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# CARDIOVASCULAR FUNCTIONS IN TWO MACRURAN DECAPOD CRUSTACEANS (*PROCAMBARUS CLARKII* AND *HOMARUS AMERICANUS*) DURING PERIODS OF INACTIVITY, TAIL FLEXION AND CARDIORESPIRATORY PAUSES

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## Summary

**Arterial hemolymph flow was measured in restrained crayfish (*Procambarus clarkii*) and lobsters (*Homarus americanus*). Implanted pulsed Doppler flow transducers were used to measure arterial flows in the anterior aorta, posterior aorta, sternal artery, lateral artery, ventral thoracic artery and ventral abdominal artery, allowing determination of flow simultaneously in several arteries over a period of 4 days. Calculated Doppler hemolymph flow showed a strong correlation ($P<0.05$) with 'pumped' hemolymph flow as determined by *in situ* calibration.**

**Arterial flow patterns remained constant during quiet conditions. In crayfish, cardiac output was 7.5±1.1 ml min$^{-1}$ (252 ml kg$^{-1}$ min$^{-1}$), of which the anterior aorta received 1.3±0.15 ml min$^{-1}$ (20.1±4.0 %), the posterior aorta received 0.8±0.1 ml min$^{-1}$ (12.3±2.7 %) and the sternal artery received 5.2±1.4 ml min$^{-1}$ (67.5±37.0 %). Mean heart frequency at rest was 125.6±5.2 beats min$^{-1}$ and stroke volume was 0.06±0.01 ml beat$^{-1}$ (1.98 ml kg$^{-1}$ beat$^{-1}$). In lobsters, cardiac output was 60.8±4.4 ml min$^{-1}$ (93.6±6.8 ml kg$^{-1}$ min$^{-1}$), with the anterior aorta receiving 7.8±0.8 ml min$^{-1}$ (12.8±2.7 %), the lateral arteries receiving 0.6±0.2 ml min$^{-1}$ (1.0±0.5 %), the posterior aorta receiving 12.6±1.0 ml min$^{-1}$ (20.7±3.3 %) and the sternal artery receiving 38.9±4.1 ml min$^{-1}$ (64.0±13.4 %). Flows in the branches of the sternal artery were 0.3±0.05 ml min$^{-1}$ (0.5±2 %) in the ventral abdominal artery and 4.0±0.1 ml min$^{-1}$ (6.5±0.3 %) in the ventral thoracic artery. Lobster heart rate was 82.5±2.9 beats min$^{-1}$ and stroke volume was 0.7±0.05 ml beat$^{-1}$.**

**Periods of constant hemolymph flow were interrupted by tail flexions (abdominal flexion) and, in lobsters, periods of cardiac/respiratory pause. Tail movement increased flow (peak height and minimum flow values) in both crayfish and lobsters, although the general wave form of hemolymph flow and pressure did not change. In lobsters, periodic respiratory pauses were observed during which all arteries received hemolymph, despite the low heart rate.**

Key words: crustacean, *Procambarus clarkii*, *Homarus americanus*, cardiovascular function, Doppler flow, lobster, crayfish.

## Introduction

A pulsed Doppler technique has been used extensively to determine arterial flows in crabs (Brachyura) (McMahon and Burnett, 1990; Airriess *et al.* 1994; Airriess and McMahon, 1994; McGaw *et al.* 1994*a*). This technique has been found to be less invasive and more useful than methods used previously to estimate cardiac output and stroke volume in crustaceans, including the Fick principle, dye-injection and thermodilution techniques (Burnett *et al.* 1981; McMahon and Wilkens, 1983). An *in situ* calibration of the pulsed Doppler flowmeter by Airriess *et al.* (1994) has shown this technique to be applicable to brachyurans. Its use has allowed a greater understanding of the complexity of cardiovascular physiology in crabs. Yet there has been little comparative work concerning the use of the Doppler technique to determine the distribution of cardiac output through the multiple arterial outlets of macruran hearts. Measurements of hemolymph velocity and arterial cross-sectional area have yielded estimates of flow in the posterior aorta of the lobster *Panulirus interruptus* (Belman, 1975), but there have been few accurate, long-term measurements of flow in macruran arteries (compared with brachyuran systems). Consequently, the division of cardiac output between the multiple arterial outputs from macruran hearts is poorly understood.

A comparison between already established brachyuran cardiovascular patterns and macruran patterns is of interest because of the more primitive macruran body design and cardiovascular anatomy. Macrurans (Astacidea) have a large muscular abdominal region used for locomotion which results in a fundamental difference in their circulatory design from the brachyuran crabs. The crayfish (*Procambarus clarkii*) and

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lobster (*Homarus americanus*) are used in the present study to compare typical macruran cardiovascular physiological patterns with those of brachyurans (both within the infraorder Astacidea and between infraorders).

The present study was designed (1) to obtain baseline measurements of arterial hemolymph distribution, heart rate ($f_H$), cardiac output ($\dot{V}b$) and stroke volume ($Vs$) in two species of macruran decapod crustaceans, (2) to verify the applicability of the pulsed Doppler technique for measuring hemolymph flow with specific regard to the macruran system, and (3) to monitor hemolymph flow patterns during periods of respiratory and cardiac pauses.

## Materials and methods

### *Animals*

Crayfish [*Prombarus clarkii* (Girard)] (mean mass ± S.D. 29.8±1.5 g, $N$=45) were obtained from Carolina Biological Supply (Burlington, North Carolina, USA). Before use, animals were placed into 60 l freshwater aquaria (10–15 animals per aquarium) and allowed to acclimate to laboratory holding conditions for 4–6 weeks under a 12 h:12 h L:D cycle at 25 °C. The crayfish were fed boiled spinach and raw liver twice a week.

Lobsters, *Homarus americanus* (L.) (mean mass ± S.D. 650±75.5 g, $N$=24), were obtained from a local supplier. The animals, trapped off the coast of New England 48 h earlier, were kept in a saltwater laboratory holding tank (210 l, 10 °C) for a maximum of 4 days before experimental use. Lobsters were fed pieces of raw fish while in the holding tank. Both crayfish and lobsters were fasted for 48 h prior to experimental use.

### *Hemolymph flow*

Arterial hemolymph velocity was measured using a directional pulsed Doppler flowmeter (Iowa Bioengineering 545C-4). Crayfish and lobsters were prepared for flow transducer implantation by drilling a 1 mm diameter hole with a dental drill through the carapace to the level of the hypodermis over the desired vessel. To prevent hemolymph loss, the hole was covered with a 5 mm square piece of rubber dental dam glued (cyanoacrylate glue) in place. Pulsed Doppler (piezoelectric ultrasound) transducers (Crystals Biotech), 1 mm in diameter, were held firmly in position adjacent to the required vessel and at a constant 45 ° by using a flared piece of polyethylene tubing (PE60, 1.5 cm in length) bent to 45 ° and glued with cyanoacrylate glue to the carapace over the hole. The tubing was glued to the carapace parallel to the vessel to be investigated and served to hold the transducer at the appropriate angle (45 °) relative to the vessel and to stabilize the implantation. In crayfish, transducers were placed over the anterior aorta, sternal artery and posterior aorta (Fig. 1) (Reiber, 1990, 1994). In the lobster, transducers were placed over the anterior aorta, right lateral artery, posterior aorta, sternal artery, ventral thoracic artery and the ventral abdominal artery. The larger size of the lobster enabled hemolymph velocity to be measured in some of the smaller arteries less accessible in the crayfish (Fig. 1).

