Dynamic synchronization analysis of venous pressure-driven cardiac output in rainbow trout

Adrienne Robyn Minerick, Hsueh-Chia Chang, Todd M. Hoagland, and Kenneth R. Olson

Dynamic synchronization analysis of venous pressure-driven cardiac output in rainbow trout. Am J Physiol Regul Integr Comp Physiol 285: R889–R896, 2003. First published June 12, 2003; 10.1152/ajpregu.00228.2003.—Measurement of venous function in vivo is inherently difficult. In this study, we used the Hilbert transform to examine the dynamic relationships between venous pressure and cardiac output (CO) in rainbow trout whose blood volume was continuously increased and decreased by ramp infusion and withdrawal (I/W). The dorsal aorta and ductus Cuvier were cannulated percutaneously and connected to pressure transducers; a flow probe was placed around the ventral aorta. Whole blood from a donor trout was then I/W via the dorsal aortic cannula at a rate of 10% of the estimated blood volume per minute, and the duration of I/W was varied from 40, 60, 80, 90, 120, 230, 240, 260, 300, and 340 s. Compliance [change in (Δ) blood vol/venous pressure] was 2.8 ± 0.2 ml·mmHg⁻¹·g⁻¹ (N = 25 measurements; 6 fish with closed pericardium) and 2.8 ± 0.3 ml·mmHg⁻¹·kg⁻¹ (N = 19 measurements, 4 fish with open pericardium). Compliance was positively correlated with the duration of I/W, indicative of cardiovascular reflex responses at longer I/W durations. In trout with closed pericardium, CO followed venous pressure oscillations with an average time lag of 4.2 ± 1.0 s (N = 9); heart rate (HR) was inversely correlated with CO. These studies show that CO is entrained by modulation of venous pressure, not by HR. Thus, although trout have a rigid pericardium, venous pressure (vis-a-tergo), not cardiac suction (vis-a-fronte), appears to be the primary determinant of CO. Estimation of venous compliance by ramp-modulation of venous pressure is faster and less traumatic than classical capacitance measurements and appears applicable to a variety of vertebrate species, as does the Hilbert transform, which permits analysis of signals with disparate frequencies.

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EURYHALINE TELEOST FISH, such as the rainbow trout, are potentially useful models for integrative cardiovascular studies. First, trout have a permeable integument yet thrive in environments that are potentially volume-loading and salt-depleting (freshwater), or volume-depleting and salt-loading (saltwater). This enables independent experimental control of fluid and ion balance. Second, the trout respiratory (gill) circulation is relatively noncompliant (27) and, because it is in-series with the systemic circulation, there is little chance of transient fluid movement between these two circuits. Third, because fish are the most "primitive" vertebrates, one might expect their cardiovascular system to be the least evolutionarily derived. For example, because fish live in a neutrally buoyant environment, they do not experience orthostatically driven venous pooling or plasma extravasation, additional factors that must be continuously dealt with by most terrestrial vertebrates.

Numerous in vitro and in vivo studies have helped identify the parameters of piscine cardiovascular homeostasis and compare them with mammalian systems. Overall, fish and mammals are quite similar with respect to signaling mechanisms and vascular responsiveness, e.g., sympathetic nervous system (20), renin-angiotensin system (21, 32), kallikrein-kinin system (5), vasotocin/vasopressin (1, 3), natriuretic peptides (19, 35), and endothelin (15, 37). Perhaps the only difference between fish and mammalian signaling systems is the apparent lack of nitric oxide release from vascular endothelium in the former (24).

There is, however, one potential disparity between fish and mammals relative to the role of the venous system in affecting venous return and thereby cardiac output (CO). Many fish, including rainbow trout, have a rigid pericardium, and it has been proposed that cardiac suction (vis-a-fronte pressure), generated by decreased pericardial pressure during atrial and ven-tricular systole, is the primary determinant of venous return (8, 9, 33). This, plus the fact that fish live in a buoyant environment and the observation that fish veins are very-thin-walled vessels, led Satchell (33) to conclude that fish veins were merely conduits for the return of blood. This supposition is in contradistinction to the well-appreciated dependence of venous return on venous tone and compliance in mammals (12, 13, 29).

