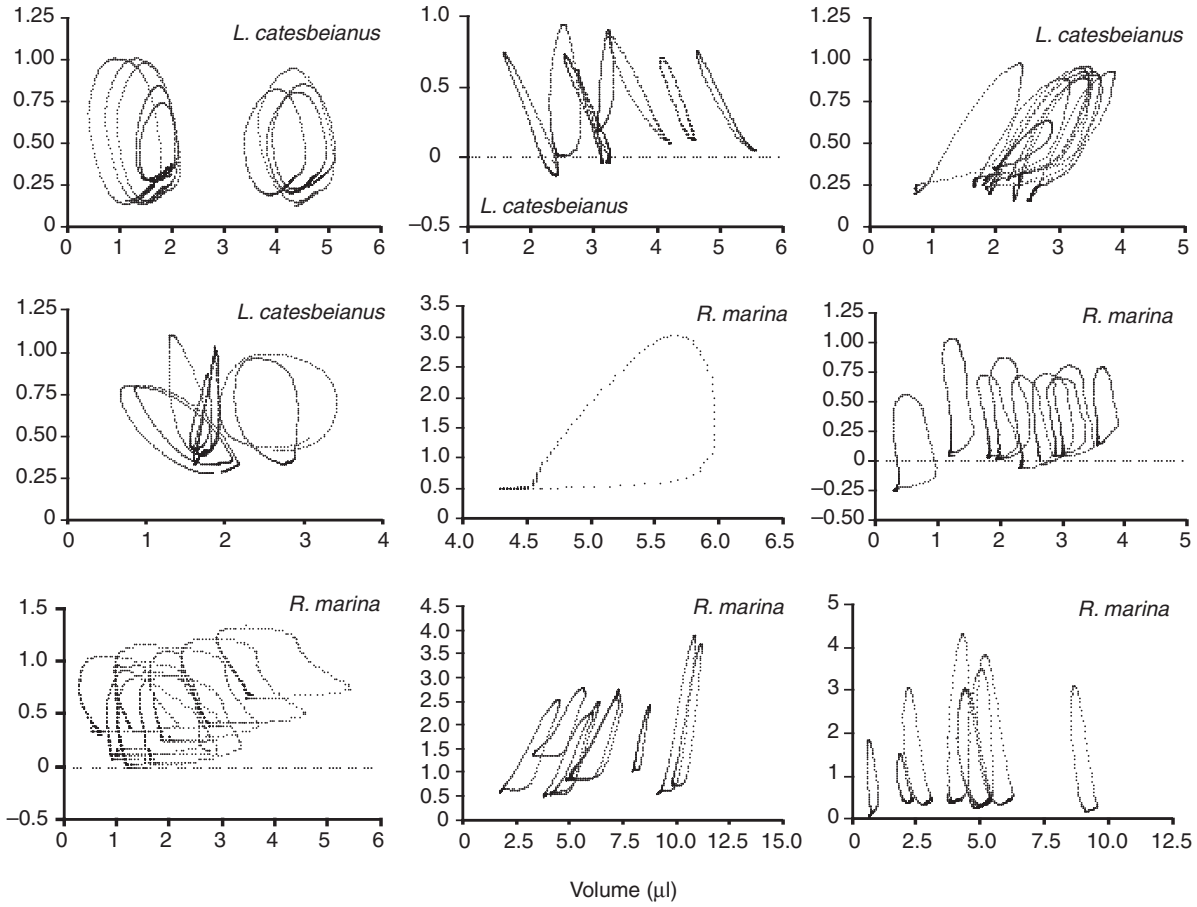

Fig. 3. Representative conductance pressure (kPa)–volume (μl) loops for four *L. catesbeianus* and five *R. marina*.