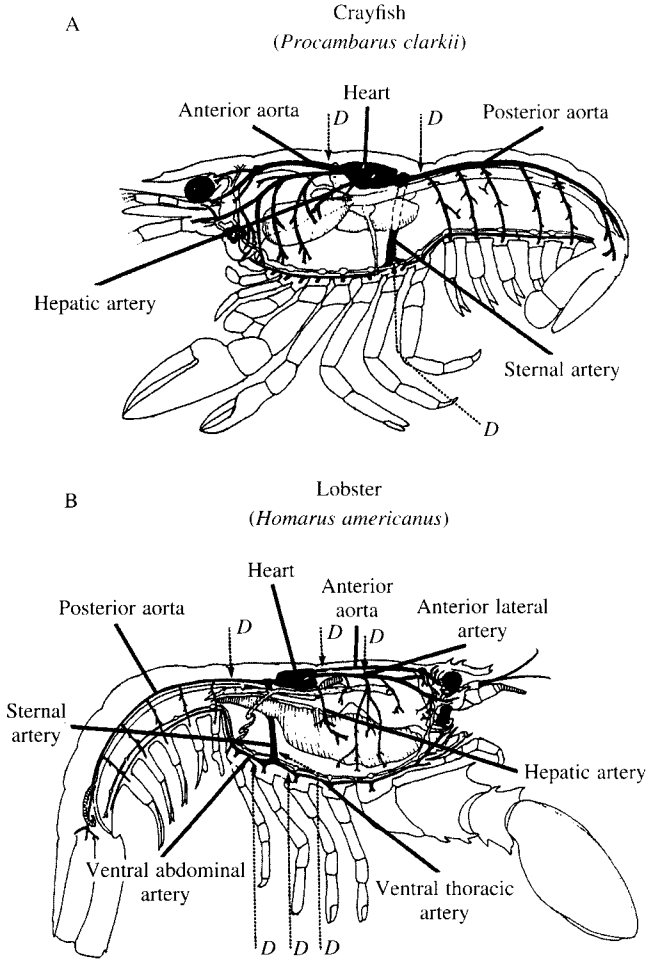


Fig. 1. Diagram of the open circulatory system of the crayfish (*Procambarus clarkii*) (A) and the lobster (*Homarus americanus*) (B). The heart, the major arteries and the location of the Doppler flow probes ($D$) are shown. (Drawings adapted from McLaughlin, 1983.)

Following Doppler transducer implantation in crayfish, a clamp attached to a ring stand was placed around the cephalothorax to restrict movement and prevent the animal from destroying the flow probes. The clamp was adjusted to allow the animals to touch the bottom of the aquarium and to move their claws, legs and tail, but it did not significantly impede the animal's ability to ventilate its branchial chamber nor did it restrict hemolymph flow (see Reiber, 1994). After transducers had been implanted in lobsters, they were secured by gluing a 1.5 cm diameter aluminum post to the anterior dorsal cephalothorax using cyanoacrylate glue. The post was then attached to a ring stand. Lobsters were suspended within the aquarium by the aluminum post and were allowed to hold onto an artificial substratum made of a 25 cm length of 8 cm diameter ridged plastic tubing coated with fine gravel and suspended beneath the lobster using rubber bands. This allowed the lobster to maintain contact with a firm substratum,

limiting tail flexion. The post was then attached to a ring stand holding the animal in a secure position.

A 6 l styrofoam-insulated freshwater aquarium was used as the experimental chamber for crayfish. This aquarium was temperature-regulated to 25 °C using a circulating water bath connected to a heat-exchange coil and was covered to prevent disturbance of the animal during the experimental period.

Lobsters were placed into a 35 l aquarium, with sea water circulating through the aquarium from the marine holding tank (10 °C) at a rate of 144 l $h^{-1}$. The experimental chamber was insulated with styrofoam and covered to prevent disturbance of the animals.

Once the crayfish or lobster had been clamped into position within its respective experimental chamber, the flow transducers were connected to the pulsed Doppler flowmeter. The probes were manipulated to a position that produced a maximal voltage by minor twisting of the transducer wires. Once the probes were so positioned, they were secured for the duration of the experiment. Probe position was verified by *post-mortem* dissection for all vessels in all animals. The Doppler signal from the anterior aorta was also fed into a biotachometer which allowed continuous display of $f_H$. Tail flexion was monitored in both crayfish and lobster by looping a thread around the tail and connecting it to a strain gauge whose signal was displayed on a chart recorder. Right scaphognathite beat frequency (equivalent to ventilation rate) was monitored in lobsters using ventilatory impedance (McDonald *et al.* 1977). Once the signals for all vessels had been established, the animal was left undisturbed for either 72 h (crayfish) or 24 h (lobster) prior to data collection. Movement of a probe relative to its vessels was not found to be a problem during experiments; once the preparation had been fixed in place, it proved to be very stable.

*Experimental protocol and data analysis*

Phasic and mean hemolymph flow signals, as well as $f_H$ and tail position, were displayed on a Narcotrace 80 programmable physiograph. The physiograph was programmed to alternate between paper speeds of 0.25 mm $min^{-1}$ for 8 min and 25 mm $min^{-1}$ for 4 s. Heart rate and mean hemolymph velocity for each vessel were measured during the 4 s periods of 25 mm $min^{-1}$ paper speed, and an average value was calculated for each animal. Tail position was monitored throughout the experiment and recorded as mean tail flexions per minute. Parameters were monitored on individual animals for a minimum of 4 h per day for four consecutive days (allowing a minimum of 16×4 s sampling periods per day).

Hemolymph velocity ($v$) was calculated directly from the Doppler shift frequency as $v=\Delta fc/(2f_o\cos\theta)$, where $v$ is the velocity of the reflector (m $s^{-1}$), $\Delta f$ is the Doppler shift frequency (Hz), $c$ is the speed of sound in the medium (m $s^{-1}$), $f_o$ is the transmitted frequency (Hz) and $\theta$ is the angle between the direction of the sound beam and the velocity vector. Substituting for the constants, the speed of sound through the medium ($c$=1500 m $s^{-1}$), the transmitted frequency ($f_o$=20 MHz) and the cosine of the angle of incidence ($\cos\theta$ =0.7071), $v=5.3\Delta f$ (where $\Delta f$ is in kHz) (Chauveau *et al.* 1985).

A parabolic velocity profile is seen during laminar flow in a circular tube; therefore, velocity is considered to be the maximum velocity attained at the center of the vessel or $v_{max}$. The average velocity ($v_{avg}$) is the integrated velocity across the vessel; a good approximation of the average velocity is calculated by dividing $v_{max}$ by 2 (Hartley *et al.* 1978). The average velocity ($v_{avg}=5.3\times\Delta f\times0.5$) multiplied by the luminal cross-sectional area of the vessel ($A$, in $cm^2$) equals laminar flow ($\dot{Q}$, in ml $min^{-1}$), where $\dot{Q}=5.3\Delta f/2A$.