A variety of recent studies, however, have suggested that veins in fish, like those of mammals, are active participants in cardiovascular homeostasis. Rainbow trout have a rigid pericardium, venous pressure (vis-a-tergo), not cardiac suction (vis-a-fronte), appears to be the primary determinant of CO. Estimation of venous compliance by ramp-modulation of venous pressure is faster and less traumatic than classical capacitance measurements and appears applicable to a variety of vertebrate species, as does the Hilbert transform, which permits analysis of signals with disparate frequencies.

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trout veins in vitro have been shown to contract or relax in response to a variety of stimuli (3). Cod, Gadus morhua, veins are innervated by adrenergic neurons (16), and both cod and rainbow trout veins are innervated by peptidergic neurons (17). Furthermore, echocardiographic analysis of ventricular filling in three teleost genera, Paralabrax, Channa, and Monopterus, was consistent with the mammalian pattern (18).

The most direct evidence for an active participation of veins in venous return is obtained through studies of venous capacitance in vivo (31). These methods have recently been applied to rainbow trout (38), and we have shown that a variety of stimuli, including arginine vasotocin (2), atrial natriuretic peptides (22), endothelin (15), and catecholamines (39), can affect venous capacitance in vivo. In fact, venous tone appears to be under constant adrenergic control in trout (39).

Measurement of venous capacitance in vivo entails repeated measurement of central venous blood pressure (P_{VEN}) during transient cardiac arrest while blood volume is manipulated above and below resting levels (30, 31). This procedure is time consuming, technically difficult, and in both mammals and trout it may be complicated by reflexes invoked by transient arterial hypotension during the period of zero CO (11, 38). In this study, we employed a novel method with which to measure venous compliance and used dynamic synchronization analysis to examine the relationship between venous function and CO in trout. Using this approach, we obtained compliance values similar to those derived previously from capacitance curves. We were also able to show that venous return drives CO and confirm that vis-a-tergo filling of the heart is a primary determinant of CO in trout. Furthermore, this method appears to be applicable for use in most, if not all, vertebrates.

**MATERIALS AND METHODS**

**Animals.** Rainbow trout (Onchorhynchus mykiss, Kamloops strain; 0.3–0.8 kg) of both sexes were purchased from a local hatchery and kept in circulating 2,000-liter tanks at 14°C and under appropriate, seasonal light-dark cycles. They were fed a maintenance diet of commercial trout pellets (Purina) up to 48 h before experimentation. All procedures have been approved by the International Animal Care and Use Committee.

**Surgery.** Trout were anesthetized in benzocaine (ethyl-paminobenzoate; 1:12,000 wt/vol), and the dorsal aorta was cannulated percutaneously through a 1-cm dorsal incision in the lateral wall of the gular cavity with heat-tapered polyethylene tubing (PE-60; see Ref. 22). The cannula was filled with heparinized [50 United States Pharmacopia (USP)/ml; 0.9% NaCl] saline. The gills were not irrigated during this brief (<1 min) procedure. Thereafter, gills were continuously irrigated with 10°C aerated water containing 1:24,000 wt/vol benzocaine during placement of the flow probe and the ductus Cuvier cannula.

A 1-cm incision was made in the lateral wall of the gular frenulum, and the ventral aorta was exposed by blunt dissection. A 2S or 3S Transonic flow probe (Transonic Systems, Ithaca, NY) was placed around the ventral aorta and connected to a Transonic T206 flowmeter. A small amount of acoustical couplant (K-Y jelly) was injected in the acoustical window of the probe, and the wound was closed with cyanoacrylate glue.

