The Journal of Experimental Biology 206, 457-467

doi:10.1242/jeb.00123

Transvascular and intravascular fluid transport in the rainbow trout: revisiting Starling's forces, the secondary circulation and interstitial compliance

Kenneth R. Olson*, Daniel W. Kinney, Ryan A. Dombkowski and Douglas W. Duff

Indiana University School of Medicine, South Bend Center for Medical Education, University of Notre Dame, Notre Dame IN 46556, USA

*Author for correspondence (e-mail: olson.1@nd.edu)

Accepted 7 October 2002

Summary

The kinetics of transvascular fluid transport across fish capillaries and redistribution of fluids between intravascular compartments in intact fish are unknown. Cannulae were placed in the dorsal aorta (DA) and caudal vein (CV) of rainbow trout *Oncorhynchus mykiss* (mass 0.45–0.85 kg) and the fish spleenectomized. The following day a peristaltic pump was fitted to complete the extracorporeal arterio-venous circulation. Hematocrit (Hct) was monitored in unanesthetized fish either manually, by collecting blood from the extracorporeal loop at 5 min intervals for a period of 1 h (groups 1 and 2), or continuously (instantaneously) with an impedance flow-cell inserted in the aortic cannula (group 3). Fish in group 1 were volume expanded by injecting a volume of saline (0.9 g% NaCl; SI) or trout plasma (PI) equivalent to 40% of the plasma volume. In group 2, 20% or 35% of the blood volume was removed, and in group 3, 35% of the blood volume was removed. Plasma volume (V_P) was calculated from an assumed blood volume of 35 ml kg^{-1} and the Hct. V_P declined mono-exponentially after SI with a half-time of 6.8 min and V_P reached a new steady state at 28.1 ml kg^{-1}; 30% of the injected volume remained in the vasculature. Volume recovery after PI was also mono-exponential, but slower (half-time=15.4 min) than SI, whereas the steady-state V_P (27.3 ml kg^{-1}) was similar and 30% of the injected volume remained in the vasculature. Thus the presence of plasma proteins delayed fluid efflux from the vasculature, but did not affect the volume lost. Transvascular fluid filtration coefficients calculated from this data were 5.5 (SI) and 4.5 ml mmHg^{-1} kg^{-1} min^{-1} (PI), and interstitial compliance was 11.8 (SI) and 9.7 ml mmHg^{-1} kg^{-1} (PI). The rate of volume recovery after 20% or 35% hemorrhage was independent of the hemorrhage volume (half-time=13.3 and 15.1 min, respectively) and similar to the half-time of PI, indicating that protein-rich interstitial fluid is returned to the vasculature. There is a nearly instantaneous change in Hct that occurs during the hemorrhage period; it is dependent on hemorrhage duration and volume and not associated with the subsequent mono-exponential recovery. This initial response is best explained by a rapid fluid shift from a large-volume (approximately 40% of total blood volume), low-hematocrit (less than half of systemic Hct) microcirculation into the higher-hematocrit macrocirculation. These studies are consistent with transcapillary fluid flux across a barrier that is highly permeable to protein, and cannot be explained by fluid shift between primary and secondary circulations, or by transcapilllary flux across a capillary bed that is impermeable to plasma proteins. The results support the hypothesis that whole-body reflection coefficients in trout are very low and that plasma oncotic pressure is not a determinant of transcapillary fluid balance. They also show that both transvascular and intravascular fluid movements are important effectors of central volume homeostasis.

Key words: fish cardiovascular system, transcapillary fluid filtration, oncotic pressure, hemorrhage, microcirculation, rainbow trout, *Oncorhynchus mykiss*.

Introduction

The vertebrate cardiovascular system had its origins in aquatic animals and later adapted to a terrestrial habitat. Many features of this system, such as myocardial function (Farrell and Olson, 2000; Farrell and Jones, 1992), venous capacitance (Olson, 1997) and vasoregulatory signaling mechanisms (Conlon, 1999; Hoagland et al., 2000; Loretz and Pollina, 2000; Russell et al., 2001; Takei, 2000; Nilsson, 1984; Wang, 1999) have been surprisingly well conserved throughout this long evolutionary process. Clearly these features are well designed to serve the basic convective needs of the organism.

Invasion of the terrestrial environment, however, added a gravitational force on body fluids and this created problems that were not anticipated in an aquatic environment. Anti-gravity responses of the mammalian cardiovascular system are well known and include an elevated arterial blood pressure to move blood above the heart and venous reflexes to prevent

blood from pooling below it (Rowell, 1993). Satchell (1991) realized this and he suggested that, because fish are neutrally buoyant relative to their environment, they needed neither high blood pressure nor active control of venous tone. With the exception of tunas, blood pressure in fish is indeed low (Olson, 1997); however, it is also evident that active regulation of venous capacitance is necessary to regulate cardiac output and to respond to alterations in blood volume and the effects of acceleration (Olson, 1997).

Attendant with an elevated arterial blood pressure and increased intravascular pressure in dependent vessels is the need to minimize fluid extravasation across the capillaries. To achieve this, mammalian capillaries act as a barrier to plasma protein and maintain a sufficient protein concentration gradient between plasma and interstitial fluid to offset the hydraulic tendency for transvascular fluid filtration. Collectively these are the well-known Starling's forces. In addition, lymph vessels return the small amount of extravasated protein back to the circulation along with excess filtered fluid (Renkin and Tucker, 1995; Aukland and Reed, 1993).

Considerable controversy surrounds our understanding of transvascular fluid balance in fish. In a review of the literature, Olson (1992) suggested that the full compliment of Starling's forces may not be applicable in fish because fish capillaries appear to be relatively permeable to protein. This conclusion was based on observations that protein concentration in peritoneal and subcutaneous fluid was similar to that in plasma (Turner, 1937; Hargens et al., 1974) and that blood volumes measured with protein indicators consistently exceeded those determined from tagged red blood cells. More recently, Bushnell et al. (1998) measured the time course of appearance of ^{125}I-albumin in trout tissues following intra-arterial injection and observed a rapid and sustained accumulation of radiolabel in many tissues, including gut and skeletal muscle, which they attributed to protein extravasation.

The extent of the so-called secondary circulation and an apparent lack of a true lymphatic system in fish is a second point of contention. There is adequate anatomical evidence for a secondary vascular system (Vogel, 1985; Steffensen and Lomholt, 1992; Olson, 1996), but estimates of the volume of the secondary circulation have been based on kinetic measurements of ^{125}I-albumin disappearance from the primary circulation (dorsal aorta), and the conclusion that the volume of the secondary circulation in rainbow trout is 1.5 times that of the primary circulation (Steffensen and Lomholt, 1992) has been questioned (Bushnell et al., 1998). Two assumptions were made in the study by Steffensen and Lomholt (1992): (1) that fish capillaries are relatively impermeable to protein, and (2) the kinetic components can accurately separate mixing within and between the different vascular compartments. To date there is little evidence to support or refute either of these assumptions. Obviously, if fish capillaries were permeable to protein and the kinetics of protein extravasation were near those attributed to mixing into the secondary circulation, the estimates of Steffensen and Lomholt (1992) would be erroneous.