In a separate set of animals (crayfish, $N$=7; lobsters, $N$=5),

Table 1. *Arterial diameters of* Procambarus clarkii *and* Homarus americanus

| Vessel | Systolic diameter, $D_s$ (mm) | Diastolic diameter, $D_d$ (mm) | $\Delta D=D_s-D_d$ (mm) | Mean diameter, $D_a$ (mm) | $d^2$ |
|---|---|---|---|---|---|
| *Procambarus clarkii* | | | | | |
| Anterior aorta | 0.65±0.1 | 0.55±0.1 | 0.1 | 0.6 | 0.36 |
| Sternal artery | 1.0±0.1 | 0.9±0.1 | 0.1 | 0.95 | 0.9 |
| Posterior aorta | 0.55±0.1 | 0.45±0.1 | 0.1 | 0.5 | 0.25 |
| Hepatic artery | ND | ND | ND | 0.1* | ND |
| *Homarus americanus* | | | | | |
| Anterior aorta | 1.2±0.1 | 0.95±0.1 | 0.25 | 1.1 | 1.2 |
| Sternal artery | 1.9±0.1 | 1.7±0.1 | 0.2 | 1.8 | 3.4 |
| Ventral abdominal artery | 0.4±0.05 | 0.3±0.05 | 0.1 | 0.3 | 0.1 |
| Ventral thoracic artery | 1.45±0.2 | 1.2±0.2 | 0.25 | 1.4 | 1.9 |
| Right lateral artery | 0.9±0.05 | 0.8±0.05 | 0.1 | 0.8 | 0.6 |
| Posterior aorta | 2.0±0.1 | 1.6±0.15 | 0.4 | 1.86 | 3.5 |
| Hepatic artery | ND | ND | ND | 0.45* | ND |

Mean arterial diameters were calculated for crayfish ($N$=7) and lobsters ($N$=5).

The square of the arterial diameter is given as $d^2$ and used in the Doppler flow equation.

Values are means ±1 S.E.M.

*The hepatic artery was too small to obtain a pulsatile diameter; the value given is from a visual determination of mean diameter; ND, not determined.

direct measurements of systolic and diastolic arterial diameters were obtained (Table 1) and a confirmation of laminar flow profiles established using a Zeiss dissecting microscope equipped with a video imaging system. The artery to be studied (anterior aorta, posterior aorta, and sternal artery in the crayfish; anterior aorta, posterior aorta, common sternal artery, right lateral artery, ventral thoracic artery and abdominal artery in the lobsters) was exposed by cutting a hole through the carapace. A piece of clear acetate, graduated with 0.5 mm divisions, was placed under the artery. The recorded image was then replayed and analyzed for systolic and diastolic diameters. A mean value for vessel diameter was used to calculate flow. The equation used to determine mean vessel diameter was adapted from Berne and Levy (1977): $D_a=D_d+T_s\ (D_s-D_d)$, where $D_a$ is mean arterial diameter (mm), $D_s$ is the arterial diameter during systole (mm), $D_d$ is the arterial diameter during diastole (mm) and $T_s$ is the duration of systole (fraction of cycle). This equation allows integrated values of mean arterial diameter to be calculated on the basis of the fraction of the cardiac cycle spent in systole.

A parabolic flow profile (laminar flow) was confirmed in each vessel at the location where flow was measured (as required for the Doppler equations) by observing video recordings of a bolus of blue-stained hemolymph (blue food coloring) passing through each vessel. In all vessels, laminar flow was observed within 1 or 2 mm downstream from the heart.

Estimates of cardiac output ($\dot{V}$b) were made by summation of arterial hemolymph flow rates (ml $min^{-1}$). In crayfish, these included the flow rates in the anterior aorta, sternal artery and posterior aorta. Right and left lateral arterial flow was not determined in crayfish as anterior aortic flow was measured posterior (upstream) to the division which gives rise to the left and right lateral arteries. Hepatic arterial flow was considered to be a negligible component of $\dot{V}$b owing to this vessel's extremely small diameter (representing a calculated theoretical maximum of 5 % of $\dot{V}$b, on the basis of hepatic vessel diameter and a maximal Doppler shift). Lobsters were separated into two groups for simultaneous measurements. The first group consisted of lobsters in which measurements were made in the anterior aorta, posterior aorta, ventral abdominal artery and ventral thoracic artery ($N$=6). The second group had flow measured in the anterior aorta, posterior aorta, common sternal artery and the right lateral artery ($N$=6). Cardiac output estimates for lobster were obtained by summation of flow rates in the anterior aorta, sternal artery, posterior aorta plus twice the flow rate in the lateral artery. Lateral arterial flow was doubled to estimate $\dot{V}$b because the flow rate was measured in only one of the two lateral arteries present. Stroke volume ($V_S$) was calculated by dividing mean $\dot{V}$b by mean $f_H$. The hepatic arterial flow rate was not measured in lobsters owing to the extremely invasive nature of the procedure; the hepatic artery is accessible only by opening the branchial chamber. Tail flexion was assessed during the entire experimental period and a mean rate of tail flexions determined per minute. The onset of tail flexion was indicated by movement of the strain gauge, resulting in a pen deflection. The time course and arterial flow changes during tail flexion were analyzed for six lobsters. All values obtained from individual animals were averaged and are presented as means ±1 S.E.M.

### In situ *calibration*

Calculated Doppler flow rates in the posterior aorta were compared with known pumped flow rates using the methods described by Chauveau *et al.* (1985). Crayfish and lobsters were prepared as described for measurement of posterior aortic hemolymph velocity. Mean hemolymph velocity was recorded continuously from the intact animal for 15 min to establish *in situ* flow values. The animal was then removed from the experimental chamber, cold-anesthetized (in iced water) and its heart exposed. Care was taken to maintain precise flow probe position relative to the posterior aorta during the cannulation procedure. The posterior aorta was cannulated *in situ* using a 5 cm piece of PE50 (crayfish) or PE90 (lobster) tubing with one end flared. The flared end was introduced into the posterior aorta through a lateral ostium of the heart and was held secure by a length of 3-0 gauge surgical silk tied around the vessel and cannula. Homogenized declotted hemolymph was then pumped through the cannula at a known rate using a peristaltic pump (McDonald *et al.* 1977). A range of pump outputs was selected to encompass the range normally recorded in the posterior aorta. These were then used to generate the calibration curve. Linear regression of pumped flow *versus* calculated Doppler flow measurement was performed using the least-squares method.

### *Fick principle estimates of cardiac output*

Fick estimates of $\dot{V}$b were determined for crayfish and were compared with calculated Doppler $\dot{V}$b (summed arterial flows). Literature values of $\dot{V}$b for the crayfish *P. clarkii* determined using the Fick method were limited in their utility, owing to the wide range of experimental conditions under which the animals were sampled. Crayfish used for Fick estimates of $\dot{V}$b in the present study were maintained under the same conditions as animals used for Doppler experiments. Cardiac outputs determined using the better-established Fick technique were used as a reference point against which a relative comparison could be made with calculated Doppler $\dot{V}$b.