have an open and extremely compliant lymph sac return, and blood hearts have a closed venous return. The passive nature of the lymph sacs cannot account for lymph return to fill dorsally located lymph hearts (Hillman et al., 2004), in contrast to the blood system, in which even the mean circulatory filling pressure will fill the heart. Movement of lymph to the posterior lymph hearts requires a combination of pressure changes initiated by skeletal muscle contraction and lung expiration (Drewes et al., 2007; Hedrick et al., 2007). Also, lymph hearts eject into vertebral veins, hence the afterload pressure is venous, as opposed to arterial as occurs in blood hearts. Functionally, lymph hearts may interspecifically differ in afterload characteristics, preload characteristics, and/or characteristics that generate intrinsic force. It is important to point out that the measurements presented here were made on fully hydrated individuals just recovered from anesthesia for the conductance experiments. Anesthesia would have interfered with both the skeletal muscle contractions (Drewes et al., 2007) and ventilation events (Hedrick et al., 2007) necessary to move lymph dorsally. Therefore, the experimental preload conditions on the lymph hearts just coming out of anesthesia may not reflect normal preload conditions for these beating hearts. Consequently, we cannot comment on potential interspecific characteristics in either transport of lymph to the lymph hearts or lymph volumes as potential additional sources of interspecific variation.

The lack of correlation between end diastolic volume or end diastolic pressure and stroke volume in lymph hearts does not fit preload Starling Law predictions for blood hearts. Hence, preload variables do not appear to be the primary determinants of stroke volume in posterior lymph hearts. The arterial elastance (end systolic pressure/stroke volume) is frequently considered the most reliable index of afterload on blood hearts (Yang et al., 1999). The renal portal elastance did not differ interspecifically, and the values fall within the range of arterial elastance for left ventricles in mice (Yang et al., 1999; Shi et al., 2001; Nishio et al., 2002). Because

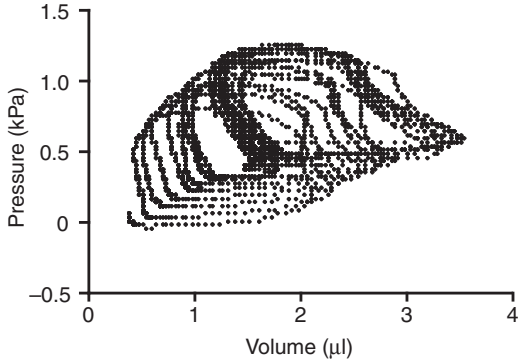

Fig. 4. A series of pressure (kPa)–volume (μl) loops as 0.8% saline is infused into the lymphatic sac surrounding a posterior lymph heart in one *R. marina*. This illustrates that the end diastolic pressure increases the end systolic pressure of the heart, but stroke volume is independent of end diastolic volume and pressure.

Table 1. Lymph heart characteristics measured by conductance manometry for *R. marina* and *L. catesbeianus*

	R. marina (*N*=6)	*L. catesbeianus* (*N*=5)	*P*
Mean lymph heart pressure (kPa)	0.53±0.01	0.47±0.01	0.7
Stroke volume (μl)	1.30±0.27	1.28±0.31	0.9
Indexed stroke volume (μl kg^{-1})	3.1±0.7	2.7±0.8	0.58
Lymph heart frequency (beats min^{-1})	53±5	53±2	0.9
Lymph heart output (ml min^{-1} kg^{-1})	0.15±0.03	0.14±0.04	0.58
d*P*/d*t* max (kPa s^{-1})	11.5±1.1	4.7±0.3	0.0001
Systolic pressure (kPa)	1.90±0.45	1.00±0.14	0.07
Stroke work (mJ kg^{-1})	0.003±0.0008	0.001±0.0005	0.1
Power output (mW kg^{-1})	2.9±0.7	1.1±0.5	0.06
Renal portal elastance (kPa μl^{-1})	0.91±0.18	0.89±0.18	0.98
Measured change in lymph heart volume per 50 μl saline infusion (μl kg^{-1})	10.6±4.9	11.0±5.3	0.9

Values are means ± s.e.m.

the volume of posterior lymph hearts of these amphibians is similar to the left ventricular volume of mice hearts, it indicates equivalent afterload resistances for both. This suggests substantial afterload resistance on the lymph hearts, even though they are emptying into relatively low-pressure renal portal veins. The combination of a lack of correlation of stroke volume to preload variables and a high afterload index may indicate that pressures in the vertebral veins are significant determinants of stroke volume in posterior lymph hearts.