In the present study we used a different approach to estimate fluid compartments and protein permeability in fish. Hematocrit was monitored in spleenectomized rainbow trout at 5 min intervals following volume loading with either saline or trout plasma, or after hemorrhage. In another group of spleenectomized fish, hematocrits were measured continuously during and after hemorrhage. These methods (1) eliminated the kinetic mixing-in of exogenous indicators, (2) provided an estimate of both the volume and rate of fluid movement within the vasculature and between intravascular and extravascular compartments, and (3) allowed us to assess the contribution of plasma protein to Starling's forces. In addition, we obtained the first estimates of interstitial compliance in any fish and found evidence for a red-blood-cell-poor vascular compartment that could be rapidly mobilized in response to hypovolemia.

Materials and methods

Rainbow trout *Oncorhynchus mykiss* Walbaum mixed Kamloops strain (mass 0.45–0.85 kg) of either sex were purchased from a local hatchery and kept in circulating 2000 liter tanks at 14°C and under appropriate, seasonal light:dark cycles. Fish were fed a maintenance diet of commercial trout pellets (Purina) for up to 48 h prior to experimentation.

Methods for cannulation of the dorsal aorta (DA) and caudal vein (CV) have been described in detail (Olson et al., 1997; Perry et al., 1999). Trout were anesthetized in benzocaine (ethyl-*p*-aminobenzoate; 1:12 000 w:v) prior to surgery. The DA was cannulated percutaneously through the roof of the buccal cavity with heat-tapered polyethylene tubing (PE 60); the gills were not irrigated during this brief (<1 min) procedure. The gills were then continuously irrigated with 10°C aerated water containing 1:24 000 w:v benzocaine, during CV cannulation and spleenectomy. The CV was cannulated with beveled PE 50 tubing *via* a lateral incision at the level of the caudal peduncle to expose the hemal arch. The wound was closed with interrupted silk sutures and the cannula was affixed to the caudal peduncle with an additional suture. Both DA and CV cannulae were filled with heparinized [100 USP (United States Pharmacopeia) ml^{-1}] saline (0.9% NaCl). The spleen was exteriorized *via* a 1 cm incision slightly right-lateral to the ventral midline and anterior to the pelvic fin. The splenic arteries were ligated with heavy silk, the spleen was removed, and the wound closed with interrupted silk sutures. The fish was then revived and placed in a black plastic tube in a 100 liter tank with free-flowing, aerated tapwater.

The following day the cannulae were connected to a peristaltic pump and blood was pumped from the DA to the CV at a rate of 5 ml min^{-1}. Duplicate blood samples were withdrawn into 25 µl microhematocrit tubes, centrifuged and read on a hematocrit reader. Samples were collected 5 min prior to and at 5 min intervals for 1 h after volume expansion or depletion. In one group of fish, blood volume was expanded by infusion of a volume of saline equivalent to 40% of the estimated plasma volume (V_{PE}). The second group of trout

were injected with an equivalent volume of trout plasma previously obtained (within 1 h prior to infusion) from donor fish. V_{PE} was estimated from an assumed blood volume (V_B) of 35 ml kg^{-1} (Duff et al., 1987) and the hematocrit of the experimental fish prior to volume expansion (Hct_0);

$$V_{PE} = V_B(1–0.01Hct_0)\,. \quad (1)$$

This volume was injected into the CV over a period of 1 min with a hand-held syringe. The third and fourth group of trout, 20% or 35% of their estimated blood volume was bled over 1 min by withdrawing blood from the CV.

Hematocrit in the fifth group of trout was continuously monitored during hemorrhage using the impedance flow-cell method developed by Tanaka et al. (1976) and modified by S. S. Hillman (personal communication). The flow cell was placed on the aspiration (DA) side of the peristaltic pump and impedance was recorded at 100 ms intervals using Labtech notebook software. 1 s block averages were archived on a computer for 10 min before and 1 h after hemorrhage of 35% of the estimated blood volume. Hemorrhage was achieved by diverting blood on the discharge side of the pump away from the fish and this usually took approx. 4 min. It was not possible to draw blood directly from the DA because this interrupted flow through the flow-cell and affected the impedance. Hematocrits were also measured manually by collecting samples from the pump discharge at 10–15 min intervals throughout the experiment, and these were used to calibrate the impedance cell. The dead space of the flow-cell and arterial cannula was 0.5 ml; the dead space of the entire extra-corporeal loop was 1.2 ml.

A sixth group of fish served as controls to ensure that the extra-corporeal pump did not affect blood volume or red cell integrity. These fish were instrumented as above and hematocrits were measured at 5 min intervals for 1 h. Hematocrit remained constant for the 60 min sampling period (Fig. 1).

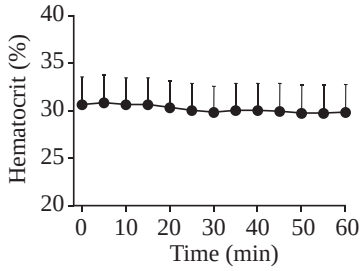

Fig. 1. Effect of 1 h extra-corporeal circulation on the hematocrit of 6 spleenectomized trout. Values are means + S.E.M.

Calculations

A curve-fitting program (Jandel) was applied, assuming a mono-exponential recovery, to determine the rate constants of the averaged data for all experiments in which hematocrit was manually determined. The equation used for volume recovery following infusion was:

$$V_{Pt} = V_{Peq} + V_{inf} \cdot e^{-kt}\,, \quad (2)$$

where V_{Pt} is plasma volume at time t, V_{Peq} is plasma volume at equilibrium, V_{inf} is volume infused, and k is the rate constant. The equation for volume recovery following hemorrhage was:

$$V_{Bt} = V_{Bh} + V_{res} \cdot (1–e^{-kt})\,, \quad (3)$$

where V_{Bt} is blood volume at time t, V_{Bh} is blood volume immediately after hemorrhage, V_{res} is fluid volume restored to the circulation.

It was not possible to apply the curve-fit analysis to data from individual fish because we could only read hematocrits down to 0.5%, and this frequently created enough variation to preclude a good fit of the curve. All curve-fitting was done on averaged volumes, except for the instantaneous hematocrit measurements (see below). If the curves for volume expansion (Equation 2) were forced through the actual volume expansion at t=0, the r^2<0.8; however, when the curves were derived at t=5–60 min, r^2=0.99. Similarly, hemorrhage curves (Equation 3) inadequately described the data points when they were forced through t=5 min. Failure to fit the curves around t=0–5 min has physiological significance and this is described in detail in the Discussion. Other values are presented as mean ± S.E.M.

The instantaneous hematocrit response to hemorrhage clearly showed that two processes were involved. The first response appeared to occur only while blood was being withdrawn and ended within 15–30 s after the end of the hemorrhage period. This rapid response was not amenable to curve-fitting. The second response became evident after the first had ended and followed a mono-exponential relationship. In order to avoid artifact from the transition between these two events, the starting point for curve-fit analysis of the second response was 1 min after hemorrhage was completed. The resultant curve was extrapolated back to the onset of hemorrhage to predict the volume contribution of each process (see Fig. 4).

Statistics

Comparisons were made with Student's *t*-test; significance was assumed at $P \leq 0.05$. Values were expressed as mean ± standard error of the mean (S.E.M.), or mean + S.E.M. in Figs 1–3.

