

# Effects of Temperature and Physical Activity on Blood Flow Shunts and Intracardiac Mixing in the Toad *Bufo marinus*

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## ABSTRACT

Blood flow in systemic ( $\dot{Q}_{\text{sys}}$ ) and pulmocutaneous ( $\dot{Q}_{\text{pul}}$ ) arteries was measured as a function of body temperature (10°, 20°, and 30°C) at rest and following enforced physical activity in conscious, adult cane toads (*Bufo marinus*). Arterial and mixed venous hemoglobin concentration ( $C_{\text{Hb}}$ ) and total oxygen content ( $\text{CO}_{2,\text{tot}}$ ) were measured in a separate group under identical conditions. Heart rate ( $f_{\text{H}}$ ) and total flow ( $\dot{Q}_{\text{tot}}$ ) increased significantly ( $P < 0.001$ ) with elevated temperature and with activity, whereas stroke volume ( $V_{\text{S}}$ ) increased ( $P < 0.001$ ) only with activity.  $\dot{Q}_{\text{tot}}$  ranged about 10-fold, from 10°C (rest) to 30°C (activity); increases in both  $f_{\text{H}}$  and  $V_{\text{S}}$  contributed to the increase in  $\dot{Q}_{\text{tot}}$ . The overall distribution of blood to the pulmocutaneous circuit (net L-R shunt) increased with both temperature and activity and was significantly correlated with  $\dot{Q}_{\text{tot}}$ . These data indicate that blood flow distribution in toads is a direct function of cardiac output, and this is linked to relative changes in resistance in the major outflow vessels. Arterial  $\text{O}_2$  saturation ( $S_{\text{a}}$ ) was high (mean = 93%) in all conditions except activity at 30°C, when it decreased to 74% and contributed to a decrease in the arteriovenous  $\text{O}_2$  difference. Venous  $\text{O}_2$  saturation ( $S_{\text{v}}$ ) was high at rest (76%) and dropped significantly during activity to about 30% at all temperatures. Intracardiac arterial-venous mixing (systemic mixing index) showed the strongest correlation with variation in  $f_{\text{H}}$  with minimal mixing (17%) occurring at about 50 beats  $\text{min}^{-1}$ . The most mixing occurred at the lowest  $f_{\text{H}}$  (13 beats  $\text{min}^{-1}$ ) and at the highest  $f_{\text{H}}$  (103 beats  $\text{min}^{-1}$ ). The results indicate that the

heart of a 0.25-kg toad becomes more efficient from an oxygen transport perspective from low  $f_{\text{H}}$  to 50 beats  $\text{min}^{-1}$  and then less efficient at higher  $f_{\text{H}}$ , contributing to an uncoupling of blood flow and metabolic rates at these high rates.

## Introduction

Anuran amphibians experience acute changes in environmental temperatures and activity states that result in obligatory metabolic adjustments. These changes in metabolism are accompanied by appropriate adjustments in systemic oxygen transport through increases of heart rate, stroke volume, and/or arteriovenous oxygen content difference. Convective cardiovascular oxygen transport appears to be the rate-limiting step for maximal oxygen consumption in anurans (see Withers and Hillman 1988). Understanding the role of cardiovascular variables in temperature- and activity-induced increases in metabolism requires direct measurements of blood flow in the major outflow vessels of the heart. Although some studies have measured blood flow and heart rate in resting, anesthetized, or pithed animals, no studies have measured pulmocutaneous and systemic blood flow in conscious animals either exposed to changes in temperature or during activity.

One complicating feature of examining the role of blood flow and oxygen transport in anurans is the effect of the undivided ventricle and partially divided bulbus cordis, characteristic of the amphibia. Three major bilateral vessels emerge from the amphibian heart and are arranged in parallel: carotid, systemic (aortic), and pulmocutaneous arteries. Carotid arterial resistance is very high relative to systemic or pulmocutaneous arteries (West and Smits 1994); therefore, its contribution to selective blood flow distribution in the major vessels is small. Cardiac output is, therefore, primarily distributed between the systemic and pulmocutaneous arteries that carry approximately 90%–95% of total cardiac output in toads (West and Smits 1994). Because blood flow in the two circuits is generated from a single ventricle, it is reasonable to expect that blood pressure in these two parallel circuits should be nearly identical. Several studies have confirmed that mean pressures in the two circuits are nearly equivalent (de Graaf 1957; Johansen 1963; Shelton and Jones 1965; Jones and Shelton 1972; West and Smits 1994).

There are two primary implications for the effects of an undivided ventricle on blood flow and oxygen transport. First, a single pressure source dictates that selective blood flow dis-

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tribution to the pulmocutaneous and systemic circuits will primarily depend on the relative resistances of the two circuits. Thus, outflow from the heart in anurans mainly follows two parallel pathways whose resistances may be altered by active regulation of vessel caliber and by the intrinsic properties of the vessels (Shelton 1970, 1976). Second, within the amphibian ventricle there may be considerable mixing of deoxygenated mixed venous blood and oxygenated pulmonary venous blood that will reduce systemic arterial oxygen content.

Measurements of intracardiac mixing in amphibian hearts have utilized blood color (Ozorio de Almeida 1923), injections of a variety of materials (Noble 1925; Foxon 1951; de Graaf 1957; Simons 1957, 1959; Johansen 1963), and oxygen partial pressure or content measurement (DeLong 1962; Johansen 1963; Johansen and Ditadi 1966; Tazawa et al. 1979; Withers et al. 1988b). The results of these studies, summarized by Foxon (1955), Johansen and Hanson (1968), and Shelton (1976), have indicated the full range of possibilities, from complete mixing to highly efficient separation. The source of the variation in arteriovenous mixing has been attributed to interindividual differences; interspecific differences relating to cutaneous reliance on gas exchange (Foxon 1964), whether or not the pericardium was intact (Tazawa et al. 1979); and relative flows in pulmonary and systemic circuits (Haberich 1965).

Shelton (1976) suggested that laminar blood flow through the amphibian ventricle plays an important role in maintaining functional separation of venous and arterial blood. This hypothesis suggests that any factor that would disrupt blood flow through the heart (e.g., turbulence) could potentially increase venous/arterial mixing and reduce separation efficiency in the heart. These two major factors, blood flow distribution and arterial-venous mixing (intracardiac mixing), are poorly understood in conscious amphibians but necessary for determining overall systemic oxygen transport. To fully characterize the impact of intracardiac mixing and blood flow distribution on oxygen transport in conscious amphibia, measurements of blood flow in the major outflow vessels should be accompanied by measurements of oxygen content in blood entering and leaving the heart.

Given the technical demands of measuring blood flow and oxygen content in several major vessels in order to characterize net shunting and intracardiac mixing in amphibians, we have divided this study into two parts. In one group of animals, we report measurements of blood flow in the systemic and pulmocutaneous arteries of *Bufo marinus* at rest and following a bout of enforced physical activity at three different body temperatures. We report measurements of arterial and venous oxygen content, hemoglobin concentration, and oxygen saturation in a second group of *B. marinus* exposed to identical protocols as in the first group. Together, the two studies have allowed us to assess the impact of two biologically relevant variables, temperature and activity, on net shunts and intracardiac mixing in the toad *B. marinus*.

