Dynamic Synchronization Analysis of Venous Pressure-Driven
Cardiac Output in Rainbow Trout

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Key Words: fish cardiovascular system; blood transit time, cardiovascular time series, vascular compliance, Hilbert Transform.

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ABSTRACT

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 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 was then I/W via the dorsal aortic cannula at a rate of 10% of the estimated blood volume per min, the duration of I/W was varied from 40, 60, 80, 90, 120, 230, 240, 260, 300, 340 seconds. Compliance (blood vol/venous pressure) was 2.8 ±0.2 ml mmHg⁻¹ kg⁻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, cardiac output (CO) followed venous pressure oscillations with an average time lag of 4.2 ±1.0 seconds (N=9); heart rate (HR) was inversely correlated with CO. These studies show that cardiac output is entrained by modulation of venous pressure, not by heart rate. 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.
INTRODUCTION

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 non-compliant (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 which 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 to 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 (1,2), 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 (22).

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. 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 ventricular systole is the primary determinant of venous return (8, 10, 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 veins in vitro have been shown to contract or relax in response to a variety of stimuli (2). 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 (3), atrial natriuretic peptides (23), 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 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 cardiac output (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 cardiac output 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 cardiac output and confirm that vis-a-tergo filling of the heart is a primary determinant of cardiac output in trout. Furthermore, this method appears to be applicable for use in most, if not all vertebrates.

MATERIALS AND METHODS

Animals.

Rainbow trout (*Oncorhynchus mykiss*, mixed Kamloops strain; 0.3-0.8 kg), of both sexes were purchased from a local hatchery and kept in circulating 2000-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 prior to experimentation. All procedures have been approved by the IACUC.

Surgery.

Trout were anesthetized in benzocaine (ethyl-\(p\)-aminobenzoate; 1:12,000, W:V) and the dorsal aorta was cannulated percutaneously through the roof of the buccal cavity with heat-tapered polyethylene tubing (PE 60; ref 23). The cannula was filled with heparinized (50 USP per 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, W:V, 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 exposed by blunt dissection. A 2S or 3S Transonic flow probe (Transonic Systems Inc., Ithaca, NY) was placed around the ventral aorta and connected to a Transonic T206 flow meter. A small amount of acoustical couplant (K-Y jelly) was injected into the acoustical window of the probe and the wound 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 PE60 was inserted 2-3 cm at a 30 degree angle, with respect to the mid-saggital 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 Gould P23 pressure transducer (Cleveland, OH). In a second group of fish the pericardium was opened via a mid-line incision and 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 7853A patient monitor (Palo Alto, CA). Digitized signals of dorsal aortic ($P_{DA}$) and central venous ($P_{VEN}$) pressure and flow (cardiac output; CO) were collected at 0.1-s intervals, and 1-s averages were stored on computer. In later experiments, the 0.1-s data was 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 was derived from the pulsatile cardiac output.

**Protocol.**

Prior to experimentation ~10 ml blood was withdrawn from a donor fish into a heparinized (50 USP per ml) syringe and placed in an infusion-withdrawal syringe pump (Model 55-2219, Harvard Apparatus, South Natick MA). The pump was then connected to the dorsal aorta cannula with PE 90.

Resting pressures and cardiac output were monitored for 1-2 h prior to experimentation to ensure stability; control parameters were recorded for a minimum of 10 min prior to the start of blood volume manipulation. Blood volume was repeatedly increased and decreased by continuous ramp infusion and withdrawal of ten percent of the estimated resting blood volume (35 ml kg$^{-1}$; ref 6) per min. The total volume perturbation was dependent on the duration of infusion/withdrawal (I/W), which was varied between 40 and 340 seconds. Thus for a 40 second duration, blood was infused for 20 seconds and withdrawn for 20 seconds. At the start of an experiment only half of the blood was withdrawn (10 seconds in the example above) to ensure that blood volume oscillated around resting levels. A total of 44 experiments were carried out on 6 different fish. In two fish the flow records were inexplicably poor and the cardiac output did not oscillate with venous pressure. Records from these fish were used as an 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 the Results section.