The index of contractility was significantly greater in *R. marina* compared with *L. catesbeianus*. Since contractility was significantly correlated with peak systolic pressure and stroke work, this suggests that *R. marina* have stronger lymph hearts capable of more work than the lymph hearts of *L. catesbeianus*. The absolute values of lymph heart contractility are 20–30% of values for ventricular contractility of blood hearts in these two species (Hillman, 1984). Our estimates of both power output and peak systolic pressure did not vary interspecifically. Previous studies with much larger sample sizes have found higher peak systolic pressures in *R. marina* compared with *L. catesbeianus* (DeGrauw and Hillman, 2004). Consequently, we feel confident that the more terrestrial anuran *R. marina* has a greater capacity to pump lymph. The maintenance of blood volume by mobilizing lymph following both hemorrhage and dehydration illustrates the capacity of lymph heart work, the volume of lymph present in the system, and the capacity to transport this lymph to the lymph hearts. Whether interspecific differences exist in these variables remains to be determined.

The mass-indexed posterior lymph heart end diastolic volume is lower than that previously reported for *R. marina* by about 50–60% (Jones et al., 1997). Jones et al. (Jones et al., 1997) reported a pressure–volume loop for lymph hearts based on a series of assumptions whereas our pressure–volume loops are based on measurements. As can be seen in Fig. 8, our measurements differ dramatically in shape, indexed diastolic volume, and indexed stroke volume from the idealized loop reported by Jones et al. (Jones et al., 1997).

End diastolic volumes of the posterior lymph heart of *R. marina* measured by conductance were 10.8±1.0 μl kg^{-1}, by ultrasound 22.0±4.1 μl kg^{-1} and 43.0±0.01 μl kg^{-1} by an injection method (Jones et al., 1997). We feel that the actual end diastolic volume probably lies somewhere in between the conductance and ultrasound determinations. The ultrasound measurements were on animals that were anesthetized, whereas the conductance measurements were on animals just recovered from the anesthesia. Consequently, contractions were sporadic in the ultrasound measurements. This sporadic activity would allow fluid to accumulate in the lymph hearts, increasing end diastolic volumes. The injection of fluid into the lymph heart to measure lymph heart volume, as used by Jones et al. (Jones et al., 1997), has a reported error estimate of 0.01 μl kg^{-1}. This method of determining the end diastolic volume may have an increased potential for movement of the injected volume from the lymph heart into the vertebral vein. Injections of 50 μl of saline into both species resulted in a conductance-determined volume increase of only

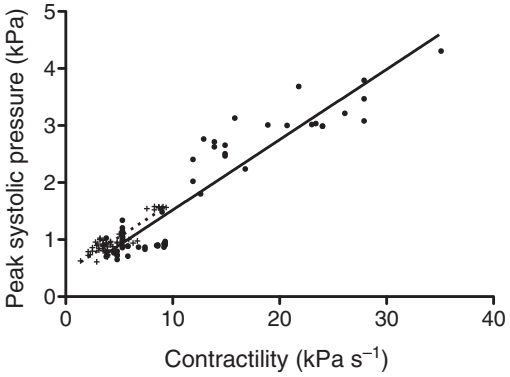

Fig. 5. The relationship between maximum contractility (kPa s^{-1}) and peak systolic pressure (kPa) of the posterior lymph hearts for *R. marina* (circles) and *L. catesbeianus* (crosses). The lines (solid for *R. marina* and dashed for *L. catesbeianus*) show a linear regression fitted to the data.