Using the Fick method, estimates of $\dot{V}$b in crayfish were calculated on the basis of the rate of oxygen uptake (ml $min^{-1}$) and the oxygen content (ml $O_2$ $ml^{-1}$ hemolymph) of arterial and venous hemolymph: $\dot{V}b=\dot{V}_{O_2}/Ca_{O_2}-Cv_{O_2}$, where $\dot{V}$b is gill perfusion rate or cardiac output (ml $min^{-1}$), $\dot{V}_{O_2}$ is the rate of oxygen uptake (ml $min^{-1}$), $Ca_{O_2}$ is total arterial $O_2$ content (ml $ml^{-1}$ hemolymph) and $Cv_{O_2}$ is total venous $O_2$ content (ml $ml^{-1}$ hemolymph).

### *Oxygen consumption*

Rates of $O_2$ consumption (ml $min^{-1}$) for use in the Fick equation were determined on 16 crayfish using closed-system respirometry. Animals were placed individually into respirometers which were filled with air-saturated water at 25 °C. The respirometer was sealed and submerged in a

waterbath regulated to 25 °C. Water samples were drawn through valved 21 gauge needles fitted with a piece of PE60 tubing ending near the bottom of the respirometer. A 0.5 ml water sample was withdrawn every 10 min and its oxygen partial pressure ($P_{O_2}$) determined using an Instrument Laboratory pH/blood-gas analyzer. Experiments in which water $P_{O_2}$ values fell below 133.3 kPa were discarded to avoid hypoxic influences on $O_2$ consumption.

### *Hemolymph total oxygen content*

Oxygen contents of arterial ($Ca_{O_2}$) and venous ($Cv_{O_2}$) hemolymph for use in the Fick equation were determined on a separate series of 12 animals. These animals were maintained at 25 °C and were prepared for paired arterial and venous hemolymph sampling. Arterial hemolymph was drawn through a 1 mm diameter predrilled hole over the heart, which was sealed with a 5 mm×5 mm piece of rubber dam. Venous samples were drawn from the infrabranchial sinus through a hole drilled through the ventral carapace medial to the last pair of walking legs, which was then covered with a triangular piece of rubber dental dam. The paired hemolymph samples were then analyzed for total $O_2$ content using Tucker's (1967) method. Collection of each pair of samples took approximately 30 s, during which time the animal was monitored for $f_H$ using an impedance technique (McDonald *et al.* 1977). Heart rate was monitored for 2 min prior to hemolymph sampling and during the sampling procedure. Crayfish $\dot{V}_{O_2}$ and paired hemolymph $Ca_{O_2}$ and $Cv_{O_2}$ values were used to determine $\dot{V}b$ using the Fick equation.

### *Cardiovascular anatomy*

Doppler probes were positioned adjacent to each artery using keyhole (blind) surgery. Fine positioning of the Doppler probe was required to obtain a maximum signal and thus the precise location of each artery was determined by prior dissection. Crayfish and lobsters were chilled in an iced waterbath prior to dissection. The animal's heart was then injected with 0.1 ml of blue food coloring diluted 10:1 with declotted hemolymph. This gave greater contrast to the vessels, allowing them to be distinguished from background material. Once the vessels had been located internally, landmarks were obtained on the carapace, enabling precise transducer implantation.

The heart and circulatory system of crayfish and lobsters are representative of the anatomical arrangement found in other macrurans and have been described previously in detail (Maynard, 1960; Blatchford, 1971; Belman, 1975; McLaughlin, 1983). The circulatory anatomy of the crayfish *P. clarkii* was found to be very similar to that of astacidean crayfish described by McLaughlin (1983). The outstanding exception to this similarity was the origin of the sternal artery, which originates from the bulbus arteriosus in an analogous manner to the circulatory anatomy of homarid and palinurid lobsters.

## Results

### *Evaluation of the Doppler technique*

Seven crayfish and five lobsters were prepared for *in situ* calibration of posterior aortic hemolymph velocity. Mean posterior aortic Doppler flow determined in five crayfish was 0.8±0.1 ml min$^{-1}$, with a range for all crayfish of 0.2–2.7 ml min$^{-1}$. Mean posterior aortic flow in the lobster was 12.6±1.0 ml min$^{-1}$, with a range for all animals of 2.6–27.7 ml min$^{-1}$.

Pumped flow values were a linear function of calculated Doppler flow values in both crayfish and lobster for a physiologically significant range [(crayfish) $r^2$=0.99, $P$<0.05, $y$=1.01$x$–0.021, where $y$ is pumped flow rate and $x$ is calculated Doppler flow rate; (lobster) $r^2$=0.97, $P$<0.05, $y$=1.1$x$–0.05] (Fig. 2). The mean deviation of Doppler flow values from pumped flow was 0.2 ml min$^{-1}$ for crayfish data and 0.8 ml min$^{-1}$ for data from lobster.

Fick estimates of $\dot{V}b$ were 11.6 ml min$^{-1}$ (391.2 ml kg$^{-1}$ min$^{-1}$) in crayfish using measured total oxygen content of arterial and venous hemolymph ($Ca_{O_2}$=0.63 vol%, $Cv_{O_2}$=0.21 vol%) and whole-animal oxygen consumption ($\dot{M}_{O_2}$=2.0±0.13 μmol min$^{-1}$). Heart rate monitored prior to hemolymph sampling was 171.7±2.4 beats min$^{-1}$ and $V_S$ was 0.07 ml beat$^{-1}$ (2.3 ml kg$^{-1}$ beat$^{-1}$) (Table 2).

### *Hemolymph flow*

*Crayfish*

Simultaneous measurements of hemolymph velocities in the anterior aorta, sternal artery and posterior aorta were made in 12 crayfish (e.g. Fig. 3).

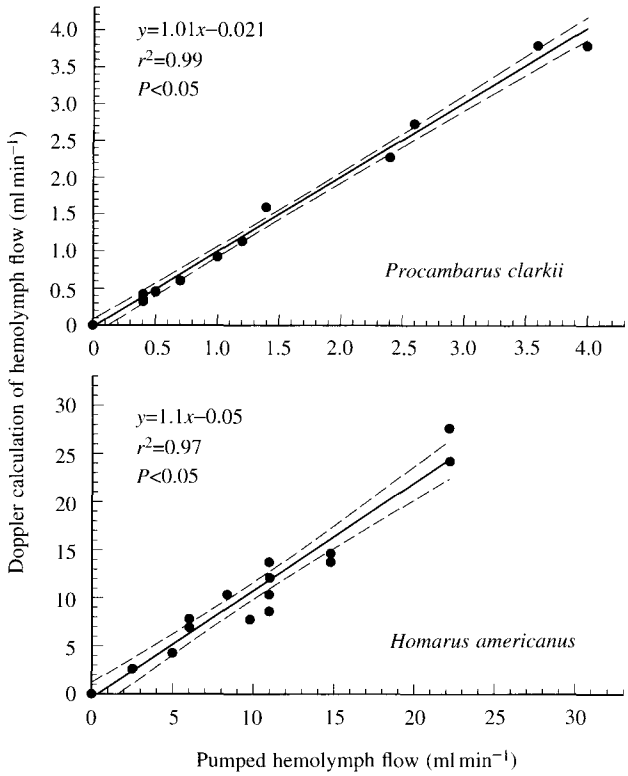


Fig. 2. *In vivo* calibration of the crayfish posterior aorta (A) and the lobster posterior aorta (B): calculated Doppler flow rate *versus* pumped flow rate (dashed lines indicate 95 % confidence intervals).