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

RESULTS

The effects of continuous ramp infusion and withdrawal of blood at 40 and 180 second intervals on dorsal aortic (P\(_{DA}\)) and central venous (P\(_{VEN}\)) pressures and cardiac output (CO) are shown in figure 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 venous pressure indicate the compliance properties of the veins. It is evident from figure 1 that both pressure and (CO) were entrained by blood volume manipulation of both 40 and 180 second 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 figure 2. Average compliance for trout with a closed pericardium was 2.8 ±0.2 ml mmHg\(^{-1}\) kg\(^{-1}\) (N=25 measurements) and for an open pericardium was 2.8 ±0.3 ml mmHg\(^{-1}\) kg\(^{-1}\) (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).

Since the infusion duration must be much slower than the heart rate due to 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 venous pressure and cardiac output time series (Fig. 3). The separation of time scales in both signals was accomplished by first determining the instantaneous amplitude, \( A(t) \), and phase, \( \varphi(t) \), of a nonharmonic signal, \( s(t) \), via Hilbert transform (28). The analytical signal, \( \tilde{s}(t) \), is a complex function of time defined as: 

\[
\tilde{s}(t) = s(t) + i\tilde{s}(t) = A(t)e^{i\varphi(t)}, \quad \text{where} \quad i = \sqrt{-1}
\]

and the function, \( \tilde{s}(t) \), is the Hilbert transform of \( s(t) \), as follows.

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

\( \text{P.V.} \) 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 \( /2 \) from the original \( s(t) \) signal. This shift is illustrated in figures 3e and 3f. As a result, the modulus or combination of the two signals, which harnesses constructive and destructive interference, yields the instantaneous amplitude, \( A(t) = \sqrt{s^2(t) + \tilde{s}^2(t)} \), or signal envelope (Figs. 3c and 3d). The
The instantaneous phase, \( \varphi(t) \), of the signal, \( s(t) \), can be obtained from: 
\[
\varphi(t) = \arctan\left( \frac{\tilde{c}(t)}{s(t)} \right).
\]
The instantaneous frequency, \( \omega(t) \), is the derivative of the phase, as follows: 
\[
\omega(t) = \frac{d\varphi(t)}{dt}.
\]
The instantaneous phase (\( \varphi(t) \)) of the low frequency component of the venous pressure and cardiac output time series is shown in figures 3g and 3h, respectively, while the instantaneous frequency (\( \omega(t) \)) of the high frequency component is shown in figures 3i and 3j.

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 venous pressure and cardiac output oscillations can be determined by examining the continuously increasing instantaneous phase (\( \varphi(t) \)) of both signal envelopes. The
difference between the $P_{VEN}$ and 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 cardiac output perturbation. These are depicted in figures 4a-c for three experiments with a blood volume manipulation of 40 second duration and a poor record of cardiac output, and 40 and 240 second manipulation durations with good cardiac output records, respectively. The average time lag can be determined by the phase lag distribution shown in figures 4 d-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 $(t)$ is estimated by an index $\tilde{\rho}_{P_{VEN},CO}$ based on the Shannon entropy, $S (36)$:

$$\tilde{\rho}_{P_{VEN},CO} = \frac{S}{S_{\text{max}}}$$

$$S = -\sum_{k=1}^{N} p_k \ln p_k$$

$$S_{\text{max}} = \ln N$$

The Shannon entropy, $S$, is found from the probability, $p$, in each bin, $k$, of the histogram shown in figure 4d-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 0 and 1. An index of 0 corresponds to a constant phase difference or Dirac delta-like probability distribution and an index of 1 indicates an irregular phase difference with a uniform probability distribution, similar to that shown in figure 4d.