Results

Fig. 1 shows the average hematocrit for six control fish during 1 h of extra-corporeal circulation. There was no apparent effect of this procedure on hematocrit, nor was there any noticeable hemolysis.

Volume expansion

The kinetics of volume restoration after saline or trout plasma infusion are shown in Fig. 2, and in Table 1. 60 min after infusion of 12 trout with 10.0±1.0 ml kg^{-1} saline (40% of the estimated plasma volume), 7.3±0.8 ml kg^{-1} of fluid had

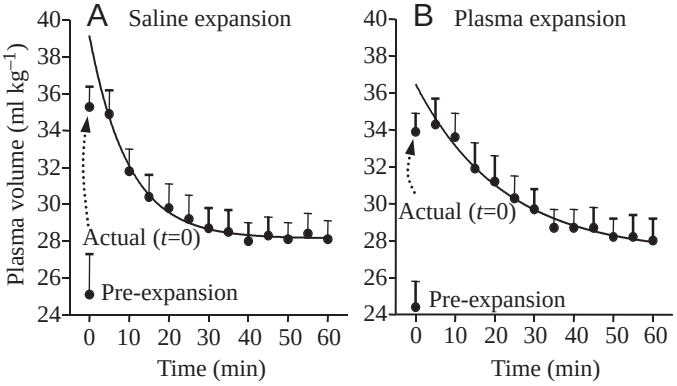

Fig. 2. Restoration of plasma volume in intact trout after 40% volume expansion with saline (A) or trout plasma (B). The solid line is a mono-exponential decay fit to data points 5–60 min after volume expansion. Pre-expansion, calculated plasma volume prior to saline or plasma infusion; actual (t=0), pre-expansion plasma volume plus volume of saline or plasma infused. Values are means + S.E.M.; N=12 for both experiments.

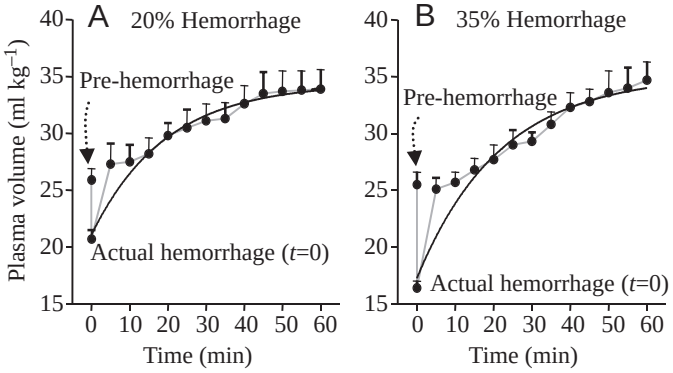

Fig. 3. Restoration of plasma volume in intact trout after 20% (A) or 35% (B) hemorrhage. Pre-hemorrhage, assumed plasma volume prior to hemorrhage; Actual hemorrhage (t=0), calculated plasma volume immediately after 20% or 35% hemorrhage. Other solid circles joined by a grey line are plasma volume calculated from the change in hematocrit. The solid line is the mono-exponential curve fit to data points (excluding t=5 min). Values are means + S.E.M.; N=9 (A), N=6 (B).

been transferred out of the vasculature and 28.1±3.6% of the injected volume remained in the circulation. In the same period after 9.6±0.5 ml kg^{-1} of trout plasma infusion (also 40% of the estimated plasma volume) into 15 trout, 5.9±0.6 ml kg^{-1} of fluid had been transferred out of the vasculature while 37.9±6.0% of the injected volume remained in the circulation. The actual volume expansion (sum of pre-expansion plasma volume and infusion volume) at t=0 after the onset of infusion was consistently less than the plasma volume predicted by Equation 2 at the same time period for both saline infusion (35.3±1.1 ml kg^{-1} *versus* 39.2 ml kg^{-1}, respectively), and after plasma infusion (33.9±1.0 ml kg^{-1} *versus* 36.5 ml kg^{-1}, respectively).

The rate constant for volume recovery following saline infusion was twice that for plasma infusion and the half-time for recovery from saline infusion was half that of plasma infusion (Table 1). However, in spite of the slower rate of plasma efflux from the vasculature, the predicted equilibrium plasma volumes (the plasma volume after fluid efflux from the vasculature had stabilized; estimated from Equation 2) for saline and plasma infusion were essentially the same (Table 1). Thus volume restoration was slower after trout plasma infusion, but the presence of trout plasma proteins did not quantitatively affect the actual volume restored.

Hemorrhage

The kinetics of volume restoration after 20% or 35% hemorrhage are shown in Fig. 3 and in Table 2. Within 5 min after either 20% or 35% hemorrhage, the plasma volume estimated from the change in hematocrit appeared to be essentially back to normal (Fig. 3). By 60 min after 20% and 35% hemorrhage, the estimated blood volumes were 41.2±1.7 and 40.9±1.8 ml kg^{-1}, respectively, or 117.7±5.0 and 116.8±5.2% of the initial blood volume. The estimated blood volume (based on hematocrit) greatly exceeded the predicted blood volume (from Equation 3) at 5 min after hemorrhage; following 20% hemorrhage the estimated blood volume was 34.5±1 ml kg^{-1}, and blood volume predicted from Equation 3 was 31.4 ml kg^{-1}; after 35% hemorrhage the estimated blood volume was 31.2±1.0 and the predicted blood volume was 27.1 ml kg^{-1}. Despite the fact that considerably more blood was removed by 35% hemorrhage compared to 20% hemorrhage (12.3 *versus* 7 ml kg^{-1}, respectively), the predicted blood volumes after recovery from 20% or 35% hemorrhage were

Table 1. *Kinetics of restoration of plasma volume following 40% plasma volume expansion with saline or trout plasma*

Infusion	Initial Hct	Plasma volume (ml kg^{-1})			k	Half-time (min)	r^2
		Initial	Expanded[a]	Equilibrium[b]			
Saline (N=12)	27.9±2.3	25.1±2.2	35.3±1.1	28.1	0.102	6.8	0.99
Plasma (N=15)	30.7±2.1	24.4±1.4	33.9±1.0	27.3	0.045	15.4	0.99

[a]Sum of initial plasma volume and volume of saline or plasma infused.
[b]Predicted plasma volume after fluid efflux from the capillary has ceased (calculated from the exponential decay curve; Equation 2).
k, rate constant; r^2, from mono-exponential curve-fit.
Values are means ± S.E.M., N = number of fish.

Table 2. *Kinetics of restoration of blood volume following 20% or 35% reduction of blood volume*

Hemorrhage (%)	Initial Hct	Blood volume (ml kg^{-1}) Recovered at 5 min[a]	Recovered at 60 min[a]	Equilibrium[b]	*k*	Half-time (min)	r^2
20 (*N*=9)	26.1±2.8	6.8±1.5	13.4±2.0	41.7	0.052	13.3	0.98
35 (*N*=6)	27.2±3.1	8.5±0.6	18.2±1.5	41.3	0.046	15.1	0.97

20% hemorrhage=7 ml kg^{-1} removed, 35% hemorrhage=12.3 ml kg^{-1} removed.
[a]Estimated fluid volume returned to vasculature based on change in hematocrit.
[b]Predicted final blood volume after fluid influx into vasculature has ceased (calculated from the exponential recovery curve; Equation 3).
k, rate constant; r^2, from mono-exponential curve-fit.
Values are means ± S.E.M., *N* = number of fish.

essentially identical, as were the rate constants for recovery and the half-times (Table 2).