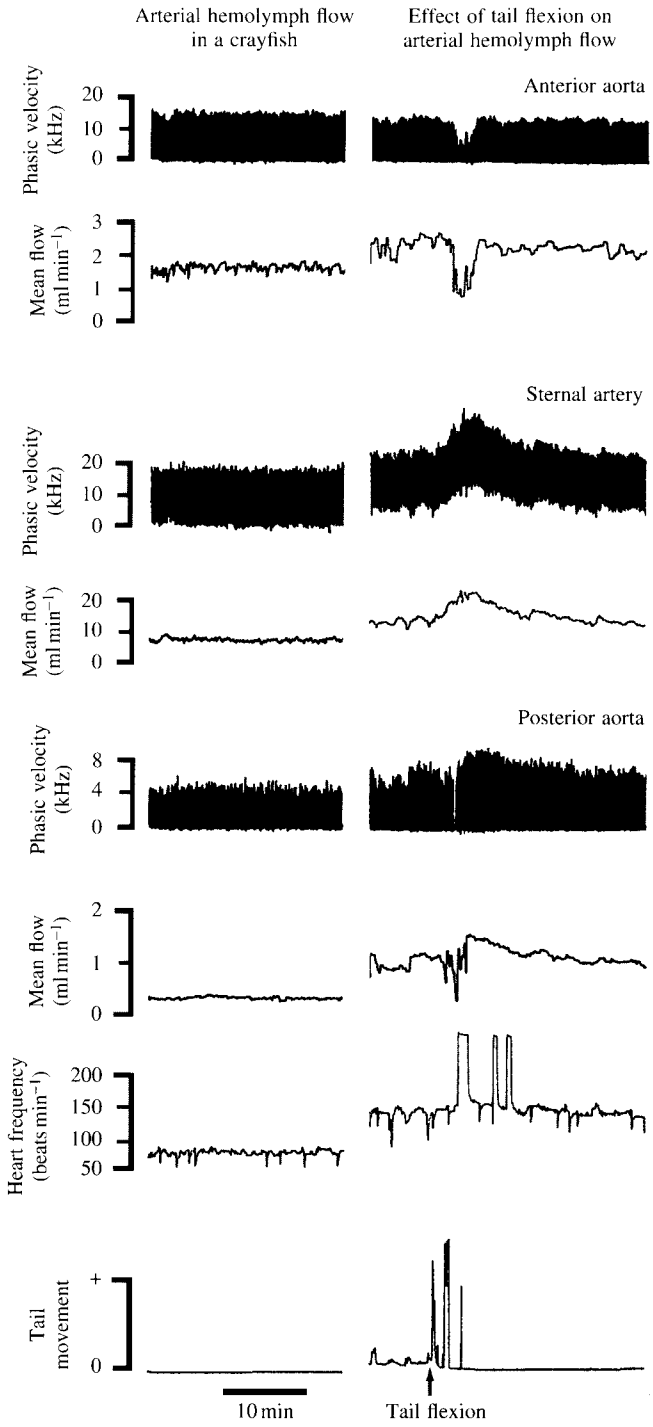


Fig. 3. Simultaneous recordings of anterior aortic, sternal arterial and posterior aortic hemolymph flow rates (both phasic velocity and mean flow rate), heart frequency and tail flexion in a crayfish. The effects of tail flexion on arterial flows and heart rate (tail position indicated by 0 for extension, + for flexion; initiation of tail flexion indicated by an arrow) are shown in the same individual in the right-hand panels.

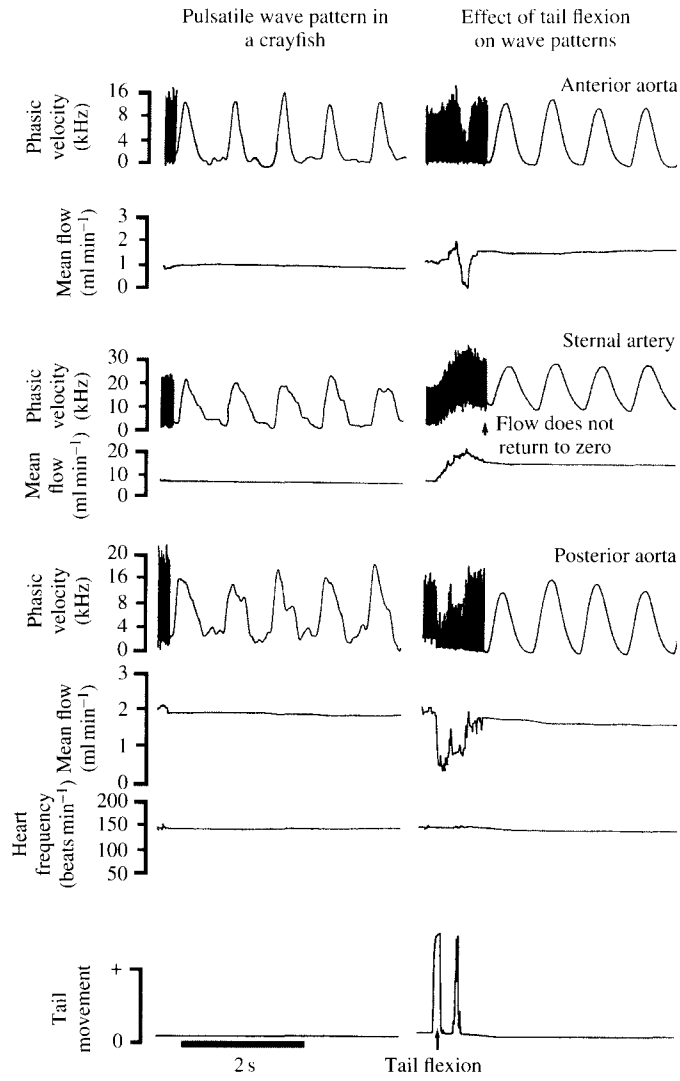


Fig. 4. Simultaneous recordings of pulsatile wave forms of anterior aortic, sternal arterial and posterior aortic hemolymph flow rates in a crayfish (both phasic velocity and mean flow rates), tail flexion and heart rate. Pulsatile wave forms of arterial flows during tail flexion (tail position indicated by 0 for extension, + for flexion; initiation of tail flexion indicated by an arrow) are shown in the right-hand pannel. Note that sternal arterial flow does not return to the baseline (zero) value during and for some time after tail flexion (right).

Mean $f_H$ was 125.6±5.2 beats min$^{-1}$, $\dot{V}b$ was 7.5±1.1 ml min$^{-1}$ (252 ml kg$^{-1}$ min$^{-1}$) with a $V_S$ of 0.06±0.01 ml beat$^{-1}$ (1.98 ml kg$^{-1}$ beat) (Table 2). The sternal artery received the greatest percentage of $\dot{V}b$ (68±37 %) with a flow rate of 5.2±1.4 ml min$^{-1}$. The anterior aorta received 1.3±0.15 ml min$^{-1}$ (20±4 %) of $\dot{V}b$. The posterior aorta received 0.8±0.1 ml min$^{-1}$ of $\dot{V}b$ (12±3 %). Mean baseline arterial flow levels remained constant over the 4 day experimental period except during spontaneous or induced activity.