With a 40 second duration and poor flow record, there is no apparent synchronization between $P_{VEN}$ and CO (Fig. 4a) and there is a uniform phase difference probability distribution (Shannon index = 0.98; Fig. 4d). This is in contrast to the 40 and 240 second signals with good flow records where $P_{VEN}$ and CO are synchronized (Figs. 4b and 4c, respectively) and the
Shannon entropy indices are lower (0.75 and 0.45; Figs. 4e and 4f, 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 ±1.0 seconds (N=9), i.e., there is a 4.2 second time-lag between a change in central venous pressure and a change in ventral aortic flow.

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

\[
C_{ij} = \frac{1}{M} \sum_{t=1}^{M} x_i(t)x_j(t) - \langle x_i \rangle \langle x_j \rangle
\]

where the indices \( i \) and \( j \) represent the first (CO or SV) and second (\( P_{\text{VEN}} \) 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 trendlines shown in figure 5 are determined directly from the dominant eigenvector. The ratio, , of the eigenvectors becomes an index of the degree of scatter of the data; the smaller the , the better the correlation.

Figures 5a-5c show normalized venous pressure cross-correlated with cardiac output for the same fish in figure 4 (40 second duration with poor flow and 40 and 240 second durations with good flow records, respectively). There is little correlation in the fish with poor flow data (Fig. 5a), whereas there is a positive trend with reduced scatter in figures 5b and 5c; showing
that $P_{VEN}$ events result in very similar events in CO seconds later, i.e., an increase in venous pressure is followed by an increase in cardiac output.

Cross correlation of the heart rate (obtained via the Hilbert transform frequency technique) and cardiac output yields a strong negative correlation for the 240 second I/W duration (Fig. 5f). The heart rate is highest during conditions of low flow, and lowest during regions of high flow. There was no apparent correlation between cardiac output and heart rate in either experiment employing a 40 second duration (Figs. 5d and 5e). The derived relationship between stroke volume (obtained by integrating the area under the flow signal for each heart beat) and heart rate (Figs. 5g-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 infusion/withdrawal (I/W) of whole blood into unanesthetized rainbow trout produces entrained oscillations of central venous pressure and cardiac output. Analysis of the relationships between infusion rate and volume versus 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 venous pressure and cardiac output oscillations during blood volume
manipulation and our results showed that over multiple beats changes in cardiac output lag
behind venous pressure by a little over 4 seconds. These studies show that venous return, not
cardiac suction is the primary determinant of cardiac output 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, 23, 30, 31, 38, 39). With this
method, cardiac output is transiently stopped by cardiac arrest or aortic occlusion and zero-flow
venous pressure (mean circulatory filling pressure; 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 venous pressure (abscissa) is
equivalent to vascular compliance. Because veins are over twenty times more compliant than
arteries (2), systemic vascular compliance is approximately equivalent to venous compliance.
While this method provides information on unanesthetized animals, it is time consuming and
there is always the chance that the transient arterial hypotension will elicit barostatic reflexes
(11, 38).

In the present studies, central venous pressure was continuously monitored while 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 (3,14, 15, 23, 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 to capacitance methods that
require 40 minutes for three points, or over an hour for 5 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
second I/W maneuver is 0.58 ml mmHg⁻¹ kg⁻¹; less than 2% of the resting blood volume. When
the duration of I/W is increased to 240 seconds, 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 (24), we anticipate that potential anti-
hypotensive reflexes would be less during the small blood volume manipulations employed in
the present experiments than those produced by reducing cardiac output to zero for more than 7-10 sec.