## Material and Methods

### Animals

Cane toads (*Bufo marinus*; 127–585 g) were obtained from a commercial supplier (Charles Sullivan, Nashville). The toads were kept in large plastic containers and given free access to water but were not fed. Experiments were conducted within 1–4 wk after the animals arrived in the laboratory.

### Surgical Procedures

A total of six animals (127–230 g) were used in the blood flow study. The animals were anesthetized in unbuffered tricane-methanesulfonate (MS 222, Sigma Chemicals) at room temperature (20°–22°C). Toads were outfitted with blood flow probes around the left systemic and left pulmocutaneous arteries the day preceding experimentation. To approach the systemic and pulmocutaneous arteries, a 1.5–2.5-cm incision was made in the lateral body wall approximately 1 cm ventral to the parotoid gland. The underlying musculature was separated by blunt dissection to expose the major outflow vessels of the heart. The carotid, systemic, and pulmocutaneous arteries were identified visually, and cuff-type blood flow probes (Iowa Doppler Products) of an appropriate size (1.3–2.0 mm i.d.) were placed around the systemic and pulmocutaneous arteries. The probes were held closed by placing a small strip of latex around the outside of the probe so that it encircled the cuff with the vessel enclosed. The ends of the latex were held together with cyanoacrylate adhesive that held the vessel within the cuff of the probe. A single suture was placed in the musculature to ensure stability of the probes, and the skin was sutured closed.

A total of eight toads (223–585 g) were anesthetized as above and outfitted with occlusive indwelling cannulae in the left systemic arch (polyethylene [PE] 90) and the ventral abdominal vein (PE 90) for sampling of systemic arterial and venous blood, respectively, the day before experimentation. Acute occlusion of one systemic arch increases arterial pressure by only a few Torr (Jones and Shelton 1972). Preliminary experiments indicated that chronic occlusion longer than 10 min of one systemic arch has no effect on total blood flow through the dorsal aorta. The left systemic arch was approached through a 1–2-cm incision in the skin posterior to the parotoid gland and lateral to the transverse processes of the vertebrae, the underlying musculature was exposed, and the external and internal abdominal oblique muscles were bisected to expose the arch. The cannula was filled with heparinized saline (0.7% NaCl) and secured in the vessel with surgical silk (Ethicon). Abdominal muscles and skin were closed separately with silk sutures. The ventral abdominal cannula was implanted via a 0.5–1.0-cm midventral incision posterior to the xiphisternum and parallel to the rectus abdominis muscle. The cannula was secured in the same manner as the systemic arch cannula, and the

wound was closed with surgical silk. Both cannulae were flushed 24 h postsurgery and after blood sampling with approximately 50  $\mu\text{L}$  of heparinized saline.

### Protocol

The procedure for varying body temperature ( $T_b$ ) and for exercising animals at each  $T_b$  consisted of placing individual animals in nonairtight covered plastic containers with 0.5 cm of tap water in the bottom at room temperature ( $T_b = 20^\circ\text{--}22^\circ\text{C}$ ) or in constant-temperature water baths for low ( $10^\circ\text{C}$ ) or high ( $30^\circ\text{C}$ )  $T_b$ . Body temperature was measured with a thermistor probe (Bailey, Bat-10) inserted into the cloaca after toads were placed at any temperature for a minimum of 2 hr.  $T_b$  was measured after blood flow measurement to avoid disturbance of the animal and to ensure resting conditions;  $T_b$  was measured after a 3-min bout of activity for each temperature regimen. Under this protocol, resting and active toads both had measured body temperatures (mean  $\pm$  SEM) of  $10.8^\circ \pm 0.3^\circ\text{C}$ ,  $20.6^\circ \pm 0.2^\circ\text{C}$ , and  $30.4^\circ \pm 0.3^\circ\text{C}$ . The enforced activity protocol involves taking advantage of a behavioral righting response that has been shown to elicit maximal rates of oxygen consumption in this species (Walton and Anderson 1988; Withers et al. 1988b).

### Blood Flow Measurements

Blood flow measurements at each  $T_b$  were taken for animals at rest and after a 3-min bout of activity as described above. Miniature pulsed Doppler blood flow probes were connected to a directional pulsed Doppler flowmeter (Iowa Doppler Products, model 545C-4). The analog output of the flowmeter interfaced with an A-D board (MacADIOS.ABO, GW Instruments), and the data were collected at 10 Hz using a commercial software package (SuperScope II, GW Instruments) for measurement of instantaneous and mean  $\dot{Q}_{\text{sys}}$  and  $\dot{Q}_{\text{pul}}$ . After measurement of  $\dot{Q}_{\text{sys}}$  and  $\dot{Q}_{\text{pul}}$  at each  $T_b$  at rest and after activity, the toad was double-pithed and its chest was opened to expose the heart and major vessels. A cannula (PE 90) was tied into the left truncus arteriosus with suture and connected to a 60-mL syringe filled with blood collected from the animal by heart puncture. The right truncus was ligated along with the left carotid artery so that perfusion by the syringe pump was restricted to the arteries in which flow was measured. Each probe was calibrated in situ at the end of each experiment by perfusing each vessel independently with flows from a syringe pump that produced a voltage output from each probe that encompassed the range of voltage outputs measured during the experiment. Because the hematocrit of the calibrating flow was 3%–5%, compared with the hematocrits in vivo of 20%–30%, we are potentially overestimating true flows when hematocrit is low (see Evans 1989). A correction factor for flow probe voltage output was made by perfusing the systemic arch with a flow

probe at normal hematocrit (28%) and at low (1%) hematocrit at the same Doppler frequency shift (20 MHz) used in the experiments. It was found that low hematocrit overestimated flow by a factor of 1.45, and all flows were corrected by this value. Calibration curves were analyzed by least-squares linear regression techniques to yield mean blood flow from output voltage for each probe. Coefficients of determination ( $r^2$ ) for calibration curves ranged from 0.79 to 0.99 (mean = 0.94).

### Blood Flow Calculations and Statistics

We assumed that flows were bilaterally equal and that flows were isometrically related to body mass; therefore, total flow ( $\dot{Q}_{\text{tot}}$ ;  $\text{mL kg}^{-1} \text{min}^{-1}$ ) was determined as  $2 \times (\dot{Q}_{\text{pul}} + \dot{Q}_{\text{sys}})$ . Heart rate ( $f_{\text{H}}$ ;  $\text{beats min}^{-1}$ ) was counted directly from the blood flow trace; stroke volume ( $V_s$ ;  $\text{mL kg}^{-1}$ ) was calculated as  $\dot{Q}_{\text{tot}}/f_{\text{H}}$ . Because carotid flow was not measured, we have slightly underestimated total cardiac output; carotid flow for resting toads at  $23^\circ\text{--}24^\circ\text{C}$  is approximately 8% of total cardiac output and does not change in response to increased cardiac output with exposure to hypercapnia (West and Smits 1994). The net shunt ( $\dot{Q}_{\text{shunt}}$ ) was calculated as  $\dot{Q}_{\text{pul}} - \dot{Q}_{\text{sys}}$ .

### Arterial and Venous Blood Hemoglobin and Oxygen Saturation Measurements

At each  $T_b$ , resting blood samples (50  $\mu\text{L}$  each sample) were taken from arterial and venous cannulae on three separate occasions over a 20–30-min period and collected in heparinized capillary tubes for measurement of hemoglobin concentration ( $C_{\text{Hb}}$ ) and blood oxygen content ( $\text{Co}_{2, \text{tot}}$ ). Care was taken to sample the interior of the capillary tube for oxygen content determinations in order to prevent air contamination of the samples. Each sample was analyzed within 2–3 min. Arterial and venous blood samples were collected on three separate occasions in the same manner as above after a 3-min bout of activity (Withers et al. 1988b).