The ductus Cuvier was cannulated percutaneously. A small puncture wound through the scales was made with a sharp-point scalpel ~1 cm dorsal to the lateral line and ~1 cm caudal to the caudal border of the operculum. Heat-tapered PE-60 tubing was inserted 2–3 cm at a 30° angle, with respect to the midsagittal plane, with the aid of a metal trochar. The cannula was attached to the skin with a suture, filled with heparinized saline, and connected to a Gould (Cleveland, OH) P23 pressure transducer. In a second group of fish the pericardium was opened via a midline incision, the flow probe was placed around the bulbus, and the ductus Cuvier was cannulated directly (39). The fish were then revived and placed in black plastic tubes immersed in a 1,500-liter aquarium with aerated, through-flowing well water at 14°C. Experiments were conducted the day after surgery.

Analog pressure signals were recorded with a Hewlett Packard (Palo Alto, CA) 7853A patient monitor. Digitized signals of dorsal aortic pressure (P_{DMA}) and central P_{VEN} and flow (CO) were collected at 0.1-s intervals, and 1-s averages were stored on computer. In later experiments, the 0.1-s data were analyzed directly. This provided better resolution of the flow signal and subsequent calculation of stroke volume but did not affect the analysis of the relationship between P_{VEN} and CO. The pressure transducer was calibrated with a water manometer, and the flowmeter was calibrated in situ at the end of the experiment by pump perfusion of the ventricle with 14°C saline at known flow rates. Heart rate (HR) was derived from the pulsatile CO.

**Protocol.** Before experimentation ~10 ml blood were withdrawn from a donor fish in a heparinized (50 USP/ml) syringe and placed in an infusion-withdrawal (I/W) syringe pump (model 55–2219; Harvard Apparatus, South Natick, MA). The pump was then connected to the dorsal aorta cannula with PE-90 tubing.

Resting pressures and CO were monitored for 1–2 h before experimentation to ensure stability; control parameters were recorded for a minimum of 10 min before the start of blood volume manipulation. Blood volume was repeatedly increased and decreased by continuous ramp I/W of 10% of the estimated resting blood volume (35 ml/kg; see Ref. 6/min). The total volume perturbation was dependent on the duration of I/W, which was varied between 40 and 340 s. Thus, for a 40-s duration, blood was infused for 20 s and withdrawn for 20 s. At the start of an experiment, only one-half of the blood was withdrawn (10 s in the example above) to ensure that blood volume oscillated around resting levels. A total of 44 experiments was carried out on six different fish. In two fish, the flow records were inexplicably poor, and the CO did not oscillate with P_{VEN}. Records from these fish were used as a example of an asynchronous system. For all fish, the I/W durations were randomly selected.

Vascular compliance was determined for each duration of I/W by dividing the volume of blood infused (3.5 ml·kg⁻¹·min⁻¹ times the infusion period) by the average change in P_{VEN} during the I/W period. The methods of Hilbert transform and dynamic synchronization analysis are presented with the data in RESULTS.

Values are reported as means ± SE. Pearson’s correlation coefficient for the relationship between vascular compliance and duration of volume perturbation was obtained with a commercial statistical program (SigmaStat; Jandel).
RESULTS

The effects of continuous ramp I/W of blood at 40- and 180-s intervals on P_{DA}, P_{VEN}, and CO are shown in Fig. 1. In both instances, P_{DA} was biased by the infusion pump and the resistance of the dorsal aortic cannula in such a way that its square-wave appearance accurately reflected the I/W periods. The ramp oscillations in P_{VEN} indicate the compliance properties of the veins. It is evident from Fig. 1 that both pressure and CO were entrained by blood volume manipulation of both 40 and 180 s duration. Similar results were observed at other durations.

Vascular compliance at different I/W durations in trout with an open or closed pericardium is shown in Fig. 2. Average compliance for trout with a closed pericardium was 2.8 ± 0.2 ml·mmHg \textsuperscript{-1}·kg\textsuperscript{-1} (N = 25 measurements) and for an open pericardium was 2.8 ± 0.3 ml·mmHg \textsuperscript{-1}·kg\textsuperscript{-1} (N = 19). The duration of I/W was not correlated with vascular compliance for trout with an open pericardium (P = 0.07), but duration was positively correlated with compliance in trout with a closed pericardium (correlation coefficient = 0.43, P = 0.03, n = 25) and with compliance for the open and closed pericardium data combined (correlation coefficient = 0.39, P = 0.008, n = 44).