The slight increase in compliance associated with an increase in duration of I/W could be
due to 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 are not linear (14, 23, 38, 39). Compliance is nearly constant at
normal and elevated blood volume, whereas it progressively increases as blood volume falls
below resting 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 due to the increased duration of volume perturbation and possibly due to the
volume perturbation itself. Tonic adrenergic 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 second I/W periods, but not observed when the I/W is only 40 seconds, (Fig. 5f and e, respectively; see below) support 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 venous pressure and cardiac output because of an intermittent systolic flow and because of heart-induced fluctuations in venous pressure. Instead, we introduced blood volume manipulations at a much lower frequency to modulate central venous pressure 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 venous pressure and cardiac output 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, viz. the low-frequency cutoff or filtering window. It also eliminates the high-frequency content and often corrupts the low-frequency signal if the cut-off function is not properly shaped. In our experiments, the low frequency signal varies over a wide range from 40 seconds to 340 seconds. 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 heart rate. We hence 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 (7-9). However, the
observations that central venous pressures are sometimes sub-ambient, fish have a rigid
pericardium, and veins do not appear to be well endowed with smooth muscle, has led to the
concept that cardiac suction (vis-a-fronte 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 (9, 33, 34). Our experiments support the hypothesis that
venous return, hence venous pressure, is a primary determinant of cardiac output in trout.

In the present experiments on trout with a closed pericardium, we observed that central
venous pressure may be either slightly negative or positive (compare Fig. 1; 180 vs 40 seconds,
respectively), yet using the low frequency signal envelope and phase information obtained along
with distribution information quantified through use of the Shannon entropy, it is clear that
cardiac output is entrained by venous pressure. 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 cardiac output 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 into 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 due to adrenergic stimulation would enhance ventricular filling and compensate for
a decreased diastolic period.

The relationship between cardiac output and heart rate (Fig. 5; Table 1) provides
additional evidence that vis-a-tergo mechanisms are operative in trout. Because there is a strong
negative correlation between cardiac output and heart rate for the 240 second I/W duration (Fig.
5f), it is evident that heart rate is reflexively responding to the change in cardiac output (or flow-
driven changes in arterial pressure) and that cardiac events are not the driving force behind
cardiac output. However, there was no apparent correlation between cardiac output and heart rate
in either experiment employing a 40 second duration (Figs. 5d and 5e), and it is not known if 1)
barostatic reflexes become uncoupled at this (40 second) 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 heart rate.

Figure 5 and Table 1 also show that there is little correlation between stroke volume and
heart rate as blood is infused and withdrawn. This is at odds with the well-known reciprocal
relationship between the two (7-9), i.e., if venous return is constant, then an increase in heart rate
will decrease stroke volume (and visa versa), because the heart pumps what it receives. As
shown in figure 5, for a 240 second I/W duration, a decrease in venous return decreases cardiac
output (Fig. 5 c), which is associated with an increase in heart rate (Fig. 5 f), the latter
presumably a reflexive response to the fall in arterial blood pressure. However, heart rate did
not appear to be inversely related to stroke volume (Fig. 5i), even though under these conditions
(low venous return) we would have expected stroke volume to be substantially reduced. The
explanation for this discrepancy is not evident, but it may be due to the fact that our data
collection intervals, 0.1 seconds and initially many of these were then averaged to 1.0 seconds, may not have been sufficient to resolve intra-beat dynamics. Clearly, additional experiments with this technique and shorter sampling intervals may be able to resolve this issue.

The approximately 4 second phase lag between an increase in venous pressure and an increase in cardiac output probably reflects the time for conduction of pressure/flow parameters through the cardiac chambers. Assuming an approximate heart rate of 50 beats per minute, 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.

ACKNOWLEDGMENTS

This work was supported by Bayer Chair Fund (H.-C.C) and the Clare Boothe Luce Fellowship Fund (A.R.M.) and by the National Science Foundation, Grant Nos. IBN-9723306 and IBN 0235223 (K.R.O.) We also thank Alison Weltner, Michelle Roeser, Katherine Brakora, and John Howard for help with data collection and analysis.
REFERENCES


Table 1. Relationships between central venous pressure ($P_{VEN}$), cardiac output (CO), heart rate (HR), and stroke volume (SV) from 9 experiments with a Shannon entropy index of 0.85 or less; infusion/withdrawal duration ranged from 40 to 240 seconds. A kappa value of 1 is indicative of asynchronization while 0 represents perfect synchronization without a phase lag (see also fig. 5); cardiovascular parameters are positively correlated when trendline slope is positive, inversely related when negative, and unrelated as slope approaches zero; mean ±SE.