The cutaneous contribution to blood  $\text{O}_2$  saturation during activity at room temperature was assessed in separate trials on a different day. Animals ( $N=4$ ) were placed individually in plastic bags that covered approximately 65% of the body surface area, and the plastic bag was sealed around the body by a large rubber band placed posterior to the front limbs and anterior to the parotoid glands. Surgical tubing connected to a tank of 100%  $\text{N}_2$  was placed between the skin and rubber band near the axillary region. The bag was filled and emptied with  $\text{N}_2$  three times and then filled and a positive pressure of  $\text{N}_2$  was kept in the bag during a 3-min bout of activity. Arterial and venous blood samples were taken as before and analyzed for  $C_{\text{Hb}}$  and  $\text{Co}_{2, \text{tot}}$ .

Arterial and venous  $C_{\text{Hb}}$  (g Hb/100 mL blood) for each blood sample was determined spectrophotometrically (Bausch and Lomb Spectronic 20) with a hemoglobin assay kit (Sigma

Table 1: Cardiovascular variables measured for resting (R) and active (A) *Bufo marinus* at 10°, 20°, and 30°C

Variable	10°C		20°C		30°C	
	R	A	R	A	R	A
$f_H$ (beats $\text{min}^{-1}$ )	13 ± 1	28 ± 2	31 ± 3	64 ± 2	51 ± 3	103 ± 1
$V_S$ (mL $\text{kg}^{-1}$ )	3.7 ± .40	6.1 ± 1.1	2.5 ± .30	5.6 ± .70	3.1 ± .30	4.9 ± .50
$\dot{Q}_{\text{tot}}$ (mL $\text{min}^{-1} \text{kg}^{-1}$ )	50 ± 7	162 ± 26	81 ± 12	358 ± 43	158 ± 21	504 ± 45
$\dot{Q}_{\text{pul}}$ (mL $\text{min}^{-1} \text{kg}^{-1}$ )	13 ± 3	96 ± 20	32 ± 9	227 ± 37	85 ± 16	362 ± 38
$\dot{Q}_{\text{sys}}$ (mL $\text{min}^{-1} \text{kg}^{-1}$ )	37 ± 7	66 ± 12	49 ± 9	131 ± 25	73 ± 15	142 ± 22
$\dot{Q}_{\text{shunt}}$ (mL $\text{min}^{-1} \text{kg}^{-1}$ )	-24 ± 9	30 ± 20	-17 ± 13	96 ± 46	12 ± 22	221 ± 42
$\dot{Q}_{\text{pul}}/\dot{Q}_{\text{sys}}$	.45 ± .13	1.61 ± .36	.76 ± .28	2.12 ± .53	1.46 ± .43	2.85 ± .49

Note. Values are means ± 1 SEM.  $N = 6$ .

Chemicals) and 20  $\mu\text{L}$  of blood. Standard curves were determined for each assay.

Total blood oxygen content ( $\text{CO}_{2, \text{tot}}$ ) for each blood sample was measured according to Tucker (1967), using a polarographic  $\text{O}_2$  electrode (Transidyne General, model 730) connected to an electrode amplifier (Chemical Microsensor, model 1202).

$\text{CO}_{2, \text{tot}}$  was divided by  $C_{\text{Hb}}$  to yield mL  $\text{O}_2/\text{g Hb}$ ; this value was divided by the Hb  $\text{O}_2$  capacity at 100% saturation (1.34 mL  $\text{O}_2/\text{g Hb}$ ; Guyton 1981) to yield saturation of arterial ( $S_a$ ) and venous ( $S_v$ ) blood. Arteriovenous  $\text{O}_2$  difference was calculated as the difference between  $S_a$  and  $S_v$ .

#### Intracardiac Mixing Calculations and Statistics

Intracardiac mixing was calculated after the equation of Tazawa et al. (1979). An isometric relationship of these variables with body mass was assumed. Systemic mixing ( $m_{\text{sys}}$ , %) is

$$m_{\text{sys}} = 100 \times (C_{\text{pv}} - C_{\text{sys}}) / (C_{\text{pv}} - C_o),$$

where  $C_{\text{pv}}$  = blood oxygen content of the pulmonary vein (assumes 100% saturation of  $C_{\text{pv}}$ ),  $C_{\text{sys}}$  = blood oxygen content of the systemic arch, and  $C_o$  = mean blood oxygen content if pulmonary ( $\dot{Q}_{\text{pul}}$ ) and systemic ( $\dot{Q}_{\text{sys}}$ ) flows were completely mixed, calculated as

$$C_o = [(\dot{Q}_{\text{pul}} \times C_{\text{pv}}) + (\dot{Q}_{\text{sys}} \times C_{\text{ven}})] / (\dot{Q}_{\text{pul}} + \dot{Q}_{\text{sys}}),$$

where  $C_{\text{ven}}$  = blood oxygen content of the ventral abdominal vein,  $\dot{Q}_{\text{pul}}$  = pulmocutaneous blood flow, and  $\dot{Q}_{\text{sys}}$  = systemic blood flow (systemic arch flow).

Mean values ± 1 SEM are presented. A two-way ANOVA with repeated measures was used to test for significant differences for variables as a function of  $T_b$  and activity (Graph Pad Prism 2.0, San Diego, Calif.). The Student-Newman-Keuls (SNK) multiple-range test was used to determine significance between mean values where differences were detected by

ANOVA (Zar 1974). Saturation data (%) were arcsine transformed before statistical analyses were undertaken. The level of significance was taken as  $P < 0.05$ .

## Results

#### Blood Flow Measurements, Heart Rate, and Stroke Volume

Cardiovascular data collected from the six animals are summarized in Table 1. Heart rate ( $f_H$ ) increased significantly with changes in  $T_b$  ( $F_{2,30} = 417$ ,  $P < 0.001$ ) and during activity ( $F_{1,30} = 431$ ,  $P < 0.001$ ). For resting toads,  $f_H$  increased significantly from  $13.3 \pm 0.8$  (10°C) to  $50.7 \pm 3.0$  beats  $\text{min}^{-1}$  (30°C); for active toads,  $f_H$  also increased significantly from  $27.5 \pm 1.6$  (10°C) to  $103 \pm 1.6$  beats  $\text{min}^{-1}$  (30°C). At each  $T_b$ , there were also significant increases in  $f_H$  with activity with an approximate doubling of  $f_H$  at each  $T_b$  (Table 1). Cardiac stroke volume ( $V_S$ ) remained constant with changes in  $T_b$  ( $F_{2,30} = 1.3$ ,  $P > 0.05$ ) in both resting and active toads, but activity had a significant effect on  $V_S$  ( $F_{1,30} = 24.5$ ) at each  $T_b$ , with increases of  $V_S$  ranging from about 1.6- to 2.2-fold during activity (Table 1).  $\dot{Q}_{\text{tot}}$  increased significantly with  $T_b$  ( $F_{2,30} = 29.5$ ;  $P < 0.001$ ) in both resting and active toads (Table 1). In resting toads,  $\dot{Q}_{\text{tot}}$  did not increase in the 10°–20°C interval but did increase significantly ( $P < 0.05$ ) in the 20°–30°C interval. Enforced activity had a significant effect on  $\dot{Q}_{\text{tot}}$  ( $F_{1,30} = 105$ ;  $P < 0.001$ ) at any  $T_b$ .