Because the infusion duration must be much slower than the HR because of both technical and physiological limitations, the physiological time series from our experiments involve two very disparate time scales. As a result, the usual Fourier analysis techniques become less satisfactory, and we employed a new analytical signal approach of the Hilbert transform (25, 26, 28) to isolate the high-frequency flow and pressure signals associated with individual heart beats from the low-frequency signals resulting from blood volume manipulation. This technique was also used to estimate the relative phases between the low-frequency component of the P_{VEN} and CO time series (Fig. 3). The separation of time scales in both signals was accomplished by first determining the instantaneous amplitude [A(t)] and phase [\psi(t)] of a nonharmonic signal [s(t)] via the Hilbert transform (28). The analytical signal [\psi(t)] is a complex function of time defined as \psi(t) = s(t) + is(t) = A(t)e^{i\psi(t)}, where i = \sqrt{-1} and the function \tilde{s}(t) is the Hilbert transform of s(t), as follows

\[
\tilde{s}(t) = \frac{1}{\pi} \text{PV} \int_{-\infty}^{\infty} \frac{s(\tau)}{t - \tau} \, d\tau
\]

PV indicates the integral is taken in the sense of the Cauchy principal value (25, 28). If the function \tilde{s}(t) is a monochromatic harmonic, its phase is shifted \pi/2 from the original s(t) signal. This shift is shown in Fig. 3, E and F. As a result, the modulus or combination of the

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*Fig. 1. Representative trace of dorsal aortic (P_{DA}; A and B) and central venous (P_{VEN}; C and D) pressures, and cardiac output (CO; E and F) from 2 trout with 3.5 ml·min\textsuperscript{-1}·kg\textsuperscript{-1} blood volume infusion/withdrawal at a duration of 40 s (A, C, and E) or 180 s (B, D, and F). P_{DA} is entrained by the infusion/withdrawal pump, whereas P_{VEN} and CO respond to the change in blood volume.*

*Fig. 2. Whole body vascular compliance of rainbow trout with closed (○) or open (▲) pericardium obtained at different rates of infusion/withdrawal (40 s = 20 s infusion, 20 s withdrawal). Data are means ± SE; no. of fish in parentheses. Dotted line shows regression of all measurements (n = 44 fish).*
two signals, which harnesses constructive and destructive interference, yields the instantaneous amplitude

\[ A(t) = \sqrt{s^2(t) + \tilde{s}^2(t)} \]

or the signal envelope (Fig. 3, C and D). The instantaneous phase \( \varphi(t) \) of the signal \( s(t) \) can be obtained from

\[ \varphi(t) = \arctan \frac{\tilde{s}(t)}{s(t)} \]

The instantaneous frequency \( [\omega(t)] \) is the derivative of the phase, as follows

\[ \omega(t) = \frac{d\varphi(t)}{dt} \]

The instantaneous phases \( \varphi \) of the low-frequency component of the \( \text{P}_\text{VEN} \) and \( \text{CO} \) time series are shown in Fig. 3, G and H, respectively, whereas the instantaneous frequency \( \omega \) of the high-frequency component is shown in Fig. 3, I and J.

The Hilbert technique can be applied to aperiodic signals with high-frequency content. As such, it establishes the instantaneous phase of an aperiodic signal and can be used to determine the instantaneous relative phase (time lag) between two such signals. The envelope modulates slowly relative to the phase and represents an isolated low-frequency component of the aperiodic signal. Its phase can once again be defined through its Hilbert transform. The particular modulation frequency captured is dependent on the length of the signal the transform sees, which is easily controlled by windowing large time series and tracking the mean of each window to preserve the slowest trends. Slight corruption of the signal while obtaining the signal envelope is caused by interference with the Fourier transform at the edges of each window. This was avoided by overlapping the windows such that the first quarter and the last quarter of each window are replaced by the middle of subsequent windows. It should be noted that the Hilbert transform does not filter out random noise.