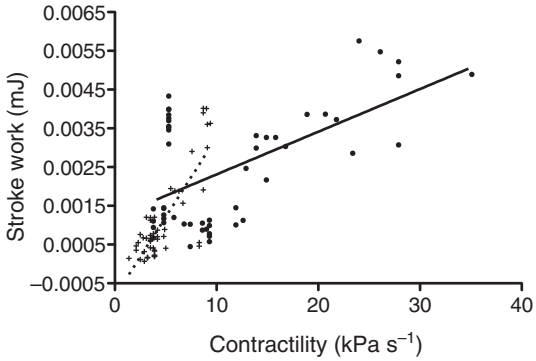

Fig. 6. The relationship between maximal contractility (kPa s^{-1}) and stroke work (mJ) of posterior lymph hearts for *R. marina* (circles) and *L. catesbeianus* (crosses). The lines (solid for *R. marina* and dotted for *L. catesbeianus*) show linear regression fits to the data.

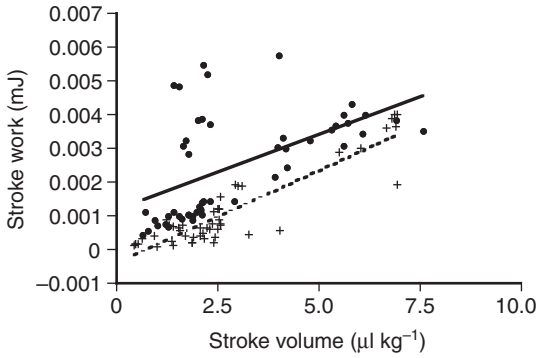

Fig. 7. The relationship between variation in stroke volume (μl kg^{-1}) and stroke work (mJ) of posterior lymph hearts for *R. marina* (circles) and *L. catesbeianus* (crosses). The lines (solid for *R. marina* and dotted for *L. catesbeianus*) show linear regression fits to the data.

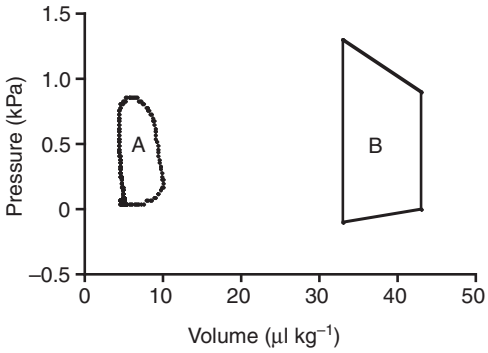

Fig. 8. A representative pressure (kPa)–volume (μl kg^{-1}) loop (A) taken from *R. marina* (this study) compared with the only previously published pressure–volume loop for a lymph heart (B) (Jones et al., 1997).

approximately 10 μl (Table 1). This indicates that 40 μl of the injected fluid presumably entered the vertebral vein during the injection period. This methodological problem could account for the discrepancy between the Jones et al. (Jones et al., 1997) study and our measurements of diastolic volumes. Based on these considerations, we feel that our reported measured values more accurately reflect end diastolic volume in the posterior lymph hearts of these species.

The stroke volumes reported by Jones et al., 1997 (8.9±1.4 μl kg^{-1}), were based on pulsatile Doppler flow measurements in the vertebral vein. A previous study in *R. marina* using lymph collection methods reported a posterior lymph heart output of 0.093 ml min^{-1} kg^{-1} which, given the reported heartbeat frequency of 48 beats min^{-1} calculates to a stroke volume of 1.9 μl kg^{-1} (Jones et al., 1992). Our conductance stroke volume estimates were 3.1±0.7 μl kg^{-1}. The Doppler flow probes of Jones et al. (Jones et al., 1997) were calibrated with a solution that had a hematocrit of 2–4%, which is about an order of magnitude lower than average in the circulation. Doppler signals are sensitive to blood hematocrit, hence by calibrating with a low hematocrit solution and measuring flow in a higher hematocrit solution (i.e. vertebral vein), stroke volume would be overestimated. Our conductance measurements were calibrated and made on a cell-free solution, similar to lymph, hence there was no systematic potential error in the methodology related to differences in conductivity.