Instantaneous hematocrit

When hematocrit was measured continuously, it was evident that removal of 35% of the blood volume produced a two-phase change in hematocrit (Fig. 4; Table 3). Within seconds after the onset of hemorrhage, hematocrit began to fall rapidly and it continued its rapid descent until shortly after the blood was withdrawn. Thereafter, there was an abrupt transition to the second, slower phase. The time course of the rapid phase appeared to be directly coupled to the duration of hemorrhage and the faster blood was withdrawn, the faster hematocrit fell. The slow phase followed a mono-exponential time course with a rate constant (0.052; Table 3) similar to those determined for plasma volume loading (0.045; Table 1) and 20% and 35% hemorrhage (0.052 and 0.046, respectively; Table 2). The rapid response accounted for approximately 25% of the total volume response and the predicted blood volume at equilibrium (40.7 ml kg^{-1}) was 16% greater than the estimated blood volume prior to hemorrhage (35 ml kg^{-1}).

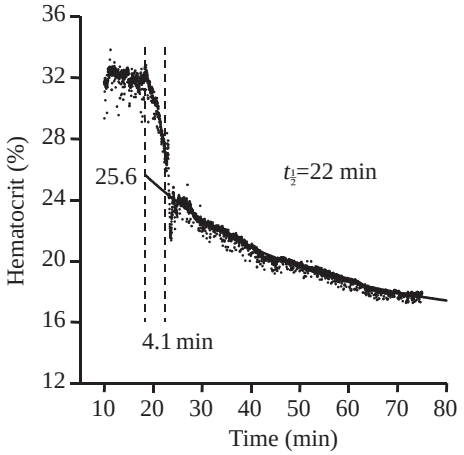

Fig. 4. Instantaneous hematocrit measured in an unanesthetized trout before, during (4.1 min period between broken vertical lines) and following hemorrhage of 35% of estimated blood volume. There is a rapid fall in hematocrit concomitant with hemorrhage and a slow decline thereafter. The slow phase can be described by a mono-exponential decay (solid line) and extrapolated back to the onset of hemorrhage in order to determine the contribution of both rapid and slow processes to the observed hematocrit. In this example, hematocrit is predicted to change from 32 to 25.6 during the fast phase and from 25.6 to 16 during the slow phase, the latter with a half-time of 22 min.

Discussion

Our experiments show that there is a rapid efflux of fluid from the circulation after trout are volume expanded with either saline or trout plasma. While the rate of efflux is decreased by the presence of plasma proteins, the volume of fluid that leaves the vasculature after expansion of the plasma volume by 40% (approximately 70% of the injected volume) is not quantitatively affected. Hemorrhage of 20% or 35% of the blood volume mobilizes fluid into the circulation with a rate constant that is independent of the volume of blood lost

Table 3. *Kinetics of restoration of blood volume following 35% reduction of blood volume*

Initial Hct	Hct after rapid recovery[a]	Blood volume (ml kg^{-1}) Rapid volume recovered[b]	Slow volume recovered[c]	Equilibrium	*k*	Half-time (min)
28.1±2.9	23.8±2.9	4.7±1.0	12.6±1.7	40.7±1.3	0.052±0.007	14.5±1.9

[a]Extrapolated to onset of hemorrhage.
[b]Estimated fluid volume returned to vasculature in initial rapid phase during hemorrhage assuming that hematocrit of this fluid is 0.
[c]Predicted fluid volume returned to circulation during the slow phase following hemorrhage.
k, rate constant for slow phase.
Values are means ± S.E.M., *N* = number of fish.

and essentially identical to the rate constant for fluid efflux from the vasculature during volume expansion with trout plasma. Mono-exponential equations provide a reasonably good fit of the observed changes in intravascular fluid volume after volume expansion or hemorrhage, except during the initial minutes of volume perturbation. Continuous measurement of hematocrit during hemorrhage showed that this initial response was temporally coupled to blood withdrawal, and we propose that this is the result of rapid fluid transfer from the red-blood-cell-poor microcirculation to the macrocirculation. Information derived from these experiments enabled us to estimate interstitial compliance and whole-body trans-capillary fluid filtration rates, and this is the first report of these parameters in any fish. Our experiments also substantiate the hypotheses that (1) trout capillaries are very permeable to plasma protein and that Starling's forces are probably irrelevant in transvascular fluid balance in these fish, and (2) use of plasma-protein-based volume indicators has led to a gross over-estimation of the volume of the secondary circulation.

Vascular and interstitial compliance and transvascular fluid filtration

The rate and degree of recovery of hematocrit following volume expansion with either saline or plasma indicate that fluid rapidly leaves the primary circulation, and that at equilibrium only 30% of the injected fluid remains in the primary circulation. As will be demonstrated below, we feel that there is sufficient justification to omit the hematocrit responses during the first 5 min after infusion when estimating vascular and interstitial compliance and transvascular filtration.

Our estimates of compliance and transvascular fluid filtration rate are based on two assumptions. First, we assume that fluid lost from the primary circulation enters the interstitial space and not the secondary circulation. Steffensen and Lomholt (1992) have shown that the flow between the primary and secondary circulations in trout is $6\,ml\,kg^{-1}\,h^{-1}$, which is less than our observed saline loss in 25 min ($6.4\pm ml\,kg^{-1}$, or $15.36\,ml\,kg^{-1}\,h^{-1}$). It could be argued that volume expansion increased the flow of saline into the secondary circulation; however, comparison of the kinetics following volume expansion with saline *versus* plasma argue against this. If saline and plasma entered the secondary circulation, the kinetics of plasma volume recovery following volume loading with saline and plasma (Fig. 2) should be identical, but they are not. In fact, the rate constant for volume recovery following volume loading with plasma is less than half the rate constant following saline loading (Table 1). It seems unlikely that the presence of plasma proteins could have such a substantial affect on the rate of fluid entry into the secondary circulation. The most plausible way to account for the delayed loss of volume from the circulation following plasma infusion is by a barrier that delays protein transit. Capillary endothelia and the interstitial matrix have this barrier, the secondary circulation does not. It is possible that transcapillary fluid efflux and entry into the secondary circulation are occurring simultaneously, although this also seems unlikely because both conditions are described by a monoexponential recovery (except for the instantaneous phase, discussed below). Furthermore, our observation that the rate constants for fluid efflux from the circulation following plasma infusion and those for fluid recovery following hemorrhage are virtually identical indicates that essentially all of the fluid influx following hemorrhage follows the same pathway as fluid efflux following volume expansion with plasma. While our experiments do not allow quantitative differentiation between transcapillary fluid movement and fluid flux between the primary and secondary compartments, we feel that the data strongly support the transcapillary route and that the contribution by the secondary circulation may be quite small.