The time lag between \( \text{P}_\text{VEN} \) and \( \text{CO} \) oscillations can be determined by examining the continuously increasing instantaneous phase \( \varphi \) of both signal envelopes. The difference between the \( \text{P}_\text{VEN} \) and \( \text{CO} \) phases, when converted to a time lag, is the time it takes for a pressure perturbation to translate through the heart and be detected as a \( \text{CO} \) perturbation. These are depicted in Fig. 4, A–C, for three experiments with a blood volume manipulation of 40 s duration and a poor record of \( \text{CO} \) and 40- and 240-s manipulation durations with good \( \text{CO} \) records, respectively. The average time lag can be determined by the phase lag distribution shown in Fig. 4, D and E. Only synchronized data are used for this estimate.

To quantify the strength of phase synchronization between two signals, the spread of the non-Gaussian distribution of \( \varphi(t) \) is estimated by an index \( \hat{p}_{\text{P}_\text{VEN},\text{CO}} \) based on the Shannon entropy \( S \) (see Ref. 36)

\[ \hat{p}_{\text{P}_\text{VEN},\text{CO}} = \frac{S}{S_{\text{max}}} \]

The Shannon entropy \( S \) is found from the probability \( (p) \) in each bin \( (k) \) of the histogram shown in Fig. 4, D–F. \( N \) is the total number of bins (50 in this case). The index includes \( S_{\text{max}} \) to account for the size of the bins and to normalize the index between zero and one. An index of zero corresponds to a constant phase difference or Dirac delta-like probability distribution, and an index of one indicates an irregular phase difference.
with a uniform probability distribution, similar to that shown in Fig. 4D.

With a 40-s duration and poor flow record, there is no apparent synchronization between PVEN and CO (Fig. 4A), and there is a uniform phase difference probability distribution (Shannon index $H_1$ of 0.98; Fig. 4D). This is in contrast to the 40- and 240-s signals with good flow records where PVEN and CO are synchronized (Fig. 4, B and C, respectively) and the Shannon entropy indexes are lower (0.75 and 0.45; Fig. 4, E and F, respectively). Averaging experimental trials with a Shannon entropy index of 0.85 and lower yields a mean transit time across the heart of $4.2\pm1.0$ s ($N=9$), i.e., there is a 4.2-s time lag between a change in central PVEN and a change in ventral aortic flow.

These parameter dependencies were examined further with cross-correlation techniques. The PVEN and CO signal envelopes were reduced down to a mean of zero and then normalized with respect to each fish's control data (the latter obtained before blood volume manipulations). To measure the scatter in the cross correlations, we defined a symmetric covariance matrix, $C_{ij}$ as follows

$$C_{ij} = \frac{1}{M} \sum_{i=1}^{M} x_i x_j (x_i x_j)$$

where the indexes $i$ and $j$ represent the first (CO or stroke volume) and second (PVEN or HR) data set, respectively. The sum is taken over all data points, $M$, of the cross product between data sets, $x_i$ and $x_j$. The eigenvectors of $C_{ij}$ point in the major and minor axis of the ellipse data. The trend lines shown in Fig. 5 are determined directly from the dominant eigenvector. The ratio ($\kappa$) of the eigenvectors becomes an index of the degree of scatter of the data; the smaller the $\kappa$, the better the correlation.

Figure 5, A–C, shows normalized PVEN cross-correlated with CO for the same fish in Fig. 4 (40-s duration with poor flow and 40- and 240-s durations with good flow records). There is little correlation in the fish with poor flow data (Fig. 5A), whereas there is a positive

$$\begin{align*}
\text{Fig. 4.} & \quad \text{A–C: instantaneous phase information for PVEN (broken line) and CO (solid line) signal envelopes for trout with 40-s blood volume manipulation and poor (asynchronous) CO record (A) and good CO records at 40 (B) and 240 (C) s blood volume manipulations.} \\
\text{D–F: phase difference probability distribution.} & \quad \text{There is a uniform phase difference distribution.} \\
\text{There is synchronization with a distinct phase difference (Shannon entropy index, } H_1 \text{ of 0.75) for the 40-s blood volume manipulation with good CO record (E), and a distinct phase difference (Shannon entropy index, } H_1 \text{ of 0.45) for the 240-s blood volume manipulation (F).}
\end{align*}$$