Calculated ejection fractions of 20–30% are obtained if the independent data of Jones et al. (Jones et al., 1997) and our conductance data are used. Ejection fractions of 40–80% are achieved if stroke volumes reported by Jones et al. (Jones et al., 1997) are used in conjunction with the end diastolic volumes from our studies. From our ultrasound measurements it was clear that the ejection fraction was a small proportion of diastolic volume (20–30%), which is also the average ejection fraction from our conductance measurements. We feel that Jones et al. (Jones et al., 1997) overestimated both end diastolic volume and stroke volume, for the reasons delineated above, whereas their ejection fraction data are in total agreement with our conductance and ultrasound values. It should be noted that our conductance values were on individuals just out of anesthesia whereas those of Jones et al. (Jones et al., 1997) were on fully recovered animals. Consequently, one possible explanation for the difference in the results might be a depression of lymph heart function in our experiments. Ejection fractions of mammalian mesenteric lymphatics are in the range of 45–65% (Ohhashi et al., 1980), which is about double the fraction of the posterior lymph heart. Mammalian lymphatic vessels contract in a peristaltic fashion (Ohhashi et al., 1980), which may account for the higher ejection fractions. There was no indication of a peristaltic pattern of contraction from our ultrasound measurements of the posterior lymph hearts. The systolic pressures generated by mammalian lymphatic vessels are in the same range as those generated by anuran posterior lymph hearts.

Our data portray lymph hearts at rest differing little in stroke flow, heart rate or indexed output between the species. This would imply equivalent rates of lymph volume flux in the two species and is consistent with equivalent rates of plasma volume turnover (Hillman et al., 2010) and whole body transcapillary filtration coefficients (Hancock et al., 2000) for these two species. Variation in both stroke volume and systolic pressure contributed to variation in work. The stroke work was approximately two orders of magnitude lower than left ventricular stroke work in mice (Yang et al., 1999; Shi et al., 2001; Nishio et al., 2002). This difference in work between mouse hearts and the posterior lymph hearts of amphibians, taken in relation to equivalent afterload indices, reinforces the potential importance of afterload determining output for posterior lymph hearts. The stroke work and power output of posterior lymph hearts are both only about 0.05% of values for amphibian blood hearts (Acierno et al., 1994). The standard metabolic rate or power output of a 1 kg toad is approximately 0.25 W (Secor, 2005). The power output of an individual posterior lymph heart was 0.0026 mW for a 1 kg toad. Therefore, the pumping cost of the posterior lymph heart represents only 0.001% of standard metabolism. The four lymph hearts of anurans are responsible for returning about 1% of the blood volume per minute. The viscosity of lymph is about one third that of blood in amphibians (Weathers, 1976). Work demands on lymph hearts are much less than the work demands of the blood heart. Consequently the low stroke work and power output of an individual heart is not unexpected.

The shape of the pressure–volume loop from our measurements is unlike the idealized loop reported by Jones et al. (Jones et al., 1997), and the standard blood heart loop of mice using equivalent techniques (Yang et al., 1999; Shi et al., 2001; Nishio et al., 2002). We have demonstrated that increasing end diastolic pressure and volume can influence the loop shape but not the stroke volume. This indicates that lymph hearts do not behave in a Starling Law manner

with increased preload volume. As volume and end diastolic pressure increase, there was an immediate decrease in volume during the initial stages of contraction. This is probably a consequence of end diastolic pressure being close to renal portal pressure. Overall, the results we report here differ from the only other published pressure–volume loop in lymph heart stroke volume, end diastolic volume and the shape of the pressure–volume loop (Jones et al., 1997).

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REFERENCES

Acierno, R., Gattuso, A., Cerra, M. C., Pellegrino, D., Agnisola, C. and Tota, B. (1994). The isolated and perfused working heart of the frog, *Rana esculenta:* an improved preparation. *Gen. Pharmacol.* **25**, 521-526.

Baldwin, A. L., Ferrer, P., Rozum, J. S. and Gore, R. W. (1993). Regulation of water balance between blood and lymph in the frog, *Rana pipiens*. *Lymphology* **26**, 4-18.