<table>
<thead>
<tr>
<th>Shannon Entropy</th>
<th>Kappa values</th>
<th>Trendline Slope</th>
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<tbody>
<tr>
<td></td>
<td>CO - $P_{VEN}$</td>
<td>CO - HR</td>
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<tr>
<td>0.67 ±0.04</td>
<td>0.154 ±0.030</td>
<td>0.340 ±0.048</td>
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FIGURE LEGENDS

Figure 1. Representative trace of dorsal aortic ($P_{DA}$; a, b) and central venous ($P_{VEN}$; c, d) pressures and cardiac output (CO; e, f) from two trout with 3.5 ml min$^{-1}$ kg$^{-1}$ blood volume infusion/withdrawal at a duration of 40 seconds (left panels) or 180 seconds (right panels). $P_{DA}$ is entrained by the infusion/withdrawal pump, whereas $P_{VEN}$ and CO respond to the change in blood volume.

Figure 2. Whole-body vascular compliance of rainbow trout with closed (circles) or open (triangles) pericardium obtained at different rates of infusion/withdrawal (40 sec = 20 sec infusion, 20 sec withdrawal). Mean ±SE; (n) = number of fish. Dotted line shows regression of all measurements (n=44).

Figure 3. Hilbert transform of data from a trout with a 240 second infusion/withdrawal duration; venous pressure in left column and cardiac output in right column. a and b) raw data trace for central venous pressure ($P_{VEN}$) and cardiac output (CO), respectively. c and d) signal envelope obtained via the Hilbert transform. e and f) enlarged view of the Hilbert $\tilde{s}(t)$, (dashed line) of the high frequency component of the raw signal, $s(t)$ (solid line). g and h) enlarged view of the instantaneous phase (modulus - to ) of the high frequency component. i and j) instantaneous frequency of the high frequency component over the duration of blood volume manipulation. k) stroke volume derived from cardiac output and heart rate.
**Figure 4.** Panels a-c show instantaneous phase information for venous pressure (dashed line) and cardiac output (solid line) signal envelopes for trout with 40 second blood volume manipulation and poor (asynchronous) cardiac output record (a), and good cardiac output records at 40 and 240 second blood volume manipulations (b and c, respectively). Panels d-f show phase difference probability distribution. There is a uniform phase difference for the 40 second asynchronous record (d), whereas there is synchronization with a distinct phase difference (Shannon entropy index, $\rho_{\text{PVEN},\text{CO}}$ of 0.75) for the 40 second blood volume manipulation with good cardiac output record (e), and a distinct phase difference (Shannon entropy index, $\rho_{\text{PVEN},\text{CO}}$ of 0.45) for the 240 second blood volume manipulation (f).

**Figure 5.** Cross-correlation between cardiac output (CO) and venous pressure ($P_{\text{VEN}}$; a-c), CO and heart rate (d-f), and stroke volume and heart rate (g-i) for fish in figure 4. The degree of scatter is obtained from the eigenvalue ratio of the co-variance matrix. A ratio ($\lambda$) of 1 signifies asynchronization while a ratio of 0 represents perfect synchronization without a phase lag; the equation for the trend line (solid line) is given in the top of each figure. For the 240 second duration, CO and $P_{\text{VEN}}$ are positively correlated and there is a negative correlation between CO and heart rate.
Figure 1.

40 seconds

40 seconds

180 seconds

180 seconds

PDA (mmHg)
PVEN (mmHg)
CO (ml/min/kg)
PDA (mmHg)
PVEN (mmHg)
CO (ml/min/kg)
Figure 2.
Figure 3.
Figure 4.
Figure 5.