Our second assumption is that the change in central venous pressure following volume expansion is an accurate indicator of simultaneous changes in capillary hydraulic pressure. Determination of the fluid filtration coefficient, vascular compliance and interstitial compliance are all based on a relative change in pressure; it is not necessary to know the absolute capillary or interstitial pressures. If we assume that arterial resistance is greater than venous resistance, then changes in venous pressure will most nearly reflect changes in capillary pressure during volume expansion. Furthermore, the net change in capillary pressure at equilibrium will reflect a similar change in interstitial pressure at equilibrium. Hoagland (2001) measured the rapid (within seconds) change in arterial and central venous pressure following volume expansion with whole blood up to 150% of blood volume in trout with an intact pericardium. These data, 2.41 mmHg at rest, 5.35 mmHg after 40% volume expansion and 3.35 mmHg at 12% volume expansion (the latter is equivalent to the equilibrium period after volume loading when only 30% of expansion fluid remains in the vasculature), can be used to predict the effect of volume expansion on the change in central venous pressure (and, therefore, the change in capillary pressure) in the present experiments. In addition, because central venous pressure is linearly related to volume over the expansion volumes employed in this study (100–150% blood volume) and because changes in volume and central venous pressure are temporally coupled within seconds (Zhang et al., 1995; Hoagland, 2001), it can be assumed that the fall in capillary pressure during the recovery period following volume expansion has the same rate constant as the volume curve (Fig. 2) and can be described by Equation 2, after substituting the appropriate pressures for volumes. Using this relationship, the vascular compliance following saline or plasma infusion is 5.5 and $4.6\,ml\,mmHg^{-1}\,kg^{-1}$, respectively. These values are close to those reported previously for unanesthetized, intact trout and in perfused whole fish (Zhang et al., 1995; Hoagland, 2001).

The rise in interstitial volume following volume expansion is inversely related to the loss of fluid from the vasculature and can be described by Equation 3, substituting interstitial volume (V_I) for blood volume and the time-dependent volumes, V_{I0} for

pre-volume loading (control period), and $V_{I_{inc}}$ for the net gain in interstitial volume at equilibrium:

$$V_{I_t} = V_{I_0} + V_{I_{inc}} \cdot (1 - e^{-kt}) . \quad (4)$$

V_{I_0} can be estimated by subtracting the initial plasma volume (Table 1) from the total extracellular fluid volume (287.1 ml kg^{-1}; D. C. Hambleton, T. M. Hoagland and K. R. Olson, unpublished observation), although its actual value is irrelevant when calculating compliance and can be omitted from the calculation. $V_{I_{inc}}$ is the increase in interstitial volume, which is calculated from the volume of saline or plasma added to the fish minus the net increase in plasma volume between t_0 and t_{eq}.

The time course for the rise in interstitial fluid pressure as intravascular pressure falls can be estimated by substituting pressures for volumes in Equation 4 and by using the rate constants determined for volume distribution:

$$P_{I_t} = P_{I_0} + P_{I_{inc}} \cdot (1 - e^{-kt}) . \quad (5)$$

The increase in interstitial pressure after volume equilibration ($P_{I_{inc}}$) can be estimated from the net change in capillary pressure (central venous pressure) between control (prior to volume loading; 2.41 mmHg) and after volume equilibration (3.35 mmHg), which is 0.94 mmHg. Because we are only interested in the relative change in pressure for a given change in volume, the initial interstitial pressure (P_{I_0}) is irrelevant and drops out of the equation. The change in interstitial volume (ΔV_I) over any period of time (t_1 to t_2) can obtained by solving Equation 4 for the two time periods and taking the difference. Similarly, the change in interstitial pressure (ΔV_I) over the same time interval can be obtained from the difference after solving Equation 5. Using these relationships, interstitial compliance ($\Delta V_I/\Delta P_I$) after saline or plasma infusion is 11.8 and 9.7 ml mmHg^{-1} kg^{-1}, respectively.

Because we used a dynamic analysis to determine vascular compliance with our time scale in minutes, the calculated vascular compliance is also equal to the transvascular fluid filtration coefficient. Thus for saline infusion, this coefficient is 5.5 ml mmHg^{-1} kg^{-1} min^{-1}, and for plasma infusion it is 4.6 ml mmHg^{-1} kg^{-1} min^{-1}. To our knowledge, this is the first estimate of the transvascular fluid filtration coefficient in an intact fish.

Although the information is quite limited (we are unaware of filtration coefficients in reptiles and birds), there appears to be a phylogenetic trend toward lower interstitial compliance and lower transvascular filtration rates in the higher vertebrates. Using a method essentially identical to ours, Tanaka (1979) found that interstitial compliance in the dog was 5.9 ml mmHg^{-1} kg^{-1}, nearly half our value for the trout. Aukland and Reed (1993) summarized more recent compliance measurements in mammals obtained through a variety of methods and in general these values were approximately one-third of those in trout. Our estimate of the transvascular fluid filtration coefficient in trout is only 50% higher than that reported by Hancock et al. (2000) for two anurans, *Bufo marinus* (3.6 ml mmHg^{-1} kg^{-1} min^{-1}) and *Rana catesbeiana* (3.2 ml mmHg^{-1} kg^{-1} min^{-1}), whereas it is five times greater than that found by the same authors for the rat (1.0 ml mmHg^{-1} kg^{-1} min^{-1}) and 15 times greater than that reported in the dog (0.3 ml mmHg^{-1} kg^{-1} min^{-1}) by Tanaka (1979).

It is tempting to speculate that the reduction in transvascular filtration coefficient and interstitial compliance in mammals was necessitated by elevated intravascular pressures, the latter attendant with adopting a terrestrial lifestyle. It may also help explain why mammals are less readily able to mobilize extravascular fluid after constant-volume or constant-pressure hemorrhage than trout (Duff and Olson, 1989). However, birds are paradoxical. They have arterial blood pressures in the mammalian range, yet respond like trout in their ability to mobilize fluid following hemorrhage (Djojosugito et al., 1968; Kovach et al., 1969; Ploucha and Fink, 1986). Clearly, it is necessary to examine these parameters in other vertebrates, especially birds and fish with high blood pressure, such as tunas (Brill and Bushnell, 2001).

Theoretically, we could have also calculated compliance and filtration coefficients from the hemorrhage studies. We did not do this because the trout vascular capacitance curve becomes non-linear as blood volume is lowered below resting levels (Zhang et al., 1995; Olson et al., 1997; Hoagland 2001), and it becomes difficult to estimate dynamic changes in venous pressure.

Rapid hematocrit responses and the role of the microcirculation

A mono-exponential curve quite accurately fits the experimental data following either volume expansion (Fig. 2), or hemorrhage (Fig. 3), except for the first 5 min after volume perturbation. Within this 5 min period, the curve appears to overestimate plasma volume (it predicted that hematocrit should have fallen to a lower level) after volume expansion and under-estimate volume (it predicted that hematocrit should have been higher) after hemorrhage. By continually measuring hematocrit during hemorrhage (Fig. 4) it became apparent that the initial decrease in hematocrit was nearly instantaneously coupled to the reduction in blood volume. There are three possible explanations for these observations: (1) transvascular fluid flux is unusually high during volume perturbation; (2) plasma is skimmed off, or returned from, the secondary circulation during expansion or hemorrhage; (3) plasma is stored in, or mobilized from, an intravascular compartment. We feel that the third explanation is the most plausible.

It seems unlikely that a transient increase in transvascular fluid flux could account for the observed changes. First, it would have to be triggered by both volume expansion and depletion. Second, it would have to be turned on and off within seconds of the start and end of addition or removal of fluid. Third, once turned off, it would have to remain off, even though part of the original volume perturbation remains. Fourth, the process would require an unrealistically high capillary permeability.