There was a significant effect of both  $V_S$  and  $f_H$  on  $\dot{Q}_{\text{tot}}$  (Fig. 1);  $f_H$  explained 78% of the variation in  $\dot{Q}_{\text{tot}}$  (Fig. 1A) compared with 32% for  $V_S$  (Fig. 1B). The relationship between  $f_H$  and  $\dot{Q}_{\text{tot}}$  was highly significant ( $\dot{Q}_{\text{tot}} = 5.2 \times f_H - 30.8$ ,  $F_{1,34} = 121.5$ ,  $P < 0.0001$ ; Fig. 1A), as was the relationship between  $V_S$  and  $\dot{Q}_{\text{tot}}$  ( $\dot{Q}_{\text{tot}} = 53.2 \times V_S - 10.8$ ,  $F_{1,34} = 16.2$ ,  $P < 0.001$ ; Fig. 1B); however,  $f_H$  is a better predictor of  $\dot{Q}_{\text{tot}}$  than is  $V_S$  in *Bufo marinus* under resting and active conditions at the three temperatures used in this study.

Systemic and pulmocutaneous blood flows also showed significant changes with  $T_b$  or with activity; however, these changes were proportionally greater for  $\dot{Q}_{\text{pul}}$  than for  $\dot{Q}_{\text{sys}}$  (Table 1). In

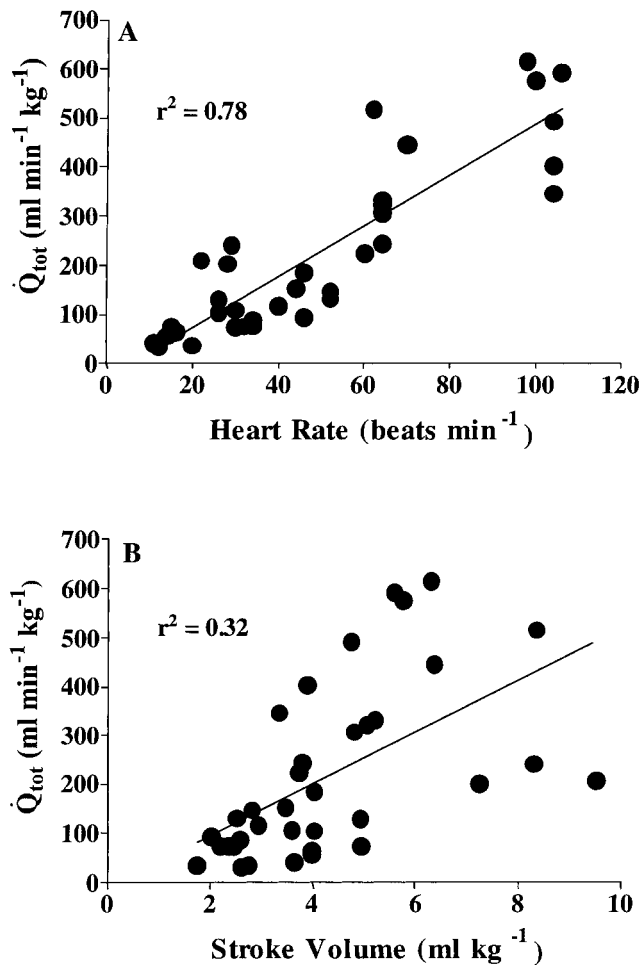


Figure 1. A, Total blood flow ( $\dot{Q}_{\text{tot}}$ ) as a function of heart rate ( $f_{\text{H}}$ ) for *Bufo marinus*; data are taken from resting and active animals at body temperatures of 10°, 20°, and 30°C. The regression equation describing this relationship is  $Y = 5.2 \times X - 30.8$  ( $F_{1,34} = 121.5$ ,  $P < 0.0001$ ,  $r^2 = 0.78$ ). B,  $\dot{Q}_{\text{tot}}$  as a function of stroke volume ( $V_{\text{S}}$ ) for the same animals as in A. The regression equation for this relationship is  $Y = 53.2 \times X - 10.8$  ( $F_{1,34} = 16.2$ ,  $P < 0.001$ ,  $r^2 = 0.32$ ). Most of the variation ( $r^2$ ) in  $\dot{Q}_{\text{tot}}$  (78%) is explained by  $f_{\text{H}}$  rather than  $V_{\text{S}}$  (32%).

resting toads,  $\dot{Q}_{\text{pul}}/\dot{Q}_{\text{sys}}$  increased significantly ( $F_{2,30} = 4.2$ ;  $P < 0.05$ ) from  $0.45 \pm 0.1$  (10°C) to  $1.46 \pm 0.4$  (30°C). There were also significant increases in  $\dot{Q}_{\text{pul}}/\dot{Q}_{\text{sys}}$  with activity ( $F_{1,30} = 16.8$ ,  $P < 0.001$ ), with mean values ranging from  $1.61 \pm 0.4$  at 10°C to  $2.85 \pm 0.54$  at 30°C (Table 1).

In resting toads, the net cardiac shunt ( $\dot{Q}_{\text{shunt}}$ ) ranged from  $-24 \pm 8.8$  (10°C) to  $11.6 \pm 22.4$  mL  $\text{min}^{-1} \text{kg}^{-1}$  (30°C), but this increase was not significant (Table 1).  $\dot{Q}_{\text{shunt}}$  increased significantly with  $T_{\text{b}}$  in active toads ( $F_{2,30} = 7.9$ ,  $P < 0.002$ ) from  $29.7 \pm 19.6$  (10°C) to  $221 \pm 42.4$  mL  $\text{min}^{-1} \text{kg}^{-1}$  (30°C) and increased significantly with activity ( $F_{1,30} = 27.9$ ) at 20°C and 30°C ( $P < 0.001$ ) but not at 10°C ( $P > 0.05$ ). With increased  $T_{\text{b}}$

or with activity, there was an overall increase in the net L-R shunt ( $\dot{Q}_{\text{shunt}}$ ; Table 1).

Figure 2 shows the relationship between  $\dot{Q}_{\text{shunt}}$  and  $\dot{Q}_{\text{tot}}$  for the toads in this study. There was a highly significant correlation ( $F_{1,34} = 62.8$ ,  $P < 0.0001$ ) between  $\dot{Q}_{\text{shunt}}$  and  $\dot{Q}_{\text{tot}}$  ( $\dot{Q}_{\text{shunt}} = 0.5 \times \dot{Q}_{\text{tot}} - 55.4$ ;  $r^2 = 0.65$ ). Most of the variability in  $\dot{Q}_{\text{shunt}}$  was explained by variations in  $f_{\text{H}}$  (52%) rather than  $V_{\text{S}}$  (17%), owing to the greater dependence of  $\dot{Q}_{\text{tot}}$  on  $f_{\text{H}}$ . The  $\dot{Q}_{\text{tot}}$  that corresponds to no net shunt ( $\dot{Q}_{\text{shunt}} = 0$ ) is approximately 110 mL  $\text{min}^{-1} \text{kg}^{-1}$  (Fig. 2), which is predicted to occur at a  $f_{\text{H}}$  of about 27 beats  $\text{min}^{-1}$ , based on the relationship between  $f_{\text{H}}$  and  $\dot{Q}_{\text{tot}}$  (Fig. 1A). The relationship between  $\dot{Q}_{\text{shunt}}$  and  $\dot{Q}_{\text{tot}}$  indicates that  $\dot{Q}_{\text{pul}}$  increased proportionally with increased cardiac output over the range of temperatures and activity levels for toads in this study.