$$\begin{align*}
\text{Fig. 5.} & \quad \text{Cross-correlation between CO and PVEN (A–C), CO and heart rate (HR; D–F), and stroke volume (SV) and HR (G–I) for fish in Fig. 4. The degree of scatter is obtained from the eigenvalue ratio of the covariance matrix. A ratio (}\kappa\text{) of 1 signifies asynchronization, whereas a ratio of 0 represents perfect synchronization without a phase lag; the equation for the trend line (solid line) is shown on top in A–I. For the 240-s duration, CO and PVEN are positively correlated, and there is a negative correlation between CO and HR.}
\end{align*}$$
trend with reduced scatter in Fig. 5, B and C, showing that PVEN events result in very similar events in CO seconds later, i.e., an increase in PVEN is followed by an increase in CO.

Cross correlation of the HR (obtained via the Hilbert transform frequency technique) and CO yields a strong negative correlation for the 240-s I/W duration (Fig. 5f). The HR is highest during conditions of low flow and lowest during regions of high flow. There was no apparent correlation between CO and HR in either experiment employing a 40-s duration (Fig. 5, D and E). The derived relationship between stroke volume (obtained by integrating the area under the flow signal for each heart beat) and HR (Fig. 5, G-I) showed a relatively high degree of correlation; however, the slopes of these relationships were essentially nil. These relationships for data with a Shannon entropy index <0.85 are summarized in Table 1.

DISCUSSION

In the present experiments, we show that periodic ramp I/W of whole blood into unanesthetized rainbow trout produces entrained oscillations of central PVEN and CO. Analysis of the relationships between infusion rate and volume vs. the measured cardiovascular parameters permits rapid determination of vascular compliance without stopping the heart, and these values are independent of an open or closed pericardium and similar to those reported previously using more laborious methods. Compliance increases as the duration of I/W increases, indicative of initiation of cardioregulatory reflexes when blood volume is manipulated over longer durations. Information derived from The Hilbert transform allowed us to separate the low (beat-to-beat)- and high (over multiple beats)-frequency components of PVEN and CO oscillations during blood volume manipulation, and our results showed that, over multiple beats, changes in CO lag behind PVEN by a little over 4 s. These studies show that venous return, not cardiac suction, is the primary determinant of CO in trout and that the Hilbert transform is a useful analytical tool for analyzing events with disparate time scales.

Vascular capacitance measurements have been the primary means of determining whole animal vascular compliance in both fish and mammals (13–15, 22, 30, 31, 38, 39). With this method, CO is transiently stopped by cardiac arrest or aortic occlusion, and zero-flow PVEN (mean circulatory filling pressure; see Ref. 12) is determined (31). The procedure is repeated after blood volume is incrementally increased or decreased, and the slope of the relationship between blood volume (ordinate) and zero-flow PVEN (abscissa) is equivalent to vascular compliance. Because veins are over 20 times more compliant than arteries (3), systemic vascular compliance is approximately equivalent to venous compliance. Although this method provides information on unanesthetized animals, it is time consuming, and there is always the chance that the transient arterial hypotension will elicit baroreflexes (11, 38).

In the present studies, central PVEN was monitored continuously, whereas blood volume was increased and decreased by repetitive ramp I/W of whole blood. The compliance values obtained with this method (2.8 ml·mmHg⁻¹·kg⁻¹) are similar to those reported previously (2–3.5 ml·mmHg⁻¹·kg⁻¹) for intact trout (2, 14, 15, 22, 38, 39) and perfused trout carcasses (38). The ramp-infusion method is considerably faster than the capacitance method, and vascular compliance can be determined within several minutes, compared with capacitance methods that require 40 min for three points, or over an hour for five points. In addition, ramp infusion probably does not result in as severe a drop in arterial pressure as the capacitance method. The actual increase or decrease in blood volume above or below resting blood volume during a 40-s I/W maneuver is 0.58 ml·mmHg⁻¹·kg⁻¹, <2% of the resting blood volume. When the duration of I/W is increased to 240 s, the blood volume increase or decrease is only 3.5 ml·mmHg⁻¹·kg⁻¹, i.e., 10% of the resting blood volume. Because trout are also able to nearly instantaneously mobilize a substantial volume of blood (~20% of total blood volume) during hemorrhage by passive recoil of the microcirculation (23), we anticipate that potential antihypotensive reflexes would be less during the small blood volume manipulations employed in the present experiments than those produced by reducing CO to zero for >7–10 s.