Central arterial flows were highly pulsatile and were initiated by the opening of the cardioarterial valves at the beginning of systole (Fig. 4). The posterior aorta showed a slight retrograde flow during early diastole that was not evident in the other two arteries. Arterial flow rates increased substantially as a result of tail flexions, and the elevated flow rates in the posterior aorta and sternal artery often continued

Table 2. *Arterial flows and their percentage of cardiac output in crayfish*

| Vessel | Doppler arterial flow (ml min$^{-1}$) | Doppler arterial flow (% of cardiac output) | Fick estimates |
|---|---|---|---|
| Anterior aorta | 1.3±0.15 | 20.1±4.0 | |
| Sternal artery | 5.2±1.4 | 67.5±37.0 | |
| Posterior aorta | 0.8±0.1 | 12.3±2.7 | |
| Cardiac output (ml min$^{-1}$) | 7.5±1.1 | | 11.6 |
| Heart frequency (beats min$^{-1}$) | 125.6±5.2 | | 171.7±2.4 |
| Stroke volume (ml beat$^{-1}$) | 0.06±0.01 | | 0.07 |

A comparison of cardiac output, stroke volume and heart frequency between the Doppler method and the Fick method is also shown.
Values are means ± S.E.M. ($N$=12).

Table 3. *Arterial flows and their percentage of cardiac output in lobster*

| Vessel | Doppler arterial flow (ml min$^{-1}$) | Doppler arterial flow (% of cardiac output) | Fick estimates |
|---|---|---|---|
| Anterior aorta | 7.8±0.8 | 12.8±2.7 | |
| Sternal artery | 38.9±4.1 | 64.0±13.4 | |
| Ventral abdominal artery | 0.3±0.05 | 0.5±0.2 | |
| Ventral thoracic artery | 4.0±0.1 | 6.5±0.3 | |
| Lateral artery (right) | 0.6±0.15 | 1.0±0.5 | |
| Posterior aorta | 12.6±1.0 | 20.7±3.3 | |
| Cardiac output (ml min$^{-1}$) | 60.8±4.4 | | 67.0 |
| Heart frequency (beats min$^{-1}$) | 82.5±2.9 | | 90.0 |
| Stroke volume (ml beat$^{-1}$) | 0.7±0.05 | | 0.7 |

A comparison of cardiac output, stroke volume and heart frequency between the Doppler method and the Fick method is also shown.
Values are means + S.E.M. ($N$=12) (Burger and Smythe, 1953).

throughout diastole for several minutes after tail flexion (Figs 3, 4).

*Lobsters*

Lobsters were separated into two groups for measurements of hemolymph velocity. In the first group ($N$=6), the anterior and posterior aortic flow rates and ventral abdominal and ventral thoracic arterial flow rates were determined. In the second group ($N$=6), the anterior and posterior aortic flow rates, common sternal arterial flow rate and right lateral arterial flow rate were monitored.

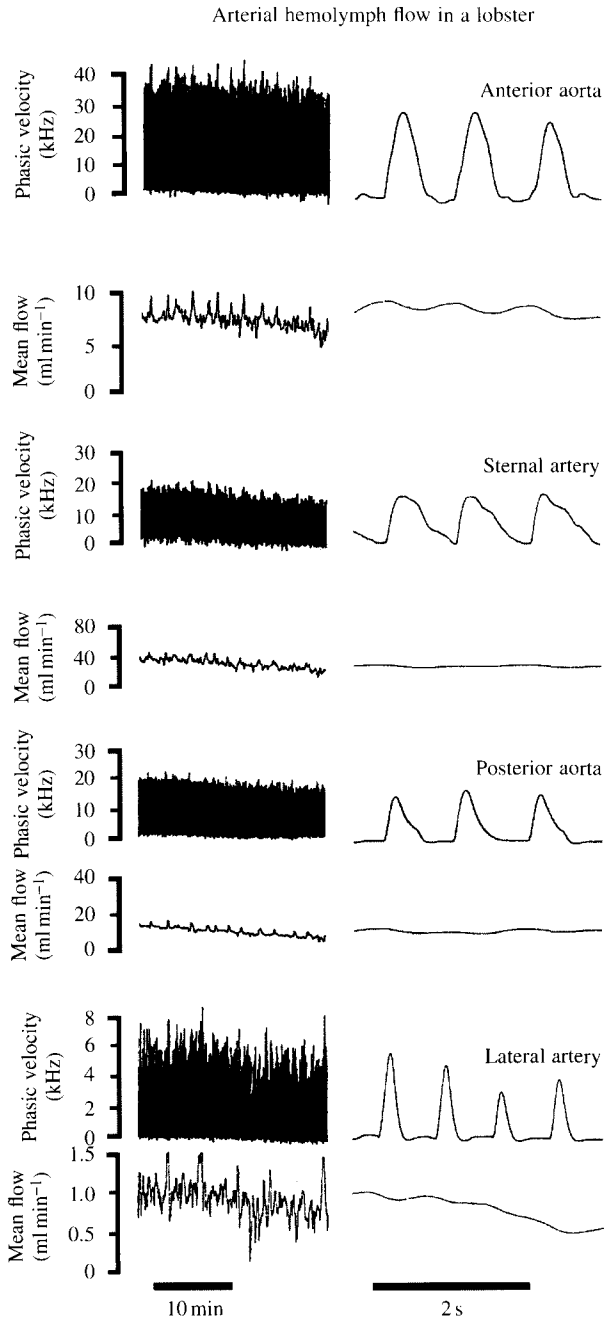


Fig. 5. Simultaneous recordings of anterior aortic, sternal arterial, posterior aortic and right lateral arterial hemolymph flow rates (both phasic velocity and mean flow rates are shown) in a lobster (left panels) and a higher-speed recording showing the pulsatile wave forms of arterial flow (right panels).