#### Arterial and Venous Blood Hemoglobin and Oxygen Saturation

Data are given in Table 2. It is apparent that the site of blood sampling (arterial vs. venous) is not critical for determination of  $C_{\text{Hb}}$  since, at any  $T_{\text{b}}$  for rest or for activity, there was no significant difference between arterial and venous  $C_{\text{Hb}}$ . In contrast, at any  $T_{\text{b}}$ , there was a significant increase in  $C_{\text{Hb}}$  from rest to activity.

In resting animals,  $S_{\text{a}}$  was 82% at 10°C and increased significantly ( $P < 0.01$ ) to 96% at 20°C (Table 2). Resting  $S_{\text{a}}$  was not different for 10°C and 30°C toads.  $S_{\text{a}}$  increased significantly with activity at 10°C ( $P < 0.01$ ) and decreased significantly with activity at 30°C ( $P < 0.01$ ). Therefore,  $S_{\text{a}}$  was lowest at the extreme ends of the activity range, as indicated by  $f_{\text{H}}$  (Table 1).  $S_{\text{v}}$  was unaffected by  $T_{\text{b}}$  in resting toads and averaged 76% (Table 2). With activity,  $S_{\text{v}}$  decreased significantly ( $P < 0.001$ ) at any  $T_{\text{b}}$  to about 30%. Arteriovenous  $\text{O}_2$  saturation difference

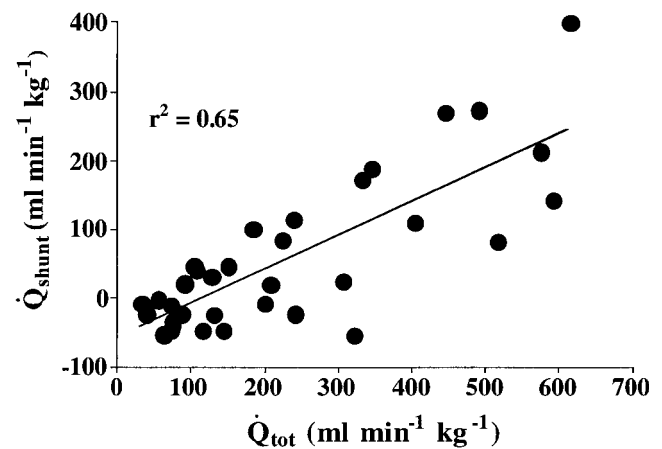


Figure 2. Net cardiac shunt ( $\dot{Q}_{\text{shunt}}$ ) as a function of  $\dot{Q}_{\text{tot}}$  for *Bufo marinus*. The regression equation describing this relationship is  $Y = 0.5 \times X - 55.4$  ( $F_{1,34} = 62.8$ ,  $r^2 = 0.65$ ,  $P < 0.0001$ ).

( $S_a - S_v$ ) ranged from 7% to 18%, with no significant effect of  $T_b$  in resting or active toads. During enforced activity, there was a significant increase in  $S_a - S_v$  ( $P < 0.001$ ), primarily caused by the large reduction in  $S_v$  (Table 2).

The cutaneous contribution to  $S_a - S_v$  was estimated from cutaneous exposure to  $N_2$  in active toads (Table 3).  $S_a - S_v$  was significantly larger ( $P < 0.001$ ) when the skin was exposed to  $N_2$ . Most of the increase is attributed to a larger decline in  $S_v$  (-12%) compared with  $S_a$  (-4%). The values of  $C_{Hb}$  for these exercised animals while they were partially enclosed in a plastic bag (Table 3) compared favorably with the activity-induced hemoconcentration observed at various temperatures (Table 2), suggesting that similar levels of activity were achieved in both groups.

#### Intracardiac Systemic Mixing Indices

The mean  $O_2$  content and flow data used to calculate  $m_{sys}$  are presented in Table 4. Because  $O_2$  content and flow data were obtained from two different groups of animals,  $m_{sys}$  was calculated using mean values. We calculated a "proportional error" for  $m_{sys}$  based on the largest error in our measurements that were used to calculate  $m_{sys}$  (i.e., content or flow). The flow data contained the largest proportional error in all conditions, ranging from 10% to 28% error of the mean (see data in Table 1). Therefore, the proportional error is based on the error in the flow measurements for that particular condition (see Figs. 3, 4). In resting toads, increased temperature decreased  $m_{sys}$  from 85% at 10°C to 17% at 30°C. Active toads had lower  $m_{sys}$  at 10°C and 20°C compared with resting animals, but  $m_{sys}$  increased from 17% to 60% in active animals at 30°C (Fig. 3). The highest  $m_{sys}$  values occurred at 10°C rest (85%) and 30°C

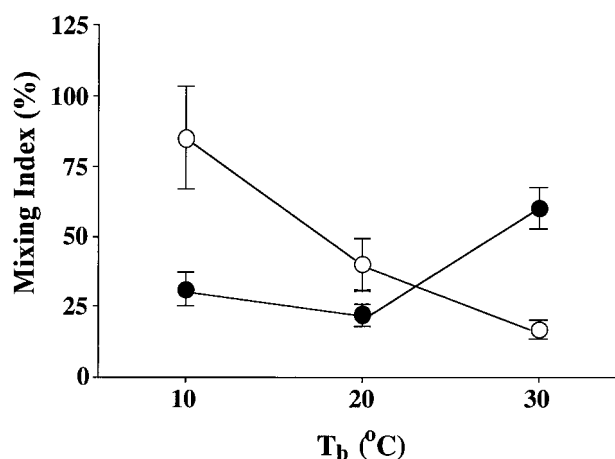


Figure 3. Mixing index (%) as it relates to body temperature ( $T_b$ ; °C) at rest (open circles) and exercise (filled circles) in *Bufo marinus*. Error bars represent the largest proportional error (see "Results") for  $\dot{Q}_{pul}$  or  $\dot{Q}_{sys}$  applied to the mean mixing index value.

active (60%) animals, with 17%–40% mixing in other conditions.