The slight increase in compliance associated with an increase in duration of I/W could be the result of the physical properties of the vascular system and/or reflexive responses. Vascular compliance obtained from static capacitance measurements in the perfused trout trunk and in intact, unanesthetized trout is not linear (14, 22, 38, 39). Compliance is nearly constant at normal and elevated blood volume, whereas it progressively increases as blood volume falls below resting

<table>
<thead>
<tr>
<th>Shannon Entropy</th>
<th>CO-PVEN</th>
<th>CO-HR</th>
<th>SV-HR</th>
<th>Trendline Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67 ± 0.04</td>
<td>0.154 ± 0.030</td>
<td>0.340 ± 0.048</td>
<td>0.021 ± 0.005</td>
<td>1.081 ± 0.260</td>
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<tr>
<td></td>
<td>−0.133 ± 0.350</td>
<td>−0.133 ± 0.350</td>
<td>−0.003 ± 0.001</td>
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Table 1. Relationships between PVEN, CO, HR, and SV from 9 experiments with a Shannon entropy index of 0.85 or less

Data are means ± SE. CO, cardiac output; PVEN, central venous pressure; HR, heart rate; SV, stroke volume. Infusion/withdrawal duration ranged from 40 to 240 s. A kappa value of 1 is indicative of asynchrony, whereas 0 represents perfect synchronization without a phase lag (also see Fig. 5). Cardiovascular parameters are positively correlated when trendline slope is positive, inversely related when negative, and unrelated as slope approaches 0.
levels. Thus one would expect that compliance would be biased upward as more blood is withdrawn during the longer I/W cycles. Longer I/W cycles could also promote active compliance changes because of the increased duration of volume perturbation and possibly because of the volume perturbation itself. Tonic adrennergic control of venous tone in trout has been demonstrated (39) and would be a likely candidate for this process. The inverse correlation between CO and HR accompanying 240-s I/W periods, but not observed when the I/W is only 40 s (Fig. 5, E and F, respectively; see below), supports such a reflexive component.

The Hilbert transform appears to be a useful tool in analysis of cardiovascular synchronization in trout. It is technically difficult to directly analyze the relationship between PVEN and CO because of an intermittent systolic flow and because of heart-induced fluctuations in PVEN. Instead, we introduced blood volume manipulations at a much lower frequency to modulate central PVEN and to help discern correlations over a time scale longer than their period. The new analytical signal approach of the Hilbert transform (25, 26, 28) conveniently isolates the high-frequency flow and heart beat signals from the low-frequency blood volume manipulation signals. We also used this transform to estimate the relative phases between the low-frequency component of the PVEN and CO time series. Low-pass filter techniques are traditionally used for filtering high-frequency components to isolate low-frequency ones. However, this technique requires a priori knowledge of the relevant filtering frequency compared with the low-frequency cutoff or filtering window. It also eliminates the high-frequency content and often corrupts the low-frequency signal if the cutoff function is not properly shaped. In our experiments, the low-frequency signal varies over a wide range from 40 to 340 s. It is hence difficult to select a properly shaped cutoff function that is suitable for all data. More importantly, the high-frequency content is an important part of our data, as it yields the HR. Hence, we do not want to eliminate it with a low-pass filter. Fortunately, the new technique of Hilbert transform allows us to obtain both the signal envelope (the low-frequency content) and the high-frequency fluctuations accurately without these disadvantages. It also does so rapidly and robustly without the need to introduce a cut-off function (window).