The secondary circulation could be a site of plasma storage

or mobilization. The narrow-bore vessels that form the entrance into the secondary circulation restrict red blood cell access (Steffensen and Lomholt, 1992; Olson, 1996), thus forming a low-hematocrit vascular reserve that could conceivably inflate or deflate with concomitant pressure changes in the primary circulation. However, it would seem that a vasculature as capacious as the secondary circulation is reported to be (Steffensen and Lomholt, 1992) would be able to provide all the post-hemorrhage fluid without need of capillary resorption, and that it would continue to serve as a fluid depot or reserve as along as fluid imbalance remained in the primary circulation. As described above, the distinctly different kinetics observed after volume expansion with saline compared to plasma indicate that this is apparently not the case.

Plasma storage or mobilization from an intravascular compartment (whose hematocrit is lower than systemic hematocrit) in response to volume expansion or depletion, respectively, appears to be the most likely scenario, and it has a precedent in mammalian studies. It is well known that in mammals the hematocrit in capillary-size vessels is considerably less than that in large vessels (Johnson, 1971), and that the ratio of the hematocrit in the whole cardiovascular system to hematocrit in large systemic vessels (Hct_w/Hct_{sys}), also called the F_{cell} ratio, is approximately 0.9 (Albert, 1971). In a recent review, Lee (2000) defined the mammalian microcirculation as all vessels with a diameter smaller than 250 μm, i.e. arterioles, capillaries and venules. Lee (2000) proposed that this microcirculation contains 40–50% of the total blood volume and collectively contributes to the low F_{cell} ratio. Because the microcirculation is compliant, when blood is withdrawn from the macrocirculation of an intact animal, there is a nearly simultaneous shift of blood from the microcirculation into the macrocirculation (Lee, 2000). The amount of fluid mobilized from the microcirculation depends on the relative compliance, volume and pressure drop in both the microcirculation and macrocirculation; however, because of the lower hematocrit in the microcirculation, the outcome is always the same, large-vessel hematocrit falls. Lee described the relationship between these parameters and the volume of the microcirculation (V_{mic}) and total blood volume (V_{total}) as:

$$F_{cell} = 1 - [1 - (Hct_{mic}/Hct_{sys}) \times (V_{mic}/V_{total})] . \quad (6)$$

Thus if one knows the F_{cell} ratio, Hct_{sys} and V_{total}, then microcirculatory hematocrit (Hct_{mic}) and V_{mic} can be estimated.

The F_{cell} ratio in fish has not been accurately measured, but it appears to be 0.8 or less, which is lower than that reported in mammals (Olson, 1992). Many estimates in fish are unreliable because they are compromised by methodological problems, especially those that use albumins as plasma volume indicators. For example, the F_{cell} ratio falls from 0.63 to 0.39 between 4 and 16 h after injection of radio-labeled albumin into trout (Bushnell et al., 1998). Failure to account for splenic sequestration of tagged red blood cells can also be problematic (Duff et al., 1987). Probably the best estimate can be derived from the report by Duff and Olson (1989), in which labeled red cell and albumin spaces were determined within 30 min after injecting the indicators. This is long enough for mixing, yet short enough to minimize albumin extravasation. The F_{cell} calculated from this study (Duff and Olson, 1989) is 0.8.

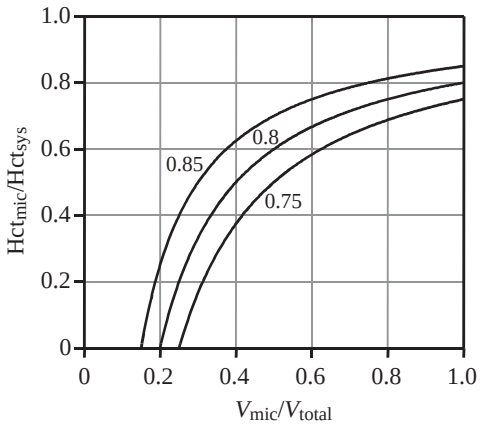

Fig. 5. Relationship between ratios for microcirculation volume (V_{mic}) *versus* total blood volume (V_{total}) and microcirculation hematocrit (Hct_{mic}) *versus* large-vessel systemic hematocrit (Hct_{sys}) for F_{cell} ratios of 0.85, 0.8 and 0.75. If V_{mic} is 40% of V_{total} and the trout F_{cell} ratio is 0.8, then Hct_{mic} will be half of that in large vessels.

In Fig. 5, the relationship between Hct_{mic}/Hct_{sys} and V_{mic}/V_{total} is calculated from Equation 6 for F_{cell} ratios of 0.75, 0.8 and 0.85. With an F_{cell} ratio of 0.8, it is evident that the volume of the microcirculation must be at least 20% of the total blood volume because Hct_{mic}/Hct_{sys} cannot fall below zero. Similarly, Hct_{mic} cannot exceed 80% of Hct_{sys} because V_{mic} cannot be greater than V_{total}. Realistically, both Hct_{mic} and V_{mic} are probably mid-way between these extremes, i.e. 40–50% of Hct_{sys} and V_{total}. If fish F_{cell} ratios turn out to be lower than 0.8, which seems quite possible, then either Hct_{mic} will be lower and/or V_{mic} will be proportionally greater than the above estimates.

Equation 6 also has implications for the volume of fluid transferred from the microcirculation to the macrocirculation. Our estimate of the volume transferred from the microcirculation after 35% hemorrhage (4.7 ml kg^{-1}; Table 3) was based on the assumption that the hematocrit of the fluid transferred from the microcirculation was zero, which is highly unlikely. From the relationship between the pre-hemorrhage (Hct_{pre}) and post-hemorrhage (Hct_{post}) systemic hematocrits (Table 3) and estimated pre-hemorrhage systemic red cell (RCS_{pre}) and plasma (PS_{pre}) spaces (assuming total blood volume is 35 ml kg^{-1}), and the post-hemorrhage contribution of red cells (RCS_{mic}) and plasma (PS_{mic}) from the microcirculation to the macrocirculation, shown in Equation 7:

$$Hct_{pre} - Hct_{post} = RCS_{pre} / (RCS_{pre} + PS_{pre}) - (RCS_{pre} + RCS_{mic}) / (RCS_{pre} + RCS_{mic} + PS_{pre} + PS_{mic}) , \quad (7)$$

we can solve for the relationship between red cell space (RCS_{mic}) and plasma space (PS_{mic}) transferred from the

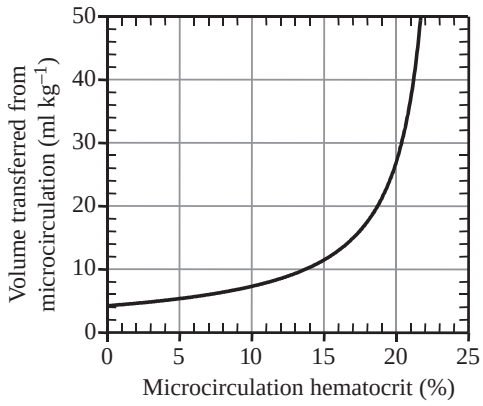

Fig. 6. Predicted relationship between microcirculatory hematocrit and the volume that must be transferred from the microcirculation in order to produce the change in hematocrit observed after 35% hemorrhage (Table 3). If microcirculatory hematocrit was 10, then over 7 ml kg^{-1} would be instantaneously transferred into the macrocirculation in order to produce the observed systemic hematocrit of 23.8 (see Table 3).

microcirculation to the systemic circulation during hemorrhage. After substituting the known physiological values into Equation 7, we obtain the relationship: $RCS_{mic}=0.31\times PS_{mic}-1.31$. Solving for RCS_{mic} over a range of PS_{mic} allows us to determine the relationship between the volume transferred from the microcirculation to the macrocirculation ($RCS_{mic}+PS_{mic}$) and microcirculatory hematocrit [$RCS_{mic}/(RCS_{mic}+PS_{mic})$] for any ($PS_{mic}$), as shown in Fig. 6.