For the results in this study,  $m_{sys}$  appeared to be most closely related to  $f_H$  in a curvilinear fashion ( $m_{sys} = 0.027 \times (f_H)^2 - 3.323 \times (f_H) + 116.1$ ;  $r^2 = 0.91$ ), with greatest mixing at the lowest  $f_H$  (13 beats  $\text{min}^{-1}$ ) and highest  $f_H$  (103 beats  $\text{min}^{-1}$ ), and minimal  $m_{sys}$  (17%) at a  $f_H$  of 51 beats  $\text{min}^{-1}$  (Fig. 4). Data from two studies that allowed calculation of  $m_{sys}$  in resting and active *B. marinus* at 20°–22°C (Withers et al. 1988b) and pithed *Rana catesbeiana* at 25°C (Tazawa et al. 1979) were used to compare with the mixing data from this study (Fig. 4). The

Table 2: Effect of  $T_b$  and activity level (rest, activity) on systemic arterial and mixed venous values of  $C_{Hb}$ ,  $S_a$ ,  $S_v$ , and  $S_a - S_v$  for cane toads

Condition	$T_b$ (°C)	$C_{Hb}$ (g%)	Saturation (%)	$S_a - S_v$ (%)
Rest:				
Arterial .....	10	4.5 ± .40	82.1 ± 3.3	...
Venous .....	10	4.6 ± .30	74.9 ± 2.5	7.3 ± 3.4
Arterial .....	20	4.7 ± .50	95.8 ± 1.8	...
Venous .....	20	4.8 ± .60	77.4 ± 5.3	18.4 ± 5.4
Arterial .....	30	6.3 ± .50	93.0 ± 1.2	...
Venous .....	30	5.7 ± .40	74.6 ± 3.3	18.4 ± 3.8
Active:				
Arterial .....	10	7.1 ± .50	94.9 ± 2.2	...
Venous .....	10	8.1 ± .50	28.9 ± 2.7	66.0 ± 4.1
Arterial .....	20	8.9 ± .50	91.8 ± 1.6	...
Venous .....	20	8.5 ± .40	37.5 ± 4.2	54.3 ± 3.7
Arterial .....	30	8.7 ± .40	74.0 ± 1.8	...
Venous .....	30	7.9 ± .40	24.3 ± 2.5	49.8 ± 1.8

Note. Values are means ± 1 SEM.  $N = 8$ .

Table 3: Effect of air and nitrogen environment on systemic arterial and mixed venous values of  $C_{Hb}$ ,  $S_a$ ,  $S_v$ , and  $S_a - S_v$  for active *Bufo marinus* at 20°C

Condition	$C_{Hb}$ (g%)	Saturation (%)	$S_a - S_v$ (%)
Air:			
Arterial .....	7.8 ± .30	83 ± 1.7	...
Venous .....	7.0 ± .20	26 ± 2.2	57 ± 3.2
Nitrogen:			
Arterial .....	7.2 ± .20	79 ± 1.9	...
Venous .....	6.7 ± .30	14 ± 1.2	65 ± 2.7

Note. Values are means ± 1 SEM.  $N = 4$ .

equation that describes the data in this study in combination with the data from Withers et al. (1988b) and Tazawa et al. (1979) is  $m_{sys} = 0.023 \times (f_H)^2 - 2.899 \times (f_H) + 118.3$  ( $r^2 = 0.41$ ).

In this study,  $m_{sys}$  was significantly related in a curvilinear fashion with increases in  $\dot{Q}_{sys}$  ( $r^2 = 0.88$ ),  $\dot{Q}_{tot}$  ( $r^2 = 0.81$ ),  $\dot{Q}_{shunt}$  ( $r^2 = 0.61$ ), and  $\dot{Q}_{pul} / \dot{Q}_{sys}$  ( $r^2 = 0.88$ ). Because all of these flow variables are linearly related to  $f_H$  ( $\dot{Q}_{sys}$ ,  $r^2 = 0.86$ ;  $\dot{Q}_{shunt}$ ,  $r^2 = 0.89$ ;  $\dot{Q}_{pul} / \dot{Q}_{sys}$ ,  $r^2 = 0.84$ ; and  $\dot{Q}_{tot}$ ,  $r^2 = 0.91$ ), the primary determinant of intracardiac shunting is probably  $f_H$ . The  $m_{sys}$  was not significantly related to  $V_s$  at rest or following activity.

**Discussion**

*Blood Flow Measurements, Heart Rate, and Stroke Volume*

$\dot{Q}_{tot}$  in resting toads at room temperature ( $20.6^\circ \pm 0.2^\circ\text{C}$ ) was  $81 \text{ mL min}^{-1} \text{ kg}^{-1}$ , which is approximately 40% higher than the value of  $57 \text{ mL min}^{-1} \text{ kg}^{-1}$  for cardiac output in resting *Bufo marinus* at 24°C reported by West and Smits (1994). This difference can be attributed to differences in  $V_s$  since  $f_H$  in both studies were similar. Heart rates for toads in this study were similar to values recorded in previous studies with changing  $T_b$  and activity (Table 1; Hedrick 1985; Withers et al. 1988b). The  $Q_{10}$  for  $f_H$  was greater at the 10°–20°C interval ( $Q_{10} = 2.3$ ) for rest or exercise than at the 20°–30°C interval ( $Q_{10} = 1.6$ ).

During exercise at 30°C,  $f_H$  reached  $103 \text{ beats min}^{-1}$ , which is the highest heart rate recorded for *B. marinus*. The factorial scope (exercise/rest) for both  $f_H$  and  $V_s$  was greater at 20°C than at 10°C or 30°C. As a consequence, the scope for  $\dot{Q}_{tot}$  was also maximal at 20°C.

*Blood Flow Distribution between Pulmocutaneous and Systemic Arteries*

Blood flow distribution, or net shunt ( $\dot{Q}_{shunt}$ ), was not significantly affected by changes in  $T_b$  in resting *B. marinus* (Table 1); however, at lower temperatures,  $\dot{Q}_{sys}$  exceeded  $\dot{Q}_{pul}$  (net R-L shunt), whereas at 30°C,  $\dot{Q}_{pul}$  was greater than  $\dot{Q}_{sys}$  (net L-R shunt). During activity at each  $T_b$ , there was a large increase of  $\dot{Q}_{shunt}$  where  $\dot{Q}_{sys}$  becomes a smaller fraction of  $\dot{Q}_{tot}$ . In this study, no net shunt occurred (i.e.,  $\dot{Q}_{shunt} = 0$ ) at a  $\dot{Q}_{tot}$  of about  $110 \text{ mL min}^{-1} \text{ kg}^{-1}$ , as predicted by the relationship between these variables (Fig. 2). Because  $\dot{Q}_{tot}$  is primarily predicted by  $f_H$  (Fig. 1A), our data predict that no net shunt would occur at a  $f_H$  of about 27  $\text{beats min}^{-1}$  for *B. marinus*. Interestingly, the study by West and Smits (1994) showed that for resting toads with a mean  $f_H$  of 30  $\text{beats min}^{-1}$ ,  $\dot{Q}_{shunt}$  was  $4.7 \text{ mL min}^{-1} \text{ kg}^{-1}$ , which is very close to the predicted  $\dot{Q}_{shunt}$  value of  $7.1 \text{ mL min}^{-1} \text{ kg}^{-1}$  from our data (see “Results”). Thus, our data suggest that  $f_H$  is a strong predictor of  $\dot{Q}_{shunt}$  in *B. marinus*.

$\dot{Q}_{shunt}$  was positively correlated with  $\dot{Q}_{tot}$  over the entire range of temperatures and activity states (Fig. 2), indicating that there were larger increases in  $\dot{Q}_{pul}$  relative to  $\dot{Q}_{sys}$ . This relationship has also been observed in turtles (Hicks et al. 1996). Because mean blood pressures are equivalent in the pulmocutaneous and systemic arteries in conscious toads (West and Smits 1994), these adjustments in blood flow imply that increased temperature and enforced activity is accompanied by a proportionally larger reduction in pulmonary resistance ( $R_{pul}$ ) than systemic resistance ( $R_{sys}$ ). Over this broad range of activity states, we estimate that  $R_{pul}$  changes by a factor of about 24-fold, compared with an estimated three- to fourfold change in  $R_{sys}$ .