Heart rate was 82.5±2.9 beats min$^{-1}$ with a $V$s of 0.7±0.05 ml beat$^{-1}$ and a $\dot{V}$b of 60.8±4.4 ml min$^{-1}$ (93.6±6.8 ml kg$^{-1}$ min$^{-1}$) (Table 3). Mean arterial hemolymph flow rates remained constant during the experimental period (Fig. 5). Hemolymph flow to the anterior aorta was 7.8±0.8 ml min$^{-1}$ (13±3 %), to the right lateral artery was 0.6±0.15 ml min$^{-1}$ (1±1 %), to the posterior aorta was 12.6±1.0 ml min$^{-1}$ (21±3 %) and to the sternal artery was 38.9±4.1 ml min$^{-1}$ (64±13 %). Hemolymph

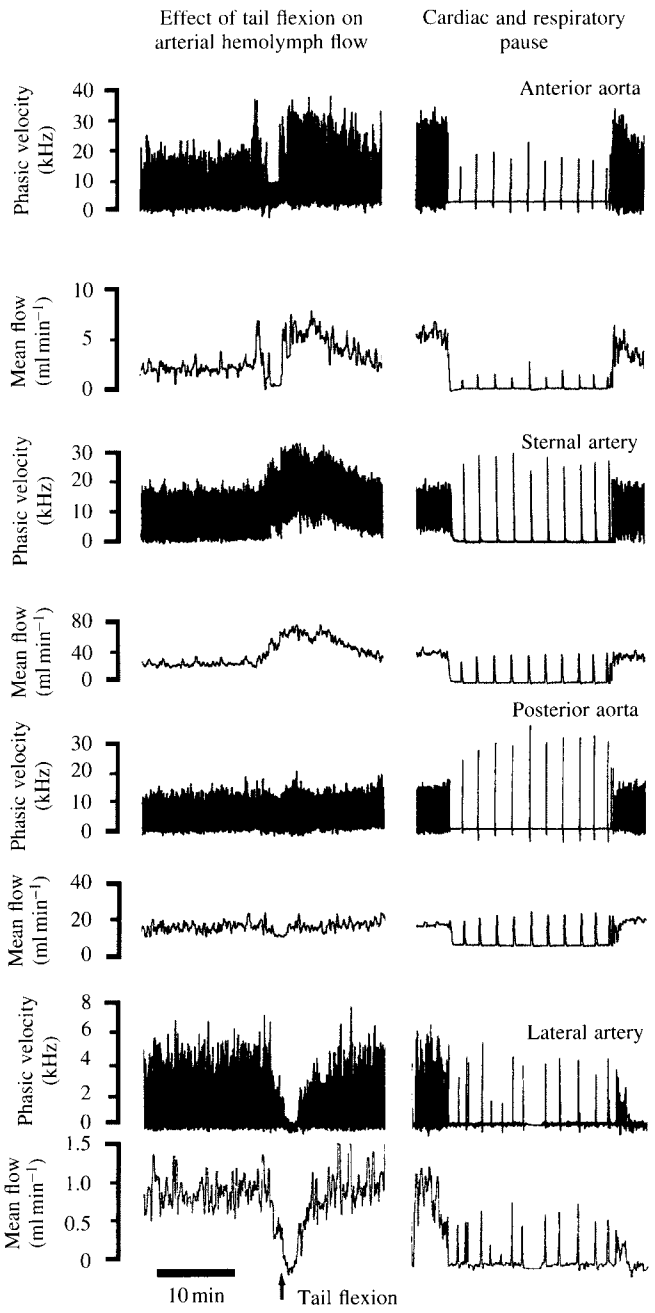


Fig. 6. Composite recording from two lobsters of anterior aortic, sternal arterial, posterior aortic and right lateral arterial hemolymph flow rates (both phasic velocity and mean flow rates are shown) during tail flexion (an arrow indicates the start of tail flexion) (left panel) and during a cardiac and respiratory pause (right panel).

flow to the ventral abdominal artery was 0.3±0.05 ml min$^{-1}$ (0.5±0.2 %) and to the ventral thoracic artery 4.0±0.1 ml min$^{-1}$ (7±0.3 %), equal to 10 % of sternal arterial flow (Table 3).

Central arterial flows recorded in lobsters showed similar waveforms to those seen in crayfish (Fig. 5). The ventral abdominal and ventral thoracic arteries showed a slight retrograde flow during early diastole (results not shown); this was not seen in the other arteries. During periods of activity (tail or leg movement), arterial flow increased, and flow in the sternal artery (Fig. 6), ventral abdominal and ventral thoracic arteries (not shown) often continued during diastole for some minutes following cessation of tail flexion.

*Tail flexion and arterial flow in lobsters*

Quiescent animals showed no consistent pattern of tail flexion events. Cardiac output, *Vs* and flow distribution changed as a result of tail flexion (Fig. 6). Heart rate was not affected by tail flexion, but $\dot{V}$b increased by 41±6 % during a maximal response (from 65.5 ml min$^{-1}$ to 91.5±1.8 ml min$^{-1}$). Stroke volume increased by 41 % from 0.8±0.05 ml beat$^{-1}$ in quiescent animals to a maximum of 1.1±0.1 ml beat$^{-1}$. Flow in the anterior aorta immediately after tail flexion decreased to near zero for 32±3 s, followed by an immediate return to pre-flexion flow rates (Fig. 6). In contrast, flow in the sternal artery increased to 118±13 % of pre-flexion values in 25±2.1 s. Peak flow in the sternal artery lasted for less than 30 s before an initial rapid decline, which then became asymptotic and approached pre-flexion values in 1.8±0.3 min. Within 10 s of a tail flexion, posterior aortic flow showed a 54±1 % decrease for 12.8±1.6 s, followed by a slightly increased flow and a return to pre-flexion flow rates in 1.4±0.3 min. Right lateral arterial flow dropped to near zero by 22±1 s after tail flexion and remained at this level for 11±2 s before returning to pre-flexion values in 48±3 s.

*Cardiac and respiratory pauses in lobsters*

Periodic cessations of ventilation and heart beat were characteristic of quiescent lobsters but not crayfish. During a pause (Fig. 6), ventilatory activity in the right branchial chamber stopped and was assumed to cease in the left branchial chamber as well. Heart rate decreased from 82.5±2.9 beats min$^{-1}$ to a regular rate of 2 beats min$^{-1}$. Pause length varied from less than 1 min to longer than 8 min. Mean flow rate in arteries decreased, but the peak height for each flow pulse in all the arteries was 15–20 % higher (Fig. 6). The termination of a pause was marked by a return to a regular ventilatory and cardiac rhythm.

## Discussion

*Evaluation of the Doppler technique in macrurans*

Previous measurements of $\dot{V}$b in macruran crustaceans (see McMahon and Wilkens, 1983) were based on the Fick principle, dye-injection or thermodilution techniques. The pulsed Doppler system described here allows extended simultaneous measurement of arterial outflows from the heart. Summation of arterial flows allows long-term assessment of $\dot{V}$b and regional blood flow in intact macruran crustaceans. A similar system has been used previously to study regional blood flow distribution in brachyuran decapods (Airriess *et al.* 1994).

*In situ* calibration of the pulsed Doppler flowmeter was carried out using flow rates in the posterior aorta of both the crayfish and the lobster. A procedure using predetermined

pumped flow rates (within physiologically relevant values for both the crayfish and lobster) was based on techniques established by Chauveau *et al.* (1985) for vertebrates and Airriess *et al.* (1994) for crustaceans. A Doppler shift frequency was obtained for each pumped flow rate and was used to calculate hemolymph flow. Pumped flow rates were then compared with calculated Doppler flow rates (Fig. 2). The correlations between pumped flow rates and calculated Doppler flow rates were very high, with the slope of the regression lines not differing significantly from 1 and the intercept not differing significantly from zero. Posterior aortic hemolymph flow calculated using the Doppler flow rates compared with pumped flow rates resulted in overestimates averaging 2.8 % and 3.7 % for the crayfish and lobster, respectively, which are low enough to be of minimal significance in terms of practical application of the technique in crustaceans.