Baustian, M. (1988). The contribution of lymphatic pathways during recovery from hemorrhage in the toad *Bufo marinus*. *Physiol. Zool.* **61**, 555-563.

Brace, R. A. and Power, G. G. (1981). Thoracic duct lymph flow and protein flux dynamics: responses to intravascular saline. *Am. J. Physiol.* **240**, R282-R288.

Chaparro, J. C., Pramuk, J. B. and Gluesenkamp, A. G. (2007). A new species of arboreal *Rhinella* (Anura: Bufonidae) from a cloud forest of southeastern Peru. *Herpetologica* **63**, 203-212.

DeGrauw, E. A. and Hillman, S. S. (2004). General function and endocrine control of the posterior lymph hearts in *Bufo marinus* and *Rana catesbeiana*. *Physiol. Biochem. Zool.* **77**, 594-600.

Drewes, R. C., Hedrick, M. S., Hillman, S. S. and Withers, P. C. (2007). Unique role of skeletal muscle contraction in vertical lymph movement in anurans. *J. Exp. Biol.* **210**, 3931-3939.

Farrell, M. P. and MacMahon, J. A. (1969). An eco-physiological study of water economy in eight species of tree frogs (Hylidae). *Herpetologica* **25**, 279-294.

Frost, D. R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C. F. B., de Sá, R. O., Channing, A., Wilkinson, M., Donnellan, S. C. et al. (2006). The amphiban tree of life. *Bull. Am. Mus. Nat. Hist.*, **297**, 1-370.

Greber, K. and Schipp, R. (1990). Early development and myogenesis of the posterior anuran lymph hearts. *Anat. Embryol.* **181**, 75-82.

Hancock, T. V., Hoagland, T. M. and Hillman, S. S. (2000). Whole-body systemic transcapillary filtration rates, coefficients, and isogravimetric capillary pressures in *Bufo marinus* and *Rana catesbeiana*. *Physiol. Biochem. Zool.* **73**, 161-168.

Hedrick, M. S., Drewes, R. C., Hillman, S. S. and Withers, P. C. (2007). Lung ventilation contributes to vertical lymph movement in anurans. *J. Exp. Biol.* **210**, 3940-3945.

Hillman, S. S. (1978). The roles of oxygen delivery and electrolyte levels in the dehydrational death of *Xenopus laevis*. *J. Comp. Physiol. B* **128**, 169-175.

Hillman, S. S. (1980). Physiological correlates of differential dehydration tolerance in anuran amphibians. *Copeia* **1980**, 125-129.

Hillman, S. S. (1984). Inotropic influences of dehydration and hyperosmolal solutions on amphibian cardiac muscle. *J. Comp. Physiol. B* **154**, 325-328.

Hillman, S. S. (1987). Dehydrational effects on cardiovascular and metabolic capacity in two amphibians. *Physiol. Zool.* **60**, 608-613.

Hillman, S. S. (1991). Cardiac scope in amphibians: transition to terrestrial life. *Can. J. Zool.* **69**, 2010-2013.

Hillman, S. S. and Withers, P. C. (1988). The hemodynamic consequences of hemorrhage and hypernatremia in two amphibians. *J. Comp. Physiol. B* **157**, 807-812.

Hillman, S. S., Zygmunt, A. and Baustian, M. (1987). Transcapillary fluid forces during dehydration in two amphibians. *Physiol. Zool.* **60**, 339-345.

Hillman, S. S., Hedrick, M. S., Withers, P. C. and Drewes, R. C. (2004). Lymph pools in the basement, sump pumps in the attic: the anuran dilemma for lymph movement. *Physiol. Biochem. Zool.* **77**, 161-173.

Hillman, S. S., DeGrauw, E. A., Hoagland, T., Hancock, T. and Withers, P. (2010). The role of vascular and interstitial compliance and vascular volume in the regulation of blood volume in the regulation of blood volume in two species of anuran. *Physiol. Biochem. Zool.* **83**, 55-67.