The large reductions in  $R_{pul}$  may result from the combined

Table 4: Blood oxygen content and flows for resting (R) and active (A) *Bufo marinus* at 10°, 20°, and 30°C

Variable	10°C		20°C		30°C	
	R	A	R	A	R	A
$C_{pv}$ (mL O <sub>2</sub> /100 mL) .....	6.0	10.0	6.3	11.5	7.9	11.0
$C_{sys}$ (mL O <sub>2</sub> /100 mL) .....	4.9	8.9	5.9	10.8	7.7	8.5
$C_{ven}$ (mL O <sub>2</sub> /100 mL) .....	4.6	3.1	4.9	4.2	5.6	2.5
$C_o$ (mL O <sub>2</sub> /100 mL) .....	4.7	6.5	5.3	8.4	6.7	6.8
$\dot{Q}_{pul}$ (mL min <sup>-1</sup> kg <sup>-1</sup> ) .....	13	96	32	227	85	362
$\dot{Q}_{sys}$ (mL min <sup>-1</sup> kg <sup>-1</sup> ) .....	37	66	49	131	73	142

Note. Oxygen content values are measured means from the same animals used in Table 2, and flow data are means from animals in Table 1.  $C_{pv}$  value assumes 100% saturation.

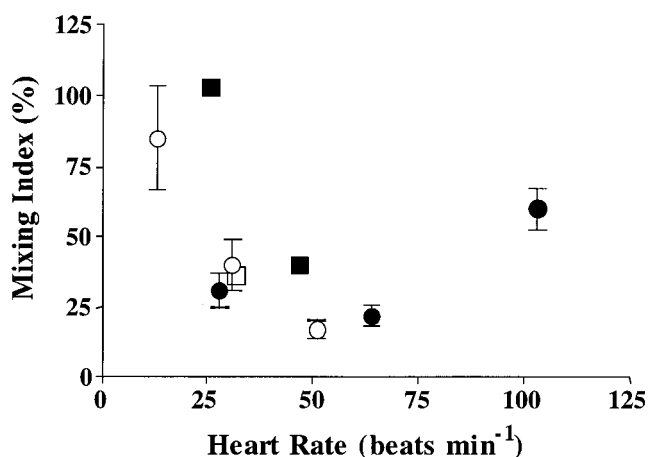


Figure 4. Effect of heart rate (beats  $\text{min}^{-1}$ ) on mixing index (%) in *Bufo marinus* at rest (open circles) and during activity (filled circles) at  $T_b$  of 10°, 20°, and 30°C. Two data points for resting ( $f_H = 26$  beats  $\text{min}^{-1}$ ) and active ( $f_H = 47$  beats  $\text{min}^{-1}$ ) *B. marinus* from Withers et al. (1988b) using our data for flow (filled squares) and the datum for *Rana catesbeiana* ( $f_H = 32$  beats  $\text{min}^{-1}$ ) from Tazawa et al. (1979; unfilled square) are included (see “Discussion”).

effects of vagal inhibition (de Saint-Aubain and Wingstrand 1979; West and Burggren 1984; Courtice 1990) and, perhaps, vasodilating effects of increased sympathetic tone as shown in turtles (Hicks 1994, 1998). In *B. marinus*, enforced activity causes an increase of plasma catecholamine levels to some two to 18 times resting levels (Tufts et al. 1987; Withers et al. 1988a), which is likely to play a role in the increased cardiac output and blood flow distribution in toads. In support of this hypothesis, systemic injection of adrenaline causes an increase in  $\dot{Q}_{\text{pul}}$  and an increase in the net L-R shunt in turtles (Hicks and Wang 1998) and toads (A. K. Gamperl and T. Wang, personal communication). In toads, as well as in turtles, there appears to be an overall increase in  $\dot{Q}_{\text{shunt}}$  that is correlated with cardiac output and activity level. This increase in  $\dot{Q}_{\text{shunt}}$  may be regulated by a balance between cholinergic and adrenergic inputs to the pulmonary vasculature (see Hicks 1998).

#### Arterial and Venous Hemoglobin and Oxygen Saturation

The exercise-induced hemoconcentration for toads in this study is consistent with other studies of anurans with exercise (Boutilier et al. 1986; Tufts et al. 1987), handling stress (Mbangkollo and de Roos 1983), hypoxia (Malvin and Wood 1991; Pörtner et al. 1991; Pinder and Smits 1993; Malvin et al. 1995), and the administration of catecholamines (Herman 1977; Mbangkollo and de Roos 1983). The mechanism for the hemoconcentration has not been established but appears to result from a combination of pressure-mediated ultrafiltration to the interstitial space (Hillman et al. 1987), osmotic fluid uptake by

active muscle fibers (Boutilier et al. 1986), and inhibition of lymphatic return (Malvin et al. 1995), rather than mobilization of stored erythrocytes from the spleen (Pinder and Smits 1993; Malvin et al. 1995). The hemoconcentration dictates that for a full understanding of oxygen transport in exercising amphibians, simultaneous measurement of both blood  $\text{CO}_{2,\text{tot}}$  and  $C_{\text{Hb}}$  is necessary for interpreting intracardiac shunting in the amphibian heart.

The values for resting  $S_a$  of 82%–96% are similar to values from previous studies of conscious *B. marinus* (Withers et al. 1988b), anesthetized *Bufo paracnemis* (Johansen and Ditadi 1966), and pithed *Rana catesbeiana* (Tazawa et al. 1979) at 25°C. Temperature increased resting  $S_a$  from 10°C to 20°C in our study, which contrasts with a report that resting  $S_a$  declines in warm (20°C)-acclimated *R. catesbeiana* compared with cold (5°C)-acclimated animals (Weathers 1976). Two factors may be important here, both acute versus chronic temperature change and stress. Our data are for acute temperature changes over 2–3 h, while Weathers’s (1976) data represent a minimum of 2 wk of acclimation. It should be noted that the resting heart rate values reported by Weathers (1976) are equivalent to heart rates of *R. catesbeiana* during enforced activity (Hillman et al. 1987); consequently, the decline in  $S_a$  reported previously may have been stress induced and similar to the decline in  $S_a$  in this study with active toads at 30°C (Table 1). The values for resting  $S_v$  were high in this study (75%–77%) and declined significantly with exercise to 24%–38%. The absolute values and pattern of change correspond well with a previous study on *B. marinus* at 20°C (Withers et al. 1988b) using oxygen partial pressure measurements. Blood sampling and measurement of  $S_v$  from open-chest preparations in conscious *B. paracnemis* (Johansen and Ditadi 1966) indicate that a certain degree of “stress” may have been involved with those techniques because the values are closer to the values obtained during enforced activity in this study. The increased  $S_a - S_v$  in this study during activity is primarily the result of increased  $\text{O}_2$  extraction and also is consistent with previous studies on the source of oxygen during periods of increased demand (Becker et al. 1979; Carey 1979; Withers et al. 1988b; Hillman 1991).

We used cutaneous  $\text{N}_2$  exposure to estimate the potential contribution of the skin to  $S_a - S_v$  during enforced activity (Table 2). Our data indicate that we are underestimating  $S_a - S_v$  by at least 8% because of cutaneous  $\text{O}_2$  uptake. This is a conservative estimate because only about 65% of the skin was exposed to  $\text{N}_2$ ; thus, some cutaneous  $\text{O}_2$  uptake would still have occurred in our experiment. However, it is clear that during activity, cutaneous  $\text{O}_2$  uptake is a small fraction of overall  $\text{O}_2$  uptake in active *B. marinus*.