It is evident from Fig. 6 that if Hct_{mic} exceeds 20, then the volume recruited from the microcirculation approaches the total blood volume, which is impossible. If we assume that 40–50% of the blood volume is in the microcirculation, as it is in mammals (Lee, 2000), and if all of it (14–17.5 ml) was transferred to the macrocirculation, then with a macrocirculatory hematocrit of 28, Hct_{mic} could not exceed 16–18. It is unrealistic to assume that the entire microcirculatory volume would be transferred to the macrocirculation, but these calculations put an upper limit on Hct_{mic}. It is also evident from Fig. 6 that as Hct_{mic} falls below approx. 14 (half that of the macrocirculation), there is less of an effect of Hct_{mic} on the volume transferred from the microcirculation.

Within the limits of Hct_{mic} (between 0 and 16), we can use Figs 5 and 6 to predict the magnitude of rapid fluid transfer from the microcirculation to the macrocirculation during hemorrhage of 35% of the blood volume, and this is summarized in Table 4. As Hct_{mic} increases, the predicted V_{mic} and the volume that is transferred from the microcirculation into the macrocirculation during hemorrhage increases. A modest decrease in the F_{cell} from 0.8 to 0.75 increases V_{mic} by 25% and lessens the impact of hemorrhage on V_{mic}, although it is clear that at least half, and more likely two-thirds, of the volume of V_{mic} is transferred to the macrocirculation, irrespective of Hct_{mic} or F_{cell}. The actual Hct_{mic} in fish is unknown. If we assume it is between 8 and 12, which seems reasonable based on mammalian studies (Johnson, 1971), then between half and two-thirds of the volume lost from the macrocirculation during 35% hemorrhage can be nearly instantaneously replaced by elastic recoil of the microcirculation. This is of obvious benefit in maintaining central venous pressure and it provides a safety factor until volume can be restored from the interstitium.

Capillary permeability to protein and the significance of Starling's forces in trout

We previously hypothesized that trout capillaries are quite

Table 4. *Predicted effects of microcirculatory hematocrit (Hct_{mic}) on rapid fluid movement from the microcirculation to the macrocirculation during hemorrhage of 35% of the blood volume*

			F_{cell}=0.8		F_{cell}=0.75	
Hct_{mic}	Volume transferred	% Volume recovered	V_{mic}	% of V_{mic} transferred	V_{mic}	% of V_{mic} transferred
0	4.2	34	7.0	60	8.8	48
2	4.6	38	7.4	63	9.5	49
4	5.1	42	8.1	63	10.2	50
6	5.6	46	8.8	64	10.9	52
8	6.4	52	9.8	65	12.3	52
10	7.3	60	10.9	67	13.7	53
12	8.5	69	12.3	70	15.4	55
14	10.3	84	14	73	17.5	59
16	13	106	16.1	80	20.3	64

All volumes are ml kg^{-1}.
Volume transferred, volume moved from microcirculation to macrocirculation (from Fig. 6).
% volume recovered, volume transferred divided by hemorrhage volume (12.3 ml kg^{-1})×100%.
V_{mic}, estimated volume of microcirculation based on Hct_{mic} and F_{cell} (from Fig. 5).
% of V_{mic} transferred, volume transferred divided by V_{mic}×100%.

permeable to plasma protein based on the distribution volumes and kinetics of labeled albumin and the appearance of labeled albumin in tissues that do not appear to have a secondary circulation (Duff and Olson, 1989; Olson, 1992; Bushnell et al., 1998). The present experiments offer a novel methodological route to the same conclusion.

Three pieces of evidence support our conclusion that plasma proteins readily cross trout capillaries. (1) Volume recovery after volume expansion with plasma is rapid, equivalent to 4.5% of the plasma volume min^{-1}. By comparison, plasma protein turnover in mammals is approx. 2% h^{-1} (Aukland and Reed, 1993); this is less than one-hundredth of the rate we observed in trout. (2) In less than 90 min the volume lost from the circulation is the same, irrespective of whether the trout were volume-expanded with saline or plasma. If trout capillaries were an effective protein barrier, then after volume expansion with plasma, the amount of intravascular protein would increase as water was hydraulically driven across the capillaries. The resultant increase in plasma oncotic pressure would cause retention of fluid in the vasculature compartment, and at equilibrium the plasma volume after plasma injection would be greater than plasma volume after saline injection. Clearly this is not the case. Because volume recovery after plasma loading is somewhat slower than recovery after saline loading, it appears that the capillaries do indeed slow the rate of fluid extravasation, albeit minimally. This is unlike the situation in the rat skin and muscle where albumin and volume flux are not coupled (Renkin et al., 1988) and volume expansion (equal to the total blood volume) with lactated Ringer does not affect the rate of blood–tissue albumin transport in intact rats (Renkin et al., 1989). (The fact that saline does not isovolumetrically replace whole blood, and neither does plasma, should also be kept in mind during experiments requiring serial blood samples and fluid replenishment.) (3) The similarity between the kinetics of volume expansion with plasma and hemorrhage indicate that similar processes, i.e. movement of fluid and protein, are involved both situations. Furthermore, because the rates are the same, it is likely that the same amount of protein is being translocated in each experiment. This implies that the interstitial protein concentration is very close to that in the plasma and that whole-body reflection coefficients in trout may approach 0, even though the vessels in certain tissues, especially gill and brain, may be relatively protein-impermeable (Bushnell et al., 1998).

Note that when ^{125}I-albumin is injected into normovolemic trout, the rate of albumin efflux from the vasculature is considerably slower (Bushnell et al., 1998) than the apparent rate of plasma protein efflux observed when trout were volume-expanded with trout plasma in the present experiments. It seems likely that in the experiments by Bushnell et al. (1998), protein extravasation was diffusion-limited, whereas in the present experiments the convective transport of protein predominated. This is different from mammalian vessels where transcapillary flux of albumin is not coupled to solvent flow (Renkin et al., 1988).

The authors wish to express their gratitude to Dr S. S. Hillman for numerous helpful suggestions, to Dr R. E. Kingsley for assistance and advice in constructing the flow cell and to K. A. Brakora for technical assistance. Supported in part by NSF Grant No. IBN 9723306.

References

Albert, S. N. (1971). *Blood Volume and Extracellular Fluid Volume*. 273p. Springfield: Charles C. Thomas.

Aukland, K. and Reed, R. K. (1993). Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol. Rev.* **73**, 1-78.