The Frank-Starling mechanism, whereby an increase in cardiac filling increases the force of contraction and thereby stroke volume, is well known in fish (7, 8, 10). However, the observations that central PVEN are sometimes subambient, fish have a rigid pericardium, and veins do not appear to be well endowed with smooth muscle have led to the concept that cardiac suction (vis-a-tergo pressure) rather than pressure derived from arterial flow and venous capacitance (vis-a-tergo pressure) are the primary factors in determining venous return and cardiac filling in fish (10, 33, 34). Our experiments support the hypothesis that venous return, hence PVEN, is a primary determinant of CO in trout.

In the present experiments on trout with a closed pericardium, we observed that central PVEN may be either slightly negative or positive (compare Fig. 1; 180 vs. 40 s, respectively), yet with the use of the low-frequency signal envelope and phase information obtained along with distribution information quantified through use of the Shannon entropy, it is clear that CO is entrained by PVEN. Therefore, regulation of venous return either through changing venous tone or compliance, or by increasing flow through the capillaries (as occurs during exercise), is predicted to be the primary determinant of CO in trout, as it is in mammals. This does not mean that the pericardium and cardiac suction are unimportant, but it suggests that they are more involved with cardiac filling on a beat-to-beat basis. For example, ventricular systole would lower pericardial pressure and assist in aspiration from the sinus venosus in the atrium and also enhance ventricular recoil during ventricular diastole (7–10). This can have significant impact in situations such as heavy exercise where an increase in contractility resulting from adrenergic stimulation would enhance ventricular filling and compensate for a decreased diastolic period.

The relationship between CO and HR (Fig. 5 and Table 1) provides additional evidence that vis-a-tergo mechanisms are operative in trout. Because there is a strong negative correlation between CO and HR for the 240-s I/W duration (Fig. 5F), it is evident that the HR is reflexively responding to the change in CO (or flow-driven changes in arterial pressure) and that cardiac events are not the driving force behind CO. However, there was no apparent correlation between CO and HR in either experiment employing a 40-s duration (Fig. 5, D and E), and it is not known if 1) barostatic reflexes become uncoupled at this (40-s) duration, 2) the volume perturbations were not sufficient to elicit reflexive responses, or 3) the data collection intervals were frequent enough to allow the Hilbert transform to accurately track HR.

Figure 5 and Table 1 also show that there is little correlation between stroke volume and HR as blood is I/W. This is at odds with the well-known reciprocal relationship between the two (7, 8, 10), i.e., if venous return is constant, then an increase in HR will decrease stroke volume (and visa versa), because the heart pumps what it receives. As shown in Fig. 5, for a 240-s I/W duration, a decrease in venous return decreases CO (Fig. 5C), which is associated with an increase in HR (Fig. 5F), the latter presumably a reflexive response to the fall in arterial blood pressure. However, HR did not appear to be inversely related with stroke volume (Fig. 5I), even though under these conditions (low venous return) we would have expected stroke volume to be reduced substantially. The explanation for this discrepancy is not evident, but it may be because of the fact that our data collection intervals, 0.1 s and initially many of these were then averaged to 1.0 s, may not have been sufficient to resolve intrabeat dynamics. Clearly, additional experiments with this
technique and shorter sampling intervals may be able to resolve this issue.

The ~4-s phase lag between an increase in $P_{VEN}$ and an increase in CO probably reflects the time for conduction of pressure/flow parameters through the cardiac chambers. Assuming an approximate HR of 50 beats/min, this would translate into one beat each for the sequential transfer of blood from the ductus Cuvier into the sinus venosus, atrium, ventricle, and finally into the bulbus/ventral aorta.

We thank Alison Weltner, Michelle Roeser, Katherine Brakora, and John Howard for help with data collection and analysis.

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DISCLOSURES

This work was supported by the Bayer Chair Fund (H.-C. Chang), the Clare Boothe Luce Fellowship Fund (A. R. Minerick), and by National Science Foundation Grants IBN-9723306 and IBN-0235223 (K. R. Olson).

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