#### Intracardiac Mixing Indices

One drawback of this study was that arteriovenous oxygen saturation and blood flow were measured in two different

groups of animals, albeit under identical conditions; thus, we are forced to use mean values. In a previous study using pithed *R. catesbeiana* and blood sampling from various sites within the vascular system, intracardiac mixing increased after the pericardium was opened (Tazawa et al. 1979). One interpretation of those results is that  $V_s$  increased, which allowed more intracardiac mixing and less arteriovenous separation.

In our study, the one variable that seems most tightly related to variation in  $m_{\text{sys}}$  was  $f_{\text{H}}$ . Minimal  $m_{\text{sys}}$  appears to be associated with intermediate  $f_{\text{H}}$  around 50 beats  $\text{min}^{-1}$ , and  $m_{\text{sys}}$  increases with either increases or decreases in  $f_{\text{H}}$  from that value (Fig. 4). Combinations of temperature and activity that produce  $f_{\text{H}}$  of about 50 beats  $\text{min}^{-1}$  are the most "efficient" from a minimal mixing perspective. Interestingly, the only other quantitative data available on systemic mixing are from the bullfrog, *R. catesbeiana* (Tazawa et al. 1979), and for the cane toad, *B. marinus* (Withers et al. 1988b). Although Tazawa et al. (1979) did not measure  $f_{\text{H}}$ , if we extrapolate from the temperature- $f_{\text{H}}$  data of Weathers (1976) for a resting bullfrog at this temperature, their systemic mixing data fit ours closely (Fig. 4). The mixing indices for *B. marinus* were calculated by Withers et al. (1988b) from measurements of  $f_{\text{H}}$ , arterial and venous  $\text{PO}_2$  and pH, a blood oxygen binding curve, and an assumption of equal  $\dot{Q}_{\text{sys}}$  and  $\dot{Q}_{\text{pul}}$ . The mixing indices recalculated for their data using our  $Q_{\text{sys}}$  and  $Q_{\text{pul}}$  data also fit closely with our  $m_{\text{sys}}$  data (Fig. 4). Consequently, one important source of variation in the myriad of available descriptive studies on the degree of mixing in amphibian hearts may very well be  $f_{\text{H}}$  variation attributable to either stress, exercise, or temperature. Although we have no data on the mechanism of how  $f_{\text{H}}$  affects mixing, it is potentially as simple as either long diastolic intervals at low  $f_{\text{H}}$  and turbulence at high  $f_{\text{H}}$  that allow for significant mixing.

What range of  $f_{\text{H}}$  might characterize a *B. marinus* in the field? Cane toads prefer a temperature of 24°C in the laboratory (Johnson 1972; Malvin and Wood 1991) and maintain  $T_b$  at or near 24°C when they are active or in shelter sites in the field (cited as unpublished data in Schwarzkopf and Alford 1996). This would define a range of  $f_{\text{H}}$  from about 35 beats  $\text{min}^{-1}$  at rest to 75 beats  $\text{min}^{-1}$  at maximal activity. Given that toads operate at activity levels around 5%–15% of their rest to maximal interval (Strussman et al. 1984; Walton 1988), the normal activity  $f_{\text{H}}$  would be about 40–45 beats  $\text{min}^{-1}$  at their preferred temperature. There appears to be a significant coadaptation of preferred temperature, activity pattern, and the efficiency of cardiac function in *B. marinus*.

#### Implications for Systemic Oxygen Transport

At any given temperature, enforced physical activity resulted in a large net L-R shunt (Table 1; Fig. 2) and reduced intracardiac mixing at lower temperatures (10°–20°C) but increased mixing at higher temperatures (30°C). Studies based on the-

oretical considerations and data for reptiles have led to the prediction that increased oxygen demand should result in a reduced R-L shunt to improve systemic oxygen transport (Hicks 1994; Hicks and Wang 1996; Wang and Hicks 1996; Wang et al. 1997). Our results with toads generally fit this prediction; however, systemic arterial saturation declined significantly (to 74%) only with the largest net L-R shunt and increased R-L shunt (i.e., mixing) in active toads at 30°C. Moreover, intracardiac mixing was largely independent of  $\dot{Q}_{\text{shunt}}$  in resting toads over a broad range of temperatures. Thus, it is unclear to what extent measurements of net shunts in amphibians have predictive value for assessing intracardiac mixing in the absence of oxygen content measurements. In this study,  $f_{\text{H}}$  was a better predictor of intracardiac mixing than was  $\dot{Q}_{\text{shunt}}$ .

Systemic oxygen transport has been implicated as the principal limitation to maximal rates of oxygen consumption in amphibians (see Withers and Hillman 1988). Maximal oxygen consumption ( $\dot{V}_{\text{O}_{2,\text{max}}}$ ) in active toads ranges from about 2 to 12 mL  $\text{O}_2 \text{ min}^{-1} \text{ kg}^{-1}$  over the temperature interval used in this study (Hedrick 1985). These values represent factorial scopes ( $\dot{V}_{\text{O}_{2,\text{max}}} / \dot{V}_{\text{O}_{2,\text{rest}}}$ ) ranging from about six- to 12-fold. Because arteriovenous oxygen content difference remains constant with activity over this temperature range, increased oxygen demand with activity must be supported by increasing rates of  $\dot{Q}_{\text{sys}}$ . With increased activity, the proportion of  $\dot{Q}_{\text{sys}}$  becomes a smaller fraction of  $\dot{Q}_{\text{tot}}$ ; therefore, the relative reduction in  $\dot{Q}_{\text{sys}}$  may limit maximal rates of systemic oxygen transport to working muscles. Systemic oxygen transport appears to be limited also by increased intracardiac mixing during high metabolic demand, as indicated by the significant reduction in  $S_a$  during activity at 30°C.

The curvilinear relationship of intracardiac mixing and  $f_{\text{H}}$  indicates that systemic oxygen transport should be compromised at low and high  $f_{\text{H}}$ . A high degree of mixing at low  $f_{\text{H}}$  is less problematic since metabolism is low and venous  $\text{O}_2$  contents are high. Increased mixing at high  $f_{\text{H}}$  introduces an inefficiency that would limit oxygen delivery and, hence, uncouple rates of systemic blood flow and rates of oxygen consumption. The net shunting data and intracardiac mixing data both predict that systemic oxygen transport would be more compromised over the 20°–30°C interval compared with the 10°–20°C interval. This should decrease the  $Q_{10}$  for activity oxygen consumption at  $f_{\text{H}}$  greater than 50 beats  $\text{min}^{-1}$  (temperatures above 20°C). Data are available for the  $Q_{10}$  of activity metabolism for five species of anurans over a temperature interval of 10°–30°C (Seymour 1973; Carey 1979). In all five species, the  $Q_{10}$  (mean = 1.56) for the 20°–30°C interval was 55% lower (range 44%–66%) than the 10°–20°C interval (mean = 2.85). The  $Q_{10}$  data for activity metabolism supports both (1) compromised systemic oxygen transport as a consequence of both intracardiac mixing and an increased net L-R

shunt and (2) the hypothesis that aerobic activity metabolism is limited by systemic oxygen transport in amphibians.

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