Brill, R. W. and Bushnell, P. G. (2002). Cardiovascular system of tunas. In *Fish Physiology* Vol. 19, *Tuna Physiology, Ecology and Evolution* (ed. B. A. Block and E. D. Stevens), pp. 79-120. San Diego: Academic Press, Inc.

Bushnell, P. G., Conklin, D. J., Duff, D. W. and Olson, K. R. (1998). Tissue and whole-body extracellular, red blood cell, and albumin spaces in the rainbow trout as a function of time: a reappraisal of the volume of the secondary circulation. *J. Exp. Biol.* **201**, 1381-1391.

Conlon, J. M. (1999). Bradykinin and its receptors in non-mammalian vertebrates. *Regul. Pept.* **79**, 71-91.

Djojosugito, M., Folkow, B. and Kovach, G. B. (1968). The mechanism behind the rapid blood volume restoration after hemorrhage in birds. *Acta. Physiol. Scand.* **74**, 114-122.

Duff, D. W., Fitzgerald, D., Kullman, D., Lipke, D. W., Ward, J. and Olson, K. R. (1987). Blood volume and red cell space in tissues of the rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* **87A**, 393-398.

Duff, D. W. and Olson, K. R. (1989). Response of rainbow trout to constant-pressure and constant-volume hemorrhage. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **257**, R1307-R1314.

Farrell, A. P. and Jones, D. R. (1992). The heart. In *Fish Physiology* Vol. XII, Part A. *The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall, and A. P. Farrell), pp. 1-73. San Diego: Academic Press, Inc.

Farrell, A. P. and Olson, K. R. (2000). Cardiac natriuretic peptides: a physiological lineage of cardioprotective hormones? *Physiol. Biochem. Zool.* **73**, 1-11.

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

Hargens, A. R., Millard, R. W. and Johansen, K. (1974). High capillary permeability in fishes. *Comp. Biochem. Physiol.* **48A**, 675-680.

Hoagland, T. M. (2001). Blood volume perturbations affect venous function and cardiovascular homeostasis in the rainbow trout (*Oncorhynchys mykiss*). 239p. PhD thesis, University of Notre Dame, Indiana, USA.

Hoagland, T. M., Weaver, L., Jr., Conlon, J. M., Wang, Y. and Olson, K. R. (2000). Effects of endothelin-1 and homologous trout endothelin on cardiovascular function in rainbow trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **278**, R460-R468.

Johnson, P. C. (1971). Red cell separation in the mesenteric capillary network. *Am. J. Physiol.* **221**, 105-111.

Kovach, A. G. B., Szasz, E. and Pilmayer, N. (1969). Mortality of various avian and mammalian species following blood loss. *Physiol. Acad. Sci. Hung.* **35**, 109-116.

Lee, J.-S. (2000). 1998 Distinguished lecture: biomechanics of the microcirculation, an integrative and therepeutic perspective. *Ann. Biomed. Eng.* **28**, 1-13.

Loretz, C. A. and Pollina, C. (2000). Natriuretic peptides in fish physiology. *Comp. Biochem. Physiol.* **125A**, 169-187.

Nilsson, S. (1984). Adrenergic control systems in fish. *Mar. Biol. Lett.* **5**, 127-146.

Olson, K. R. (1992). Blood and extracellular fluid volume regulation: role of the renin-angiotensin system, kallikrein-kinin system, and atrial natriuretic peptides. In *Fish Physiology* Vol. XII, Part B. *The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 136-232. San Diego: Academic Press, Inc.

Olson, K. R. (1996). The secondary circulation in fish: anatomical organization and physiological significance. *J. Exp. Zool.* **275**, 172-185.

Olson, K. R. (1997). The cardiovascular system. In *The Physiology of Fishes* (ed. D. H. Evans), pp. 129-154. Boca Raton: CRC Press LLC.

Olson, K. R., Conklin, D. J., Farrell, A. P., Keen, J. E., Takei, Y., Weaver, L., Jr, Smith, M. P. and Zhang, Y. (1997). Effects of natriuretic peptides

and nitroprusside on venous function in trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **273**, R527-R539.

Perry, S. F., Fritsche, R., Hoagland, T. M., Duff, D. W. and Olson, K. R. (1999). The control of blood pressure during external hypercapnia in the rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **202**, 2177-2190.

Ploucha, J. H. and Fink, G. D. (1986). Hemodynamics of hemorrhage in the conscious rat and chicken. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **251**, R846-R850.

Renkin, E. M., Gustafson-Sgro, M. and Sibley, L. (1988). Coupling of albumin flux to volume flow in skin and muscles of anesthetized rats. *Am. J. Physiol. Heart Circ. Physiol.* **255**, H458-H466.

Renkin, E. M., Rew, K., Wong, M., O'Loughlin, D. and Sibley, L. (1989). Influence of saline infusion on blood-tissue albumin transport. *Am. J. Physiol. Heart Circ. Physiol.* **257**, H525-H533.

Renkin, E. M. and Tucker, V. L. (1995). Integration of capillary, interstitial and lymphatic function. In *Interstitium, Connective Tissue, and Lymphatics* (ed. R. K. Reed, N. G. McHale, J. L. Bert, C. P. Winlove and G. A. Laine), pp. 250-270. London; Portland Press.

Rowell, L. B. (1993). *Human Cardiovascular Control*. 500p. New York: Oxford University Press, Inc.

Russell, M. J., Klemmer, A. M. and Olson, K. R. (2001). Angiotensin signaling and receptor types in teleost fish. *Comp. Biochem. Physiol.* **128A**, 41-51.

Satchell, G. H. (1991). *Physiology and Form of Fish Circulation*. 235p. New York: Cambridge University Press.

Steffensen, J. F. and Lomholt, J. P. (1992). The Secondary Vascular System. In *Fish Physiology* Vol. XII, Part A. *The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 185-213. San Diego: Academic Press, Inc.

Takei, Y. (2000). Structural and functional evolution of the natriuretic peptide system in vertebrates. *Int. Rev. Cytol.* **194**, 1-66.

Tanaka, Y. (1979). Whole body transvascular filtration coefficient and interstitial space capacitance. *Jap. J. Physiol.* **29**, 181-193.

Tanaka, Y., Morimoto, T., Watari, H. and Miyazaki, M. (1976). continuous monitoring of circulating blood hematocrit. *Jap. J. Physiol.* **26**, 345-353.

Turner, A. H. (1937). Serum protein measurements in the lower vertebrates. II. In marine teleosts and elasmobranchs. *Biol. Bull.* **73**, 511.

Vogel, W. O. P. (1985). Systemic vascular anastomoses, primary and secondary vessels in fish, and the phylogeny of lymphatics. In *Cardiovascular Shunts: Phylogenetic, ontogenetic, and clinical aspects* (ed. K. Johansen and W. Burggren), pp. 143-159. Copenhagen: Munksgaard.

Wang, Y., Olson, K. R., Smith, M. P., Russell, M. J. and Conlon, J. M. (1999). Purification, structural characterization, and myotropic activity of endothelin from the trout, *Oncorhynchus mykiss*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **277**, R1605-R1611.

Zhang, Y., Jenkinson, E. and Olson, K. R. (1995). Vascular compliance and mean circulatory filling pressure in trout: effects of angiotensin converting enzyme inhibition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **268**, H1814-H1820.