The scatter around the mean values for calculated Doppler flows may be due to several factors involved in the calculation of these flows. The variables in the equations that may account for such scatter are $\theta$, the angle between the direction of the sound beam and the velocity vector, and $A$, the luminal cross-sectional area of the vessel. The remaining parameters are either known or constant (Chauveau *et al.* 1985).

The technique used in the present study to measure vessel diameter allowed the calculation of integrated values for arterial diameters based on the fraction of the cardiac cycle spent in systole. Precise determination of real-time *in situ* arterial diameter is not possible; however, our technique may lead to either under- or overestimates of mean diameter because of (1) changes in vessel transmural pressure resulting from the invasive surgical procedure, and (2) changes in systolic/diastolic hemolymph pressure as a result of trauma to the animals during exposure of the vessel. Error could have been introduced into calculated Doppler flows as a result of such errors in mean arterial diameter.

The angle between the direction of the sound beam and the velocity vector was maintained as close to 45 ° as possible through the use of a guide constructed from angled polyethylene tubing, which fixed the transducer at this angle relative to the vessel. Once the preparation was secure, the angle between the transducer and the vessel should remain constant.

The variance around the regression lines in Fig. 2 could be attributed to one or both of these sources of error. However, given the linearity of the calibration curves for both crayfish and lobsters, the calculated Doppler flow values are likely to be as accurate as those from other techniques currently employed. The technique is also inherently more useful because of the small size of the transducers and its ability to give long-duration measurements of hemolymph flow.

Cardiac outputs obtained by summation of arterial outflow agreed closely with values obtained (on a separate set of crayfish) using the Fick method. Fick estimates were approximately 35 % higher than Doppler estimates. This difference can be considered of little significance given the differences in the level of disturbance to the animals between the two techniques. The critical difference between the two techniques is the ability to measure arterial flows, $V_S$, $\dot{V}b$ and $f_H$ simultaneously provided by the Doppler technique.

Cardiac output in the lobster determined by the Doppler technique fell within the range (100–144 $ml\,kg^{-1}\,min^{-1}$) reported previously for *H. americanus* (Burger and Smythe, 1953; McMahon and Wilkens, 1975, 1983; Butler *et al.* 1978). Literature values for resting $f_H$ (92–100 $beats\,min^{-1}$) are slightly higher than those determined using the Doppler method (Burger and Smythe, 1953; McMahon and Wilkens, 1975).

### *Hemolymph flow measurements*

Cardiac output determined by summation of arterial hemolymph flows was similar to that reported previously for the crayfish *Pacifasticus leniusculus* at 20 °C (236±47 $ml\,kg^{-1}\,min^{-1}$) (Rutledge, 1981) and fell within the reported range of $\dot{V}b$ for decapod Crustacea (McMahon and Wilkens, 1983). The distribution of $\dot{V}b$ through the arterial system also remained relatively constant during periods of inactivity, with most of the hemolymph going to the sternal artery, and smaller proportions going to the anterior aorta and posterior aorta. A relatively large percentage of $\dot{V}b$ was therefore directed towards neural tissues. The anterior aorta and sternal artery together received 80 % of $\dot{V}b$, supplying the eyes, subesophageal ganglia, ventral nerve cord, mouthparts and a portion of the abdomen. The posterior aorta, which supplies the gut, the midgut gland, the pleopods, the uropods and the abdominal musculature, received the lowest percentage of $\dot{V}b$. A similar pattern is observed in the brachyuran *Cancer magister*, where hemolymph flow through the sternal and anterolateral arteries represents 87 % of $\dot{V}b$ (Airriess and McMahon, 1994).

Flow was altered substantially as a result of tail flexion in both lobster and crayfish. Contraction of the abdominal muscles during tail flexion increased $V_S$ substantially and caused a redistribution of $\dot{V}b$, but had no effect on $f_H$. Cardiac output was redistributed during tail flexion, increasing the blood supply to the abdominal muscles and thus serving the increased metabolic demand during activity. Blood supply also increased to the limbs, the mouthparts and the muscles supplying the scaphognathites. Of particular importance would be perfusion of the muscles supplying the scaphognathite because they would have an increased metabolic demand during activity. A similar shunting of flow towards active tissues was noted during struggling in the crab *C. magister* by Bourne and McMahon (1989).

Respiratory pauses associated with extreme bradycardia were observed only in lobsters. These pauses were often prolonged and could be associated with the development of internal hypoxia (Wilkens *et al.* 1974; McMahon and Wilkens, 1975, 1977; Wilkens, 1976). Internal hypoxia could be ameliorated by utilization of the substantial venous reserve. The slow maintained heartbeat during pausing could thus be beneficial by delivering occasional amounts of arterialized

hemolymph to the central nervous system and by allowing slow turnover of venous-reserve hemolymph. The absence of a pronounced recovery response, such as that occurring after long-term hypoxia in *H. americanus* (Reiber, 1990, 1995; McMahon *et al.* 1989; Reiber *et al.* 1992), argues against the development of tissue hypoxia in the central nervous system and other regions during a pause.

Few direct measurements of macruran crustacean arterial hemolymph flows have been made previously. Hemolymph velocities were reported for the lobsters *Panulirus interruptus* (Belman, 1975) and *H. americanus* (Jorgenson *et al.* 1982). The specific distribution of $\dot{V}$b through the arterial system has been demonstrated in only one other decapod crustacean, *Cancer magister* (Airriess and McMahon, 1992; Airriess *et al.* 1994; McGaw *et al.* 1994*a*,*b*; McGaw and McMahon, 1995). A fundamental difference between Brachyura and Macrura is the large muscular abdominal region used for swimming in the latter. This region, perfused primarily by the posterior aorta and to a lesser extent by the ventral abdominal artery, accounts for 12.3 % and 20.7 % of $\dot{V}$b in crayfish and lobster, respectively, whereas the crab posterior aorta only receives 4 % of $\dot{V}$b. During periods of tail activity, flow to this region increases greatly (by 50 %) in crayfish (Reiber, 1994) and to a lesser extent in lobster (by approximately 20 %, this study). Increased perfusion to this region during activity would help maintain oxygen delivery and removal of metabolic waste. Anterior aortic flow is a significant component of $\dot{V}$b in both macruran species (20.1 % and 12.8 % in crayfish and lobster, respectively). Crabs pump much less hemolymph through the anterior aorta than either macruran species, with only 5 % of $\dot{V}$b going to the anterior aorta. Macrurans dramatically reduce flow through the anterior aorta during tail flexion.

The general design of the circulatory system in macrurans differs from that in brachyurans, with a large vessel supplying the muscular abdominal region. During periods of locomotion (tail flexion), this region requires a high rate of perfusion to maintain $O_2$ delivery. Brachyurans use their walking legs for locomotion or these may be modified as swimming paddles (e.g. in the Portunidae). These locomotory patterns both demand a circulatory system designed to supply their ventral region with arterialized blood to support muscular activity of the legs. This difference between macrurans and brachyurans is apparent when comparing their anatomy and cardiovascular physiology. In spite of the differences between posterior aortic flows and the patterns of perfusion of the anterior aorta, both macrurans and brachyurans also show fundamental similarities. Distribution of $\dot{V}$b in both is apparently well regulated, with complex patterns of arterial perfusion resulting from increased demands.

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