Jones, J. M., Wentzell, L. A. and Toews, D. P. (1992). Posterior lymph heart pressure and rate and lymph flow in the toad *Bufo marinus* in response to hydrated and dehydrated conditions. *J. Exp. Biol.* **169**, 207-220.

Jones, J. M., Gamperl, A. K., Farrell, A. P. and Toews, D. P. (1997). Direct measurement of flow from the posterior lymph hearts of hydrated and dehydrated toads (*Bufo marinus*). *J. Exp. Biol.* **200**, 1695-1702.

Kampmeier, O. F. (1969). *Evolution and Comparative Morphology of the Lymphatic System*. Springfield, IL: Thomas.

Kawaguti, S. (1967). Electron microscopic study on the cross striated muscle in the frog lymph heart. *Biol. J. Okayama Univ.* **13**, 13-32.

Lindner, E. and Schaumburg, G. (1968). Zytoplasmatische Filamente in den quergestreiften Muskelzellen des kaudalen Lymphherzens von *Rana temporaria*. L. *Z. Zellforsch. Mikrosk. Anat.* **84**, 549-562.

Malvin, G. M., Macias, S., Sanchez, M., Dasalla, R., Park, A. and Duran, M. (1995). Lymphatic regulation of hematocrit in the toad *Bufo woodhousei*. *Am. J. Physiol.* **269**, R814-R821.

McClanahan, L. (1967). Adaptations of the spadefoot toad, *Scaphiopus couchii* to desert environments. *Comp. Biochem. Physiol.* **20**, 73-90.

Nishio, R., Sasayama, S. and Matsumori, A. (2002). Left ventricular pressure-volume relationship in a murine model of congestive heart failure due to acute viral myocarditis. *J. Am. Coll. Card.* **40**, 1506-1514.

Ohhashi, T., Azuma, T. and Sakaguchi, M. (1980). Active and passive mechanical characteristics of bovne mesenteric lymphatics. *Am. J. Physiol.* **239**, H88-H95.

Rumyantsev, P. and Shmantzar, I. A. (1967). Ultrastructure of muscle fibers of the frog lymph heart. Citologiya **9**, 1129-1136.

Schipp, R. and Flindt, R. (1968). Zur feinstruktur und innervation der lymphherzmuskulatur der Amphibien (*Rana temporaria*). *Z. Anat. Entwicki Gesch.* **127**, 232-253.

Secor, S. M. (2005). Physiological responses to feeding, fasting and aestivation for anurans. *J. Exp. Biol.* **208**, 2595-2608.

Shi, J., Larson, D. F., Yang, B., Hunter, K., Gorman, M., Montes, S., Beischel, J. and Watson, R. R. (2001). Differential effects of acute ethanol treatment on cardiac contractile function in young adult and senescent mice. *Alcohol* **24**, 197-204.

Shoemaker, V. H. (1964). *Physiological Effects of Water Deprivation in a Toad,* Bufo marinus. Ph.D. Dissertation, University of Michigan, USA.

Shoemaker, V. H. and Nagy, K. A. (1977). Osmoregulation in amphibians and reptiles. *Annu. Rev. Physiol.* **39**, 449-471.

Thorson, T. B. (1955). The relationship of water economy to terrestrialism in amphibians. *Ecology* **36**, 100-116.

Thorson, T. B. and Svhila, A. (1943). Correlation of the habitats of amphibians with their ability to survive the loss of body water. *Ecology* **24**, 374-381.

Weathers, W. W. (1976). Influence of temperature on the optimal hematocrit of the bullfrog (*Rana catesbeiana*). *J. Comp. Physiol. B* **105**, 173-184.

Yang, B., Larson, D. F. and Watson, R. (1999). Age-related left ventricular function in the mouse: analysis based on *in vivo* pressure-volume relationships. *Am. J. Physiol.* **277**, H1906-H1913.

Zwemer, R. L. and Foglia, U. G. (1943). Fatal loss of plasma after lymph heart destruction in toads. *Proc. Soc. Exp. Biol. Med.* **53**, 14-17.